

Review

EFFECTS OF ENERGY AND MACRONUTRIENT INTAKE ON COGNITIVE FUNCTION THROUGH THE LIFESPAN

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This paper reviews effects of both insufficient and excessive energy and macronutrient intake on cognition over the lifespan, including the prenatal period, infancy, childhood, and adulthood. In the brain, the hippocampus is particularly vulnerable to both protein malnutrition and energy undernutrition in utero or infancy, resulting in impaired neurogenesis and deficits in memory and spatial learning, reduced anxiety and increased impulsivity, reflected in impairments of neurotransmitter systems. Conversely, higher protein intake is associated with improved motor development but not mental development, whereas breastfeeding benefits mental development. Nevertheless, pre- and post-natal protein-energy supplementation has been shown to improve cognition in later childhood, and in adulthood, above energy alone. Similarly, n-3 essential fatty acid intake during pregnancy may have long-lasting effects on children's cognitive abilities. However, over-nutrition, particularly during gestation, may lead to metabolic programming that increases risk of cognitive impairment. In undernourished school children, breakfasts can benefit cognitive performance. In normally nourished children, benefits of breakfast are less clear; nevertheless, acutely, breakfasts lower in glycaemic index can benefit memory, whereas higher glycaemic breakfasts may improve vigilance. This difference depends on the impact of the carbohydrate on cortisol release. In adolescents and adults, moderate carbohydrate loads can benefit cognition, provided that glucoregulation is efficient. Protein intake may benefit memory by restraining task-related cortisol release. Chronically high intake of saturated fats may contribute to cognitive decline, whereas n-3 fatty acids, and perhaps reduced energy intake, may be protective. With ageing populations, dietary prediction of cognition in adults is a key area for research.

Key words: *glucose, protein, fatty acids, memory, dementia, cortisol, breakfast, cognition.*

INTRODUCTION

This review will briefly consider the impact on cognitive development and function, of variation in intake (insufficient or excessive) of the macronutrients, protein, carbohydrate and fat, and, where its effects can be separated, energy, over the human lifespan. For each macronutrient, attention will be paid to what is known about the role of specific forms for cognition and brain function. This review is mainly concerned with evidence in human beings, and emphasis is placed on highlighting current understanding of the evidence, rather than an extensive review of the literature.

All the macronutrients can provide energy, albeit with differing priorities and target organs, as well as carbon for structural functions, and many forms are fundamental to cellular processes governing growth, signalling and survival (Mann and Truswell, 2012). For the brain specifically, carbohydrates provide glucose as the primary energy source, amino acids in proteins provide the precursors for synthesis of neurotransmitters and create their receptors, while fatty

acids provide the structural basis of neuronal cell membranes. These properties point to ways in which macronutrient intake may be crucial for optimal brain function.

Firstly, the importance of adequate intake of energy and protein, both for the pregnant mother and thus the intrauterine nutritional environment for the foetus, and for the neonate and infant child, will be considered. Breastfeeding will briefly be compared to formula feeding, as the quantity and quality of fats and protein may have implications for cognitive development.

Next, the review will discuss evidence for the benefits of breakfast, and other meal types, for cognitive function in school children, adolescents and adults, comparing effects of different forms of breakfast, including the nature of the carbohydrates and the glycaemic index of the meal. For adolescents and adults, there is extensive literature on the effects of glucose loads on cognition, particularly memory and attention: this literature will be summarised, and the strengths and weaknesses of the evidence, and its implications, discussed.

Finally, evidence will be presented for the impact of different sorts of fat, and of total energy intake, on cognition in adults, both young and old; including discussion of the impact of diet over the lifespan on risk of cognitive decline in old age. Consideration will be given to possible mechanisms that might explain observed effects of diet on cognition, as well as limitations of current interpretations of the evidence.

MACRONUTRIENT INFLUENCES ON PRENATAL DEVELOPMENT AND INFANT AND CHILD COGNITION

The gestational and neonatal periods are crucial for brain growth, with the majority of neural development occurring within the first year after birth. Although environment and interventions can counteract early developmental disadvantage to some extent, early childhood cognitive function has been shown to predict later school performance (Grantham-McGregor *et al.*, 2007). Regrettably, approximately 200 million children around the world still suffer retarded development due to either malnutrition, as well as other effects of extreme poverty (Grantham-McGregor *et al.*, 2007). Data from the World Health Organisation on prevalence of nutritional status in preschool children from developing countries show that about 10% are wasted (loss of body tissue especially muscle), 17% are underweight and 29% are stunted (Anonymous, 2012). These children are often born small for gestational age, having suffered *in utero* from the effects of malnutrition and disease on their mothers, including during their mothers' own infancy (Victora *et al.*, 2008), and are most likely to suffer cognitive impairments too (Grantham-McGregor *et al.*, 2007). However, it is difficult to determine from these natural observations in epidemiological studies just what deficiencies are responsible for what consequences, as these children will typically be suffering from multiple deficiencies, both of macronutrients and energy, and key micronutrients such as iodine and iron, as well as frequent infections, diarrhoea and dehydration, and all the psychosocial impairments associated with extreme poverty (Grantham-McGregor *et al.*, 2007). Nevertheless, there have been some important intervention studies that have included indicators of cognitive development; these are now considered.

Protein-energy malnutrition. The major nutritional insult to infants and children worldwide is protein-energy malnutrition (PEM), which refers to insufficient intake of energy and protein (the two invariably occur together in 'natural' malnutrition) such that development is retarded. There are three forms: (a) marasmus (primarily an energy deficiency), characterised by severe wasting, but with good appetite; (b) kwashiorkor (primarily a protein deficiency), often seen in slightly older infants, is distinguished by presence of oedema and loss of appetite. By contrast with marasmus, the pathological signs may be more concerning (Mann and Truswell, 2012); (c) marasmic kwashiorkor, describes the presence of severe wasting and stunted growth, but also oedema, and so is likely to reflect both lack of energy and protein.

Although there is little consensus as to why children may develop either marasmus or kwashiorkor, the mortality rate for the latter is far higher than the former (Mann and Truswell, 2012). However, a recent study investigated whether the outcome of PEM for a given infant is determined by pre-existing metabolic differences that might arise from differing intrauterine nutritional environments. Specifically, Forrester *et al.* (2012) examined medical records of Jamaican children with diagnoses of kwashiorkor ($n = 391$), marasmus ($n = 383$), and marasmic kwashiorkor ($n = 375$): children of lower birth weight were more likely to have developed marasmus, whereas those with higher birth weights developed the more maladaptive kwashiorkor, with marasmic-kwashiorkor children having intermediate birth weights. This was interpreted as supporting maternal nutritional 'programming' of the child's metabolism *in utero* via epigenetic effects.

Alamy and Bengelloun (2012) recently reviewed evidence from effects of pre- and post-natal rodent models of protein and/or energy malnutrition on brain morphology and function, and on behaviour. An important conclusion was that both protein malnutrition and energy undernutrition in these models produce similar effects on health, brain and behaviour to those seen in malnourished children in developing countries. In fact, both forms of nutritional insult can damage the hippocampus, and result in deficits in learning and memory (Zhang *et al.*, 2010); postnatal and post-weaning malnutrition may be at least as damaging to the brain as that occurring prenatally, perhaps because the foetus may gain some protection by sacrificial changes in the mother's nutritional status (Alamy and Bengelloun, 2012).

The hippocampus is also part of the brain's emotional circuitry known as the limbic system: thus, it is not surprising that malnutrition has been found to reduce anxiety-related behaviour, and increase impulsivity. These effects may be mediated by changes in GABAergic, noradrenergic and serotonergic transmitter systems (Alamy and Bengelloun, 2012), and possibly also by the hunger-related hormone, ghrelin, which is known to increase novelty seeking and risk taking (Symmonds *et al.*, 2010; Hansson *et al.*, 2011).

How reversible are these deficits? The animal literature suggests that nutritional rehabilitation may allow recovery of body weight and even brain weight, but deficits in learning and memory and other behaviours tend to persist despite a return to adequate nutrition (Alamy and Bengelloun, 2012).

For infants, both intervention and observational studies suggest that higher intake of animal protein benefits growth and motor development, but may not improve mental function, in contrast to effects of breastfeeding (Kuklina *et al.*, 2004; Morgan *et al.*, 2004). One randomised controlled trial (RCT) in 294 pregnant Taiwanese women found that motor, but not mental, development of the infant offspring was improved by a protein-calorie supplement during pregnancy and breast feeding (Margolis, 2010).

However, mental benefits of early supplementation seem to emerge in older children. A particularly influential long-term study, the Oriente Longitudinal Study (Institute of Nutrition of Central America and Panama, 1969 to 1977), has produced a wealth of information on effects of protein-energy supplementation in mothers and children on mental and physical development (Engle and Fernandez, 2010). The intervention targeted all children under eight years in four villages in rural Guatemala, which were allocated one of two supplement drinks: 'Atole', containing 11.5 g protein and 682 kJ energy per cup, and 'Fresco', containing no protein and 247 kJ per cup; both drinks also contained vitamins and minerals. By adolescence, Atole resulted in superior cognition vs. Fresco, most evidently in children from lower socioeconomic status (SES) families (Pollitt, Gorman, Engle, Rivera and Martorell, 1995). However, the design does not exclude the possibility that the benefit of Atole is partly due to the extra energy, as much as to the protein, since the former may be more limiting in those villages: in correlational analyses, both greater energy intake and growth were positively related to cognitive performance (Engle and Fernandez, 2010). Importantly, these data have led to a more complex theoretical interpretation of how supplements improve cognition: that is, there is a positive feedback model in which supplementation hastens physical development and promotes proactive, appetitive and prosocial behaviours, which in turn allow for greater opportunities for social support, interaction, exploration and learning, leading to faster cognitive development (Engle and Fernandez, 2010).

Some other studies have been able to show relations between pre- or post-natal diet and adult cognition; however, the specific effects on cognition vary with the form of deficit. For example, data from 737 adults in their late fifties from the Dutch Famine Cohorts, who were *in utero* during famine in World War II, showed significantly impaired selective attention (de Rooij *et al.*, 2010). However, they showed no deficits in memory or perceptual motor skills tests. By contrast, in a large epidemiological study of 18,436 Chinese adults aged at least 50 (Guangzhou Biobank Cohort), recalled frequency of meat eating during childhood was strongly positively associated with verbal memory after adjusting for likely confounding factors (Heys *et al.*, 2010).

In summary, insufficiency of either protein or energy or both, pre- and/or post-natally, can impair psychomotor development and aspects of cognitive function. The relative importance of protein vs. energy for specific aspects of cognitive development is less clear, at least in human beings.

Polyunsaturated fatty acids in pregnancy, infancy and childhood. Long-chain polyunsaturated fatty acids (PUFA), specifically the n-6 PUFA arachidonic acid (AA), and the n-3 series PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are major structural and functional components of the CNS. These PUFA are synthesised from the dietary fatty acids linoleic acid (n-6) and alpha-linolenic acid (n-3), and are available from the diet in sources such as many plant or seed oils (for AA) and animal tissues espe-

cially oily fish (AA, EPA, DHA). These are essential fatty acids (EFA), with a wide range of biochemical and physiological functions, including as precursors for immune regulatory and anti-inflammatory chemicals that convey cardiovascular health benefits (Calder *et al.*, 2010). However, their role as major components of phospholipid bilayers in cell membranes, and in promotion of neurogenesis and neuroprotection, intracellular signalling and ion channel regulation, makes them of particular interest as contributors to CNS function and therefore cognitive development. The balance of n-3 to n-6 fatty acids may be critical for maintaining membrane fluidity and thus receptor expression. Thus, there has been considerable attention paid to the importance of such PUFAs for cognitive development in infants and young children (Calder *et al.*, 2010; Karr, Alexander and Winningham, 2011).

The rapid development of the brain during the third trimester of gestation and the first few months after birth make this period particularly sensitive to supply of both n-3 and n-6 EFA. The foetus depends on delivery via the placenta and the neonate via breast milk (or formula if not breast-fed), so the maternal EFA status should be a key factor in neonate brain development. Indeed, there is a striking protective effect of maternal oily fish consumption, or supplementation with EPA and/or DHA, against premature birth or miscarriage (Cetin and Koletzko, 2008), as well as predicting greater visual and motor development and verbal intelligence quotient (verbal IQ) (Helland *et al.*, 2003; Daniels *et al.*, 2004) and improved social behaviour (Hibbeln *et al.*, 2007). An emerging complexity is that polymorphisms associated with the fatty acid desaturase (FADS) gene cluster can affect the synthesis of EFA, especially for n-6 fatty acids in the foetus, as well as levels in breast milk, likely adding to variation in associations with brain development (Lattka *et al.*, 2013).

Delivery of EFA to neonates via breast milk might contribute to the beneficial effects of breastfeeding on cognitive development, though not all studies support an association (Keim *et al.*, 2012), perhaps because of confounding by maternal and neonate FADS genotypes (Steer *et al.*, 2010). Breastfeeding is often confounded with maternal IQ and socioeconomic status (Holme *et al.*, 2010), especially in countries such as the UK where infants breastfed even for 6–8 weeks are in the minority (Department of Health, 2011). Nevertheless, the results of a large-scale cluster-randomised controlled breastfeeding promotion, conducted in 31 maternity units in Belarus, support a clear beneficial effect of exclusive breastfeeding on children's IQ (Kramer *et al.*, 2008).

By contrast, interventions involving EFA supplementation of breast milk or formula, and in diets of older children, have suggested either no, or only weak, benefit (Karr *et al.*, 2011). A plausible conclusion is that the main benefit from higher intake of PUFAs is to be found during rapid brain development *in utero* and early infancy, although methodological weaknesses may contribute (Meldrum *et al.*, 2011). The existence of a critical window for dietary impact on

cognitive development has been underlined by recent evidence from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort: a 'health-conscious' diet (salad, rice, pasta, fish, fruit) at age 3, but not later ages, was associated with significantly improved IQ at age 8.5 years, whereas children eating more of a 'processed' diet (high in sugar and fat) tended to have lower IQ (Northstone *et al.*, 2012). This suggests that well-designed interventions are worthwhile and may yet produce clearer benefits.

Carbohydrates, breakfast and cognitive function in school-age children. Breakfast has potential to be 'the most important meal of the day' in young school-age children, by improving the benefit children receive from education at school. Moreover, it is widely accepted that the brain glucose utilisation rate in children is twice that of adults, with evidence from positron emission tomography (PET) that this high rate is maintained from age 4 to 10 years, then gradually declines to adult levels after age 16 (Chugani, 1998). Thus, primary school years should theoretically be a critical period for cognitive function being sensitive to energy supply and perhaps to carbohydrate-rich breakfasts in particular. Detailed consideration of the effects of breakfast vs. no breakfast on cognition is beyond the scope of this review, since it would not address mechanisms specific to particular macronutrients. To date, the consensus is that a benefit of breakfast on cognitive function in healthy children is disappointingly small and inconsistent, but greater in children with a history of suboptimal nutritional status (Hoyland *et al.*, 2009). Also, for performance on a few tests, particularly involving speed of information processing, there is evidence that fasted children do better than those receiving breakfast (Gibson and Green, 2002), a finding that also emerges from studies in adults, and presumably reflects differential effects of acute nutritional status on arousal (Gibson, 2007).

Variation in glucose utilisation rates with age could have contributed to variable results reported by Wesnes *et al.* (2003): 9–16 year old children were given either 38 g of glucose in a drink or two cereal-based breakfasts (29 g or 38 g carbohydrate) or no breakfast. Performance in spatial and working memory (but not attention) declined in the no-breakfast group during the morning, but even more so after glucose, at least for word recall. By contrast, performance after either of the cereals had not declined at all by midday, suggesting that the form rather than amount of carbohydrate may be critical.

Thus, there has been considerable interest in whether the glycaemic index (GI) and/or glycaemic load (GL, the product of % GI and weight of carbohydrate) of breakfasts differentially affects performance. Several studies have supported a benefit of low-GI/GL breakfasts on cognition, especially memory, in 6–11 year-old children compared to high-GI/GL breakfasts (Mahoney *et al.*, 2005; Benton *et al.*, 2007; Ingwersen *et al.*, 2007).

The interpretation of these findings, and practical implications, are complicated by evidence that increases in blood

glucose for three hours after breakfasts varying in carbohydrate content and form are determined more by GL than by GI, although insulin levels are influenced by GI (Micha and Nelson, 2011). With the exception of the study by Benton *et al.*, (2007), the impact of GL was not assessed. However, in an observational study of the impact of reported breakfast GI and GL on cognition in 60 11–14 year-old children, performance on different cognitive domains varied by GI, GL or both (Micha *et al.*, 2010). However, limitations of this study include the self-reported meal information, and categorisation of GI and GL by median split (medians were 61 and 27, respectively).

To try to account for this sort of variation in findings, Gibson and Green (2002) argued that the impact of these sorts of dietary manipulations on cognitive function "is likely to reflect individual differences in neurohormonal influences on glucose synthesis and uptake, interacting with task involvement and stress reactions, as well as nutritional state" (Gibson and Green, 2002, p. 186). For example, it is well established that food and performance testing alter autonomic nervous system (ANS) activity, which in turn can alter blood glucose levels and arousal state (Gibson and Green, 2002; Rogers and Lloyd, 1994). Gibson (2007) has further developed a theory that proposes that a meal-induced increase in blood glucose can interact with the stress of task performance to provoke a particularly marked increase in release of the glucocorticoid hormone cortisol, via activation of the limbic hypothalamic pituitary adrenal (HPA) axis, which in turn will alter cognitive function. This is based on evidence that a glucose drink prior to a stressful task elicits a large stress-induced cortisol release that correlates with post-drink blood glucose levels, and that high cortisol is associated with poorer memory (Kirschbaum *et al.*, 1997).

A recent study, in 74 11–14 year-old children, explicitly set out to investigate the relationships between breakfasts varying in GI and GL, cortisol, mood and cognitive function, using a 2 × 2 high/low-GI by high/low-GL design, as already validated by Micah *et al.*, (2011). The high- and low-GI breakfasts used cornflakes and muesli respectively, and GL was varied by using different amounts of the breakfast cereals and with or without apple juice. High-GI breakfasts (irrespective of GL, and energy levels) resulted in higher cortisol levels both immediately before and after the cognitive testing. Relative to low-GI breakfasts, high-GI meals impaired verbal memory but improved speed of information processing, similar to reports in adults (Gibson, 2007). If verbal memory is more important for learning at school than information processing speed, then low-GI breakfasts would be the preferred nutritional preparation for school (Micha *et al.*, 2011). Of course, most studies comparing GL/GI differences also vary the other macronutrients, and perhaps total energy, particularly for meals differing in GI, so mechanistic interpretations need to be made cautiously.

In adolescents aged 14–17 years, recall of words after a 60-min delay was slightly impaired after a high-GI (and higher GL) breakfast, but at 100 min was improved, whereas per-

formance after a low-GI breakfast remained unchanged (Smith and Foster, 2008). These times coincided, respectively, with the peak change in blood glucose and the point at which blood glucose had returned to baseline. In line with the theory discussed earlier, it is possible that the impairment at 60 min reflects a greater increase in cortisol induced by the high-GI breakfast (and so higher blood glucose) in combination with a demanding task, but this was not assessed.

MACRONUTRIENT EFFECTS ON COGNITION IN YOUNG ADULTS

Facilitation of cognitive function by glucose. The majority of research relevant to macronutrients and cognition in young adults has concerned the effects of glucose drinks, and there have been several recent reviews of this glucose facilitation effect (Gibson, 2007; Hoyland *et al.*, 2008; Smith *et al.*, 2011). This section will briefly summarise the consensus, interpret recent new evidence and discuss the implications.

The idea that ingesting glucose could improve cognitive function emerged from the knowledge that glucose is the primary energy source for the brain, and hypoglycaemia is associated with cognitive impairment, although even in the early studies there were indications that the relationship may be more complex. For example, it became clear that the nature of the task (cognitive domain and difficulty), the dose of glucose and the glucoregulatory efficiency of participants could all influence the outcomes (Gibson and Green, 2002; Meikle *et al.*, 2004; Riby *et al.*, 2004); moreover, the association between changes in peripheral blood glucose and delivery of glucose to neurones is loose at best, not least because glucose (and blood) supply to neurones is mediated by astrocytes and oligodendrocytes (Lee *et al.*, 2012).

A systematic review of the impact of glucoregulation on cognition concluded that it was clearly associated with poorer performance, especially for memory and in older people (Lamport *et al.*, 2009); moreover, ingesting glucose was more likely to benefit those with poor glucoregulation. Indeed, the effect of glucoregulation can obscure any treatment benefit from glucose (Messier *et al.*, 2011). These findings could again suggest a role for elevated cortisol levels. Furthermore, dissociation of task difficulty from task domain suggests that glucose facilitation of cognition is dependent more on domain (e.g. memory) than on task difficulty (Riby *et al.*, 2006).

Given this non-linear relationship between blood glucose and performance across individuals, it is perhaps not surprising that several studies found an inverse-U shaped dose-response curve, with the most beneficial dose seeming to be about 25 g glucose, although this varied somewhat with the nature of the task (Gibson, 2007). In a recent study involving administration of 0 g, 15 g, 25 g, 50 g or 60 g glucose, there was some support for a peak benefit at 25 g, particularly for longer-delay verbal memory tasks, but this dose relationship was less apparent in others, such as working memory tasks (Sünram-Lea *et al.*, 2011). One moderating factor was again glucoregulation, such that participants with high blood glucose responses to the higher doses were less likely to show benefits from those doses. The basis of the narrow effective dose range may relate to the brain's management of energy supply (Peters *et al.*, 2004), where oversupply of glucose could provoke greater inhibitory neuronal activity (Gibson, 2007), and/or a trade-off between speed of learning and speed of recall (Sünram-Lea *et al.*, 2011). However, moderation by counter-regulatory hormones or other neuroendocrine responses may also play a part (Gibson, 2007; Rogers and Lloyd, 1994) (see Fig. 1).

To some extent, another study has shed light on the question of whether the glucose dose is dependent on energy

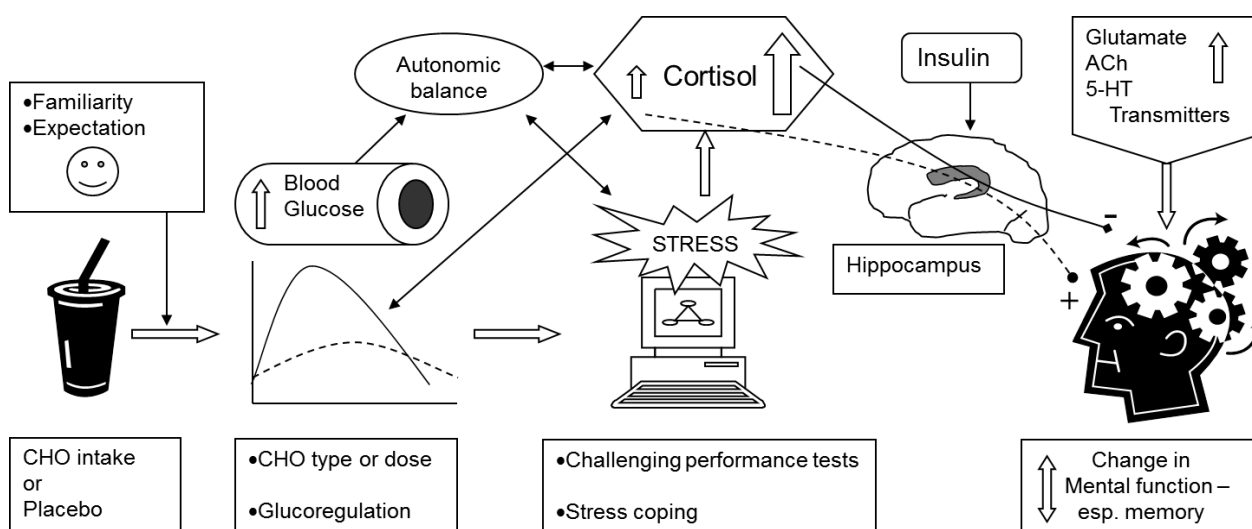


Fig. 1. This diagram summarises putative pathways linking carbohydrate intake and mental function, including various influences that can prevent or reverse an otherwise beneficial effect. Moderate increases in blood glucose are more likely to improve mental function, especially memory. Conversely, higher glucose increases, especially if accompanied by poor glucose tolerance, may enhance release of cortisol during the challenging performance tests, which could result in relatively impaired cognition (CHO, carbohydrate; ACh, acetylcholine; 5-HT, 5-hydroxytryptamine or serotonin). Reproduced from Gibson (2007) with permission of the author.

status. Thus, Owen *et al.* (2012) examined the interaction of a 12-hr vs. 2-hr fast on cognitive function following either a 25-g or 60-g dose of glucose (or placebo): the higher dose benefited performance after the longer fast, and vice-versa for the shorter fast, suggesting that the dose-response curve is shifted to the right by energy deprivation. Interestingly, regardless of the drink condition, those participants with better glucoregulation appeared to be calmer and to perform better: this might implicate higher levels of counter-regulatory hormones such as adrenaline or cortisol, which would raise blood glucose, being present in poor glucoregulators and perhaps impairing performance on some tasks. A further complication by is that a high-GI vs. low-GI evening meal can improve memory following a high-GI breakfast the next day (Lamport *et al.*, 2011).

Acute effects of meals varying in macronutrient content on cognition in adults. Whereas eating breakfast at all may benefit memory (Gibson and Green, 2002), this section briefly considers a series of studies where the macronutrient content, and/or GI or GL of the breakfasts was varied. Clearly, the results for breakfasts of differing GI and GL in children, discussed above, suggest that the relationships between these factors and cognitive performance are not straightforward. Benton *et al.* (2003) compared 'breakfasts' consisting of decaffeinated tea or coffee with skimmed milk, a sugar-free orange drink, and either 50 g of a plain biscuit (low GI) or 50 g of a cereal bar (high GI) on verbal memory performance. Significant differences in global memory scores were only apparent for performance at 150 and 210 min after breakfast (by which time blood glucose had returned to baseline, but cortisol may have been raised); participants recalled more words at these times after the low-GI food than after the high-GI food.

In another study, in participants with good glucose tolerance, variation in breakfast GL had little impact on their verbal memory; by contrast, poor glucoregulators performed worse on the word recall test after 30 and 50 g carbohydrate breakfasts compared to a 15 g carbohydrate meal (Nabb and Benton, 2006). This again suggests that a large rise in blood glucose has a negative effect on memory. Conversely, higher blood glucose was associated with improved performance on vigilance and reaction time tests. These differential findings are similar to those reported recently in children (Micha *et al.*, 2011), and it is therefore not surprising that glucose ingestion or high-GI food also impairs memory in Type II diabetics (Papanikolaou *et al.*, 2006).

In a comparison of muesli (low GI) versus cornflakes (high GI) versus an overnight fast, participants' reaction times on a Stroop colour-word test were quickest when fasted (cp. arousing effect of deprivation discussed above), intermediate after cornflakes and slowest after muesli (Gibson, 2007); however, the 'Stroop effect' of failure of response inhibition to incongruent colour-word stimuli did not differ between breakfasts, suggesting that the effect was specific to information processing speed. On a word recall test, number of words remembered did not differ across breakfasts, al-

though after muesli participants produced more new words not listed (false positives); also, only this muesli group resisted deterioration in mood from before to after testing.

For macronutrient content, there is surprisingly little evidence to support a consistent pattern of effects (Hoyland *et al.*, 2008). However, in one carefully designed study, Fischer *et al.* (2001) fed subjects different 'breakfasts' consisting of one of three 400-kcal vanilla creams with that contained either protein, fat or carbohydrate. The fat condition produced the most reliable benefit for cognitive performance, including memory, attention and choice reaction time relative to protein or carbohydrate intake. This was attributed to the comparative absence of glycaemic and hormonal (insulin, glucagon and cortisol) perturbations in the 3 hr following fat ingestion, relative to the other two macronutrients. In a subsequent study using similar methods, effects on performance and hormones were assessed after 400-kcal meals varying in carbohydrate and protein in the respective proportions of 4:1, 1:1, and 1:4 (Fischer *et al.*, 2002). Effects varied over time, with an early benefit from the high-carbohydrate food on attention and decision times, whereas after the first hour, the higher protein conditions produced greater improvements in performance.

These later effects from protein maybe related to changes in availability of neurotransmitter precursor amino acids, such as tryptophan (TRP) and tyrosine (TYR), to the brain. Typically, high-protein results in less TRP being available to the brain via competitive transport across the blood brain barrier, thus reducing brain serotonin synthesis, and possibly increasing alertness and thus influencing cognition (Gibson and Green, 2002). In support of this, studies comparing high-protein/low-carbohydrate vs. low-protein/high-carbohydrate lunches have found that high protein intake was associated with faster responses and more alertness, although also greater susceptibility to distraction (Lieberman *et al.*, 1986; Smith *et al.*, 1988).

In a recent well-designed cross-over study, comparing macronutrient effects on cognition, Jones *et al.*, (2012) tested the effects of isocaloric 300-ml drinks (145 kcal) containing either 38 g glucose (contrary to the 25 g stated in the abstract; E. Jones, personal communication), 36 g (whey) protein or 16 g vegetable oil (in emulsion), or a non-caloric placebo (aspartame), on memory and attention. The results differed from some previous findings, but did share similarities with the macronutrient proportion effects reported by Fischer *et al.*, (2002): glucose enhanced attention and glucose and fat slightly increased speed of processing at 15 min after the drink, but glucose impaired working memory at 60 min. By contrast, protein enhanced working memory at 15 min and episodic memory at 60 min. Rather than consequences of initial nutrient absorption, the early effects might reflect expectations in response to sensory differences, which would have been apparent even though the drinks were taken through a straw from opaque containers, and all flavoured with lemon juice; for example, alertness was rated as higher at 10 min than at baseline and later, for all drinks including sweet placebo. Quenching of thirst is a

possibility for this effect, as the authors suggested. However, the effects might result from neurohormonal responses to sensory or early nutrient detection. At 60 mins, differential effects reflecting absorbed nutrients are more likely; the impaired memory after 38 g glucose is similar to the effect of the same dose of glucose in children, and is in line with previous evidence that large increases in blood glucose are associated with memory impairment.

However, these results do not yet allow for reliable predictions of acute cognitive effects from macronutrient intake, let alone understanding of mechanisms. As pointed out by a systematic review of the area, there are too few studies comparing effects of protein, fat and carbohydrate manipulations on cognition to date (Hoyland *et al.*, 2008).

What is the relationship between such findings comparing variations in macronutrient intakes and the effects of glucose drinks discussed above? One difference may be the absolute energy values concerned; that is, the optimal dose of 25 g glucose contains just 94 kcal energy, so greater levels of energy from breakfasts or macronutrient loads may invoke quite different neurohormonal consequences that in turn alter cognitive function in different ways and with different temporal effects (Gibson and Green, 2002). Indeed, larger doses of glucose might well be sedating, via increased TRP entry to brain (Gibson, 2007).

Dietary fats and cognitive function in young adults. A recent longitudinal epidemiological study has found evidence for cognitive decline in adults as early as young middle-age: a cohort of 10,308 British civil servants aged 45–70 (the Whitehall II study) was assessed for cognitive function on three occasions over ten years (Singh-Manoux *et al.*, 2012). Cognitive decline was apparent in all age groups, even in those aged 45–49 at baseline; moreover, the effect was probably underestimated due to practice effects. Such cognitive decline in a relatively healthy group is most likely to be due to lifestyle factors including diet, perhaps interacting with genetic susceptibility. Yet, detectable cognitive decline is thought to result from changes taking place over 20–30 years (Launer, 2005); therefore it is possible that chronic dietary factors may already be affecting the cognition of younger adults.

There is already good evidence (see the next section) that chronic intake of dietary saturated fats can adversely affect cognitive function in the elderly (Kalmijn *et al.*, 2004; Kanoski and Davidson, 2011). This includes the finding that the fat intake of 40–50 year-olds predicted cognitive decline 20 years later (Eskelinen *et al.*, 2008). Gibson *et al.*, (2011) tested the hypothesis that habitual consumption of dietary fats could be predictive of cognitive function in women aged 25–45 years (N = 38). The women's dietary fat intakes, particularly trans fats and the saturated fat to PUFA ratio, were negatively related to learning and memory performance: no associations with carbohydrate or protein intake were found.

EFFECTS OF MACRONUTRIENTS AND ENERGY INTAKE ON COGNITION IN MIDDLE-AGED AND ELDERLY ADULTS

Impact of glucose and glucoregulation on cognitive function in older adults. The negative impact of poor glucoregulation on cognitive function seems if anything to be more marked in older adults, perhaps in part because of lower baseline performance. A systematic review of studies relating glucoregulation to cognition considered 23 studies of which 20 contained middle-aged or elderly participants (Lamport *et al.*, 2009). Twelve of these studies assessed impaired glucose tolerance (IGT) using an oral glucose tolerance test (OGTT): most of these did not support a relationship between glucose tolerance and cognition; however, the tests were mostly quite crude assessments of cognitive impairment such as the Mini Mental State Examination (MMSE). Also, the one study that measured glucose tolerance longitudinally did find a negative effect of impaired glucose tolerance (Vanhanen *et al.*, 1998), with hyperinsulinaemia being a significant predictor of impairment.

The remaining studies examined relations between glucose tolerance within the normal range and cognition: whilst most of these studies used more sensitive measures of cognition, the assessment of glucose tolerance was inconsistent and usually cruder than using an OGTT. Even so, the majority of the studies found evidence for a negative relationship between impaired glucose tolerance and cognitive performance, particularly for memory-based tests, although there were some inconsistent differences between sexes. Several of these studies also examined the impact of ingesting glucose on cognition. In 57 non-diabetic men and women aged 55–84, poor glucoregulators performed worse on tests of working memory, verbal memory and executive function, with the more elderly (over 72) being worst affected (Messier *et al.*, 2003).

Another study addressed whether the GI of foods might differentially affect cognition in the elderly: in ten men and ten women aged 60–82 years, without diabetes, Kaplan *et al.* (2000) compared the effects of three carbohydrate-rich foods varying in GI (glucose, potato, barley, or placebo) on cognitive performance. The foods did not differ in their effects overall, nor did changes in blood glucose arising from eating them, but those with baseline indices of poor glucoregulation benefited more from the foods vs. placebo, except on tests of attention.

A subsequent study by Kaplan *et al.* (2001), in men and women aged 61–79, compared effects of pure glucose, whey protein or fat emulsion in isocaloric 774 kJ drinks with a placebo non-caloric drink. All the macronutrients improved memory compared to placebo, despite blood glucose only rising appreciably after glucose. On other tests, there was some variation between macronutrients, such as improved visuomotor performance after glucose, and better attention 60 min after the fat drink. This suggests that there is an acute benefit from energy intake independent of blood

glucose changes, but also some differential effects of macronutrients.

Dietary fats and cognitive function in older adults. Both experimental studies in animals and epidemiological studies in older humans supported a link between high saturated fat intake and cognitive decline, or suggested a protective effect of higher PUFA intake (Kalmijn, 2000). However, there were exceptions: in 2002, results from a large 6-year prospective study of 5,395 Dutch adults (the Rotterdam Study), which included a semi-quantitative food frequency questionnaire, and monitoring for dementia, did not find any association between either high intakes of saturated and trans fats, and cholesterol, or low intakes of MUFA and PUFA, and risk of developing dementia, after adjusting for likely confounds (Engelhart *et al.*, 2002). Nevertheless, only 3.6% had been diagnosed with dementia over that period, and more subtle declines in cognitive function were not assessed. In a three-year prospective study of 482 women aged 60 or more, no association between cognitive decline and intake of saturated and trans fats was found; however, greater MUFA intake was associated with less decline (Naqvi *et al.*, 2011).

Nevertheless, evidence continues to grow that supports dietary fats being important risk factors for cognitive health in older adults. For example, in a cross-sectional study of 1613 adults aged 45–70 (Kalmijn *et al.*, 2004), intake of n-3 PUFA, EPA and DHA was associated with reduced cognitive decline; by contrast, dietary cholesterol and saturated fats were associated with increased decline, after adjusting for confounders including energy. In a six-year longitudinal study of 2560 American adults aged 65 or over, high intakes of saturated and trans fats, and low intake of MUFA and PUFA/saturated fat ratio, were associated with cognitive decline (Morris *et al.*, 2004). In the Women's Health Study, in 6,183 older participants over less than a decade, higher intake of saturated fat, and lower intakes of MUFA, were associated with worse global cognition and verbal memory (Okereke *et al.*, 2012).

Beyond mild cognitive decline, there is growing interest in risk factors for dementia, given the world's increasingly elderly population. Over the last decade or so, several observational epidemiological studies, including thousands of participants, have examined the relationship between fish consumption, and/or n-3 fatty acid intake, or blood or erythrocyte membrane levels of n-3 fatty acids and incidence of dementia (Brown, 2012). The majority reported a protective effect of higher fish or n-3 intake, or higher membrane levels of n-3 EFAs; the negative studies tended to be smaller sample sizes, the exception being data from the Rotterdam cohort study discussed earlier. There are also some reports of increased risk of dementia associated with high n-6:n-3 fatty acid ratio (Conquer *et al.*, 2000). An additional complication is that people vary in genetic predispositions affecting metabolism and functions of dietary fats: for example, carriers of the $\epsilon 4$ allele for apolipoprotein-E have deficiencies in lipid transport and metabolism and increased risk of dementia including Alzheimer's disease (Martins *et*

al., 2006); they also seem to be more resistant to potentially beneficial changes from n-3 EFA supplementation (Barberger-Gateau *et al.*, 2011), although there is one report of this phenotype benefiting from n-3 EFA supplements (van de Rest *et al.*, 2008). Moreover, FADS genotypes (discussed above) may influence the availability of n-3 fatty acids from the diet to the brain (Brown, 2012).

The logical progression from observational evidence that does not allow causal conclusions is to conduct RCTs of n-3 EFA supplementation. A limited number of such studies have been conducted, although they vary considerably in both intervention period (6–40 months) and treatment doses (0.2–1.8 g EPA; 0.4–2 g DHA). Treatments were effective in increasing biomarkers of EFA incorporation, but by and large did not show significant benefits to cognitive function across a range of performance domains, nor differences in cognitive decline compared to placebo. However, subgroup analyses suggest that benefits can be detected if those with adequate EFA intake and participants with more than mild cognitive impairment at baseline are excluded (Brown, 2012). Nevertheless, a recent Cochrane Database review of three of the longer interventions (2–3 years) concluded that there was no evidence overall that n-3 EFA supplements either prevented dementia or benefited cognition in healthy older adults, although only one of the studies used sensitive tests over a range of cognitive functions (Sydenham *et al.*, 2012).

EFFECTS OF CHRONIC ENERGY RESTRICTION ON COGNITIVE FUNCTION IN ADULTS

In animal models, especially studies in rodents, but also non-human primates, long-term caloric restriction, in the region of 25–40% below standard energy intake, has been shown to increase longevity and indices of health, including amelioration of age-related decline in learning and memory (Mattson, 2010). Likely mechanisms include enhancing brain energy efficiency, particularly in the hippocampus, for example by increasing insulin sensitivity via glucagon-like peptide 1 (GLP-1) activity (Mattson, 2010). Beneficial effects on synaptic plasticity by increasing brain derived neurotrophic factor (BDNF) may also be a mechanism (Stranahan and Mattson, 2011). Nevertheless, not all animal studies have been able to show benefits to cognition following chronic caloric restriction in rodents (Bellush *et al.*, 1996).

It has been argued that cognitive function in human beings might similarly benefit from restricted energy intake, given the global predominance of excess energy and fat intake, and obesity (Kanoski and Davidson, 2011). However, there are few studies that have tested this hypothesis in human adults, and these mainly involve quite short-term restrictions, use of overweight or obese participants, as well as variation in the ketogenic effects of the diets (Kretsch *et al.*, 1997; Krikorian *et al.*, 2012).

In 49 moderately overweight 50–80 year-old men and women, an intervention to reduce energy intake by 30% improved memory after three months, compared to either a group attempting to increase unsaturated fat intake or a control group (Witte *et al.*, 2009). The improvement in memory was significantly related to the reduction in fasting insulin in the caloric restriction group. These and other encouraging findings have led to the start of a long-term intervention, the CALERIE study (Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy) (Rochon *et al.*, 2011), that will investigate outcomes including cognition, in non-obese adults, following two years on a 25% energy reduction diet.

SUMMARY AND CONCLUSIONS

It is clear that both macronutrient and energy intake can have substantial impacts on cognitive function at all stages of the lifespan, particularly where there is obvious insufficiency or excess. The number of children at risk of diet-related retarded development globally remains disturbingly high. The hippocampus is particularly vulnerable to protein and energy deficiencies, resulting in impaired neurogenesis and deficits in memory and spatial learning, reduced anxiety and increased impulsivity, reflected in impairments of neurotransmitter systems. Pre- and post-natal protein-energy supplementation has been shown to improve cognition in later childhood above energy alone; developmental assessment in very young children may be biased to detecting psychomotor rather than cognitive improvements. Both maternal undernutrition and low protein intake during childhood can impair cognitive function in adulthood.

Higher maternal intake of n-3 EFA during pregnancy has been associated with better cognitive, behavioural and psychomotor outcomes of offspring. Nevertheless, EFA supplementation in young children has not produced consistent evidence of benefits, perhaps indicating a sensitive period for the need for EFA during early brain development. Yet, a diet dominated by energy-rich, high-fat/sugar foods and low in EFA specifically in early childhood may impair cognitive development.

In undernourished school children, breakfasts can benefit cognitive performance. In normally nourished children, adolescents and adults, benefits of breakfast are less clear; nevertheless, acutely, breakfasts lower in GI can benefit memory, whereas higher glycaemic breakfasts may improve vigilance or speed of information processing. This difference depends on the impact of the carbohydrate on cortisol release, with higher GI breakfasts being more likely to result in increased cortisol levels during challenging tasks. These effects interact with differences in glucoregulation, particularly in older adults. Protein intake may benefit memory, perhaps by restraining task-related cortisol release.

Chronically high intake of saturated fats may impair memory, even in relatively young adults. In time, this is likely to contribute to cognitive decline and dementia, whereas in-

take of n-3 fatty acids may be protective. There is also preliminary evidence that a reduction in energy intake may improve cognition, at least in older adults, possibly mediated by improved insulin sensitivity. Dietary prediction of cognition in adults is a key area for research, especially given our increasing lifespans and the cost to society of caring for our elderly population.

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ENERĢIJAS UN BARĪBAS VIELU UZŅĒMŠANAS IETEKME UZ KOGNITĪVAJĀM FUNKCIJĀM DZĪVES LAIKĀ

Šajā rakstā tiek apskatīta gan pārmērīga, gan nepietiekama barības vielu un enerģijas uzņemšana cilvēka dzīves laikā, ietverot grūtniecības periodu, zīdaiņa vecumu, bērnību un pieaugušā vecumā. Hipotalams ir jutīgs pret olbaltumvielu un enerģijas nepietiekamu uzņemšanu gan prenatālajā periodā, gan agrā bērnībā, izraisot traucētu neiroģenēzi, atmiņu un telpisko uztveri, palielina impulsivitāti, kas atspoguļojas neiromediatoru sistēmā. Turpretim, pastiprināta olbaltumvielu uzņemšana tiek saistīta ar uzlabotu motoro attīstību, bet ne ar garīgo attīstību. Tomēr krūts barošana labvēlīgi ietekmē garīgo attīstību. Ir pierādīts, ka olbaltumvielu un enerģijas papildus lietošana pirms un pēc dzimšanas uzlabo kognitīvās funkcijas vēlāk bērnībā, bet pieaugušo vecumā tās uzlabo tikai enerģija. Omega-3 taukskābju lietošanai grūtniecības laikā varētu būt ilgstoša iedarbība uz bērnu kognitīvajām spējām, lai gan jāatzīmē, ka pārmērīga to uzņemšana, sevišķi grūtniecības laikā, var izraisīt vielmaiņas traucējumus, kas kognitīvos traucējumus var palielināt. Skolas vecuma bērniem, kas nesaņem pietiekamu uzturu, brokastis var uzlabot kognitīvos rādītājus. Bērniem, kas saņem pietiekamu uzturu, brokastu ievērošanas ietekme nav tik skaidra, tomēr brokastis ar samazinātu glikēmisko indeksu, var labvēlīgi ietekmēt atmiņu, savukārt brokastis ar augstu glikēmisko indeksu var palielināt modrību. Pieaugušā vecumā mērena ogļhidrātu uzņemšana var labvēlīgi ietekmēt kognitīvās funkcijas ar nosacījumu, ka glikoregulācija ir optimāla un atbilstoša normai. Olbaltumvielu uzņemšana var labvēlīgi ietekmēt atmiņu. Palielinoties senioru vecuma iedzīvotāju skaitam, diētas ietekme uz kognitīvajām spējām pieaugušā vecumā ir viena no galvenajām pētījuma jomām.

Mini Review

HEALTHY ALTERNATIVES OF THE MEDITERRANEAN DIET IN LATVIA

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The milestone of illness prophylaxis is a healthy lifestyle, which is composed of regular physical activity and a healthy diet. Following the Mediterranean diet for two years has been shown to have significant decrease in cardiovascular death by 9%, cancer by 6%, Parkinson's and Alzheimer's by 13%. This diet helps to control the perfect weight, improves lipid profile and diminishes the risk of diabetes. The Mediterranean diet consists of extra virgin olive oil, vegetables and fruit, wholegrain products, legumes, nuts and seeds, dairy products (with no other sources of fat other than milk fat), fish (at least twice a week), poultry, veal, pork in limited amount, and eggs — 0–4 per week. It is possible to adapt this kind of alimentation in the Nordic countries, but it is important to find products grown there with similar nutritional characteristics. Nowadays, fresh fruits and vegetables can be bought all year round, but it is essential to use seasonal products. In Latvia, at this point, attention should be brought to more efficient storage and conservation. We have a vast variety of legumes and cereals. The selection of dairy products should be bigger and of higher quality, because you rarely see local cheeses made in an artisanal manner at the market-places. There is good availability of saltwater fish in the cities, but in the countryside the only fish one can buy is salted and smoked, having exaggeratedly high amounts of salt. Consumption of meat and its products should be lowered to a maximum of three times per week. A special attention should be brought to game (such as deer), because it contains low levels of cholesterol and higher amounts of unsaturated fatty acids due to the alimentation of wild herbs. Unfortunately, there is a lack of good quality oil in Latvia, because no other product can be compared to the nutritious components of extra virgin olive oil and its effects on cardiovascular health. Consumption of high amounts of olive oil decreases the incidence of stroke by 41%. Education should be conducted widely to promote tradition and gastronomic heritage as a cultural aspect. Healthy lifestyle has to be visible to everyone at any time as a constant reminder of its importance.

Key words: cardiovascular disease prevention, healthy lifestyle, Mediterranean diet.

INTRODUCTION

It is known worldwide nowadays that the most frequent cause of death is cardiovascular disease (CVD); about 4.3 million deaths in Europe annually (de Lorgeril *et al.*, 1999). In Latvia the incidence is among the highest in Europe, for men in particular, being more than 130 deaths in 100 000 people and 20 to 50 for women (Anonymous, 2006). Many uncontrollable factors influence the formation of CVD, such as age, genetics, race, but the primary cause of CVD is unhealthy lifestyle, such as bad diet, visceral obesity, smoking, little or no physical activity, which are all modifiable aspects for preventing the risk.

HEALTH BENEFITS AND CULTURAL IMPORTANCE OF THE MEDITERRANEAN DIET

After industrialisation, many refined foods have become available, industrial farming with substances of chemical

synthesis has spread widely and physical activity for people has decreased significantly with the availability of transportation and sedentary work, encouraging an unhealthy lifestyle. People often complain about lack of time to prepare meals at home, meaning that sausages, sandwiches or frozen dumplings are their first choice. The problem here is not so much the little free time, but the insignificant value given to food. Considering eating as something important and cultural makes you organise your daily life more efficiently in order to be able to dedicate time for a meal. The Mediterranean way of life is a great example where food still today has great importance.

Since 2010, the Mediterranean diet has been inscribed for the UNESCO Representative List of the Intangible Cultural Heritage of Humanity. Based on century old traditions of Spain, Italy, Morocco and Greece, it has become very popular in the whole world and has been considered as one of the healthiest diets to follow.

Latvia is one of the few countries left in Europe that still has a good concentration of small non-intensive farms, large areas covered with forests, many rivers and lakes, and the longest part of the boarder has access to the sea. We have a great potential for obtaining high quality products. This fact confirms the possibility to adapt a diet as healthy as the Mediterranean also in Latvia.

The Mediterranean diet “is a set of traditional practices, knowledge and skills passed on from generation to generation and providing a sense of belonging and continuity to the concerned communities.” (UNESCO)

The climate of the Mediterranean Sea is very favourable for cultivation of grains, vegetables, fruit and most importantly the olive tree. It is essential to keep in mind that when we talk about the Mediterranean diet, we must consider not only what foodstuffs are used. It is a whole culture of techniques of production, conservation, preservation and conviviality, meaning time, place and people whom to share a meal with (Fundación Dieta Mediterránea). A part of the diet still remains touched only a little by the industrialisation.

As mentioned before, the Mediterranean diet is based mainly on wholegrain products (bread, pasta, couscous), extra virgin olive oil, vegetables and fruit. Fish and meat are consumed in a moderate amount and meals are accompanied by wine or herbal infusions. A lot of condiments and spices are used, which means that salt is added in limited quantities, because spices exalt the salty taste.

People tend to cook a lot at home even now when everything is readily available at the supermarket, so refined foods and carbonated soft drinks are not the first choice.

FOOD CATEGORIES LOOKED AT CLOSER. MEDITERRANEAN DIET AND LATVIAN ALTERNATIVES

Wholegrain products — rich in fibre, improve digestion and have a low glycemic index. Bread is mostly made from unrefined stone-milled flour. Pasta in Italy is consumed “al dente”, digested slower than fully cooked pasta, letting the glucose levels in blood rise slowly. Rice is more healthy when consumed unrefined. Complex carbohydrates are the major part of the Mediterranean diet. Wholegrain category of foodstuffs is essential for patients with diabetes.

Cereals — wheat, rye and oats historically are the main cultivated plants in Latvia, so this category of diet is fully available locally.

Fruit and vegetables — full of vitamins, fiber, and phytonutrients. High consumption of tomatoes containing lycopene has been proved a good antioxidant. Cruciferous plants, like cabbages, broccoli, horseradish and other vegetables containing sulfurous compounds are anti-carcinogenic and some can be conserved for wintertime. Consumption of different vegetables and fruits should be a part of each meal.

Since the summer period is very short in Latvia, vegetable preserves can be made. More care should be brought to conservation of fresh produce. It is essential not to forget that salt is necessary only in the right amount for conservation.

Latvia has a wide variety of berries, which are excellent for summertime, like blackberries, cranberries, wild strawberries and raspberries. For winter, apples are a good choice if conserved properly. People who have a garden very often throw away the fruit that they are not able to consume until the end of summer. Instead of wasting a perfectly good product, juices and jams with low sugar content can be made, with low pH to keep them well preserved.

Greens and legumes. In the Mediterranean region a lot of salad is consumed, which contains bitter compounds of different chemical compositions that work as nutraceuticals. Chicory is a good example of bitter greens. The bigger the variety of green leafed plants consumed, the more the chance to consume enough microelements to improve one's health. Rocket, that can be bought at the market or grown in one's garden, have a tingling property that is due to an anti-carcinogenic compound. Spinach is a good source of iron.

Sometimes when a Latvian is asked, how often he consumes legumes, the response is: “At Christmas as the traditional dish of marble peas with bacon.” Since one's daily diet should consist of 50% carbohydrates, legumes are the perfect source to substitute potatoes or rice. The variety is big — peas, chickpeas, different kinds of lentils, different kinds of beans.

Legumes are rich in soluble and insoluble fibre, they help digestion and are great for intestinal microbiota.

Lipids. High consumption of extra virgin olive oil rich in monounsaturated fatty acids and tocopherol fights free radicals and raises low-density cholesterol in blood. Little or no consumption of butter and no consumption of margarine is needed, as is sweet pastry and bread usually made with olive oil or butter, not margarine or tropical fruit oils.

There are many possible choices for oils in Latvia, such as linseed, sunflower, rapeseed oil, but most of them are refined, meaning that all the possible beneficial vitamins are long gone, but the high quality ones are expensive. It has been shown in many studies that extra virgin olive oil is the best, so this is one of the few products that only with difficulty can be replaced with anything else. Cold pressed extra virgin olive oil should be used for cooking and for fresh consumption, not plain olive oil that contains refined oil.

Dairy. Low consumption of milk and fatty cheese, moderate consumption of yoghurt and other fermented milk products is recommended.

Yoghurt in Greece is a very important part of breakfast served with honey, nuts and fruit making it a wholesome breakfast.

In Latvia people consume fatty milk products — sour cream, full fat cheese, butter, cottage cheese.

Sour cream is used widely, even in soups. Kefir, however, is an excellent fermented milk product even for lactose intolerant people, because during fermentation all the lactose is consumed by bacteria and it has a significantly lower fat content than sour cream.

Spices and herbs. Adding more spices and herbs to a dish, like mint, basil, thyme, rosemary, makes it tastier and reduces the need for excessive salt. They also contain phytonutrients that can improve one's health. Chili, for example, contains capsaicin that has been seen to reduce inflammation. Rosemary is exceptionally rich in salicylic acid, which is the active compound of aspirin. Many patients suffering from heart disease are prescribed aspirin as an anti-inflammatory drug. Garlic and onions contain sulphurous compounds that lower blood pressure.

The above mentioned plants are used a lot in Latvia for any preparation.

Salt. Salt is used while cooking to season food, as elsewhere in the world, but the Mediterranean people consume less processed foods full of hidden salt that is the main worry for high blood pressure. The addition of pepper or chili makes the taste receptors more sensitive to salty taste, therefore less salt is needed.

Unfortunately, consumption of processed foods in Latvia is quite high, meaning that consumption of excessive salt is inevitable. One in ten males in Latvia add salt to their dish even before tasting it.

Wine. Moderate amounts of red wine is a good source of polyphenols (resveratrol). These antioxidants are found in the skin of red grapes, so white wine does not have the same characteristics. As the French paradox tells us, red wine is good for the cardiovascular system, but it is important to remember that wine is used during healthy meals not in excessive doses — products taken out of their context may not have the same desirable effect.

It is difficult to substitute wine with any other product available in Latvia, even though there are some people who are trying to produce it in cooler climates. White grapes definitely grow better more in the north than red, but polyphenols are found only in the skin of red grapes.

High quality beer could have some similar benefits as wine because of the presence of hop which has bitter compounds that work as an antibacterial. "High quality" should be underlined, meaning non-industrial beer with no extra additives and preservatives. No beer made from corn. Always keep in mind, that consumption of alcohol is not to be encouraged and should be done in moderation.

Fish. In Latvia very little fish is consumed, even though there is a variety of salt and sweet water species.

There is good availability of saltwater fish in the cities, but in the countryside the only fish one can buy is salted and smoked, having inappropriately high amounts of salt (Anonymous, 2008).

In the Mediterranean diet the small fish such as anchovies and sardines are widely used. Many small fish are also available in Latvia and they can substitute them. They have a short maturation period, so they are more sustainable than tuna and salmon, and they are very rich in Omega-3 fatty acids, one of the main reasons for fish consumption. Since they have a short life cycle, they are less polluted with heavy metals.

Sweet water fish is not as rich in Omega-3 as salt water fish; this depends mostly on the habitat it has lived in and on its alimentation. Still it is a good protein source as an alternative to meat.

Meat. Consumption of meat and its products should be lowered to a maximum of three times per week.

Latvians consume too much pork in the form of processed meat. The positive aspect of meat consumption is that recently game is becoming more and more popular.

Game is shown to be very low in fat and cholesterol, high in iron and unsaturated fatty acids, making it a great healthier choice. The composition of meat depends on the animal's diet. Wild animals feed on everything they find in the forests, which could be anything from lichens to berries. This is the reason for the high level of unsaturated fatty acids, if compared to a farm animal fed with corn silage and soy. Physical activity for animals living in the wild increases the amount of iron present in the meat.

It is true that game usually has to be cooked longer than farm-grown livestock, but a lot of prepared products are available, like cured venison. It is essential to keep in mind that when eating cured or smoked meats with bread it should not be accompanied with butter, because it has already enough saturated fatty acids. Wild boar is also a wonderful choice, even though its preparation might be long, but if made in the oven, it occupies no work at all.

In the Mediterranean diet a lot of preparations are composed of long sautéing or oven roasting on a low heat. Grilling should be kept to minimum because of the possible carcinogens that might form when fat is burned on open fire.

Dried fruit and seeds — full of unsaturated fatty acids. A lot of different types of edible seeds, nuts and dried fruit can be found in Latvia. Since the vegetal period is quite short, this sort of snack is great if fresh fruit is not available. Care must be taken with consumption of dried sweet fruit because they are full of sugar.

Non-alcoholic drinks. In the Mediterranean countries a lot of herbal infusions are prepared, especially after dinner to help the digestion. There are a lot of different plants that our great-grandmothers once used to collect every Midsummer

Night's Eve (*Jāņu nakts*), such as peppermint, chamomile, nettle, cowslip and many others. Not only helpful after a hearty meal, they can also improve weight control.

Dietary supplements. In cases when not enough micronutrients can be ingested, there is always the possibility for dietary supplements. Many studies have been conducted on resveratrol, lycopene and others, but still we do not have enough evidence for benefits for using dietary supplements. It is always best to take in nutrients with a healthy varied diet, also because nutrients work in synergy.

LATVIAN EATING HABITS

Latvians are very fond of fatty foods and usually their diet is quite unbalanced. Most people consume boiled sausages that are filled with little meat and many additives. Anything fatty like cheese or meat is consumed on bread with a layer of butter or margarine. The intake of vegetables is quite poor, mostly potatoes with meat or fish dishes, fresh salad with the typical ingredients, like tomato, lettuce and cucumber, all covered with sour cream. Most of the fat and protein consumed are from the animal kingdom. People cook a lot with full fat cream that can be substituted with sauces based on vegetable stock.

Only 49% of Latvians consider their daily food as wholesome, 39% found it unhealthy.

Men in general have worse eating habits — low vegetable, whole grain products consumption (Anonymous, 2006).

For a nation living at the sea, it is shocking that only 1% consumes fish, about 2–4 times per week (Anonymous, 2008). Individuals, who consume fish very often, prefer it smoked which usually contains large amounts of salt.

As mentioned before, Latvia has a lot of small family farms. Unfortunately, the legislation concerning food production is very severe and the subsidies given are not sufficient, making the end product very expensive, if compared to the industrial one. Good quality meat cattle is raised for export only and meat available in the market is of lower quality. White bread at a supermarket does not have less than seven ingredients, when all it needs is water, flour, yeast and salt.

The consumer is not educated enough or willing to read the label to make sure it is healthy. This means that a producer will use any raw material to make production cheaper, since no one is there to protest. We have to keep in mind that, with the production technology, this is the first time in human history that food costs are this low for the way we live. People are willing to choose quantity over quality.

A lot of work should be done on the way supermarkets and markets work to introduce other healthy, local, seasonal products. It is a long process to make an evolution in the market and it is not a one person's job, but still there are products available even now consumed in low quantities that should be revalued.

People should be more educated and more responsible of their diet, intended as eating habits, and lifestyle.

Following the Mediterranean diet for two years has been shown to have a significant decrease in deaths concerning cardiovascular disease by 9%, incidence of cancer by 6%, Parkinson's and Alzheimer's by 13%. This is because a correct diet helps control the perfect weight and improves lipid profile. The risk of diabetes diminishes because of the decrease of sugar metabolism disturbances, and there is an important fall of inflammation and tumour markers (Sofi *et al.*, 2008). Usage of high amounts of extra virgin olive oil decreases the incidence of stroke by 41% (Anonymous, 2011).

It is not only what you eat that is important in the Mediterranean diet, but everything around it and how the dish came to your table. In Latvia there is a possibility to adapt this kind of lifestyle in a certain way. Old traditions should be brought back to life with the use of forgotten products, cooking and preservation of food. More attention should be brought to education of children in schools, kinder gardens, patients in the doctor's office. Good quality healthy food should be considered as pleasure.

CONCLUSIONS

Although cardiologists can tell very much about the diet failure of their CVD patients, we need a further nutrition-based research to move us closer to our long-sought goal of developing and implementing dietary strategies that have a major beneficial impact on CVD risk.

A lot of changes should be done in mass production and distribution of food in Latvia, but without a doubt there are products available to have a wholesome diet at any age. The key factor is variety and seasonality.

Education should be done widely to promote tradition and gastronomic heritage as a cultural aspect, as it is thought of in the Mediterranean countries. Healthy lifestyle has to be visible to everyone at any time as a constant reminder of its importance, meaning that teachers have to set an example to children, the doctors to their patients and so on. No credibility of a good diet can come from an obese doctor talking about healthy eating.

If you feel good about your lifestyle yourself, you are more likely to share it with others!

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VESELĪGAS ALTERNATĪVAS VIDUSJŪRAS DIĒTAI LATVIJĀ

Slimību profilakses pamatā ir veselīgs dzīvesveids, ko veido regulāra fiziskā aktivitāte un veselīga diēta. Ļoti svarīgi ir balansēt uzņemto kaloriju daudzumu ar adekvātām fiziskām aktivitātēm. Pēdējo gadu laikā tieši Vidusjūras diēta tiek uzskatīta par visveselīgāko dzīvesveidu sirds un asinsvadu slimību profilaksē. Sekojot Vidusjūras diētai divu gadu garumā, ir novērota mirstības mazināšanās no kardiovaskulārajām saslimšanām par 9%, no vēža par 6%, no Parkinsona un Alcheimera slimībām — par 13%. Pozitīvie rezultāti ir skaidrojami ar to, ka veselīga diēta palīdz kontrolēt svaru un uzlabot lipīdu profilu. Samazinās arī risks saslimt ar cukura diabētu, jo uzlabojas glikozes vielmaiņa. Ievērojami samazinās iekaisuma un audzēja marķieri. Vidusjūras diētu definē produktu grupas: auksti spiesta olīveļļa — svarīgs mononepiesātināto taukskābju avots; daudz augļu un dārzeņu; pilngraudu produkti, pākšaugi, rieksti un sēklas; pazemināta tauku saturs piena produkti (bez pievienotiem augu taukiem); zivis (vismaz divas reizes nedēļā); putnu gaļa, liellopa gaļa un cūkgaļa nelielos daudzumos; olas — 0–4 nedēļā. Šāda veida uzturu ir iespējams pielāgot arī ziemeļvalstīs, atrodot produktus ar līdzīgu uzturvērtību. Ļoti svarīgi ir pievērst uzmanību svaigo produktu sezonālībai. Tā kā Latvijā ir ļoti īss veģetatīvais periods, nepieciešams ir uzlabot konservēšanas un uzglabāšanas metodes ziemas mēnešiem. Ziemējos ir pieejama liela graudaugu un pākšaugu izvēle. Jāpievērš lielāka uzmanība piena produktu kvalitātei un jāveicina zemnieku ražoto produktu patēriņš, kā arī jāsamazina rūpnieciski ražoto produktu patēriņš. Gaļas produktu īpatsvars diētā būtu jāsamazina līdz trīs reizēm nedēļā, dodot priekšroku mājās dzīvnieku gaļai, kas ir bagāta ar nepiesātinātajiem taukskābēm un dzelzi, bet tajā ir zemāks piesātināto tauku saturs. Latvijā ziemā netiek ražotas augstas kvalitātes augu eļļas. Auksti spiestas olīveļļas (*extra virgin*) uzturvielas ir grūti salīdzināmas un aizvietošanas ar citām, tās ievērojami spēj uzlabot kardiovaskulāro veselību. Augsts šīs olīveļļas patēriņš spēj samazināt insulta risku līdz pat 41%.

Mini Review

FEEDING INFANTS WITH COW'S MILK AND SOY ALLERGY: SOCIAL AND ECONOMIC ASPECTS OF EFFICACY

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Allergy to cow's milk protein and/or soy is common among allergic diseases in infants. They appear at an early infant stage and remain important in clinical practice from one up to three years. According to clinical research, cow's milk allergy affects about 1.9–4.9% of babies and infants, respectively, and in addition some of them also suffer from soy protein allergy. Dietary prevention of allergic protein by its elimination in food is a significant part of treatment, and allows adequate development of babies and restricts the risk of progressive allergic diseases. Securing exclusive breastfeeding is one of the basic principles in successful therapy treatment. However, there are cases when breastfeeding does not prevent the development of cross milk protein allergy. Only adequate special feeding formulas can provide both energy needs and sufficient quantity of proteins (8.9–11.5%) in food when breastfeeding is not possible. Knowledge of effective compensation mechanisms become apparent by analysing the situation in Europe and USA in the area of different available feeding formulas using both the medical insurance system and randomised formula providing tolerance of the mixture at about 90–95%. The goal of research was to determine the correlation between the availability of a special mixture, parental adherence and treatment outcomes. Applying special formulas is a routine part of treatment, and there is no doubt about its efficacy. No compensation mechanisms exist in present-day Latvia, and the current complicated economic situation in Latvia reduces the ability of parents to choose and buy appropriate formula food. Therefore, a substantial part of therapy treatment is unavailable to infants. Dietary prevention of allergic diseases in infants and small children in Latvia needs special consideration also because of poor knowledge of parents regarding the real situation.

Key words: cow's milk protein allergy, soy allergy, breastfeeding, special formulas, reimbursement system.

INTRODUCTION

Cow's milk protein and/or soy allergies are common in infants. They appear at an early infant stage and remain important in clinical practice from one up to three years. This problem has arisen through a historical tradition to diversify infant feeding with cow's milk. One of the causes for this rarely observed allergy was the rapid increase in the use of artificial feeding formulas in the 20th century, accompanied by aggressive marketing policy of their manufacturers. As a result, the importance of breastfeeding was compromised and the percentage of breast-fed infants fell dramatically. For example, in the 1970s in the USA, about 75% of babies were formula fed. From this time, the incidence of allergic disease amongst early babies stages grew noticeably. Now two tendencies have become urgent: dietary intervention for primary allergy prevention and providing appropriate feeding in the cases of allergic manifestation (Mavroudi *et al.*, 2011). The last wide report made by Cohrana highlights the

efficacy of exclusive breastfeeding in both prevention and treatment of allergy (Host *et al.*, 2008).

Statistics on the estimated incidence of cow's milk protein allergy in infants varies in different counties. The European Paediatrician Association performed a Europe-wide study on clinical practice for ambulatory treatment in cases of cow's milk protein based allergy. It was found that during prime medical care, 47% of doctors selected the diagnosis of "allergy caused by cow's milk proteins"; on the other hand, allergy specialists have found that this type of allergy affects only an estimated one to three percent of babies. Confusingly, the amount of parents considering their infants suffering of cow's milk or other kind of allergy is between 5 and 20 percent (Venter *et al.*, 2006). No other native milk instead of cow's milk can be used effectively, despite regularly arising promising prospects (D'Auria, 2011). Soy is a genetically modified product provoking cross allergy and food tolerance in infants (Kattan, 2011). Food allergy is a

cause of bullying in behaviour of children with negative consequences in their future life quality (Schemech, 2012).

NOURISHMENT-PROVOKED ALLERGY — REAL OR TEMPORARY, INAPPROPRIATE NUTRITION CAUSING PROBLEMS

According to data of some other clinical research, cow's milk protein allergy prevalence in infants and babies ranges from 1.9 to 4.9 per cent. In addition, some of them also suffer from soy protein allergy. Nourishment-provoked allergy also could be connected with raised IgE or allergy specific secretion of IgG. It has been strongly shown that allergic disease impedes the quality of a babies' life, and the negative effect can increase for teenagers and families as a whole. Therefore, the correct diagnosis and appropriate therapy becomes one of the most significant problems for paediatricians. Detection of protein causing allergy, and its elimination, is an important part of treatment. Use of hypoallergenic formulas combined with restricting dietary intervention, chosen by parents singly, can cause insufficiency of nutrition, causing problems in future physical development of children (Carafelli, 2010).

The problem of nourishment allergy in infants in Latvia has not been widely studied. A research group at the University of Latvia conducted an inquiry of 500 parents with children under the age of 5. In this study, in children feeding pathology symptoms of the skin, respiration system, digestive apparatus were identified in about 25% of cases (Rumba *et al.*, 2009).

The elimination of the protein provoking allergy from the diet has a significant role in the treatment of allergy. It helps to provide the adequate development of children and to diminish the risk of prolongation of allergy diseases. Therefore, the correct diagnosis is needed for choosing an appropriate treatment, including special formula fed in accordance with guidelines of ESPACI and ESPGHAN (Host *et al.*, 1997).

ELIMINATIVE DIETS, BREASTFEEDING SUPPORT

Supporting and promotion of breastfeeding is the first step providing necessary nourishment to infants (Niel *et al.*, 2010). However, there are cases when breastfeeding does not prevent the development of cross milk protein allergy (Brill, 2009). When breastfeeding is not possible, only use of appropriate feeding formula can cover an infants' energetic requirements, which can provide the sufficient amount of protein in food (from 8.9 to 11.5 per cent). It is important to take into consideration that when inflammation factors increase (because of skin or intestinal canal injuries) connected with decreased absorption of nutrients, a dangerous possibility of malnutrition arises even in the case of special nourishing. When the diagnosis has been incorrect or it has not been proven later, the elimination method and use of

feeding formula is expensive and non-effective (Meyer *et al.*, 2012).

The situation of Latvia in the field of cow's milk protein and soy protein allergy diagnosis is comparable with data given by research of the European Paediatrician Association. Only a limited part of patients obtain a confirmed diagnosis from an allergy specialist. Mainly, diagnosis is defined during prime medical care, and is based on anamnesis and clinical manifestation. After the prime medical care, as a rule, dietary prescriptions are given to the mother and breastfeeding is promoted; in some cases feeding formula is recommended, but often it is without any result due to lack of parents' being informed and training. Dietary prevention of allergic diseases in infant and small children in Latvia needs special consideration also because of poor knowledge of parents of the real situation and their wrong beliefs (Hays, 2012).

FORMULA FEEDING COMPENSATION POSSIBILITIES: COMPARATIVE OVERVIEW

Formula fed products are expensive and in combination with non-correct diagnosis their efficacy is low (Remberga, 2009).

The average cost of medical feeding formula ranges from 0.30 to 1.27 Ls per 100 ml of usable mixture.

During the first half-year of life, an infant needs about 850–900 ml of usable mixture average per day and 600–700 ml of usable mixture per day. This amounts to 81.00 to 342.90 Ls for one baby.

According to data of the Latvian Central Statistics Department (11.06.2012), food expenses per one member of household per month were 50,47 Ls. Visibly non-useful and probably dangerous expenses should be excluded.

Thus, it is possible to conclude that high expenses of feeding formula combined with a low information level amongst parents, leads to non-use of feeding formula, or that it is used side by side with cow's milk or soy protein-containing food; so the treatment efficacy planned could not be gained.

The trends described above exist for more than some decades, not only in the USA and developed members of the European Union, but also in Estonia, where formula fed products are included in lists of medicaments and remedies to be compensated. In Estonia, for example, medical feeding formula has been compensated for 2000 children for a period from birth till 12 months of age, corresponding to diagnosis by International classification of diseases — SSK10–K52.2; K90.4; L20.8; L27.2. Parents have only to prepay 1.27 Euro for a doctor's prescription, when 10 units are prescribed simultaneously. Diagnosis is arranged by the family doctor or by a paediatrician. No additional examination is required for clinic manifestation and a family's history is sufficient. The system described above is easily real-

ised and sufficiently effective, but hyper-diagnostic risks are still possible.

When analysing the situation in accordance with accessibility of feeding formula to parents in Europe and the USA, effective compensative mechanisms exist through the medical insurance system and randomised prescription providing tolerance of formula fed at 90–95% (Niggemann *et al.*, 2008).

In Netherlands, on the other hand, the eliminative protocol completed by provocation test has been confirmed. Those who work in the area of primary care need to meet the requirements of said protocol; only later formula for compensative mixture could be prescribed (or not prescribed). If it is necessary to clarify the diagnosis, a patient can be sent to a specialist. In Bulgaria only specialists (allergologists or gastroenterologists) are allowed to write prescriptions. In Germany expenses are covered by means of the medical insurance system. In Lithuania only synthetic amino acid formula mixtures are included in the list of compensative medicaments; the recipe could be prescribed only by a narrow list of specialists allowed to write prescriptions for compensative medical formula fed, and annual quota exist.

PARENTS AND ALLERGY SPECIALISTS: COLLABORATIVE ACTIVITIES; INFORMATION ACCESSIBILITY AND FEEDBACK

The role of the educational level of parents and their participation in treatment of allergies cannot be underestimated. Research conducted in the USA in 2008/2009 showed that the role of parents is very high not only in treatment of infants' allergy, but also during the school years. In total, 2495 respondents took part in the inquiry fully, and 558 respondents participated in different interrogatories partially. The best knowledge and most participation was amongst those of parents, who were visiting an allergy specialist regularly, in comparison with those who preferred prime care doctors (Gupta *et al.*, 2010).

Taking into account the high expenses of feeding formula and insufficient knowledge and level of parents' participation creates a situation whereby the infant is not able to receive sufficient nutrition. As a result it becomes possible to predict an increase of allergy diseases in the nearest future, especially in cases of real cow's protein allergy — not only milk intolerance. A special feedback providing algorithm is necessary due to prevailing empiric healing consequences (Meyer, 2008).

It would be necessary to implement a unified system for cow's milk and soy protein allergy diagnosis in Latvia in accordance with the important needs of infants, excluding unsuitable use of special feeding formula when allergy due to milk and soy proteins has not been confirmed. On the other hand, when the treatment of young children depends on elimination of proteins, feeding formulas can protect children from future progress of allergy.

Now special formula compositions are available for consumers in Latvia not only in pharmacies but in also supermarkets and shops for children's goods — in contradiction to other states of European Union, where special formula fed compositions can be bought only in pharmacies, as medically prescribed products.

We analysed the experience of parents in using special fed formula compositions. The summary was based on enquiry of parents within the frame of the state research programme "Scientific study on main pathologies and factors exposing to danger the lifelong and life quality of Latvian inhabitants by means of multidiscipline research consortium". The work took part as the sub-project "Modern early diagnosis, prophylaxis and therapy of diseases provoking invalidity and mortality of children". We came to the conclusion that parents, when making a decision about which feeding formula composition to use, including special ones, act on the advice of relatives and friends; thereby, the used formula compositions change, parents begin feeding and assess the results themselves, without being consulted by specialist. An allergy nutrition compensating mechanism for babies in Latvia does not exist, thereby restricting a parents' ability to buy the appropriate medical formula compositions, which causes future manifestation of allergy diseases. Also, the connection between all participants — prime care specialists, allergists and parents supported by continuous feedback and state thoughtful policy could be able to significantly control food allergy effects.

CONCLUSIONS

In most cases parents in Latvia lack understanding about possible diseases of infants and the principles of their treatment; they are more convinced about the effectiveness of medicaments than about the importance of nourishment.

Cooperation between prime care specialists, allergists and parents could evade hyper diagnostics of allergy and unproved applying of an elimination diet.

A babies' allergy nutrition compensating mechanism in Latvia does not exist, thereby restricting a parents' ability to buy appropriate medical formula fed compositions; this causes future manifestation of allergy diseases.

It would be preferable to apply the experience of other countries and to diagnose cow's milk and soy protein food allergy in good time using unified criteria. This approach could provide the possibility of using special fed formula in cases when it is necessary, creating a mechanism of supplying free medical feeding for infants under the age of one year.

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BĒRNU AR GOVS PIENA UN SOJAS ALERĢIJU ĒDINĀŠANAS EFEKTIVITĀTES SOCIĀLI EKONOMISKIE ASPEKTI

Govs piena olbaltuma alerģija un/vai sojas alerģija ir viena no pirmajām alerģisko slimību izpausmēm, kas sākas jau agrīni zīdaiņa vecumā un ir klīniski nozīmīga līdz 1–3 gadu vecumam. Saskaņā ar klīnisko pētījumu datiem govs piena olbaltuma alerģijas izplatība ir no 1,9 līdz 4,9% zīdaiņu un mazu bērnu vidū, daļai no šiem bērniem ir arī sojas olbaltuma alerģija. Ārstēšanas nozīmīga daļa ir izraisošā olbaltuma alergēna eliminēšanai no uztura, lai nodrošinātu adekvātu bērna attīstību un mazinātu risku tālākai alerģisko slimību attīstībai. Ekskluzīvas zīdīšanas nodrošināšana ir viens pamatnosacījumiem veiksmīgai uztura terapijai. Gadījumos, kad tas nav iespējams, tikai adekvāta ārstnieciskā maisījuma lietošana mākslīgi ēdinātiem bērniem spēj nodrošināt enerģētiskās vajadzības un pietiekamu olbaltuma daudzumu no 8,9 līdz 11,5% uzturā. Eiropas un ASV situācijas analīze par maisījumu pieejamību vecākiem parāda efektīvu kompensācijas mehānismu darbību, gan izmantojot veselības apdrošināšanas sistēmu, gan receptūru, kas pierādītas alerģijas gadījumā nodrošina maisījumu toleranci par 90–95%. Ārstnieciskā maisījuma lietošana ir ikdienas terapijas daļa, tās lietderība netiek apšaubīta. Latvijā kompensācijas mehānisms neeksistē, un valstī esošā ekonomiskā situācija ierobežo vecāku iespēju iegādāties maisījumus, līdz ar to bērnam nav iespēju saņemt nepieciešamo terapijas daļu.

Mini Review

OVERVIEW OF LINSEED PRODUCTION IN BOSNIA AND HERZEGOVINA

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Linseed (Linum usitatissimum L.), as one of the richest sources of omega-3 fatty acids, anti-tumoral phytoestrogens and plant mucilage, has great importance for human health. For this reason, the Agricultural Institute of Republic of Srpska promotes linseed production and processing through practical research activities. This paper provides an overview of linseed production and research activities on linseed since 2004, when this crop was returned into production in Bosnia and Herzegovina. The most important findings regarding linseed production and quality in recent years are discussed. So far, it seems that organic linseed production has better perspective in mountain regions compared to lowlands, mainly due to lack of invasive weeds 500 m above sea level. Until now, linseed diseases that can spoil the quality of seed, have not been recorded, due to extensive linseed production. The first comprehensive technological analyses of cold extracted linseed oil from the mountain region Petrovac showed unique quality characteristics. This mountain region is well known for its virgin nature and sunny microclimate. This has resulted in increased interest for linseed oil, as well as raised the price of domestic linseed oil.

Key words: *linseed, linseed oil, organic production, unique quality, human health.*

INTRODUCTION

Flax/linseed is an ancient crops that has been grown in many regions as an important fibre, oil and food source. The oldest flax findings date from Kavkaz 35 000 years ago (Kvavadze *et al.*, 2009). It is fascinating that some of these fibres were coloured and cut, indicating “advanced” technologies in the dawning of mankind. Using molecular methods, Danish scientists have proved growing of fibre and oil flax (linseed) forms in Denmark even 1600 years BC (Al-laby *et al.*, 2005; Runge and Henriksen, 2007).

The health traits of flax/linseed have been known to almost all civilizations. Flax clothing dominated in warm regions of ancient Mesopotamia and Egypt, as it offered ideal skin protection. Lignin in flax fibres is an excellent absorbent of ultraviolet radiation (Zimniewska *et al.*, 2004). Numerous inscriptions in Sumerian clay tablets show that flax (sumerian “gu”) had great importance for dressing, diet and healing. The multifunctional role of flax/linseed has been recorded on papyrus and wall drawings dating from all Pharaoh dynasties. Hippokrates (460–377 BC) successfully healed intestinal diseases with linseed. Respecting the im-

portance of linseed oil for Russian dietary and healing needs, the emperor Peter the Great banned linseed exports.

Before the 1960’s, flax/linseed was grown throughout the Balkan region organically. In that period, organic production was easier than today due to availability of rural labour. Unfortunately, flax/linseed production in the Balkan region was discontinued in the 1960’s when cheap synthetic fibres replaced natural ones.

The interest for linseed products has been increasing after the recognition of the irreplaceable role of essential fatty acids and their acid derivatives (DHA, EPA) for the structure and function of the neuro- and cardiovascular systems. About one half of population in the Balkan region suffers mortality due to consequences of irregular lipid status, which is (among other factors) the result of essential fatty acid deficit. Flax is the richest source of linolenic fatty acid (omega-3), which plays a key role for proper neuro- and cardiovascular functions.

The beneficial influence of linseed/flax products on human health has been an important subject of many multidisciplinary studies (Prasad, 1997; Simopoulos, 1999; Dahl *et*

al., 2005). Linseed contains 600-800 times more lignans than other oil crops (Westcott *et al.*, 2001). Lignans belong to phytoestrogens, which in a natural way compensate estrogen deficit in menopause, reducing risk of mammal cancer (Thompson, 1998). Flax fibre (linen) can increase the alfa-immunoglobulin content in humans and result in lower miographic tension of muscles, lower body temperature and sounder sleep (Kozlowski, 2001; Zimniewska *et al.*, 2004). An extended list of scientific studies about linseed/flax curative traits confirms the importance of this crop, which has been neglected in Balkan region for half of one century.

The FAO International Conference “Natural Fibres for Healthy Life”, held in Banja Luka in October 2004 had a key role for launching of research activities on linseed in Bosnia and Herzegovina. This Conference was organised by the Agricultural Institute of Republic of Srpska and the Institute for Natural Fibres in Poznan, where coordination centre of the FAO European Cooperative Research Network on Flax and other Bast Plants is located.

In the frame of organising the Conference, linseed was returned into production in Bosnia and Herzegovina. The title of this scientific event symbolically defines the directions of the research and practical activities in the future. All these activities will aid health benefits through increased consumption of domestic linseed products. The processing of flax fibres is presently limited, as the domestic textile industry has not yet adapted to this product.

The research activities, as well as an educational programme conducted by the Agricultural Institute of Republic of Srpska, have raised interest of farmers and buyers for domestic linseed and linseed products. Recently, cold extracted linseed oil has attracted farmer interest due to its

high market price. In spring 2013, a few animal farms started growing linseed in order to produce meat and eggs with increased content of omega-3 fatty acids.

LINSEED PRODUCTION AND RESEARCH ACTIVITIES IN 2004

In 2004, the linseed variety ‘Olin’ was introduced into production in Bosnia and Herzegovina through a network of demonstration trials. These trials occurred at 20 locations, representing different agroecological conditions from 100 to 700 m above sea level. More detailed data were available from 16 locations (Table 1). The size of production plots varied from 0.5 to 1 ha. Two Czech fibre flax varieties (‘Jitka’ and ‘Venica’) were included in demonstration trials at locations Banja Luka and Drinić in 2004. The agrotechnology was adapted for each trial depending on climate conditions and soil fertility (pH, humus content, available P₂O₅, and K₂O). The nitrogen treatment varied from 15 to 62 kg/ha. Field observations were focused on basic agronomic traits and the most important factors in practical production (weeds, harvest). Herbicides (bentazon) were recommended for plots where risk of weed development was observed. This treatment was particularly needed in the Brčko region, where the land had not been cultivated for a longer period due to migration of farmers in the war and post-war period.

The highest linseed yield (2.5 t/ha) was obtained at Šereg Ilova (Table 1). Excellent seed purity at this location was due to high herbicide efficiency. In the organic production system used in the neighboring village Velika Ilova, the yield was only 500 kg/ha with 10% weed seed impurity.

Table 1

RESULTS OF DEMONSTRATION LINSEED/FLAX TRIALS IN BOSNIA AND HERZEGOVINA IN 2004

No.	Municipality	Location	Alt.	Yield, kg/ha	Cd mg/kg	Problem
1.	Banja Luka	Agricultural School	150	1080	-	difficult harvesting
2.	Banja Luka	Agricultural Institute	150	Fiber variety Jitka-800 kg/ha, fiber variety Venica-810 kg/ha		
3.	Banja Luka	Stričići	700	1320	0.50	high soil acidity
4.	Prnjavor	Šereg Ilova	240	2500	0.54	-
5.	Prnjavor	Velika Ilova	220	500	0.39	<i>Ambrosia artemisifolia</i>
6.	Prnjavor	Potočani	300	1220	0.58	harvesting
7.	Prnjavor	Vijačani	330	1000	0.16	grass weeds
8.	Prnjavor	Štrpci	100	500	0.57	lodging, grass weeds
9.	Brčko	Boderište	140	1390	1.06	-
10.	Brčko	Donje Dubrave	120	1380	0.54	<i>Ambrosia artemisifolia</i>
11.	Brčko	Omerbegovača	120	1270	0.92	<i>Ambrosia artemisifolia</i>
12.	Brčko	Bukvik	120	870	0.44	<i>Ambrosia artemisifolia</i>
13.	Brčko	Sandići	100	900	-	<i>Ambrosia aremisifolia</i>
14.	Brčko	Cerik	150	700	-	<i>Ambrosia artemisifolia</i>
15.	Brčko	Brezovo polje	96	200	-	various weeds
16.	Drinić	Drinić (fibre variety)	700	Fiber variety Jitka-810 kg/ha		
17.	Petrovac	Vrtoče	600	1.000	-	disabled harvesting
18.	Gradiška	Kučiste	100	400	-	<i>Ambrosia artemisifolia</i>
Average yield—cultivar Olin				1014		

Significant lodging (80%) was observed at Štrbci, while at other locations it was recorded as 50%. Because of old combines, harvest was quite difficult at seven locations. Two-phase harvest caused loss of seed by 10–30%.

Fibre flax varieties at Banja Luka and Drinić were sampled for grain yield (Table 1), fibre yield and quality. The Czech fibre variety 'Jitka' produced the highest average straw yield (5800 kg/ha) in micro trials for the period 2008–2010, which was significantly higher than the average of all trials (Table 2).

Table 2

THE STRUCTURE OF FATTY ACIDS (%) IN THE COLD PRESSED LINSEED OIL-PANONIAN CULTIVAR

FFA	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0
0.57	5.71	0.65	3.68	17.62	14.25	57.55	0.16

Micro trials in 2004 included 26 genotypes placed in four replications randomly. Plot size was 5 m². The average linseed yield in the micro trials was 1074 kg/ha. It is interesting that the highest linseed yield of 2500 kg/ha, obtained at Šereg Ilova, was significantly higher than the average (1074 kg/ha) and maximal yield (1740 kg/ha) in the micro trial in Banja Luka in that year (Garić and Mandić, 2004). Usually, under similar agroecological conditions and similar agrotechnology, seed yields in micro trials significantly exceed seed yields in demonstration trials. Therefore, as the obtained rank in yield is illogical, reasons for this trend need to be answered. Possibly, this was due to very different soil traits and increased activity of microelements in producing yield.

The fertile alluvial soil in Banja Luka has neutral reaction (pH in water, 7.1), 2.6% humus content, very high concentration of available P₂O₅ (35 mg/100 g) and high K₂O concentration (45 mg/100 g). The soil in Šereg Ilova is pseudoclay with moderate acidity (pH in water, 5.3), 3.4 % humus content, very low concentration of available P₂O₅ (2.3 mg/100 g) and high K₂O concentration (21.7 mg/100 g). Moderate acid soils (pH 5-6) with lower concentration of available P₂O₅, as in Šereg Ilova, contain higher concentration of available zinc, which has a very positive effect on linseed/flax yield (Moraghan, 1980; Jankauskiene, 2001). Most microelements are more available in acid soils, which occupy about 60% of the arable land in Bosnia and Herzegovina. For this reason, the effect of microelements on yield of flax should be studied more detailed. In the 2004, the economically profitable yield was over 1.000 kg/ha.

High concentration of the heavy metal cadmium in food is a serious risk factor for renal disease (Vapa, M. and Vapa Lj., 1997). In 2004, the cadmium concentration was determined in the products to provide information for debates in the international conference held in Banja Luka. According to EU regulations, the acceptable level of cadmium in edible oil is 0.50 mg/kg. Cadmium concentration in linseed oil from 10

locations (cultivar 'Olin') in Bosnia and Herzegovina in 2004 ranged from 0.16–1.06 mg/kg (Table 1). In that same year, in the micro trial with 26 linseed genotypes at Banja Luka, the concentration of cadmium in oil ranged from 0.31–0.60 mg/kg. Higher accumulation of cadmium was observed in vegetative plant parts (fibres). In some cases, higher cadmium concentration in fibres can present a serious risk for skin health (Lukipudis, 2001).

LINSEED PRODUCTION CONSTRAINTS

Weeds have been the most important problem in the practical linseed production since 2004. Linseed yield and seed purity mainly depended on the weed spectrum and development. The period for herbicide application of linseed is quite short, when crop height is from 8 to 12 cm, which is an additional problem.

In the period 2005–2010, a few farmers attempted organic linseed production in the lowlands, but weeds resulted in a halt of this. *Ambrosia artemisifolia*, *Convolvulus arvensis* and *Cirsium arvense* have been the most common weeds in lowland regions. In contrast to lowlands, in the mountain regions over 600 m a.s.l., organic linseed production has been easier, mainly because of the absence of *Ambrosia artemisifolia* (Nožinić *et al.*, 2009; Nožinić *et al.*, 2012). In 2008, the variety Olin was grown organically at the mountain location Sitnica (750 m). The soil was enriched by sheep manure, and the problem of weed patches was solved by manual weeding.

Spread of thermophile invasive weeds has been promoted by climate warming in the recent two decenniums (Trkulja *et al.*, 2010; Nožinić *et al.*, 2012). Similar changes of weed distributions have been observed in Poland during a 30-year period (Heller, 2001).

Climate change effects have become more and more evident on linseed/flax production. Unfavourable weather conditions in 2007 caused lack of linseed in subsequent years. April 2007 was recorded as the driest (5 l/m²) in Banja Luka since 1961. Because of drought, linseed emerged unevenly with significantly reduced number of plants. Mean temperature in April (14.2 °C) and May (19.2 °C) in that year was the warmest in meteorological records at Banja Luka.

Late frosts present risk for linseed production in mountain valleys. It was encouraging that linseed (in the phase of coleoptiles) at Vrtoče (600 m) survived (with small damage) at a minimal temperature of –8 °C (April 10, 2012). The local authors noted that young linseed plants can withstand temperatures to –5 °C (Jevtić, 1986; Kocjan, 1999).

The main problem is the lack of the high yielding linseed cultivars. So far, Bosnia and Herzegovina has not adopted the EU cultivar list, which makes seed exchange difficult.

BETTER TIME FOR LINSEED AND LINSEED OIL PRODUCERS

In 2011, organic linseed and linseed oil production was carried out successfully in the mountain valley Vrtoče in the municipality Petrovac (600 m). The variety originated from Srbija, but the name of the variety was not known. The production of the linseed was based on liquid cow manure.

The advantages for organic production in the Petrovac region are: unpolluted soils, moderate temperatures during the vegetation period, over 2.000 sunny hours per year, high precipitation (1.200–1.300 l/m²), frequent winds and absence of the invasive weed *Ambrosia artemisiifolia*.

The content of free fatty acids (FFA) in the cold extracted oil was 0.57% (Table 2), which is significantly lower than the maximum allowed content of 3%. A very low peroxide concentration of 0.65 mmol O₂/kg confirmed the excellent quality of cold extracted oil (Table 3). The analysis of microbiological oil status showed absence of bacteria.

Table 3

THE PARAMETERS OF LINSEED OIL QUALITY-PANONIAN CULTIVAR

Characteristic	Determined	Maximum allowed	Method
Peroxide concentration number	0.65 mmol O ₂ /kg	10 mmol O ₂ /kg ulja	ISO 3960
Water and other evaporable substances	0.02%	do 0.4%	ISO 662:1998
Phosphorus	7.90 ppm	No limits	A.O.C.S. Ca 12-55
Non soapy substances	5.16 g/kg	15 g/kg	ISO 3596-2:1998
The content of insoluble impurity	0.01%	0.05%	ISO 663

Due to environmental advantages and proper agrotechnology, weeds did not appear, and thus the linseed was exceptionally pure. Weed seeds and impurities (soil dust, gasses absorbed during harvesting) can change the taste and smell of cold extracted oil.

So far, stronger infestation with common linseed diseases, like *Fusarium* wilt, has not been recorded in Bosnia and Herzegovina, which is extremely important for linseed and linseed oil quality. This is mainly due to the current extensive linseed production at several distant locations (regions). This status of uncontaminated seed should be maintained as long as possible. Flax disease was described by local authors during the period when flax occupied a large area in Balkan countries (Panjan, 1968).

The organic production on the farm in Vrtoče, which was associated with high quality traits, as well as and educational programme (by the Agricultural Institute of Republic of Srpska) led to a price of 20 euros per litre of linseed oil.

Bearing in mind the obtained yields of 1.4 t/ha in 2011 and 2012, this yield had very high economic value.

The sowing area of linseed in spring 2013 will be higher than in previous years due to the good results on the farm in Vrtoče. It seems that raised awareness of the importance of omega three fatty acids, phyto estrogens as well as other linseed healing components has led to a better time for linseed producers.

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PĀRSKATS PAR EĻĻAS LINU AUDZĒŠANU BOSNIJĀ UN HERCEGOVINĀ

Rakstā dots pārskats par eļļas linu audzēšanu un zinātniskajiem pētījumiem par eļļas liniem kopš 2004. gada, kad šo kultūru atsāka audzēt Bosnijā un Hercegovinā. Organisko linsēklu audzēšanai labākas perspektīvas, salīdzinājumā ar zemiēm, ir kalnu rajonos. To var izskaidrot galvenokārt ar nezaļu neesamību 500 m augstumā virs jūras līmeņa. Pateicoties ekstensīvai linu audzēšanai, līdz šim nav novērotas linu slimības, kas varētu ietekmēt linsēklu kvalitāti. Pirmās visaptverošās tehnoloģiskās analīzes par auksti ekstrahētas linsēklu eļļas kvalitāti no kalnu reģiona Petrovacas uzrādījušas unikālas kvalitātes īpašības. Šim kalnu reģionam raksturīga neskarta daba un saulains mikroklimats. Pētījumu rezultāti vairojuši interesi par linsēklu eļļu un paaugstinājuši pašmāju linsēklu eļļas cenu.

Mini Review

HEALTH PROMOTING CHEMICAL COMPONENTS OF ORANGE JUICE

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Citrus fruit or juice can be an excellent source of health-promoting substances at breakfast. A 150–200 ml glass of orange juice daily provides many nutrients required for good human health. As has been reported, vitamin C, thiamine (vitamin B₁), riboflavin, vitamin A, vitamin D, vitamin E, pantothenic acid, vitamin B₆, folate are present in oranges. Citrus juices also provide minerals — calcium, potassium, iron, zinc, magnesium, copper, and phosphorous, which are part of the vital enzyme system of the human body. In addition, several compounds — flavonoids and other health-promoting substances are present in citrus fruit. There are hundreds of useful products and substances with properties, which have origin in citrus products. There are also many patents for helpful products to be made from citrus substances. Treatment of major inflammation-related ailments target on phytochemicals involved in oxidative stress, metabolic syndrome (diabetes), cardiovascular diseases, bone health (osteoporosis), skin aging, cognitive function and brain diseases, aging, allergy and immune function and cancer. A clinical study published shows that orange juice and hesperidin increase nitric oxide production in human. Orange juices have been shown to provide several important health benefits, particularly for the cardiovascular system, bone and skin health, brain health, cognitive functions, aging, and also cancer. However, the number of clinical studies available remains limited and significant efforts are necessary to provide irrefutable proof of these benefits in human.

Key words: naringin, hesperidin, orange juice, nitric oxide, vitamins.

INTRODUCTION

Citrus is the world's most popular fruit. Citrus products have always been valued as excellent sources of human nutrition. Nutrition is the ability to engender growth, and many of the components of citrus products contribute to the growth and well-being of the human body. It is well established that citrus and citrus products are a rich source of vitamins, minerals, flavonoids and dietary fibre NSP (non-starch polysaccharides) and that are essential for normal growth and development and overall nutritional well-being. For example, the recommended dietary allowances for average adults in the United States and India in terms of nutrients available in citrus juices are given in Table 1.

In addition, citrus contains no fat or sodium and, being a plant food, no cholesterol. The average energy value of fresh citrus is also low (see Table 2), which can be very important for consumers concerned about putting on excess body weight. For example a medium orange contains 60 to 80 kcal, a grapefruit 90 kcal and a tablespoon (15 ml) of lemon juice only 4 kcal (Whitney and Rolfes, 1999).

CARBOHYDRATES

Citrus fruits contain carbohydrates in the form of sugars: sucrose, glucose, and fructose as well as citric acid, which can also provide a small amount of energy. Total soluble solids in juice consist mainly of sugars. Fibrous rag and many other polysaccharides, which may also provide calories, are also eaten when fresh citrus fruit is consumed (Ladaniya, 2008). A reasonable goal for dietary NSP/fibre intake is 25 to 30 g/day, but in many developed countries the actual average intake is closer to 15 g (Cleveland *et al.*, 1996). With one medium orange containing approximately 3.0 g of NSP, citrus fruit can make a valuable contribution to meeting the daily fibre goal (Whitney and Rolfes, 1999). An average-size orange (7–8 cm diameter) can provide 0.8 g of fibre in the diet. Drinking of a cup of fresh orange juice provides 0.3 g of fibre. A half grapefruit eaten at breakfast adds 0.2 g of fibre to the meal, thus replacing fibre that has been removed from the breakfast cereal by the milling process (Church and Church, 1970). Fibre has its own importance for the people of industrialized nations who eat high-fat, low-fibre diets full of highly refined and processed carbohy-

Table 1

RECOMMENDED DIETARY ALLOWANCES FOR AVERAGE ADULTS IN THE UNITED STATES AND INDIA IN TERMS OF NUTRIENTS AVAILABLE IN CITRUS JUICES (one glass, 100 g)

Nutrient	Recommended dietary allowance		Content in citrus juices		
	essential in human nutrition (USRDA)	for average adult Indian (60 kg weight)	orange	tangerine	grapefruit
Vitamin C	90 mg	40 mg	45–50 mg	30–31 mg	30–35 mg
Vitamin A	5000 IU	600 µg retinal	190–400 IU	350–420 IU	21 IU
Vitamin D	200 IU	-	-	-	-
Vitamin E	15 mg	25 µg	100 µg	-	-
Thiamin (B ₁)	1.5 mg	1.4 mg	50–80 µg	50–80 µg	30–40 µg
Riboflavin	1.7 mg	1.6 mg	20–40 µg	20–40 µg	-
Niacin	20 mg	-	300–600 µg	200–250 µg	200 µg
Calcium	1g	400–1000 mg	10–11 mg	-	9–10 mg
Iron	18 mg	28 mg	0.1–0.2 mg	-	-
Pyridoxine (B ₆)	2 mg	2.0 mg	47–66 µg	40–50 µg	18–20 µg
Folic acid	0.4 mg	100 µg	34 µg	21 µg	8 µg
Vitamin B ₁₂	5–6 µg	1 µg	-	-	-
Phosphorus	1g	-	14–20 mg	16–18 mg	15–18 mg
Iodine	150 µg	-	0.25 µg	-	-
Magnesium	400 mg	-	8–12 mg	10–15 mg	8–10 mg
Zinc	15 mg	-	25–30 µg	-	-
Copper	2 mg	-	50–160 µg	-	-
Biotin	0.3 mg	-	-	-	-
Pantothenic acid	10 mg	-	130–150 µg	-	280–300 µg

Source: Anonymous (2004a); Gopalan *et al.* (1999). USRDA, United States Recommended Daily Allowance

Table 2

NUTRITIONAL FACTS ON CITRUS FRUIT

Weight (g)	Orange 131	Grapefruit 236	Tangerine 84
Energy (kcal)	62	78	37
Fibre (g)	3.1	2.5	1.7
Ascorbic acid (mg)	70	79	26
Folate (µg)	40	24	17
Potassium (mg)	237	350	132

Source: Guthrie and Picciano, 1995.

drates that are more slowly absorbed through the intestines. In fresh citrus fruit, fibre contains cellulose, hemicellulose, lignin, and pectin — all found in citrus segments, membranes and other parts of the albedo (Ladaniya, 2008). Dietary fibre also adsorbs calcium and may increase its availability (Nishimura *et al.*, 1992). Citrus fruits are not considered a major source of protein; but proteins are a constituent of citrus juices. In citrus fruits, seeds are very rich in protein. Citrus seeds contain 18.2% protein on a dry weight basis (in whole seeds) (Ladaniya, 2008).

MINERALS

Citrus juices also provide minerals that are part of the vital enzyme system of the human body. Citrus fruits have very high potassium (K) content (300 mg in 178 ml of orange juice and 200 mg in grapefruit juice), while the sodium content is relatively low (3–4 mg/178 ml orange juice and 4.5 mg/178 ml tangerine juice). The ratio of K and sodium

(Na) in orange juice plays an important role in maintaining electrolyte balance. Dietary levels are 50–150 meq (milli-equivalent) per day for potassium (Araujo, 1977). The daily requirement of potassium is approximately 2000 mg and, while frank deficiency of potassium is rare, there is some concern that a high sodium-to-potassium intake ratio may be a risk factor for chronic disease. Increased consumption of citrus fruits and juices is a good means of increasing potassium intake. One medium orange and one 225 ml glass of orange juice provide approximately 235 mg and 500 mg of potassium, respectively (Whitney and Rolfes, 1999). The RDA for calcium in the diet of adult Indian men is 400 mg per day; one orange provides about 2% of this amount. In addition to K and Na, calcium and phosphorus, and magnesium are required in higher amounts. Like calcium and phosphorus, magnesium is sequestered in bone. Magnesium is present in mitochondria and other enzymes important in energy transfer. Zinc, manganese, and copper are also important for the body and supplied by citrus fruits. Ascorbic acid and citric acid increase the absorption of calcium and other minerals. A study indicated that ascorbic acid was not the only organic acid responsible for promoting the effects of citrus fruit juices on iron absorption. Iron absorption from laboratory orange juice containing 100 ml water, 33 mg ascorbic acid, and 750 mg citric acid was significantly better than that from 100 ml water and 33 mg ascorbic acid alone (0.097 and 0.059 g, respectively). There was a close correlation between iron absorption and ascorbic acid content. A weaker but still significant correlation with the citric acid content also was observed (Ballot *et al.*, 1987). A glass of chilled orange juice or few fresh oranges or mandarins are very refreshing in summer and also provide required

electrolytes. One orange (200 g) provides about 2 mg of iron. Two oranges a days can give 4 mg, which would be more than 10% of the Recommended Dietary Allowance (RDA) in the USA. The RDA is set assuming a 10% rate of intestinal absorption (Ladaniya, 2008).

VITAMINS

Ascorbic acid (vitamin C) is the most important nutrient in orange juice. Vitamin C is essential for the synthesis of collagen, the most abundant protein in mammals. Collagen is the major fibrous element of skin, bone, blood vessels and teeth. A lack of vitamin C leads to scurvy, which causes loss of teeth, bleeding skin and ulcers. Vitamin C is sometimes suggested to have anticancer effect by its reaction with and inactivation of free radicals in the body. It is estimated that a glass of orange juice (177.4 ml; Ladaniya, 2008) provides about 100% of the recommended daily allowance of vitamin C to the average American diet. The RDA set in 2000 for an average adult of good health is 75–90 mg (Anonymous, 2004). Too much vitamin C (above 500 mg), generally seen with very high levels of supplementation, may be dangerous, especially for those at risk of iron overload (Fleming *et al.*, 1998). Consuming five servings of fruits and vegetables each day can result in an intake of about 200 mg of vitamin C. Citrus fruits are a particularly good source of vitamin C, with one medium orange or grapefruit providing approximately 70 mg and 56 mg, respectively. A 225 ml glass of orange juice contains approximately 125 mg of vitamin C (Whitney and Rolfes, 1999).

Among other vitamins present in fresh citrus fruit are compounds of the vitamin B complex. Folic acid, which is heat-sensitive and lost in food processing, can be obtained from fresh oranges, mandarins, and grapefruits. Folic acid or folate is required for the multiplication and maturation of red blood corpuscles; its deficiency can result in a type of anaemia. Folic acid plays a role in metabolic pathways by the formation of purine and pyrimidine nucleotides as well as certain amino acid conversions. Since growth requires proteins and nucleic acid synthesis, growing children and pregnant women are more sensitive to its deficiency (Ladaniya, 2008). Folate has also been associated with a reduced risk of heart disease by lowering blood serum homocysteine levels. Women at child-bearing age and those at the greatest risk of coronary heart diseases need to take sufficient folate in their diet (Widmer and Stinson, 2000). In the United States, recommended daily intake of folate is 180 mcg for females and 200 mcg for males. Over the past decade, however, it has become clear that higher levels of folic, 400 mcg, are associated with the prevention of neural tube defects, a severe birth defect (Center for Disease Control and Prevention, 1992). Thiamin (Vitamin B₁) content ranges from 90 to 280 mcg in a serving of 177 ml of oranges, juice, which is 6–18 % of the USRDA (United States Recommended Daily Allowance)*.

* http://1stholistic.com/nutrition/hol_nutrition-RDA.htm

Citrus fruits are also a source of the B₆ vitamins, known as pyridoxal, pyridoxamine and pyridoxine. These are interchangeable in the body. Some types of stomatitis and a type of anemia have been shown to be cured by the administration of pyridoxine. The average intake of 0.6–2.5 mg of B₆ is considered sufficient for all age groups of Indians (Gopalan *et al.*, 1999).

PHYTOCHEMICALS

The effects of flavonoids from citrus juices, particularly those found in oranges and grapefruit, on blood circulation, as well as their anti-allergenic, anti-carcinogenic, and antiviral properties have been discussed by Rice-Evans *et al.* (1997), Filatova and Kolesnova (1999), who reviewed the antioxidant properties of phenolic compounds.

The effects of the citrus bioflavonoid naringin were tested by using it as a supplement in a high-cholesterol diet (Shin *et al.*, 1999). A research team, from the University of East Anglia, UK, reported that women consuming the highest amounts of flavanones, a subclass of flavonoids that are found in especially high levels in citrus fruits, were associated with up to a 19% reduction in stroke risk, compared to those in the group who consumed the lowest amount (Cassidy *et al.*, 2012).

ROLE OF CITRUS FRUIT IN REDUCING RISK OF HUMAN DISEASES

Dietary fibre is reported to lower the incidence of ischemic heart diseases (Trowell, 1972). Synthetic fibrous substances have been utilised to promote the lowering of blood cholesterol from the intestinal lumen. Garvin *et al.* (1965) and Resnicow *et al.* (1991) reported that a type of strict vegetarian diet that is typically very low in saturated fat and dietary cholesterol and high in fibre can help children and adults maintain or achieve a desirable blood lipid level. Each 1% reduction in blood cholesterol yields an approximately 2% reduction in risk of coronary heart disease; thus a 7.6% decrease translates to a 15% decrease in the risk of coronary heart disease. Thus, without drastically changing lifestyle and/or diet, cholesterol levels can be lowered, and it also has been established that atherosclerosis is retarded in pigs fed a grapefruit pectin atherogenic diet (Attaway and Moore, 1992). These findings are important for people who have established atherosclerosis and whose only remedy at present is bypass surgery. These studies all indicated beneficial aspects of eating oranges and tangerines, showing that certain compounds, mostly flavonones, are promising for lowering low density lipoprotein cholesterol (so-called “bad cholesterol”), without causing any harmful side effects. Studies conducted by USDA with KGK Synergise Inc, Canada, have also shown that antioxidant compounds (called polymethoxylatedflavours, or PMF) are the most potent in reducing cholesterol (Anonymous, 2004b). Diets rich in citrus and citrus-based products have been negatively correlated with the risk of cardiovascular disease. Studies have

been conducted to determine whether naringenin and hesperetin, two major flavanones in citrus plants, influence endothelium nitric oxide production (Liu *et al.*, 2008). Limonoids (a group of triterpenoids) in citrus fruits possess important biological activity. The similar chemical structure of citrus limonoids to those of recognised anti-tumour agents led to their evaluation as potential anti-tumour agents in mammalian systems. Studies in mice showed that citrus limonoids induced a significant amount of the chemical carcinogen detoxifying enzyme system glutathione-S-transferase in the liver and intestinal mucosa. *In-vitro* tests with human breast cancer cells have shown that limonoids have significant anti-tumour activity (Manners and Hasegawa, 2000). Consumption of peel products is associated with reduced risk of squamous-cell carcinoma of the skin, as reported by I. A. Hakin and R. B. Harris of the Arizona Cancer Center (Anonymous, 2004c). Modified citrus pectin was also observed to interfere with the metastasis of other cancer cells. The alcohol of citrus peel is active in inducing apoptosis in tumour cells without affecting normal cells and can revert tumour cells back to a differentiated state. Applying peel extracts can inhibit the growth of melanoma and other skin cancer cells (Anonymous, 2004c).

Orange juice has also been reported to prevent the formation of kidney stones, due to the presence of citrates (Anonymous, 2006). Kidney stones are formed when urine is too concentrated, causing minerals and other chemicals in the urine to bind together. The studies of Dr. Clarita Odvima, UT, South-Western Medical Centre, indicated that orange juice increased the level of citrates in urine and reduced crystallisation of uric acid and calcium oxalate (Anonymous, 2006). The study indicated that potassium citrate, a generally prescribed medicine, had gastrointestinal side effects in some patients and therefore orange juice was the better option.

CONCLUSIONS

Orange juices have been shown to provide several important health benefits, particularly for the cardiovascular system, bone and skin health, brain health and cognitive functions, aging and also cancer. However the number of clinical studies available remains limited and significant efforts are necessary to provide irrefutable proof of these benefits in human.

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VESELĪBU VEICINOŠI ĶĪMISKIE KOMPONENTI APELSĪNU SULĀ

Citrusaugļi un citrusaugļu sulas ir lielisks veselības avots — veselību veicinošu vielu uzņemšana brokastīs. 150–200 ml glāze apelsīnu sulas nodrošina daudzas veselībai labas uzturvielas, kuras nepieciešams uzņemt katru dienu. Apelsīni satur C vitamīnu, tiamīnu, riboflavīnus, A vitamīnu, D vitamīnu, E vitamīnu, pantotēnskābi, B₆ vitamīnu, folijskābi. Citrusaugļu sulas satur minerālvielas — kalciju, kāliju, dzelzi, cinku, magniju, varu, fosforu, kas ir daļa no svarīgās enzīmu sistēmas cilvēka organismā. Turklāt citrusaugļu sula satur arī vairākus savienojumus — flavonoidus un citus veselību veicinošas vielas. Ir simtiem noderīgu produktu un vielu ar šādām īpašībām, un to pirmavots ir citrusaugļi. Ir daudz patentu par veselīgiem produktiem, kuru izgatavošanā izmantotas citrusaugļu vielas. Svarīgu ar iekaisumiem saistītu slimību ārstēšanā izmanto fitoķīmiskās vielas, saistībā ar tādām slimībām kā oksidatīvais stress, metabolais sindroms (diabēts), sirds un asinsvadu slimības, kaulu slimības (osteoporoze), ādas novecošana, kognitīvo funkciju un smadzeņu slimības, novecošana, alerģijas, imūnsistēmas funkcijas un vēzis. Klīniskajos pētījumos iegūtie un publicētie dati liecina, ka apelsīnu sula un hesperidīns veicina slāpekļa oksīda ražošanu cilvēkā. Ir pierādīts, ka apelsīnu sula nodrošina vairākus būtiskus veselības uzlabojumus, galvenokārt, saistībā ar sirds un asinsvadu slimībām, kaulu un ādas veselību, smadzeņu darbību, kognitīvajām funkcijām, novecošanu un vēzi. Tomēr pieejamo klīnisko pētījumu skaits ir ierobežots, ir nepieciešami jauni pētījumi, lai sniegtu neapgāžamus pierādījumus šiem cilvēka veselības uzlabojumiem.

NUTRITION-RELATED DISEASES IN THE MEDICINES REIMBURSEMENT SYSTEM OF LATVIA

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In Latvia, the medicine reimbursement system for outpatient treatment operates under conditions of limited resources, and the state budget financing has been reduced in recent years. Under such circumstances, a necessity to identify additional possibilities to optimise resources allocation to health care programmes and prevention activities is important. The aim of this research was to identify nutrition-related diagnoses in the Latvian medicine reimbursement system and to determine potential efficiency of the resource allocation to complementary nutrition programmes. To meet the aim defined, theoretical research methods (analysis of legislative documents and special literature analysis) were used alongside empirical research methods (data collection: documentary analysis, statistical database analysis). The results of this research showed that the largest amount of reimbursed diagnoses, such as cardiovascular diseases, diabetes, cancer, digestive diseases, and osteoporosis, are identified as nutrition-related diagnoses in the review of special literature. Determination of the efficiency of complementary nutrition programmes is a crucial issue in the context of the evidence-based medicine, both in terms of direct and indirect costs to society and government, and in terms of disability-adjusted life years and other health determinants directly related to patients. For this reason, possibilities for the evaluation of relative efficiency of the nutrition programmes for reimbursed diagnoses are investigated in depth.

Key words: *health care, allocation of resources, allocative and technical efficiency.*

INTRODUCTION

The Latvian medicine reimbursement system for outpatient treatment has developed starting from the 1990s. The 2006 Regulations No. 899 of the Cabinet of Ministers of the Republic of Latvia, "Procedures for the Reimbursement of Expenditures for the Acquisition of Medicinal Products and Medicinal Devices Intended for Out-patient Medical Treatment" (hereafter – Regulations No. 899), regulate this system at the present. The procedures for reimbursement are a set of measures that provide patients with an opportunity to acquire medicinal products and medical devices, the expenditures for the acquisition of which are completely or partially covered by funds from the state budget for the current year, granted for the reimbursement of expenditures for the acquisition of medicinal products in accordance with these regulations, or by the funds granted in accordance with the Social Security Network Strategy (Anonymous, 2006).

Nineteen groups of diagnoses are reimbursed in accordance with the Regulations No. 899 (groups of diagnoses are classified by the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10)). Expenditures for the reimbursement of medicinal products

are covered under the following reimbursement categories (Anonymous, 2006):

- Category I – reimbursement in the amount of 100%, if it has been determined that a patient has a chronic, life-threatening disease or a disease, which causes serious irreversible disability and the medical treatment of which requires the use of the respective medicinal products in order to maintain the patient's vital functions;
- Category II – reimbursement in the amount of 75%, if it has been determined that a patient has a chronic disease, in the medical treatment of which the maintenance of the patient's vital functions is made difficult or which causes serious disability without the use of the respective medicinal products; and
- Category III – reimbursement in the amount of 50%, if it has been determined that a patient has a chronic or acute disease, in the medical treatment of which the use of the respective medicinal products is necessary in order to maintain or improve the patient's state of health or in the case where vaccines are paid for from the funds granted for reimbursement.

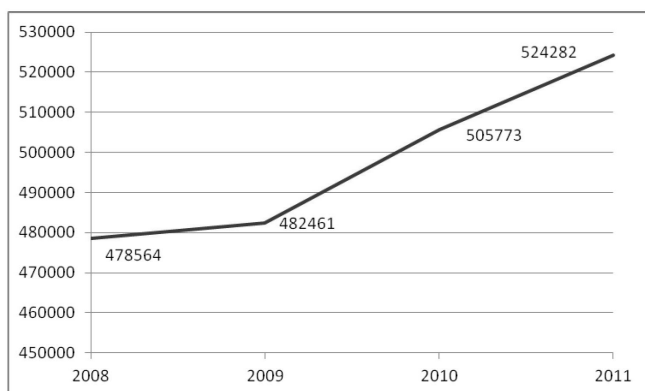


Fig. 1. Number of patients treated in the reimbursement system for outpatient treatment in Latvia, 2008–2011 (Anonymous, 2012).

The number of patients treated through the reimbursement system has increased yearly (Fig. 1). The biggest number of patients is treated through the reimbursement system in the group of diagnoses “Diseases of the circulatory system”, “Endocrine, nutritional and metabolic diseases” and “Diseases of the respiratory system”, and the number of patients is rising annually (excluded diagnoses “Mental and behavioural disorders” in which the number of patients has declined in 2011) (Fig. 2).

In spite of a relatively low number of patients with diseases of the digestive system, this group is one of the more important in mortality data (Table 1). Table 1 includes data of mortality in Latvia and in neighbouring Baltic States.

The largest part of the health care budget resources for the reimbursement system is allocated for diseases of the circulatory system, endocrine, nutritional and metabolic diseases and neoplasms (Fig. 3). However, the data on total expenditure for reimbursed medicinal products (budget allocation and patients’ co-payment) show that the patients’ co-payment has increased in circumstances of reduced budget

Table 1

DEATHS BY CAUSE PER 100 000 POPULATION IN ESTONIA, LATVIA AND LITHUANIA BY MAJOR GROUPS OF CAUSES (Anonymous, 2010)

Cause of death	Deaths by cause per 100 000 population in Baltic States		
	Estonia	Latvia	Lithuania
Diseases of the circulatory system	652.9	727.1	718.8
Neoplasms	269.0	273.6	251.2
Injury, poisoning and certain other consequences of external causes	84.7	94.1	123.1
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	23.7	85.0	16.7
Diseases of the digestive system	45.9	46.7	67.5
Diseases of the respiratory system	31.7	29.6	38.9

allocation, as the need for treatment, based on growing patients number, increases independently of budget resources allocated (Fig. 4). In these circumstances, taking into account the limited state budget for the medicine reimbursement system and patient resources, the aim of this research was to identify additional possibilities to optimise resource allocation to the health care, based on nutrition-related diagnoses in the medicine reimbursement system and potential efficiency of complementary nutrition programmes.

MATERIALS AND METHODS

To meet the aim defined, theoretical research methods (analysis of legislation, special literature analysis and analysis of electronic resources) were used alongside empirical research methods (data collection: document analysis, statistical database analysis). For data processing and analysis, statistical analysis methods (comparison, grouping, calculation of averages) and methods of economic analysis were used.

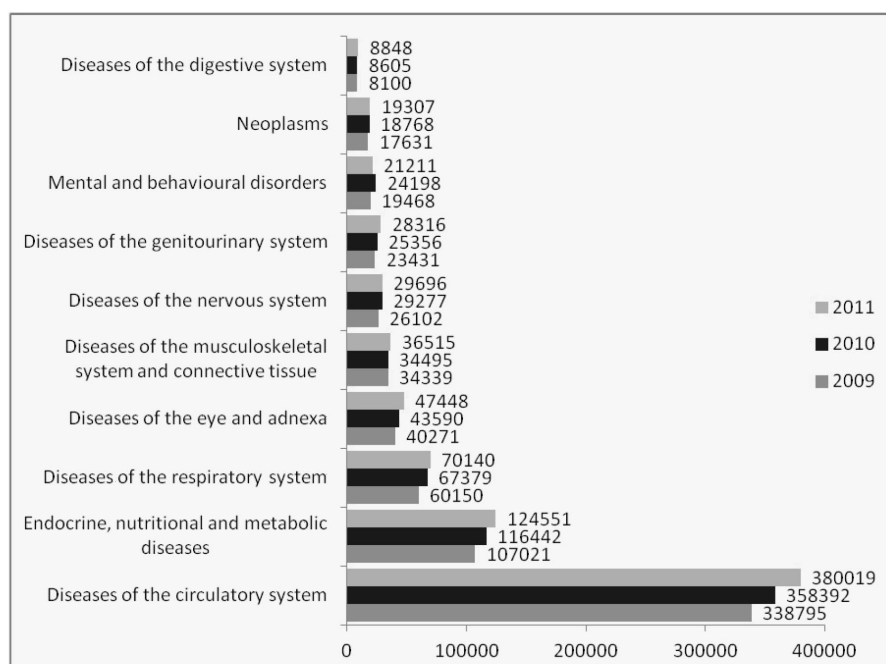


Fig. 2. Number of patients treated in the reimbursement system for outpatient treatment by the ten biggest diagnoses groups in Latvia, 2009–2011 (Anonymous, 2012).

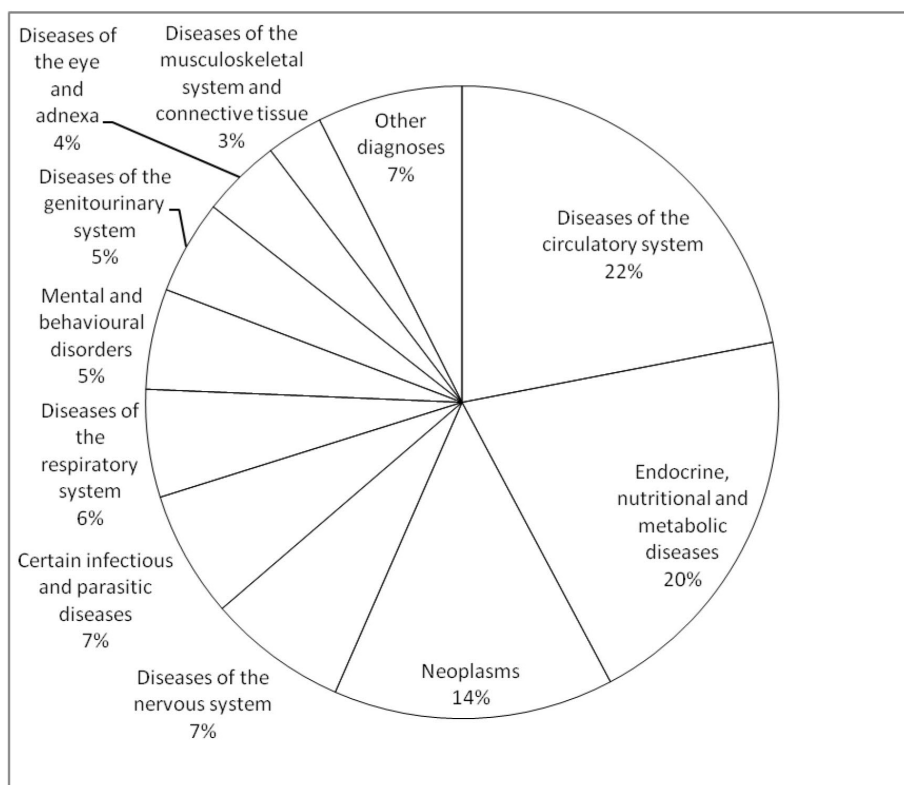


Fig. 3. Health care budget expenditure by groups of diagnoses in Latvia, 2011 (Anonymous, 2012).

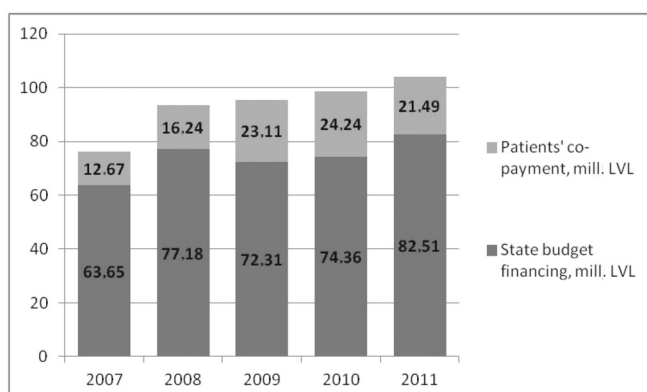


Fig. 4. State budget recourses allocated for the reimbursement system and patients' co-payment in Latvia, 2007–2011 (Anonymous, 2012).

RESULTS

The World Health Organisation in the Report of a Joint WHO/FAO Expert Consultation “Diet, Nutrition and the Prevention of Chronic Diseases”, 2003, indicated that chronic diseases are largely preventable diseases. Although more basic research may be needed on some aspects of the mechanisms that link diet to health, the available scientific evidence provides a sufficiently strong and plausible role of prevention. Beyond the appropriate medical treatment for those already affected, the public health approach of primary prevention is considered to be the most cost-effective, affordable and sustainable course of action to cope with the chronic disease epidemic worldwide. The above mentioned report reviewed the evidence on the effects of diet and nutrition on chronic diseases and made recommendations for public health policies and strategies that encompass socie-

tal, behavioural and ecological dimensions. In setting out ways to decrease the burden of chronic diseases (such as obesity, type 2 diabetes, cardiovascular diseases (including hypertension and stroke), cancer, dental diseases and osteoporosis), the report proposed that nutrition should be placed at the forefront of public health policies and programmes.

In the present study, a literature review provided sufficient evidence that the largest part of the reimbursed diagnoses in Latvia are nutrition-related diseases (in the following list the directly nutrition-related diagnoses are marked in bold and the indirectly nutrition-related diagnoses in italic):

- I. *Specific infectious and parasitic diseases*
- II. **Neoplasms**
- III. Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism
- IV. **Endocrine, nutritional and metabolic diseases**
- V. *Mental and behavioural disorders*
- VI. *Diseases of the nervous system*
- VII. *Diseases of the eye and adnexa*
- VIII. Diseases of the ear and mastoid process
- IX. **Diseases of the circulatory system**
- X. Diseases of the respiratory system
- XI. **Diseases of the digestive system**
- XII. *Diseases of the skin and subcutaneous tissue*

- XIII. *Diseases of the musculoskeletal system and connective tissue*
- XIV. *Diseases of the genitourinary system*
- XV. *Pregnancy, childbirth and the puerperium*
- XVI. *Specific conditions originating in the perinatal period*
- XVII. *Congenital malformations, deformations and chromosomal abnormalities*
- XVIII. *Injury, poisoning and certain other consequences of external causes*
- XIX. *Factors influencing health status and contact with health services*

The diagnosis of diabetes mellitus, as an example, justifies the approach of the direct and indirect relation with nutrition.

There are two main types of diabetes mellitus:

- Type 1 diabetes (T1DM) usually develops in childhood and adolescence and patients require lifelong insulin injections for survival;
- Type 2 diabetes (T2DM) usually develops in adulthood and is related to obesity, lack of physical activity, and unhealthy diets. This is the more common type of diabetes (representing 90% of diabetic cases worldwide (Diabetes Programme, WHO, 2012), as well as in Latvia (Anonymous, 2012g) and treatment may involve lifestyle changes and weight loss alone, or oral medications or even insulin injections.

In Latvia the direct treatment of type 2 diabetes patients (more than 70 000 patients) consumes almost 15% of the overall state budget for the reimbursement system (Anonymous, 2012g). At the same time, diabetes mellitus causes a lot of complications, such as following (Anonymous, 2012a):

- Diabetes mellitus increases the risk of heart disease and stroke — 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke).

- Combined with reduced blood flow, neuropathy in the feet increases the chance of foot ulcers and eventual limb amputation.
- Diabetic retinopathy is an important cause of blindness, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind, and about 10% develop severe visual impairment.
- Diabetes mellitus is among the leading causes of kidney failure — 10–20% of people with diabetes die of kidney failure.
- Diabetic neuropathy is damage to the nerves as a result of diabetes, and affects up to 50% of people with diabetes mellitus. Although many different problems can occur as a result of diabetic neuropathy, common symptoms are tingling, pain, numbness, or weakness in the feet and hands.
- The overall risk of dying among people with diabetes mellitus is at least double the risk of their peers without diabetes mellitus.

In 2011, the treatment of diabetes mellitus complications was ensured for 53 400 patients, there were 314 direct disability cases and 486 deaths caused by diabetes in Latvia (Anonymous, 2012g). In the author's opinion, the calculation of the Potential Years of Life Lost (PYLL) and Disability-Adjusted Life Years (DALY) are the most appropriate methods to evaluate the economic impact of this disease, but for this purpose, data of mortality by age and gender should be used, which are not available on the statistic database websites.

Data on morbidity by groups of age and gender show that under age of 50 years the diabetes mellitus prevalence is higher for males, but beginning from 50 years the morbidity sufficiently increases with higher prevalence for females. The number of patients is particularly high in the 70–80-year age group of (Anonymous, 2012b). According to provisional results of the Central Statistic Bureau, the average life expectancy of inhabitants will rise (Anonymous, 2012b), and taking into account the population age structure (Fig. 5), an increase in the number of patients needed to

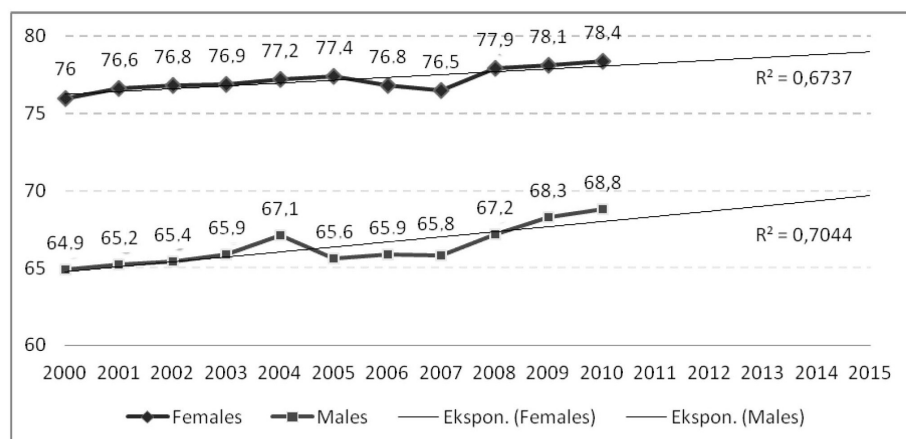


Fig. 5. Life expectancy at birth in Latvia, 2000–2010, and forecasts (Anonymous, 2011).

treat is forecast not only for diabetes mellitus, but also for the other chronic diseases.

In these circumstances the search and assessment of alternative treatments, based on prevention, become important.

DISCUSSION

The author, by analysis of the medicine reimbursement system from allocative and technical efficiency points of view, notes that there has been a trend towards technical efficiency, rather than overall economic efficiency (Fig. 6). Figure 6 is based on the classic framework of the Farrell model, which makes it possible to decompose overall efficiency into technical and allocative efficiency. The output of the model is the treated patient, which is the outcome of two input variables (medicines, alternatives). Under the assumption that the production function is linearly homogeneous, the efficient unit isoquant shows all technically efficient combinations. In circumstances of a limited budget, represented by isocost line A1A2, the reimbursement result point is located at point S on line A1A2, which insures allocative efficiency, but not technical and overall efficiency, and therefore the patient does not receive complete treatment. There is a tendency to attempt to achieve technical efficiency at point R by increasing the budget assignation for medicine reimbursement (isocost line B1B2), but the treatment alternatives (nutrition programme, physician's time by consultation or manual therapy etc.) are not evaluated sufficiently. It should be taken into account that the overall economic efficiency would be achieved by the evaluation and wider use of the alternatives (point Q on line A1A2). Any point on the isoquant line has technical efficiency, but only Q receives technical efficiency at minimum cost, by combination with allocative efficiency, to achieve the overall efficiency — the product of technical and allocative efficiency.

In the author's view, at least for individual reimbursement in the case when standard medicine therapy does not achieve the desired therapeutic effect, both the relative effectiveness of comparable medicines and other health technology assessment should be considered. In accordance with the International Society for Pharmacoeconomics and

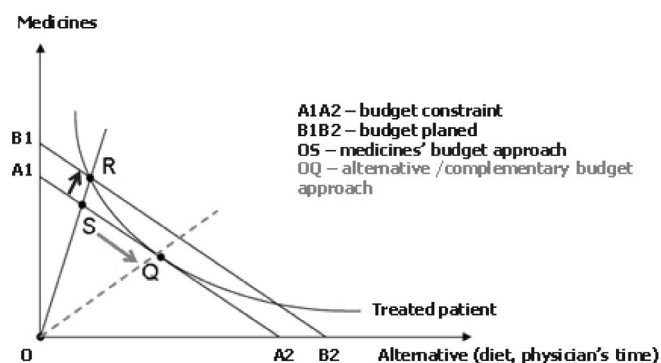


Fig. 6. Allocative, technical and overall efficiency in health care (Anonymous, 2008).

Outcomes Research, the goal of health technology assessment (HTA) is to provide policymakers with information on policy alternatives. Health care technology within the concept of HTA is defined broadly as consisting of medicines, biologicals, devices, equipment, supplies, medical and surgical procedures, support systems, organisational, delivery and managerial systems (Anonymous, 2003). For example, in the United Kingdom, competent authorities carry out complex HTA, which includes medicines, medical devices, vaccinations, screening programmes, medical staff services, procedures and public health programmes (Garrido *et al.*, 2008). In Latvia, the evaluation of the economic efficiency is centered on assessment of medicines and medical devices, but there is not a competent authority for evaluation of the comparative efficiency of all applicable health technologies.

The essential role of the alternatives, for example, in the case of above mentioned type 2 diabetes, is justified by several randomised controlled trials (RCT), which show that lifestyle interventions based on diet and exercises, reduce the proportion of people with glucose tolerance that would otherwise develop T2DM. The results of RCT collected and evaluated in an academic dissertation on Assessment of Risk and Prevention of Type 2 Diabetes in Primary Health Care (Saaristo, 2011) showed that the 1–6 years incidence of diabetes was reduced in lifestyle trials by 30–60% compared with a control group. At the same time, the preventable use of *Metforminum* without lifestyle interventions showed less than 30% relative risk reduction of T2DM. Follow-up studies of the major lifestyle trials in prevention of type 2 diabetes in seven-year period showed a relative risk reduction of T2DM by 34–43%, compared to the control group. Thus, these trials showed that T2DM can be prevented or delayed by lifestyle interventions. In scope of above mentioned academic dissertation, the effect of lifestyle interventions on weight and its association with glucose tolerance was evaluated in individuals at high risk for diabetes in a one-year follow-up. In total 17.5% of them lost more than 5% of weight. Their relative risk for diabetes decreased 69% compared with the group that maintained their weight (Saaristo, 2011).

Coming back to the issue of the limited budget and patient resources for the medicines reimbursement system, in the author's opinion a discussable question is also, data from the Central Statistical Bureau of Latvia that shows, based on the composition and structure of average consumption expenditure per household member, one third of the income of Latvian inhabitants is spent for food (Anonymous, 2012e). Thus, the rational diet is both the potential for disease prevention and saving resources for a healthy lifestyle.

The main conclusions of this research are following:

- The largest number of reimbursed diagnoses, such as cardiovascular diseases, diabetes, cancer, digestive diseases, osteoporosis, are identified as nutrient-related diagnoses by a special literature review.

- The nutrient-related diagnoses contribute more than half of the state budget resources allocated to the reimbursement system in Latvia.
- The budget approach could be changed from medicine-based to an alternatives-based approach.
- There are not sufficient data to calculate indicators such as Potential Years of Life Lost (PYLL) and Disability-Adjusted Life Years (DALY), which can be used in economic assessment of treatment and preventive programmes.
- Prevention programmes for potential patients and educational programmes for patients should be encouraged.
- Data on composition and structure of consumption expenditure average per household member show that one-third of income is spent for food, and thus the Hippocrates' quote "Let food be thy medicine and medicine be thy food" can be important and useful also in the present epoch.

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UZTURA ATKARĪGĀS SLIMĪBAS ZĀĻU KOMPENSĀCIJAS SISTĒMA LATVIJĀ

Latvijā ambulatorajai ārstēšanai paredzēto zāļu un medicīnisko ierīču iegādes izdevumu kompensācijas sistēma darbojas stingri ierobežotu valsts budžeta līdzekļu apstākļos, līdz ar to aktuāls kļūst jautājums par papildus iespēju izvērtēšanu līdzekļu izlietojuma optimizācijai, ņemot vērā arī preventīvo pasākumu nozīmīgumu. Pētījuma mērķis ir identificēt uztura atkarīgās slimības zāļu iegādes izdevumu kompensācijas sistēmā un novērtēt iespējas noteikt papildinošo uztura programmu potenciālo efektivitāti. Mērķa sasniegšanai izmantotas teorētiskās izpētes metodes (normatīvo dokumentu un speciālās literatūras analīze) un empīriskās izpētes metodes (statistikas datu vākšana, apkopošana un analīze). Pētījuma rezultāti liecina par to, ka lielākā daļa kompensējamo diagnožu, tādas kā sirds un asinsvadu slimības, diabēts, onkoloģiskās slimības, gremošanas sistēmas slimības, osteoporoze, ir uztura atkarīgās slimības, un tām tiek izlietota vairāk kā puse zāļu kompensācijas sistēmā atvēlētajā budžeta. Turklāt ir virkne slimību, kas ir netieši atkarīgas no uztura, bet tās rodas kā uztura atkarīgo slimību izraisītās komplikācijas. Uz pierādījumiem balstītās medicīnas laikmetā īpaši aktuāla kļūst ārstēšanas un preventīvo pasākumu ekonomiskās efektivitātes novērtēšana. Speciālajā literatūrā ir dati par uztura programmu pozitīvo nozīmi smagu slimību novēršanā vai ietekmes mazināšanā, tomēr jāatzīmē, ka nav izstrādāta precīza metodoloģija, kas ļautu veikt medikamentozās ārstēšanas un preventīvo pasākumu salīdzinošo ekonomiskās efektivitātes novērtēšanu, lai sagatavotu ar pierādījumiem pamatotu informāciju lēmumu pieņemšanai par līdzekļu sadali. Līdz ar to šis ir turpmākās izpētes objekts.

MICROBIOLOGICAL RISK ANALYSIS IN CATERING ESTABLISHMENTS

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The epidemiological data suggest that the food preparation process in public catering establishments involves the risk of food microbiological contamination. To develop a preventive food safety assurance system based on HACCP (Hazard Analysis and Critical Control Points) principles, adequate identification, monitoring and communication of food safety hazards are important considerations. The aim of the research was microbiological risk analysis of catering establishments. Statistical analysis was used in microbiological risk assessment and to ensure science-based proposals for control of microbiological contamination and prevention of outbreaks of food-borne infections. The results on microbiological testing of 17 192 food samples and 17 604 surface swab samples were analysed using the SPSS 13.0 and MS EXCEL software packages. Statistically significant differences in microbiological contamination of food and environmental surfaces with regard to Aerobic Plate Count, coliforms and S. aureus were found. Impact of technological processing on safety of ready-to-eat foods was demonstrated. Petrifilm rapid test methods were tested for use as self-control purposes. The results of the research demonstrate characteristic trends in contamination of foods and environmental objects in catering establishments and ensure scientific justification for setting priorities with regard to relevant control measures during technological processing and serving of food.

Key words: risk analysis, microbiological risk assessment, catering food safety, HACCP.

INTRODUCTION

Improvement of food quality and safety is the overall aim of food policy to achieve a higher level of protection of consumer health. Food should not contain contamination that can create risk to human health. The primary responsibility with regard to implementation of food safety legislation rests with food business operators. To fulfil legal requirements, food establishments need to establish appropriate food safety management procedures based on HACCP (Hazard Analysis and Critical Control Points) principles (Farber *et al.*, 2000; Luning *et al.*, 2006).

The epidemiological data suggest that food preparation processes in catering establishments are often associated with increased food microbiological contamination risk (Griffith and Clayton, 2005; Koppanen *et al.*, 2005; Szabo, 2005; Bohm *et al.*, 2007). Pathogenic microorganisms can enter food due to unhygienic food handling procedures, and can multiply in food due to inadequate food storage, chilling, thawing and processing parameters (Pourkomailian, 2005; Scmhid *et al.*, 2007).

Development of preventive food safety assurance systems comprises both the identification of important food safety hazards and the introduction of regular monitoring measures in critical control points of technological processes. It is widely recognised that management of technological processes should be based on detailed analysis of product characteristics and process conditions to assess the potential impact on quality and safety of the ready-to-eat foods (Kārklīņa *et al.*, 2005; Burlingame and Pineiro, 2007; Schaffner, 2007).

According to global food safety standards, 'risk' means a function of the probability of an adverse health effect consequential to a hazard, and 'risk analysis' means a process consisting of three interconnected components: risk assessment, risk management and risk communication (Anonymous, 2006). It should be emphasized that implementation of risk management and risk communication measures should always be based on identification of objective risk factors (Stringer, 2005; Špoģis, 2005; Rivža *et al.*, 2007). Microbiological risk assessment can be used as a supportive tool for establishment of scientifically justified control mea-

asures within HACCP to improve the efficiency of risk management and communication procedures (Reij M.W *et al.*, 2004; Sprenger, 2004; Anonymous, 2005; Buchanan, 2005; Tebbutt, 2007; Hugas *et al.*, 2007; Melngaile and Karklina, 2007; 2006; Lammerding, 2007; Fretz, 2007; Rodgers, 2005). The concept of risk analysis should be applied for development of a food safety assurance strategy of both the food businesses and the governmental food safety surveillance and control institutions (Andersen *et al.*, 2007). It should be mentioned that catering establishments still face serious problems in relation to identification of food safety hazards and implementation of adequate monitoring procedures (Bolton *et al.*, 2008; Eves and Dervisi, 2005; Hielm *et al.*, 2006).

The aim of the research was to investigate the application of microbiological risk analysis in public catering establishments. In order to achieve the aim of the research, the following tasks were identified a) to assess microbiological contamination risk that results from technological processing of foods in public catering establishments, including food cross-contamination risk; b) to highlight proposals for further improvement of microbiological risk management and communication procedures in public catering establishments.

MATERIALS AND METHODS

Microbiological testing of food samples. Results on microbiological testing of 17 192 food samples from catering establishments obtained in the frame of the state surveillance programme and available in the Database of the Food and Veterinary Service of Latvia, including 3152 Aerobic Plate Count tests, 4481 coliform tests, 2138 *Staphylococcus aureus* tests, and 5283 *Salmonella* spp. tests, were statistically analysed.

Microbiological testing of surface swab samples. Results on microbiological testing of 17 604 surface swab samples from catering establishments that were obtained in the frame of the state's surveillance programme and available at the Database of the Food and Veterinary Service of Latvia, including 8934 coliforms' tests, 5113 *S. aureus* tests, and 3385 *Salmonella* spp. tests, were statistically analysed.

Testing methods. The following testing methods were used in the frame of the state surveillance programme: a) Aerobic Plate Count (APC) in foods was determined according to the standard method LVS ISO 4833:2003 "Microbiology. General directions for enumeration of microorganisms. Colony counting method at 30 °C"; b) *Enterobacteriaceae* count in foods and environmental surface swab samples was determined according to the standard method ISO 21528-2:2004 "Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 2: Colony – count method"; c) *Staphylococcus aureus* count in food samples was determined according to the standard method LVS EN ISO 6888-1/A1-2003 "Microbiology of food and animal

feeding stuffs – Horizontal method for enumeration of coagulase positive *Staphylococcus aureus* – Part 1: Method, using environment"; d) Coliforms in surface swab samples were determined in accordance with the test method VVMDC-T-012-010.5-2000 "Methods for microbiological analysis of surface swabs – Part 2: Detection of coliforms"; e) *Staphylococcus aureus* in surface swab samples was tested in accordance with the test method VVMDC-T-012-010.5-2000 "Method for microbiological analysis of surface swabs – Part 5: Detection of *Staphylococcus aureus*".

Approbation of Petrifilm rapid test method. The Petrifilm rapid test method was tested for monitoring of cleaning efficiency – disinfection procedures in catering establishment, using ready-to-use selective media for cultivation of microorganisms. Swabs were taken from 20 visually clean surfaces, taking into account known significant trends in distribution of microbiological contamination. 3M Microbiology Products (USA) for enumeration of microorganisms were used for detection of aerobic plate count (APC), *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, yeast and mould counts in swab samples.

Data arrangement for mathematical analysis. For statistical analysis, the following encoding of data was made:

- 1) food samples were grouped into 14 identification classes, 31 groups and 142 types, taking into account the food main components and characteristic methods of technological processing;
- 2) surfaces were grouped into 16 identification classes, 90 groups and 187 types, taking into account the characteristic application of equipment, utensils, constructions and other objects;
- 3) methods of technological processing were grouped into 18 groups taking into account the way of technological processing, including characteristic sequence of technological processes during food preparation processes.

Statistical analysis. Microbiological contamination of foods and surfaces of equipment and utensils was described by features: Aerobic Plate Count (APC), coliforms, *Staphylococcus aureus* and *Salmonella* spp. The statistical data were processed using software package SPSS 13.0.

Testing results on coliforms, *Staphylococcus aureus* and *Salmonella* spp. were qualitative parameters. Presence was designated as "1" and not detected as "0". The qualitative features were ranked with regard to the following factors: identification classes, groups and types of foods; identification classes, groups and designations of surfaces; methods of technological processing; and types of microorganisms. Significant differences ($P < 0.05$) in empirical and theoretical distributions were determined using the Chi-Square test.

Aerobic Plate Count was expressed quantitatively as colony-forming units (CFU) g⁻¹. Analysis of variance ANOVA was used to test for differences in APC among public catering establishments, identification classes, groups and types

of foods and methods of technological processing. Homogeneity of variance was tested. The post hoc Scheffe criterion was used in cases with similar variances and Dunnet's T3 criterion when variances were not similar.

MS EXCEL software was used for graphical description of the data. Proportions were shown as pie charts. Other data were depicted as bar charts and histograms.

RESULTS

Microbiological risk assessment with regard to ready-to-eat foods. The results of the statistical analysis indicate that the mean value of APC in ready-to-eat foods, as well as probability of the presence of coliforms and *S. aureus* in ready-to-eat foods, significantly depended on the method of food technological processing ($P = 0.000$) (Figs. 1 and 2). Increased microbiological contamination risk was proven for foods that were prepared using mechanical processing, for both thermally unprocessed foods and thermally processed and chilled foods. Characteristic trends in microbiological contamination were also detected for thermally processed foods.

The APC of ready-to-eat foods significantly differed among the identification classes of ready-to-eat foods (Fig. 3), as well as among certain groups and types of foods ($P = 0.000$). The mean value of APC was significantly lower for thermally processed foods, such as hot soups, grain and vegetable foods, fish foods and meat foods. The mean value of APC was considerably higher for chilled foods, e.g. chilled entry foods, pastry foods, salads and dairy products.

The APC significantly differed for farinaceous foods, salads and pastry foods, with higher mean APC for foods with meat components. According to the results of microbiological risk assessment, ready-to-eat foods were grouped into four categories:

- thermally processed foods, the APC of which was usually less than 1000 CFU g^{-1} : thermally processed soups, grain foods, seafood, vegetable foods and meat foods;
- ready-to-eat foods, the APC of which was usually $1000\text{--}5000 \text{ CFU g}^{-1}$: thermally processed pasta foods, farinaceous foods and different dessert foods. A relatively large standard deviation was found for pasta foods (2.9), farinaceous foods (3.0), and different dessert foods (3.1);

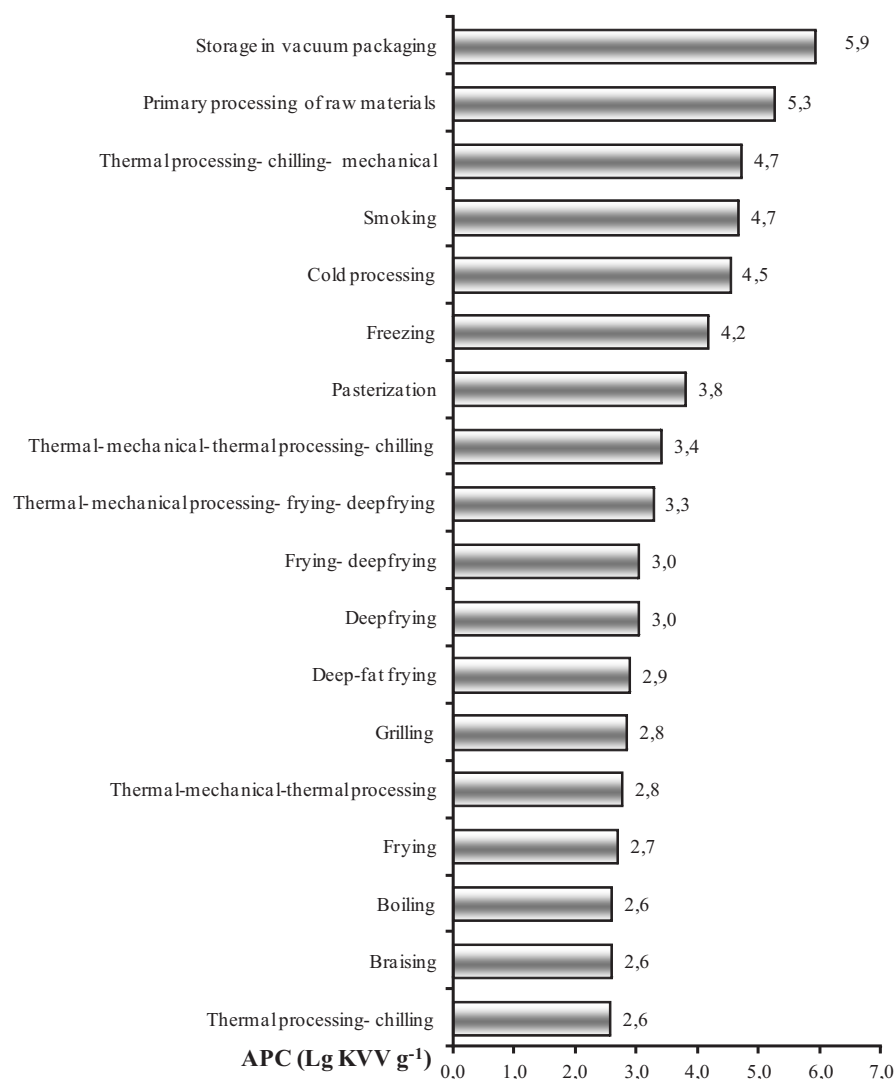


Fig. 1. Impact of the technological processing on APC (Aerobic Plate Count) of ready-to-eat foods ($P = 0.000$).

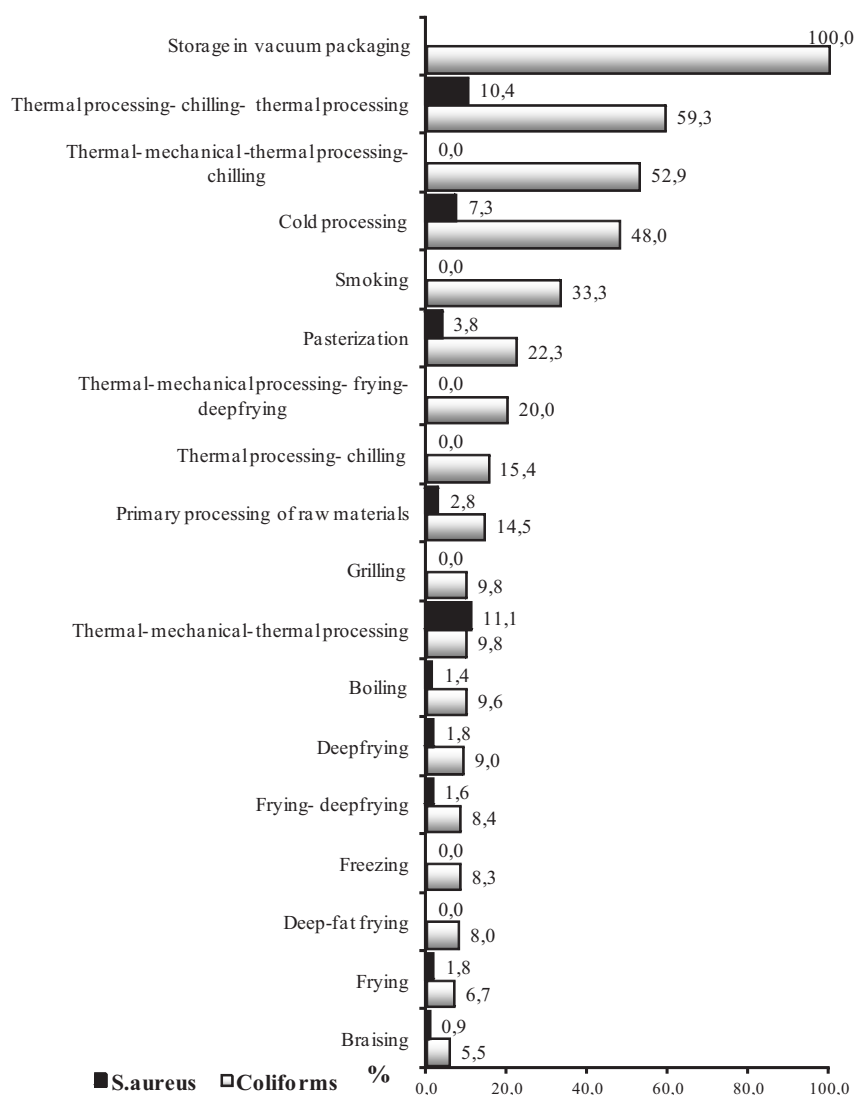


Fig. 2. Impact of the technological processing on the probability of presence of coliforms and *S. aureus* in ready-to-eat foods ($P = 0.000$).

- ready-to-eat foods, the APC of which was usually 5000–10 000 CFU g⁻¹: curd foods, egg foods, cold entry foods, milk product foods. A relatively large standard deviation was found for curd foods (3.3), egg foods (5.3), and chilled entries (3.8);

- ready-to-eat foods, the APC of which was usually more than 10 000 CFU g⁻¹: pastry foods and different salads (standard deviation 3.1 and 3.2, respectively).

Significant differences were observed in probability of presence of coliforms and *S. aureus* in foods of different identification classes (Fig. 4), groups and individual types of ready-to-eat foods ($P = 0.000$). APC and occurrence of coliforms and *S. aureus* in food samples were relatively low.

Relatively higher contamination risk with regard to coliforms and *S. aureus* was shown for the following chilled food groups: vegetable salads and salads containing components of animal origin, desert sweets, chilled meat entries and chilled curd foods. In the case of thermally processed foods, pasta and farinaceous foods had higher contamination risk.

Taking into account the mean APC value, as well as probability of presence of coliforms and *S. aureus* in different foods, higher microbiological contamination risk was observed for certain types of ready-to-eat foods: fried and braised meat and offal foods, especially fried poultry and fried minced-meat foods; pasta foods with meat components; pancakes with meat or curd stuffing; soups prepared on milk basis and rissole soup; salads containing raw vegetable components and salads containing cooked vegetable components; meat and fish entry foods; certain dessert sweets — dessert creams, mousse, sweet porridges; and for pastry foods with cream stuffing.

Microbiological risk assessment with regard to direct and indirect food contact surfaces. Significant differences were found in probability of presence of coliforms and *S. aureus* on different surfaces that come into direct or indirect contact with foods ($P = 0.000$) (Fig. 5). Relatively higher contamination risk was shown for utensils used for storage, display and serving of ready-to eat foods (e.g. bowls, pots, servers, ladles, spoons used for salads and dessert foods, pastry vessels, juice glasses), for utensils and equipment used for preparation of chilled foods (e.g. vegetable cutting boards, knives, graters), as well as for surfaces

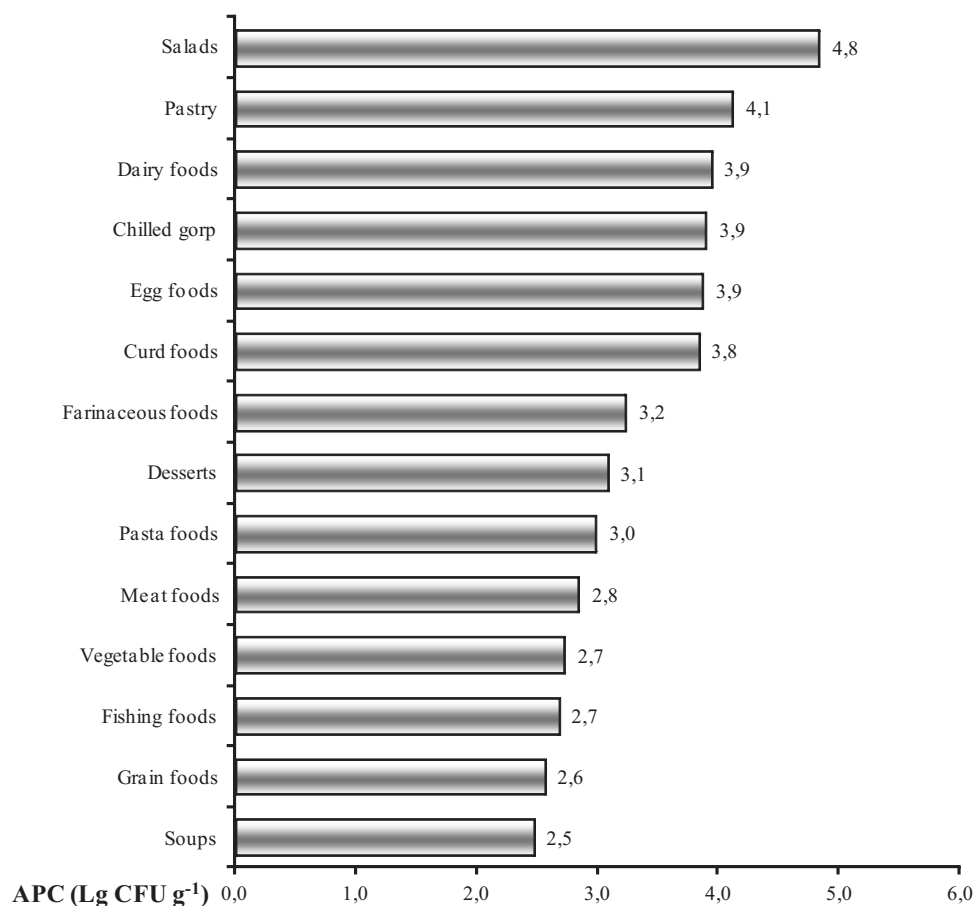


Fig. 3. Mean APC (Aerobic Plate Count) within identification classes of ready-to-eat foods ($P = 0.000$).

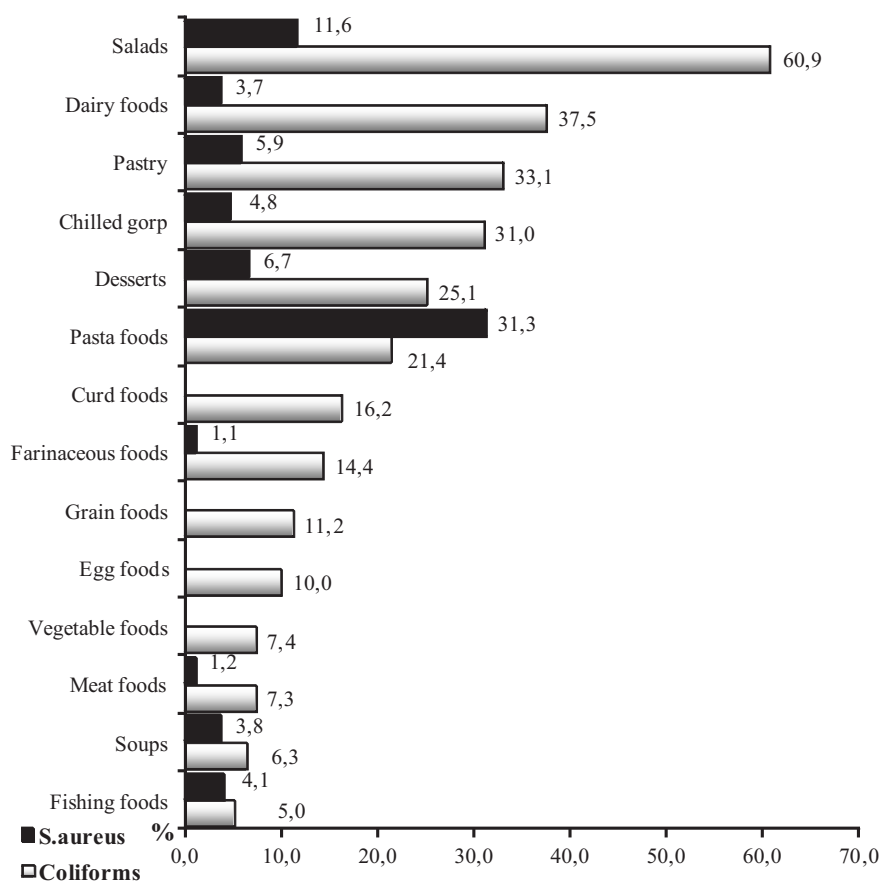


Fig. 4. Probability of presence of coliforms and *S. aureus* within the identification classes of ready-to eat foods ($P = 0.000$).

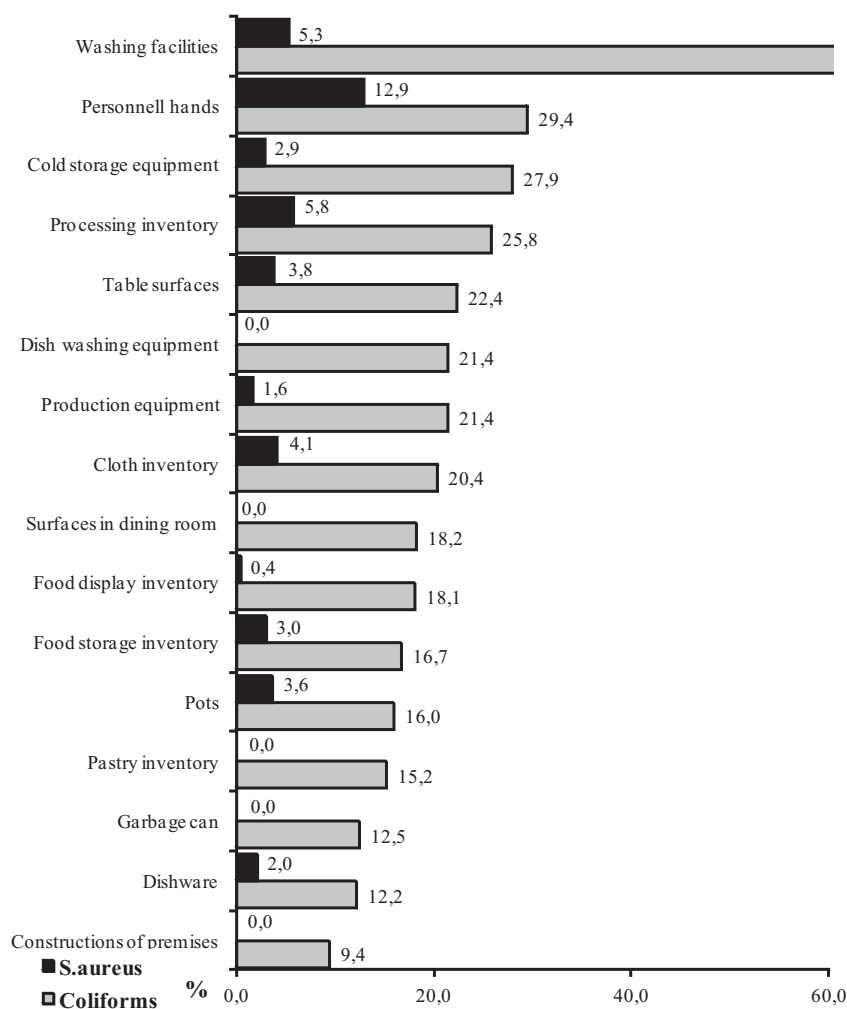


Fig. 5. Probability of the presence of coliforms and *S. aureus* on direct and indirect food contact surfaces ($P = 0.000$).

of certain work-tables (e.g. work-tables for salad and dessert preparation), sink taps and personnel hands. The results indicated that coliforms and *S. aureus* were most often found on surfaces that come into contact with ready-to-eat foods; surfaces that come into direct contact with personnel hands; and surfaces that come into contact with clean dishes. In total, the presence of coliforms was detected on 118 different contact surfaces (63% of surfaces examined), and the presence of *S. aureus* on 34 contact surfaces (18% of surfaces under investigation).

Taking into account that rapid hygiene tests have been recommended for verification of self-control measures in different areas of food industry (Park *et al.*, 2001; Schoeller and Ingham, 2001; Ferrati, 2005; Silva *et al.*, 2005; Sousa, 2005; Nyachuba and Donnelly, 2007), the approbation of *Petrifilm* rapid test methods in catering establishments was carried out during the study. Swabs were taken from surfaces for which the highest contamination risk was suggested by the statistical analysis. Significant microbiological contamination (APC), including *Enterobacteriaceae*, *E. coli*, *S. aureus*, yeasts and moulds was detected on 20 visually clean surfaces that come into contact with foods and hands of food service personnel. For example, the biggest APC values were observed in swab samples that were taken from surface of hands of food service personnel (1.1×10^4

and 4.2×10^3 CFU cm⁻²), drink containers (6.5×10^3 CFU cm⁻²), dessert bowls (2.8×10^3 CFU cm⁻²) and salad bowls (1.7×10^3 CFU cm⁻²). All tested microorganisms, i.e. *Enterobacteriaceae*, *E. coli*, *S. aureus*, yeasts and moulds were detected on visually clean surfaces of salad bowls and vegetable cutting knives. Presence of *S. aureus* was detected on surfaces of 12 different utensils and equipment, due to presence of those bacteria on surface of hands of two food service personnel.

DISCUSSION

It is widely recognized that adequate preventive measures should be put in place to eliminate or reduce microbiological contamination of ready-to-eat foods in catering establishments. According to the research results, the main risk factors that lead to microbiological contamination of foods are: cross-contamination of foods due to poor hygiene practice, survival of bacteria due to insufficient temperature-time regime during thermal processing of foods, as well as multiplication of bacteria due to inadequate food chilling and storage conditions. Similar risk factors are mentioned in publications of other authors (Soriano *et al.*, 2002; Evans *et al.*, 2004; Beumer and Kusumaningrum, 2005; Koppanen *et al.*, 2005; Medus, 2005; Bohm *et al.*, 2007; Scmhid *et al.*, 2007). It should be emphasized that,

unlike previous studies, the distribution of microbiological contamination was examined by statistical analysis. The results of research carried out by the authors suggest that implementation of preventive hygiene measures is a critical step that may have substantial impact on catering food safety; unfortunately, the efficiency of hygiene procedures is not adequately controlled.

According to findings of the research the presence of hygiene indicator-microorganisms in ready-to-eat foods can be also detected in cases when overall APC is relatively low. Taking into account that even small amounts of pathogenic microorganisms can cause illness of food consumers, trends in distribution of microbiological contamination should be analysed and used to develop adequate control arrangements in a timely manner.

The statistical data obtained showed significant differences in distribution of microbiological contamination with regard to foods and food contact surfaces, and therefore, can be successfully used for improvement of procedures for both the technological processing of foods and the cleaning and

disinfection of contact surfaces. The results on microbiological risk assessment can be successfully used for ranking of foods and food contact surfaces with regard to microbiological contamination, as well as for purposeful modelling of food microbiological contamination risk with regard to impact of certain technological processes. For example, the results on risk assessment suggest that there is high probability of cross-contamination of salads and desserts during preparation and serving of them in catering establishments, as we observed a probability of presence of coliforms and *S. aureus* (Fig. 6; Fig. 7). It should be mentioned that food and swab samples are rarely analysed within self-control procedures of catering establishments (e.g. only one ready-to-eat food sample or aggregated swab sample during period of one-two years) and do not provide suitable information on the actual distribution of microbiological contamination within the course of technological processing of foods.

The data obtained in frame of the state's surveillance and control programmes suggest that control of *Salmonella spp.* in ready-to-eat foods and on food contact surfaces almost

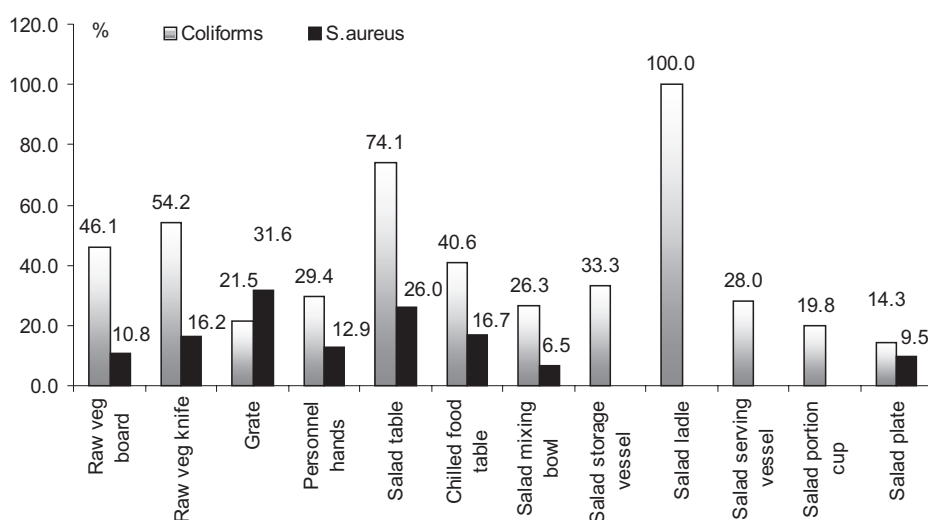


Fig. 6. Risk assessment with regard to food contact surfaces that contribute to cross-contamination of fresh vegetable salads.

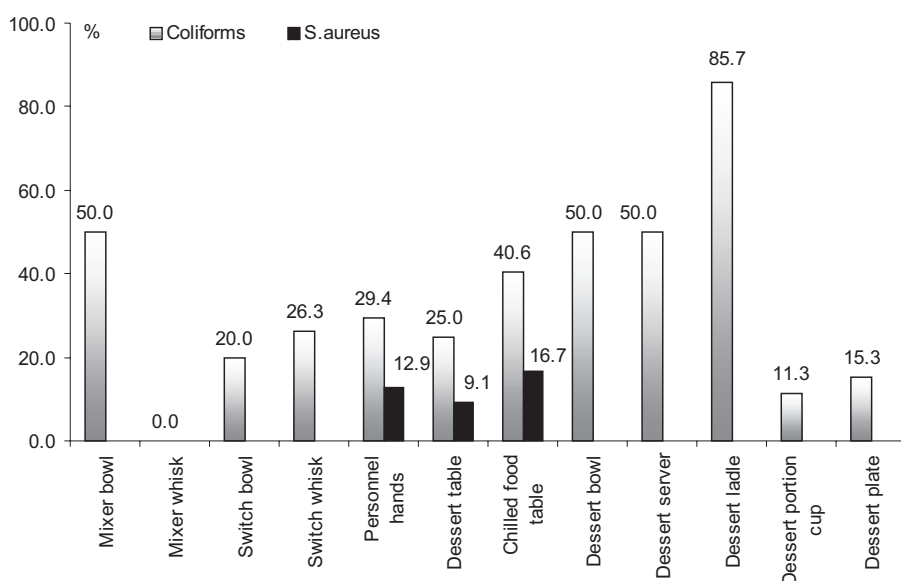
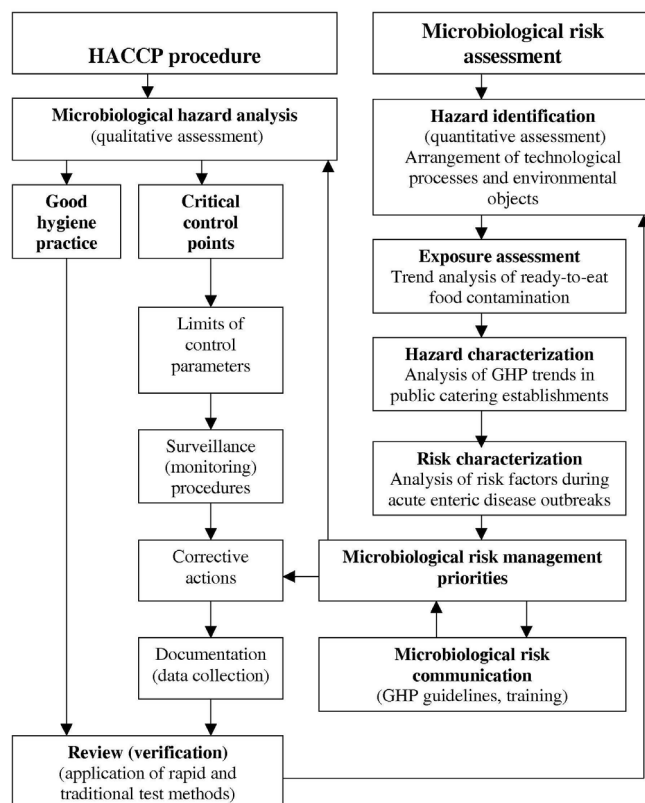


Fig. 7. Risk assessment with regard to food contact surfaces that contribute to cross-contamination of desserts

2. Application of methods of mathematical statistics for microbiological risk assessment helps to reveal important trends in contamination of ready-to-eat foods and food contact surfaces and ensures science-based information for setting of priorities with regard to control of microbiological hazards in ready-to-eat foods.



7. Microbiological risk analysis can be applied in catering establishments to ensure objective data on distribution of microbiological hazards and to implement purposeful risk management and communication procedures in the frame of HACCP procedures to prevent outbreaks of food-borne diseases.

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MIKROBIOLOĢISKĀ RISKĀ ANALĪZE SABIEDRISKĀS ĒDINĀŠANAS UZŅĒMUMOS

Epidemioloģiskie dati liecina, ka ēdienu gatavošanas process sabiedriskās ēdināšanas uzņēmumos ietver ēdienu mikrobioloģiskās piesārņošanas risku. Lai izveidotu profilaktisku, uz HACCP principiem balstītu pārtikas nekaitīguma nodrošināšanas sistēmu, ir būtiski veikt atbilstošu pārtikas drošuma apdraudējumu identifikāciju, monitoringu un komunikāciju. Pētījuma mērķis bija izpētīt mikrobioloģiskās riska analīzes metodoloģijas pielietojanu ēdināšanas uzņēmumos. Lai veiktu mikrobioloģiskā riska novērtēšanu un izstrādātu zinātniski pamatotus priekšlikumus mikrobioloģiskā piesārņojuma kontrolei un pārtikas izcelsmes infekciju uzliesmojumu novēršanai, izmantotas matemātiskās statistikas metodes. Lietojot SPSS 13.0 un MS EXCEL programmatūras, analizēti 17 192 ēdienu un 17 604 virsmu nomazgājumu paraugu mikrobioloģiskās testēšanas rezultāti. Noskaidrotas statistiski nozīmīgas ēdienu un vides virsmu mikrobioloģiskā piesārņojuma atšķirības saistībā ar kopējo mikrobioloģisko piesārņojumu un zarnu nūjiņu grupas baktēriju un *S. aureus* klātbūtni. Atklāta tehnoloģiskās apstrādes ietekme uz gatavo ēdienu nekaitīgumu. Lai īstenotu paškontroli, aprobētas *Petrifilm* ātrās testēšanas metodes. Pētījuma rezultāti liecina par raksturīgām ēdienu un vides objektu mikrobioloģiskā piesārņojuma tendencēm ēdināšanas uzņēmumos un nodrošina zinātnisku pamatojumu prioritāšu izvirzīšanai, lai ēdienu tehnoloģiskās apstrādes un pasniegšanas laikā ieviestu nepieciešamos kontroles pasākumus.

EATING HABITS OF CHILDREN AND ADOLESCENTS WITH EPILEPSY IN LATVIA

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The aim of the study was to evaluate the eating habits of epileptic children and adolescents. Forty one patients (12 girls and 14 boys aged 0–10 years, and 10 girls and 5 boys aged 11–18 years) were enrolled in the study. A survey on consumption rate of different dietary products was conducted. The diet groups considered were carbohydrates, fat, protein, fibre and liquid. We found that 35–58% of patients aged 0–10 years used soda drinks, juices and popcorn regularly. The proportion was more than two times in adolescents. Consumption of sweets in both age groups was 83–100%. Half of children aged 0–10 years said that they ate chips and fried potatoes regularly and 80–100% of adolescents consumed these products at least once a week. Half of the boys and almost all younger girls consumed various milk products daily; adolescent epileptic boys consumed milk products two times more than girls. Among older girls, 40% did not drink milk. The youngest girls consumed meat and fish 1.5 to 3.5 times more than boys, while the number of boys consuming these products increased with age. It was estimated that 75–100% of patients ate fruits and vegetables daily. Only two children and two adolescents were found to drink lots of water. In conclusion, children and adolescents with epilepsy preferred meals rich in carbohydrates and fat, rather than protein; a high number of the evaluated patients drank soda drinks and juices several times a week; only a few had intake of water daily; and all patients ate fruits and vegetables daily, thus compensating unhealthy eating habits.

Key words: children and adolescents, epilepsy, growth and maturation, hormonal changes, antiepileptic drugs, bone metabolism.

INTRODUCTION

Epilepsy is a fairly prevalent neurological disorder in children and adolescents and is defined as a proneness to recurrent epileptic seizures. An epileptic seizure arises as a result of changed electrical activity in the brain, and is manifested as alterations in sensation (vision, hearing, touch, smell, taste), behaviour or consciousness (Neal and Cross, 2010). The incidence of epilepsy is 5–7 children in 100 000 (about 3–4%) aged 0–15 years (Nettekoven *et al.*, 2008). There are still no available precise data regarding the frequency of epilepsy among children and adolescents in Latvia.

Although epilepsy is defined as a condition of recurrent spontaneous seizures not directly related to a specific event, many patients also report that seizures are occasionally provoked by external or internal stimuli (Asadi-Pooya and Sperling, 2007). The most common precipitating factors in adults and children and adolescents include sleep deprivation, consumption of alcohol among adolescents, premature awakening, menstruation, psychological stress, and visual stimulation. Other factors such as reading, thinking, writing,

calculating, and playing musical instruments have been reported as well (Frucht *et al.*, 2000; Spector *et al.*, 2000; Sousa *et al.*, 2005). Knowledge of factors promoting seizure precipitants is helpful, as it can modify patient's behaviour and, perhaps, lead to a reduction in seizure frequency.

Nutrition is fundamental in regulating the activity of electrical discharge in the brain. Unfortunately, there has been quite little research regarding nutrition and related eating habits in children and adolescents with epilepsy.

Already 1000 years ago, the great Iranian scientist Avicenna recommended that patients with epilepsy should avoid excessive eating and some foods such as cow and sheep meat, fish, onion, garlic, celery, cauliflower, and carrot (Avicenna, 1991). The ketogenic diet, which is compatible with avoiding excessive eating, is a high-fat, low-carbohydrate and low-protein regimen that has been used successfully for more than 70 years in thousands of patients (Asadi-Pooya *et al.*, 2004). It has been calculated that this diet provides 75–100 kcal/kg body weight and 1–2 g of dietary protein/kg body weight per day. Such strict caloric re-

quirements can be adjusted to minimise weight gain and to maximise ketonemia (excessive amount of keton bodies in the blood) (Nordli and De Vivo, 2001). A recent study suggested that caloric restriction reduces neuronal excitability in rats and that it has anticonvulsant effect, perhaps by an increase of fast inhibition (Bough *et al.*, 2003).

There are several possible reasons why specific foods may lead to seizures. Firstly, some foods may lower the seizure threshold (food-disease interaction) (Gallagher *et al.*, 1968; Bhagavan *et al.*, 1971; Miwa *et al.*, 2001). Such a food-disease interaction has been observed in adult patients with migraine headache, in some of whom certain foods induced attacks, such as chocolate, hot dogs, smoked meats, aged cheese, orange, and monosodium glutamate (MSG). Some theories suggest that migraine may be triggered via an allergic or chemical reaction that affects the vascular system (Burns and Carr-Davis, 1996; Victor and Ropper, 2001).

Secondly, a food-drug interaction has been observed with carbamazepine: the bioavailability and plasma concentration of carbamazepine was increased in patients who consumed grapefruit juice and dairy products (Garg *et al.*, 1998). Moreover, among patients who used carbamazepine, the belief and experience with a food-seizure relationship were reported more often than using phenobarbital or valproic acid.

There are several reports suggesting hazardous effect of certain foods on the brain by triggering seizures, including the occurrence of generalised convulsions after consumption of large amounts of Ginkgo nuts (Miwa *et al.*, 2001). There are also reports about decrease of the seizure threshold in rats by intake of excessive amino acids (Gallagher *et al.*, 1968), induction of convulsions by MSG in rats (Bhagavan *et al.*, 1971), and the induction of *status epilepticus* by star fruit intoxication in patients with chronic renal disease (Tsai *et al.*, 2005).

Noteworthy research regarding the preferences for food items in epileptic children and adolescents, compatible and incompatible with the ketogenic diet, has been conducted in the USA (Amari *et al.*, 2007). Enrolled patients were asked to choose a food item from a list of high-fat and high-carbohydrate food items. The researchers observed that the most popular item among children with seizures was bacon, and among the control group, noodles. The obtained data supported the prediction that children with seizures would exhibit higher preferences for high-fat than high-carbohydrate foods, compared with controls. This was a novel finding, without precedent in the literature.

It has been hypothesised that humans and animals may prefer to choose certain items to compensate for physiological and metabolic imbalances (Weingarten and Elston, 1990). For instance, significantly increased sodium-seeking behaviour and hedonic preference for salty foods were observed among adolescents with congenital adrenal hyperplasia (Cohli *et al.*, 2005). Another study demonstrated that carbohydrates are selectively craved by people who experience a

depressed mood (Wurtman and Wurtman, 1986; 1988; Christensen and Pettijohn, 2001).

It has been proved that consumption of fat versus carbohydrates as the primary source of energy is highly effective in controlling seizures in individuals, as well as improving behaviour, alertness, and mood. Compared to carbohydrates, consumption of fat in epileptic patients has positive consequences — reduced abnormal electrical discharge activity in the brain, increased alertness, and improved mood (Amari *et al.*, 2007).

Norwegian researchers evaluated nutritional habits in 12–19-year-old youth with epilepsy who consumed unhealthy food (candy, coffee/tea, potato chips, French fries or soda pop) at least once a day. They found that being male and never eating breakfast were the strongest predictors for eating „unhealthy food”. The authors also found that the significant increase among youth with epilepsy was associated with greater psychosocial problems within the adolescents (Clench Aas *et al.*, 2006).

In other work, Iranian scientists questioned 125 families with children who had epilepsy (the mean age 8.4 ± 4.3 years) in order to identify the beliefs and experiences associated with specific dietary restrictions (Asadi Pooya and Ghafari, 2004). It was observed that most of the families believed in such relationship — one-third had personal experience with seizure occurrence after consumption of specific foods. Dairy products, sour foods (like vinegar and lemon juice), fruits and vegetables, and fish and meat were the most common foods reported as the responsible ones (Asadi-Pooya and Hossein-Zade, 2005).

Conversely, research on adult patients in the USA ($n = 193$; mean age 40.3 ± 16 years), identified that only 11 patients (5.7%) reported foods as precipitating factors for seizures (Asadi-Pooya and Sperling, 2007). Moreover, there was no relationship between gender, education, seizure control, and certain food items. Thereby, authors suggested that reports of seizure precipitants were subjective impressions, which could easily be influenced by cultural beliefs or patterns of thought or superstitions. However, careful scientific studies are needed to establish the true incidence of food-induced seizures. It is also the responsibility of physicians and health care workers to provide patients and their families with appropriate and correct information regarding future changes in their lifestyle.

PATIENTS AND METHODS

In total, 41 epileptic patients (26 children and 15 adolescents) were included in the study, conducted at the Children's Clinical University Hospital in Riga. Patient groups were made according to gender and age of overall physical maturation: (1) 12 girls and 14 boys aged 0–10 years; and (2) 10 girls and 5 boys aged 11–18 years. The survey consisting of detailed questions about the consumption rate of different dietary products (milk, kefir, yoghurt,

sour cream, curd; pork, beef, poultry, fish; vegetables and fruits; sweets, soda drinks, water, coffee, tea; popcorn, potato chips, fried potatoes/French fries, and cereal products). Five dietary groups were made according to above mentioned dietary products: carbohydrates, fat, protein, fibre and liquid. All of the enrolled children had a written agreement from their parents or responsible persons, and the study was approved by the Ethical Commission.

RESULTS

Of the enrolled children, three girls (25%) aged 0–10 years were on ketogenic diet for more than three months. Of these girls, one was fed via nasogastric tube and one girl could not eat herself. Three boys aged 0–10 years (21.4%) had difficulties in consuming food: one boy (7.1%) had hypersensitivity (urticaria) from consuming potatoes, carrots, and apples, 1 boy (7.1%) had difficulty in chewing meat and complained of nausea after he had eaten an egg, 1 boy (7.1%) could not put a spoon in his mouth, and had difficulties in chewing and swallowing solid food items. One 11-year-old girl (10%) consumed food in small portions, because she was constantly having nausea.

On average, 3–4 meals per day were consumed by 71.4% boys and 83.3% girls in the youngest age group, and 100% boys and 60% girls in the oldest age group. 40% of girls aged 11–18 years had three and more snacks in between meals, which was twice higher than in boys (20%). However, more young boys (28.6%) had four or more snacks than girls (8.3%), a 3.4 times difference. Parents of boys from the youngest patient group mentioned increased appetite 2.6 times more often than the parents of girls (21.4% and 8.3%, respectively); one 14-year-old boy complained of increased appetite (20%), whereas 4 boys (28.6%) and two girls (16.6%) aged 0–10 years, as well as two girls (20%) aged 11–18 years complained of reduced appetite. One 15-year-old and one 11-year-old girl (20%) did not eat breakfast.

Carbohydrates. Of patients aged 0–10 years, 35–58% used soda drinks and juices regularly. With increase of age, the proportion rose more than two times (70–100%). Similar proportions by gender of children in the youngest age group consumed sweets (chocolate cheese, chocolate, candies, cookies, cakes) regularly and ten girls (83.3%) and ten boys (81.7%), and nine (90%) adolescent girls and five (100%) boys consumed sweets every day. Two times more younger girls than boys consumed soda pop 1–3 times per month (58.3% versus 21.4%), and in adolescents with epilepsy — three boys (60%) and seven girls (70%). Every second boy (57.1%) aged 0–10 years did not eat popcorn, and in adolescent patient group 40% of boys and 30% of girls did not eat popcorn. Consumption of high-carbohydrate food items is shown in Figures 1 and 2.

Fat. Up to 66.6% girls and 50% boys aged 0–10 years and more adolescent girls (80%) and 100% boys consumed potato chips at least once a week. 35.7% boys and 83% girls in the youngest patient group, and 10% girls aged 11–18 girls did not eat potato chips. 66.6% girls and 64.3% boys with epilepsy aged 0–10 years consumed fried potatoes at least once per week, and adolescents with epilepsy — considerably more often (100% girls and 80% boys, respectively). One (10%) girl in the oldest patient group drinks cocoa every day, but in the youngest age group — three epileptic girls (25%) drinks cocoa 1–3 times per week. Consumption of high-fat food items is shown in Figures 1 and 2.

Protein. Every second boy and almost all younger girls (90%) consumed different dairy products (milk, kefir, yoghurt, curd, sour cream) daily, and almost two times more adolescent epileptic boys than girls (60% girls and 100% boys). 40% older girls did not drink milk. 75% girls and 50% boys aged 0–10 years consumed meat 2–4 times per week; these proportions were 70% in adolescent girls and 1.6 times more (80%) in boys. Girls aged 0–10 years consumed fish 3.5 times more than boys (50% versus 14.3%, respectively), and in adolescents with epilepsy: 20% girls versus 40% boys. Consumption of high-protein food items is shown in Figures 3 and 4.

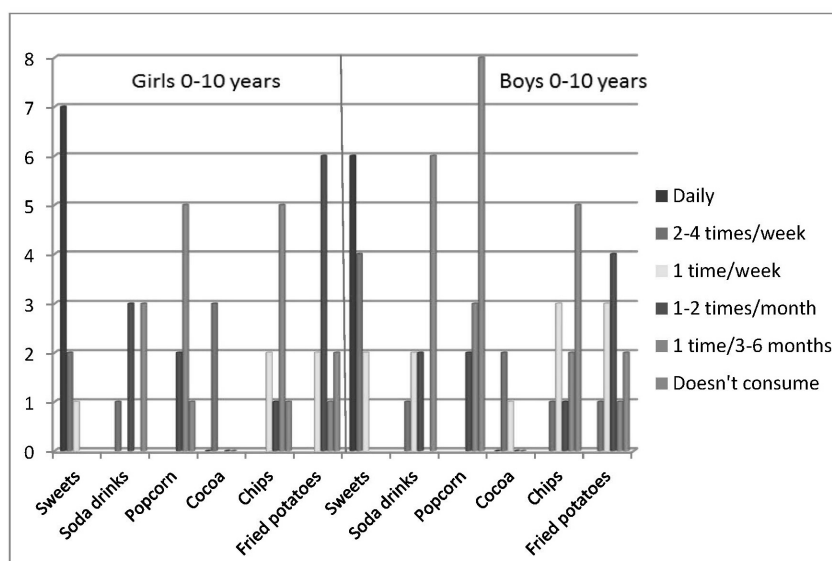


Fig. 1. Mean values of carbohydrate- and fat-rich dietary product consumption in 0–10-year-old patients with epilepsy.

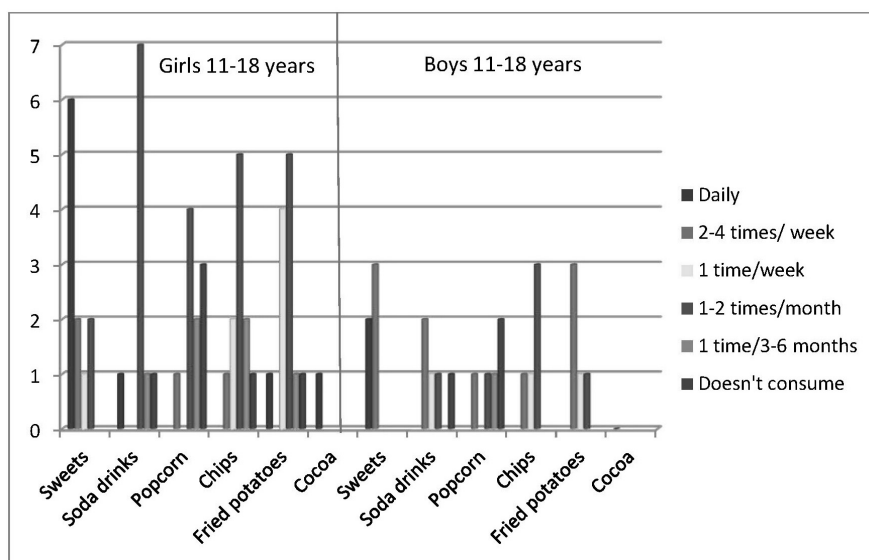


Fig. 2. Mean values of carbohydrate- and fat-rich dietary product consumption in 11–18-year-old patients with epilepsy.

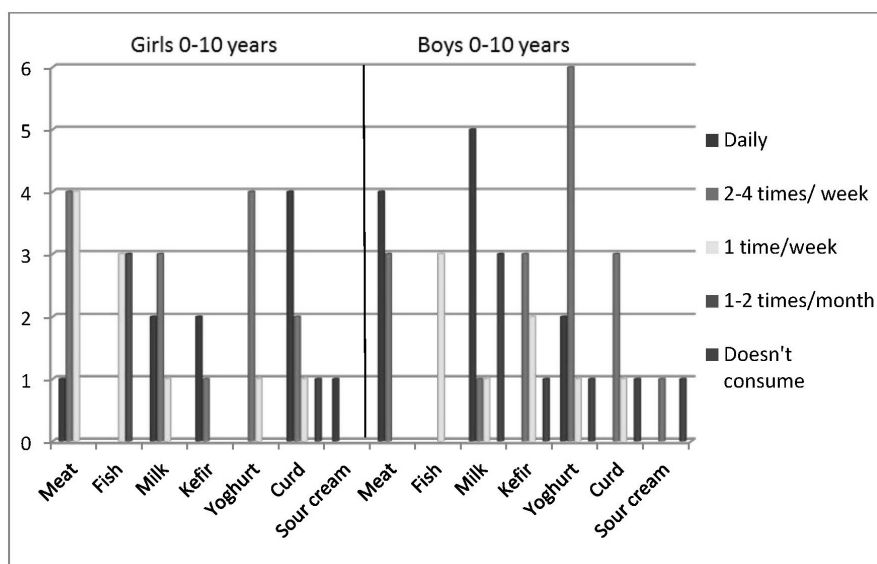


Fig. 3. Mean values of protein-rich dietary product consumption in 0–10-year-old patients with epilepsy.

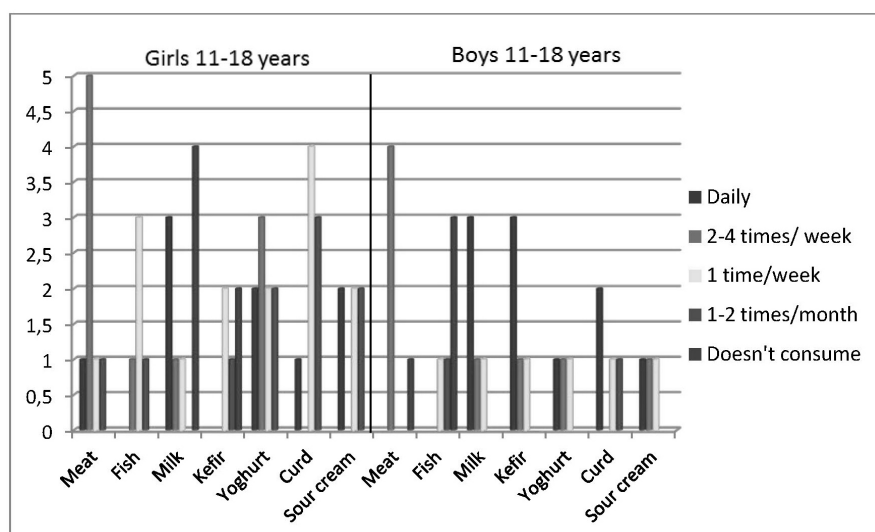


Fig. 4. Mean values of protein-rich dietary product consumption in 11–18-year-old patients with epilepsy.

Fibre. Fruits were used daily among girls in both age groups, whereas boys — slightly less at the age of 0–10 years (85.7%) and all of boys aged 11–18 years. Younger boys with epilepsy more often than girls consumed both

fresh and heat-treated vegetables (100% boys and 75% girls, respectively), and up to 100% patients of both genders aged 11–18 years consumed vegetables (fresh and heat-treated) equally. Only one 5-year-old boy (7.1%) consumed

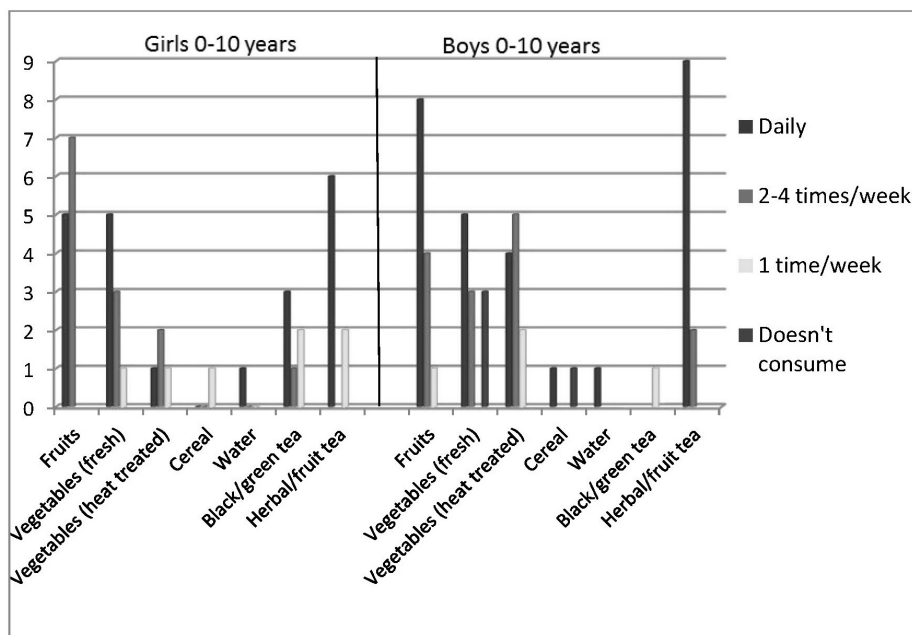


Fig. 5. Mean values of liquid and fiber-rich dietary product consumption in 0–10-year-old patients with epilepsy.

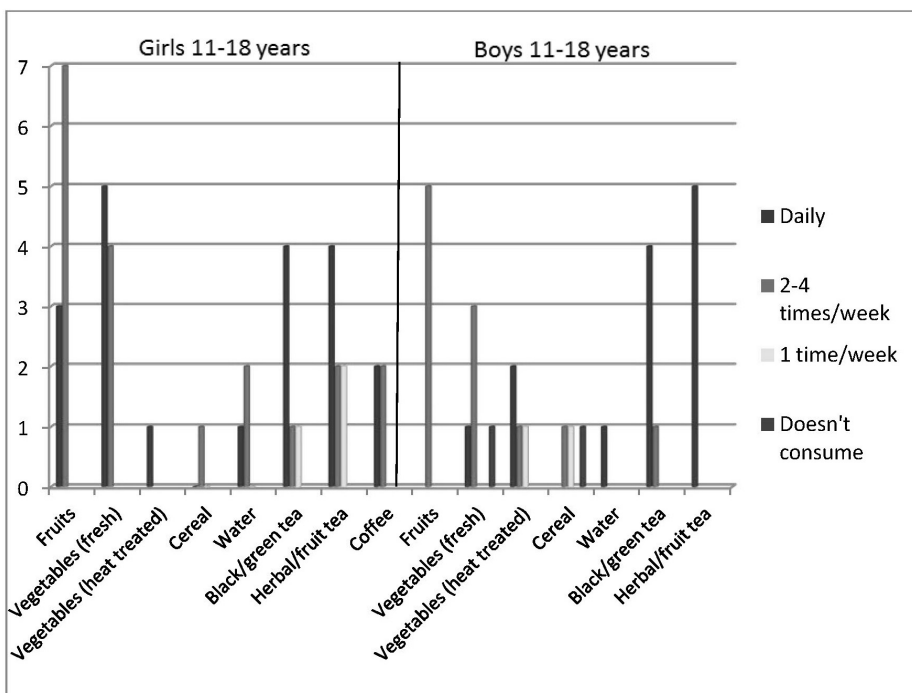


Fig. 6. Mean values of liquid and fiber-rich dietary product consumption in 11–18-year-old patients with epilepsy.

porridge every day, and one 15-year-old girl (10%) and one 13-year-old boy (20%) consumed wholemeal flakes or porridge every other day. Consumption of fibre-rich food items is shown in Figures 5 and 6.

Liquid. On average, every second child with epilepsy (50% girls and 64.3% boys), and 75% girls and 100% boys in the older age group drank herbal (peppermint, camomile, sweet-briar, caraway etc.) and fruit tea daily. 25% of 0–10 year old girls drank green or black tea daily, and adolescents consume this product 2–4 times more (50% girls and 80% boys, respectively). Adolescent boys two times more than girls drank coffee. Only two children aged 0–10 years and two adolescents with epilepsy aged 11–18 years drank lots of water daily. Consumption of liquid is shown in Figures 5 and 6.

DISCUSSION

In the study we observed that in general children with epilepsy consumed healthier food than adolescents with epilepsy. Most often, the choice of food was determined by family nutritional habits, food items that are included in the kindergarten and school menu, as well as the commercials in the mass media whose basic target audience often is children and adolescents. Interestingly, when questioning parents of the enrolled children about their eating habits, it was reported that only a small number of parents drink water, herbal or fruit tea, make fresh salad, buy different fruits and vegetables, dairy products, and cook fish foods regularly themselves. On average, every second child admitted that he or she would consume the above mentioned food item if their parents and other relatives were doing the same.

Studies have shown that eating habits of adolescents are greatly influenced by their peers (Bisset *et al.*, 2007), food eaten outside the house, and concepts of the youth themselves about their body, weight and beauty standards in the world of today (Cusatis and Shannon, 1996).

We observed that children and adolescents with epilepsy preferred to have high-carbohydrate and high-fat product items. In contrast to what might be expected, that older boys (11–18 years) would consume a rather small amount of dairy products, the results showed that adolescent girls with epilepsy are the ones who choose not to drink milk and kefir in lesser amounts because of personal prejudice.

The research also demonstrated that younger patients eat meat and fish more often than adolescents. Also, older girls generally ate meat and fish less times per week than boys because of different considerations (for example, fear of gaining weight).

Noteworthy, some adolescent girls with epilepsy did not eat breakfast. In most cases, this is associated with concerns of gaining weight. A quite recent study in a number of European countries indicated that skipping breakfast is one of preconditions of obesity (Szajewska and Ruszczynski, 2010).

It has been shown that children and adolescents who eat breakfast have a reduced risk of obesity and reduced body mass index (BMI), compared to those who skip breakfast (Szajewska and Ruszczynski, 2010). In this study, we did not analyze in detail how often children and adolescents skip breakfast, what they consume at all, and why they make such choices. This article only summarizes general hallmarks of eating habits. We have not evaluated changes in individual BMI according to each patient's eating habits.

It is possible that epileptic children and adolescents in Latvia, as has been shown in the USA, prefer high-fat to high-carbohydrate food items (Amari *et al.*, 2007). Therefore, future studies are required in this field. Epileptologists and child neurologists should also educate relatives of patients about features of epilepsy and encourage them to make changes in their eating habits.

In summary, the observed 41 children and adolescents with diagnosed epilepsy: 1) chose to have meals rich of carbohydrates and fat, rather than protein; 2) a large number of the evaluated patients drank soda drinks and juices daily; 3) only several of the evaluated patients drank water daily; 4) all patients ate fruits and vegetables daily, thus compensating unhealthy eating habits.

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ĒŠANAS IERADUMI BĒRNIEM UN PUSAUDŽIEM AR EPILEPSIJU

Pētījumā tika iekļauts 41 pacients: 12 meitenes un 14 zēni vecumā no 0 līdz 10 gadiem, 10 meitenes un 5 zēni vecumā no 11 līdz 18 gadiem. Tika veikta anketēšana par dažādu pārtikas produktu lietošanas biežumu uztura grupās: ogļhidrāti, tauki, olbaltumvielas, šķiedrvielas, šķidrums. Darba mērķis bija ēšanas paradumu izvērtēšana un noteikšana bērniem un pusaudžiem ar epilepsiju. No novērotajiem bērniem 33–58% vecumā no 0 līdz 10 gadiem ikdienā dzer gāzētos dzērienus, ēd popkornu, savukārt pusaudžiem šī proporcija palielinās 1,2–2,4 reizes. Saldumus regulāri ēd 83%–100% bērni un pusaudži. Vidēji katrs otrais bērns (0–10 gadi) vismaz vienu reizi nedēļā ēd kartupeļu čipsus, bet pusaudži ar epilepsiju — no 80% līdz 100%. Katrs trešais bērns vecumā no 0 līdz 10 gadiem un 80–100% pusaudžu regulāri ēd ceptus kartupeļus. Katrs otrais zēns un gandrīz visas meitenes no 0 līdz 10 gadiem regulāri ēd dažādus piena produktus, savukārt vecākajā pacientu grupā zēni piena produktus lieto divas reizes biežāk nekā meitenes, 40% meiteņu nedzer pienu. Jaunākās meitenes gaļu un zivis ēd 1,5–3,5 reizes biežāk nekā zēni. Augļus un dārzeņus ikdienā ēd 75–100% bērni un pusaudži ar epilepsiju. Tikai divi bērni un divi pusaudži ikdienā dzer daudz ūdens. Kopumā bērni un pusaudži ar epilepsiju: 1) labprātāk izvēlas ēst ar ogļhidrātiem un taukvielām bagātus pārtikas produktus, nevis olbaltumvielas saturošus; 2) ievērojams skaits epilepsijas slimnieku ikdienā dzer gāzētus dzērienus vai saldinātas sulas; 3) ūdeni dzer tikai daži; 4) visi ēd šķiedrvielām bagātus augļus un dārzeņus, tādējādi kompensējot neveselīgos ēšanas paradumus.

MONITORING OF EATING HABITS AND PHYSICAL ACTIVITY LEVELS AS A BASIS FOR A NEW NONCOMMUNICABLE DISEASE PREVENTION PROGRAMME IN THE REPUBLIC OF MACEDONIA

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Research evidence on health determinants has led to development of a national programme for noncommunicable disease (NCD) prevention. Numerous studies have confirmed the role of nutrition and physical activity in preventing main NCDs: cardiovascular disease, type 2 diabetes, cancer and chronic respiratory disease. The aim of the study was to analyse and evaluate the results obtained from the new "cross-sectional" study in 2012 offer monitoring of eating habits and physical activity levels monitoring related to socioeconomic status of the participants in 2012. The study was conducted in five regions of the Republic of Macedonia and included 1600 respondents aged 10 to 64 years. Monitoring was conducted using self-designed questionnaire. Eating habits were surveyed using questionnaires to monitor the frequency of consumption of main food groups during a week. Leisure time physical activity was a priority measure in the study. Also, in the study a model was applied for strategic planning in NCD prevention, based on the results of the health behaviour survey. The model was analysed using a software package. The results indicate that NCDs prevention through improving physical activity levels and changes in eating habits should be focused on changes in food content including availability of whole grain bread, guidelines and policy on food labelling and health claims, marketing and city planning that facilitates an active life.

Key words: NCDs prevention, eating habits, physical activity levels, socioeconomic differences.

INTRODUCTION

Four major noncommunicable diseases (NCDs) are the greatest public health problem in the WHO European region, with approximately 86% of all deaths in the region caused by this broad group of diseases that includes cardiovascular disease (CVD), cancer, type 2 diabetes (T2DM) and chronic respiratory disease. Nearly four out of five deaths (80%) from NCDs occur in low- or middle income countries. Also, NCDs result in mortality at a younger age in low and middle-income countries — on average, ten years younger than in high-income countries (Anonymous, 2011).

NCDs have multifactorial etiology and they are a result of complex interaction between individual characteristics (gen-

der, age, ethnicity, genetic predisposition) together with social, economic and environmental determinants (education, income, living and working condition). They are a consequence of a transition in lifestyles that leads to increased risk of NCDs. The most prominent NCDs are linked to common risk factors: an unhealthy diet and lack of physical activity, tobacco use and harmful use of alcohol. Six of the seven key determinants of mortality in developed countries are related to how we eat, drink and move. Diet and physical activity, together with tobacco and alcohol, are key determinants of contemporary public health. Nutrition is one of the main determinants (Anonymous, 1990).

A large percentage of NCDs can be prevented by reducing the main risk factors. The greatest potential for NCDs pre-

vention and control lies in population based integrated preventive interventions targeting lifestyle risk factors: diet, physical activity and tobacco (Anonymous, (2008). The study of dietary habits and physical activity levels in the community action programmes both as major health determinants and also as important public health issues for which accurate, reliable and comparable information is needed as a basis for the development of evidence-based strategy and programmes in the field of NCDs prevention. The burden of NCDs is increasing rapidly in the developing world, very much as a result of changes in lifestyles. In addition to changes in tobacco use and physical activity, major changes are taking place in diets, which is contributing greatly to the growing epidemic of NCDs. Thus, a huge global public health challenge is how to influence the trends in diet and nutrition for effective global NCDs prevention.

The Republic of Macedonia needs to address its growing NCDs epidemic through health promoting partnerships, strategies and programmes. High-level political commitment, inter-sectoral coordination, and community mobilisation are important in developing a successful, national, multi-sectoral programme for the prevention and control of NCDs. An efficient multi-sectoral mechanism is also crucial at the stage of monitoring, evaluating enforcement of strategy, and analysing impact of multi-sectoral initiatives on reducing NCDs burden in the country.

Country-level surveillance and monitoring systems, including surveys that are integrated into existing national health information systems and include monitoring exposure to risk factors, outcomes, social and economic determinants of health, and health system responses are needed, recognising that such systems are critical in appropriately addressing NCDs (Puska *et al.*, 1995).

The first Plan of Action for Integrated Community and Primary Care Based Prevention and Control of NCDs as well as the Cancer Prevention Programme of Republic of Macedonia were implemented in years 2002–2005 (Anonymous, 2003).

This paper presents the link between food habits and physical activity levels in socioeconomic groups in the Republic of Macedonia. Disparities in food habits are defined as the differences in food consumption based on education and/or occupation/income in population. Food consumption is measured as frequency of consumption of the following items or groups: fruit and vegetables, milk and dairy products, meat and type of bread (Ross *et al.*, 1999).

We need more scientific knowledge on how socioeconomic differences in eating behaviour and physical activity among the Macedonian population can be explained. To establish the second health-risk behaviour surveillance system is a useful tool to obtain more specific data for strategic planning on regional and local levels related to socioeconomic status of population and regional or local situation in food and nutrition.

This study focuses on individual social determinants, such as education and incomes. It is known that education might provide potential cognitive resources that influence an individual's healthy choices. In addition, educational success may forecast future success: better jobs, higher income, good living area and better housing etc. Assessing how socioeconomic differences in food habits and physical activity levels can be explained, we can more efficiently promote healthier nutrition and physical activity among those with low socioeconomic position in the Republic of Macedonia.

Our hypothesis was that the differences in eating habits and physical activity levels are based on socioeconomic factors such as education, income, occupation/profession, poverty, ethnicity, locality and gender. Socioeconomic status affects the healthiness of the diet.

The general objective of the study was to obtain information and to describe socioeconomic differences in eating habits and physical activity levels.

The specific aims were:

- to analyze and evaluate the results obtained from the new “cross-sectional” study for socioeconomic differences: relationships of educational levels and income with the consumption of the main health-related foods and physical activity levels in the Republic of Macedonia, in 2012;
- to set-up a model that describes how multi-sectoral action can effectively address such challenges through mainstreaming NCDs prevention into national health and development programmes.

MATERIALS AND METHODS

The new “cross-sectional” population-based study was conducted at the Faculty of Technology and Technical Sciences-Veles at the University St. “Clement of Ohrid” in Bitola. The study was carried out between March and May 2012 in five regions in the Republic of Macedonia, and included 1600 respondents aged 10 to 64 years.

Eating habits were measured as frequencies of consumption of the following food groups: fruit and vegetables, milk and dairy products, meat and type of bread from responses in a food frequency questionnaire (FFQ).

Physical activity levels were measured as leisure time physical activity (LTPA). The questionnaire included a comprehensive list of examples of brisk walking and cycling, known as physical activity with moderate intensity as well as organized sports known as strenuous activity. Leisure time physical activity (LTPA) is a personally chosen activity undertaken in an individual's free time that can be motivated by several reasons, such as health benefits, aesthetics, social contacts and fun. Exercise and sports is a form of LTPA that is more planned, structured and aims at more specific objectives, for example the improvement of physical fitness.

Several socioeconomic measures were linked from the national population register. Indicators of socioeconomic inequalities in health behaviour, including nutrition and physical activity levels by gender and age groups were education and income. Socioeconomic differences in food groups' consumption and physical activity levels were analysed.

Educational levels were often grouped according to a standard 5-level classification developed by the OECD (Anonymous, 1997). For practical reasons, educational levels were grouped as basic (elementary), secondary and higher education (university degree).

Income was measured as family monthly income divided by the number of family members. Income was grouped in three classes: up to 75 Euros, from 75 to 150 Euros and over 150 Euros per month.

In the study a model was applied for strategic planning for NCD prevention and health promotion using software package for data analyzing (Martinovski, 2012). This model was chosen for two reasons. The first was to show the application of the proposed concept in public health, food technology, nutrition and economy. The second reason was that

the Faculty of Technology and Technical Sciences-Veles had been created a database from its current research on the socioeconomic impact on a healthy diet and physical activity level.

The results were presented in percentages (%) of total numbers of the participants in the study by education and income.

RESULTS

Eating habits. A considerable part of the health behaviour study dealt with monitoring of food habits in Macedonian population, in 2012.

Bread. The most remarkable result has observed was regarding the usage of black and whole grain bread; people with better income consumed more frequently black and whole grain bread in comparison with those with low socioeconomic status (Fig. 1).

Also, use of these types of bread was more prevalent among higher educated people (Fig. 2).

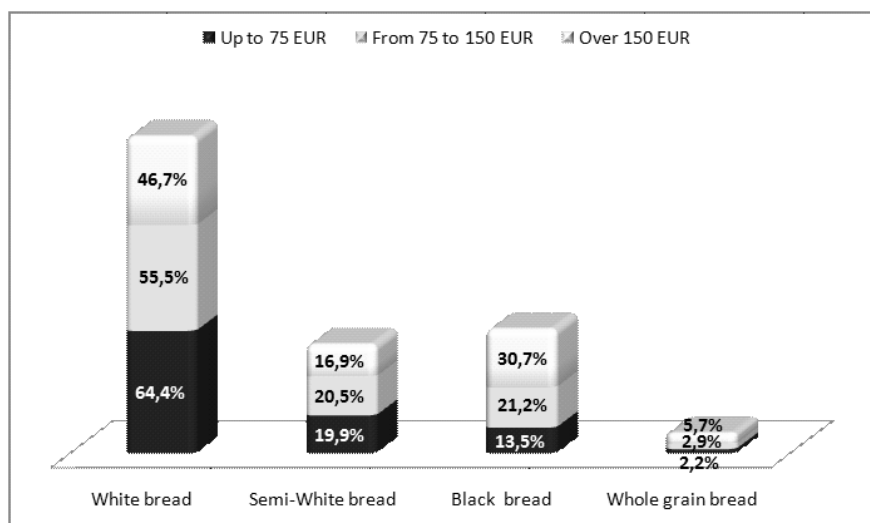


Fig. 1. Proportion (%) of participants using different type of bread by income in 2012.

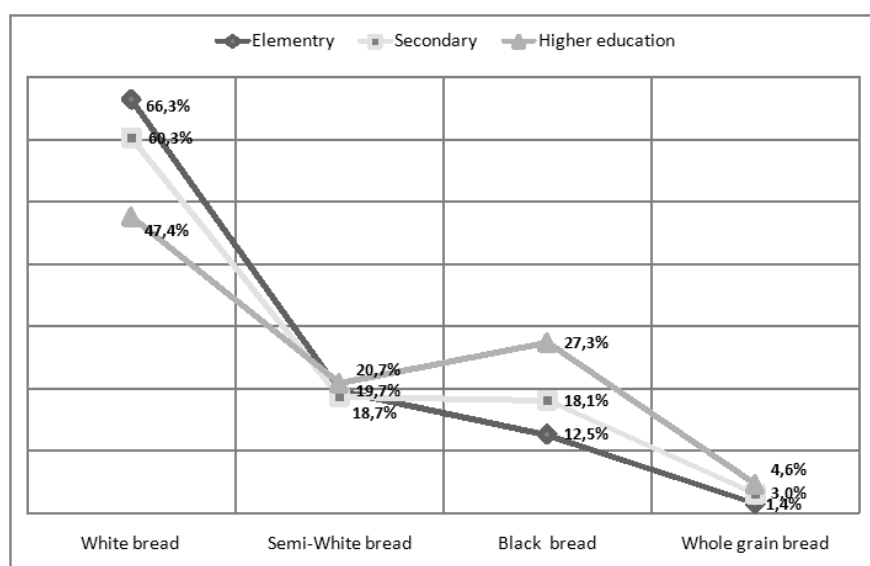


Fig. 2. Proportion (%) of participants using different type of bread by education levels in 2012.

Milk and dairy products. Education and monthly income of the participants had high impact on the frequency of consumption of milk and dairy products.

Less frequently consumption of selected foods in Republic of Macedonia in 2012 were related to lower income and less education (Figs. 3 and 4).

Regarding content of fat in milk and dairy products more frequently low-fat were used by people with higher education.

In general, we noticed less frequent consumption of dairy products that were enriched with vitamins and minerals.

Perhaps, media education regarding food labelling and health claims are needed.

Vegetables and fruit. Education and family monthly income had little impact on the frequency of consumption of fruits and vegetables (Figs. 3 and 4).

The main reason was the relatively low price, availability and relative good production of vegetables and fruits in the Republic of Macedonia.

Meat. Regarding consumption of meat and meat products, most of the study participants consumed more frequently chicken and pork, but less fish. One of the reasons for this is the low price of chicken in the country.

In general, participants with higher education and higher income, more frequently used fish and beef meat.

Physical activity levels. The results showed that monthly income had little impact on physical activity with low intensity such as walking. 35% of all participants from families with different monthly income walked regularly.

The proportion of sedentary participants who had only lower education was 40% of all study participants. Physical activity with moderate and higher intensity such as organized sports activities were related to the higher income.

DISCUSSION

This is the first national “cross-sectional” study in the Republic of Macedonia conducted with the aim to analyse and evaluate data on socioeconomic differences in educational levels and income on the consumption of the main health-related foods and physical activity levels of the population (10–64 years).

On the European level, the differences in food consumption in relation to education and income were examined in an adult population (18–65 years) in 1985–1997 as part of the FAIR-97-3096 project “Compatibility of The Household and Individual Nutrition Survey in Europe and Disparities in Food Habits” (Roos *et al.*, 1999). Forty seven studies

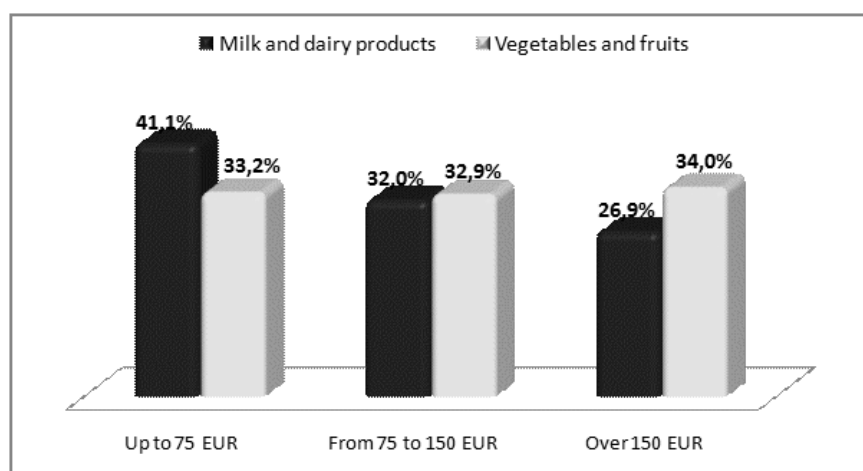


Fig. 3. Proportion (%) of participants using less frequently consuming milk and dairy products by income in 2012.

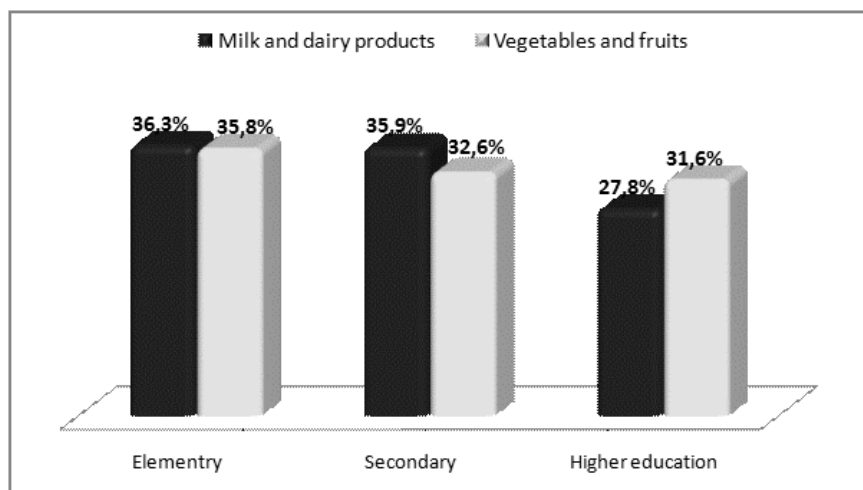


Fig. 4. Proportion (%) of participants less frequently consuming milk and dairy products by education in 2012.

from 15 countries were reviewed. These studies were mainly large-scale national dietary, household budget and health behaviour surveys. The meta-analysis showed that people belonging to higher social classes have healthier diets. Those with higher education, with the exception of inhabitants of the southern Europe, tended to consume more vegetables and fruit, and less fat. The socioeconomic differences in food consumption were not homogenous across Europe. The patterns varied by food groups and region.

Project results of food-related disparities observed in the present study were used in planning food and nutrition policy, programmes and dietary interventions aimed at promoting health among lower social classes and risk groups.

Clinical and epidemiology studies conducted over the past ten years in Republic of Macedonia have shown a reduced level of physical activity was found in 64.5% of the adult population and in 50% of girls aged from 7 to 12 years independent of educational level (Simovska, 2002). In the mentioned study conducted in the WHO European region (Currie *et al.*, 2012) 31% of participants were sedentary.

In accordance to UN Political Declaration on the Prevention and Control of Non-communicable Diseases (Anonymous, 2011), the Government should implement programmes that tackle the social determinants of NCDs with particular reference to the health in early childhood, the health of the poor, fair financing, and equitable access to primary health care services and essential medicines.

Also, governments should adopt the evidence-based global strategies developed by the WHO including the Global Strategy on Diet, Physical Activity and Health (Anonymous, 2004) and other programmes such as the Disease Control Priorities Project, as the foundation of future evidence-informed policies to reduce the burden of NCDs.

Various socioeconomic groups are differently affected by political and economics changes. Assessment of the trends in health and health behaviour of different socioeconomic groups in the Republic of Macedonia is essential for planning and evaluation of the NCDs prevention programme. Also, the research study provides results for health-policy decision-making and can be used for evaluating specific health promotion and NCDs prevention programmes. Changes in food content, guidelines and policy on food labelling and health claims, marketing, city planning that facilitates active transport (as opposed to motorised) are among the measures that will help to keep the NCDs epidemic under control. Projected future ratio of bread's economic status estimated using Buhlmann-Straub statistical method are presented in Table 1.

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Table 1

ECONOMIC STATUS OF BREAD: PROJECTED FUTURE RATIO
(Standard Credibility Method: Buhlmann-Straub)

Number of participants before 2012	Economic status (income per family member)	Actual excepted ratio	Credibility Factor Z	Number of participants 2012	Buhlmann empirical Bayesian Estimate PFR
White bread					
550	Up to 75 EUR	85.27%	0.79	469	88.40%
502	From 75 to 150 EUR	96.02%	0.78	482	96.89%
248	Over 150 EUR	92.74%	0.64	230	95.36%
Total 1300				Total 1181	
Semi White Bread					
138	Up to 75 EUR	105.07%	0.65	145	103.28%
150	From 75 to 150 EUR	118.67%	0.66	178	112.40%
63	Over 150 EUR	131.75%	0.59	83	118.78%
Total 351				Total 406	
Black Bread					
80	Up to 75 EUR	122.50%	0.60	98	113.51%
184	From 75 to 150 EUR	100.00%	0.70	184	100.00%
135	Over 150 EUR	112.59%	0.60	152	107.60%
Total 399				Total 434	
Whole grain bread					
11	Up to 75 EUR	145.45%	0.38	16	117.08%
14	From 75 to 150 EUR	178.57%	0.42	25	132.89%
15	Over 150 EUR	186.67%	0.36	28	130.86%
Total 40				Total 69	

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18 September 2012

ĒŠANAS PARADUMU UN FIZISKĀS AKTIVITĀTES LĪMEŅA MONITORINGS KĀ PAMATS JAUNAI NEINFEKCIJAS SLIMĪBU PROFILAKSES PROGRAMMAI MAĶEDONIJAS REPUBLIKĀ

Pētījumā tika novērtēti 2012. gadā veikti „šķērsgriezuma” pētījumi par ēšanas paradumu un fizisko aktivitāšu ietekmi uz neinfekcijas slimībām, no kurām galvenās ir sirds un asinsvadu slimības, 2. tipa diabēts, vēzis un hroniskas respiratorās slimības. Pētījums tika veikts piecos Maķedonijas republikas reģionos un ietvēra 1600 respondentus vecumā no 10 līdz 64 gadiem. Tika izmantota aptaujas anketa par ēšanas paradumiem, kurā bija jautājumi par nedēļas laikā patērētajām galvenajām produktu grupām. Pētījumā tika mērītas brīvā laika fiziskās aktivitātes. Pētījuma rezultāti parādīja, ka neinfekcijas slimību profilaksei, līdz ar fizisko aktivitāšu līmeņa paaugstināšanu un ēšanas paradumu maiņu, ir jāfokusējas uz uztura sastāvu, ietverot pilngraudu maizes pieejamību, kā arī uz pārtikas etiķetēšanas politiku, veselīguma prasībām, mārketingu un pilsētu plānošanu, kas veicina aktīvu dzīvesveidu.

DIETARY BEHAVIOUR IN STUDENTS WITH DIFFERENT BODY FAT PERCENT

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Eating habits with uniform food preferences and increased energy intake can contribute to increased gain of body fat. An adequate diet, body self-evaluation, and recognition of unhealthy nutrition patterns should promote appropriate corrective actions. The aim of the present study was to determine whether energy intake, food diversity and corrective modification of body mass differed among student groups with low, normal and high body fat percentage. The study involved 737 (158 male and 579 female) students of the Rīga Stradiņš University (age 18–49 years). Dietary behaviour was determined using self-administered questionnaire. Body fat percentage was determined with a Tanita MC-180 bioimpedance analyser. Fluid and food intake, as well as physical activity before the test was restricted. The results showed that 15% of students in the low, 38% in the normal and 62% in the high body fat percentage groups considered that they eat too much. In the low, normal and high body fat percentage groups of students, 27%, 37% and 42%, respectively, agreed that they do restrict food intake. There were no significant differences in normalised energy intake and food diversity indexes between these student groups. Students in the high body fat percentage group more frequently admitted eating too much, and their corrective behaviour was associated more with reduced amounts of eaten food rather than minimisation of energy intake and increased food diversity. In all fat percentage groups, female students more frequently admitted that they eat too much and more often tended to restrict food intake than male students.

Key words: body fat percent, eating habits, energy intake, food diversity.

INTRODUCTION

Incidence of obesity, especially in children and young adults, is increasing. This indicates a the need to determine the main causal factors and to advise on effective prevention (Chizuru, 2004). The adiposity in this age can predispose persons to diabetes mellitus, cardiovascular diseases, degenerative joint diseases, certain cancers and other health risks in further life (Anonymous, 2000). Energy balance of energy intake and energy expenditure enables to maintain normal body weight.

Nowadays energy intake is influenced by high availability of energy dense food, rich with fat and added sugars, while intake of complex carbohydrates, dietary fibers, as well as fruits and vegetables is reduced (Anonymous, 2003). This situation stimulates increased energy intake, but energy expenditure is markedly decreased due to low physical activity.

In such conditions the natural body weight regulation mechanisms in some cases are not able to provide effective body weight control, and therefore the importance of conscious activities to maintain normal body size is increased

(Lowe, 2003). Eating habits with uniform food preferences and increased energy intake can contribute to increased gain of body fat. An adequate diet, body self-evaluation, as well as recognition of unhealthy nutrition patterns, can promote appropriate corrective activity. Adoption of healthy dietary behaviour is an effective way to maintain normal body weight and composition (Roach *et al.*, 2003).

The aim of the present study was to determine whether energy intake, food diversity and body mass corrective modification differ among students within low, normal and high body fat percentage groups.

MATERIALS AND METHODS

All students who were residents of Latvia and who attended a physiology course in Rīga Stradiņš University from 2009–2012 were invited to participate in the investigation. The investigation involved 737 (158 male and 579 female) students of the Rīga Stradiņš University with age 18–49 years. Persons were involved voluntarily and the investigation was conducted according to the requirements of Ethical Committee of the Rīga Stradiņš University.

At onset all of the students were asked to identify their eating habits by completing a self-administered questionnaire. The questionnaire consisted of 64 questions examining eating style, food diversity and product preferences. In the questionnaire, groups of questions were selected that characterise energy intake and food diversity. The acquired score in each group of questions was expressed as energy intake index and food diversity index, respectively. Higher energy index indicates a tendency to consume products with higher caloric values. Higher food diversity index is associated with using a diet with higher variety of products, including fibre-rich and low-energy dense food.

Then, body height of participants was measured to the closest 0.1 cm using a standard wall-mounted height measuring device. Body weight to the closest 0.05 kg and fat percentage with accuracy 0.1% were estimated using a multi-frequency body composition analyser MC-180MA (Tanita, Japan). To obtain more precise results, fluid and food intake, as well as physical activity level of the participants before the test were limited. Students with metal implants and pregnant female students were excluded from the investigation. Body fat percentage was evaluated according to the predicted values (Gallagher *et al.*, 2000) (Table 1). The students were divided into three groups – low fat %, normal fat % and high fat %.

Table 1

CHARACTERISTICS OF THE STUDIED POPULATION (mean \pm SD)

Parameter	Males	Females
Age (years)	20.8 \pm 3.0	21.3 \pm 3.7
Height (cm)	181.8 \pm 7.2	168.7 \pm 5.7
Weight (kg)	75.4 \pm 10.8	61.3 \pm 10.1
Fat percent (%)	14.4 \pm 5.7	24.7 \pm 5.8

The obtained data were analysed using SPSS software version 17.0 (SPSS Inc., Chicago). Alpha level $P < 0.05$ was considered as statistically significant. Differences in distributions between both genders were evaluated using a Chi square test.

RESULTS

Of the studied population, 75% males and 67% females had a normal fat percentage. 10% males and 25% females had a low fat percentage, and 15% males and 8% females had an increased fat percent (Figs. 1 and 2)

The frequency distributions of *yes* or *no* answers to the statement „In my opinion I eat too much” differed significantly between student groups with low, normal and high fat percentages. According to the questionnaire, 52% males and 67% females with high fat percentage thought that they eat too much. In the group of students with normal fat percent this answer was given by 17% males and significantly more (45%) females ($P < 0.05$ for gender differences). Surprisingly, also 6% males and 16% females in the group of students with a low fat percentage thought that they eat too much (Fig. 3).

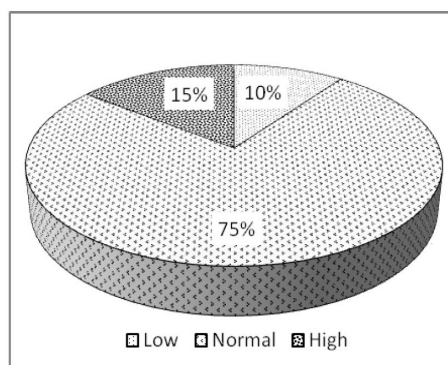


Fig. 1. Frequencies of low, normal and high fat percentage among males.

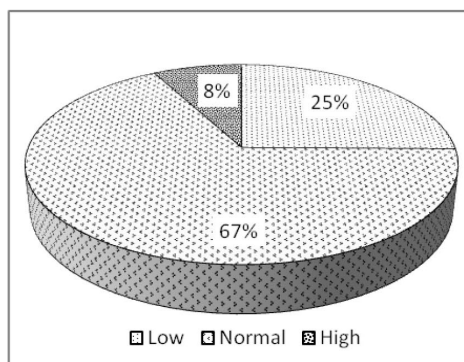


Fig. 2. Frequencies of low, normal and high fat percentage among females.

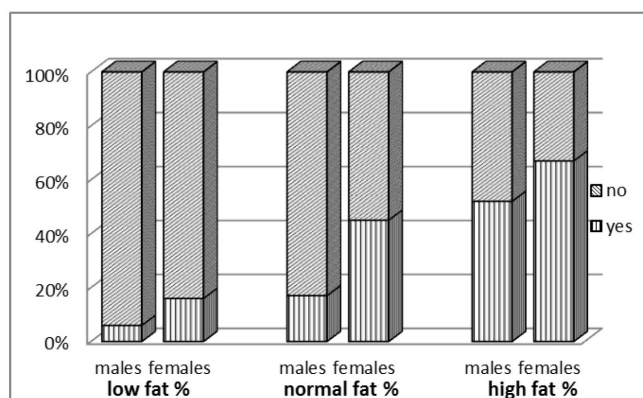


Fig. 3. Percentage of males and females in the low, normal and high fat percent groups, who answered *yes* or *no* to the statement „In my opinion I eat too much”

Significant ($P < 0.05$) difference between low, normal and high fat percent groups according to the Chi square test.

Statistically significant differences were observed between the groups of low, normal and high fat percentage groups according to the answers in the statement “I restrict my food amount”. 33% males and 47% females with a high fat percentage agreed that they restrict their food amount. In the group of students with normal fat percentage only 27% males and 40% females agreed. Even 30% of females with a low body fat percentage agreed that they restrict their food amount (Fig. 4).

On average most of the students were in the low or medium tertile of energy intake index. There were no statistically significant differences in index of energy intake between males and females with different fat percentages. However, the energy intake index of females with normal and high fat percentages was lower than in males ($P < 0.05$) (Fig. 5).

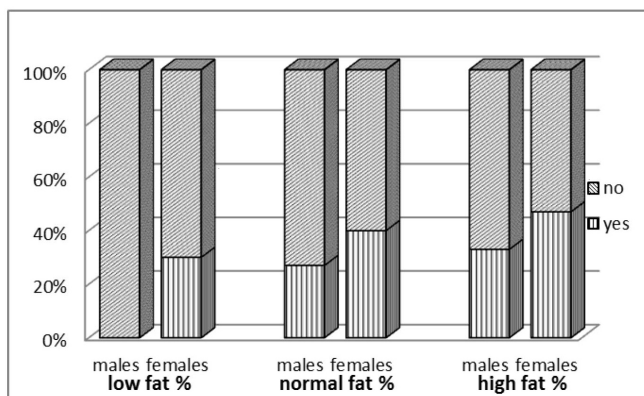


Fig. 4. Percentage of males and females in the low, normal and high fat percentage groups who answered *yes* or *no* to the statement „I restrict my food amount”.

Significant ($P < 0.05$) difference between low, normal and high fat percent groups according to the Chi square test.

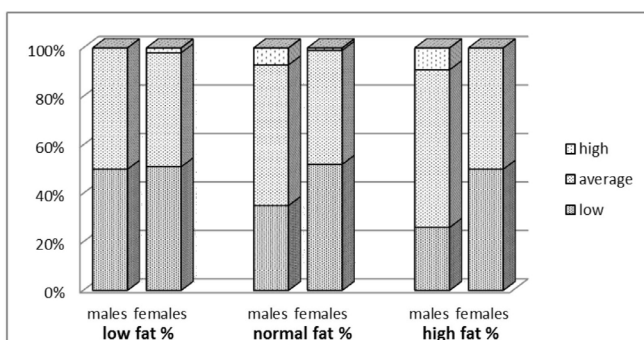


Fig. 5. Percentage of males and females in low, normal and high fat percentage groups according to the tertile of energy intake index.

No significant ($P > 0.05$) difference between low, normal and high fat percent groups according to the Chi square test; significant ($P < 0.05$) difference between males and females in normal and high fat percent groups according to the Chi square test.

Generally, most of the students were in the high or medium tertile of food diversity index. Distribution of students in the three tertiles among groups of low, normal and high fat percentage groups was quite similar. No significant differences in distribution of this index were observed between males and females in any of these groups (Fig. 6).

DISCUSSION

The average fat percentage of students involved in the study was normal. In this, as well as in previous studies (Blumfelds *et al.*, 2008), females prevailed in the group of low fat percentage, while males in the group of high fat percentage. These data are consistent with other reports about young adults (Mohsen, 2007); however, they show higher rates of overweight and obese men than in our study.

It is known that females are more exposed to eating disorders such as anorexia nervosa and bulimia nervosa (Hilbert *et al.*, 2012). However, binge eating disorder and sub-threshold binge eating disorder is more common in males than females (Hudson *et al.*, 2007; Sepulveda *et al.*, 2008).

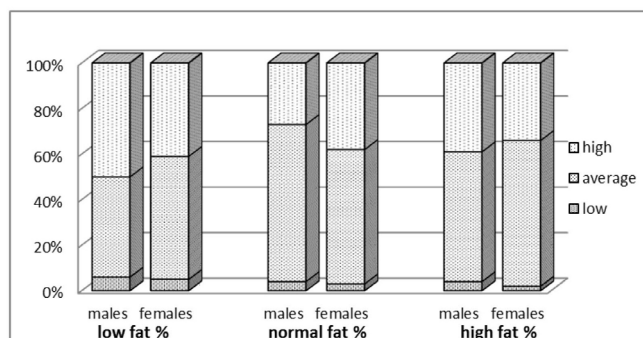


Fig. 6. Percentage of males and females in low, normal and high fat percent groups according to the tertile of food diversity index.

No significant ($P > 0.05$) difference between low, normal and high fat percent groups according to the Chi square test.

The ability to evaluate one's own body size is important when weight management activities are initiated. The self-evaluation of body size is not very accurate, especially when the body mass index (BMI) and fat percent are below or above normal range. Persons with higher BMI and fat percentage tend to underestimate their BMI, and persons with lower BMI overestimate it (Blumfelds *et al.*, 2011). In another study involving students from 22 countries in which, body mass index was used to characterise the weight/height relationship, a steady increase in perceived overweight with increase of BMI decile was observed (Wardle *et al.*, 2006).

A low body fat percentage might be the indication of engagement in sports and fitness (Hetland *et al.*, 1995; 1998) or impending, or borderline eating disorder (Probst *et al.*, 1996). Students with a low fat percent who answered that they are overeating most probably could be linked with the latter.

It is not surprising that people with a high fat percentage chose restriction of food intake as the first activity for body weight control. The higher the fat percent, the higher is the urge to implement some corrective activities in one's life. In studies of the general population of the USA and Germany, it was found that females restrict their food intake more than males (Striegel-Moore *et al.*, 2009). Young women are pursuing extremely low ideal body weight and are risking their mental and physical health. They more frequently perceive themselves overweight and engage in different weight control activities that are irrational and even hazardous (Crawford and Owen, 1994; Patton *et al.*, 1997; Wardle *et al.*, 2006). A total of 44 to 81% of USA college females with normal and increased BMI, respectively, report consciously eating less than they want to promote their weight loss. This dietary behaviour, similar to that in our study, was more frequent in groups of overweight and obese females (Malinauskas *et al.*, 2006).

Students who considered some corrections of body weight, more often tended to decrease the amount of food eaten and thus caloric intake rather than increasing product diversity. Females have been observed to be more concerned about their diet than males, in our and also in other studies

(Mikulan *et al.*, 2012). In people with a borderline high body fat percentage, a decrease of amount of food eaten may be effective to normalise body weight, in persons with a high fat percent it may be ineffective by only decreasing intake of products with high caloric value, if these are not substituted with alternatives with lower energy density (Prentice and Jebb, 2003).

The lack of significant differences in energy intake index across low, normal and high fat percentage groups in this study most probably could be related to composition of the questions used for the index analysis. This study did not consider weight of each separate question used in the questionnaire and each question was not studied specifically.

In conclusion, students in the high fat percentage group more frequently admitted eating too much, and their corrective behaviour was more associated with restriction of eaten food amount rather than reduction of energy intake and increase of food diversity.

Female students more frequently admitted that they eat too much and tended more to restrict food intake than male students in all fat percentage groups.

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ĒŠANAS PARADUMI DAŽĀDA VECUMA STUDENTIEM AR DAŽĀDU RELATĪVO ĶERMEŅA TĀUKU DAUDZUMU

Ēšanas paradumi ar vienveidīga uztura izvēli un pārmērīgu enerģijas uzņemšanu var veicināt palielinātu ķermeņa tauku uzkrāšanos. Savas diētas un ķermeņa adekvāts pašvērtējums, kā arī neveselīgu ēšanas paradumu atpazīšana varētu veicināt piemērotu koriģējošu rīcību. Šī pētījuma mērķis bija noteikt, vai enerģijas uzņemšana, uztura dažādība un ķermeņa masu regulējoša rīcība ir atšķirīga studentu grupās ar zemu, normālu un augstu ķermeņa tauku procentu. Pētījumā brīvprātīgi tika iesaistīti 737 (158 vīrieši un 579 sievietes) Rīgas Stradiņa universitātes studenti vecumā no 18 līdz 49 gadiem. Uztura paradumu pētīšanai tika lietota anketa, ko studenti aizpildīja patstāvīgi. Ķermeņa tauku daudzums dalībniekiem tika izmērīts ar bioimpedances analizatoru Tanita MC-180. Pirms mērījuma studentiem tika noteikti šķidruma, uztura uzņemšanas un fiziskās aktivitātes ierobežojumi. Rezultāti parādīja, ka 15% studentu zema, 38% normāla un 62% augsta ķermeņa tauku procentu grupā uzskata, ka viņi ēd par daudz. Attiecīgi — zema, normāla un augsta ķermeņa tauku procentu grupā — 27%, 37% un 42% studentu piekrita apgalvojumam, ka viņi ierobežo savu uztura daudzumu. Normalizētie enerģijas uzņemšanas un uztura daudzveidības indeksi būtiski neatšķīrās studentiem ar dažādu relatīvo ķermeņa tauku daudzumu. Studenti augsta ķermeņa tauku procentu grupā biežāk nekā pārējie uzskatīja, ka viņi ēd par daudz, un viņu koriģējoša rīcība galvenokārt bija saistīta ar uzņemtā uztura daudzuma ierobežošanu, nevis uzņemtās enerģijas apjoma samazināšanu un uztura daudzveidības palielināšanu. Visās ķermeņa tauku procentu grupās sievietes biežāk nekā vīrieši atzina, ka ēd par daudz un cenšas ierobežot savu uztura daudzumu.

NUTRITIONAL SUPPLEMENTS IN OPTIMAL HUMAN NUTRITION

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*The basic requirements for human health and life quality improvement are wholeness, variety and moderation in food choices. In industrial countries the numbers of adipose inhabitants is constantly growing. People consume insufficiently vegetables, fruits and fish. The missing nutrients must be augmented with the help of functional foodstuffs. The objective of the study was to compare the impact of food supplements and local products rich in fibre and polyunsaturated fatty acids on the human body. The research was conducted at the Riga Heart Consulting Room in 2010. For two months, 60 volunteers in three groups: using the food supplement "Wellness" in their diet (Wellness group), or Jerusalem artichoke (*Helianthus tuberosus* L.) and linseeds (*Linum usitatissimum* L.) (Nature group) and Control group were monitored. The participants' weight, girth, amount of glucose and level of cholesterol were measured by standard methods. Data acquired were analysed using Microsoft Excel and SPSS (level of significance $P < 0.05$). The cholesterol level was reduced by 14% in the Nature Group. Glucose normalised to 5.8 mmol/l. Weight of participants was reduced by 2 to 7 kg. The results show that by enriching the daily diet with food supplements, both as natural products and as a special complex, it is possible to reduce body weight, reduce cholesterol and glucose levels in the blood, and reduce the risks of obesity and coronary heart disease.*

Key words: food supplements, Jerusalem artichoke, linseeds, cholesterol level.

INTRODUCTION

A wholesome and balanced diet, and safe and qualitative foodstuffs, are the basic requirements for human health and life quality improvement. The main criteria for qualitative food are wholeness, variety and moderation. The amount of food is less significant than its biological value, as determined by the presence of all necessary nutrients in the right proportions. The amount of food consumed must correspond to daily energy use. If the amounts of the consumed and expended energies are disproportionate for a prolonged time, the human body is not able to maintain its optimum weight, and deviation takes place. If, in previous centuries the reason for dietary imbalance was due to insufficient amounts of food, then nowadays the higher risk of illness is due to too much food, overly rich in calories.

The industrialisation of food production has changed accessibility to food, and the amounts now consumed cause many health problems. In Latvia there are very high levels of heart and coronary diseases, and the number of adipose inhabitants is constantly growing. Latvian inhabitants do not consume enough fresh vegetables, fruits and fish (Pudule *et al.*, 2007; Mintāle *et al.*, 2010). The missing fibre and polyunsaturated fats must be consumed additionally with the help of functional foodstuffs or special food supplements (Kauliņš *et al.*, 2001; Bekers *et al.*, 2004).

Nowadays obesity is epidemic worldwide. In the United States, 20% persons suffer from obesity, in Europe, 15% (Willcox *et al.*, 2001). Every year the number of overweight people increases. Latvia makes an effort to not fall behind in this aspect. According to a questionnaire administered in 2004, 52.7% of Latvians were normal weight, overweight — 27.4%, but obese — 19.9% (Erglis *et al.*, 2007).

The close connection between sickness prevention and food consumption has turned scientist attention to recommendations for a beneficial and balanced diet designed to reduce the risk of illness.

Food with high energy value but low nutritive value, combined with low physical activity, contributes to extra weight and obesity. Most people do not know or do not observe the principles of a healthy diet. The arrival of international eating traditions in Latvia has caused the consumption of atypical foods and, thus, metabolic disturbance. These factors dictate the necessity of replacing the missing food elements in the basic inferior diet with assorted food supplements.

Often weight reduction can be the decisive factor in normalising the level of fat in blood and stop the progress of sickness. Reduction of body mass mainly means a diet with lowered calorie intake and increased calorie use at high physical loads.

A typical visitor to the Rīga Heart Health Consulting Room is a person with an immobile lifestyle who is overweight and has an unbalanced diet, and who has a cholesterol level deviating from the norm. The inferior, unbalanced diet combines lack of fibre with too many fast carbohydrates and fats, as well as lacking microelements, natural vitamins, unsaturated fats and other biologically active compounds. If such a person is not motivated to correct his or her lifestyle, then dislipidemia progresses and drug-induced treatment becomes the only solution. Most people at this stage could successfully correct their risk factors with the help of diet, but do not yet realise the danger of their condition. On learning about heightened cholesterol levels, this person will try to change daily eating habits, however, soon finds it too difficult to resist favorite products in the long-term, and therefore, gives up any efforts to maintain his or her health. Some behaviours, especially where diet is concerned, are almost impervious to modification, and therefore, rapid change cannot be expected. So one of the possible options, to change one's diet completely, is not achievable for most people.

Another option is to supplement one's daily diet with nutrients that are significant for the human organism but are not present in sufficient amounts in food. To enrich one's diet would be the easiest lifestyle change.

Diet can be enriched both with foodstuffs containing the missing elements and with specially enhanced food supplements containing fibre, microelements, vitamins and bioactive compounds from vegetables. It is advisable to consume about 30–35 grams of fibre, since products rich in fibre have been proven to lower cholesterol levels.

Food enrichment can improve human well-being overall: working capacity, maintain health and improve the quality of life by reducing the risks of coronary heart disease, cholesterol levels, triglyceride and glucose measures, and weight reduction.

An alternative option is to enrich diets with natural local products that can supplement the diet with missing food elements and which are preferable for three reasons — commercial food supplements are comparatively expensive, some people prefer to use only natural foodstuffs, and many people prefer local agricultural products. In the research it was decided to use linseeds as a familiar foodstuff that is rich with polyunsaturated fats and lignans, as well as Jerusalem artichoke (Rickard and Thompson, 1997; Mazur *et al.*, 1998). Jerusalem artichoke (*Helianthus tuberosus* L.) tubers are known to be health-promoting. They contain inulin instead of starch as a carbohydrate reserve. Inulin and its degraded byproduct, oligofructose, are major compounds of interest to the food industry as functional and low-calorie food materials. Many scientists have discovered that inulin has a beneficial effect on gastro-intestinal activity, stimulating reproduction of beneficial bacteria (Kaur and Gupta, 2002; Roberfroid, 2005). Jerusalem artichoke powder made from Jerusalem artichoke roots also is a valuable product,

rich in inulin, as well as vitamins and minerals (Praznik *et al.*, 2002; Cieslik *et al.*, 2005; Gedrovica, 2012).

One final correction of dietary inadequacies can be addressed by ensuring sufficient water consumption.

Using different kinds of food supplements it is possible to reduce cholesterol and glucose levels, to reduce weight and to decrease the hazard of coronary heart diseases and obesity. The objective of this research was to compare the impact of food supplements and local products rich in fibres and polyunsaturated fatty acids on the human body.

MATERIALS AND METHODS

The research was conducted in 2010 at the Rīga Heart Health Consulting Room (HHCR) in collaboration with the Latvia University of Agriculture, Faculty of Food Technology, and involved 60 volunteers. Qualitative methods — observation and document analysis — were used in the research.

The visitors to the Consulting Room are chiefly people who do not have chronic diseases, who have not visited health care institutions for a long time, and who would like to know if their health indicators meet the norms.

Standardised examination was done by specially trained employees at the HHCR.

Anthropometric data – height, weight and girth. Height was measured by a Kirchner & Wilhelm GmbH+Co Kawe height measuring instrument, precision 0.5 cm. Weight was measured by a medical scale made by Omron, precision 0.1 kg (weight was measured without footwear and in light clothes). Girth was estimated with a special measuring-tape, precision 0.1 cm. Waist measurement was taken in the area from the pelvic bone to below the rib cage, with the abdomen relaxed, at exhalation phase.

After estimating weight and length, **body mass index** (BMI) was calculated according to the following formula: $BMI = \text{weight (kg)} / \text{height (m}^2\text{)}$. The following data was also collected:

Physiological data – blood pressure, pulse.

Biochemical data – estimated cholesterol (H), triglycerides and glucose concentration in the blood plasma using an Accutrend GCT device made by Roche and test strips – Accutrend Cholesterol, Accutrend Glucose, and Accutrend Triglycerides.

Analyses were taken on an empty stomach (no food 8 h before measuring).

Subjective information was gathered in the form of a questionnaire about additional risk factors:

- adverse heredity factors: heart attack, insult, hypertension (some respondents' next of kin had diabetes mellitus before they reached 55 years of age);

- smoking: currently or in the past. If the answer is positive then information about how many cigarettes per day and how many years of smoking;
- degree of physical activity: intensive, moderate, immobile;
- diagnosed and treated with medication for diseases such as: diabetes mellitus, arterial hypertension, fibrillation, stenocardia, heart attack / insult, dislipidemia, adiposity;
- regularly increased level of stress;
- attitude towards advice about healthy food: accepts most, accepts some, does not accept any.

For this research three homogeneous and equivalent groups with 20 participants in each group were created. All participants had increased weight/girth, and aberrant cholesterol levels. Their life style did not conform to healthy diet recommendations.

A balanced diet should contain optimum protein, fat and carbohydrate ratios, with protein comprising 10–15%, fat 25–30%, and carbohydrates 55–60%, assuming a person of average physical activity ingesting 2000–2200 kca/d. It is possible to ensure this by consuming 100 g of protein, 80 g of fat, and 400–500 g of carbohydrates daily. Human health also requires 30–35 g fibre, and 3–5 g omega 3 daily (Erglis *et al.*, 2007).

The average research participant consumed 3120–3700 kcal/d. The total caloric intake included 13–15% protein, 40–48% fat, and 44–48% carbohydrates, consumed as 100–174 g protein, 145–174 g fat, and 366–417 g carbohydrates. Fresh fruits and vegetables were consumed once per week by 24% of participants, 2–3 times per week by 43%, daily by 17%, 2–3 times daily by 5%, and not at all by 11%. Fish was consumed 2–3 per week by 14% of participants, once per week by 38%, 2–3 times per month by 28%, several times per year by 6%, and not at all by 14%. As half of participants did not consume enough fish, the use of omega 3 supplements would compensate in maintaining a balanced diet in these cases. Fish oil was consumed year-round by 13%, 2–3 times per year by 18%, once a year by 12%; in the event of illness, 12% remembered using fish oil; fully 45% of participants regarded fish oil as completely unnecessary. Necessary to human health is the consumption of 1.5–2 litres drinking water daily. Of the research participants, only 3% achieved this optimal amount, 18% managed 1 litre, 46% felt 1–2 glasses of water daily was sufficient, and 24% regarded water as necessary only when taking medication. Most unsettling was the finding that 9% did not use drinking water at all, taking liquids only in the form of coffee, tea, soft drinks or beer. Insufficient fibre and polyunsaturated fat intake was recorded for 79% of participants.

The criteria of volunteer choice were: BMI > 25 < 40, H > 5 mmol/l, unbalanced diet (insufficient consumption of vegetables, fruits and water), and motivation to change lifestyle.

Division into groups was determined by their responses to the question: “Do you plan to change your lifestyle?” People who wanted to use food supplements were added into Wellness Group. People who wanted to use natural products to supplement their diet were added into Nature Group. The Control Group was comprised of people sharing similar habits and health indicators, and who wanted to change their lifestyle but could not motivate themselves. All participants had to keep to their usual eating habits and were not allowed to start new diets during this two-month research period.

Food supplements used were: Wellness Pack (multivitamin & mineral, astaxanthin, Omega3) and Natural Balance Shake (pea protein, apple powder, whole egg, whey, ground sugar beet fibre, natural vanilla flavour) by Wellness from Oriflame (Sweden). In addition to daily food intake, the Wellness Group used:

- 1 package of Wellness Pack vitamins after lunch;
- 2 portions of Nature Balance Shake before lunch and dinner (18 g = 1 measurement spoon mixed with 150 ml of water, juice or milk);
- 1 litre of noncarbonated drinking water, in doses of 200–250 ml 30 minutes before food.

The Nature Group products were: linseeds and linseed oil by „Viza Oil Group” (Latvia), and Jerusalem artichoke powder by “Herbe” (Latvia). The Nature Group additionally used daily:

- 20 g Jerusalem artichoke powder (10 g inulin) = 1 table-spoon mixed into drink or food;
- 10 g linseeds = 1 teaspoon;
- 10 ml linseeds oil = 1 teaspoon;
- 1 litre of noncarbonated drinking water, in doses of 200–250 ml 30 minutes before food.

The Control Group used daily food without change.

Each research participant received a code ensuring anonymous participation and a registration card containing HHCR standard research data:

- anthropometric – length, weight and girth;
- physiological – blood pressure, pulse;
- biochemical – glucose, cholesterol and triglycerides;
- completed questionnaire about eating habits;
- volunteer’s written agreement to participate in the research project.

During the two-month research period, food supplement usage data was collected repeatedly at HHCR using the same instruments and recording the results on each participant’s card.

Data acquired during the research was processed using Microsoft Excel and SPSS.

All results were evaluated using a level of significance $P < 0.05$.

The author has received the permission of the RSU (Rīga Stradiņš University) Ethics Committee to conduct biomedical research, and each volunteer subject has given his or her written, informed consent to participate in this research.

RESULTS

Measurements at the beginning of research: The average body mass of the participants in the beginning of the control period was 83.5 ± 1.7 kg. Body mass was similar for both experiment groups. In the Wellness Group the average weight was 80.9 ± 3.5 kg, in the Nature Group – 81.1 ± 2.2 kg, and in the Control Group the body mass was slightly higher — 88.4 ± 2.1 kg; however, body mass fit within the allowed deviation of 10%. All the participants (weight ranging from 57 up to 116 kg) were overweight and some were already obese. BMI for all participants exceeded 25 — in Wellness Group the average BMI was 28.7, in the Nature Group the average BMI was 29.7. The maximum BMI was registered for two participants in the Wellness Group — 38.4. Girth is one of the most significant indicators signalling clinical obesity and heart/coronary disease risk. On average, the girth of research participants came to 100 cm. The girth in the Wellness Group and Nature Group varied from the accepted 88 centimeters up to 114 centimeters.

Cholesterol levels in the blood in the Wellness Group were, on average, 6.07 ± 0.07 mmol/l (5.28–7.41), and in the Nature Group, on average 6.0 ± 0.11 mmol/l (5.48–6.81).

For some of the research participants, along with dislipidemia, initial glucose tolerance disturbances with slightly increased readings (5.9–6.4 mmol/l) were also detected. Experience has shown that quite often excess weight and dislipidemia facilitate development of diabetes mellitus type 2. Also, triglyceride levels for several participants were too high, on average — 2.1 mmol/l instead of the recommended 1.7 mmol/l.

Measurements at the conclusion of study period were repeated, and new body mass indicators were obtained: in the Wellness Group — 77.2 ± 3.4 kg (53–105 kg), in the Nature Group — 76.4 ± 2.1 kg (61–99 kg), and in the Control Group — 88.35 ± 2.9 kg (67–117 kg) (Fig. 1). During the research period, the average body mass loss in Nature Group for each participant was about 4.7 kg. The biggest loss of a single individual's weight was 7 kg, and in general participants lost from 2 to 7 kg of excess weight.

Seven people reached normal BMI < 25 , but six participants reduced their weight, on average to BMI of 27.9. Representatives of the Nature Group on average lost $4.35 \text{ cm} \pm 0.25 \text{ cm}$.

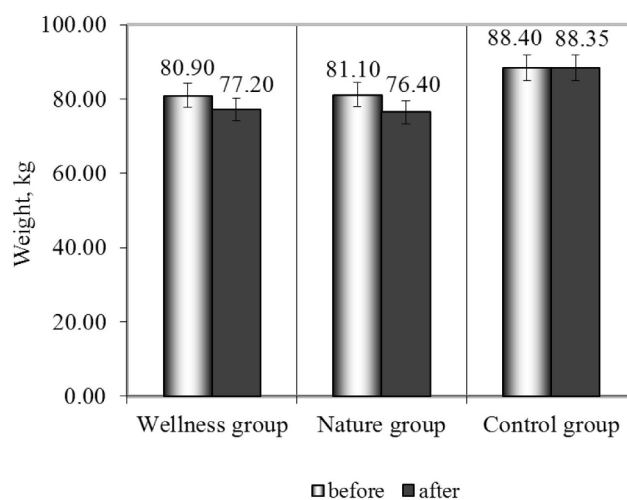


Fig. 1. Body mass at the beginning and end of the study period.

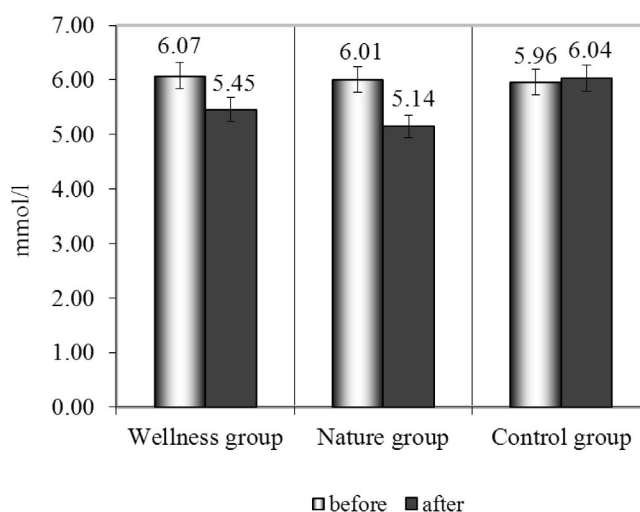


Fig. 2. Cholesterol level at the beginning and end of the study period

Cholesterol readings were reduced most in the Nature Group, on average by 0.86 mmol/l, which proves the close connection between weight reduction and regulation of lipid levels. The average cholesterol measurement in the Nature Group was $5.14 \text{ mmol/l} \pm 0.07$. As a result, all groups left the risk group. Also, in the Wellness Group there was a reduction by 0.62 mmol/l, to average cholesterol level of $5.45 \text{ mmol/l} \pm 0.11$ (Fig. 2). No glucose disturbances were registered for any of the participants.

Results obtained during the research were registered in individual protocols, entered into a computer and analyzed with methods of mathematical statistics. The methods used were: analyses of case absolute and relative frequency, tests of independent selections and pair selection, analyses of variance (ANOVA), linear correlation and regression analyses.

At the onset of the research, the groups were similar in body mass, with a variations coefficient of 5–10%. The difference is not big, which indicates the similarity of body mass. Since the groups at onset were similar, it is possible to compare the average changes during the research period.

The parametric Pearson method of paired linear correlation analyses indicated a relationship between body mass and girth and BMI in all groups. Correlation analyses showed a strong correlation between body mass, girth and BMI at the outset of the research, both in the Wellness Group ($r = 0.81$; $P = 0.01$), Nature Group ($r = 0.78$; $P = 0.01$ and $r = 0.67$; $P = 0.01$) and in the Control Group ($r = 0.76$; $P = 0.01$ and $r = 0.78$; $P = 0.01$). The same correlation was observed at the end of the study period ($r = 0.99$; $P = 0.01$).

Cholesterol levels showed explicit correlation between cholesterol level at the beginning of the research and at the end of the research, both in the Wellness Group ($r = 0.85$; $P = 0.01$) and Nature Group ($r = 0.96$; $P = 0.01$). A not so explicit correlation was showed by girth ($r = 0.43$; $P = 0.05$). Correlation of cholesterol level and body lacked statistical significance ($P > 0.05$), indicating that a person can carry excess weight for a long time before metabolic changes start, developing over time into dislipidemia.

The indicators of glucose at the end of the period were correlated with glucose indicators at the onset of the research ($r = 0.69$; $P = 0.01$), and slightly with cholesterol indicators in the beginning ($r = 0.47$; $P < 0.05$) and in the end ($r = 0.56$; $P = 0.01$), as disturbances of glucose norms are related to cholesterol production in the liver.

DISCUSSION

During the two-month study period, the participants' average results improved in all aspects. At the conclusion of the research, improvement in cholesterol levels was by 14% in the Nature Group and 10% in the Wellness Group.

Body mass loss by the end of the study in the Nature Group was by 6%, in the Wellness Group, 5% from the initial weight. Summarising the results, it is obvious that in the Nature Group the average results improved in all readings and the increases were statistically valid ($P < 0.05$). Expressed by absolute numbers this 10–14% loss corresponds to 6–8 kg of mass, which actually is not large, but, considering the fact that such results were achieved without any diets restricting calories and amount of food, the success is convincing.

At the conclusion of the study, the difference between average results was statistically significant but the percent improvement in the Nature Group in comparison to the Wellness Group is higher in three parameters.

The total body mass loss was less than 11%, which, evaluated separately is not a convincing result, as weight was still in excess in all groups. However, they are optimistic as the results came closer to the recommended norms for health. Furthermore, loss of 10% excess weight reduces heart disease and diabetes mellitus development risk by 20%. Glucose had normalised for all participants to 5.8 mmol/l, corresponding to the norm.

All observed changes are statistically significant for body mass and cholesterol reduction, but change in cholesterol level was not significantly correlated with body mass loss. This indicates that changes of cholesterol level cannot be regarded as a consequence of regular overeating but as a kind of organism illness manifestation. This is confirmed by comparing the changes between the experimental research groups and Control Group at the end of this experimental research. At the beginning of the study there were no statistical differences between the Nature Group, Wellness Group and Control Group, but at the end of the study differences were observed in three indicators.

Using food supplements, Wellness products and natural products, led to significant changes in the studies indicators ($P < 0.05$). The food supplement "Wellness" and local products rich in fibre and unsaturated fatty acids (Jerusalem artichoke and linseeds) demonstrated positive effect on human health and in a natural way reduced adiposity and dyslipidemia. The results acquired during the study confirmed the hypothesis of this paper, i.e. by enriching daily diet with food supplements both as natural products and as a special complex it is possible to reduce body weight, reduce cholesterol and glucose levels in blood, and reduce the hazards of coronary heart disease and obesity.

Several research participants expressed interest in continuing the use of the supplements they learned about through the research in their daily diet and even to reconsider the option of changing their diet altogether. After two months, the idea of health improvement through healthy food was not so scary anymore. Observing the results of the other two groups, and knowing that this method is neither difficult nor complex for users, some of the research participants expressed an interest in starting the use of food supplements and healthy products in their own daily diets. Most convincing was their own negative result.

Dietary habits often are mechanisms of psychological problem reduction, and rapid change is regarded as a violent interruption that raises stress levels and provokes a desire to stop lifestyle corrections. Unlike the traditional weight loss approaches, participants were not restricted from their habitual foods and all volunteers were very cooperative during the study. Most probably a gradual change in old habits will give positive long-term result. The best indicators of the success of this approach were shown by Nature Group, which used powdered Jerusalem artichoke together with food, linseeds and linseed oil – they not only lost 4 kg on average, but also reduced cholesterol levels until the norm was achieved, and completely balanced their glucose levels. The most significant result was achieved in preventive education, since the greater number of the participants expressed their wish to continue the improvements in their lifestyles both by continuing consumption of Jerusalem artichoke, linseeds and vitamins and by analyzing their diet and making healthy corrections in it — more water, vegetables, fruits and fish. This study allowed people to understand that less food can be sufficient and that changes are not so difficult. In this case, the researchers can regard the stated tasks

as fulfilled. Furthermore, the mission of the Health Centre food consultant is also fulfilled because the most difficult task is to make people think and act.

The obtained results show that comparing the impact on the human organism of the "Wellness" dietary supplement to the impact of local products rich in fibre and polyunsaturated fats — artichokes and linseeds — both appear to benefit and strengthen human health, resulting in the reduction of obesity and dislipidemia.

The conclusion is that since local products – artichoke, linseeds, linseed oil – both as consumables and as food enrichment products are much less expensive than commercially prepared supplements, it would appear to be more important to promote local products, and hope that people can be convinced to use them in their daily diets.

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24 September 2012

UZTURA BAGĀTINĀTĀJI CILVĒKA OPTIMĀLA UZTURA IZVEIDĒ

Mūsdienu cilvēka uzturs nav sabalansēts, jo ar pārtikas produktiem uzņemtais šķiedrvielu un polinepiesātināto taukskābju daudzums ir nepietiekams. Sabalansētu uzturu nodrošināt cilvēkam ir vieglāk, savu diētu papildinot ar uztura bagātinātājiem, tādējādi uzņemot trūkstošās vai nepietiekamā daudzumā organismā esošās uzturvielas. Pētījums veikts Rīgas Sirds veselības kabinetā 2010. gadā, iekļaujot 60 brīvprātīgas personas, kurām reģistrēts palielināts svārs/apkārtmērs, holesterīna līmeņa novirzes un veselīga uztura ieteikumiem neatbilstošs dzīvesveids. Divu mēnešu ilgā eksperimentā, lietojot uzturā divu veidu uztura bagātinātājus — "Wellness" un ar šķiedrvielām un nepiesātinātām taukskābēm bagātus vietējos produktus — linsēklas (*Linum usitatissimum* L.), no tām gatavotu eļļu un topinambūra (*Helianthus tuberosus* L.) pulveri — konstatēts, ka, lietojot vietējās izcelsmes uztura bagātinātājus, eksperimentā iekļauto dalībnieku masa samazinājās par 4 līdz 7 kg, bet ar uztura bagātinātāju "Wellness" — no 3 līdz 8 kg. Pētījuma laikā eksperimenta dalībniekiem samazinājās holesterīna un glikozes līmenis. Būtiski, ka vietējās izcelsmes uztura bagātinātāji ir lētāki, salīdzinot ar rūpnieciski ražotajiem uztura bagātinātājiem „Wellness”. Rezultāti parādīja, ka vietējās izcelsmes uztura bagātinātājus ir iespējams veiksmīgi izmantot uzturā ar mērķi organismā samazināt holesterīna un glikozes līmeni asinīs, kā arī cīņai ar lieko svaru, tādējādi samazinot sirds asinsvadu slimību un aptaukošanās risku.

NUTRITION VALUE OF WILD ANIMAL MEAT

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One of the most valuable foods is meat, due its nutritional value, largely determined by the essential amino acids, fatty acids, vitamins, minerals, etc. At the same time, concern needs to be given to health of consumers by used products with less calories, which can be ensured by greater variety of game animals, including also deer grown in captivity. The aim of our investigation was to compare the nutrition value of elk, wild deer, farm deer, roe deer, and wild boar that were killed during hunting in Latvia. Meat samples (m. logissimus lumborum) were collected in the autumn–winter season. The results of the conducted research suggest that game meat samples have higher protein and essential fatty acid content in comparison with domestic animals. The amount of fat in all analysed samples did not differ greatly, although the fatty acid content in wild game meat differed significantly. The fatty acid profile was used to calculate the atherogenicity index (AI), which has a positive correlation with the risk of cardiovascular disorders. The formula $AI = [C12 : 0 + (C14 : 0 \times 4) + C16 : 0] / (\text{Total unsaturated fatty acids})$ was used to calculate the ratio of total saturated fatty acids, ω -6 and ω -3. Regarding the microelement content of meat there were great differences in iron and manganese concentration among animals.

Key words: *hunting animals meat, healthy food.*

INTRODUCTION

In recent years, in the Latvian population, public attention has been paid to the health and the value of it, which can be achieved using wholesome food. One of the most valuable foods is meat. In the “food pyramid” meat is one of the products that needs to be used moderately — about one-fifth of all food. It provides the organism with wholesome, highly digestible proteins.

Protein varies among the meat animal species, and its content ranges between 13 and 23% of the fresh weight (Honikel, 2009). Our body uses 97% meat protein and 25% connective tissue.

The amino acid profile is important because some amino acids cannot be synthesised by humans and therefore must be supplied by the diet. Amino acids are divided into two groups: essential and substitutable. Essential amino acids are not synthesised in the body and therefore must be taken with food. If the diet lacks even one of the essential amino acids (such as tryptophan, lysine, methionine) the required protein synthesis is not possible. Meat is rich in the so-called essential or indispensable amino acids — lysine, leucine, isoleucine, and sulphur-containing amino acids — and in this sense meat has highly-quality protein (Young *et al.*, 2001). Meat is also rich in B vitamins and is an important

source of iron, phosphorus, copper and manganese. Depending on the type of meat fat content is 1–10% fat.

Meat has been identified, often wrongly, as a food having a high fat content and an undesirable balance of fatty acids. In fact, lean meat is very low in fat, with a content of 20–50 g/kg (Wood and Enser, 2004). Game animals have a favourable balance between polyunsaturated and saturated fatty acids (P : S) and wild ruminants produce muscle with a desirable ω -6: ω -3 polyunsaturated fatty acid ratio.

Wild game meat is considered significant in the diet, and its share in consumption in recent years has increased. Currently, among consumers there is increased interest in meat from animals kept in conditions as close as possible to the natural ones. Such a requirement is undoubtedly fulfilled by game that is characterised by high nutritional value and specific sensory properties desired by consumers (Vergara *et al.*, 2003; Soriano *et al.*, 2006).

Game animals, such as elk (*Alces alces*), deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa scrofa*), every autumn and winter provide an excellent investment and diversification of many consumer meals. The statistics show that there are about 33 000 hunters in the Latvian register, of which 17 000 are active hunters. During the annual hunt, about 2789 tons (2712 – auxiliary service) of game meat is provided in Latvia (Data on

number of hunted animals in 2010–2011 are from the State Forest Service official statistics).

The human body is composed of a broad range of chemical elements and their combinations, which must be in balance for us to feel good and be healthy. There are negative consequences for both under- and over-intake of a trace element (causing immune status and increased susceptibility to infections). The content of a trace element in domestic meat products depends on the animal species and the quality of animal nutrition. Lack of minerals can be caused by animal age and physiological state, feed intake and housing conditions (Kalafova *et al.*, 2003). In contrast, game animals move within an unlimited territory, and thus animal products contain all trace elements in sufficient quantities, but which varies according to the functional status of the body. The aim of our investigation was to compare the nutrition value of elk, wild deer, farm deer, roe deer, and wild boar that were killed during hunting in Latvia.

MATERIALS AND METHODS

Chemical analyses were conducted on a total of 54 meat samples (*m. logissimus lumborum*): elk (8), deer (18), roe deer (16), wild boar (12) collected after hunts throughout Latvia in the autumn–winter season (2008–2012). The analyses were conducted at the laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine „Siga”. In the studied samples, protein, amino acids, fat, fatty acids, cholesterol content and micronutrient concentrations were determined. Samples were prepared within 48 hours after slaughtering or hunting. Meat samples of about 300 g were homogenised with a BÜCHI B-400 (ISO 3100-1).

Protein concentration was determined as total nitrogen content by Kjeldahl method and using coefficient 6.25 for calculation (ISO 937:1974).

For determination of amino acids concentration, dried, defatted meat samples were treated with constant boiling 6N hydrochloric acid in an oven at around 110 °C for 23 h. Hydrolysate was diluted with 0.1% formic acid. Samples (2 ml) were filtered using a syringe filter with a 0.45 µm nylon membrane. Amino acid concentrations were determined using a reverse-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 µm, 1 × 100 mm): mobile phase (90% acetonitrile: 10% deionized water) 0.5 ml min⁻¹, column temperature, 40 °C. Data acquisition was conducted using the programme Empower pro.

Intramuscular fat concentration was determined by Soxhlet method with hydrolysis procedure (boiling in hydrochloric acid) using SoxCap 2047 and SOX TEH 2055 equipment (FOSS) (LVS ISO 1443:1973).

Cholesterol concentration was determined by Blur colorimetric method using a spectrometer (Шманенков и Алиев, 1973).

Fatty acid analysis of meat. Homogenised meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by derivatisation with (trimethylsilyl) diazomethane using the method of Aldai *et al.* (2006). An ACME, model 6100, GLC (Young Lin Instrument Co.) equipped with a flame ionisation detector, and an Alltech AT-FAME analytical column (fused silica 30 m × 0.25 mm i.d.) was used. The individual FAMES (fatty acid methyl esters) were identified according to similar peak retention times using standard mixture Supelco 37 Component FAME Mix.

From the fatty acid profile, the atherogenicity index (IA), a parameter proposed by Ulbricht and Southgate (1991), which has a positive correlation with risk of cardiovascular disorder, was calculated. The calculation was as follows:

$$AI = [C12 : 0 + (C14 : 0 \times 4) + C16 : 0] / (\text{Total unsaturated fatty acids}).$$

The relative proportions of total saturated fatty acids and unsaturated ω-6 and ω-3 fatty acids were calculated.

Micronutrient concentrations in meat were measured according to ISO 6869-2002. The methods are based on flame atomic absorption using a spectrometer AAnalyst 200.

The experimental design was randomised and data were evaluated by analysis using SPSS 17. One-way ANOVA was used for comparison of mean values. Statistical significance was set at $P < 0.05$. The biochemical composition (protein, fat, cholesterol, fatty acids, amino acids and trace elements) of wild game meat was determined as the amounts in 100 g of meat. Beef and pork samples were included for comparison (previously determined in the Research Institute of Biotechnology and Veterinary Medicine in Latvia SIGRA, unpublished data).

RESULTS

Proteins are an important part of our diet. All animal species given in Table 1 are excellent high-quality sources of protein. Protein concentration in game meat samples varied from 22.36 till 22.92%. The results of the statistical analysis showed that the total protein concentration in the ruminants meat did not differ significantly ($F = 1.286$; $P > 0.05$).

All of the animal species in the table has a low fat concentration (1.33–1.90%) in meat samples (*m. logissimus*

Table 1
BIOCHEMICAL COMPOSITION OF GAME MEAT

Group	n	Protein, %	Fat, %	Cholesterol, mg 100 g ⁻¹
Elk	8	22.72	1.33	64.41
Deer	18	22.36	1.90	70.57
Roe deer	16	22.82	1.59	67.92
Wild boar	12	22.92	2.82	95.07
Beef	15	19.61	1.48	76.31
Pork	15	21.32	2.77	67.85

lumborum), not only in ruminants, but also in wild boar meat 2.82%.

Cholesterol found in muscles is free cholesterol and it is not related to that in animal blood, i.e. “good” or “bad” cholesterol. It is found in both fat and muscle in the range 64.41 to 95.07 mg 100 g⁻¹ among animal species (Table 1). From a nutritional point of view, in terms of cholesterol, none of ruminant meats is superior (preferred) over others. The exception is wild boar meat, in which higher cholesterol levels can be explained by the high level of adrenaline under stress during the hunting season. The cholesterol levels statistically differed between animals ($F = 2.55$, $P < 0.05$)

The human body requires the essential amino acids lysine, isoleucine, phenylalanine, tryptophan, leucine, methionine, threonine, and valine. Each of these amino acids have a role in the human body. The concentrations of essential amino acids in game meat samples are shown in Figure 1.

Game muscle protein contains all essential necessary amino acids. Wild deer, roe deer and elk meat samples are similar in the summed concentrations of essential amino acids, suggesting that the diet of ruminants is sufficient in late autumn to winter (Fig. 1). Wild boar and pork samples, differed most in the summed concentrations of essential amino acids: 24.14 and 12.35 g 100 g⁻¹, indicating the relatively higher biological protein value of wild boar over pork.

Meat contains most of the essential fatty acids. Higher concentrations of saturated fatty acids are found in ruminant meat: 44% in cow and sheep. Lower concentrations of saturated fat are found in pork. These fat content of beef is greater than moose and roe deer meat (41%) (Rule *et al.*, 2002).

Composition of dietary fat is more significant for consumers than is total fat content. Therefore, composition of fatty acids, sum of saturated, monounsaturated, and polyunsaturated fatty acids was investigated (Table 2).

Results of investigation showed that lowest content of saturated fatty acids has meat samples of wild boar and elk meat samples — 34.79 and 35.75, respectively. The highest sum of saturated fatty acids was found in deer meat samples (42.13%), which is in agreement with Petkov (1986). Saturated fatty acids other than myristic, palmitic, and stearic

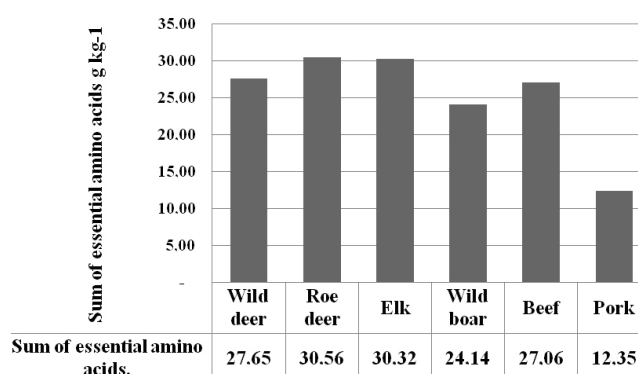


Fig. 1. Comparison of sums of essential amino acids in meat samples.

Table 2

FATTY ACID COMPOSITION OF MEAT

Fatty acid class	Elk	Deer	Roe deer	Wild Boar	Beef	Pork
Sum saturated (SFA)	35.75	42.13	37.54	34.79	40.11	37.46
Stearic (C 12: 0)	0.19	0.30	0.01	0.11	0.24	0.15
Myristic (C 14: 0)	2.44	4.57	1.32	2.92	3.62	1.48
Palmitic, (C 16: 0)	18.08	21.02	18.72	23.12	21.43	26.65
Sum mono-unsaturated (MUFA)	34.09	26.57	28.96	35.63	34.60	50.87
Sum polyunsaturated (PUFA)	18.97	23.47	25.38	17.25	15.36	5.95
Sum Omega-3*	6.81	6.20	8.23	2.89	2.65	0.44
Sum Omega-6**	11.73	17.05	17.04	13.89	12.71	5.51
Sum unsaturated	53.06	50.04	54.34	52.88	49.96	56.82
ω-6 : ω-3	1.72	2.75	2.07	4.81	4.8	12.52
P : S ratio	0.53	0.68	0.68	0.50	0.38	0.16
Atherogenicity index AI	0.53	0.79	0.44	0.66	0.82	0.58

* Linoleic and arachidonic acids

** Linolenic acid

acids are found in extremely small amounts in meats. Conversely, myristic and palmitic acids may have a negative effect on cardiovascular health (Medeiros *et al.*, 2002). Thus, myristic, palmitic, and stearic acids are the only saturated fatty acids presented in Table 2. Regarding the concentrations of myristic acid (C 14 : 0) and palmitic (C 16 : 0), lower concentrations of myristic acid (1.32%) were found in roe deer meat compared to 4.57% and 3.62% in deer and beef meat, respectively. Palmitic acid concentration was the lowest in elk (18.08%), followed by deer meat (18.72%). Palmitic acid concentration was similar in deer and beef meat: 21.2% and 21 : 43%, respectively. Results of statistical analysis confirmed that summed SFA did not differ significantly ($P > 0.05$) among animals. The results showed that the PUFA:SFA ratio in game meat samples varied from 0.50 to 0.68, and the ratio of 1-6:1-3 fatty acids varied from 1.72 to 2.75.

The iron concentration in meat depends on the amount of fat and blood. Fe compounds in meat are utilised well by the human body. Meat samples of wild animal species contained iron from 1.300 to 5.04 mg kg⁻¹. Manganese occurred at up to two times in game animal meat (0.006 to 0.27 mg kg⁻¹) than in beef and pork samples (0.09 mg kg⁻¹) (Fig. 3). The highest concentrations of zinc were observed in wild boar samples (2.560 to 4.200 mg kg⁻¹) (Fig. 2). Copper concentrations were higher in beef (0.39 mg kg⁻¹) and pork (0.410 mg kg⁻¹) (Figs. 2, 3). All meats sampled were found to be excellent sources of trace elements.

DISCUSSION

The results of our investigation are similar with those of other studies: protein concentration in raw deer meat samples was reported as 21.7%, in boar meat samples 21.9% (Paleari *et al.*, 2003). Meat has most of the essential fatty

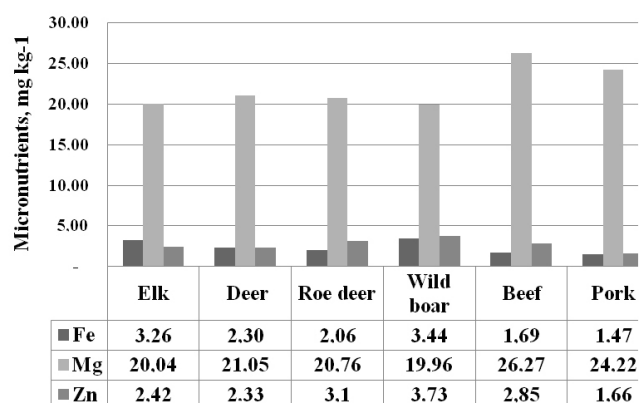


Fig. 2. Iron, magnesium, and zinc micronutrients composition of meat.

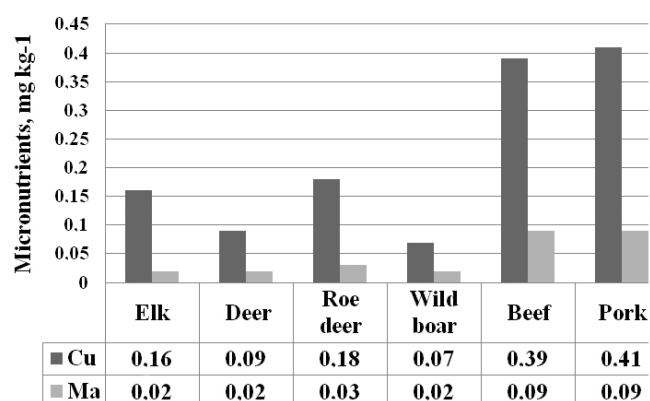


Fig. 3. Copper and manganese micronutrients composition of meat.

acids. Greater concentration of saturated fatty acids (44%) is found in ruminant meat — cow, sheep. Less saturated fat is found in pork. The fat concentration in beef is greater than in moose and roe deer meat (41%) (Rule *et al.*, 2002). Wild game meat fats are more favourable to the human body, as the saturated fatty acid concentration is less, and polyunsaturated fatty acid concentration is higher (Cordan *et al.*, 2002).

There are four interrelated factors that have important health ramifications: (1) the total fat content; (2) distribution of specific fatty acids; (3) the ratio of PUFA:SFA; and (4) the ratio of ω -6: ω -3 fatty acids. Each of these dietary lipid elements has been shown to influence the development of coronary heart disease (Cordan *et al.*, 2002).

All types of meat are excellent sources of monounsaturated fatty acids, which can reduce cholesterol levels in human blood. The most important monounsaturated fatty acids in meat are oleic acid and palmitoleic acid. A diet rich in polyunsaturated FA (PUFA), especially long chained ω -3 FA (\geq C20), has beneficial effects on human health, e.g. in prevention of arteriosclerosis. Game meat is a potential food source that is both lean and rich in ω -3 PUFA (Sampels, 2005). Currently high intakes of fat containing high amounts of SFA and MUFA in modern Western diets are associated with adverse effects on human health, such as cardiovascular diseases, obesity and diabetes (Mann, 2000).

It is not only the amount of PUFA in the food that is important, but also the ratio between n-6 and n-3 PUFA, for which values of 1 to 4 have been recommended (Simopoulos, 2002; Anonymous, 2003). In our study, in samples of game ruminants meat this ratio varied from 1.72 in elk meat till 2.75 in deer meat, but was higher in wild boar meat Medeiros *et al.* (2002) reported a ratio ω -6: ω -3 in deer meat of 3.45. In bovine animals (hogs and pigs), the ratio is above 4. Thus, it can be concluded that wild animal (except wild boar) is favourable from the point of view of healthy nutrition, and that it is less associated with a variety of health problems. Improving the dietary ratio by decreasing the ω -6 fatty acid concentration and increasing the ω -3 fatty acid concentration is essential for brain function and for the management of cardiovascular disease, arthritis and cancer (Simopoulos, 2002).

WHO and Wood reported that the recommended ratio PUFA : SFA must be higher than 0.4 and that in domestic animals it is too low 0.1 (Wood *et al.*, 2003). High relative proportions of PUFA are characteristic of all wild ruminant muscle tissue, whereas the relative proportion of PUFA in muscle tissue of wild boar is lower than in wild ruminants. In the present investigation, the PUFA:SFA ratio was higher than 0.4 in all game meat samples (range from 0.50 till 0.68). Medeiros *et al.* (2002) reported that the PUFA:SFA ration of beef samples was 0.38.

To calculate the atherogenicity index (IA), Ulbricht and Southgate (1991) used the fatty acid profile. This index has a positive correlation with the risk of cardiovascular disorders. Increases in dietary levels of saturated fat, particularly 12 : 0, 14 : 0 and 16 : 0 (palmitic acid) have been identified as the major dietary factor responsible for raising total and LDL serum cholesterol concentrations (Howell *et al.*, 1997). Therefore, these fatty acids are included in the formula.

Consumers are interested in the environment and the products which are produced with sustainable farming methods. Hunting of wild moose, deer, roe deer and wild boar can provide food that perfectly complements and diversifies the daily assortment of meat, which is in line with current health and dietary recommendations, low-fat and high content of essential amino acids. All meat samples are excellent sources of trace elements

ACKNOWLEDGEMENTS

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SAVVAĻAS DZĪVNIEKU GAĻAS UZTURVĒRTĪBA

Rakstā analizēts viens no vērtīgākajiem pārtikas produktiem — gaļa, tās uzturvērtību nosaka neaizstājamās aminoskābes, taukskābes, vitamīni, minerālvielas. Darba mērķis bija salīdzināt uzturvērtību nomedītu savvaļas dzīvnieku — aļņu, savvaļas briežu, audzētāvos audzētu briežu, stirnu, mežacūku un mājdzīvnieku — ar liellopu un cūku gaļas paraugiem (pētījumā tika izmantoti gaļas paraugi no muguras garā muskuļa (*m. longissimus lumborum*)). Veikto pētījumu rezultāti liecina, ka savvaļas dzīvnieku gaļas paraugi, salīdzinājumā ar mājdzīvnieku gaļu, ir ar augstāku olbaltumvielu un būtiski vērtīgāku taukskābju sastāvu. Savvaļā nomedītu aļņu, briežu, stirnu un mežacūku gaļa var lieliski papildināt un dažādot ikdienas gaļas sortimentu, saskaņā ar pašreizējiem veselīga uztura ieteikumiem — zemu tauku un augstu neaizvietojoamo aminoskābju saturu. Visi medījuma gaļas veidi ir lielisks mikroelementu avots.

NUTRITIONAL BENEFITS OF *Bifidobacterium lactis* IN DAIRY PRODUCTS

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Bifidobacteria are one of the most important probiotics in dairy products. They have positive effects on human health. Nutritional benefits of bifidobacteria are genetically determined and can be promoted with addition of prebiotics. The aim of the present study was to examine the properties of Bifidobacterium lactis in dairy products. Pasteurised milk, freeze-dried starter culture Bb-12 (Bifidobacterium lactis, Chr. Hansen, Denmark), syrup of lactulose (Duphalac®, the Netherlands), and inulin ("Raftiline®HP", ORAFI, Belgium) were used in the experiments. The optimal concentrations of lactulose (2%) and inulin (4%) were established in preliminary studies, based on quality indices and nutritional value of fermented dairy products (Beitane, 2008). Amino acids, carbohydrates, such as lactose, lactulose and inulin, as well as cholesterol were determined during the study using appropriate analytical procedures. The enzymatic activity of bifidobacteria determines nutritional value of the fermented dairy products. Addition of 2% lactulose resulted in significant increase of some amino acid concentrations, such as leucine, phenylalanine, lysine and arginine concentrations ($P < 0.05$), compared with those in other treatments. The presence of prebiotics caused a decrease of cholesterol level by 35% and lactose content by 31% in fermented milk samples. The enzymatic activity of bifidobacteria should be promoted with addition of lactulose and inulin to increase nutritional value of functional dairy products.

Key words: *bifidobacteria, lactulose, inulin, lactose, amino acids, cholesterol.*

INTRODUCTION

Bifidobacteria are one of the most important probiotics in dairy products (Parvez *et al.*, 2006; Russell *et al.*, 2011). Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Oliveira *et al.*, 2011). Bifidobacteria have positive effects on human health: (1) synthesis of vitamins (Crittenden *et al.*, 2003; Beitane and Ciprovica, 2011); (2) immunostimulation (Dong *et al.*, 2010); (3) cholesterol reduction (Ziarno *et al.*, 2007; Beitane, 2008); (4) lactose hydrolysis (He *et al.*, 2007); and (5) prevention of infectious diarrhoea (Qiao *et al.*, 2002). Nutritional benefits of bifidobacteria are genetically determined. Bifidobacteria are saccharolytic and produce organic acids (Russell *et al.*, 2011). They release various enzymes, such as leucine, valine and cystine aminopeptidases, α -, β -galactosidases, α -, β -glucosidases, *N*-acetyl- β -glucosaminidase, esterases during multiplication (Martinez-Villaluenga and Gomez, 2007), which improve nutritional value of the end product: higher content of amino acids, and lower content of cholesterol and lactose in fermented dairy products. Thereby milk products are acceptable for patients with hypercholesterolemia and lactose

intolerance. The growth of bifidobacteria can be promoted with addition of prebiotics (Rastall and Maitin, 2002).

Prebiotics are non-digestible food ingredients that beneficially effect the host by selective stimulating the growth and/or activity of one or a limited number of bacteria in the colon (De Vrese and Schrezenmeir, 2001). Lactulose and inulin are one of the widely used prebiotics for promotion of bifidobacteria growth in milk and the gastrointestinal tract (Szilagyi *et al.*, 2001; Palframan *et al.*, 2002; Holzapfel and Schillinger, 2002; Aider and de Halleux, 2007; Bouhnik *et al.*, 2007). Lactulose is mostly used in Europe, Japan and the United States (Tuohy *et al.*, 2005). It is a synthetic disaccharide created by the isomerisation of lactose (Aider and de Halleux, 2007). Fructo-oligosaccharides are widely used as commercial prebiotics, which contain a varying number of fructose monomers connected by β -2-1-glycosidic bonds (Russell *et al.*, 2011). Evaluation of the suitability of prebiotics for promotion the growth of bifidobacteria in milk showed that the appropriate concentration were 2% lactulose and 4% inulin (Beitane and Ciprovica, 2008). The aim of the present study was to examine the specific properties of *Bifidobacterium lactis* in dairy products.

MATERIALS AND METHODS

Pasteurised milk with fat content 2.5% and freeze-dried *Bifidobacterium lactis* culture (Bb-12, Chr. Hansen, Denmark) were used for the experiments. During the experiments, the culture was maintained at -18°C . As additions, the following prebiotics were used: inulin “Raftiline[®] HP” (ORAFI, Belgium) with polymerization degree ≥ 5 and degree of purity 99.5% and syrup of lactulose (Duphalac[®], the Netherlands) with the following composition (%): lactulose — no less than 67, lactose — less than 6, galactose — less than 10.

2% lactulose and 4% inulin were added individually to 100 g of milk. The concentrations of prebiotics were chosen according to previous study, in which the optimal bifidogenic effect of lactulose and inulin was established (Beitane and Ciprovica, 2008). *Bifidobacterium lactis* was inoculated with 2 ml of milk suspension (10^6 cfu ml⁻¹) and cultured at 37°C for 16 hours. The control sample was prepared without prebiotics for comparison.

The content of amino acids was determined with an automatic amino acids analyser AAA 339 (Microtechna Praha, Czech Republic), according to requirements of „Amino acid standard solution for protein hydrolysates — 0.5 $\mu\text{moles per ml}$ ”.

The level of cholesterol was determined according to AOAC Official Method 976.26A.

The concentrations of lactose and lactulose were determined by IDF standard 147B:1998 procedure, the content of inulin by AOAC Official Method 999.03 and by AACC Official Method 32.32.

Significant differences were determined by analysis of variance (ANOVA) and t-tests at P value 0.05.

RESULTS

The concentrations of amino acids in milk, control and fermented milk samples with 2% of lactulose and 4% of inulin are shown in Table 1.

The obtained results showed that the addition of lactulose increased amino acid concentration in fermented product more than with inulin; the highest total amino acids after fermentation in fermented dairy product was 2.829 g 100g⁻¹.

ANOVA indicated significant increase of isoleucine, leucine and lysine concentrations ($P < 0.05$) in fermented milk with 2% of lactulose, compared with other treatments. A significant decrease of leucine, phenylalanine, lysine and arginine concentrations ($P < 0.05$) were observed in fermented milk with 4% inulin, indicating promotion of glycolysis and suppression of proteolysis in fermented milk with *B. lactis*. Lactulose facilitated the proteolytic properties of bifidobacteria.

Table 1

CONCENTRATIONS OF AMINO ACIDS IN FERMENTED MILK SAMPLES WITH LACTULOSE AND INULIN, g 100 g⁻¹

Amino acids	Milk	Control	Fermented milk with 2% of lactulose	Fermented milk with 4% of inulin
Threonine	0.107	0.095	0.112	0.094
Valine	0.130	0.115	0.123	0.101
Methionine	0.061	0.064	0.075	0.052
Isoleucine	0.095	0.087	0.108	0.078
Leucine	0.254	0.232	0.271	0.207
Phenylalanine	0.115	0.114	0.093	0.095
Tyrosine	0.131	0.123	0.121	0.094
Lysine	0.197	0.181	0.208	0.151
Aspartic acid	0.233	0.212	0.223	0.201
Serine	0.133	0.115	0.154	0.109
Glutamic acid	0.620	0.569	0.711	0.366
Proline	0.248	0.221	0.277	0.178
Glycine	0.051	0.039	0.052	0.044
Alanine	0.086	0.069	0.094	0.073
Histidine	0.100	0.108	0.108	0.108
Arginine	0.089	0.092	0.099	0.087
Total	2.650	2.436	2.829	2.038

The ability of *B. lactis* to assimilate cholesterol during milk fermentation in the presence of prebiotics was studied. The levels of cholesterol in milk, control and in fermented milk samples with 2% of lactulose and 4% of inulin are presented in Figure 1.

The results showed that fermentation of milk with *B. lactis* decreased cholesterol concentration by 25% in samples, indicating promotion of this activity by prebiotics. Both prebiotics showed similar results. Significant differences were observed between milk and fermented milk samples with and without prebiotics ($P < 0.05$). Differences between control and fermented milk samples with prebiotics were insignificant ($P > 0.05$).

The lactose concentrations in samples with and without prebiotics before and after fermentation are presented in Figure 2. Decrease of lactose concentration during fermen-

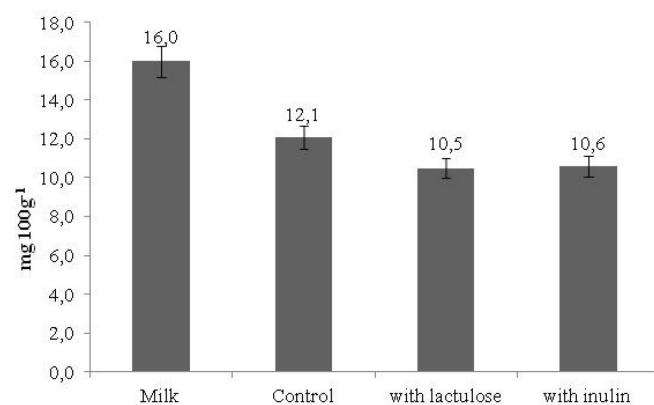


Fig. 1. Level of cholesterol in milk, control and in fermented milk samples with 2% lactulose and 4% inulin.

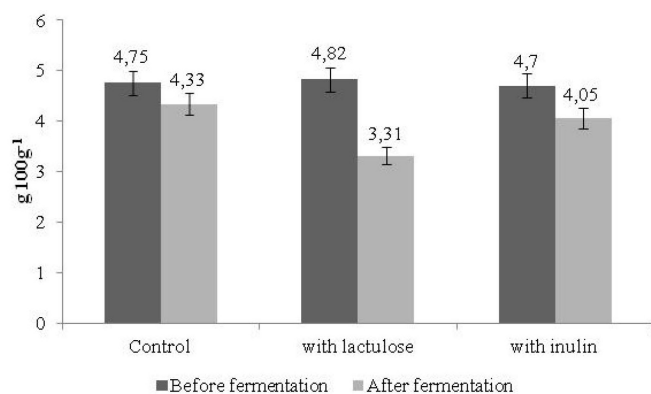


Fig. 2. Concentration of lactose in milk before and after fermentation with and without prebiotics (increasing of lactose content in milk samples before fermentation is associated with the presence of lactose in lactulose syrup).

tation occurred in all samples. The highest reduction of lactose content was observed in samples with 2% lactulose, i.e. $1.51 \text{ g } 100 \text{ g}^{-1}$. However, no significant decrease of lactose were established ($P > 0.05$) between fermented milk sample with 2% lactulose and other fermented milk samples with prebiotics. Changes of lactose content during fermentation in samples with and without prebiotics were significant ($P < 0.05$).

Regarding the ability of *B. lactis* to hydrolyse with addition of prebiotics, the results showed that bifidobacteria have the ability to ferment up to 50% of added lactulose (from $1.34 \text{ g } 100 \text{ g}^{-1}$ to $0.66 \text{ g } 100 \text{ g}^{-1}$) during fermentation. However, the decrease of inulin concentration was negligible (from $3.98 \text{ g } 100 \text{ g}^{-1}$ to $3.60 \text{ g } 100 \text{ g}^{-1}$) in fermented milk samples. In the present study, inulin with a polymerisation degree ≥ 5 and degree of purity 99.5% was used, which could explain the obtained results.

DISCUSSION

The growth of the starter bacteria in milk depends on their proteolytic activity to hydrolyse casein (Thomas and Mills, 1981), where casein is the main source of amino acids in milk (Walstra *et al.*, 2006). It is known that proteolytic properties of bifidobacteria are quite weak (Sgorbati *et al.*, 1995), however, they release aminopeptidases during multiplication (Martinez-Villaluenga and Gomez, 2007). They are vital for metabolic pathway regulation, cell maturation and protein turnover (Herrera-Camacho *et al.*, 2007). Much research indicates that bifidobacteria are able to synthesise a considerable amount of amino acids (Matteuzzi *et al.*, 1978; Ballongue, 2003), which is in contrast with the present results. Regarding concentrations of amino acids in milk and control (Table 1), there was a decrease of amino acid concentrations, except histidine and arginine, in fermented milk samples. However, *B. lactis* has the ability to synthesise a remarkable amount of specific amino acids, such as threonine, methionine, tyrosine, lysine and aspartic acid, compared with lactic acid bacteria (Table 2). The differences of amino acids concentration in milk and fermented

Table 2

CONCENTRATIONS OF AMINO ACIDS IN FERMENTED MILK SAMPLES USING DIFFERENT STARTER CULTURES, $\text{g } 100 \text{ g}^{-1}$

Amino acids	<i>B. lactis</i>	<i>L. lactis</i> C15	<i>L. bulgaricus</i> HP1	<i>L. helveticus</i> MP12
Threonine	0.095	0.005	0.035	0.011
Valine	0.115	0.096	0.138	0.092
Methionine	0.064	0.006	0.040	0.048
Isoleucine	0.087	0.003	0.254	0.144
Leucine	0.232	0.037	0.266	0.297
Phenylalanine	0.114	0.092	0.142	0.294
Tyrosine	0.123	0.022	0.022	0.020
Lysine	0.181	0.006	0.074	0.036
Aspartic acid	0.212	0.032	0.041	0.161
Serine	0.115	0.061	0.215	0.234
Glutamic acid	0.569	0.677	1.130	2.051
Proline	0.221	0.202	1.007	1.281
Glycine	0.039	0.032	0.057	0.158
Alanine	0.069	0.093	0.454	0.543
Histidine	0.108	0.050	0.174	0.232
Arginine	0.092	0.007	0.094	0.024
Total	2.436	1.421	4.143	5.465

After Simova *et al.*, 2006

milk can be explained by the need of amino acids for bifidobacteria growth (Ballongue, 2003).

B. lactis can release high activity leucine and valine aminopeptidases (Martinez-Villaluenga and Gomez, 2007). This can be explained by the chemical composition of milk, method of preparation and storage conditions of experimental samples. We observed an increase of aminopeptidase activity by addition of 2% lactulose, which led to a significant increase ($P < 0.05$) of isoleucine, leucine and lysine. Amino acids released by bifidobacteria influenced the nutritional value of fermented products.

Bifidobacteria can decrease the serum cholesterol in blood (Xiao *et al.*, 2003), which can be promoted by addition of prebiotics (Pereira and Gibson, 2002). The cholesterol-lowering effect of probiotics can be explained by, for example, enzymatic deconjugation of bile acids by bile salt hydrolase of probiotics (Kumar *et al.*, 2012). The decrease of cholesterol concentration in fermented milk in the present study can be explained by the activity of esterases, which was determined as intermediate activity for *B. lactis* (Martinez-Villaluenga and Gomez, 2007). Esterases have the capacity for hydrolysis and synthesis of esters by esterification of fatty acids and ethanol (Liu *et al.*, 2004). Esterases from lactic acid bacteria release preferentially short-chain fatty acids, and furthermore, several strains possess more than one esterase (Oliszewski *et al.*, 2007). The decrease of cholesterol level in milk depends mainly on the bacteria species used for fermentation (Oberman and Libudzisz, 1998). *Lactobacillus acidophilus* and *Bifidobacterium* spp. in fermented milk are able to assimilate cholesterol from 18% to 38% in fermented milk (Ziarno *et al.*, 2007). The results of

the present study showed that *B. lactis* can assimilate cholesterol by 25% in milk, whereas by 35% with addition of the studied concentrations of lactulose and inulin. Thus, the presence of prebiotics in milk promotes *B. lactis* esterase activity.

Two-thirds of the world's adult population suffers from lactose maldigestion (Vesa *et al.*, 2000), which is caused by reduction of β -galactosidase activity in the small intestine after weaning (Russell *et al.*, 2011). Jiang *et al.* (1996) indicated that β -galactosidase improves lactose utilization for lactose intolerant patients. Martinez-Villaluenga and Gomez (2007) reported that β -galactosidase activity for mostly bifidobacteria strains was high, but *B. lactis* had intermediate activity. β -galactosidase is widely employed for reduction of lactose content in milk products (Rhim *et al.*, 2010), thereby fermented milk products are acceptable for patients with lactose intolerance. Lactose is split by β -galactosidase into glucose and galactose (Kandler, 1983). The activity of β -galactosidase released from *B. lactis* can be described as intermediate (Martinez-Villaluenga and Gomez, 2007), which explain the results of the present study. Evaluating the ability of *B. lactis* to assimilate added lactulose and inulin, it was obvious that lactulose was most suitable substrate for growing of bifidobacteria in milk.

This confirmed the results mentioned in literature about the bifidogenic effect of lactulose in certain concentrations (Palframan *et al.*, 2002; Bouhnik *et al.*, 2004) and the ability of bifidobacteria to assimilate lactulose (Saarela *et al.*, 2003). Semjonovs *et al.* (2004) reported that *B. lactis* is not able to assimilate inulin. Biedrzycka and Bielecka (2004) indicated that inulin assimilation decreases the inulin polymerisation degree and the degree of purity.

In conclusion, the enzymatic activity of bifidobacteria determines nutritional value of fermented dairy products. The addition of 2% lactulose resulted in significant increase of some amino acids, such as leucine, phenylalanine, lysine and arginine ($P < 0.05$), compared with the other treatment. The presence of prebiotics resulted in decrease of cholesterol level by 35% and lactose content by 31% in fermented milk samples. The enzymatic activity of bifidobacteria can be promoted by addition of lactulose and inulin to increase the nutritional value of functional dairy products.

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Bifidobacterium lactis RAUDZĒTU PIENA PRODUKTU UZTURVĒRTĪBAS PAAUGSTINĀŠANA

Bifidobaktērijas ir vienas no nozīmīgākajām un plašāk lietotajām probiotikām piena produktu ražošanā, pateicoties to daudzveidīgajai labvēlīgajai ietekmei. Bifidobaktērijām vairojoties, substrātā tiek izdalīti šādi enzīmi: aminopeptidāzes, galaktozidāzes, esterāzes, kuru darbības rezultātā iegūst raudzētu piena produktu ar paaugstinātu aminoskābju, zemāku holesterīna un laktozes saturu. Bifidobaktēriju enzimatiskā aktivitāte pienā nosaka raudzētā produkta uzturvērtību. Tā kā dažādu enzīmu aktivitāte ir atšķirīga, tad, pievienojot prebiotikas, ir iespējams veicināt enzīmu darbību, tādējādi palielinot vai samazinot atsevišķu uzturvielu saturu gatavajā produktā. Pievienojot pienam 2% laktulozes, tika panākts būtisks ($P < 0.05$) atsevišķu aminoskābju (leicīna, fenilalanīna, lizīna un arginīna) satura pieaugums raudzētajā produktā. Prebiotiku klātbūtne nodrošināja holesterīna satura samazinājumu par 35% un laktozes satura samazinājumu par 31% piena fermentēšanā. Pētījuma rezultāti pierādīja, ka bifidobaktēriju enzimatisko aktivitāti ir iespējams veicināt, pievienojot noteiktās koncentrācijas laktulozi un inulīnu, paaugstinot raudzēto piena produktu uzturvērtību.

SALT AND BREAD: LATVIA'S EXPERIENCE

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Much research has shown that reduction in salt intake can have a very positive impact on human health, as salt intake plays a critical role in regulating blood pressure. According to the World Health Organization, populations with low salt intake, all other factors being equal, usually have lower blood pressure level, in that way decreasing the risk of cardiovascular disease. The main source of salt in the diet is ready-made food, and only about 11% of overall salt intake is the salt people add to their food themselves. A common EU framework for salt reduction has been developed, which describes a common vision for a general European approach towards salt reduction. Bread has been identified as an important contributor to the daily salt intake in many countries. On the other hand, salt has important technological functions in bakery products. It improves the qualities of dough, fermentation, and flavour of bread. The aim of this paper was to evaluate the amount of salt in Latvian bread and to assess the need for decreasing the amount of salt in bread. The majority of Latvian bread contains 1.1 to 1.2% salt in flour, but in some other kinds of bread it is even less.

Key words: salt, bread, sodium chloride.

INTRODUCTION

Common salt or sodium chloride is a chemical compound NaCl. Salt is a natural mineral halite, which exists as deposits in many world countries, and is the compound of sea water and salt lakes (Kurlansky, 2003). Salt is a significant element in the diet not only for humans, but also for animals and many plants. Salt is one of the most effective and popular food preservatives. Sodium is a mineral, an inorganic compound, and it performs a biological function in the human organism. Ions of sodium are located mainly outside cells, maintaining the amount of water outside cells in balance. Salt takes part in maintaining the balance of liquids and transmitting nervous impulses, and it affects the contraction and relaxation of muscles (Baltess, 1998). Salt participates in regulating the amount of water in the human organism. A strong desire for salt may be related to a lack of minerals, particularly sodium chloride, in the organism. Ions of sodium and chloride act in the function of kidneys. Sodium “seizes” water and together with chloride “holds” water in tissues. Water is absorbed from intestines by means of NaCl. Salt is the central regulator of water exchange in this system. Ions of sodium and chloride are important components of digestive juices. Both ions maintain the tonus of tissues. The sodium-potassium exchange pump is required to maintain cell pressure (Denton, 1983).

The human organism contains approximately 70 g of Na, and its amount has to be regularly replenished in small quantities. We ingest the necessary amount of sodium in the

form of salt. Salt is the main source of sodium in the diet and is used as a seasoning that nowadays is usually consumed too much. A too large intake of salt affects the human organism very negatively, as it burdens physiological functions. For example, excessive salt will need to be excreted via kidneys. Too large intake of salt increases the amount of salt in the organism, which promotes hypertension, increases the risk of heart diseases or cerebral thrombosis, as well as promotes kidney diseases (Lejins and Kalvelis, 2011). Studies have shown that individuals consume on average 10–15 g of salt daily, which is much more than the 5 g recommended by dietary doctors. Salt is added to food bought in almost all supermarkets, not only to canned food and sausages, but also to bread, spaghetti, cheese, and even yogurt. The content of salt is so high in food products that the amount ingested in one day would suffice for many days. Consumption of products with a high content of salt can result in dropping/swollen eyelids. If salt is regularly consumed too much, more serious health problems might arise. Regarding salt, women have to be very careful during menopause, as its excessive consumption causes greater excretion of potassium into urine, which may make bones fragile to a dangerous extent. There is a view that excessive intake of sodium chloride can promote asthma, stomach ulcer, duodenal ulcer, and cancer (Großklauss *et al.*, 2009).

The mechanism by means of which salt causes hypertension is not completely known, but doctors consider that the kid-

neys of patients suffering with hypertension are not able to excrete sodium as much as necessary. Blood pressure affects fluctuations of the Na level in blood plasma. With a decrease in the amount of Na in blood plasma, blood pressure also falls. Serum Na also determines the volume of extracellular fluid, which also affects blood pressure; yet, changes in blood pressure are determined by the sodium level, not changes in the volume (Valtnera, 1995).

Research has confirmed that nowadays salt is consumed through food too much, and that its consumption has to be reduced. A decrease of salt in the diet positively affects human health. It reduces blood pressure and improves the condition of heart and blood vessels. A decrease in the blood pressure of individuals, which is caused by a reduction in salt consumption, depends on age — in older individuals, blood pressure can decrease to a greater extent. Given the fact that older people more often have cerebral thrombosis and heart failure, they benefit more from a decrease in their blood pressure. Limiting salt in the diet of this group of people is of great importance (Lejins and Kalvelis, 2011).

Bread is one of the products that is consumed almost every day. To bake bread, the following raw materials are needed: flour, yeast, water, and salt. Salt is a very important seasoning in bread. Salt affects biochemical, colloidal, and microbiological processes in bread dough (Fig. 1). In water, salt splits into ions and a dissociated solution forms — sodium ions are positively charged, while chlorine ions are negatively charged. This affects the properties of gluten, i.e. gluten strengthens, the activity of ferments is hindered and the life processes of yeast are affected (Kunkulberga and Seglins, 2010). Salt obstructs the growth of undesired microorganisms and the structure of bread is more porous; it assists in keeping the shape of bread loaf and stabilises starch. The most significant property of salt is the improvement of taste, as bread without salt is tasteless and insipid.

Salt is added to dough in small quantities — from 1 to 2% of the total amount of flour. Without salt, dough is sticky and flabby, it rises fast during fermentation and does not maintain its form, and the bread is insipid and has a pale and hard crust. However, an excessive amount of salt — more than 2% relative to the weight of flour — delays fermentation processes or dough does not rise at all, it is wet and hard to processed, and the bread has an unpleasant salty taste.

According to the recommendations of the European Commission, the amount of salt in producing bakery products

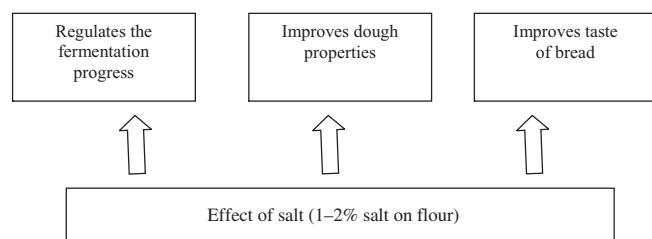


Fig.1. Technological role of salt in breadmaking.

should be reduced at least to 1.5%, and for some products to 1.8% per 100 g of flour. For instance, it is planned to reduce the amount of salt in dough from 2.2% to 1.8% per 100 g of flour in Ireland, Italy, Spain, and Great Britain during the coming years, while bread producers in Germany do not plan to take any special salt reduction measures (Galeone, 2009; Kleinert *et al.*, 2009).

Scientists and practitioners of many countries have investigated the effects of salt on dough and bread baking processes. One disadvantage appears in dough with a low salt content if the dough is processed and formed at a higher temperature or during an extended period of fermentation. It is hard to precisely determine the critical amount of salt that negatively affects technological processes, as different equipment is exploited in industrial bread production. It is easier to adapt to the production of bread with a lower salt content in the sector of small and medium bakeries where dough is processed manually. According to Finnish specialists, it is necessary to convince bread producers to reduce the amount of salt in this sector, as processing dough with a low salt content may cause problems to industrial bread producers (Salovaara, 1982a; Salovaara *et al.*, 1982).

A governmental system for proposals for voluntary salt consumption reduction has been introduced in the European Union, which is supported by action plans, to meet the recommendations of the member states and the World Health Organisation. The approaches of the EU member states need to be adapted to all socio-economic societal groups for salt consumption reduction in foods of all price groups. Also, the international aspect of changing salt content in food products and the role that might be played by the existing forums of interested individuals, for instance, the EU Action Platform on Diet, Physical Activity and Health, have to be considered. A significant role is played by food marking that provides visible, clear, and easy-to-understand information to consumers, thus enabling them to choose products with a lower salt content. The working group has decided to use the term “salt”, instead of “sodium”, as salt is the basic substance targeted by the mentioned recommendation, the term “salt” is easier to understand, and it is the main way of how to add sodium to food (Großklaus *et al.*, 2009).

Given the statistical data on diseases of heart and blood vessels, the EU Council’s working group on food products has decided to appeal for reducing salt consumption in the EU member states. It is known that excessive salt consumption is the main contributor to hypertension, which, in its turn, causes diseases of heart and blood vessels. These diseases cause 42% of all deaths in Europe, and the treatment of these diseases costs EUR 192 billion. The European Food Safety Agency stated that the majority of Europeans consume 8–11 grams of salt a day, which is much more than the recommended amount. The largest part of salt or up to 75% of its daily portion is consumed by people through ready-made food. Mainly people of industrially developed countries consume approximately 80% of salt through products to which salt has been added during their industrial

production. Natural products contain a small amount of sodium and its salts, and some salt is added by us ourselves when we cook food or add it during eating our meals. According to studies, in natural products the amount of salt accounts for approximately 12% of the total intake of salt a day, ready-made food produced industrially and food from public caterers provides 77%, while salt that is knowingly added by us accounts for only 11% (of which approximately 5% is additionally added to meals and 6% is added during cooking home meals) (Johnston, 2009).

The member states are requested to actively implement their salt consumption reduction programmes. These may include, for example, activities to inform the public and to encourage the food industry to reduce the amount of salt in food and ready-made meals as much as possible. The European Commission permanently supports the efforts of the member states regarding these issues. Together with the member states, the European Commission regularly identifies the lowest salt contents reached in various food categories in the European Union. Such a survey allows to determine the extent to which salt content can be reduced in products that are the main sources of salt in daily diets. The EU stresses the need for controlling the activities related to changes in the content of products, for informing the public on the consumption of salt by individuals to enable them to regularly analyse and review their actions, and for familiarising the general public with the corresponding findings. The EU appeals to the member countries for strengthening their coordinated and sustainable national nutrition policy as well as for elaborating a policy if one has not been yet introduced, which also includes salt consumption reduction programmes aimed at reducing intake to an appropriate level (Galeone, 2009).

Given the fact that the European Commission has established a high-level working group on diets and physical activities, one of the objectives of which is to reduce the salt content in food products, member states have to analyse their national statistics on salt consumption and the present amounts of salt in food. The research data show that bread is one of the key sources of salt in the diet of individuals; therefore, a salt consumption reduction plan has been elaborated in many countries, which envisages a 16% reduction in salt consumption over a period of four years. According to the European Commission recommendations, the member states have to make discussions with their food producers and local federations of food enterprises and hold activities for informing the public on the salt content in food and its reduction; therefore, it is important to ascertain the knowledge of consumers on salt consumption and their salt consumption behaviour.

No salt reduction programme has been elaborated in Latvia, and studies are needed to identify the salt content in bread baked in Latvia. No detailed information is available on the amounts of salt added to bread during its production process. Therefore, the aim of the present paper was to determine the salt content in bread produced in Latvia, to identify the possibilities for reducing the salt content, and to

analyse the intake of salt through bread by Latvia's consumers.

MATERIALS AND METHODS

The study analyzes two types of bread, one — baked in the laboratory with different salt contents, other — industrially produced bread. Wheat bread with various salt content was experimentally baked at the Bread Technological Laboratory of the Faculty of Food Technology, Latvia University of Agriculture (LLU). The basic recipes for bread are presented in Table 1. The amounts of salt relative to the weight of flour were selected as follows: 0.5%, 1%, 1.5%, and 2% as well as no salt at all. The dough production technology and the baking of bread complied with the guidelines for experimental baking.

The dough was prepared in a dough mixer *Varimixer bear AR10 2*. The time of dough mixing: 2 minutes at Speed 1 and 4 minutes at Speed 2. After mixing the dough, it was fermented for 10 minutes, then it was divided and into pieces of 220 g, the pieces were shaped and fermented in a post-fermentation camera for 45 minutes at a temperature of 36 °C and a relative humidity of 75%. The bread was baked in a convection oven *Sveba Dahlen S8*, steam was supplied for 9 seconds, and the duration of baking was 18 minutes at a temperature of 200 °C. After a period of 24 hours, the bread was tested for its organoleptic indicators, and the salt content was identified according to a chloride determination method by titrating it with mercury (II) nitrate.

The bread moisture content was determined according to ISO 6496:1999 method.

The salt content of some samples was determined at the Food and Environmental Test Laboratory of company *BIOR Ltd* according to the GOST 5698_51 method. At the Scientific Laboratory of Agronomic Research of LLU, the sodium content was determined by Atomic Absorption Spectrophotometry AACC Method 40-71.

In the present study, 103 wheat bread, rye bread, seed bread and toast samples produced in various Latvian bakeries, with different package labels, as well as information on the contents of salt and sodium available on the labels were analysed.

Table 1

WHEAT BREAD FORMULA WITHOUT SALT

Raw materials	Amount, kg
Wheat flour, Type 550	0.500
Yeast	0.015
Sugar	0.010
Water	0.300
Dough total	0.825

Standard deviations and errors were calculated for the data by using the Microsoft Office package, Microsoft Excel (XLS), and SPSS.

RESULTS

To verify the effect of salt on the quality of bread and to determine the content of salt in the bread samples having a known amount of salt added to the dough, wheat bread was experimentally baked. The samples of bread were tested for organoleptic, physical, and chemical indicators. The acidity of the bread samples titrated ranged within a pH range of 1.8–2.0 and the moisture was 36–38%, which corresponded to usual parameters of wheat bread. The dough prepared without salt was sticky and flabby and rose fast, and the bread was pale with a hard crust and tasteless and insipid. Salt content affected colour of the crust by becoming darker. According to the scientific literature, salt not only regulates the fermentation process, but it also improves the properties of proteins, which enhances the porosity and elasticity of the soft part of bread, the size of bread and what is the most important — its taste (Salovaara, 1982b).

Most often, salt is added to dough at an amount from 1.2 to 2% relative to the weight of flour. It is important to consumers to know the percentage of salt per 100 g of bread as well as the weight of sodium, in mg, per 100 g of bread; therefore, an experiment was carried out to calculate correlation between the salt content, as percentage of the weight of flour, and the salt content as percentage in bread. The salt content in the bread samples was determined by the titration method, and the results obtained are presented in Table 2. A linear correlation existed between the percentage of salt content in bread and salt content calculated in flour ($r = 0.9984$). The regression equation derived from the results (Fig. 2) was:

Table 2

SALT AND SODIUM (%) IN BREAD SAMPLES

Bread sample	NaCl of flour, %	NaCl in bread, %
1.	0	0.1 ± 0.03
2.	0.5	0.48 ± 0.04
3.	1	0.74 ± 0.02
4.	1.5	1.08 ± 0.02
5.	2.0	1.36 ± 0.04

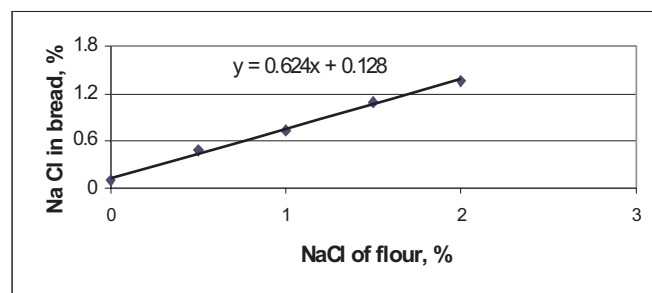


Fig. 2. Linear regression between the percentage of salt content in bread and in flour.

$\text{NaCl \% in bread} = \text{NaCl \% relative to the weight of flour} \times 0.6 + 0.1$.

However, this equation is precise only for bread baked according to the given recipes and the mentioned technological parameters of baking bread at the given output of dough; therefore, in relation to other kinds of bread, this equation can produce approximate values. Even if the same recipes are used, but the duration or temperature of baking is changed, the loss of bread weight is greater and the result is not precise; in this case, a correction has to be made. By using such equations, it is possible to easily and quickly calculate a salt content in bread if the amount of salt added to dough relative to the weight of flour is known.

Analysis of the information on the package labels of 103 bread samples regarding contents of salt and sodium in various kinds of bread showed that the salt contents in wheat, rye, seed, and toaster breads do not differ significantly, and per 100 g of bread, and according to package labels, varied from 1.1 to 1.2%. The content of sodium is within the range from 0.4 to 0.5% per 100 g of bread.

According to the present study, Latvian bread producers show correct information on package labels, in accordance with Cabinet Regulation No. 964 of 23 November 2004 “Regulations regarding the Marking of Food Products”. Although showing the energy and nutritional values on any package label of food products is mandatory only if a special note on the energy or nutritional value or the impact on health has to be made, it has to be noted that bread producers provide such information in 99% cases. On package labels, more and more often information is provided on the recommended daily portion of nutrients present in a product, as no occurs in 60.4% cases. One can conclude that bread producers understand that salt is a significant mineral, and they try to inform consumers about salt content in a product. It has to be noted that such information is not available on the package labels of other products, such as cheese, sausage, and salty meat snacks.

Six ryc bread samples (R1–R6) were tested for salt content at the Food and Environmental Test Laboratory of BIOR Ltd and at the Scientific Laboratory of Agronomic Research of LLU, which confirmed that the salt amount shown on the package label provided sufficiently precise information on its content (Table 3).

Table 3

SALT AND SODIUM CONTENT OF BREAD, %

Bread sample designation	Indicated on packaging, %		Salt content, %	Na content, %	Salt content by calculation from the Na, %
	Salt	Na			
R1	0.9	0.3	0.9 ± 0.1	0.31 ± 0.02	0.78
R2	1.2	0.48	0.9 ± 0.1	0.37 ± 0.01	0.94
R3	1.0	0.4	-	0.34 ± 0.03	0.86
R4	2	-	1.2 ± 0.1	0.43 ± 0.04	1.09
R5	1.0	0.4	-	0.37 ± 0.02	0.94
R6	-	0.8	-	0.41 ± 0.01	1.04

The daily intake of salt through bread at a daily bread consumption of 100, 200, and 300 g is shown in Table 4. On average, 1 slice of “Live-Seed Bread” produced by Fazer Bakeries Ltd weighs 15 g, meaning that 100 g of the bread corresponds to approximately 6.5 slices of bread, and 1 slice of “Latvian Rye Bread” weighs on average 35 g, thus 100 g of the bread comprises three slices. On average, 1 slice of “Special Rye Bread” from Liepkalni Ltd weighs 40 g, thus 100 g of this bread comprises about two slices. If an adult consumes on average 250 g of bread a day, the salt content of which is 1.2%, the salt intake is 3 g, which accounts for 50% of the recommended daily salt portion. Yet, if bread contains only 0.9% salt, the daily intake of salt is 2.25 g by consuming the same amount of bread, which accounts for 26.6% of the recommended daily salt portion. If bread contains 1.6% salt, the salt intake is 4 g by consuming 250 g of bread, which accounts for 80% of the recommended daily salt portion.

DISCUSSION

Salt plays a very important role in the human organism. It takes part in regulating the amount of water and in kidney function; Na and Cl as ions are the main electrolytes of blood. People are not able to live without salt, whereas an excessive intake of salt negatively affects their health.

Adding salt to products is partially determined by technological needs, but salt is mainly used for improving the taste of products. Therefore, there is a real possibility to reduce the amount of salt in products added during their production. This can also apply to bread production. In Latvia, it is important to conduct studies on the lowest possible salt content in food products, which would include information on consumption of food products and would allow to elaborate legal acts regarding an optimal use of salt in food production, including bread baking.

The majority of bread producers show the content of both salt and sodium in bread on the package label. Some producers showed salt content (R4) relative to the weight of flour on their package label, which provides imprecise information and misinforms consumers, as the salt content in bread is lower. In accordance with European Council Regulation No. 1924/2006 (20 December 2006) regarding information on nutritional values and health that has to be shown on package labels of food products, the amounts of components have to be shown for the final product.

Every day people eat different food products, including bread, and every product contains some salt. Bread is a

valuable food product, yet, according to statistical data, the consumption of bread declined in Latvia from 70 to 48 kg per capita in the period 2004–2009. This trend may be explained not only by the economic situation, but also by a change in food consumption behaviour (Noort *et al.*, 2010).

Many dietary specialists recommend increasing the consumption of bread up to 250 g a day, including the World Health Organisation. On average 27% of dietary fibres are provided by consuming the recommended amount of wheat bread, while rye bread provides 54%.

In most cases, Latvian bread producers add salt to dough at an amount of 1.2–2% relative to the weight of flour. Yet it is important to consumers to know the percentage of salt in 100 g or in 1 slice of bread as well as the amount of sodium in mg in 100 g or in 1 slice of bread. The result of research shows that correlation between salt content as a percentage relative to the weight of flour and the salt content as a percentage in bread was $r = 0.9984$.

In 99% cases, bread producers provide information on the special energy value of bread, showing the contents of salt and sodium in bread on their package labels, and 60.4% of bread producers provide information on their package labels about the recommended daily portion of nutrients present in their product.

If an adult consumes on average 250 g of bread a day, the salt content of which is 1.2%, the salt intake is 3 g, which accounts for 50% of the recommended daily salt portion. Yet, if bread contains only 0.9% salt, the daily intake of salt is 2.25 g by consuming the same amount of bread, which accounts for 26.6% of the recommended daily salt portion.

Analysis of the salt amounts in bread baked in Latvia indicates that it was in the range of 0.8–1.2%, which did not exceed the amount (1.0–1.2%) recommended by the European Commission. Given the fact that nowadays food is over-saturated with salt, it is necessary to regularly hold informative campaigns for the public and discussions with food producers about reduction of salt content in food.

ACKNOWLEDGEMENTS

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Table 4

INTAKE OF SALT THROUGH BREAD CONSUMPTION, g

Type of bread and salt content, %	100 g bread	200 g bread	300 g bread
Live-Seed Bread 0,9%	0.9	1.8	2.7
Latvian Rye Bread 1,2%	1.2	2.4	3.6
Special Rye Bread 1,6%	1.6	3.2	4.8

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SĀLS UN MAIZE: LATVIJAS PIEREDZE

Pēc Eiropas Komisijas ieteikumiem, dalībvalstīm ir jāanalizē nacionālie dati par sāls patēriņu un pašreizējo sāls daudzumu pārtikā. Maize ir viens no galvenajiem uztura produktiem Eiropā, un ar to tiek uzņemta 20–25% no ikdienas sāls devas. Cilvēka organismam sāls ir nepieciešams, jo tas veic svarīgas bioloģiskas funkcijas, savukārt pārmērīga tā lietošana ir riska faktors dažāda veida slimībām — hipertensijai, nieru slimībām. Pētījumā ar dažādām metodēm maizes paraugos noteikts sāls un nātrija saturs un secināts, ka Latvijā ražotā kviešu, rudzu, sēklu un tostermaize pēc sāls satura būtiski neatšķiras un tas ir robežās no 1,1 līdz 1,2%. Veikta maizes iepakojuma informācijas analīze un aprēķini par uzņemto sāls saturu, apēdot noteiktu maizes daudzumu. Šāds aprēķins ļauj novērtēt uzņemto sāls daudzumu pēc patērētā maizes apjoma, ko noderīgi zināt katram patērētājam. Pēc iedzīvotāju domām, Latvijā ražotajai maizei nav nepieciešams samazināt sāls daudzumu, arī pēc iepakojumā norādītās informācijas un veiktajiem pētījumiem sāls saturs maizē ir ieteicamās normas (1,0–1,2%) robežās. Tomēr jāatzīmē, ka nepieciešams plašāk organizēt sabiedrības un pārtikas produktu ražotāju informēšanas pasākumus par sāls saturu un tā samazināšanas nepieciešamību pārtikas produktos.

CONTENT OF ALKYLRESORCINOLS IN CEREALS GROWN IN LATVIA DETERMINED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV

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Cereal alkylresorcinols (ARs) are a group of phenolic lipids mainly found in the outer parts of grains of rye and wheat. They have been suggested for use as selective biomarkers for intake of whole grain and bran products of these cereals. Consumption of whole grains and whole grain products has been associated with reduced risk of developing chronic diseases, such as cardiovascular disease, diabetes type 2, obesity and some types of cancer. In this article a sensitive and rapid method of High Performance Liquid Chromatography with UV detection for quantitative determination of ARs in the cereals grown in Latvia is described. Instrument detection limits (IDL) were determined for C17:0, C19:0 and C21:0 homologues (coefficient of variation < 3%). According to the results of these studies, ARs were found in rye (87.1–112.0 mg/100 g), wheat (24.0–40.2 mg/100 g), triticale (32.1–74.4 mg/100 g), and in small amounts in barley (2.2–3.7 mg/100 g), but not in oats.

Key words: alkylresorcinols, HPLC-UV, whole grain cereal.

INTRODUCTION

Epidemiological studies have associated a diet rich in whole grain and cereal products with decreased risk of several chronic diseases, e.g. coronary heart disease (Liu *et al.*, 1999), diabetes type 2 (Munter *et al.*, 2007), obesity (Fung *et al.*, 2001) and some cancers (Jacobs *et al.*, 1998), although the mechanisms are poorly understood (Slavin, 2003). Also, there are concerns about the methodology used to determine dietary intake in epidemiological studies (Bingham *et al.*, 2003). Studies are further complicated by the fact that consumers might have difficulty in identifying whole grain products (Lang and Jebb, 2003). Whole grain intake assessment also shares the common weaknesses, such as poor precision and bias, with assessment methods for other dietary constituents (Kaaks, 1997; Bingham *et al.*, 2003). The use of a biomarker can establish complementary or alternative measurements of whole grain intake and is expected to overcome some of these obstacles (Ocke and Kaaks, 1997; Ross *et al.*, 2004a). Alkylresorcinols (ARs) have been proposed to be biomarkers of whole grain wheat and rye intake (Ross *et al.*, 2004b; Linko and Adlercreutz, 2005).

Alkylresorcinols are amphiphilic 1,3-dihydroxybenzene derivatives with an odd-numbered alkyl chain at position 5 (Fig. 1). The alkyl chain in cereal ARs varies from 15 to 27 carbon atoms. They are located in the intermediate layers

between pericarp and testa in the grain (Landberg *et al.*, 2008), and are found in high concentrations only in whole grain and/or bran products of wheat (31.7–143.9 mg/100 g dry matter, DM) and rye (36.0–320.0 mg/100g DM), and in very small amounts in refined flour (white bread, most breakfast cereals, pasta) or in any other commonly consumed foods (Ross *et al.*, 2003; Ross *et al.*, 2004a; Ross and Kochhar, 2009; Annica *et al.*, 2010). They are also present in triticale (43.9–64.7 mg/100 g DM), and in small amounts in barley (4.2–5.1 mg/100 g DM), but not in oats (Ross *et al.*, 2003).

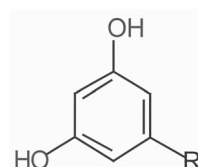


Fig. 1. Structures of alkylresorcinols commonly found in cereal grains.

R	ARs names	Short names	Molecular weight
C ₁₅ H ₃₁	5-n-pentadecylresorcinol	C15:0	320
C ₁₇ H ₃₅	5-n-heptadecylresorcinol	C17:0	348
C ₁₉ H ₃₉	5-n-nonadecylresorcinol	C19:0	376
C ₂₁ H ₄₃	5-n-heneicosylresorcinol	C21:0	404
C ₂₃ H ₄₇	5-n-tricosylresorcinol	C23:0	432
C ₂₅ H ₅₁	5-n-pentacosylresorcinol	C25:0	460

In vitro, ARs have been reported to have anticancer, enzyme-inhibiting, and DNA-cleaving properties (Kozubek and Tyman, 1999). ARs are reported to be antioxidants (Winata and Lorenz, 1996), although they are weak antioxidants *in vitro* compared with α -tocopherol (Kamal-Aldin *et al.*, 2001; Ross *et al.*, 2004b; Korycińska *et al.*, 2009). Another *in vitro* study (Ross *et al.*, 2004c) showed that ARs significantly inhibited the conversion of γ -tocopherol to its water-soluble hydroxychroman metabolite, indicating that ARs may increase γ -tocopherol's levels via inhibition of tocopherol metabolism *in vivo*. A recent *in vitro* study (Stasiuk *et al.*, 2008) showed that ARs isolated from rye grain decreased the enzymatic activity of acetylcholinesterase of erythrocytes. These findings are of interest due to the growing evidence that acetylcholinesterase could participate in the pathological processes of Alzheimer's disease. The biological activities of dietary and purified ARs have been previously reviewed (Kamal-Aldin *et al.*, 2001; Ross *et al.*, 2004b).

Different chromatographic methods for analysis of ARs in whole grain and cereal products have been developed over years. Gas chromatography — mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) are commonly used for the routine quantitative determination of ARs (Ross *et al.*, 2001; Mattila *et al.*, 2005; Landberg *et al.*, 2006). For the rapid determination of total ARs content in cereal grain products, colorimetric method after formation of intense red complex with fast blue salt (the diazonium salt Fast Blue B) has been used (Mattila *et al.*, 2005; Kulawinek and Kozubek, 2008). Fast Blue B has been used for quantitative determination of ARs in cereal grain extracts, but without reported validations of the methods used (Andersson *et al.*, 2008). Recently, the HPLC method for the analysis of intact ARs in cereals using coularray detection (HPLC-CA) has been suggested. Use of the CA detector allowed detection of low concentrations of ARs in white wheat flour, which had not been reliably detected using previous methods (Ross and Kochhar, 2009).

In this paper we present a rapid and sensitive HPLC method for the analysis of ARs in whole grain cereals with ultra violet (UV) detector. This new method was developed based on method by Ross *et al.* (2001). HPLC-UV method is rather simple and does not require derivatization or any other sample pretreatment. However, validation parameters should be performed in next research.

MATERIALS AND METHODS

Chemicals and standards. The Alkylresorcinol standard C17:0 used had $\geq 95\%$ purity as determined by the company from where it was purchased (Sigma-Aldrich, St.Luis, USA). Homologues C19:0 and C21:0 were of $> 98\%$ purity as determined by the company from where they were purchased (Reseachem Life Science, Burgdorf, Switzerland). All standards were prepared as stock solutions at 2.5 mg/ml in methanol/ethanol mixture and were stored in a freezer at $-18\text{ }^{\circ}\text{C}$. All solvents were of HPLC-grade (Sigma-Aldrich, St.Luis, USA) and were used without further purification.

Cereal samples. Rye samples ($n = 2$) and triticale samples ($n = 2$) were provided by the State Priekule Plant Breeding Institute (Priekule, Latvia), and grains were harvested in 2010. Common spring wheat samples ($n = 2$) and barley samples ($n = 2$) were provided by the State Stende Cereals Breeding Institute (Stende, Latvia), and grain were harvested in 2010. Common winter wheat samples ($n = 2$), triticale samples ($n = 1$), rye samples ($n = 4$) and oat samples ($n = 2$) were also provided by the State Stende Cereals Breeding Institute (Stende, Latvia), and grains were harvested in 2011.

Extraction of samples. According to a slightly modified method of Ross *et al.* (2001), alkylresorcinols were extracted from 1.00 g cereal grains (coarsely ground in a coffee grinder, then milled with a mortar and pestle) with 40 ml of ethyl acetate for 24 h with continuous shaking at room temperature. The extracts were centrifuged at 4400 rpm for 10 min and the supernatants (4 ml) were then evaporated to dryness in a rotary evaporator (Heidolph Laborata 4001 Efficient System, USA). Methanol (1 ml) was added and samples were filtered through $0.45\text{ }\mu\text{m}$ filters before injection into the HPLC. All samples were extracted in duplicates, and the results are reported on a basis of fresh weight (FW). Alkylresorcinol homologues C17:0–C21:0 were quantified using an external standard method. Calibration curves were prepared with the following concentrations: 0, 5, 20, 50, 70 and 100 $\mu\text{g/ml}$. Alkylresorcinol homologues C15:0, C23:0 and C25:0 were identified according to their spectra (Kulawinek and Kozubek, 2008) and determined semiquantitatively by method of internal standard, with C21:0 homologue as an internal standard (10 $\mu\text{g/ml}$). Relative response factors for C15:0, C23:0 and C25:0 homologues were calculated by taking into account the amount of homologue (based on molecular weight) injected into chromatographic column versus homologue C21:0. All quantifications were based on peak area.

HPLC-UV analysis. Method development and analysis were carried out on a Shimadzu Prominence HPLC with a SPD-20A Diode Array Detector scanning between 260 and 295 nm. Separation of alkylresorcinol homologues was done with a Symmetry C-18 ($4.6 \times 150\text{ mm}$, $5\text{ }\mu\text{m}$) column from Waters. The gradient programme was used at a flow rate 1.00 ml/min starting with a mobile phase of methanol/water (80/20) for 5 min, followed by methanol/water (99.2/0.8) for 25 min. The temperature of the column oven was set on $30\text{ }^{\circ}\text{C}$ and 40 μl of each sample were injected into the chromatographic column. Instrument detection limits (IDL) were determined for C17:0 homologue (0.48 $\mu\text{g/ml}$), for C19:0 homologue (0.44 $\mu\text{g/ml}$) and for C21:0 homologue (0.47 $\mu\text{g/ml}$), with a coefficient of variation $< 3\%$. IDL was determined to be signal (peak area) that is greater than the system noise. Within a specified probability, IDL was calculated according to the formula (Wells *et al.*, 2011):

$$X_{\text{IDL}} = t_{\alpha,n} \cdot \text{relative standard deviation} \cdot \text{amount standard} / 100\%, \text{ where}$$

$$t (\alpha = 0.05; n = 8) = 2.3646 \text{ (Student T-table).}$$

RESULTS

In the present study, a developed HPLC-UV method for quantitative determination of ARs in the cereals is described. A good homologue separation (an appropriate gradient), IDL lower than 0.5 mg/100 g and a relatively short time of total analysis, showed that this method is sensitive and rapid (Fig. 2).

The UV spectrum of the homologues in methanol is shown on Fig. 3. All the homologues showed characteristic spectra with maximum absorbance at $\lambda = 275$ nm and $\lambda = 280$ nm.

Quantification of C15:0, C23:0 and C25:0 alkylresorcinol homologues was problematic due to the lack of these standards. We attempted to overcome this by using C21:0 as an internal standard. All data were converted to fresh weight (FW), considering that the moisture content of cereals is

about 14% (Anonymous, 1992). Alkylresorcinols were found in rye, wheat, triticale, and barley, but not in oats (Table 1).

DISCUSSION

Results of the analysis of rye grains showed that ARs levels varied from 87.1 to 112 mg/100 g FW. These values are comparable to literature: 61.9–65.5 mg/100 g FW by Ross *et al.* (2003), 92.7 mg/100 g FW by Mattila *et al.* (2005), 134.2 mg/100 g FW by Landberg *et al.* (2009).

Average ARs concentration in the wheat samples analysed in this study was 34.8 mg/100 g FW. This value is comparable to the previously reported data: 28.8–42.5 mg/100 g FW by Chen *et al.* (2004), 42.0–42.1 mg/100 g FW by Landberg *et al.* (2009). However, our results for wheat samples are

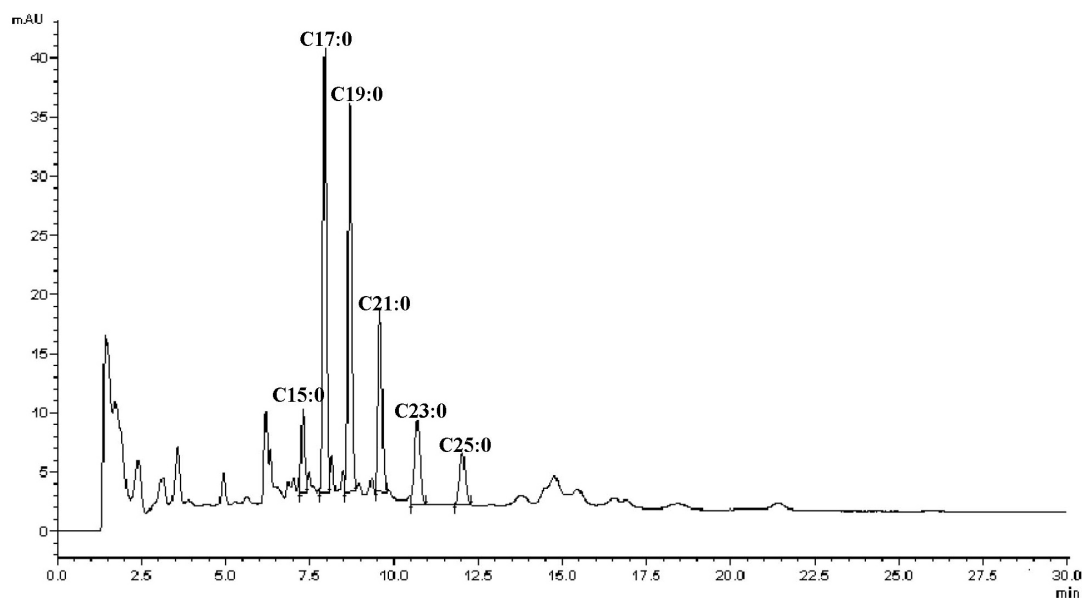


Fig. 2. HPLC-UV chromatogram of alkylresorcinol homologues in rye (Amilo) after extraction with ethyl acetate for 24 h at room temperature, centrifuged, evaporated and redissolved in methanol (1 ml). See text for HPLC conditions.

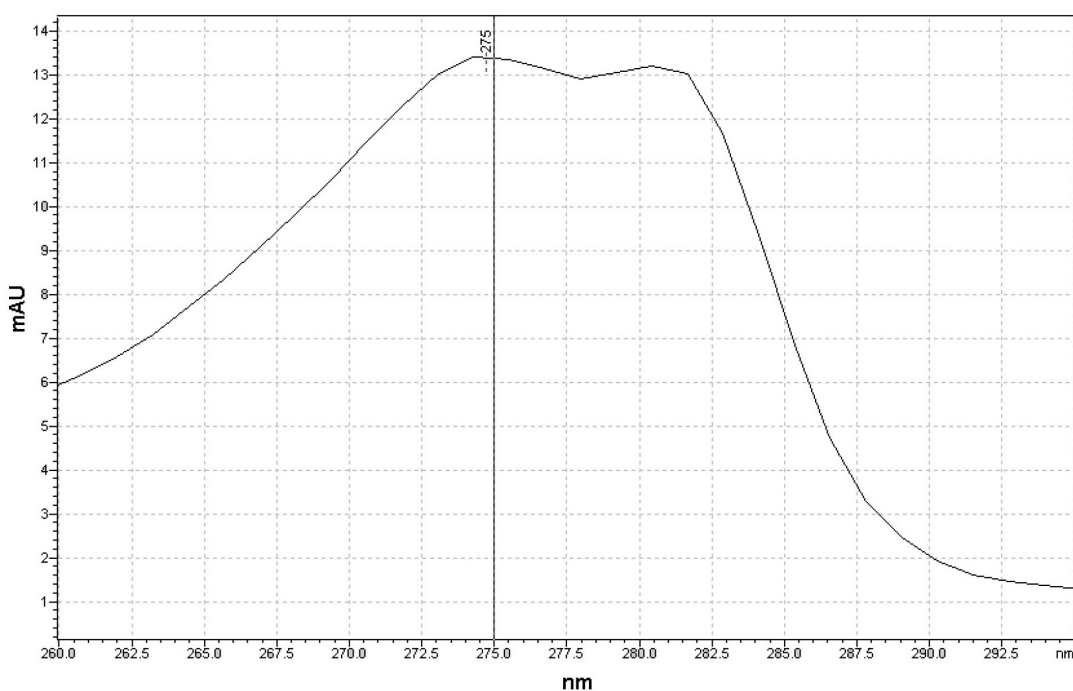


Fig. 3. The characteristic spectrum of alkylresorcinol homologues in methanol.

Table 1

CONTENT OF ALKYLRESORCINOLS IN CEREAL GRAINS (mg/100 g of fresh weight)¹

Cereal	Cultivar	C15:0	C17:0	C19:0	C21:0	C23:0	C25:0	Total ARs
Rye	04014-4	0.8 ± 0.4 ²	24 ± 4	28 ± 2	21.8 ± 0.3	21 ± 2 ²	12 ± 1 ²	107.6
	Amilo	2.18 ± 0.05	30.5 ± 0.7	26.8 ± 0.5	14.3 ± 0.5	9.6 ± 0.7	4.6 ± 0.5	88.0
	Kaupo	1.14 ± 0.07	31.8 ± 0.1	29.5 ± 0.1	19.7 ± 0.2	14.6 ± 0.7	8.9 ± 0.1	105.6
	Dankovskie Diamant	2.8 ± 0.3	34 ± 2	30 ± 2	19 ± 1	14.5 ± 0.3	8.8 ± 0.6	109.1
	9918	1.75 ± 0.02	25 ± 2	25 ± 1	16.3 ± 0.8	10.9 ± 0.9	8.1 ± 0.4	87.1
	Picaso	2.0 ± 0.4	34 ± 4	31 ± 2	22 ± 2	14 ± 2	9 ± 1	112.0
Common spring wheat	L920 Uffo	nd ³	1.96 ± 0.05	13.7 ± 0.2	20.2 ± 0.6	4 ± 1	0.38 ± 0.03	40.2
	L934 Eminent	nd	nd	5 ± 1	11 ± 2	7.7 ± 0.2	0.343 ± 0.004	24.0
Common winter wheat	Spelta (ID)	nd	3.9 ± 0.1	8.3 ± 0.6	18.3 ± 0.4	5.8 ± 0.2	0.97 ± 0.09	37.3
	96-58	nd	5 ± 1	13.6 ± 0.2	16.7 ± 0.1	2.1 ± 0.1	0.34 ± 0.02	37.7
Triticale	Falmero	nd	nd	3.2 ± 0.3	9.5 ± 0.7	16 ± 3	3.4 ± 0.2	32.1
	Nazaret	nd	4 ± 2	12 ± 4	15 ± 2	11.1 ± 0.4	2.0 ± 0.2	44.1
	9402-3	nd	12.1 ± 0.7	23 ± 1	21.0 ± 0.7	15 ± 2	3.28 ± 0.01	74.4
Barley	Jet	nd	nd	nd	1.5 ± 2	0.17 ± 0.02	2.0 ± 0.8	3.7
	1196	nd	nd	nd	1.2 ± 0.8	nd	1.0 ± 0.7	2.2
Oats	S-156	nd	nd	nd	nd	nd	nd	nd
	Arta	nd	nd	nd	nd	nd	nd	nd

¹ Values are average ± standard deviation² C15:0, C23:0 and C25:0 – semiquantitative results.³ nd = not detected (IDL of C17: – C21:0 < 0.5 mg/100 g).

lower than those obtained by Ross *et al.* (2003) — ARs levels in common wheat grains varied from 42 to 122.9 mg/100 g FW, by Mattila *et al.* (2005) — whole wheat flour contained 75.9 mg/100 g FW of ARs and, also by Kulawinek and Kozubek (2008) ground wheat grains contained 57.8 mg/100 g FW of ARs (for C15:0–C 19:0 homologues). This variation is probably due in part to differences in analytical methods and also the ARs content of cereals appears to be highly variable, depending on cultivar and environmental conditions.

Alkylresorcinol concentration in triticale varied from 32.1 to 74.4 mg/100 g FW, and is comparable to those reported previously (37.8–55.6 mg/ 100g FW) by Ross *et al.* (2003). Unfortunately, this cereal is not commonly consumed by humans.

The total ARs concentration in barley varied from 2.2. to 3.7 mg/100 g FW. These values are in line with those reported by Ross *et al.* (2003 — from 3.6 to 4.4 mg/100 g FW, by Mattila *et al.* (2005) — 3.2 mg/100 g FW, although lower than reported by Landberg *et al.* (2009) — 7.7 mg/100 g FW. The difference is probably because of use of different extraction solvents and, of course, different environmental conditions. Also, a cereal specific correction factor was used by Landberg *et al.* (2009). The factor was calculated by taking into account differences in molecular weight and the average relative homologue composition (determined by GC) of the particular cereal. None was found in oats.

According to our results, the main ARs homologues in rye are C17: 0 and C19:0; in wheat are C19:0 and C21:0; in triticale are C21:0 and C23:0; in barley are C21:0 and

C25:0. In addition, the mean total content of ARs in analyzed grain samples was in the following sequence: rye > triticale > wheat > barley > oats. Nonetheless, more data need to be collected about ARs concentration in different cereals breed in Latvia.

This HPLC-UV method is appropriate for ARs determination in cereals, due to its simplicity, rapidity and sensitivity.

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ALKILREZORCĪNU SATURA NOTEIKŠANA LATVIJĀ AUDZĒTOS GRAUDOS AR AUGSTI EFEKTĪVU ŠĶIDRUMU HROMATOGRĀFIJU AR ULTRAVIOLETU DETEKTORU

Graudaugu alkilrezorcīni (AR) ir savienojumu grupa, kur viena molekulas daļa ir fenola atlikums, bet otra ir lipīdu atlikums. AR lielā daudzumā atrodas grauda ārējās slāņos (graudapvalkos, aleirona slānī) galvenokārt rudzu un kviešu graudos, kā arī nelielos daudzumos miežos. Rafinētos produktos AR ir nēcīgos daudzumos vai vispār nav. Tieši tāpēc AR tiek piedāvāts lietot par selektīvu pārtikas biomarkieri, lai patērētājiem būtu vieglāk saprast, kurš produkts ir pilngraudu vai satur pilngraudus. Pilngraudu produktu patērēšana ir saistīta ar samazinātu risku saslimt ar dažādām hroniskām slimībām: aptaukošanās, 2. tipa diabēts, sirds slimības un vēzis. Šajā publikācijā tiek prezentēta ātra un jutīga augsti efektīvu šķidrumu hromatogrāfija ar ultravioletu detektoru – metode alkilrezorcīnu noteikšanai Latvijā audzētos graudaugos. Instrumenta detektēšanas robeža tika noteikta C17:0, C19:0 un C21:0 homologiem (variācijas koeficients < 3%). AR saturs rudzos bija robežās no 87,1 līdz 112,0 mg/100g, kviešos no 24,0 līdz 40,2 mg/100g, tritikālē no 32,1 līdz 74,4 mg/100g, nedaudz miežos no 2,2 līdz 3,7 mg/100g, bet auzās netika atrasts.

SELENIUM EFFECT ON RYE MALT QUALITY

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Rye (Secale cereale L.) is the most important cereal crop after wheat, rice and maize. A substantial part of the rye yield is used for bread making, especially in European countries. There have been numerous studies on grain enrichment with selenium (Se), as it is known that selenium is a fundamental trace element essential for human health and in the form of selenoproteins plays key structural and enzymic roles. The main aim of this study was to investigate the effect of different selenium concentrations on quality indices of rye malt — the content of malt extract, diastase activity, selenium and total phenol content in malt. Rye grain of 95% viability was soaked and germinated at temperature $+6 \pm 2$ °C for three days $+18 \pm 2$ °C, using sodium selenate Na_2SeO_4 solutions (Se concentration 3 mg l^{-1} , 5 mg l^{-1} , 10 mg l^{-1}), and dried in an oven for 24 hours at temperature $+70 - 112$ °C. Germination of grain with deionised water served as a control. The obtained results showed that an increase of selenium concentration caused increase of malt extract concentration (from 74 to 80%), selenium concentration (from 0.0139 to $0.3251 \text{ mg kg}^{-1}$) and total phenol concentration (from 3.13 to $3.63 \text{ mg GAE g}^{-1} \text{ DW}$) in rye malt, while diastase activity decreased from 330 to 216.

Key words: selenium, rye, malt extract, diastase activity, total phenols.

INTRODUCTION

Rye (*Secale cereale* L.) is the most important cereal crop after wheat, rice and maize. A substantial part of the rye yield is used for bread making, especially in European countries (Michalska *et al.*, 2008). Rye malt has amazing healing properties. It was well known to our ancestors. Porridge, bread drink and soup made from rye malt were actively used for human consumption as an effective means of winter-spring beriberi; treatment of exhausted long serious illness; and during farming work, raised strength and endurance (Смирнова и др, 1989). Rye malt is widely used in bread production today. Rye malt contains a much active enzyme (diastase) and is used for promotion of rye bread scald candying (Aly *et al.*, 2004). Rye malt is a natural food product that is made from the best varieties of rye with a coefficient of germination not lower than 96%. Its nutritional value lies on high content of protein, oligopeptides; digestible polysaccharides (glucose, maltose, dextrins), polyunsaturated fatty acids, minerals, vitamins (Michalska and Zieliński, 2006; Donkor *et al.*, 2012).

The microelement selenium is needed for normal function of the human body, as it is a constituent of some enzymes and hormones, interacts with vitamins, participates in oxidising processes, and metabolism of proteins, carbohydrates, and fats (Duma *et al.*, 2011). Selenium occurs in en-

zyme glutathione peroxidase, the main part of the antioxidative defence system in living cells (Lyons *et al.*, 2005). Therefore, selenium and its compounds have notable antioxidative properties (Surai, 1999). The selenium content in foodstuffs depends mainly on its content in plant and animal raw materials, and this is affected by the content of selenium in the soil.

It has been reported that cereals and cereal products have a wide range of selenium concentration, between 0.0010 and 0.550 mg kg^{-1} (Anonymous, 2001).

Germination of grains improves the nutritional value of grain — the content of protein, amino acids, and vitamins as well as activity of enzymes are changed. It is known that germination may influence the level and bioavailability of the bioactive compounds (Lintschinger *et al.*, 1997; 2000; Katina *et al.*, 2007; Tian *et al.*, 2010).

There have been numerous studies that showed the effects of selenium (Se) on wheat, barley and oats sprouting activity and on raising concentration of biologically active substances and vitamins in germinated grain (Dūma, 2010).

The aim of this study was to investigate the effect of different selenium concentrations on quality indices of rye malt — malt extract, total phenols and selenium concentration, and diastase activity, in rye malt.

MATERIALS AND METHODS

Plant material. The research object was rye grain (variety 'Kaupo') from Ltd. "Naukšēni", harvested in 2011. Rye grain were soaked and germinated at temperature $+6 \pm 2$ °C for three days, using sodium selenate Na_2SeO_4 solutions. The concentrations of selenium used were 3, 5, and 10 mg l^{-1} . Germination of grain with deionised water served as a control. After germination, all sprouts were dried for 24 h at a temperature of $+70 - 112$ °C; then they were ground. Moisture of malt samples ranged from 7.10% till 8.97%.

Determination of Se in germinated rye grains. The selenium concentration was determined by standard method AOAC 996.16, based on wet digestion with nitric and perchloric acids, reaction with 2,3-diaminonaphtalene (DAN) reagent and fluorimetric determination at excitation wavelength of fluorimeter at 375 nm and emission at 525 nm.

Determination of malt extract concentration. Concentration of unfermented malt extract was determined by hot extraction method.

200 ml of 47 °C warm distilled water was added to 50 g ground malt, maintained for 30 minutes at 45 °C in a water bath regularly stirring, then heated to 70 °C, and 100 ml distilled water at 70 °C was added. Stirring constantly for 1 hour, distilled water was added till 450 ml, mixed, and followed by filtering. The density of filtrate was determined and the mass of extract e , % was found.

Concentration of malt extract was calculated on the basis of air-dried substance E_1 %, as follows:

$$E_1 = \frac{e(W + 800)}{100 - e}, \text{ where}$$

e – extract mass of the filtrate, %;

W – malt moisture, %

Determination of diastase activity with Phadebas. The unit of diastase activity, the Gothe unit, is defined as that amount of enzyme that will convert 0.01 gram of starch to the prescribed end-point in one hour at 40 °C under the conditions of test. Results are expressed in Gothe units per gram of malt (Sak-Bosnar and Sakac, 2012).

The diastase activity of samples was measured by the Phadebas method (Sak-Bosnar and Sakac, 2012). A tablet of an insoluble blue-dyed, cross-linked starch was used as the substrate for the degradation reaction. After dissolving 1.00 g of malt in acetate buffer in a volumetric flask, 5.0 ml of malt solution was transferred to the test tube and incubated in a water bath at 40 °C for a few minutes. A blank was prepared by adding 5.0 ml of acetate buffer solution and then treated in the same manner as sample solutions. After placing Phadebas tablets into both test tubes, a timer was started. The tubes were quickly removed from the water bath, stirred and then returned to the water bath. After 30 min, the reaction was terminated by adding 1.0 ml 1 M sodium hydroxide solution. The mixture was stirred again and filtered. The absorbance of the sample was measured at 620

nm with deionised water as a reference. The absorbance of the blank was subtracted from that of the sample solution ($_{DA620}$). The diastase activity, expressed as DN or diastase number, was calculated from the absorbance measurements, respectively:

$$\text{DN} = 28.2 \times \Delta A_{620} - 2.64$$

Diastase activity was referred to as DN in the Schade scale, which corresponds to the Gothe scale number, or g, of starch hydrolysed per hour at 40 °C per 100 g of malt.

Determination of total phenol concentration (TPC). Total phenol determination started with preparation of extracts from rye malt. Rye malt was finely ground in a laboratory mill CIATRONIC KSW 2669. Four grams of ground samples were extracted 10 min in an ultrasound bath (ULTRASON, SELECTA P) with 40 ml of solvent (7/7/6 ethanol/acetone/water (v/v/v) mixture). After centrifugation at 3000 min^{-1} for 10 min using a centrifuge MEDITRONIC BL-C, the supernatant was removed and the extraction was repeated once more. The supernatant was collected in a 50 ml volumetric flask and refilled with solvent to previous volume. The TPC of the malt extract was determined according to the Folin-Ciocalteu spectrophotometric method with some modifications (Singleton *et al.*, 1999). First, 0.25 ml of sample was transferred to a 25.0-mL volumetric flask containing 6 mL H_2O , to which 1.25 mL of undiluted Folin-Ciocalteu reagent were subsequently added. After 1 min, 3.75 ml of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 ml with H_2O . The control sample contained all the reaction reagents except the extract. After 2 h incubation at 25 °C, absorbance was measured at 760 nm using a spectrophotometer JENWAY 6300 (Dabīņa-Bicka, 2011). Total phenol concentration was expressed as gallic acid equivalents (GAE).

Statistical analysis. The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant values were calculated. P -values < 0.05 were regarded as significant.

RESULTS

The selenium concentration in rye malt differed significantly ($P < 0.05$) between germination solutions with different selenium concentrations (Fig. 1). The concentration of selenium in rye malt using solution where selenium concentration was 3 mg l^{-1} was 5.4 times higher than in the control. Accordingly, when the concentration of selenium in solution was 5 mg l^{-1} the increase in grain was by 11.7 times. At a selenium concentration of 10 mg l^{-1} , selenium uptake increased by 22.4 times.

The influence of different selenium concentrations on the content of rye malt extract is shown in Figure 2. All Se concentrations used promoted increase of rye malt extract concentration, and the highest value was observed when the selenium concentration was 5 mg l^{-1} . In this case, the

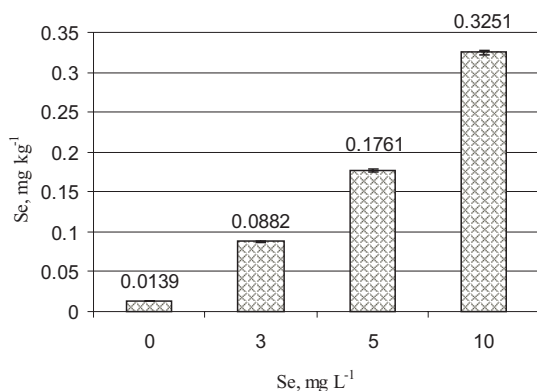


Fig. 1. Se concentration in malt, depending on the concentration of Se in solution.

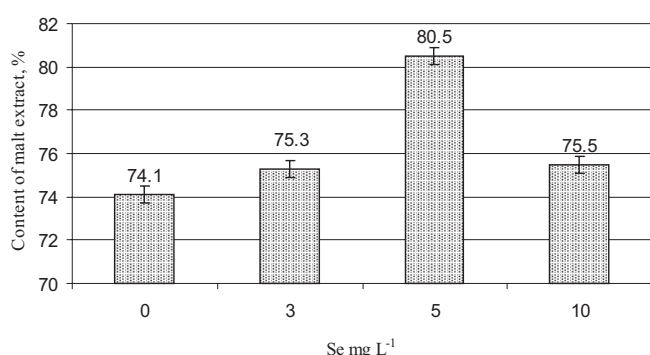


Fig. 2. Content of rye malt extract depending on Se concentration.

concentration of malt extract increased by 6.4%, compared with the control sample. There is an insufficient amount of information in the literature about the effect of selenium on concentration of rye malt extract.

Change of diastase activity in rye malt in relation to Se concentration in solution are shown in Figure 3. Rye malt diastase activity depended on selenium concentration in solution — when selenium concentration was 3 mg l⁻¹ and 5 mg l⁻¹, diastase activity increased by 33% and 5.6%, respectively. At a selenium concentration of 10 mg l⁻¹ rye malt diastase activity decreased by 12.9%, compared with the control sample.

The use of selenium treatment also significantly increased ($P < 0.05$) the total phenol concentration (Fig. 4). When concentration of selenium was 3 mg l⁻¹, the content of total phenols increased by 6.1%, at concentration 5 mg l⁻¹ the increase was by 14%, and when 10 mg l⁻¹ the concentration of total phenol increased by 16%, compared with the control sample.

DISCUSSION

It is known that cereals belong to Se non-accumulating plants due to a limited ability of selenium uptake from the soil and incorporation in compounds (Terry *et al.*, 2000). Selenium concentration in wheat, hull-less barley, hull-less

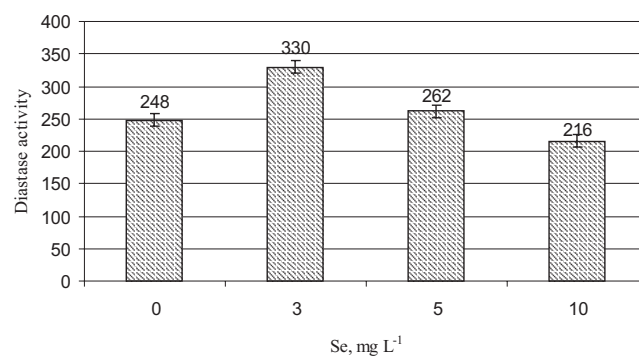


Fig. 3. Diastase activity (Gothe units) in rye malt depending on Se concentration in solution.

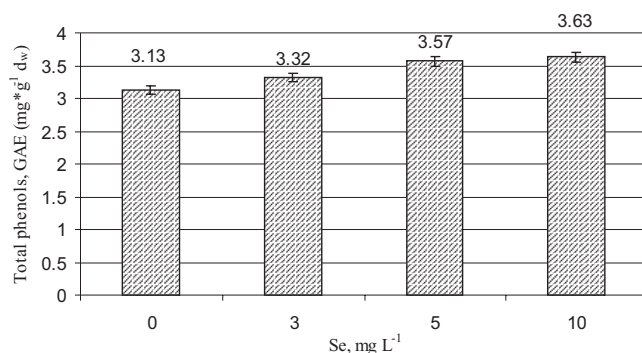


Fig. 4. Total phenol content in rye malt depending on Se concentration.

oat and rye grain harvested in Latvia is within the range from 0.01533 mg kg⁻¹ to 0.03533 mg kg⁻¹, in addition, the variety of cereal has a significant effect on the content of selenium ($P < 0.05$). The concentration of selenium is lower in hull-less grain (barley and oats) (Dūma, 2010). In comparison, the concentration of selenium in cereals cultivated in Finland is five times, and in Germany 25 times higher than in Latvia (Eurola, 1990; Combs, 2001). The obtained results showed that all analysed Se (VI) concentrations promoted uptake of selenium in rye malt. With increasing Se concentration in solution, the concentration of selenium in malt also significantly ($P < 0.05$). increased. This allows to conclude that selenium accumulation in malt is proportional to its concentration in solution and it is practically linear. These results are similar to those found previously (Lintschinger *et al.*, 1997)

The most important parameter in the evaluation of the malting usability of rye grain is concentration of malt extract, estimated as the proportion of extractive substances obtained from malt produced from a given grain under optimal technological conditions. Concentration of malt extract varies in wide range, primarily depending on grain quality (Błażewicz *et al.*, 2007). Concentration of malt extract in unfermented malt can be up to 80% (Смирнова и др., 1989). Variability in concentration of malt extract may be due to diversified water utilisation, affecting the yield and protein content of grain as well as efficacy of agricultural practices (Błażewicz *et al.*, 2007).

Malting includes germination and drying of cereal seeds and its prime aim is to promote the development of hydrolytic enzymes that are not active in raw seeds. The main enzymes produced during germination are starch hydrolases (Yamasaki, 2003; Traoré *et al.*, 2004). Diastases are a group of starch-digesting enzymes including α - and β -amylase (Sak-Bosnar and Sakac, 2012). The amount of enzyme depends on various factors, such as type of cereal, environment, duration of germination and temperature. For example, the diastase activity of barley malt has been estimated to be about 190 CU/g. (Helland *et al.*, 2002; Mark *et al.*, 2008; Kádár *et al.*, 2011).

Plant phenolics, including, flavonoids and phenolic acid and tocopherols, are known to protect plants against tissue injuries, high levels of oxygen, free radicals and reactive oxygen species formed by the by-products of photosynthesis. These molecules also play an important role in the protection of food against lipid oxidation and in human health, by counteracting the risk of cardiovascular diseases, cancer and cataract, among other degenerative diseases of aging (Bondia-Pons *et al.*, 2009). The concentration of total phenol in barley malt varieties has been reported to range from 2.51 to 3.45 mg GAE g⁻¹ DW (Dabiņa-Bicka, 2011).

In conclusion, the concentration of selenium in rye malt increased with increasing concentration of selenium in water solution by 5.4 times (3 mg L⁻¹) to 22.4 times (10 mg L⁻¹). All analysed selenium concentrations increased the concentration of rye malt extract and the highest results were obtained using selenium concentration 5 mg L⁻¹. The activity of rye malt diastase is in inverse ratio to the concentration of selenium in solution. The concentration of total phenol increased with increasing concentration of selenium.

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SELĒNA IETEKME UZ RUDZU IESALA KVALITĀTI

Rudzi (*Secale cereale* L.) ir viena no svarīgākajām labības kultūrām pēc kviešiem, rīsiem un kukurūzas. Eiropas valstīs rudzus plaši izmanto maizes ražošanā. Pasaulē ir veikti vairāki pētījumi par graudu bagātināšanu ar selēnu (Se), bet ir maz pētījumu par selēna ietekmi uz rudzu iesala kvalitāti. Ir zināms, ka selēns ir mikroelements, kuram ir būtiska loma cilvēka veselībā, pie tam selēns ir daudzu selēnproteīnu vai selēnenzīmu svarīga sastāvdaļa. Pētījuma galvenais mērķis bija izpētīt selēna ietekmi uz ekstraktvielu daudzumu, diastāzes aktivitāti, selēna un fenolu saturu rudzu iesalā. Rudzu graudi ar 95% dzīvotspēju tika diedzēti trīs dienas $+6 \pm 2$ °C temperatūrā dažādu koncentrāciju Se šķīdumos (3 mg L^{-1} , 5 mg L^{-1} , 10 mg L^{-1}), un kaltēti 24 stundas $+70 - 112$ °C temperatūrā. Kontrole — graudi tika mērcēti dejonizētā ūdenī. Iegūtie rezultāti parādīja, ka, palielinoties selēna koncentrācijai šķīdumā, palielinās iesala ekstrakta saturs (no 74 līdz 80%), selēna (no 0.0139 līdz 0.3251 mg kg^{-1}) un fenolu (no 3.13 līdz 3.63 $\text{mg GAE g}^{-1} \text{ DW}$) daudzums rudzu iesalā, bet diastāzes aktivitāte samazinās no 330 līdz 216 vienībām.

CHARACTERISATION OF PHYSICAL AND BIOCHEMICAL TRAITS OF HULLESS SPRING BARLEY GRAIN IN THE LATVIAN BREEDING PROGRAMME

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Incorporation of whole grain, such as hulless barley, as a part of a balanced diet can help reduce the risk of coronary heart diseases, diabetes and specific cancers, and provide other health benefits as well. The objectives of this study were to determine the physical and chemical characteristics of grains of hulless barley genotypes from the Latvian spring barley programme. Field experiments were carried out in 2010–2011 at the State Stende Cereal Breeding Institute. Grain samples of 29 hulless spring barley breeding lines were analysed for grain physical traits (1000 kernel weight, test weight), macronutrients (crude protein, starch, β -glucans), and micronutrients (total phenolic content, DPPH antiradical scavenging activity and α -tocopherol content). The variation of physical traits and macronutrients was mainly determined by genotype ($\omega^2 = 32$ –62%) and year ($\eta^2 = 8$ –42%) and to a lesser extent by interaction of factors. The difference between hulless genotypes, as indicated the coefficient of variation, was rather high for all micronutrients ($V\% = 13.4$ –25.6). Significant ($P < 0.05$) positive correlation was detected between radical scavenging activity and total phenolic content ($r = 0.519$) and starch content ($r = 0.530$). The best hulless breeding lines '1185' and 'IC 360' (variety 'Kornelija' submitted for DUS and VCU tests) were selected for future usage in clinical investigations.

Key words: hulless barley, physical and chemical traits, total phenolic content, scavenging activity, α -tocopherol.

INTRODUCTION

Although barley (*Hordeum vulgare* L.) has a long history as food, it must compete with oats, rice, and wheat, which are already well-accepted food commodities in the market. At present, extensive food research has demonstrated the versatility and adaptability of barley for much more food products than pearled barley. The advent of new barley varieties, starch types, and hulless genotypes has presented to the cereal food industry an opportunity to develop new barley products, which are exciting and have many health benefits (Newman and Newman, 2008).

In covered barley, the flowering glumes are fused and adhere strongly to seed with a cementing substance. In hulless barley, fusion of the flowering glumes does not occur and the hull falls off during threshing (Bhatty, 1999). The seed coat of hulless barley is loosely attached and easily removed during harvesting, resulting in grain with a test weight and physical appearance similar to wheat (Evers and Millar, 2002).

Kernel size or plumpness can become important measures of quality in food barley. Plump grains contain significantly more starch, less protein, and less fibre than thin kernels. Barley kernels vary widely in size due to genotype, position on the spike, and environmental growing conditions. Typical hulless kernels usually weigh between 25 and 35 mg (Newman and Newman, 2008). Thousand kernel weight provides a measure of average kernel weight, and this criterion is widely used in barley breeding to describe grain plumpness.

Comparison of covered and hulless barley has shown that the later generally contains more protein, starch and β -glucan. This is due to removal of the fibrous hull, which has a dilution effect on these components (Bhatty, 1999). Two large-molecular-weight polysaccharide soluble fibres (arabinoxylan and β -glucan) are structural components of the thick cell walls of the aleurone. The cell walls of the starchy endosperm contain the same two polysaccharides found in the cell walls of the aleurone tissue, but the amount of β -glucan is greater than of arabinoxylan (Newman and

Newman, 2008). Barley kernels contain high levels of β -glucans, and much of the research on the health-promoting properties of barley has focused on mechanisms involving β -glucans (Pins and Kaur, 2006).

The interest in the role of natural antioxidant compounds in human health has increased recently. Barley grains in general contain different types of phytochemicals, which are sometimes referred to as phytonutrients, and in barley the most studied compounds are sterols, tocopherols, tocotrienols and phenolic compounds (Newman and Newman, 2008). Phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activity of cereal and are considered with great interest for the development of functional foods. The antioxidant activity of polyphenols has been reported to be mainly due to their redox properties, which can play an important role in neutralising free radical and quenching oxygen or decomposing peroxides (Kahkonen *et al.*, 1999).

Vitamin E is a complex of eight isomers, four tocopherols and four tocotrienols, collectively called tocopherols. The tocopherols are associated with lipid components in aleurone, endosperm, and embryo tissue (Newman, Newman, 2008). The tocopherols in barley germ are predominantly tocopherols (about 97%), whereas those in the endosperm are predominantly tocotrienols (80–90%). Therefore, the total amounts of tocotrienols are greater than those of tocopherols in barley oil prepared from ground barley kernels (Charalampopoulos *et al.*, 2002; Moreau *et al.*, 2007). Both genotype and environment affect total tocopherol content (Cavarello *et al.*, 2004). Specific forms of tocopherols and tocotrienols accumulate with different kinetics during barley grain development (Falk *et al.*, 2004).

The objective of this study was to describe the physical and chemical characteristics of grains of hulless barley genotypes from the Latvian spring barley programme.

MATERIALS AND METHODS

Grain samples of 29 hulless spring barley breeding lines of Latvian origin were studied, including 26 lines from breeding programmes of the State Stende Cereals Breeding Institute (Stende) and three lines from the State Priekule Plant Breeding Institute (Priekule). One line with waxy endosperm from Priekule was characterized.

Field experiments were carried out at the State Stende Cereal Breeding Institute for two seasons during 2010–2011. The soil was sod-podzolic sandy loam with humus content 21 (2010)–24 (2011) mg kg⁻¹, soil pH KCl 5.4 (2010) — 5.8 (2011), available phosphorus P₂O₅ 137.0 (2010) — 158.8 (2011) mg kg⁻¹, and potassium K₂O 211.0 (2010) — 175.7 (2011) mg kg⁻¹. The pre-crop in both years of investigation was a mixture of peas and spring wheat. The barley was sown with a compact drill 'Hege 80' in a well prepared seedbed at a rate of 500 germinating seeds per m². The yield was harvested by a combine 'Hege 140'.

The following grain physical traits were evaluated: 1000 kernel weight/TKW (g) by ISTA method, test weight/TW (g L⁻¹) by automatic grain analyser Infratec Analysis 1241. Macronutrients crude protein content/CP (g kg⁻¹), starch content/ST (g kg⁻¹), β -glucans content/BGL (g kg⁻¹) were determined by an automatic grain analyzer Infratec Analysis 1241.

For the determination of total phenolic content (TPC) and radical scavenging activity (RSA), 1.5 gram grain flour was mixed with 30 ml of 50% (vol.) ethanol in an Erlenmeyer flask (250 ml). The mixture was stirred at 40 °C for 15 minutes and then at room temperature for an hour. After extraction the supernatant was filtered through filter paper. The filtrate was used for the analysis of TPC and RSA.

TPC was determined according to colorimetric method described in literature (Ragaee *et al.*, 2006) with some modifications. This method is based on the reduction of the Folin-Ciocalteu reagent by phenol to a mixture of the reaction products, having absorbance maximum at 765 nm. 1 ml of extract was mixed with 5 ml of 10% Folin-Ciocalteu's reagent in distilled water and 4 ml 7.5 % Na₂CO₃ solution, stirred for 30 min at room temperature and the absorbance at 765 nm was measured with a spectrophotometer UVIKON 930 (Kontron Instruments, Italy). TPC was calculated using gallic acid as a standard (0.0075–0.060 mg/mL) ($R^2 = 0.9999$) and expressed as mg gallic acid equivalents per 100 g of grain dry weight (DW).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) assay was performed according to the modified method prescribed in literature (Ragaee *et al.*, 2006). 0.4 ml of extract (sample) or 50% (vol.) ethanol (control sample) was added to 3.6 ml of 10⁻⁴ M DPPH solution in ethanol and mixed. The mixture was vigorously shaken and left to stand for 20 min. The absorbance at 517 nm was measured against 50% (vol.) ethanol as a blank. The DPPH radical scavenging activity (%) was calculated using equation (1):

$$RSA(\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \cdot 100\% \quad \text{where} \quad (1)$$

$A_{control}$ is the absorbance of the control sample; A_{sample} is the absorbance of the sample.

Mean samples from all replications were taken and milled for α -tocopherol (α -T) determination in the Laboratory of Food and Environmental Investigations Institute of Food Safety, Animal Health and Environment "BIOR" using the high-performance liquid-chromatography method. The method is a standard for detection of α -tocopherol in diet samples. Absorption was measured at 292 nm. Chromatography was carried out on a C18 column using methanol/water (98/2 v/v).

The growing seasons of 2010 and 2011 were characterised by temperature above to the long-term average and some extreme periods of drought. In 2010, low precipitation and

high temperature occurred in the third decade of June and the first decade of July. In 2011, hot and dry conditions occurred during the third decade of May and in first decade of June.

ANOVA were used for statistical data analysis. The obtained results were statistically processed using methods of descriptive statistics. In the analysis of variance omega squared (ω^2) was calculated to measure the effect size or relative contributions of factors (genotype, year, genotype \times year) using equation (2) (Grissom and Kim, 2012.):

$$\omega^2 = [SS_{\text{Effect}} - (df_{\text{Effect}})(MS_{\text{Residual}})] / (SS_{\text{Total}} + MS_{\text{Residual}}). \quad (2)$$

The significance of the effect of factors was determined at probability level of 0.05. The value of genotypic variability for traits was determined and expressed by standard deviation (s) and coefficient of variations (V%) values. Pearson correlation coefficients between two year phenotypic means were calculated. The best lines according to food grain physical and chemical traits were selected using selection criterion — trait values \geq mean + s.

RESULTS

Physical traits of grain. ANOVA showed that physical traits of grain for hulless barley lines in the tested breeding material were significantly ($P \leq 0.05$) influenced by two main factors (genotype, year) and their interaction (Fig. 1).

The variation of thousand kernel weight (TKW) was mainly determined by genotype ($\omega^2 = 52\%$) and year ($\omega^2_{\text{hulled}} = 31\%$). TKW for hulless barley breeding lines was 40.7 and 43.8 g in 2010 and 2011, respectively. Significantly higher ($P \leq 0.05$) values of this trait were observed in 2011, but a greater difference between minimum and maximum value of this traits was found in 2010, as indicated also coefficient of variation (Table 1). In both years, some hulless breeding lines had significantly higher TKW than those of the hulless standard variety ‘Irbe’ (+3.9 and 6.9 g compared with hulless standard variety ‘Irbe’; $Rs_{0.05} = 1.39\text{--}1.81$ g).

The main effect of variation of TW was due to year and genotype (42% and 32%, respectively) and to a lesser extent

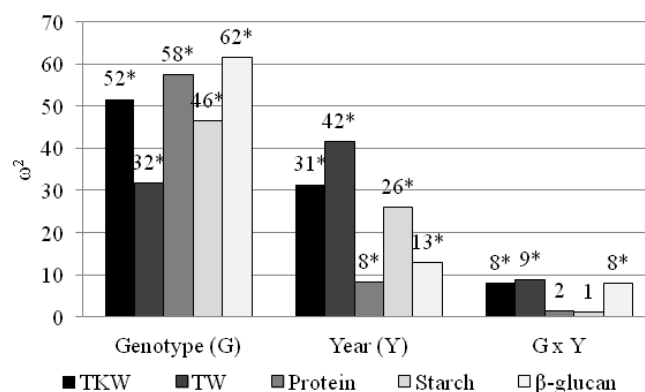


Fig. 1. Relative contribution of factors for hulless barley grain quality traits (* $P \leq 0.05$) (TKW, thousand kernel weight, TW, test weight).

by interaction of factors for both physical traits (Fig. 1). There was a significant ($P \leq 0.05$) difference between years of investigation in mean value of TW for hulless breeding lines, and a higher test weight was observed in 2010 (Table 1). Grains of hulless genotypes were characterised by higher TW than that of hulled barley standard variety ‘Ansis’. The best hulless breeding lines, according to TW value, were on the level of that of standard ‘Irbe’.

Macronutrient content. ANOVA indicated that the variation of all macronutrients (crude protein, starch and β -glucan) content was mainly determined by genotype ($\omega^2 = 46\text{--}62\%$) and to a lesser extent by year and by interaction of factors (Table 1).

The difference between minimum and maximum value of crude protein was rather high and varied from 133.0 to 188.2 g kg^{-1} in 2010 and from 145.1 to 183.2 g kg^{-1} in 2011. A significantly higher crude protein for grain of hulless barley lines was observed in 2011. In both years, there were some hulless breeding lines with significantly higher crude protein content (+30.4–47.9 g kg^{-1} , compared to the hulless standard variety ‘Irbe’; $Rs_{0.05} = 14.25\text{--}17.32$ g kg^{-1}).

There was a significant ($P \leq 0.05$) difference between years of investigation in mean value of starch content for grains

Table 1

CHARACTERISTICS OF GRAIN PHYSICAL TRAITS AND MACRONUTRIENT CONTENT FOR HULLESS SPRING BARLEY LINES AND HULLESS (HB) AND COVERED (C) STANDARDS (2010–2011)

Trait ¹	2010					2011				
	Breeding lines			Standards, mean	$Rs_{0.05}$	Breeding lines			Standards, mean	$Rs_{0.05}$
	mean	min/ max	V%			mean	min/ max	V%		
TKW, g	40.7b ³	33.9/ 44.7	5.8	40.8/ 46.1	1.39	43.8a	38.7/ 48.8	4.9	41.9/ 48.1	1.81
TW, g L^{-1}	783.8a	724.8/808.5	2.7	807.8/682.8	17.88	749.3b	711.0/777.5	2.0	764.5/ 648.5	14.25
CP, g kg^{-1}	153.0b	133.0/ 188.2	6.4	140.3/116.5	17.32	162.0a	145.1/ 183.2	5.8	152.8/ 140.7	14.74
ST, g kg^{-1}	619.0a	602.1/ 632.0	1.2	630.5/612.8	11.89	606.8b	596.3/618.0	1.1	615.8/ 602.0	13.70
BGL, g kg^{-1}	46.0b	40.4/ 57.2	9.4	44.8/ 38.8	3.75	50.0a	41.0/ 63.0	11.7	47.3/ 43.3	3.34

¹ TKW, thousand kernel weight; TW, test weight; CP, crude protein; ST, starch; BGL, β -glucans

² HB, ‘Irbe’; C, ‘Ansis’

³ Trait means followed by different letters are significantly different between the years at the level of $P \leq 0.05$

of hulless breeding lines and a higher test weight was observed in 2010. The best hulless breeding lines according to starch content did not exceed that of the standard 'Irbe' level.

As indicated by the coefficient of variation ($V\% = 9.4 - 11.7$), the difference between minimum and maximum value of β -glucan for grains of hulless breeding lines was rather high and varied from 40.4 to 57.2 g kg⁻¹ in 2010 and from 41.0 to 63.0 g kg⁻¹ in 2011. Significantly higher ($P \leq 0.05$) mean value of this trait and higher phenotypic variation within breeding lines were observed in 2011. Hulless barley genotypes showed substantially higher β -glucan content compared to that of the hulled standard variety 'Ansis'. According to results in both years, there were hulless breeding lines with significantly higher β -glucan content than that of hulless standard variety 'Irbe' (+12.4 and 15.7 g kg⁻¹ compared to hulless standard variety 'Irbe'; $R_{s0.05} = 3.34 - 3.75$ g kg⁻¹) (Table 2).

Micronutrient content. DPPH radical scavenging activity in hulless spring barley breeding material had rather high variation in antioxidant activity, with a minimum value of 44.6% and maximum value of 80.1% (Table 2).

Table 2

VARIATION OF MICRONUTRIENT CONTENT OF HULLESS SPRING BARLEY LINES, 2010¹

Indices	Radical scavenging activity, %	Total phenolic content, mg GAE 100 g ⁻¹ DW	α -tocopherol, mg kg ⁻¹
Mean value	64.8	202.6	5.5
Min	44.6	143.6	3.3
Max	80.1	262.1	8.7
s	9.0	27.1	1.4
V%	13.9	13.4	25.6
'Irbe' (HB)	64.3	177.9	7.9
'Ansis' (C)	49.7	187.5	6.5

The hulled variety 'Ansis' had lower radical scavenging activity (49.7%) than that of the hulless variety 'Irbe' (64.3%). Total phenolic compound amount expressed as gallic acid equivalent (GAE) in hulless barley breeding material had high variation: from 143.6 to 262.1 GAE mg 100 g⁻¹ with coefficient of variation 13.4%. There was no notable difference between hulled and hulless standard varieties in content of total phenolic compounds in grain. The coefficient of variation of α -tocopherol among hulless genotypes was high and varied from 3.30 to 7.78 mg kg⁻¹. The genotypic variation in α -tocopherol content in grain of hulless barley breeding lines was also high ($V\% = 25.6$). The content of α -tocopherol varied from 3.3 to 8.7 mg kg⁻¹. Hulless standard variety 'Irbe' had a higher α -tocopherol content in grains than the covered variety 'Ansis' (Table 2).

There were significant correlations between physical and chemical composition variables for hulless barley genotypes. In both years of investigation significant negative correlation was found between starch content and 1000

grain weight ($r_{10} = -0.480$; $r_{11} = -0.483$) and crude protein ($r_{10} = 0.720$; $r_{11} = -0.868$). Positive correlation was found between crude protein and β -glucan, and in 2010 this relationship was statistically significant (Table 3).

Table 3

PHENOTYPIC CORRELATION BETWEEN PHYSICAL AND BIO-CHEMICAL TRAITS OF HULLESS BARLEY¹

Traits ²	TKW	TW	CP	ST	BGL	TPC	RSA	α -T
TKW	1	0.057	0.319	-0.483*	-0.128	x	x	x
TW	0.177	1	-0.431*	0.475	0.148	x	x	x
CP	0.357	-0.138	1	-0.868*	0.375	x	x	x
ST	-0.480* ³	0.346	-0.720*	1	-0.209	x	x	x
BGL	-0.218	0.038	0.404*	-0.004	1	x	x	x
TPC	-0.307	0.082	-0.195	0.255	0.201	1	x	x
RSA	-0.476*	-0.148	-0.347	0.530*	0.327	0.519*	1	x
α -T	-0.002	-0.149	0.081	-0.176	-0.171	-0.471*	0.356	1

¹ Data of 2010 below diagonal, data of 2011 above diagonal

² TKW, thousand kernel weight; TW, test weight; CP, crude protein; ST, starch; BGL, β -glucans; TPC, total phenolic content; RSA, radical scavenging activity; α -T, α -tocopherol

³ Significant at 0.05 probability level ($r_{0.05;n-2} = 0.381$)

Significant ($P < 0.05$) positive correlation was detected between total DPPH radical scavenging activity and starch content ($r = 0.530$). There was a positive significant relationship between the content of total phenolic content in barley grain dry matter and radical scavenging activity ($r = 0.519$; $r_{0.05} = 0.381$).

A summary of genotypes with food grain physical and chemical trait values that are above the mean levels are shown in Table 4.

Several hulless barley lines provided good results according to several grain quality traits simultaneously (indicated in bold). Hulless line 'IC 360' characterized with heightened 1000 kernel weight (46.2 g), crude protein (177.3 g kg⁻¹) and β -glucan content (53.6 g kg⁻¹) as well as total phenolic content (221.6 mg GAE 100 g).

Table 4

EVALUATION RESULTS OF HULLESS BARLEY GENOTYPES USING SELECTION CRITERIA, mean+s (2011–2012)

Traits	mean+s	Lines with trait values \geq mean+s
TKW, g	44.4	IC 360 ; IC 361; 1194; 1236
TW, g L ⁻¹	787.2	1175; 1194; 1213; PR 4651
CP, g kg ⁻¹	168.2	IC 360 ; IC 361; 1216; 1226; 1244; 1217
ST, g kg ⁻¹	623.7	IC 363; 1185 ; 1214; PR 4651; 'Irbe'
BGL, g kg ⁻¹	52.4	IC 360 ; 1196; 1226; PR 4651; PR 3440(w)*
TPC, mg GAE 100 g ⁻¹	229.8	IC 363; 1165; 1196; PR 3440(w) (IC360/221.6 ; 1185/222.3)
RSA, %	73.8	1167; 1185 ; 1189; PR 3440(w)
α -T, mg kg ⁻¹	6.9	IC 364; 1166; 1185 ; 1216; 1226; 'Irbe'

* (w) – waxy endosperm

Hulless barley line '1185' was selected due to high starch content (624.5 g kg^{-1}) micronutrient levels — total phenolic content ($222.3 \text{ mg GAE } 100 \text{ g}^{-1}$), radical scavenging activity (80.1%) and α -tocopherol content (6.9 mg kg^{-1}). Hulless line 'PR 3440' with waxy endosperm showed good results of food grain quality according to grain β -glucan (59.9 g kg^{-1}) content in grains, total phenolic content ($234.7 \text{ mg GAE } 100 \text{ g}^{-1}$) and radical scavenging activity (76.9%).

DISCUSSION

Genetic variability in the material of a breeding programme is an important resource for creation of new variety ideotypes with desired traits for increased crop production and quality.

The absence of a hull results in a 10–13% average dry weight loss, and therefore hulless genotypes are commonly known to have a lower 1000 kernel weight, higher test weight, starch content and beta-glucan content in grains than covered ones (Bhatty, 1999). The data presented in this paper demonstrate notable variation among the spring barley breeding lines in all investigated grain physical traits as well as in macronutrient and micronutrient content in the grain dry matter.

In this study, significantly higher TKW, crude protein and β -glucan was observed in 2011 than in 2010. This can be explained by differences between these two years in periods of high temperature and low precipitation. Grain weight can be influenced by grain set, which should reflect conditions around anthesis and can be also influenced by earlier conditions as well. A reduction in TKW may be not only due to abiotic and biotic stress during grain filling, but a lower grain weight could indicate also the plasticity of yield components (Fisher, 2007). In 2010, during the first part of grain filling, the weather conditions were hot and dry, which could have influenced the final grain weight. In 2011, the critical period for plant development during the generative phase occurred at the end of May and beginning of June, when set of grain number in spikes were forming. Later, this development was a prerequisite for higher grain weight due to compensation during the grain filling period when moisture conditions became optimal. Barley protein usually varies inversely with the starch content, and exhibits the greatest fluctuation among the main nutrients. Accumulation of starch is more sensitive to high temperature than is accumulation of nitrogen, which frequently determines the grain nitrogen proportion and thus protein content (Schelling *et al.*, 2003). This effect of temperature was observed in 2011. Also, β -glucan content increases gradually during grain development, depending on variety and growing season (Aman *et al.*, 1989).

Several types of polyphenol extraction have been described, as well as various techniques have been used for the determination of polyphenolic compounds. For barley, total polyphenol contents measured by spectrophotometric method

and expressed in gallic acid equivalent/GAE have been published in the literature (Singleton *et al.*, 1999; Zhao *et al.*, 2008; Dabina-Bicka *et al.*, 2011). The present study indicated that hulless barley can be a good source of phenolic compounds as TPC content ranged from 143.6 to $262.1 \text{ mg GAE } 100 \text{ g}^{-1}$. The obtained values are similar to those reported by H. Zhao *et al.* (2008) but higher than those reported by Singleton *et al.* (1996). Also, similar values for TPC content of hulless barley genotypes of Latvian origin were obtained by I. Dabina-Bicka *et al.* (2011) and TPC content did not significantly differ between covered and hulless barley.

The results showed a positive significant relationship between total phenolic content and radical scavenging activity. Total phenolic content showed strong correlation with DPPH radical scavenging activity in spring barley (Zhao, 2008). This confirms that metabolites like phenolic compounds possess antioxidant activity and can play a preventive role in the development of cancer, heart and age related diseases. They have also been reported to be chemopreventive agents by lowering cholesterol levels and repairing damaged cells (Kahkonen *et al.*, 1999).

Significant ($P < 0.05$) positive correlation was observed between DPPH radical scavenging activity and grain starch content. Whole and unpolished grain of hulless barley consist mainly of starchy endosperm, and hence the ratio of amylose and amylopectin content can effect not only the quality of obtained baking products but also the antioxidant activity. For example, it has been shown that high-amylose corn has better antioxidant activity than typical corn genotypes (Li *et al.*, 2007). In our study, the only genotype 'PR 3440' with waxy or high-amylopectin endosperm also showed higher radical scavenging activity and total phenolic content. In the future, wider investigation of genotypes with different amylose and amylopectin ratios is needed to find donors with high content of natural antioxidant compounds.

Among cereals, barley can be a good source of tocopherols and tocotrienols, which are important phytochemical compounds with antioxidant activity and potential benefits for human health (Colombo, 2010). The hulless barley genotypes included in this study showed rather high variability in α -tocopherol content ($3.3\text{--}8.7 \text{ mg kg}^{-1}$). These results were similar to those reported by Ehrenbergerova *et al.* (2006) study in the Czech Republic, in which the average α -tocopherols content was 6.7 mg kg^{-1} . In Italy, for hulless varieties the α -tocopherol content was higher ($8.4\text{ to }10.1 \text{ g kg}^{-1}$) and for covered varieties — $8.4\text{--}8.6 \text{ g kg}^{-1}$ (Cavarello *et al.*, 2004). Genotype and growing conditions are known to significantly affect the content of α -tocopherol (Zilienski *et al.*, 2001; Ehrenbergerova *et al.*, 2006).

The major aim of this study was determination of hulless barley genotypes with high nutritional value. Genotypic potential exists within the hulless spring barley material included in this study. This genetic variation can be used for the development of the improved barley breeding material

specifically designed for food with high nutritional value. The study concludes that hulless barley has antioxidant activity, which is related to the total phenolic content of the grain dry matter samples. Hence, cereal species can be a potential source of natural antioxidant, which could be useful in physiological and pathological medicine, and of interest to food manufacturing industries. The best hulless breeding lines 'IC 360' and '1185' were selected for future use in clinical investigations. Genotype 'IC 360', by the name 'Kornelija', in 2012 was submitted for DUS and VCU tests.

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FIZIKĀLO UN BIOĶĪMISKO PAZĪMJU RAKSTUROJUMS KAILGRAUDU VASARAS MIEŽU GRAUDIEM LATVIJAS SELEKCIJAS PROGRAMMĀ

Pilngraudu izejvielu, kā piemēram, kailgraudu miežu izmantošana veselīgas diētas sabalansēšanai var veicināt profilaksi sirds un asinsvadu saslimšanu, diabēta un specifisku vēža formu ierobežošanai. Pētījuma mērķis bija novērtēt kailgraudu paraugus no Latvijas miežu selekcijas materiāla pēc graudu fizikālajām un bioķīmiskajām pazīmēm. Lauka izmēģinājumi iekārtoti Valsts Stendes Graudaugu selekcijas institūtā no 2010. gada līdz 2011. gadam. Pētījumā iekļautajām 29 vasaras miežu kailgraudu līnijām analizēta 1000 graudu masa, tilpummasa, kopproteīna, cietes, β -glikānu, kopējais fenolu saturs, α -tokoferolu saturs un antiradikālā aktivitāte. Graudu fizikālo un bioķīmisko pazīmju mainību galvenokārt ietekmēja genotips ($\omega^2 = 32\text{--}62\%$) un gads ($\omega^2 = 8\text{--}42\%$), bet mazākā mērā – šo faktoru mijiedarbība. Kailgraudu miežu materiālam konstatēta augsta genotipiskās mainība pēc kopējā fenolu satura, antiradikālās aktivitātes un α -tokoferolu satura graudos ($V\% = 13.4\text{--}25.6$). Būtiska ($P < 0.05$) pozitīva korelācija noteikta starp antiradikālo aktivitāti un kopējā fenolu ($r = 0.519$) un cietes saturu graudos ($r = 0.530$). Labākos rezultātus pēc analizētajām pazīmēm nodrošināja kailgraudu miežu selekcijas līnijas '1185' un 'IC 360' (iesniegta AVS un SĪN pārbaudei ar šķirnes nosaukumu 'Kornelija'), kas iekļautas klīniskajos pētījumos.

INVESTIGATION OF THE OIL AND MEAL OF JAPANESE QUINCE (*Chaenomeles japonica*) SEEDS

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Various extracts of Japanese quince (Chaenomeles japonica) seeds obtained using organic solvents were studied for their polyphenol content and antiradical activity. It was established that petroleum ether, hexane, ethyl acetate, acetone, as well as toluene and chloroform extracts, in comparison to synthetic antioxidant butylated hydroxytoluene (BHT), demonstrate better (or comparable) activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methods for detoxification of seeds, meals and press-cakes are proposed. Phenolic composition of different extracts (80% ethanol, 70% acetone), both acid and alkali hydrolysates of seeds, as well as seed oil methanol/water extract were analysed by means of high performance liquid chromatography (HPLC): chlorogenic acid was found for the first time in seed extract; protocatechuic acid predominated in all extracts. The content of other major phenolic acids was detected; it was found that seed oil contains syringic acid. It was determined that Japanese quince seeds contain almost ten times more α -tocopherol than barley grain. Due to the presence of α -tocopherol and phenolic compounds, seed oil and lipophilic extracts of seeds could serve as antioxidants.

Key words: Japanese quince seeds, polyphenols, amygdalin, antioxidant, oxidative stability.

INTRODUCTION

Japanese quince (*Chaenomeles japonica*) is cultivated mainly in Baltic and Scandinavian countries. The fruits of Japanese quince are mostly used to produce juice, jelly, candied fruits, purée, aroma-extracts, syrup, carbonated soft drinks, liqueur, wine; and fruit sugar extract has proven to be excellent as a flavouring in ice cream (Seglina, 2001; Hellin *et al.*, 2003; Jordan *et al.*, 2003).

From the economical viewpoint seeds and seed oil are interesting objects for investigation, as fruits have up to 10 wt% seeds, which contain up to 6% (Granados *et al.*, 2003) to even 20% (Mierina *et al.*, 2011) of oil. The iodine value of seed oil is 94–105 g I₂/100 g, acid number — 2.2–2.5 mg KOH/g (Granados *et al.*, 2003). The two main fatty acids are linoleic (45% (Ruisa, 1996), 50% (Granados *et al.*, 2003), 56% (Дейнека и др., 2005), 58% (Gora and Kurowska, 1979)) and oleic (~27% (Gora and Kurowska, 1979), 34% (Дейнека и др., 2005), 38% (Granados *et al.*, 2003; Ruisa, 1996)) acids, which together form about 90% of the fatty acid composition (Gora and Kurowska, 1979; Ruisa, 1996). The main saturated fatty acid of Japanese quince seed oil is palmitic acid — 8% (Дейнека и др., 2005) to 10% (Gora and Kurowska, 1979). The main triglycerides are trilinolenate and linolenate-linolate-oleate (together ~17%), dilinoleate-oleate and linolenate-dioleate (both together almost 30%), linolate-dioleate (19%),

dilinolate-stearate, linolate-oleate-palmitate and linolenate-oleate-stearate (all three together constitute about 10%), dilinolate-palmitate, linolenate-linolate-stearate and linolenate-oleate-palmitate (the last three form about 10%) (Deineka and Deineka, 2004). The total concentration of phospholipids (about 0.5%) and phytin (~1.4%) in seeds has also been previously determined (Mukhamedova *et al.*, 1979); the total concentration of phosphorous is about 4% (Gora and Kurowska, 1979). Seeds contain phytosterols (0.015%) and α -tocopherol (0.1%) (Gora and Kurowska, 1979). Till now only one publication (Sokolowska-Wozniak *et al.*, 2002) has been devoted to analysis of free and bound phenolic acids in Japanese quince seeds: composition of 80% methanol extracts was studied.

Previously we found (Mierina *et al.*, 2011) that hydrophilic extracts of Japanese quince seeds, due to the presence of phenolic compounds, can be used to improve stability of vegetable oils; we showed by high performance liquid chromatography (HPLC) that Japanese quince seeds contain amygdalin and observed that extracted seed oil or mechanically pressed oil lacks this compound and its degradation products. The aim of this study was to determine the phenolic concentration and profile as well as tocopherol concentration of seed oil and different seed extracts and to evaluate the antioxidative activity of Japanese quince seeds, seed meal and oil.

MATERIALS AND METHODS

Japanese quince seeds. Fruits of Japanese quince were cut and seeds and pulp were separated. In order to remove damaged seeds, they were washed with water. Then seeds were air-dried at 40 ± 2 °C with forced air circulation (oven Orakas, Finland). The water content of the seeds was 5.74%. The seeds were packed under vacuum in 2 kg portions in bags made of polypropylene; they were stored at 18 ± 2 °C in dark until further experiments.

Japanese quince seed oil. Japanese quince seed oil was obtained by two methods:

- 1) seeds (finely ground in a coffee grinder and sieved by particle size $d < 0.069$ mm) were extracted with organic solvent in Soxhlet apparatus;
- 2) ground seeds were extracted with organic solvent by mixing (at boiling or room temperature).

Determination of total concentration of cyanogenic compounds. Total concentration of cyanogenic compounds was determined by argentometry (Fend *et al.*, 2003). Total concentration of cyanogenic compounds was expressed as mg of hydrogen cyanide (HCN) per 1 kg of sample.

Analysis of polyphenol extracts of Japanese quince seeds. Total polyphenol content (TPC) was determined according to a modified method (Singleton *et al.*, 1999) using Folin-Denis reagent. Absorption was measured with a spectrophotometer (Camspec M501, Single Beam Scanning UV/Visible Spectrometer) at 765 nm. TPC was expressed as gallic acid equivalents (GAE) per 100 g of sample.

Preparation of polyphenol extracts. Seeds were defatted before extraction: 30 g of ground Japanese quince seeds were mixed with 100 ml hexane at room temperature 15 min, then the mixture was filtered. Extraction of seed meal was repeated twice. Hexane filtrates were combined, distilled in vacuum and used for preparation of extract E.

Extracts A1 and A2: 2.5 g defatted seed meal was mixed with 50 ml 80% ethanol for 1 h at room temperature (extract A1) or heated at boiling temperature (extract A2). The mixture was filtered and organic solvent removed in vacuum. The residual water solution was acidified with 6 M HCl up to pH~2 and after that extracted with 2×25 ml ethyl acetate. The ethyl acetate layer was dried with anhydrous Na_2SO_4 , filtered and solvent removed in vacuum. The extract was stored at 4 °C until further analysis.

Extracts B1 and B2 were prepared analogically as A1 and A2, but 70% acetone was used for extraction instead of 80% ethanol.

Extract C: 10 ml 6 M HCl were added to 2.5 g defatted seed meal and the mixture was heated for 1 h at 75–80 °C and filtered. 6 M KOH solution was added to filtrate to reach pH~2, followed by extraction with ethyl acetate, as in the case of extracts A1 and A2.

Extract D: 30 ml 2 M KOH were added to 2.5 g defatted seed meal and mixed for one hour at room temperature in dark using an orbital shaker. The solution was filtered, filtrate acidified with 6 M HCl up to pH~2, followed by extraction with ethyl acetate, as in the case of extracts A1 and A2.

Extract E: 2 g of seed oil, obtained in the defatting process (extraction of seeds with hexane), were dissolved in 1 ml of hexane. 2 ml of methanol:water (6:4, v:v) were added to this solution and centrifuged to separate the methanol/water layer; the process was repeated twice. Methanol/water layers were combined and three times washed with 5 ml of hexane; methanol and water were removed in vacuum.

HPLC analysis. The chromatographic analysis was performed on an Agilent Technologies HPLC system (model 1200) equipped with an UV/Vis detector. A reverse phase Gemini NX C18 column (3 μm , 4.6×100 mm) was used to separate the compounds. The mobile phase consisted of 1% acetic acid solution (solvent A) and methanol (solvent B). The gradient profile was 90% A at 0 min, 30% A at 30 min, 0% A at 35 min and 90% A at 40 min. Flow rate was 0.9 ml/min. Chromatograms were recorded at 280 nm. Polyphenol extracts A-E were dissolved in methanol (10 mg/ml). Injection volume was 5 μl . (+)-Catechin hydrate, gallic, chlorogenic, caffeic, ellagic, protocatechuic, vanillic, syringic and *p*-coumaric acid, quercetin hydrate, phlorizin hydrate, phloretin were used as analytical standards.

Tocopherol analysis in Japanese quince seed oil. The oil was obtained by extraction of ground seeds with hexane at boiling temperature.

A normal phase Zorbax RX-SIL column (5 μm , 4.4×250 mm) was used to separate tocopherols. The mobile phase consisted of 0.8% solution of isopropanol in hexane (v/v). Analysis was carried out in isocratic mode. Flow rate was 1 ml/min, injection volume 10 μl . Chromatograms were recorded at 292 nm. Samples were prepared by dissolving 17–22 mg oil in 1 ml hexane. Tocopherols were detected using α - and δ -tocopherols as standard compounds.

Detection of antioxidant activity. Radical scavenging activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test (Mierina and Jure, 2010). Absorption of solutions was measured with a Camspec M501, Single Beam Scanning UV/Visible spectrometer at 517 nm.

Statistical analysis. Statistical analysis of data was carried out using the Microsoft Excel software package. The total concentration of phenolic compounds and tocopherols was calculated from calibration curves ($R^2 = 0.9983$ (gallic acid), $R^2 = 0.9993$ (chlorogenic acid), $R^2 = 0.9994$ (caffeic acid), $R^2 = 0.9997$ (protocatechuic and sinapic acid), $R^2 = 0.9998$ (syringic acid and α -tocopherol) and $R^2 = 0.9999$ (vanillic and *p*-coumaric acid)); each calibration curve consisted of five points and was linear in the range of obtained results. Standard deviation was calculated by linear least

squares regression. Total amount of cyanogenic compounds and TPC was measured at least twice; the average values and standard deviations are provided.

RESULTS

Detoxification of Japanese quince seeds, meal and press-cake. Detoxification of both intact and ground seeds (which contained ~260 mg HCN/kg) was carried out by heating at 105–110 °C or 160–180 °C, as well as treatment with water steam. While heating at 105–110 °C was unsuccessful, heating at 160–180 °C completely removed cyanides after 3 h in the case of intact seeds and within 2 h in the case of ground seeds. Treatment with steam (which can promote glycosidase enzymatic reaction and result in a greater removal of the HCN) appeared applicable only for ground seeds — within 3 h seeds could be detoxified.

Japanese quince seeds can be detoxified very simply by pre-treatment of ground seeds with water, ethanol or water-ethanol (1:1 v/v) solution: 15 min mixing with hydrophilic solvent at room temperature is sufficient, if it is followed by two times washing of seed material with the corresponding solvent.

Seed meal obtained after oil extraction (carried out by 2 h heating at boiling temperature) with different solvents (petroleum ether, toluene, hexane, chloroform, ethyl acetate, acetone, ethyl acetate:hexane (1:1 v/v)) contained about the same amount of cyanogenic compounds (217–265 mg HCN/kg) as tested seeds (254 mg HCN/kg). Seed meal was detoxified easily by mixing with distilled water (1:10 w/v) at room temperature for 15 min; after that the mixture was centrifuged and washed again with water (1:5 w/v) — triple washing was necessary to complete detoxification.

Press-cakes of blends of Japanese quince seeds and oil plant seeds also contained cyanogenic compounds. Oil plant seeds contained cyanides (see Table 1); the cyanide concentration in Japanese quince seeds used in these experiments was 256.6 mg HCN/kg. Press-cakes were successfully detoxified by treatment (mixing) with water for 30 min (in the case of rapeseed/Japanese quince seed and linseed/Japanese quince seed) or for 15 min (in the case of hempseed/Japanese quince seed) at room temperature and following two times washing with water.

Total polyphenol content and identification of phenolic compounds in Japanese quince seeds, oil and meal. We obtained Japanese quince seed oil by extraction of seeds with organic solvents of different polarity — petroleum ether, toluene, hexane, chloroform, ethyl acetate, mixture ethyl acetate:hexane (1:1), and acetone. Polyphenols were extracted from oil by 80% ethanol. TPC was higher in oil samples obtained with more polar solvents, e.g., chloroform or ethyl acetate — 286 and 352 mg GAE/100 g oil, correspondingly (see Table 2). In contrast, the highest yield of oil was obtained in the case of lipophilic solvents (hexane and toluene) 12.5 and 12.2%, correspondingly. Total polyphenol

Table 1

CYANIDE CONCENTRATION IN OIL SEEDS AND PRESS-CAKES OF SEED BLENDS AND TOTAL POLYPHENOL CONTENT (TPC) IN OIL EXTRACTS OF JAPANESE QUINCE SEEDS

Analysed material	Content of cyanides, mg HCN/kg	TPC, mg GAE/100 g oil
Oil seeds		
Rapeseeds	48.6* ± 1.6 ^a	NM
Hempseeds	64.8 ± 3.8 ^a	NM
Linseeds	126.9 ± 12.6 ^a	NM
Blends of oil seeds and Japanese quince seeds		
R + 0% Q	51.3 ± 1.6 ^a	3.7 ± 0.5 ^a
R + 3% Q	54.0 ± 6.6 ^b	5.3 ± 0.5 ^a
R + 4% Q	62.7 ± 12.3 ^b	6.0 ± 1.8 ^c
R + 5% Q	78.3 ± 3.4 ^a	6.0 ± 2.5 ^c
R + 10% Q	91.8 ± 0.3 ^a	6.7 ± 0.6 ^a
H + 0% Q	62.1 ± 3.8 ^a	26.3 ± 4.5 ^b
H + 3% Q	59.4 ± 2.7 ^a	26.7 ± 0.3 ^a
H + 4% Q	62.1 ± 3.8 ^a	27.4 ± 4.0 ^b
H + 5% Q	64.8 ± 3.8 ^a	29.5 ± 0.8 ^a
H + 10% Q	67.5 ± 11.5 ^b	35.3 ± 7.2 ^b
L + 0% Q	121.5 ± 13.4 ^b	16.0 ± 6.7 ^c
L + 3% Q	124.2 ± 4.8 ^a	16.7 ± 0.7 ^a
L + 4% Q	156.7 ± 11.3 ^a	16.9 ± 1.9 ^a
L + 5% Q	164.7 ± 16.3 ^a	17.2 ± 0.1 ^a
L + 10% Q	178.2 ± 2.3 ^a	18.6 ± 3.6 ^a

* Each value is the mean of two measurements, ± standard deviation (SD). Values marked with the same letter are within the confidence interval (^a*P* < 0.05, ^b*P* < 0.1, ^c*P* < 0.2); Q, Japanese quince seeds; R, rapeseeds; H, hempseeds; L, linseeds; NM, the parameter was not measured.

Table 2

TOTAL POLYPHENOL CONTENT (TPC) AND FREE RADICAL SCAVENGING ACTIVITY (IC₅₀)* OF OIL OF JAPANESE QUINCE SEED OIL AND MEAL

Solvent used for oil extraction	Oil yield, %	TPC, mg GAE/100 g oil	IC ₅₀ , mg/ml	TPC, mg GAE/100 g meal	
				96.5% ethanol**	70% acetone**
Petroleum ether	11.6*** ± 2.0 ^b	171 ± 3 ^a	0.28	193 ± 10 ^a	NM
Toluene	12.2 ± 0.4 ^a	275 ± 11 ^a	0.30	205 ± 7 ^a	2 ± 0 ^a
Hexane	12.5 ± 2.8 ^b	186 ± 1 ^a	0.33	208 ± 3 ^a	44 ± 6 ^b
Chloroform	11.0 ± 1.4 ^b	286 ± 10 ^a	0.26	232 ± 11 ^a	104 ± 6 ^a
Ethyl acetate: hexane (1:1)	9.2 ± 1.2 ^b	284 ± 0 ^a	0.10	NM	NM
Ethyl acetate	7.6 ± 0.7 ^a	352 ± 4 ^a	0.09	NM	NM
Acetone	8.0 ± 1.4 ^a	199 ± 17 ^a	0.40	NM	NM

* IC₅₀ was detected for ethanol extract of seed oil (obtained by corresponding solvent); ** solvent used for extraction of the polyphenols from the seed meal left after extraction of the oil; *** each value is the mean of 2 measurements, ± SD. Values marked with the same letter are within the confidence interval (^a*P* < 0.05, ^b*P* < 0.1); NM, the parameter was not measured.

Table 3

PHENOLIC COMPOUNDS (mg/kg DEFATTED SEEDS) IN EXTRACTS OF JAPANESE QUINCE SEEDS

Phenolic acid	Extract					
	A1	A2	B1	B2	C	D
Proto-catechuic	20.36* ± 0.21 ^a	46.14 ± 0.33 ^a	14.20 ± 0.12 ^a	36.93 ± 0.30 ^a	24.91 ± 0.21 ^a	32.63 ± 0.27 ^a
Chloro-genic	4.69 ± 0.14 ^a	5.32 ± 0.07 ^a	NF	NF	NF	4.34 ± 0.06 ^a
Vanillic	1.41 ± 0.06 ^a	2.55 ± 0.08 ^a	1.40 ± 0.03 ^a	2.24 ± 0.06 ^a	4.57 ± 0.04 ^a	2.32 ± 0.03 ^a
Syringic	2.06 ± 0.05 ^a	3.01 ± 0.06 ^a	1.14 ± 0.02 ^a	0.97 ± 0.05 ^a	0.46 ± 0.04 ^a	0.34 ± 0.02 ^a
<i>p</i> -Cou-maric	1.12 ± 0.04 ^a	0.75 ± 0.05 ^a	0.55 ± 0.02 ^a	1.64 ± 0.04 ^a	0.44 ± 0.03 ^a	1.19 ± 0.02 ^a
Gallic	1.44 ± 0.18 ^b	4.64 ± 0.23 ^a	0.40 ± 0.10 ^c	2.00 ± 0.18 ^a	1.72 ± 0.13 ^a	NF
Caffeic	NF	NF	NF	NF	0.61 ± 0.09 ^b	1.14 ± 0.05 ^a

* Each value is the mean of two measurements, ± standard deviation (SD). Values marked with the same letter are within the confidence interval (^a*P* < 0.05, ^b*P* < 0.1, ^c*P* < 0.2); NF, the compound was not found in the extract.

content was determined for blends of oils (see Table 1) obtained by cold-pressing of mixtures of Japanese quince and oil plant seeds (Poiss, 2004). Polyphenols were extracted from oils with 80% ethanol.

Identified compounds of six extracts are given in Table 3 – (+)-catechin, quercetin, phlorizin, phloretin and ellagic acid were not found in extracts. Two peaks, which were observed at 7.2 min and 11.9 min in HPLC chromatograms of all seed extracts, were not identified; the last peak was absent in the chromatogram of extract C, but corresponded to the main component in extracts B2 and D. A considerable peak (corresponding to 90% of extract composition) at 3.7 min in the chromatogram of extract C was unidentified. The main polyphenol (found in all extracts of Japanese quince seeds) was protocatechuic acid (see Table 3) — its concentration was higher in extracts prepared by heating in organic solvent (extracts A2 and B2). Extracts prepared by mixing at room temperature (extracts A1 and B1) contained less protocatechuic acid. All extracts (except extract D) contained gallic acid. Ethanol (80%) extract A2, prepared by heating contained more chlorogenic, vanillic and syringic acid. Higher concentration of *p*-coumaric acid was found in ethanol (80%) extract A1, prepared at room temperature. Acetone (70%) extract B2 (prepared by heating) contained more vanillic and *p*-coumaric acid, while extract B1 contained more syringic acid. Comparison of ethanol and acetone extracts (extracts A1 and B1, A2 and B2) showed that ethanol extracts had higher content of vanillic and syringic acid. Ethanol extracts A1 and A2, in contrast to acetone extracts, contained chlorogenic acid. Organic solvent extracts A2 and B2, obtained by heating, contained higher concentration of identified phenolic compounds almost in all cases, in comparison with extracts A1 and B1, which were prepared at room temperature.

Both acid and alkali hydrolysates (extracts C and D, correspondingly) of defatted seeds contained caffeic acid, which was not present in ethanol and acetone extracts. Higher concentration of protocatechuic, caffeic and *p*-coumaric acid was observed in alkali hydrolysates (extract D). In comparison, acid hydrolysates (extracts C) contained more syringic and vanillic acid. The concentration of vanillic acid in acid

hydrolysate was the highest (4.57 mg/kg defatted seeds) among all six extracts studied.

The polyphenol profile of Japanese quince seed oil was investigated, too. Hydrophilic extract E of oil contained two compounds: one was identified as syringic acid (0.06 mg/100 g oil), but the other was unidentified — it corresponded to the peak (3.6 min) of the main component observed in extract C.

Detection of tocopherols in Japanese quince seed oil. α -Tocopherol and δ -tocopherol was analyzed in Japanese quince seed oil by HPLC. Seed oil contained a lot of α -tocopherol (85.96 ± 0.97 mg/100 g oil or 109.20 ± 1.23 mg/kg seeds). Thus, oil and lipophilic extracts can serve as antioxidants.

4. Evaluation of antioxidative properties of Japanese quince seed oil. Extracts of Japanese quince seeds obtained using organic solvents were studied for their antiradical activity. Petroleum ether, hexane, ethyl acetate, acetone, as well as toluene and chloroform extracts, in comparison to synthetic antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT), demonstrated better (or comparable) activity against DPPH (see Table 2). The concentration of synthetic antioxidants that inhibits 50% of DPPH was estimated: IC₅₀ in the case of quercetin was 0.02 mg/ml, ascorbic acid — 0.02 mg/ml, ascorbyl palmitate — 0.03 mg/ml, *tert*-butylhydroquinone — 0.04 mg/ml and BHT — 0.22 mg/ml.

The highest activity was observed for seed oil obtained with ethyl acetate (IC₅₀ = 0.09 mg/ml) and with the mixture ethyl acetate:hexane (1 : 1) (IC₅₀ = 0.10 mg/ml) — both samples were more active than BHT.

DISCUSSION

According to the composition of fatty acids, *Chaenomeles japonica* seed oil is similar to apple (Tian *et al.*, 2010) and melon seed (Maria *et al.*, 2001; Ismail *et al.*, 2010) oils, which is known for its antioxidative properties and belongs to the group of oils low in palmitic acid and high in oleic and linoleic acids (Granados *et al.*, 2003). The unique fatty

acid composition of Japanese quince seed oil may be of interest for the food and cosmetics industry.

Previously we determined (Mierina *et al.*, 2011) that the total concentration of cyanogenic compounds in Japanese quince seeds is 0.69 mg/g — such a cyanide concentration is similar to apple (*Malus domestica*) seeds (0.6 mg/g) and is low compared with bitter almond and apricot kernels (Barceloux, 2009). The oral lethal dose of hydrogen cyanide for human is 0.5–3.5 mg/kg body weight or about 50–250 mg for a typical adult human (Ballhorn, 2011): toxicity from the ingestion of Japanese quince seeds would be unusual. In order to use seed meal and press-cake in food industry or cosmetics, plant material should be detoxified. For this there are two possibilities: pre-treatment of seed material before oil production or detoxification of seed material after deoiling. In this study, the effective pre-treatment methods were developed for detoxification of seeds and further oil extraction. Ground seeds should be extracted with water 0.25 h at room temperature, followed by triple washing with water, which provides detoxified seed material that can be used for oil extraction with petroleum ether or hexane (oil yield in this case reaches 14.8–18.7%). Detoxification of seed meal and press-cake can be carried out similarly.

The concentration of polyphenols was observed to reach 352 mg GAE/100 g in seed oil and 232 mg GAE/100 g in seed meal; the best results can be achieved with ethyl acetate in case of seed oil, but in the case of meal — ethanol; extraction should be carried out at the boiling temperature of solvent (duration 2–6 h). As Japanese quince seed oil and seed extracts contain natural antioxidants (various polyphenols), the oil could be used for stabilisation of other oils — similar examples of application of other plant seeds have been described (Yanishlieva and Marinova, 2001; Poiana, 2012; Rubilar *et al.*, 2012); stabilisation by linseed oil has been studied more often (Omar *et al.*, 2010; Michotte *et al.*, 2011).

Till now only one publication (Sokolowska-Wozniak *et al.*, 2002) has been devoted to analysis of phenolic composition of Japanese quince seeds: it was found that 80% methanol extracts of seeds contain protocatechuic, caffeic, gallic, 4-hydroxybenzoic, *p*-coumaric, ferulic, syringic, vanillic, salicylic, 4-hydroxyphenylacetic and ellagic acid.

In this study, the phenolic profile of Japanese quince seed extracts was analysed by HPLC method; composition of polyphenols ((+)-catechin hydrate, quercetin hydrate, phlorizin hydrate and phloretin) and phenolic acids (gallic, chlorogenic, caffeic, ellagic, protocatechuic, vanillic, syringic and *p*-coumaric acid) was investigated. Phlorizin was chosen as reference compound because it is known as the predominant (79–92%) phenolic compound in apple seeds (Fromm *et al.*, 2012) and is present in some other plant species of the Rosaceae family. As Japanese quince is a member of the Rosaceae family, we thought that phlorizin or phloretin should be present in the seeds.

Japanese quince seeds contained high concentration of α -tocopherol, which was greater than in some other species of the Rosaceae family, e.g., concentration in *Cydonia oblonga* Miller seeds were: α -tocopherol (16.03 mg/100 g dry seed), β -tocopherol (0.15), γ -tocopherol (0.32) and total tocopherol (16.49 mg/100 g dry seed) (Nogala-Kalucka *et al.*, 2010). High concentration of E vitamin in Japanese quince seeds has been reported only in one publication (Gora and Kurowska, 1979). In comparison with an outstanding α -tocopherol source — barley grain oil — Japanese quince seed oil often contains more of this valuable compound (concentration of α -tocopherol in barley oil is 56.3–128.9 mg/100 g oil (Moreau *et al.*, 2007), but the content of α -tocopherol expressed per seed weight is almost ten times higher in the case of Japanese quince seeds in comparison with barley grain (8.2 up to 12.7 mg/kg grain (Panfili *et al.*, 2008)).

As Japanese quince seeds appeared to be a good source of polyphenols and tocopherol, oil blends were prepared by cold-pressing method from mixtures of Japanese quince seeds and oilseeds. Addition of Japanese quince seeds increased TPC of blends only by 3–9 mg GAE/100 g; this parameter in the case of linseed oil increased by 2.6, for rapeseed oil — 3.1 and in the case of hempseed oil by nine units. Nevertheless, lipophilic extracts of Japanese quince seeds demonstrated notable free radical scavenging properties in the DPPH test: it was established that activity of petroleum ether, toluene, hexane and chloroform extracts (IC_{50} = 0.33–0.26 mg/ml) is only somewhat lower than that of synthetic antioxidant BHT (IC_{50} = 0.22 mg/ml), and but the activity of the ethyl acetate extract even exceeded it — IC_{50} is 0.09 mg/ml.

It can be concluded that Japanese quince seeds produced as an agro-industrial by-product can serve as a valuable source of high-linoleic and high-oleic oil and natural antioxidants (phenolic compounds, α -tocopherol), and therefore, *Chaenomeles japonica* seeds, seed oil and meal may be of interest for the food industry (for oil blends and stabilisation) and non-food uses.

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KRŪMCDONIJU (*Chaenomeles japonica*) SĒKLU EĻĻAS UN SPRAUKUMU PĒTĪJUMI

Krūmcdoniju (*Chaenomeles japonica*) sēklu dažādu organisko šķīdinātāju ekstraktiem noteikts kopējais polifenolu saturs un antiradikāļu aktivitāte. Petrolētera, heksāna, etilacetāta, acetona, kā arī toluola un hloroforma ekstraktu spēja inhibēt 2,2-difenil-1-pikrilhidrazilu (DPPH) ir augstāka (vai salīdzināma) nekā sintētiskajam antioksidantam BHT (butilēts hidroksitoluols). Izstrādātas cianīdus saturošo krūmcdoniju sēklu, spraukumu un spiedpalieku detoksifikācijas metodes. Izmantojot augstas izšķirtspējas šķīdumhromatogrāfijas metodi (HPLC), analizēta krūmcdoniju sēklu dažādu ekstraktu (80% etanola un 70% acetona), skābo un bāzisko hidrolizātu, kā arī eļļas metanola/ūdens ekstrakta polifenolu kompozīcija: sēklu ekstraktā pirmo reizi konstatēta hlorogēnskābe. Visos ekstraktos kā dominējošais savienojums atrasta protokatehīnskābe; noskaidrots arī citu galveno fenolskābju daudzums, un sēklu eļļā atrasta sīringskābe. Eļļā ir daudz α -tokoferola, kura saturs krūmcdoniju sēklīs ir gandrīz desmit reizes lielāks nekā miežu graudos. Tā kā sēklu eļļa un lipofilie ekstrakti satur α -tokoferolu un polifenolus, ekstraktus var izmantot kā antioksidantu avotus.

CONTENT OF PHENOLIC COMPOUNDS IN VARIOUS SEA BUCKTHORN PARTS

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*All parts of the sea buckthorn (*Hippophae rhamnoides* L.) plant are considered to contain large amounts of compounds that are believed to have beneficial health effects. Till now, different parts of sea buckthorn plant have been used for the treatment of diseases in traditional medicine in various countries. Nevertheless, sea buckthorn parts would be a good raw material not only for medicinal properties but also for food products with functional properties; therefore, the aim of the research was to determine the concentration of different phenolic compounds and antioxidant activity in various sea buckthorn parts. The study was conducted on parts of female and male sea buckthorn bushes. Phenolic compounds (total phenols, total flavonoids and condensed tannins) and antioxidative activity (ferric reducing antioxidant power (FRAP) free radical scavenging activity (using 2,2-diphenyl-1-picrylhydrazyl, DPPH)) in ethanolic extracts of leaves, shoots, flowers, and berries were determined using various spectrophotometric methods. The study showed that concentration of phenolic compounds differed among parts of sea buckthorn plant and among gender. Leaves of female plants proved to be the most valuable, as they contained the highest total phenol concentration (165.76 mg/g) and antioxidant activity (220.97 mg/g for FRAP and 43.76 mg/g for DPPH), while lowest values were found in young shoots of male plants (84.94 mg/g, 94.24 mg/g and 24.63 mg/g, respectively). The significant differences in chemical composition and biological activity of sea buckthorn leaves, shoots, berries, and buds indicate a need for detailed studies of their extracts, specific fractions and compounds during a whole vegetative season.*

Key words: leaves, shoots, berries, flowers, gender, flavonoids, phenols, tannins, antioxidant activity.

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L.) is a thorny shrub, native to Europe and Asia (Saggu *et al.*, 2007). Berries of sea buckthorn have been much studied and their chemical composition and positive qualities are well documented. This has led to the outcome that mainly berries of this particular plant are used as a raw material for food processing (Guliyev *et al.*, 2004) in jams, beverages, candies, etc. Nevertheless all parts of sea buckthorn plant have been considered valuable, as they contain large amounts of different bioactive substances, including a wide range of polyphenols, tocopherols, carotenoids, fatty acids, sterols, vitamins, minerals, etc. (Saggu *et al.*, 2007; Jain *et al.*, 2008; Kumar *et al.*, 2011). Till now, different parts of sea buckthorn (other than berries) have been used in traditional medicine for the treatment of diseases, such as flu, colitis and enterocolitis, also cardiovascular illnesses, mucosal injuries, dermatological, and digestion disorders (Guliyev *et*

al., 2004; Kumar *et al.*, 2011). These therapeutic effects mostly are related to the high amount of compounds possessing antioxidant activity (Saggu *et al.*, 2007; Kumar *et al.*, 2011). This is in accordance with many reports that have reported a positive correlation between daily intake of fruit, vegetables and other products of plant origin and health improvement (Michel *et al.*, 2012).

Therefore, in recent years there is a growing interest and demand for natural substances, mainly phenolics derived from plants, which exhibit this property and could be used as food components or pharmaceuticals (Uttara *et al.*, 2009; Wannes *et al.*, 2010). Aromatic and medicinal plants are a good source of natural antioxidants, because of large amounts of secondary metabolites, such as polyphenols and essential oils (Wannes *et al.*, 2010), which are produced by plants for normal growth and development (Kondakova *et al.*, 2009) to defend against plant pathogens, UV stress or to attract pollinators (Michel *et al.*, 2012).

However, only some studies have conducted analyses of phenolic compounds and their activity in sea buckthorn leaves and shoots, and no information can be found regarding concentrations in flowers, green berries, and regarding effects of gender and harvest time on these qualities. Therefore, the aim of the research was to determine the concentrations of various phenolic compounds and antioxidant activity in various sea buckthorn parts.

MATERIALS AND METHODS

Plant material. Sea buckthorn material was collected in 2011, in Dobeles, Latvia. Flowers of male plants were harvested at time of pollination (late April), while leaves, shoots, unripe berries of female plants and leaves, shoots of male plants after 7 weeks of blossoming (middle of June). Equal amounts (100 ± 0.1 g) of every sea buckthorn part were dried by lyophilisation (FreeZone, Labconco, USA) at -50 ± 2 °C under 0.056 mBar pressure for 16 h.

Extraction procedure. Dried parts of sea buckthorn were pulverised in a laboratory mill (Knifetec 1095, Foss, Sweden). Extraction was performed in three steps. First, 40 ml 80% ethanol was added to 1 g of the sample, shaken for 1 min, placed in the ultrasonic bath (Sonorex RK 510 H, Bandelin electronic GmbH & Co. KG, Germany) for 15 min at 50 °C and centrifuged (Eppendorf Centrifuge 5804R, Eppendorf AG, Germany) at 10 000 g for 10 min at 4 °C. Clear supernatant was separated from sediments and placed in an 100 ml flask. The second step was performed by taking the sediments, adding 30 ml 80% ethanol and repeating the above described procedure. Thirdly, when 20 ml 80% ethanol was added to sediments obtained from the second extraction step. When all of the supernatant was collected, the flask was filled to 100 ml, mixed well and centrifuged (10 000 g, 10 min at 4 °C).

The concentration of obtained ethanolic extracts was 0.01 g/ml, and due to high concentration of bioactive substances, prior to analyses it was diluted 100 times for total phenol assay, 10 or 25 times for total flavonoid analyses, 30 times for ferric reducing antioxidant power assay, 10 times for free radical scavenging activity and two times for condensed tannin analyses.

Analyses of phenolic compounds

Total phenol concentration. The total phenol concentration was determined according to Singleton *et al.* (1999). Folin-Ciocalteu reagent (FCR) was prepared from Folin-Ciocalteu stock solution (10 ml) and water (90 ml). The sea buckthorn extract (1 ml) was added to FCR (5 ml) and Na_2CO_3 solution (4 ml), mixed and kept for 1 h in the dark. The absorption was measured at 765 nm using a spectrophotometer (UV – 1650 PC, Shimadzu, Japan). Results were expressed as mg gallic acid equivalents per 1 g of dry matter (mg GAE/g DM).

Total flavonoid concentration. Total flavonoid concentration was determined using a spectrophotometric method

previously described by Kumar *et al.* (2011) and Upadhyay *et al.* (2010). The extract (1 ml) was mixed with water (2 ml), 5% NaNO_2 (0.15 ml) and left to react for 6 minutes. Then 10% AlCl_3 (0.15 ml) solution was added, mixed and allowed to react for another 6 minutes. Then 4% NaOH (2.0 ml) was added to the mixture, shaken and left for 15 minutes. The absorption was read at 510 nm. Results were expressed as mg rutin equivalents per 1 g of dry matter (mg RUE/g DM).

Condensed tannin concentration. Condensed tannin concentration was determined by a method described by Michel *et al.* (2012). Sea buckthorn part extract (0.2 ml) was added to 4% vanillin–ethanolic solution (2.4 ml) and concentrated HCl (1.2 ml) and allowed to stand for 15 min. The absorption was detected at 500 nm by a spectrophotometer. Results were expressed as mg catechin equivalents per 1 g of dry matter (mg CE/g DM).

Antioxidant activity

DPPH free radical scavenging assay. The free radical scavenging activity was determined according to Brand-Williams *et al.* (1995) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent prepared by mixing with methanol to reach absorption about 0.9 at wavelength of 515 nm. The extract (0.131 ml) was mixed with DPPH solution (2.9 ml) and left to stand for 30 min in a dark place. The absorption of samples was read at 515 nm using a spectrophotometer. Results were expressed in mg Trolox equivalents per 1 g of dry matter (mg TE/g DM).

Ferric reducing antioxidant power assay. Reducing antioxidant power evaluation was carried out by a spectrophotometric method described by Benzie and Strain (1996) and Kumar *et al.* (2011). Before analysis, fresh ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine made in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in the ratio of 10 : 1 : 1 (v/v/v), respectively, and then warmed at 37 °C in a water bath. Each extract (0.210 ml) was added to FRAP reagent (3.8 ml) and allowed to stand for 10 min in the dark. The absorption was detected at wavelength 593 nm. Results were expressed in mg Trolox equivalents per 1 g of dry matter (mg TE/g DM).

Statistical analysis. Data were analysed using SPSS Statistics 17.0 software. The results were expressed as means \pm standard deviations for at least three experimental measurements. One-way analysis of variance (ANOVA), Duncan multiple range test and Pearson correlation tests were used. Significance was set at $P \sim 0.05$. The Pearson correlation test was performed to determine relationships between chemical parameters.

RESULTS

There were significant differences ($P < 0.05$) in the concentrations of total phenols, total flavonoids and condensed tannins among parts of sea buckthorn plant (Table 1).

Table 1

PHENOL CONTENT IN VARIOUS SEA BUCKTHORN PARTS

Plant gender	Part	Total phenol concentration, mg GAE/g DM	Total flavonoid concentration, mg RUE/g DM	Condensed tannin concentration, mg RUE/g DM
Female	Leaves	165.76 ± 2.52 ^a	47.76 ± 0.43 ^c	1.32 ± 0.28 ^e
	Shoots	119.63 ± 3.67 ^c	79.57 ± 0.75 ^b	22.47 ± 0.12 ^b
	Green berries	89.34 ± 1.32 ^d	39.04 ± 0.83 ^d	23.29 ± 0.49 ^a
Male	Leaves	123.78 ± 2.53 ^b	36.47 ± 0.27 ^e	1.03 ± 0.16 ^e
	Shoots	84.94 ± 0.91 ^e	47.01 ± 1.69 ^c	11.00 ± 0.52 ^d
	Flowers	120.16 ± 0.92 ^c	81.72 ± 0.76 ^a	18.94 ± 0.73 ^c

Different letters in a column show significant differences between samples within each analysed parameter at $P \sim 0.05$. Results are expressed as mean ± standard deviation ($n = 4$). GAE, gallic acid equivalent; RUE, rutin equivalent; CAE, catechin equivalent; DM, dry matter.

The leaves of female and male plants were the best sources of total phenols (165.76 mg/g DW and 123.78 mg/g DW, respectively). The concentration of total flavonoids had the highest level in male flowers (81.72 mg/g DW) and the lowest in male leaves (36.47 mg/g DW). The shoots and green berries of female sea buckthorn plant contained the highest concentrations of condensed tannins, reaching 22.47 and 23.29 mg/g DW which was about 20 times higher than in leaves of both genders.

Similar to phenolic compounds, also antioxidant activity significantly differed ($P \sim 0.05$) between sea buckthorn leaves, shoots, green berries, and flowers. The activity de-

termined by the FRAP method (Fig. 1) showed higher values than those by DPPH radical assay (Fig. 2).

The highest antioxidant activity by both methods was observed in leaves of female plants (220.97 mg/g DW for FRAP and 43.76 mg/g DW for DPPH assay), followed by male leaves, female and male shoots.

Pearson correlation coefficients were strong and significant between total phenol and FRAP ($r = 0.945$), and between total phenols and DPPH ($r = 0.883$).

DISCUSSION

As sea buckthorn leaves and shoots are considered to have a great potential in food processing due to biologically active substances (Shunguang *et al.*, 2003), and especially if collected in summer (as for herbal medicine) when shoots are still green, tender and non-woody. Together with the leaves and shoots, also small green berries were picked. Male flowers were harvested in the middle of spring, because at that time they are rich in pollen, which is thought to contain high amount of bioactive substances.

Although all parts of sea buckthorn plant are considered to contain high levels of hydrophilic (polyphenols, chlorophyll, vitamin C) and lipophilic (carotenoids, tocopherols, vitamin E) compounds (Jain *et al.*, 2008; Saggiu and Kumar, 2008; Kumar *et al.*, 2011), only leaves have gained great interest during the last decades.

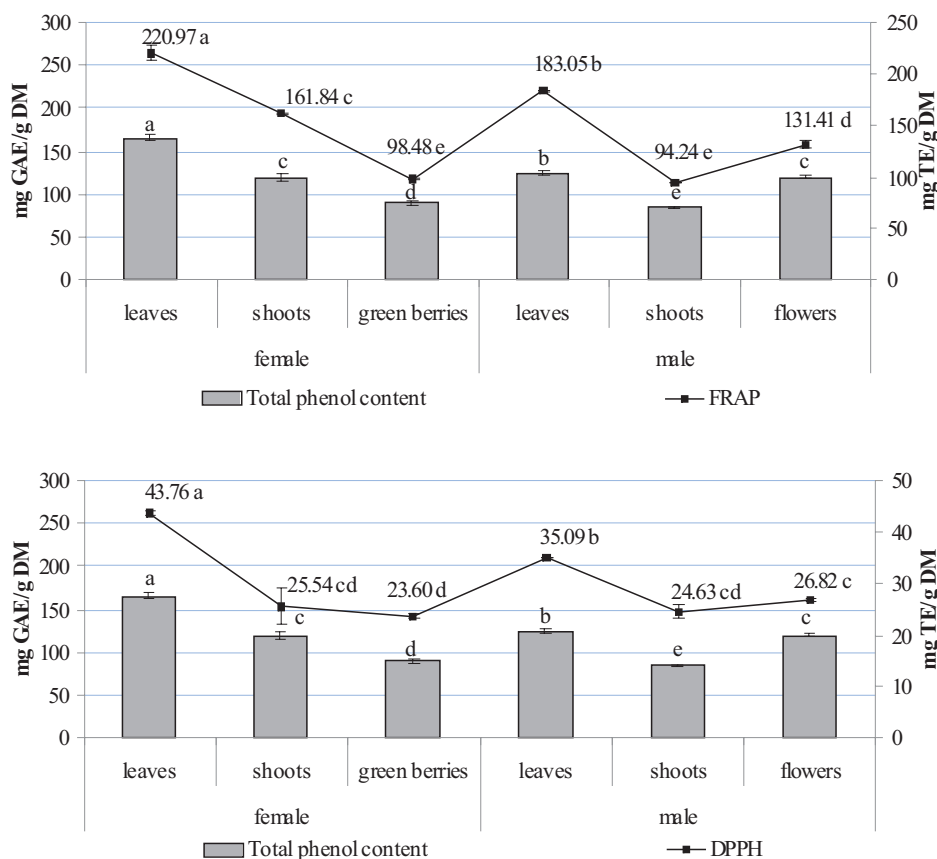


Fig. 1. Ferric reducing antioxidant power (FRAP) and total phenol concentration of sea buckthorn parts. Different letters show significant differences between samples within each analysed parameter at $P \sim 0.05$. Results are expressed as mean ± standard deviation ($n = 4$). TE, trolox equivalent; GAE, gallic acid equivalent; DM, dry matter.

Fig. 2. Free radical scavenging activity (DPPH) and total phenol concentration of sea buckthorn parts. Different letters show significant differences between samples within each analysed parameter at $P \sim 0.05$. Results are expressed as mean ± standard deviation ($n = 4$). TE, trolox equivalent; GAE, gallic acid equivalent; DM, dry matter.

In our study, the highest values for total phenol, total flavonoid and condensed tannin concentration were observed in leaves of female plants — 165.76 mg/g, 47.76 mg/g and 1.32 mg/g, respectively. These values are lower than those reported by Upadhyay *et al.* (2010) for sea buckthorn leaf samples collected in the North-West Himalayas: 56.28 mg/g total phenols and only 20.76 mg/g total flavonoids. However, the values in our study were lower than in leaves collected from west Pamirs where flavonoid content varied from 31 to 123.8 mg/g (Singh, 2006). Michel *et al.* (2012) in their research found that leaves extracted by ethanol (as in our study) contain 65 mg/g total phenols and even 13 mg/g condensed tannins, but the authors used different extraction methods, solvents, and solvent-to-sample ratios, and even small differences can significantly affect the final value. In contrast to the study by Upadhyay *et al.* (2010), where autumn leaves were studied, we used leaves collected in the beginning of summer. Therefore, seasonal variation should be taken into consideration together with plant age, genetic, agronomic and storage variations. According to Kondakova *et al.* (2009), fruits of berry plants grown in a colder climate and under a shorter vegetation period have higher levels of phenolic compounds. These differences, governed by geographical region, probably also occur for other plant parts.

Phenolic compounds (flavonoids, phenolic acids, and tannins) are considered to be the major plant compounds possessing antioxidant activity (Upadhyay *et al.*, 2010). Therefore, for better characterisation of sea buckthorn leaves, shoots, berries and flowers, two commonly applied antioxidant activity testing methods were used: ferric reducing antioxidant power and free radical scavenging activity (DPPH). The findings in the current study show that sea buckthorn leaves of both gender shrubs contained the highest amount of total phenols and also possessed the highest antioxidant activity. This was also indicated by Pearson correlation analysis, by a strong and significant relationships between total phenol concentration and FRAP/ DPPH activity in all tested sea buckthorn samples.

The study showed that different parts of sea buckthorn are rich in phenolic compounds and exhibit antioxidant activity, and that the most valuable part with regard to total phenol content and antioxidant activity is leaves from female tree, and the less valuable — shoots from male trees. There are differences in chemical composition and activity of various sea buckthorn parts, and more detailed investigation of their extracts, specific fractions and compounds and their activity during the whole vegetative season is needed.

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FENOLSAVIENOJUMU SATURS DAŽĀDĀS SMILTSĒRKŠĶU AUGA DAĻĀS

Rakstā aplūkots fenolsavienojumu saturs dažādās smiltsērķšķu auga daļās un to antioksidatīvā aktivitāte. Kā pētījuma objekts izvēlētas sievišķo un vīrišķo augu lapas, jaunie dzinumi, zaļās ogas, kas ievāktas jūnija vidū, un ziedi, kas ievākti aprīļa beigās. Paraugiem analizēts kopējo fenolu, kopējo flavonoīdu un kondensēto tanīnu saturs, kā arī antioksidatīvā aktivitāte ar FRAP (*ferric reducing antioxidant power*) un DPPH (*2,2-diphenyl-1-picrylhydrazyl*) metodi. Vērtīgākā auga daļa izrādījās sievišķā auga lapas, kurām konstatēts augstākais kopējo fenolu saturs un antioksidatīvā aktivitāte, lietojot abas metodes, savukārt vismazākie minētie rādītāji bija jaunajiem dzinumiem no vīrišķā auga. Rezultāti norāda uz būtiskām atšķirībām smiltsērķšķu auga daļu ķīmiskajā sastāvā un bioloģiskajā aktivitātē, tādēļ nepieciešami padziļināti pētījumi par ekstraktiem, to frakcijām vai atsevišķiem savienojumiem un to izmaiņām visā veģetācijas sezonā.

CHANGES OF PHYSICALLY-CHEMICAL PARAMETERS OF 'NANTE' CARROT HYBRIDS DURING STORAGE IN TRADITIONAL CONDITIONS

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The current research focuses on changes of physically-chemical parameters of 'Nante' carrot hybrids during storage in traditional conditions. Quality parameters during carrot storage for six months at temperature $8 \pm 2^{\circ}\text{C}$ and relative air humidity $85 \pm 1\%$ were evaluated using standard methods: soluble solids (digital refractometer), dietary fibre (AOAC 985.29), ascorbic acid (iodometric) and firmness (TA.XT.plus Texture Analyser). Before storage, hybrids 'Nante/Berlikum', 'Nante/Maestro' and 'Nante/Bolero' had higher soluble solid content: 1.6 times higher comparing with hybrid 'Nante/Forto' and 1.2 times higher compared with hybrid 'Nante/Champion'. These hybrids also had higher ascorbic acid content: 2.0 times higher than in 'Nante/Forto' and 'Nante/Champion'. Significant differences were not found in dietary fibre content and firmness of non-stored carrots. Substantial differences were observed in soluble solid content in the hybrid 'Nante/Berlikum' during six-month storage — the content decreased 2.0 times. However, non-significant decrease of soluble solids content decreases was found for the hybrid 'Nante/Forto'. Changes in hybrid firmness and dietary fibre amount were not significant during storage for four months. Ascorbic acid content of the carrot hybrids during storage decreased by 3.4 times.

Key words: carrot, soluble solids, dietary fibre, ascorbic acid, firmness, storage.

INTRODUCTION

Carrot is a globally important vegetable crop that is a source of important nutritional compounds (including pro-vitamin A) through their carotenoid content, and adds flavour and texture to many diets across the world (Rakcejeva *et al.*, 2012). Vegetables are an important part of our diet. They provide not only the major dietary fibre component of our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds (Rashidi, 2011; Rakcejeva *et al.*, 2012; Singh *et al.*, 2012). It is known that growth conditions and management practices affect the concentrations of these compounds, and thus also nutritional value and taste (Singh *et al.*, 2012). As consumers move toward functional foods with specific health effects, scientists and food manufacturers have also taken an interest in the potential of the antioxidant constituents of carrots to maintain health (Yen *et al.*, 2008). Considering that carrot is one of the primary vegetables in many countries and can adequately supply most, if not all, of the vitamin A daily requirement of humans (from a 100 g serving of raw carrot (Singh *et al.*, 2012)), it is important to enhance the nutritional status of carrot when possible.

Carrots are an important source of vitamin C in the human diet, but the content can decrease during storage (Matejkova

and Petrikova, 2010). Compared to other horticultural crops, it has been reported that New Zealand-grown carrots have higher vitamin C content than New Zealand-grown grapes, nectarines, pears and plums (Leong, 2012). Carrots can be consumed fresh or cooked, either alone or with other vegetables, in the preparation of soups, stews, curries and pies. Fresh grated roots are used in salads and tender roots are pickled (Soria *et al.*, 2009; Rashidi, 2011). Ascorbic acid (vitamin C) is used extensively in the food industry, not only for its nutritional value but also for its many functional contributions to product quality. Leong in 2012 reported that the human body is unable to synthesise vitamin C due to the lack of enzyme L-gulonolactone oxidase. Vitamin C helps to prevent scurvy, reduce the risk of cancers and cardiovascular diseases and enhance the absorption of iron to prevent anaemia. In plants, the active form of vitamin C, i.e. L-ascorbic acid (L-AA) acts as an enzyme cofactor, electron donor or acceptor in the proton electron transport system and as a substrate for oxalate and tartrate biosynthesis. The stability of vitamin C in carrots can be influenced by factors from pre-harvest (including cultivars and environment conditions) to harvest and post-harvest handling (Leong, 2012). Some scientists believe that vitamin C acts as an antioxidant in plants and that its levels are sensitive to a variety of environmental or stress factors, for

example, light, temperature, salt and drought, atmospheric pollutants, metals or herbicides (Singh *et al.*, 2012). Fresh vegetables have a short durability, and are exposed to conditions that destroy their superior quality in a short period of time, before cooking and consumption (Giannakourou and Taoukis, 2003). Cultivar and soil climatic conditions affect the concentrations of compounds in carrot roots, which are important for both human nutrition and taste (Smoleń and Sady, 2009).

Fruits and vegetables are good sources of dietary fibre (DF). DF is a group of food components, which are resistant to hydrolysis by human digestive enzymes. The health benefits of dietary fibre have led to increased consumption of fibre-rich products (Chantaro *et al.*, 2008). Fibres are an integral part of the foodstuffs we consume daily; the main sources in which these fibres occur are plants, vegetables, cereal grains, woody plants, fruits, legumes, leguminous plants, etc. Based on their simulated intestinal solubility, dietary fibres are either classified as insoluble or soluble fibres. Insoluble fibres include lignin, cellulose, and hemicelluloses; soluble fibres include pectins, beta-glucans, galactomannan gums, and a large range of non-digestible oligosaccharides including inulin (Rodriguez *et al.*, 2006; Chantaro *et al.*, 2008; Slavin, 2008). At present, there are still many aspects about DF properties and functions that remain unclear. Botanists define fibre as a part of the plant organs, chemists as a group of chemical compounds, consumers as a substance with beneficial effects on human health, and for the dietetic and chemical industries DF is a subject of marketing (Rodriguez *et al.*, 2006). DF is part of a plant matrix which is largely intact. Non-digestible plant carbohydrates in foods are usually a mixture of polysaccharides that are integral components of the plant cell wall or intercellular structure (Slavin, 2008). Post-harvest storage of fresh fruits and vegetables needs to be such that the organoleptic (colour, texture and flavour) and nutritional properties of the final product are maintained in optimal conditions. Changes in fibre quantity and quality depend to a great extent on storage conditions (Rodriguez *et al.*, 2006). DF concentrates obtained after dehydration of fresh fruit and vegetables can be used in the food industry as functional ingredients with excellent results. DF from cereals is more frequently used than fibre from fruit or vegetables. However, fruit and vegetable fibre has better quality due to its higher soluble DF content, better water and oil holding capacities and better colonic fermentability, as well as lower phytic acid contents and energy (Eim *et al.*, 2008). Some scientists believe that DF acts as a protective agent against cardiovascular diseases, diverticulosis, constipation, irritable colon, colon cancer and diabetes (Chau *et al.*, 2004; Eim *et al.*, 2008; Slavin, 2008; Brownlee, 2011). Five specific methods of DF analysis and the certified values in dried carrots used as European Reference Material (originally certified as BCR-515) are shown in the Table 1 (Emons, 2011).

Soluble solids in carrot roots consist mostly of soluble sugars, and therefore affect sensory sweetness impression (Gajewski *et al.*, 2010). Soluble solid content has a pro-

Table 1
CONTENT OF DIETARY FIBRE IN DRIED CARROTS (Emons, 2011)

Nr.	Mass fraction g · kg ⁻¹ (*)	Dietary fibre according to
1	311	AOAC, 1990, 985.29
2	271	Englyst by GC
3	298	Uppsala 994.13
4	295	AOAC, 1992, MES-TRIS, 991.43
5	252	Englyst by Colorimetry

(*) The values are expressed on dry mass basis.

found influence on the storage period length, mechanical properties and quality characteristics of fruits and vegetables (Rashidi, 2011). The sensory qualities of fresh vegetable such as carrot depend on their texture, which correlate well with the freshness of the produce. The cutting test is one of the common methods for determining textural changes in food products. It is generally applied with maximal cutting force as an attribute of the studied material quality (Rawson *et al.*, 2012).

Texture is a major quality characteristic of fruits and vegetables. The notion of “texture engineering” has been introduced in the context of texture optimisation of processed fruits and vegetables (Roeck *et al.*, 2010). Many earlier researchers employed instrumental texture measurement (also known as the hardness) to quantify product quality. Hardness is often defined as the peak force corresponding to the first compression of the sample. Product firmness and tissue elasticity are provided by pectic substances. Pectin is a major component present in middle lamella. Solubilisation of pectic compounds in the middle lamella results in intercellular weakening and cell separation leading to texture degradation. Under controlled conditions, the action of endogenous pectinases, such as pectinmethylesterase (PME) and polygalacturonase (PG), on the pectin is highly correlated with texture degradation of fruits and vegetables (Rastogi *et al.*, 2008). Traditionally the harvesting of carrots in Latvia starts from middle of summer, however, the main harvest occurs in autumn. Worldwide carrots can have purplish, yellow, green, white and black colour, but in Latvia mainly bright orange. The main carrot variety is ‘Nante’ and its hybrids (Rakcejeva *et al.*, 2012). Unfortunately, the brittleness that accompanies crisp texture tends to have a negative impact on the “durability” of carrots in mechanical harvesting and washing (Prohens *et al.*, 2007).

The aim of the study was to determine changes of physically-chemical parameters of ‘Nante’ carrot hybrids during storage in traditional conditions.

MATERIALS AND METHODS

The study was carried out in the Department of Food Technology at the Latvia University of Agriculture. Carrots (*Daucus carota* L.) grown in Latvia and harvested in Zemgale region from four farms in the first part of October

2011 were immediately used for experiments. Serotinous 'Nante' carrot hybrids 'Nante/Berlikum', 'Nante/Maestro', 'Nante/Forto', 'Nante/Bolero' and 'Nante/Champion' were used. Quality parameters of carrots were determined during six-month storage at temperature $+8 \pm 2$ °C and relative air humidity $85 \pm 1\%$ using standard methods. Carrots were sampled in October, December 2011 and February, April 2012 (sampling interval of two months). Meteorological data were obtained from the Latvian Environment, Geology and Meteorology Centre. Meteorological conditions of 2011 were characteristic with relatively high temperatures in the first two months of summer of 2011. June and July months were also warm, with average air temperature in June $+24.5$ °C and maximum air temperature $+32.3$ °C. In summer 2011, average rainfall was 274 mm, i.e. close to optimal precipitation. Autumn 2011 in Latvia was warm and relatively dry. All autumn months were warmer and drier than normal. The autumn temperature was 1.9 degrees above normal. Autumn precipitation was 67% of the average level.

Ascorbic acid. Ascorbic acid content was determined by titration with 0.05M iodine solution (Moor *et al.*, 2005). Samples of 25 g carrot were mixed with $100 \text{ mL} \cdot 6 \text{ g} \cdot 100 \text{ g}^{-1}$ oxalic acid solution and homogenised for 60 s, and then the sample was filtered. 10.0 ml of the filtrate and $2.0 \text{ mL} \cdot 1 \text{ g} \cdot 100 \text{ g}^{-1}$ starch solution was titrated until the endpoint was reached (first sign of blue colour that persisted after 30 s). The titration was repeated in triplicate for each sample. The ascorbic acid content was calculated as:

$$x = \frac{V_{\text{sample}} \times 5000}{V_{\text{standard}} \times g}, \text{ where}$$

V_{sample} – iodine amount for sample titration, ml;

V_{standard} – iodine amount for vitamin C standard solution titration, ml;

g – sample weight, g.

Total dietary fibre. The total dietary fibre in samples was determined according to the AOAC approved method No. 985.29. The experiments were carried out by using a FOSS Analytical Fibertec E 1023 system with enzymatic processing by incubation in a thermostatic shaking water bath; residue filtration was conducted using a Filtration Module, and

protein determination by Kjeldahl (Method 46–12, 1995) nitrogen equipment. The analyses were performed in three repetitions. The samples were defatted and dried with a particle size less than 0.5 mm. Then each sample was enzymatically digested with α -amylase incubation at $+100$ °C, and with protease and amyloglucosidase incubation at $+60$ °C. After digestion, the total fibre content was precipitated by adding $95 \text{ g} \cdot 100 \text{ g}^{-1}$ ethanol. The solution was then filtered and fibre was collected, dried and weighed. The protein and ash content were determined for correction of fibre content (Prosky, 1990).

Soluble solids content (SSC). The soluble solid content ($^{\circ}\text{Brix}$) was determined at temperature $+20 \pm 2$ °C with a digital refractometer (deviation of measuring instrument $\pm 0.1\%$) by standard method ISO 2173:2003.

Firmness. A structure analyser “TA.XT.plus texture Analyser” (Stable Micro Systems Ltd., Surrey, UK) and measuring probe HDP/BSK (blade set with knife, supplied with the Texture Analyser) were used for firmness determination. The system was equipped with a compression cell of 50 kg and software Texture Exponent 32. Firmness was measured as the maximum penetration force (N) reached during tissue breakage. The measuring parameters were: pre-test speed $2 \text{ mm} \cdot \text{s}^{-1}$; test speed $2 \cdot \text{mm} \cdot \text{s}^{-1}$; post-test speed $10 \text{ mm} \cdot \text{s}^{-1}$; penetrating distance 23 mm into the carrot. The measurement was triggered automatically at 0.04903 N (Rakcejeva *et al.*, 2012).

Statistical analysis. Data were expressed as mean \pm standard deviation; for mathematical data processing the value of $P < 0.05$ was regarded as statistically significant. One-way analysis of variance (ANOVA) was used to determine the significance of differences. In cases of statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test at the level of confidence $\alpha = 0.05$. The statistical analyses were performed using Microsoft Excel 2007.

RESULTS

Vitamin C. Vitamin C content ($P < 0.05$) (Fig. 1) significantly differed between fresh carrot hybrids. The highest vitamin C contents occurred in hybrids 'Nante/Maestro',

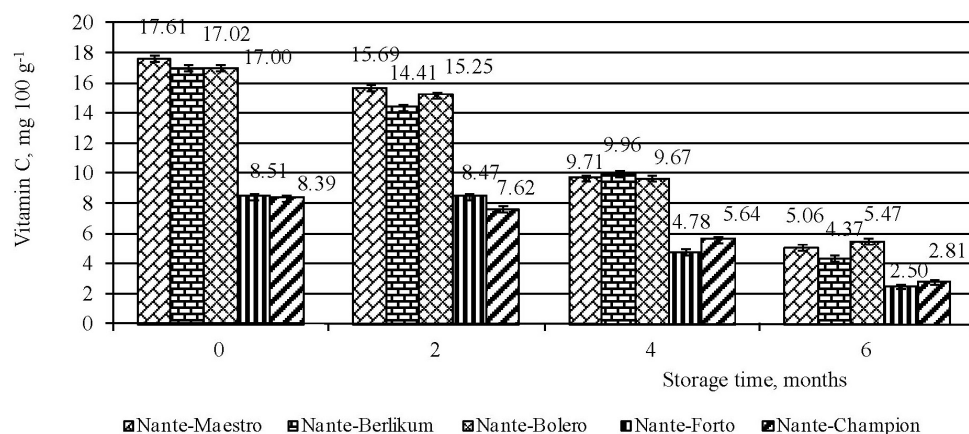


Fig. 1. Vitamin C content in variety 'Nante' carrot hybrids during storage.

'Nante/Berlikum' and 'Nante/Bolero' (17.61 ± 0.17 , 17.02 ± 0.01 and 17.00 ± 0.01 mg 100 g^{-1} , respectively), which were significantly higher ($P < 0.05$) than in hybrids 'Nante/Forto' and 'Nante/Champion' (8.51 ± 0.01 and 8.39 ± 0.17 mg $\cdot 100\text{ g}^{-1}$, respectively). Vitamin C decreased significantly during carrot storage. After two-month storage, the decrease in ascorbic acid content was not significant ($P < 0.05$): ~ 1.1 times compared with initial C vitamin content. However, during carrot storage for four months, vitamin C content decreased by ~ 1.7 times, and after six-month storage by up to 3.3 times, particularly in hybrid 'Nante/Berlikum' — 3.9 times less comparing with vitamin content in fresh carrots. These changes mainly could be explained by physically-chemical reactions in carrots during storage.

Total dietary fibre. It was found that the content of total dietary fibre in 'Nante' hybrid carrots ranged from 25.78 ± 1.54 to $34.25 \pm 5.79\text{ g} \cdot 100\text{ g}^{-1}$. Higher total dietary fibre content was observed in non-stored hybrids 'Nante/Bolero', 'Nante/Maestro' and 'Nante/Berlikum': 34.25 ± 5.79 , 29.80 ± 0.47 and $29.49 \pm 0.36\text{ g} \cdot 100\text{ g}^{-1}$, respectively (Fig. 3).

Soluble solids content and firmness. The soluble solid content (SSC) in fresh carrot hybrids °Brix (Fig. 3) was within the range from 10.30% in 'Nante/Maestro' to 6.1% in 'Nante/Forto'. Soluble solid content was similar in 'Nante/Maestro', 'Nante/Bolero' and 'Nante/Berlikum': $10.30 \pm 0.09\%$, $9.97 \pm 0.08\%$ and $9.70 \pm 0.09\%$, respectively. The lowest soluble solid content was observed in fresh carrots hybrids 'Nante/Champion' and 'Nante/Forto' ($8.16 \pm 0.18\%$ and $6.11 \pm 0.07\%$, respectively).

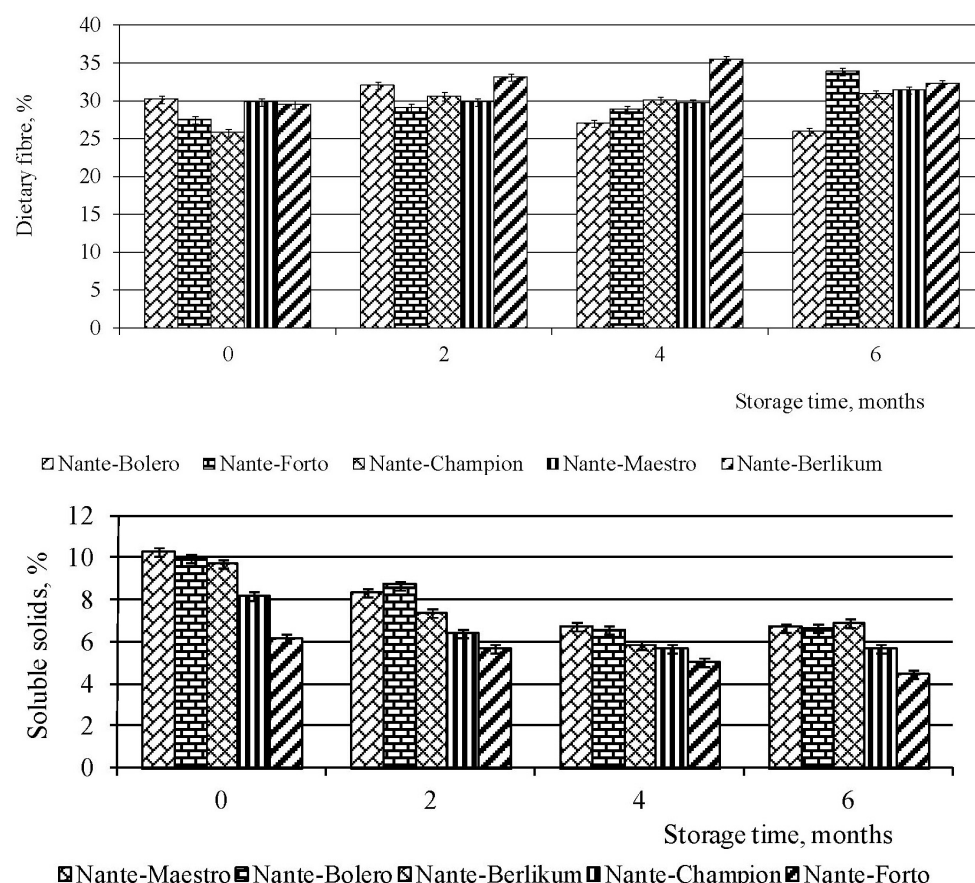


Fig. 2. Dietary fibre content in variety 'Nante' carrot hybrids during storage.

Fig. 3. Soluble solids content in variety 'Nante' carrot hybrids during storage.

Table 2

FIRMNESS (N) OF CARROT BEFORE AND AFTER STORAGE

Carrot hybrids	Before storage	After six-month storage	Whole period
	average \pm SD	average \pm SD	$X_{\min}-X_{\max}$
Nante/Bolero	95.09 \pm 21.01	100.41 \pm 7.50	72.76–112.17
Nante/Maestro	103.98 \pm 16.19	87.99 \pm 9.03	67.24–105.43
Nante/Berlikum	82.24 \pm 14.78	95.65 \pm 9.59	53.62–109.96
Nante/Forto	81.28 \pm 14.54	114.47 \pm 9.91	81.46–126.56
Nante/Champion	82.93 \pm 15.08	71.74 \pm 5.28	62.08–95.11
Total	89.70	94.05	67.43–109.85

The firmness of carrot hybrids before and after storage is summarized in Table 2. Samples of 'Nante/Bolero', 'Nante/Berlikum' and 'Nante/Forto' required higher force for cutting than fresh carrot samples. During storage, 1.05 times higher cutting force needed to be applied than for fresh carrots. This was expected, as carrots contain about 90% water, which gives rigidity to the texture. Significant differences ($P < 0.05$) during the whole period of storage in firmness of carrot hybrids were found.

DISCUSSION

The retention of vitamin C is often used as an estimation of the overall nutritional quality of vegetables (Gamboa-Santos *et al.*, 2012), as it is by far the least stable nutrient; it is highly sensitive to oxidation and leaching into water-soluble media during storage. It begins to degrade immediately

after harvest and degrades steadily during prolonged storage (Ajibola *et al.*, 2009). In the case of vitamin C, the range of observed values was very wide, reflecting the differences known to exist between various cultivars (Matejkova and Petrikova, 2010). In the present study carrot cultivars were autumn carrots; this is strongly evidenced by the higher total vitamin C content in Nante carrots (Leong and Oey, 2012). Vitamin C acts as an antioxidant in plants and its level is responsive to a variety of environmental or stress factors, for example light, temperature, salt and drought, atmospheric pollutants, metals or herbicides (Smoleń and Sady, 2009; Singh *et al.*, 2012). Literature reports the vitamin C content in carrot roots as 0.25 to 3.50 mg 100 g⁻¹ (Singh *et al.*, 2012); 5.70 mg 100 g⁻¹ (Schwedt, 2007); 4.40–6.50 mg · 100 g⁻¹ (Fineli, 2011), 7.00 mg · 100 g⁻¹ (Frede, 2006) and 8.00 mg · 100 g⁻¹ (Belitz, *et al.*, 2008). These levels are very similar with those of the present study. However, black carrot juice contains high amounts of ascorbic acid: 26.4 mg · 100 ml⁻¹ (Kirca *et al.*, 2007). Researchers from Turkey have reported that such results can be explained by individual hybrid properties, such as chemical composition, and growing conditions (Smoleń and Sady, 2009). Vitamin C is the L-enantiomeric form of ascorbic acid, which also encompasses the oxidation product of dehydroascorbic acid with a different oxidizing agent. Oxygen is the most destructive ingredient causing degradation of vitamin C in carrot. Many chemical reactions contribute to the loss of storage life of vitamin C and hence chemical deterioration of fruits. The majority of these reactions are enzymatically driven while others are chemical reactions that occur because of senescence (aging) processes (Ajibola *et al.*, 2009). The decrease in vitamin C content with storage duration was attributed to oxidation of ascorbic acid to dehydro-ascorbic acid by the enzyme ascorbic acid oxidase (Jany *et al.*, 2008). Matejkova and Petrikova (2010) reported vitamin C losses after 14 days storage at 4 °C to be by 15%. After 30 days of storage, there was an average decrease in vitamin C content by 47%.

Researchers from Thailand reported that the average fibre content in fresh carrots is 45.45 ± 0.41 g · 100 g⁻¹ (Chantaro *et al.*, 2008), which is slightly higher than the levels obtained in the present study (Fig. 2). Physiological effects of dietary fibre are greatly dependent on the physicochemical properties of the ingested material, e.g. the water-binding capacity, the molecular weight distribution and the viscosity. Another factor of great nutritional importance is plant cellular structure, which may also play a role in storage stability and sensory characteristics (Nyman and Svanberg, 2002). During storage, toughening takes place, associated with an increase of fibrousness, which devalues the quality of the final product. This quick hardening is mainly located in the basal portions of the spears, and is related to modifications of fibre components by deposition of lignin, cellulose and hemicelluloses (Rodriguez *et al.*, 2006).

In the study by Rashidi, soluble solids content was found to vary even more — from 8.5 to 12.5%, and soluble solid content was similar after four and six-month storage

(Rashidi, 2011). After four- and six-month storage, soluble solid content decreased on average from 1.48 to 1.46 times. A lower content of soluble solid content was observed after storage for all cultivars of carrots in a study conducted in Poland (Gajewski *et al.*, 2010). Soluble solids exert a profound influence on the storage period length, mechanical properties and quality characteristics of fruits and vegetables. It is known that, for example, when fruits are harvested unripe, although physiologically mature, they must be left to ripen (conversion of the stored starch into soluble solids) before consumption (Rakcejeva *et al.*, 2012).

Water uptake phenomena and the resulting moisture content change in food products affect their shelf-life through undesirable modifications of their physical, sensory and microbial qualities. Therefore, it is very important to determine the moisture content of carrots to predict shelf life in general (Rakcejeva *et al.*, 2012). However, there have been recent reports on texture increase in carrot tap roots during the first months of storage (Galindo *et al.*, 2004). In the pectic part of cell walls, the content of uronic acids and their degree of methylation, and hence ionic cross-linking, do not change during storage, and therefore, is a mechanism unlikely to have an effect on storage-induced firmness of carrots (Galindo *et al.*, 2004).

In conclusion, our results demonstrate that higher soluble solid content occurs in non-stored hybrids 'Nante/Berlikum', 'Nante/Maestro' and 'Nante/Bolero': 1.6 times higher compared with hybrid 'Nante/Forto' and 1.2 times higher compared with hybrid 'Nante/Champion'. Higher ascorbic acid content was found in the same hybrids, which was 2.0 times higher than in 'Nante/Forto' and 'Nante/Champion'. Significant differences were not found in dietary fibre content and firmness of the carrots before storage. Significant differences occurred in soluble solid content in hybrid 'Nante/Berlikum' during six-month storage — the content decreased 2.0 times; however, a non-significant soluble solid content decrease was found for the hybrid 'Nante/Forto'. Changes of hybrid firmness and dietary fibre amount were not significant during storage for four months. A decrease of ascorbic acid content of the analysed carrots hybrids during storage was 3.4 times compared with initial levels.

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FIZIKĀLI ĶĪMISKO PARAMETRU IZMAIŅAS 'NANTE' BURKĀNU HIBRĪDOS UZGLABĀŠANAS LAIKĀ TRADICIONĀLOS APSTĀKĻOS

Pētījuma mērķis bija izvērtēt vēlās burkānu šķirnes 'Nante' hibrīdu 'Nante/Forto', 'Nante'/Čempion', 'Nante/Berlikum', 'Nante/Maestro' un 'Nante/Bolero' fizikāli ķīmiskos rādītājus pēc ražas novākšanas un tradicionālajos uzglabāšanas apstākļos. Burkāni tika uzglabāti sešus mēnešus 8 ± 2 °C temperatūrā, $85 \pm 1\%$ relatīvā gaisa mitrumā. Kvalitātes parametru izmaiņas tika noteiktas, izmantojot standarta metodes: šķīstošā sausa (ar digitālo refraktometru), diētiskās šķiedrvielas (AOAC 985.29), vitamīns C (jodometrijas metode) un stingrība (TA.XT.plus Texture Analyser). Pētījumos analizēto burkānu hibrīdos vitamīna C saturs uzglabāšanas laikā samazinājās 3,4 reizes, salīdzinot ar sākotnējiem rādītājiem. Būtiskas atšķirības novērojām šķīstošās sausnas saturā vēlā burkānu šķirnes 'Nante/Berlikum' hibrīdos. Pēc sešu mēnešu uzglabāšanas tas samazinājās 2,0 reizes. Stingrības un diētiskās šķiedrvielas saturā būtiskas izmaiņas burkānu hibrīdos uzglabāšanas laikā līdz četriem mēnešiem netika konstatētas. Pētījumā iegūtie rezultāti dod pārskatu par Latvijā audzētu burkānu fizikāli ķīmisko sastāvu un to piemērotību minimālās apstrādes produktu ražošanai.

EVALUATION OF ROWANBERRY AND ROWANBERRY-PUMPKIN SAUCES

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Rowanberries (Sorbus aucuparia) are small orange-red fruits of a rowan tree. They have been described as an important source of flavonoids, and their antioxidant activity affects reactive oxygen species and lipid peroxidation. Cultivars of sweet rowanberries and hybrids with other species are sweeter and less astringent than wild rowanberries. The aim of the current study was to determine physical and chemical properties of sauces from various cultivars of wild and sweet rowanberries, and mixes of sweet rowanberries with pumpkins, and to assess their degree of acceptance. The experiments were carried out at the Faculty of Food Technology of the Latvia University of Agriculture. The sauces were made from purees of wild rowanberry and sweet rowanberry cultivars and purees of sweet rowanberry with apple or pumpkin using different species. The content of total dry matter, soluble solids, total carotene, pH, colour $L^ a^* b^*$ values and texture parameters were determined in samples of the sauces. The results showed large variability between the rowanberry and rowanberry-pumpkin sauces. The total dry matter content of sauce samples varied from 25.92 to 31.38%, the total carotene content from 3.94 ± 0.40 to 7.34 ± 0.57 mg 100 g^{-1} DM, and firmness from 0.5 to 2.2 N. According with sensory evaluation the panellists liked ($P < 0.05$) sauces samples made from rowanberry 'Michurinskaya krasnaya' puree and from 'Granatnaya' and pumpkin purees.*

Key words: rowanberry, sauce, physical and chemical parameters, sensory evaluation.

INTRODUCTION

Sauces and dressings are commonly used in the everyday life of many consumers. The main advantage of sauces is their ability to improve the taste of food. For example, hot sauces add a piquant taste to various dishes, meats, and vegetables; sweet sauces are favoured on pancakes, rice, desserts and ice-creams. Sweet and sour sauces find application in meat dishes and are very often applied in Asian kitchens. Dressings of different taste are commonly used in American kitchens, bringing greater attractiveness to meat dishes (Sikora *et al.*, 2008).

A variety of sauces, such as tomato, chilli, oyster, and white sauce, are used as seasoning to help enhance the taste of food. In general, colour, viscosity and stability are characteristics that contribute to the acceptance of a sauce. The main problem associated with quality of sauces is a tendency for separation of solids and liquid phase during storage. To prevent this the manufacturers usually use native starch (i.e. corn and rice starch), modified starch and hydrocolloids (i.e., gum, locust bean gum) as thickeners and stabilisers of sauces (Rengsutthi and Charoenrein, 2011).

Consumers are demanding high-quality healthy products, and therefore, it is very important to choose raw material

with high content of bioactive compounds. There is still much potential to improve product quality using new, non-traditional fruits and vegetables with high contents of bioactive compounds as raw materials for production of sauces. Such raw materials include also rowanberries and pumpkins.

Rowanberries (*Sorbus aucuparia* L.) belong to the subfamily *Maloideae* of the family *Rosaceae*. Their berries have been promoted as a health-food and can be a source for health-promoting components. Ripe wild rowanberries are picked in the autumn and they are eatable, but very tart in flavour and taste, although they contain lots of sugar. Rowanberries have been traditionally used to make purees, juices or wine, but their use as a food ingredient has been less popular because of their bitter taste (Wang, 2007). Cultivars of sweet rowanberries and hybrids with other species are sweeter and less astringent than wild rowanberries. According to food composition and nutrition tables, sweet rowanberry *S. aucuparia* L. var. *edulis* contains 98.0 mg 100 g^{-1} vitamin C and 2.5 mg 100 g^{-1} total carotenoids (Souci *et al.*, 2008). In comparison with wild rowanberries, the sugar content in fruits of cultivars of sweet rowanberries is 1.2–2.1 times higher (Navys, 2001). The content of reducing sugars (i.e. total amount of glucose and fructose) in

the cultivars has been estimated to be 5–18 g 100 g⁻¹ (Eder *et al.*, 1991; Souci *et al.*, 2008). The content of sorbitol, a sweetening agent that diabetics can tolerate, was high and varied from 3.5 to 12.0 mg 100 g⁻¹ (Eder *et al.*, 1991; Стрельцина и др., 2010).

Pumpkin is a vegetable that meets the requirements of healthy nutrition. It is a tasty and valuable vegetable crop, contains many biologically active substances and is distinguished for its dietary qualities. There are three common types of pumpkin world-wide: *Cucurbita pepo* L., *Cucurbita maxima* L. and *Cucurbita moschata* L. (Lee *et al.*, 2002). Pumpkins provide a valuable source of carotenoids and ascorbic acid, which have major roles in nutrition as provitamin A and as an antioxidant, respectively (See *et al.*, 2007). Pumpkins are consumed in a variety of ways, such as fresh or cooked vegetables, and products are commonly stored frozen or canned (Figueredo *et al.*, 2000).

The stability of many bioactive compounds is dependent on pre-treatment of the raw material, processing operations of the product, and storage conditions. In many fruits, carotenoids and flavonoids, which are located predominantly in epidermal tissues, are removed by peeling operations, which can greatly reduce concentrations of bioactive compounds in processed products. Removal of seeds can result in losses of phenolics (e.g. ellagitannins) (Howard, 2008). High temperature also causes the loss of important nutrients. At a temperature just above 50 °C, degradation of several phenolic compounds occurs (Rózek *et al.*, 2010). During thermal processing, 50–70% losses of ascorbic acid can occur. The losses of this vitamin can be used as an indicator of food quality. Carotenoids after thermal processing are more easily extracted from plant tissues due to tissue softening and destruction of the membrane-protein complex (Howard, 2008).

Colour is one of the most important parameters to which consumers are sensitive when selecting foods. Colour stability of fruit and fruit products is influenced by many factors. At least three factors can cause colour deterioration: the loss of red anthocyanin pigment, formation of brown pigments, and discoloration through various factors such as heavy metal contamination. The fruit cultivar, temperature, pH, presence of oxygen and time of processing were found to exert a great influence on colour stability of fruit products (García-Viguera *et al.*, 1998; Kopjar *et al.*, 2009).

The aim of current research was to determine physical and chemical properties, and degree of acceptance, of sauces made from various cultivars of rowanberries and from sweet rowanberries with pumpkins.

MATERIALS AND METHODS

Experimental design. The research was carried out at the Faculty of Food Technology, Latvia University of Agriculture, in 2012. The object of the research was rowanberry and rowanberry-pumpkin sauces from wild and sweet row-

Table 1

THE DESCRIPTION OF ROWANBERRY CULTIVARS USED FOR MAKING SAUCES

No	Rowanberry cultivar	Sort characteristics	Description of fruits
1	'Granatnaya'	Hybrid rowanberry × hawthorn (<i>Sorbus aucuparia</i> × <i>Crataegus sanguinea</i> Pallas)	Dark red or brown coloured fruits with sweet and sour taste
2	'Michurinskaya krasnaya'	Hybrid rowanberry × hawthorn (<i>Sorbus aucuparia</i> × <i>Crataegus sanguinea</i> Pallas)	Dark red coloured fruits with sweet and sour taste
3	'Moravica'	Moravian group variety of <i>Sorbus aucuparia</i>	Orange-red coloured fruits with sweet and sour taste
4	<i>Sorbus aucuparia</i>	Wild rowanberry	Orange or bright red coloured fruits with bitter taste

anberry cultivars and pumpkin (*Cucurbita maxima*) purees. Description of the used fruits of rowanberry cultivars are given in Table 1. The rowanberries were harvested in the Pure Horticultural Research centre. The pumpkin was obtained from the household "Alejas" (Ilzene's rural municipality, Latvia).

Rowanberry puree was prepared from frozen and thawed rowanberries that were pressed through a sieve, and pumpkin puree made from cut pieces of pumpkin, boiled (in 100 °C) and pressed through a sieve. The rowanberry sauces (further – samples A) were prepared according with the technological scheme given in Figure 1 and dried dill and basil was added. Rowanberry-pumpkin sauces (further – samples B) were prepared also according to this, but using rowanberry and pumpkin puree in weight ratio 1:1, and with addition of herbs: fresh garlic, cumin seeds, coriander seeds and dried rosemary (sample B1); dried ginger (sample B2); fresh garlic and dried basil (sample B3); and dried parsley, mustard seeds and cayenne pepper (sample B4).

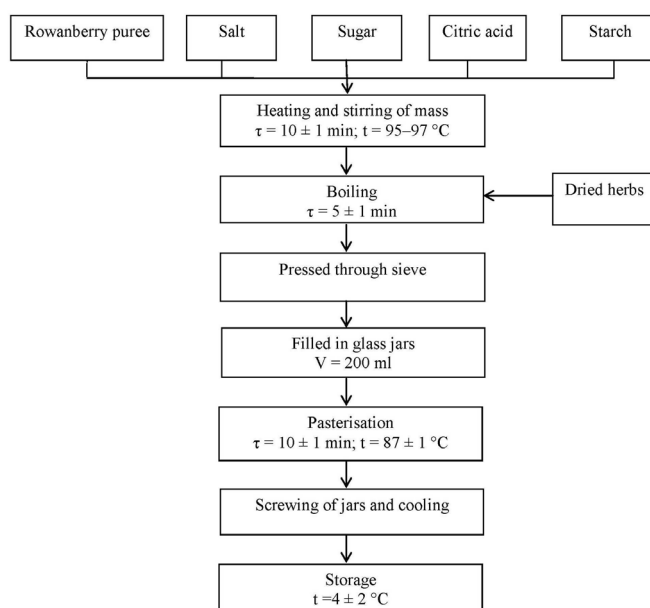


Fig. 1. Technological scheme of rowanberry sauces treatment

Four samples of rowanberry sauces and four samples of rowanberry-pumpkin sauces were prepared.

Analysis. The total dry matter content of sauce samples was determined after drying in an oven (Mattila *et al.*, 2006). A vacuum drying oven VD53 (Binder) and analytical scales BP-210s (Sartorius) were used. Measurements were carried out in three replications.

The soluble solid content ($^{\circ}\text{Brix}$) was measured using a digital refractometer Rx-5000 α (Atago) by standard method ISO 2173:2003. Measurements were carried out in five replications.

pH was measured by a pH-meter (FieldLabpH), using standard method LVS ISO 5542:2010. Measurements were carried out in three replications.

The total carotene content was determined by spectrophotometric method at 440 nm (Ермаков, 1987) after extraction with petroleum ether (boiling temperature range 80–110 $^{\circ}\text{C}$). An UV-VIS-NIR spectrophotometer UV-3100PC (Shimadzu) with 10 mm cuvettes was used. The carotene equivalent (KE) was estimated, using a calibration curve with $\text{K}_2\text{Cr}_2\text{O}_7$. Measurements were carried out in two replications for each sample.

The colour of sauces was measured in a CIE $L^*a^*b^*$ colour system using a ColorTec-PCM/PSM (Accuracy Micro-sensors Inc.). Before measurement, the colorimeter was calibrated using a white reference tile and a light trap (black tile). Ten random areas were measured through the plastic pockets and mean values were reported for each sample. Using this method, CIELAB coordinates show the degree of brightness (L), the degree of redness (+a) or greenness (–a), and the degree of yellowness (+b) or blueness (–b) (Coulate, 2002; Chakraborty *et al.*, 2011).

The textural analysis of sauces was conducted at room temperature (20 ± 2 $^{\circ}\text{C}$ temperature) with a Texture Analyser TA.XT.plus (Stable Micro Systems Ltd.) instrument according to the method described in literature (Bourne, 2002; Sikora *et al.*, 2007; Cardona *et al.*, 2010). The system was equipped with a compression cell of 50 kg and software Texture Exponent 32. A back extrusion test was carried out in a cylindrical container with 50-mm internal diameter filled with sauce, in which the 40 mm diameter compression disc with an extension bar moved with a speed of 2 mm s^{-1} , at distance 20 mm. Analyses were triplicated. The system was equipped with a compression cell of 50 kg and software Texture Exponent 32. A back extrusion test was carried out in a cylindrical container with 50-mm internal diameter filled with sauce, in which the 40 mm diameter compression disc with an extension bar moved with a speed of 2 mm s^{-1} , at distance 20 mm. Analyses were triplicated.

Sensory evaluation of the sauce samples was performed in the Laboratory of Sensory Evaluation at the Faculty of Food Technology of the Latvia University of Agriculture. All sauce samples were evaluated by 25 trained panellists

(18 females and 7 males, mean age 32). Rowanberry and rowanberry-pumpkin samples were evaluated by degree of liking using a 9-point hedonic scale. The 9-point hedonic scale (9 – extremely like, 5 – neither like nor dislike, and 1 – extremely dislike) methods used were based on ISO 4121:2003 “Sensory analysis – Guidelines for the use of quantitative response scales”.

The results are expressed as means \pm standard deviations. Significant differences were tested by analysis of variance (ANOVA) and the Tukey’s test. Statistical differences with $P < 0.05$ were considered as significant.

RESULTS

The physical parameters (the content of total dry matter and soluble solids, and pH) of the rowanberry sauce samples are given in Table 2. There were significant differences ($P \leq 0.005$) in the total dry matter content and in the soluble solids among the samples of sauce samples. This can be explained by the use of different rowanberry cultivars and different composition of recipes for sauce samples A and B.

The rowanberry and rowanberry-pumpkin sauce samples had pH levels ranging between 2.68 ± 0.01 and 3.23 ± 0.01 (Table 2), the levels significantly different between sauces ($P < 0.05$).

Total carotene content significantly differed ($P < 0.005$) between the rowanberry and rowanberry-pumpkin sauces (see Fig. 2). The highest total carotene content ($7.75 \pm 0.51 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) was in sample A2 (made from ‘Michurinskaya krasnaya’ puree), and the lowest ($3.94 \pm 0.40 \text{ mg } 100 \text{ g}^{-1} \text{ DW}$) in sample B4 (made from ‘Moravica’ and pumpkin purees). The content of total carotene of rowanberry-pumpkin sauces was lower (23.0–32.7%) than that of rowanberry sauce made only from cultivars of rowanberry. The content of total carotene of sauces was

Table 2
THE PHYSICAL PARAMETERS OF THE ROWANBERRY AND ROWANBERRY-PUMPKIN SAUCE SAMPLES

Sample designation	Total dry matter, %	Soluble solids content, $^{\circ}\text{Brix}$	pH
A1	30.65 ± 0.12^a	28.30 ± 0.16^a	2.92 ± 0.04^c
A2	28.35 ± 0.17^b	25.29 ± 0.11^c	3.23 ± 0.01^a
A3	26.35 ± 0.13^c	23.44 ± 0.10^d	2.98 ± 0.01^b
A4	31.38 ± 0.16^a	26.95 ± 0.05^b	3.02 ± 0.01^b
B1	25.92 ± 0.46^c	21.05 ± 0.03^d	2.89 ± 0.07^a
B2	27.03 ± 0.11^b	22.42 ± 0.02^b	3.00 ± 0.01^a
B3	27.16 ± 0.45^b	21.52 ± 0.06^{ca}	2.95 ± 0.01^a
B4	30.18 ± 0.25^a	26.30 ± 0.05^a	2.68 ± 0.01^b

* – values, marked with the same superscript letters in a column, are not significantly different ($P > 0.05$).

Rowanberry cultivars used for sauce preparation: A1 – ‘Granatnaya’; A2 – ‘Michurinskaya krasnaya’; A3 – ‘Moravica’; A4 – wild rowanberry *S. aucuparia*; B1 – ‘Michurinskaya krasnaya’; B2, B3 – ‘Granatnaya’; B4 – ‘Moravica’.

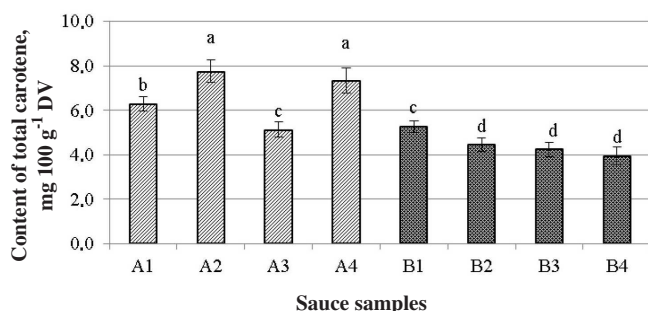


Fig. 2. Content of total carotene of rowanberry and rowanberry-pumpkin sauces

* – values, marked with the same small letters in a column, are not significantly different ($P > 0.05$).

Rowanberry cultivars used for sauce preparation are described below Table 2.

lower when rowanberry puree was mixed with pumpkin puree.

The results of colour L^* a^* b^* measurements of the rowanberry and rowanberry-pumpkin sauces are shown in Table 3. The colour values significantly differed between samples ($P < 0.005$). Rowanberry sauce sample A1 (made from 'Granatnaya' puree) had the darkest colour ($L^* = 25.98 \pm 1.73$) and sample A2 (made from 'Michurinskaya krasnaya') was the reddest sauce sample ($a^* = 24.49 \pm 1.84$). The fruits of these cultivars have dark red colour. Rowanberry-pumpkin sauce sample B4 (prepared from orange-red coloured rowanberries of 'Moravica' and pumpkin puree in proportion 50:50) was the most yellow ($b^* = 40.05 \pm 4.12$).

The textural parameters of the rowanberry sauces are shown in Table 4. Firmness and cohesiveness as well as consistency and index of viscosity of the sauce samples significantly differed among the samples ($P < 0.005$). Sauce sample B4 (made from 'Moravica' and pumpkin purees) had high values for all of these parameters. Firmness of

Table 3

COLOUR L^* a^* b^* VALUES OF ROWANBERRY AND ROWANBERRY-PUMPKIN SAUCES

Sample designation	Colour L^* a^* b^* values		
	L^*	a^*	b^*
A1	$25.98 \pm 1.73a$	$18.86 \pm 2.57b$	$19.09 \pm 2.29b$
A2	$29.06 \pm 2.52a$	$24.49 \pm 1.84c$	$30.24 \pm 1.24a$
A3	$43.88 \pm 1.73c$	$13.22 \pm 1.42a$	$30.13 \pm 2.21a$
A4	$36.01 \pm 2.78b$	$15.50 \pm 1.75ab$	$21.67 \pm 2.34b$
B1	33.10 ± 2.02	17.29 ± 0.74	25.94 ± 1.35
B2	30.80 ± 1.94	16.31 ± 1.07	27.72 ± 3.34
B3	29.25 ± 2.41	17.70 ± 1.24	26.53 ± 2.53
B4	42.58 ± 1.48	13.74 ± 0.41	40.05 ± 4.12

* – values, marked with the same small letters in a column, are not significantly different ($P > 0.05$).

Rowanberry cultivars used for sauce preparation are described below Table 2.

Table 4

TEXTURAL PARAMETERS OF THE ROWANBERRY AND ROWANBERRY-PUMPKIN SAUCES

Sample designation	Firmness, N	Consistency, N s	Cohesiveness, N	Index of Viscosity, N s
A1	0.61 ± 0.03^b	5.36 ± 0.23^b	-0.66 ± 0.06^a	-1.11 ± 0.14^a
A2	0.49 ± 0.06^b	4.24 ± 0.45^b	-0.44 ± 0.05^a	-0.82 ± 0.21^a
A3	2.15 ± 0.07^a	18.26 ± 0.54^a	-2.89 ± 0.11^c	-4.09 ± 0.10^c
A4	1.85 ± 0.17^a	15.38 ± 1.03^a	-1.81 ± 0.09^b	-2.66 ± 0.26^b
B1	1.75 ± 0.18	13.55 ± 1.30	-1.79 ± 0.23	-2.16 ± 0.13
B2	1.56 ± 0.09	12.02 ± 0.95	-1.60 ± 0.18	-2.08 ± 0.12
B3	1.39 ± 0.09	12.26 ± 0.74	-1.83 ± 0.13	-2.49 ± 0.11
B4	3.11 ± 0.23	19.73 ± 1.18	-3.14 ± 0.31	-2.98 ± 0.16

* – values, marked with the same small letters in a column, are not significantly different ($P > 0.05$).

Rowanberry cultivars used for sauce preparation are described below Table 2.

rowanberry and rowanberry-pumpkin sauce samples varied from 0.49 ± 0.06 (sample A2, made from 'Michurinskaya krasnaya' puree) to 3.11 ± 0.23 N (sample B4, made from 'Moravica' and pumpkin purees).

The hedonic evaluation scores of the rowanberry sauce are summarised in Figure 3. The results of the ANOVA showed significant differences ($F_{\text{calc}} = 22.60 > F_{\text{crit}} = 2.72$) in the degree of liking among the rowanberry sauce samples. The hedonic scores of rowanberry sauce samples were within the scale interval from „dislike moderately” to “like slightly” (3.0–6.0). There was no significant differences between samples A1 (made from 'Granatnaya' puree) and A2 (made from 'Michurinskaya krasnaya' puree); these sauces had lower bitterness, which the panellists liked better (see Fig. 3). The panellists liked sauce sample A4 made from wild rowanberry *S. aucuparia* puree) the least ($P < 0.05$), as it was too bitter and the used mountain ash gave an expressed aftertaste. Some of the panellists noted sample A1 (made from 'Granatnaya' puree) as bitter and unacceptable, but for other panellists this sauce was acceptable. Based on the results of sensory evaluation, we can conclude that wild rowanberries *S. aucuparia* fruits, which have a

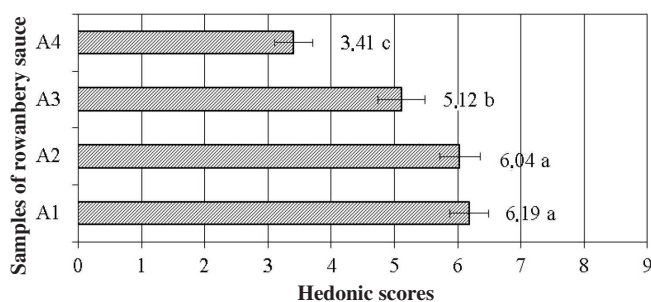


Fig. 3. Degree of liking of rowanberry sauces samples.

* – values, marked with the same small letters in a column, are not significantly different ($P > 0.05$).

Rowanberry cultivars used for sauce preparation are described below Table 2.

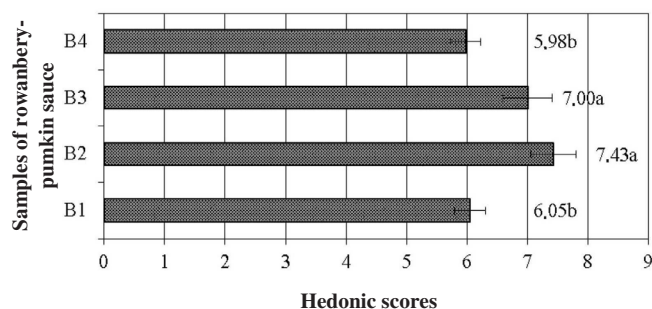


Fig. 4. Degree of liking of rowanberry-pumpkin sauces.

* – values, marked with the same superscript letters in a column, are not significantly different ($P > 0.05$).

Rowanberry cultivars used for sauce preparation are described below Table 2.

distinct bitter taste, should not be recommended for sauce preparation.

ANOVA shows that the hedonic scores of rowanberries-pumpkin sauces significantly different between samples ($F_{\text{calc}} = 7.87 > F_{\text{crit}} = 2.67$). The panellists liked rowanberry-pumpkin sauces samples B2 and B3 (both made from ‘Granatnaya’ and pumpkin purees), and between these samples there was no significant difference (Fig. 4).

DISCUSSION

In comparison with content of total dry matter of rowanberry and rowanberry-pumpkin sauce samples (25.92–31.38 %; Table 2), the content in commercial ketchup samples, which are sweetened with sucrose, and light ketchup, sweetened with aspartame, was 31.21 ± 0.18 % and 27.21 ± 0.27 %, respectively (Bannwart *et al.*, 2008). The content of soluble solids of rowanberry and rowanberry-pum Table 2) was similar to that of chili sauce, which ranges from 22.80 ± 0.28 to 23.65 ± 0.21 °Brix (Rengsutthi and Charoenrein, 2011), and with the content of soluble solids of light ketchup — 26.68 ± 0.46 % (Bannwart *et al.*, 2008).

Among the parameters analyzed for the sauce quality, pH is very important because acidity influences the thermal processing conditions required for producing safe products. However, pH of tomato products not exceeding $\text{pH} < 4.5$ are generally classified as acid foods (pH 4.5), which require moderate conditions of processing to control microbial spoilage and enzyme inactivation (Hayes *et al.*, Lehkoživová *et al.*, 2009). The rowanberry and rowanberry-pumpkin sauce samples had lower pH and can be classified as a high acid food item, such as tomato ketchup (Bannwart *et al.*, 2008) and chilli sauce (Rengsutthi and Charoenrein, 2011).

The results showed that colour L^* and b^* values of sauces was increased by the replacing of part of rowanberry puree with pumpkin puree. The rowanberry-pumpkin sauce samples had lighter colour and more yellowness than rowanberry sauces.

Firmness of sauces was increased by replacing of part of rowanberry puree with pumpkin puree. In comparison with rowanberry sauce, rowanberry-pumpkin sauce was prepared with less salt and sugar. The parameters of sauce consistency, cohesiveness and index of viscosity were also higher. According to sensory evaluation remarks, the panellists did not like firmness of sauce samples A3, A4 and B3 (in range from 1.85 ± 0.17 to 3.11 ± 0.23 N).

According to panellists, the evaluated sauces were sweet and salty, and they did not like colour of sample A3 (made from ‘Moravica’ puree), which was made from orange-red coloured rowanberries.

To obtain a softer taste and structure that would have a typical ketchup consistency, the panellists recommended a mix of several rowanberry cultivars or addition of other fruit puree.

The summary of sensory evaluation questionnaires and the colour L^* , a^* and b^* values of rowanberry sauces indicated that the panellists liked colour of samples A1 (made from ‘Granatnaya’ puree) and A2 (made from ‘Micurinskaya krasnaya’ puree). It is possible that the red colour of the sauce is acceptable, as it has the colour of tomato and cranberry sauce. Sample A3 (made from ‘Moravica’ puree) was liked best by panellists (Fig. 3), but the colour of this sauce was not acceptable to them. This can be explained by the available and most popular sauces appearance in the market, i.e. the colour of the brown-orange sauce was “alien” for consumers.

The conclusions are:

1. There were significant differences ($P < 0.01$) in physical and chemical parameters among samples of rowanberry and rowanberry-pumpkin sauces. The total dry matter content of sauce samples varied from 25.92 ± 0.46 to 31.38 ± 0.16 %, soluble solids from 21.05 ± 0.03 to 28.30 ± 0.16 °Brix, and pH from 2.68 ± 0.01 to 3.23 ± 0.01 .
2. Total carotene content differed ($P = 0.000$) between samples of rowanberry and rowanberry-pumpkin sauces, and varied from 7.34 ± 0.57 mg 100 g^{-1} DW (made from ‘Michurinskaya krasnaya’ puree) to 3.94 ± 0.40 mg 100 g^{-1} DW (made from ‘Moravica’ and pumpkin purees).
3. Sensory evaluation showed that wild rowanberries *S. aucuparia* fruits, which have a distinct bitter taste, should not be recommended for sauce preparation. The sensory evaluation of sauce samples from rowanberry × hawthorn hybrid ‘Granatnaya’ and pumpkin purees showed that this sample had the highest value (7.43).
4. There were significant differences ($P = 0.000$) in colour L^* , a^* , b^* values among the sauce samples. Colour L^* values varied between 25.98 ± 1.73 and 43.88 ± 1.73 , and the darkest sauce was from rowanberry × hawthorn hybrid ‘Granatnaya’.

5. The firmness and cohesiveness as well as consistency and index of viscosity of the sauce samples significantly differed ($P = 0.000$) among samples, and these texture parameters were higher in the sauce made from rowanberry puree with pumpkin puree. The firmness of sauce samples varied from 0.49 ± 0.06 (sample A2, made from 'Michurinskaya krasnaya' puree) to 3.11 ± 0.23 N (sample B4, made from 'Moravica' and pumpkin purees).

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PĪLĀDŽU UN PĪLĀDŽU-ĶIRBJU MĒRČU NOVĒRTĒJUMS

Pilādži (*Sorbus aucuparia*) ir mazi oranžsarkanas krāsas pilādžu koku augļi, kas literatūrā raksturoti kā nozīmīgs flavonoīdu avots, un to antioksidatīvā aktivitātē ietekmē reaktīvo skābekli un lipīdu peroksidāciju. Pilādžu šķirņu un to starpģinšu hibrīdu augļi ir saldāki un ar mazāk savelkošu garšu nekā savvaļas pilādži. Pētījumu mērķis bija izvērtēt dažādu šķirņu pilādžu un pilādžu-ķirbju mērces paraugu fizikālās un ķīmiskās īpašības, kā arī izpētīt to patikšanas pakāpi. Eksperimenti tika veikti Latvijas Lauksaimniecības universitātes Pārtikas tehnoloģijas fakultātē. Mērces tika pagatavotas no savvaļas un dažādu šķirņu saldo pilādžu biezēņiem, kā arī no saldo pilādžu un ķirbju biezēņiem. Mērces paraugiem noteikts kopējā sausnas, šķīstošās sausnas un kopējo karotīnu saturs, pH, krāsas $L^* a^* b^*$ vērtības un struktūras parametri, kā arī novērtēta paraugu patikšanas pakāpe, izmantojot hedonisko skalu. Pētījuma rezultāti parādīja, ka būtiski atšķiras pētīto pilādžu un pilādžu-ķirbju mērces paraugu rādītāji. Tika noteikts, ka pilādžu un pilādžu-ķirbju mērces paraugu kopējās sausnas saturs bija 25.92–31.38%, kopējo karotīnu saturs – no 3.94 ± 0.40 līdz 7.34 ± 0.57 mg 100 g⁻¹ sausnas, un cietība – 0.5–2.2 N. Saskaņā ar sensorās novērtēšanas rezultātiem, vērtētājiem vislabāk bija pieņemami ($P < 0.05$) mērces paraugi, kas tika pagatavoti no pilādžu šķirņu ‘Michurinskaya Krasnaya’ un ‘Granatnaya’ biezēņiem, daļu pilādžu biezeņa aizstājot ar ķirbju biezeni.

DETERMINATION OF OXALATE AND SOME INORGANIC ANIONS IN GREEN AND BLACK TEA

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Communicate by Maija Dambrova

Oxalate concentration differs in various daily consumed food products. The role of oxalic acid in the human body is very significant, as its compounds are responsible for the stability of biological membranes. However, insoluble calcium and magnesium oxalates can be accumulated in the body in the form of kidney stones. Oxalate concentration has been measured by high performance liquid, gas after derivatization and ion chromatography (IC). The most effective method for the simultaneous determination of oxalate and inorganic anions is ion chromatography with conductometric detection. Here, we report the results of the measurement of oxalic acid in bleak and green tea samples. Separation was performed by IC on an anion-exchange column Shodex IC SI-90 with surface-layer sorbent and conductimetric detection. The main analytical features of the method were: limit of detection of oxalic acid 0.03 mg/l, linear range 0.1–20 mg/l, correlation 0.9998, relative standard deviation 1%. The method did not need specific sample treatment and was successfully applied to the analysis of black and green tea samples. Oxalic acid was determined in the ranges 16.7–84 mg/l for green tea and 63–116 mg/l for black tea. Green tea contained lower oxalate ions concentration than black tea. The IC method has a lower detection limit for oxalate ions than HPLS and GC, ten and two times less, respectively.

Key words: tea, oxalic acid, ion chromatography, kidney stones.

INTRODUCTION

Oxalate can be found in food products such as sorrel, chocolate, cacao and tea (Schroder *et al.*, 2011). After water, tea is the second most popular beverage, but it contains high levels of oxalic acid (Sang *et al.*, 2011). In the human body, oxalic acid is formed by the metabolism of glycine and ascorbic acid (Rassam *et al.*, 2005). The role of oxalic acid in the human body is very significant, because its compounds are responsible for the stability of biological membranes (Melnik, 1999). However, insoluble calcium and magnesium oxalates may be accumulated in the body in the form of kidney stones. Therefore, it is necessary to control the concentration of oxalic acid in food and the human body (Hodgkinson *et al.*, 1968). Nowadays, oxalate ions can be identified in complex objects using titration, colorimetry, capillary electrophoresis (CE) (Fu *et al.*, 1999), chemiluminescence (Rubinsteln, 1983), high performance liquid chromatography (HPLC), gas (GC) (Kawamura *et al.*, 2010) and ion chromatography (IC) (Lachenmeir *et al.*, 2005). However, these methods have disadvantages. CE has low reproducibility due to migration times of ions. GC involves difficult sample preparation. Since oxalic acid is a strong organic acid ($pK_{a_1} = 1.27$; $pK_{a_2} = 3.80$), the most ef-

fective method for the determination of oxalate ions is IC with conductometric detection (Geng *et al.*, 2008). In this work, an approach for the simultaneous determination of oxalate, chloride, nitrate, phosphate, sulphate ions is proposed and applied to the analysis of these compounds in tea samples of different brands and manufacturers. We aimed to identify the optimal conditions for determination of oxalate ions in drinks, to determine interference of inorganic anions, describe the comparative characteristics of the method and to apply the method for determination of oxalate ions in tea samples.

MATERIALS AND METHODS

The chromatographic system HPLC LC-20 Prominence (Shimadzu, Japan) and conductivity detector were used. An anion-exchange column Shodex IC SI-90, 250 mm × 4 mm, 9 µm of particle size and column was filled with KanK-ASt, 120 mm × 5 mm, 14 µm of particle size. The column oven was maintained at 33 °C. Loop volume was 20 µl.

An isocratic elution was performed, using a mobile phase of 1.9 Na₂CO₃+2.4 mM NaHCO₃ at a flow rate of 1.5 ml/min,

and a column was filled with KanK-AS_t and 1.8 mM Na₂CO₃+1.7 mM NaHCO₃ at a flow rate of 1.0 ml/min for column Shodex.

Oxalic acid dihydrate, sodium bicarbonate, sodium digidrokarbonat were HPLC grade. Sodium chloride, sodium nitrate, sodium phosphate, sodium fluoride were analytical grade. Water was purified with an aquaMAXTM Ultra 370 Series – Ultra Water Purification System (Young Lin Instrument Co, Korea). Standard solutions were prepared and diluted in deionised water. Oxalic acid solution was daily diluted.

Samples of black and green tea were purchased from Krasnoyarsk outlets, produced by Russia, Ceylon, China. Assay solutions were obtained by refluxing 2.0 g of tea with hot water (100 °C) for 6 min. The obtained extract was filtered through a disposable 0.45 µm filter, diluted 10 times and injected into the chromatographic system.

RESULTS

In preliminary experiments, the conditions for the separation of the most common inorganic anions (fluoride, chloride, phosphate, nitrate, sulfate) in biological and food products were optimised. It was found out that the best separation conditions for the chromatographic system using a column filled with KanK-AS_t was obtained with eluent consisting of 1.9 mM Na₂CO₃ + 2.4 mM NaHCO₃ at a flow rate of elution of 1.5 ml/min. The retention time of oxalate ions in these conditions was $t_R = 13.73 \pm 0.01$ min. Previous reports (Rassam *et al.*, 2005) and the experimental data suggested interference from sulfate ions on oxalate ion determination. A chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions is shown in Figure 1. Resolution of the peaks was $R_s = 1.16$.

In these conditions quantitative identification of oxalate is unreasonable. Therefore, the effects of different factors on the degree of separation of the peaks were studied. The in-

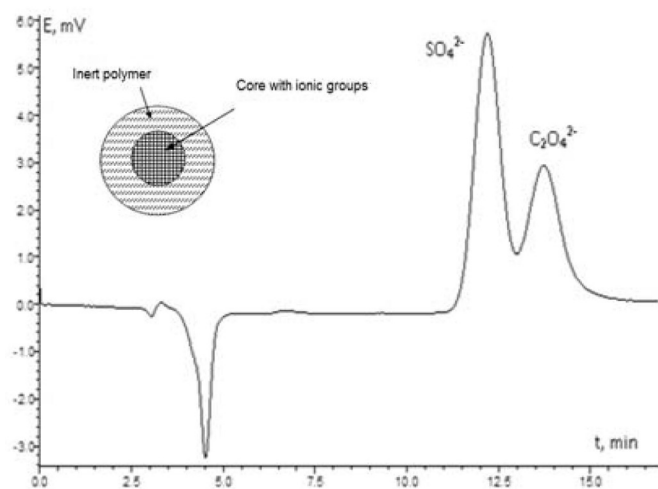


Fig. 1. Chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions. Conditions: column was filled with KanK-AS_t bipolar central-localised sorbent; eluent 1.9 mM Na₂CO₃ + 2.4 mM NaHCO₃; flow rate 1.5 ml/min.

fluence of various factors (flow rate and composition of the eluent) on the degree of separation of the peaks were studied by a full factorial experimental design. The data showed that a column was filled with KanK-AS_t gave the best results, but was not sufficient to separate of sulfate and oxalate ions. Sorbent KanK-AS_t is a bipolar central-localised sorbent, in which the act of sorption and desorption runs inside the grain. The diffusion inside the grain when anions are strongly retained anions, multiply charged and when hydrated ions have large size, is very difficult (Dolgonosov, 1984; Dolgonosov *et al.*, 1988). This can explain the observed eroding peaks. Sulfate and oxalate ions have similar constants of ion exchange and retention times, and probably for this reason a satisfactory separation of sulfate and oxalate ions in this system is impossible.

The anion-exchange column Shodex IC SI-90 with surface-layer sorbent was also applied for the separation of sulfate and oxalate ions. Under the selection experimental conditions, the retention time for the oxalic ions was 15.83 ± 0.01 min. Figure 2 shows a chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions. The selectivity of the column Shodex IC SI-90 is correct, as because the peaks showed resolution 1.5 for all of the determined compounds. In further work, we used a column Shodex IC SI-90.

Table 1 gives the resolutions of sulphate and oxalate ions at different concentrations. The column Shodex allowed to separate the peaks at all sulphate:oxalate ratios used.

The accuracy of the method was evaluated from recovery assays, preparing spiked samples of tea in triplicate at three levels of concentrations higher than the limit of quantitation (LOQ). The obtained values ranged between 98% and 102% (Table 2). The linearity of the analytical response, calibration curve, limit of detection (LOD) and LOQ were examined (Table 3).

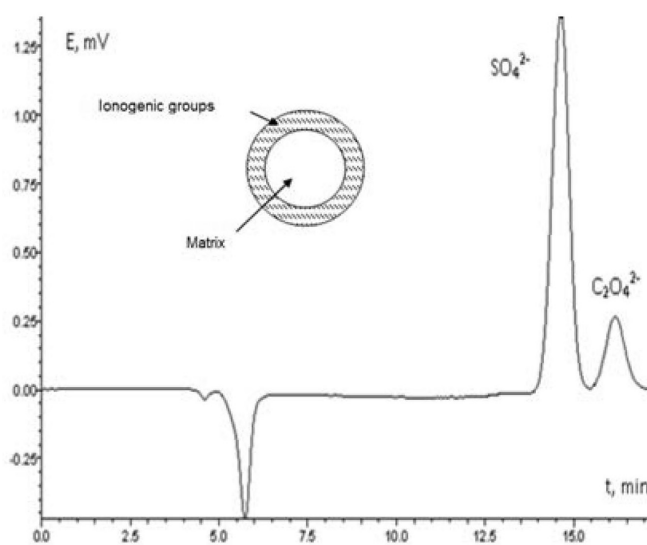


Fig. 2. Chromatogram of a standard mixture of equivalent amounts of sulfate and oxalate ions. Conditions: column Shodex IC SI-90 with surface-layer sorbent; eluent 1.8 mM Na₂CO₃ + 1.7 mM NaHCO₃; flow rate 1.0 ml/min; $R_s = 1.64$.

Table 1

RESOLUTIONS OF SULPHATE AND OXALATE IONS AT DIFFERENT RATIOS OF CONCENTRATIONS

Relation of concentrations $\text{SO}_4^{2-}/\text{C}_2\text{O}_4^{2-}$	Resolution, R_s	Concentration ratio $\text{SO}_4^{2-}/\text{C}_2\text{O}_4^{2-}$	Resolution, R_s
1	1.64	20	1.58
2	1.61	30	1.13
5	1.59	50	1.01

Table 2

RESULTS OF RECOVERY OF OXALIC ACID IN TEA SAMPLES

Added, mg/l	Found, mg/l	Recovery, %
5.0	4.9	98.0
10.0	10.2	102.0
20.0	19.7	98.5

Table 3

RESULTS OF SENSITIVITY AND LINEAR RANGE OF OXALATE ANIONS

Migration time, min	Linearity range, mg/l	Calibration curve	r	LOD, mg/l	LOQ, mg/l
15.83 ± 0.01	0.1–20.0	$C = 0.0002 \cdot S + 0.04$	0.9998	0.03	0.1

To check the precision of the method, seven replicate analysis of a standard solution were performed on different days. The precision expressed as R.S.D. remained 1%.

Chloride, nitrate, phosphate, sulphate and oxalate ions were determined in tea samples using the method proposed. The

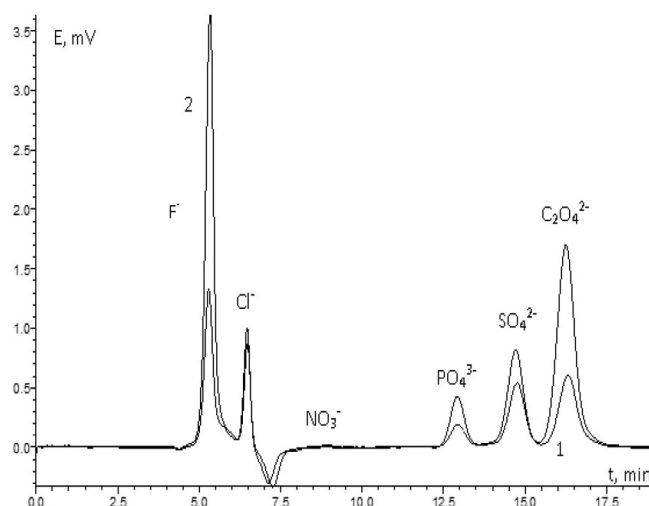


Fig. 3. Chromatograms of green (1) and black (2) tea samples brand "Tess". Conditions: column Shodex IC SI-90; eluent 1.8 mM Na_2CO_3 + 1.7 mM NaHCO_3 ; flow rate 1.0 ml/min.

presence of these compounds was confirmed by comparing their retention times with those of standard solutions. The results are summarised in Table 4 and the chromatograms of the green and black tea samples are shown in Figure 3.

DISCUSSION

As can be observed in chromatograms of samples the fluoride ion peak has an asymmetric shape, indicating the presence of several anions having similar retention times with fluorine; therefore, the separation between these peaks could not be obtained. According to the literature it is

Table 4

CONTENTS OF ANIONS IN TEA SAMPLES

Sample	Class tea	Concentration of ions (average \pm standard error) *, mg/l				
		Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	$\text{C}_2\text{O}_4^{2-}$
Rowford	Black	45 ± 2	1.48 ± 0.04	38 ± 2	42 ± 2	89 ± 4
	Green	28 ± 1	0.95 ± 0.03	25 ± 1	46 ± 2	53 ± 2
Riston	Black	31 ± 1	Nd	42 ± 2	42 ± 2	99 ± 5
	Green	20 ± 1	1.11 ± 0.03	35 ± 1	39 ± 2	84 ± 4
Ahmad	Black	23 ± 1	Nd	24 ± 1	21 ± 1	63 ± 3
	Green	14.5 ± 0.7	0.24 ± 0.01	6.7 ± 0.3	22 ± 1	34 ± 2
Princess Nuri	Black	30 ± 1	0.85 ± 0.02	45 ± 2	46 ± 2	98 ± 5
Princess Yava	Green	28 ± 1	1.37 ± 0.04	28 ± 1	60 ± 3	66 ± 3
Tess	Black	35 ± 1	0.34 ± 0.01	41 ± 2	43 ± 2	113 ± 5
	Green	26 ± 1	0.44 ± 0.01	18.9 ± 0.9	44 ± 2	43 ± 2
Indu	Black	29 ± 1	0.35 ± 0.01	38 ± 2	48 ± 2	114 ± 5
	Green	21 ± 1	0.72 ± 0.02	14.8 ± 0.7	37 ± 2	50 ± 2
Curtis	Black	26 ± 1	Nd	50 ± 2	55 ± 2	116 ± 5
	Green	15.6 ± 0.8	0.56 ± 0.02	17.9 ± 0.9	46.9 ± 2.3	16.7 ± 0.8
Da Hong Pao	Black	35 ± 1	0.51 ± 0.02	25 ± 1	37 ± 2	64 ± 3
Tie Guan Yin	Green	16.5 ± 0.8	0.25 ± 0.01	9.5 ± 0.5	28 ± 1	21 ± 1

*Average of five analysis

Nd, not detected

known that these ions can be acetate and formate. Development of a method for quantification of fluoride ions requires further research. The presence of nitrate, phosphate, sulfate ions in tea samples can be explained by the use of various fertilisers and pesticides in cultivation. The most common class of pesticides is organochlorine and organophosphorus compounds. They easily dissolve in rain water and enter the soil. They are the most dangerous substances, causing of cancers, allergies and other diseases (Flaten, 2002; Tatarchenko *et al.*, 2003).

Oxalic acid was present in the ranges 16.7–84 mg/l for green tea and 63–116 mg/l for black tea. Green tea contained lower oxalate ions concentration than black tea. It may be because green tea lacks oxidative processes. The lowest amount of oxalate ions was found in black tea brand Ahmad tea (black sheet tea “English breakfast”, Ceylon) (63±3 mg/l) and green tea brand Curtis “Original green tea” (Chinese green long leaf tea, Russia) (16.7 ± 0.8 mg/l). A high concentration of phosphate ions in all samples was indicated.

Finally, the approach developed was compared with other existing methods (Table 5). Table 5 shows that the IC method has a lower detection limit for oxalate ions than HPLS and GC, ten and two times less, respectively.

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Table 5

COMPARISON OF METHODS FOR DETERMINATION OF OXALIC ACID

Method of determination	Detection time, min	Linearity range, mg/l	LOD, mg/l	R.S.D., %	References
HPLC	3	-	0.3	-	(Judprasong, 2006)
CE	6	0.05–90.00	0.01	4	(Fu, 1999)
GC	15	0.2–5.0	0.06	1.3	(March, 2003)
IC	20	0.1–20.0	0.03	1	Approach developed

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OKSALĀTU UN DAŽU NEORGANISKO ANJONU NOTEIKŠANA ZAĻAJĀ UN MELNAJĀ TĒJĀ

Skābeņskābei ir nozīmīga loma cilvēka organismā, jo tās atvasinājumi ietekmē bioloģisko membrānu stabilitāti. Tomēr nešķīstoši kalcija un magnija oksalāti var uzkrāties organismā nierakmeņu formā. Oksalātu koncentrācijas mērījumiem izmantoja augstefektīvo šķidrums hromatogrāfiju, gāzhromatogrāfiju un jonu hromatogrāfiju. Jonu hromatogrāfija ar konduktometrisku detektoru bija visefektīvākā metode oksalātu un neorganisko anjonu noteikšanai. Pētījums veltīts skābeņskābes mērījumiem melnās un zaļās tējas paraugos. Sadalīšanai izmantoja jonu hromatogrāfu ar anjonu apmaiņas kolonnu *Shodex IC SI-90* ar virsmas slāņa sorbentu un konduktometrisku detektoru. Skābeņskābes noteikšanas robeža bija pie 0,03 mg/l, lineārais apgabals no 0,1–20 g/l, korelācija 0,9998, relatīvā standartnovirze 1%. Metodei nebija vajadzīga specifiska paraugu apstrāde, un to labi varēja izmantot melnās un zaļās tējas paraugu analīzei.

ANTIOXIDANT CHARACTERISTICS OF LATVIAN HERBAL TEA TYPES

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*The aim of the study was to characterise antioxidative properties and antiradical activity of the herbal tea collection in Latvia. High-pressure liquid chromatography and spectrophotometry methods were used to characterise antioxidant properties of herbal tea. Antiradical activity was determined spectrophotometrically. The antiradical scavenging activity was measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) reaction. The herbal tea antiradical scavenging activity was estimated as the broken down quantity of DPPH on 100 grams of the herbal tea. Individual polyphenols in the herbal tea were identified and determined by liquid chromatography. Antiradical scavenging activity of the herbal tea was found to be $104 \text{ mol } 100 \text{ g}^{-1} \text{ tea}$. The following polyphenols were identified chromatographically in herbal tea: gallic, caffeic, chlorogenic, ferulic acids, rutin, catechin, vanillin, and epicatechin. Altogether 12 different varieties of herbal tea samples were analyzed. The results showed that different types of herbal tea substantially differed in composition. Polyphenol content of herbal teas was in the range of 1 mg in 100 g of tea up to 8 g per 100 g of tea. In the case of virus-related disease, infections and weakness of the organism, it is recommended to use tea with high content of rutin, as in *Verbascum thapsiforme* Schr., *Alchemilla vulgaris* L., *Comarum palustre* L. herbal tea.*

Key words: *herbal tea, polyphenols, antiradical scavenging activity.*

INTRODUCTION

Herbal teas are widely used in traditional medicine. Antioxidant effect is one of the major descriptive characteristics of organic and medicinal activity.

In the body mitochondrial activity, during metabolism or as a result of environmental factors, such as pollution, radiation, smoking, pesticides, etc. exposure, leads to free radicals — atoms, molecules or ions with unpaired electrons — superoxide, hydrogen peroxide, singlet acid, hydroxyl radical. These are highly reactive, unstable and can lead to oxidative stress — a chain reaction that causes cell and tissue damage, contributing to aging and increased risk of developing various diseases, such as cancer, heart disease, cataracts, etc. (Sailaja *et al.*, 2011).

During oxidative stress, specific molecules actively react with proteins, lipids, and nucleic acid molecules, adding the missing electron, and thus creating other unstable molecules and inducing a chain reaction, such as via hydroxylated aromatic amino acids in protein molecules and oxidised-SH groups forming disulfide bridges — SS-bonds (Vīgants, 2008).

Unsaturated fatty acid-containing lipids with a large number of double bonds are readily oxidised. A variety of oxidation

products, such as peroxides, hydroperoxide, alkanols, aldehydes, carboxylic acids, etc., can inactivate the enzyme activity by binding to the active centres. Linoleic and arachidonic acid form malondialdehyde, as well as 4-hydroxynonenal, which is very toxic. Also cholesterol can be oxidised, resulting in total destruction of the cell membrane.

Nucleic acids participate in purine base guanine hydroxylation to form 8-hydroxy guanine (Vīgants, 2008).

Oxidative stress can reduce the body's antioxidant defense system and increase concentrations of oxygen and nitrogen in the active form (Vīgants, 2008).

Damage by free radicals can be reduced by antioxidant — molecules that react with them, eliminating the unpaired electron, or making the active radical less active, terminating the chain reaction.

The endogenous system in the body is composed of several antioxidant enzymes — superoxiddismutase, catalase, glutathionperoxidase, glutathionreductase etc.

Well-known exogenous (with dietary imbibed) antioxidants are vitamins C and E, lycopene, lutein, carotenoids, such as β -carotene, selenium, flavonoids, polyphenols, lignans,

coenzyme Q10, and others. Most of them are vegetable products (Sailaja *et al.*, 2011).

Polyphenols are chemical compounds with phenol rings. There is a big difference in the phenolic compounds regarding chemical structure, and they can be monomers or complex polymers.

Phenolic compounds of plants can be divided into two broad categories: 1) phenolic acids (oxometallic) and their derivatives, and 2) flavonoids (polyphenols). Phenolic acids and their derivatives, mainly esters, have more basic structures. Phenolic compounds constitute a wide group of representatives of caffeic acid, kumarin acid, vaniline, and gallic acid.

The flavonoid class of compounds has a more complex molecular structure, which is usually heterocyclic with attracted phenolic ring(s). Flavonoids include antocyanidines (water-soluble pigment, which are oxidised flavonoles), catechins, isoflavones and proanthocyanidin (Robarts *et al.*, 1999).

Polyphenols are the main antioxidant source from food. They are found in legumes, fruits and berries, and vegetables, red wine, chocolate, green tea, cereals and many herbal teas. Phenolic compounds constitute 50–70% of the weight of green tea, which is a key indicator of quality teas (Naghma and Hasan, 2007).

Plants consist of flavonoids, including catechins, proanthocyanins, anthocyanins, flavones, flavonoles and their glycosides, which have antioxidant properties. The weight of these compounds can reach up to 30% of leaf dry matter (Naghma and Hasan, 2007).

The aim of the research was to describe the antioxidative characteristics of Latvian herbal teas by antiradical scavenging activity method and to determine the content of the main polyphenols.

MATERIALS AND METHODS

In this study, the antioxidative properties of Latvian herbal teas were estimated regarding polyphenol content and antiradical activity. Twelve varieties of herbal teas were analysed:

Origanum vulgaris L., *Hypericum perforatum* L., *Chelidonium majus* L., *Achillea millefolium* L., *Calluna vulgaris* L., *Artemisia vulgaris* L., *Plantago major* L., *Comarum palustre* L., *Alchemilla vulgaris* L., *Calendula officinalis* L., *Verbascum thapsiforme* Schr., *Rubus idaeus* L. (Rubine and Eniņa, 2004) (Table 1). All these plants are widely distributed in Latvia and are used every day in the form of herbal tea. They include a variety of polyphenols, which act as radical and nitrite neutralisers in the body, they may show antiviral effects (including HIV), and enzyme induction, such as in the cytochrome P-450 system and phase II conjugation systems. They are important in the human body, as they

Table 1

HERBAL TEA VARIETIES ANALYSED IN THE STUDY

No.	Tea sample	No.	Tea sample
1	Plantain (<i>Plantago major</i> L.)	7	Common origanum (<i>Origanum vulgaris</i> L.)
2	Marsh cinquefoil (<i>Comarum palustre</i> L.)	8	Common St. John's wort (<i>Hypericum perforatum</i> L.)
3	Lady's mantle (<i>Alchemilla vulgaris</i> L.)	9	Greater celandine (<i>Chelidonium majus</i> L.)
4	Calendula (<i>Calendula officinalis</i> L.)	10	Milfoil (<i>Achillea millefolium</i> L.)
5	Wool mullein (<i>Verbascum thapsiforme</i> Schr.)	11	Wild wormwood (<i>Artemisia vulgaris</i> L.)
6	Red raspberry (<i>Rubus idaeus</i> L.)	12	Heather (<i>Calluna vulgaris</i> L.)

aid to avoid polyunsaturated fatty acid autoxidation (Higdon and Frei, 2012).

The scavenging activity of tea samples for the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described (Meda *et al.*, 2005) with some modifications.

1 g of dry tea was extracted in 50 mL methanol. Extraction was carried out for 1 hour at 25 °C. The filtrate of the extract was used for further analysis. A volume (1.5 ml) of each tea extract solution was mixed with 3 ml DPPH solution in methanol (0.02 mg·ml⁻¹), with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and then the absorbance was measured at 517 nm.

As mentioned above, in the control analysis, methanol was used instead of tea extract. Based on the DPPH solution and sample solution concentration, light absorption was used to calculate the break down amount of DPPH (μmol per 100 g of tea), in relation to the control solution.

Chromatographic determination of individual polyphenols in tea samples was made by liquid chromatography. Eight polyphenols in teas were determined: rutin, gallic acid, epicatechin, catechin, ferulic acid, chlorogenic acid, vanillin and caffeic acid.

The analyses were carried out using a Shimadzu liquid chromatography LC-20AD, with a DAD (diode array detector: SPD M20A) using analytical C18 column chromatography. The eluting solvent was methanol (A, 20%), water (B, 78.4%) and acetic acid (1.6% C) in gradient mode: 17:50 minutes – 58.5% B concentration, C concentration of 1.2%; 35 minute – end of analysis.

The sample injection into the chromatograph, using an automatic sample injection system SIL – 20AC. Fluent flow rate 1.0 ml/min.

Determination of polyphenols was made by using multiple wavelengths: 253, 268, 278 and 298 nm. 253 nm wavelength was used to determine rutin, and 263 nm — gallic acid, 278 nm — catechin, caffeic acid and vanillin, and 298 nm — chlorogenic acid, epicatechin and ferulic acid.

RESULTS

A significantly higher concentration of rutin was observed in *Hypericum perforatum* and *Calluna vulgaris* tea (Fig. 1).

The highest concentration of gallic acid occurred in *Rubus idaeus* tea (Fig. 2). *Rubus idaeus* tea had a relatively higher concentration of catechin (Fig. 3).

Alchemilla vulgaris, *Calluna vulgaris* and *Hypericum perforatum* teas had higher concentrations of caffeic acid (Fig. 4).

Vanillin is known as a spice, and is used in a variety of foods. Vanillin is also found in tea. A relatively higher vanillin concentration was found in *Alchemilla vulgaris*, *Calendula officinalis* and *Artemisia vulgaris* tea (see Fig. 5).

A relatively higher concentration of chlorogenic acid was found in *Comarum palustre*, and *Achillea millefolium* tea (Fig. 6). High chlorogenic acid concentration was also found in *Alchemilla vulgaris* and *Hypericum perforatum* teas.

The epicatechin concentration in teas was relatively low, and in the range from $0.07 \text{ mg} \cdot 100^{-1} \text{ g}$ to $3.6 \text{ mg} \cdot 100^{-1} \text{ g}$ of tea. Higher epicatechin concentration was found in *Alchemilla vulgaris* and *Calendula officinalis* teas ($2.6 \text{ mg} \cdot 100^{-1} \text{ g}$ and $3.6 \text{ mg} \cdot 100^{-1} \text{ g}$).

Ferulic acid had relatively high concentrations in *Origanum vulgare* tea (Fig. 7). Higher concentration of ferulic acid was also observed in *Alchemilla vulgaris*, *Verbascum thapsiforme* Schr. and *Achillea millefolium* tea.

In order to assess the total anti-oxidant tea value, they were assessed by total content of polyphenols in tea, as well as by

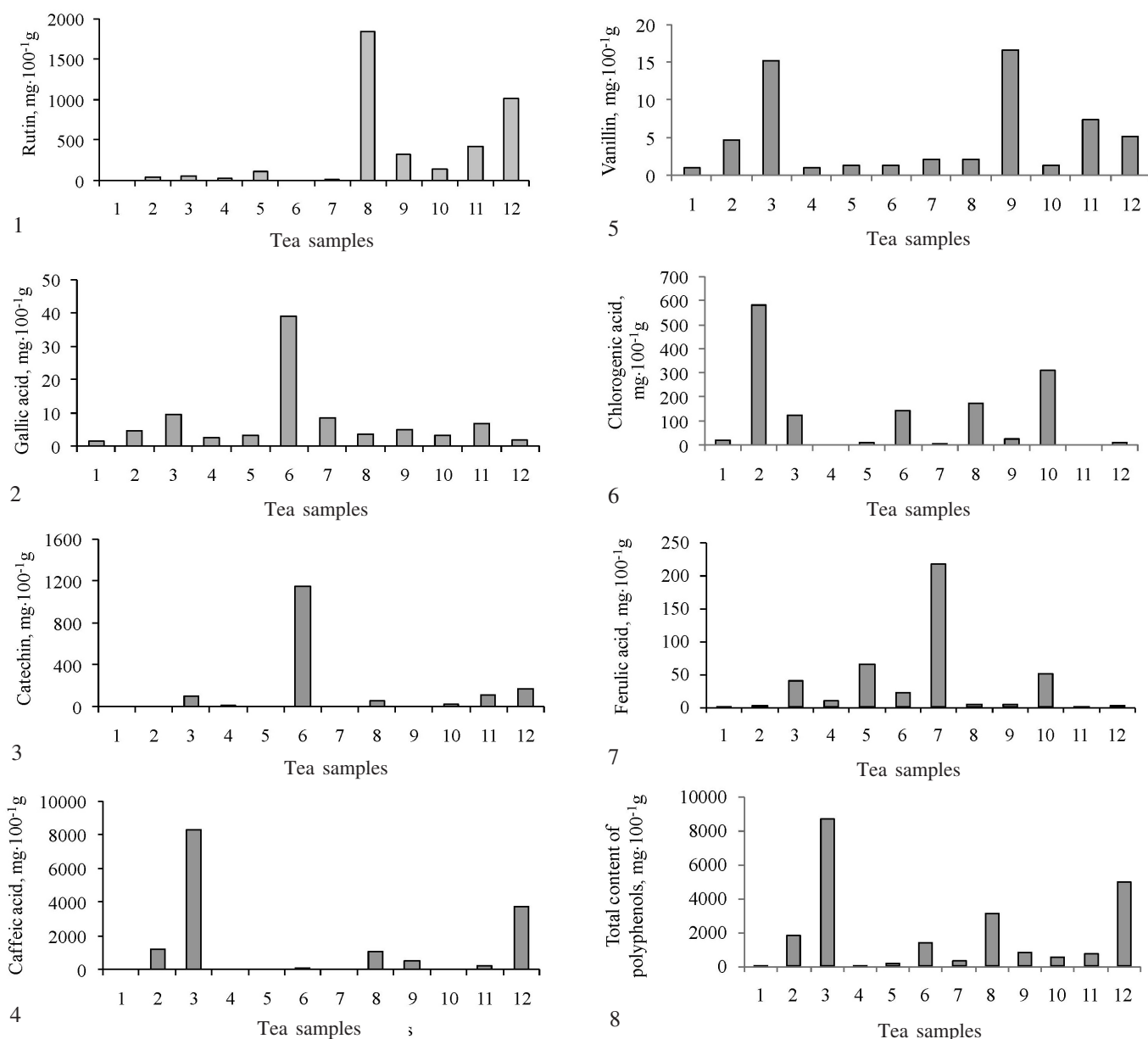


Fig. 1. Concentration of polyphenols in tea samples (numbers of tea samples identified in Table 1): 1) rutin; 2) gallic acid; 3) catechin; 4) caffeic acid; 5) vanillin; 6) chlorogenic acid; 7) ferulic acid; 8) total polyphenols.

certain antiradical activity (Fig. 8). The highest polyphenol concentration was found in *Alchemilla vulgaris* and *Calluna vulgaris* tea, and somewhat lower in *Comarum palustre*, *Rubus idaeus* and *Hypericum perforatum* tea.

The antiradical scavenging activity in the analysed tea ranged from 6 to 104 $\mu\text{mol}\cdot 100^{-1}$ g. The highest activity was in *Calluna vulgaris* tea (104 μmol break down DPPH reagent per 100 g of tea). In other teas, the antiradical activity was in the range of 21 to 38 $\mu\text{mol}\cdot 100^{-1}$ g.

DISCUSSION

It is known that rutin provides a more complete intake of vitamin C in the body. Therefore, it is used as a food supplement in combination with other substances. Rutin also has good anti-oxidant properties (Guardia *et al.*, 2001).

Catechins have antibacterial properties and it is able to destroy viruses and bacteria. Similarly, it is a good natural antioxidant (Higdon, *et al.*, 2003).

Caffeic acid has a spasmolytic and mitigating decreasing effect. Caffeic acid stimulates bile secretion. Chlorogenic acid has anti-bacterial, anti-inflammatory and antiviral properties. It also has antioxidant properties. Epicatechin is a powerful antioxidant. This is one of the rare flavonoids that in biologically active form is capable of reaching the brain (Mou-Tuan Huang *et al.*, 1991).

Ferulic acid is widely used in cosmetics as an antioxidant and as a protective measure against the adverse effects of UV rays. Ferulic acid inhibits skin aging (Graf, 1992).

High antiradical activity assessed by total polyphenols content was found only in heather tea. However, teas have many other polyphenols, which were not analyzed. Also, anti-oxidant activity can be attributed not only to polyphenols, but also to specific vitamins, amino acids, etc.

To conclude, our results demonstrate that increased antioxidant concentrations were found in *Alchemilla vulgaris*, *Calluna vulgaris*, *Comarum palustre*, *Rubus idaeus* and *Hypericum perforatum* teas.

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LATVIJĀ VĀKTO ZĀĻU TĒJU ANTIOKSIDATĪVO ĪPAŠĪBU RAKSTUROJUMS

Darbā ir pētītas dažādu Latvijā vāktu zāļu tēju antioksidatīvās īpašības, izmantojot augstspiediena šķidrums hromatogrāfijas un spektrofotometrijas metodes. Tika analizētas 12 tējas: parastā raudene (*Origanum vulgare* L.), divšķautņu asinszāle (*Hypericum perforatum* L.), lielā strutene (*Chelidonium majus* L.), parastais pelašķis (*Achillea millefolium* L.), sila virsis (*Calluna vulgaris* L.), parastā vibotne (*Artemisia vulgaris* L.), lielā ceļteka (*Plantago major* L.), purva vārnkāja (*Comarum palustre* L.), parastais rasaskrēsliņš (*Alchemilla vulgaris* L.), ārstniecības kļīņģerīte (*Calendula officinalis* L.), deviņvīru spēks (*Verbascum thapsiforme* Schr.), meža avene (*Rubus idaeus* L.). Tēju antiradikālā aktivitāte tika noteikta spektrofotometriski, izmantojot DPPH (2,2-difenil-1-pikrilhidrazils) reaģentu. Iegūtie antiradikālās aktivitātes rezultāti izteikti uz noārdītā DPPH reaģenta daudzumu 100 g tējas. Atsevišķi fenolu savienojumi — galluskābe, kafijskābe, hlorogēnskābe, ferulskābe, rutīns, katehīns, vanilīns un epikatehīns — identificēti, izmantojot šķidrums hromatogrāfiju. No iegūtajiem rezultātiem var secināt, ka palielināts antioksidantu saturs ir rasaskrēsliņu, viršu ziedu, asinszāļu, aveņu tējām. Dažādu infekciju, vīrusu saslimšanu un organisma vājuma gadījumā ieteicams lietot tējas, kas satur rutīnu, hlorogēnskābi un katehīnus. Tējas ar palielinātu ferulskābes saturu ieteicams lietot kosmētikā kā līdzekli pret ādas novecošanos un UV staru kaitīgo iedarbību.

In cases of virus diseases, infections and weakness it is recommended to use tea to increase rutin, chlorogenic acid and catechin intake.

Tea with increased ferulic acid content can be used to prevent against skin aging and UV destructive exposure.

The estimated total antioxidant content is not the only parameter that should be used in tea evaluation, and other polyphenol and antioxidant concentrations should be considered.

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DETERMINATION OF BIOACTIVE COMPOUNDS AND MINERAL SUBSTANCES IN LATVIAN BIRCH AND MAPLE SAPS

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*Birch and maple saps contain carbohydrates and organic acids, B complex vitamins and vitamin C, tannins, flavonoids, glycosides and mineral substances. The aim of the study was to quantitatively determine the concentrations of bioactive compounds and mineral substances in Latvian birch (*Betula pendula* Roth.) and maple (*Acer platanoides* L.) saps. Electrical conductivity was determined (629 and 967 S/cm in birch and maple saps, respectively) to characterise the total amount of mineral substances. In birch and maple saps the titratable acidity (0.50 and 0.70 mmol of NaOH per litre of sap, respectively) and formol number (0.25 and 0.20 mmol NaOH per litre of sap, respectively) were determined. The protein concentration was found to be higher in maple sap (171 and 127 mg/l, respectively). The antioxidant concentration, determined using quercetin as a standard, was 0.35 mg of quercetin equivalents (QE)/l in birch sap and 0.77 mg QE/l in maple sap. In conclusion, Latvian maple sap contains more bioactive and mineral compounds than birch sap. Latvian birch sap contains up to 20% more glucose and fructose than birch sap produced in Finland, but Latvian maple sap contains 10 to 40% less sucrose than sap produced in North America.*

Key words: birch sap, maple sap, bioactive compounds, mineral substances.

INTRODUCTION

Birch and maple saps are rich in carbohydrates, mineral salts, proteins, organic acids including aminoacids. These saps also contain B complex vitamins, vitamin C, phenolic compounds, tannins, flavonoids and glucosides. Birch and maple saps are pleasant and freshening dietary drinks and both are used to produce syrup. Both saps are also known to possess some curative properties. Saps have been shown to be effective in the treatment of stomach ulcer, bronchitis, radicular pain, arthritis, removal of some potentially unwanted substances from the body and stimulation of overall metabolism (Drozdova *et al.*, 1995; Deslauriers, 2000; Jiang, 2001; Sehm, 2007; Perkins and van den Berg, 2009). Extracts of maple sap and syrup have been shown to possess some antiproliferative effects on the colon cancer cells (González-Sarrias *et al.*, 2012).

In most studies birch and maple saps produced from trees growing in Finland and North America, respectively, have been used to characterise chemical composition and properties of the respective sap. In Finland birch sap is used to produce syrup (Kallio *et al.*, 1987; Kallio and Ahtonen, 1987b; Kallio, 1989; Kallio *et al.*, 1987; 1989; 2006). Several studies have described the chemical composition of birch sap: concentration of organic acids (Kallio *et al.*, 1985; Kallio and Ahtonen, 1987a), amino acids (Ahtonen and Kallio, 1989; Patzold and Bruckner, 2005), proteins

(Kallio *et al.*, 1995; Jiang *et al.*, 2001), carbohydrates (Kallio *et al.*, 1985; Kallio and Ahtonen, 1987b), and micro- and macroelements (Harju and Hulten, 1990). Similarly, the chemical composition of maple sap has been described: concentration of mineral substances (Robinson *et al.*, 1989), seasonal changes in amino acid concentration (Kozłowski and Pallardy, 1997), sucrose concentration varied from 0.5–10%, and malic acid was observed to be the most common organic acid (Gaucher *et al.*, 2003; Wong *et al.*, 2003; Perkins and van den Berg, 2009). Various phenolic compounds have been found in maple sap and the concentration of these compounds could reach up to 0.1 mg/l. Dominant compounds are sinapic and p-coumaric acids, and syringic and coniferic acids. In lesser amounts also vanillic, syringic, homovanillic and ferulic acids are present. Maple sap contains a number of flavonoids, like catechins, campherol and its glucosides, quercetin and its glucosides and others (Kermasha *et al.*, 1995; Deslauriers, 2000; Abou-Zaid, 2008; Perkins and van den Berg, 2009). Some studies have tested the antioxidant, antiradical and antimutagenic activities of maple sap and syrup (Theriault *et al.*, 2003; Legault *et al.*, 2010).

Although Latvia is known as a producer and consumer of birch and maple saps, up to date no studies have characterised chemical composition and properties of sap obtained from trees growing in Latvia. Previously we have character-

ised only the total content of phenolic compounds in birch and maple saps from Latvia. It was found that maple sap contains on average by 48% more total phenolic compounds than the birch sap (Kuka *et al.*, 2010). The aim of the present study was quantitative determination of bioactive compounds and mineral substances in birch (*Betula pendula* Roth.) and maple (*Acer platanoides* L.) saps collected from trees grown in Latvia.

MATERIALS AND METHODS

Saps were obtained from birch (*Betula pendula*) and maple (*Acer platanoides*) trees growing in sandy soil away from roads near towns of Ozolpils (56.685507N 23.731600E), Jelgava (56.649137N 23.651331E) and Tukums (56.8553437N 23.0115635E). Saps were collected in the early spring 2010 (end of March maple sap, beginning of April birch sap) during the most intense period of their production (first week of sap production).

Determination of titratable acidity and electrical conductivity. Titratable acidity was determined by potentiometric titration as described previously (Tanner, 1987). In brief, 25 ml of sap was titrated with 0.25 M NaOH solution till pH 8.1, as measured using electrode SenTix 97T for potentiometric titration connected to a pH-meter pH 538 from WTW. Titratable acidity was calculated as NaOH mmol/l of sap. Electrical conductivity was determined to characterise the total content of mineral substances using an electrode TetraCon 325 connected to a conductometer inoLab pH/Cond 720 from WTW.

Determination of content of mineral substances. Concentration of mineral substances was determined by microprocessor photometer MPM 3000 from WTW and commercially available reagent kits from MERCK according to manufacturer's protocol. The concentration of calcium ions was determined according to MERCK method Nr. 1.14815.0001. The concentration of potassium ions was determined according to MERCK method Nr. 14562. The concentration of iron was determined according to MERCK method Nr.14761/2. The concentration of copper (II) ions was determined according to MERCK method Nr. 14767. The concentration of manganese ions was determined according to MERCK method Nr. 14770. The concentration of nickel ions was determined according to MERCK method Nr. 14785. The concentration of ammonium ions was determined according to MERCK method Nr. 1.14752.0001 and the result was calculated as concentration of nitrogen in ammonia. The concentration of sulphate ions was determined according to MERCK method Nr. 1.14791.0001. The concentration of phosphate ions was determined according to MERCK method Nr. 1.14848.0001. The concentration of chloride ions was determined according to MERCK method Nr. 1.14897.0001. The concentration of nitrate ions was determined according to MERCK method Nr. 14556 and the result was calculated as concentration of nitrogen in nitrate ions. The concentration of nitrite ions was determined according to MERCK method Nr.

N4/25 and the result was calculated as a concentration of nitrogen in nitrite ions.

Determination of formol number and protein amount.

The formol number, which characterises the concentration of free amino acids, was determined as described previously (Tanner, 1987). In brief, to neutralise weak organic acids, 0.25 M NaOH solution was added to 25 ml of sap till pH 8.1, as determined by potentiometric titration. Then, formaldehyde solution (35%) was added and the solution was titrated with 0.25 M NaOH solution till pH 8.1 was reached. Results were calculated as the volume of 0.1 M NaOH in ml required to neutralise hydrogen ions liberated from 100 ml of sample solution treated with water solution of formaldehyde.

The protein amount was determined by Lowry procedure (Lowry *et al.*, 1951) using albumin as a standard. Results were calculated as mg of protein per litre of sap.

Determination of carbohydrate content by HPLC. Concentration of carbohydrates in sap was determined by high performance liquid chromatography (HPLC), (Shimadzu LC-20 prominence) as described previously (Kūka, 2008). In brief, calibration was made using standard solutions of fructose, glucose, sucrose and maltose at the following concentrations: 0.05 g/ml, 0.01 g/ml and 0.025 g/ml. Samples of sap (10 µl) were injected into the chromatograph and analysed using CH₃CN:H₂O (70:30 volume/volume, %) as a mobile phase by flow rate 1.3 ml/min.

Determination of ascorbic acid and antioxidant content.

The ascorbic acid concentration was determined using iodometry. 50 ml of sap were titrated with 0.01 M iodine solution in the presence of starch as an indicator. The results were calculated as mg of ascorbic acid per litre of sap.

The antioxidant concentration was determined with 2,2-diphenyl-1-picrylhydrazyl (0.02 mg/ml) measuring absorbance at 517 nm using UV/VIS spectrophotometer Jenway UV 6405. Quercetin was used to produce the standard curve (concentrations 0.5–4.0 µg/ml). The antioxidant content was expressed as quercetin equivalents (QE) – mg/l of sap (Theriault *et al.*, 2006).

Data analysis. The results are presented as the mean ± standard deviation. Data analysis was performed using in-built analysis of Microsoft Excel 2007.

RESULTS

Titratable acidity and electrical conductivity. Titratable acidity was determined using birch (Ozolpils) and maple sap (Ozolpils) samples obtained in the first week of sap production. Average titratable acidity in the maple sap samples was 40% higher than in the birch sap samples (Table 1). Electrical conductivity was determined in birch (Ozolpils, Jelgava) and maple sap (Ozolpils, Tukums) samples. The electrical conductivity in maple sap was higher than in birch

Table 1

VARIOUS PARAMETERS OF BIRCH (*Betula pendula* Roth.) AND MAPLE (*Acer platanoides* L.) SAPS

Sap	Titrateable acidity, NaOH mmol/l	Formol number, 0.1 M NaOH ml/100 ml	Protein, mg/l	Ascorbic acid, mg/l	Antioxidant content QE, mg/l
Birch	0.50 ± 0.01	0.25 ± 0.01	127 ± 2	3.2 ± 0.01	0.35 ± 0.03
Maple	0.70 ± 0.02	0.20 ± 0.01	171 ± 8	8.8 ± 0.03	0.77 ± 0.09

Data are presented as mean ± SD.

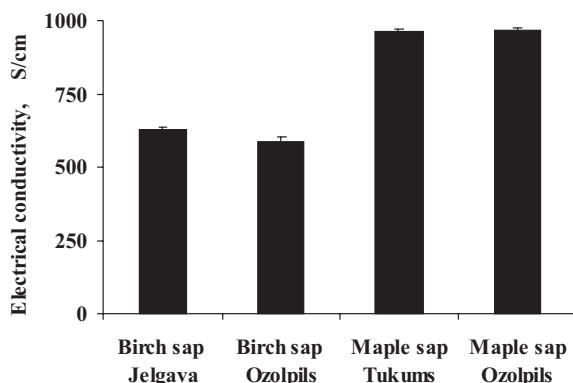


Fig. 1. Electrical conductivity of birch (*Betula pendula* Roth.) and maple (*Acer platanoides* L.) saps. Data are presented as mean ± SD.

sap on average by 58% (Fig. 1), indicating greater concentration of strong electrolytes in maple sap samples.

Content of mineral substances. The predominant macroelements in the birch and maple saps were found to be Ca^{2+} and K^+ ions and the prevailing trace elements were Mn^{2+} ions, while the dominant anions were sulphate ions (Table 2). Nitrate and nitrite concentrations in both birch and maple sap samples were negligible.

Formol number and protein amount. The formol number, which characterizes the concentration of free amino acids, was determined in birch (Ozolpils) and maple sap (Ozolpils) samples. The concentration of free amino acids was similar and negligible in both birch and maple sap samples (Table 1). The concentration of proteins was by 34.6% higher in maple sap than in birch sap (Table 1).

Carbohydrate content. Carbohydrate concentration was determined using HPLC in birch (Ozolpils) and maple sap (Ozolpils) samples. The monosaccharides glucose and fructose and disaccharide sucrose were identified in sap samples. The concentration of sucrose in the maple sap was 31-fold higher than in birch sap, while the concentration of fructose and glucose was 10-fold and 3-fold higher, respectively, in birch sap than in maple sap (Table 3). Other carbohydrates were below the identification threshold.

Content of ascorbic acid and total content of antioxidants. Concentration of ascorbic acid was determined using birch (Ozolpils) and maple sap (Ozolpils) samples. Concen-

Table 2

CONCENTRATION OF MINERAL SUBSTANCES IN BIRCH (*Betula pendula* Roth.) AND MAPLE (*Acer platanoides* L.) SAPS

Mineral substances	Birch sap, mg/l		Maple sap, mg/l	
	Jelgava	Ozolpils	Tukums	Ozolpils
Ca^{2+}	53.3 ± 0.1	41 ± 1	61 ± 1	91 ± 1
K^+	41.1 ± 0.5	66.4 ± 0.1	70.1 ± 0.2	78.9 ± 0.1
Mn^{2+}	0.5	0.520 ± 0.006	-	0.24 ± 0.01
Ni^{2+}	-	0.03 ± 0	0.15 ± 0.02	0.13 ± 0
Cu^{2+}	0.04	-	-	-
Fe^{3+}	0.10	-	-	-
$\text{NH}_4^+ - \text{N}$	0.050 ± 0.001	-	0.080 ± 0.001	-
SO_4^{2-}	36.10 ± 0.06	34.0 ± 0.1	39 ± 1	62.6 ± 0.5
$\text{PO}_4^{3-} - \text{P}$	2.06 ± 0.01	2.36 ± 0.02	4.13 ± 0.09	-
Cl^-	-	9.0 ± 0.1	9.0 ± 0.1	12.0 ± 0.1
$\text{NO}_3^- - \text{N}$	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01	3.40 ± 0.01
$\text{NO}_2^- - \text{N}$	0.008 ± 0	0.012 ± 0	-	0.115 ± 0

Data are presented as mean ± SD.

Table 3

CONCENTRATION OF CARBOHYDRATES IN BIRCH (*Betula pendula* Roth.) AND MAPLE (*Acer platanoides* L.) SAPS

Sap	Fructose, g/l	Glucose, g/l	Sucrose, g/l
Birch	5.39 ± 0.05	4.46 ± 0.04	0.58 ± 0.01
Maple	0.55 ± 0.01	1.33 ± 0.03	17.95 ± 0.05

Data are presented as mean ± SD.

tration of ascorbic acid in maple sap was by 56% higher than in birch sap (Table 1).

Total concentration of antioxidants was characterised using quercetin equivalents and the obtained results show that maple sap samples contained around two times higher concentration of compounds with antioxidant properties than in birch sap samples (Table 1).

DISCUSSION

The present study quantitatively determined the concentration of bioactive compounds and mineral substances in saps obtained from birch (*Betula pendula*) and maple (*Acer platanoides*) trees growing in Latvia. The obtained results indicate that Latvian maple sap contains significantly more bioactive and mineral compounds than birch sap. The concentration of mineral substances in sap obtained from birch (*Betula pendula*) trees reflects the chemical composition of the soil where trees grow (Harju and Hulten, 1990). In addition, our results indicate that a similar tendency can be observed in sap obtained from maple (*Acer platanoides*) trees (Table 2). Overall, the concentration of mineral substances was higher in maple than in the birch sap (Table 2). These findings were in compliance with the results of electrical conductivity measurements (Fig. 1), which showed that maple sap contains higher concentrations of strong electrolytes

and thus the concentration of mineral substances must be higher. It should be noted that the concentration of mineral substances much varies in maple sap and increases during the period of sap production (Robinson *et al.*, 1989; Perkins and van den Berg, 2009). Dominant mineral substances are calcium and potassium. Maple sap is also rich in magnesium and manganese, while only small amounts of zinc, copper, iron, sodium and aluminium can be found. Birch sap mainly contains salts of potassium, calcium, magnesium, manganese and zinc (Harju and Hulden, 1990).

Although the overall concentration of bioactive compounds was higher in maple sap, the concentrations of free amino acids were similar and both birch and maple saps contained very low concentration of free amino acids (Table 1). This could be explained by the use of the first-week saps for the study. It has been shown that the concentration of free amino acids increases during the birch sap production period (up to 100–500 mg/l) and that the most prevalent amino acids are glutamine, citrulline and glutamic acid (Ahtonen and Kallio, 1989; Jiang *et al.*, 2001). In both birch and maple saps, the concentration of proteins increases in the last stage of the season (Kallio *et al.*, 1995; Kozłowski and Pallardy, 1997; Jiang *et al.*, 2001). Similarly, the total content of all organic acids found in the saps increases during the production period and the predominant acid in both birch and maple saps is malic acid (Kallio *et al.*, 1985; Kallio and Ahtonen, 1987a; Perkins and van den Berg, 2009). Glucose, fructose and sucrose were the only carbohydrates identified in the sap samples (Table 3). Although birch and maple saps contain other carbohydrates, their content is negligible and they were not quantified. It has been shown that carbohydrate content does not depend on species or variety of the tree and the amount of carbohydrates in birch sap decreases in the following order: glucose, fructose, sucrose and galactose (Kallio and Ahtonen, 1987b). Sucrose is the dominant carbohydrate in the maple sap, while other carbohydrates like glucose and fructose are in small quantities, but the concentrations of stachyose, raffinose and xylose are negligible (Wong *et al.*, 2003; Perkins and van den Berg, 2009). Latvian birch sap contains up to 20% more glucose and fructose (Table 3) than birch sap produced in Finland (Kallio and Ahtonen, 1987b), but Latvian maple sap contains up to 10 to 40% less sucrose (Table 3) than sap produced in North America (Kozłowski and Pallardy, 1997).

In conclusion, our results demonstrate that the overall content of Latvian maple (*Acer platanoides*) sap is richer in bioactive substances and mineral salts as compared to birch (*Betula pendula*) sap. The dominant carbohydrates in birch sap are glucose and fructose, while the sucrose is predominant in maple sap.

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BIOĻĢISKI AKTĪVO SAVIENOJUMU UN MINERĀLVIELU NOTEIKŠANA BĒRZU UN KĻAVU SULĀS

Bērzu un kļavu sulas satur ogļhidrātus un organiskās skābes, B grupas vitamīnus un C vitamīnu, flavonoīdus, glikozīdus un minerālvielas. Pētījuma mērķis – noteikt bioloģiski aktīvo savienojumu un minerālvielu saturu Latvijas bērzu (*Betula pendula* Roth.) un kļavu (*Acer platanoides*) sulās. Lai raksturotu minerālvielu kopējo daudzumu, tika mērīta elektrovadītspēja (bērzu un kļavu sulās atbilstoši 629 un 967 $\mu\text{S}/\text{cm}$). Minerālvielu saturs tika noteikts spektrofotometriski. Bērzu un kļavu sulās tika noteikti kopējais skābums (atbilstoši 0,50 un 0,70 mmol NaOH uz litru sulas) un formaldehīdskaitļi (atbilstoši 0,25 and 0,20 mmol NaOH uz litru sulas). Kļavu sula satur vairāk proteīnus nekā bērzu sula (atbilstoši 171 and 127 mg/l). Fruktozes, glikozes un saharozes saturs tika noteikts, izmantojot augstefektīvu šķidrums hromatogrāfiju. Askorbīnskābes saturs tika noteikts jodometriski. Kā standartvielu izmantojot kvercetinū, tika raksturots antioksidantu saturs un bērzu sulā tas bija 0,35 μg kvercetīna ekvivalentu (QE)/l, bet kļavu sulā 0,77 μg QE/l. Kopumā Latvijas kļavu sula satur vairāk bioloģiski aktīvo savienojumu un minerālvielu nekā bērzu sula.

CONSUMER ATTITUDE AND SENSORY EVALUATION OF MARSHMALLOW

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Marshmallow is sugar-candy with foam-like structure. Many studies have revealed the high nutritional value of bee pollen. Due to the nutritional composition of pollen, and according to non-scientific studies, dried bee pollen has been used as an additive in human diets, which provides a well-being sensation and contributes to functional and harmonious balance of the body. Sensory evaluation and market research are an important part of developing a new product. The research was aimed to clarify the situation in the Latvian market, to summarise Latvian consumer attitude on marshmallow, and to determine the sensory properties and quality of marshmallows with bee pollen. A questionnaire was developed — 900 respondents answered questions about their consumption patterns, opinions on quality indices, and possible types of marshmallow packaging. Marshmallow was enriched with three different pollen concentrations: 1.0; 1.5 and 2.5%. Sensory evaluation was carried out to estimate the optimal concentration of bee pollen that which should be added to marshmallow. Sensory evaluation of experimental marshmallow was characterised by using a 9-point hedonic scale (determination degree of liking). The obtained results showed that consumers liked the marshmallow with 1.5% bee pollen best of all.

Key words: marshmallow, bee pollen, sensory evaluation, consumer attitude.

INTRODUCTION

The meaning of the term ‘confectionery’ is different in every country and may cover a very large amount of different products manufactured in various manners. Chocolate confectioneries include bars, blocks, and bonbons, and sugar confectionery encompasses boiled sweets, toffees, fudge, fondants, jellies, and pastilles. Confectionery goods are a very heterogeneous group of products made with milk powder and other dairy products; cocoa and chocolate products; sugar, honey, syrups or sweeteners; nuts, fruits or jams; starches, gelatine, pectin or other thickeners; egg albumen; spices, colours, flavours or acidulates (Cakebread, 1975; Konkel, 2001; Vaclavik and Christian, 2008; Kawo and Abdulmumin, 2009).

The confectionery industry is enormous, ranging from small shops to branches of the largest companies in the food industry. The sweets are divided into three classes: chocolate, flour and sugar confectionery (Manley, 1998; Quinton and Kennedy, 2002). Sugar confectionery has been developed over the centuries with increasing sophistication, and it exists in countless formats with different degrees of sweetness, flavours and aromas, textures and mouth feel. Confectionery serves a very simple purpose; the rush of sweetness coupled with pleasant flavours, and aromas. The feeling in the mouth provokes almost instantaneous feeling of

well-being and happiness. Sugar confectionery includes by definition products that contain predominantly one form or another of the following sugars: sucrose (usually cane or beet sugar); dextrose (otherwise known as glucose, usually corn sugar); fructose (often referred to fruit sugar) or lactose (otherwise known as milk sugar) (Zumbé *et al.*, 2001).

The specific kind of confectioneries considered here has foam-like structures (marshmallows, soufflés, and nougats). Marshmallows are simply described as air bubbles surrounded by sugar syrup. The sugar syrup, made of sucrose, corn syrup, and water, is cooked at appropriate temperature to reach the desired water content, which allows air to be whipped into the matrix by mechanical agitation. Proteins, such as gelatine, gum Arabic, egg albumen, agar-agar, pectin, milk or soy protein, are typically added to the sugar syrup to stabilise the foam. During whipping, the density of the product decreases as the syrup and foam mixture expands into a light, fluffy marshmallow. Marshmallows may be either non-grained or grained crystal aggregate, depending on the ratio of sucrose to corn syrup (Sucharzewska *et al.*, 2003; Ergun *et al.*, 2010). Certain glassy sugars tend to be hygroscopic, rapidly picking up moisture from the air, which causes significant changes that lead to the end of shelf life. These products need to be protected from moisture uptake during storage (Ergun *et al.*, 2010).

The aim of quality properties is to provide a safe, inoffensive, as well as nutritionally rich food. Pollen is important in bee life cycle, and it is understandable that people's interest in its composition and applicability as a dietary supplement is high (Graikou *et al.*, 2011; Feás *et al.*, 2012).

Bee honey has been used by man since the beginning of humanity. Although this is the most common beehive product, there are other goods, such as bee pollen, royal jelly, propolis and beeswax. These natural goods are well appreciated by consumers due to the high number of quality checks they go through, as well as for their dietetic and therapeutic qualities. The major components of bee pollen are carbohydrates, crude fibres, proteins and lipids at proportions ranging between 13 and 55%, 0.3 and 20%, 10 and 40%, 1 and 10%, respectively. Other minor components are minerals and trace elements, vitamins and carotenoids, phenolic compounds, flavonoids, sterols and terpenes. In fact, bee pollen is referred to as the "only perfectly complete food", since it contains all the essential amino acids needed for the human organism (Silva *et al.*, 2005; Carpes *et al.*, 2009; Le Blanc *et al.*, 2009; Graikou *et al.*, 2011; Feás *et al.*, 2012).

Since in a healthy diet sweets are allocated less than 5% of the daily food consumption, they must be high quality in every respect and attractive for consumers. Enriching sugar confectionery with pollen would supplement the diet with biologically active substances.

The formulation and marketing of consumer products today have become very complex operations in which both sensory testing and market research have important parts to play. Both disciplines are united by a common goal to produce goods with an optimum combination of product attributes, compatible with manufacturing costs that will sell successfully and profitably in the market place. Despite of this common goal, the different requirements of technical and marketing personnel have led to the separation and interaction between the two disciplines. There are, however, techniques and approaches used by both the sensory and market researcher, which when combined, can offer improved product testing (Wilton and Greenhoff, 1988; Moskowitz *et al.*, 2006).

Food choice is not determined solely by the perceived properties of a foodstuff. It depends on personal attitude towards the attributes a product possesses. Consumer responses are vital in the development of a product if it is to compete successfully in the market place (Frances and Piggott, 1991–1992). There is a wide variety of market research procedures that could be used to elicit information about product attributes, and to measure attitudes and beliefs. These include unstructured spontaneous techniques, such as interviews and projective methods, and also highly structured methods, such as dissimilarity scaling where appropriate statistical procedures are used to obtain product spaces (maps) and identify salient product dimensions (McEwan and Thomson, 1989).

Sensory evaluation techniques are frequently used; however, applied sensory is most often used within the private industry. Basic sensory techniques can be an invaluable aid to research on nutritional or functional benefits of natural products, such as whole fruits, nuts and vegetables (through varietal selection, breeding, (etc.) in addition to clinical trials of botanicals). As part of the effort to discover, develop and market healthier products, understanding nutritional and functional benefits is a key aspect. Of additional importance is highlighting and refining the products' sensory properties in an attempt to ultimately appeal to consumers: no matter how healthy and nutritious a food is, if it does not appeal to its intended end user, it is unlikely to succeed in today's marketplace (Civille and Oftedal, 2012).

The aim of this study was to determine the Latvian consumer attitude on marshmallow packaging presentation, and the potential marshmallow market expandability. Furthermore, sensory evaluation was carried out to estimate the degree of liking of pollen-rich marshmallow and to determine the optimal desired pollen concentration.

MATERIALS AND METHODS

The research was aimed to assess consumer attitude to marshmallow as well as its quality. The study was organized in two stages:

1. A consumer questionnaire was developed to analyze the situation in the Latvian market and to determine Latvian consumer attitude concerning their consumption patterns, opinion on quality indices, and possible packaging types of marshmallows. The questionnaire was used as a basic tool, which consisted of ten questions answered by consumers. Respondents were asked to answer both multiple-choices and open-type questions. The survey was designed using website www.visidati.lv, which gives an opportunity to obtain a quick and effective survey distribution and data collection. In order to obtain a link between the average population answers and the web-based questionnaire, it was also sent to people. The data consisted of the answers of all 900 respondents who completed questionnaires.

2. Experiments were performed in the International Food exhibition "Riga Food 2011", to assess the degree of liking and optimal desired pollen in marshmallow. Consumers evaluated the degree of liking of marshmallow samples that had additions of 1.0, 1.5 and 2.5% bee pollen. The control sample was marshmallow without bee pollen. The marshmallow used consisted of: sugar, glucose syrup, water, apple puree (apple, preservatives E202, E211), gelling agent — pectin, protein powder, acidity regulator — lactic acid, bulking — sodium lactate, and vanillin (0.05%). The 9-point hedonic scale (9, extremely like, 5, neither like nor dislike, and 1, extremely dislike) method was used based on ISO 4121:2003 "Sensory analysis — Guidelines for the use of quantitative response scales". One hundred and thirty consumers (age 14 to 66 years) were involved in the evaluation.

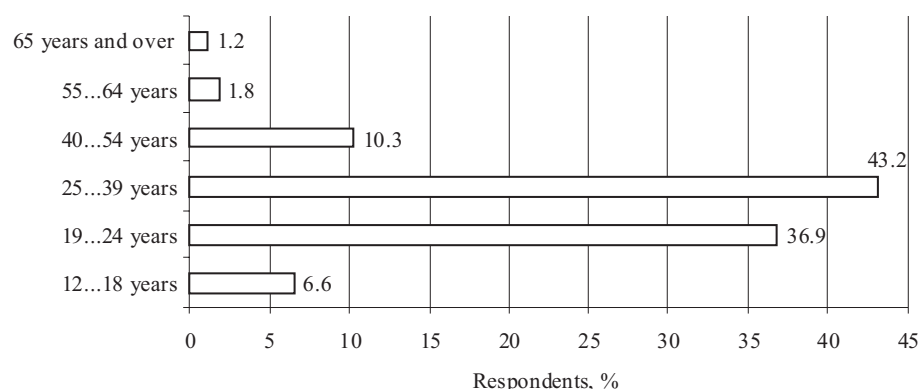


Fig. 1. Respondent division according to age, %.

Most respondents were up to 40 years, who were also considered as the main body of consumers who were likely to consume marshmallow (Fig. 1).

The test results were processed using software SPSS package 16. P value 0.05 was considered as a significant difference. One-way and two-way factor analysis of variance (ANOVA) as well the Tukey's test were used to test for significant differences.

RESULTS

The 900 respondents (28% men and 72% women) answered ten questions regarding how well-recognized is marshmallow, and the main features for this kind of sweets for choice by consumers. One of most important questions was to understand the consumer opinion about sweets, mainly the type of marshmallow packaging for marketing, and the market turnover. Respondents were asked to evaluate the consumer demand for marshmallow, likeness and the packaging options. Of the ten questions associated to the product (marshmallow), two were aimed to obtain basic information about the consumer.

All 900 respondents liked marshmallow. The majority (96%) of consumers asserted that marshmallow is tasty, and 4% of respondents considered this product as unpalatable and too sugary; some consumers did not like the structure of marshmallow.

According to frequency of purchase of marshmallow (Fig. 2), 35% of respondents infrequently purchased marshmallow, 17.4% — at least once a week, 46.8% — monthly and

0.8 % admitted they did not routinely use marshmallow, but consumed this sweet when available as a treat.

The respondents mentioned that they purchased marshmallow in various cases (Fig. 3). The majority favoured buying marshmallow daily, indicating a demand for fresh marshmallow in small appropriate packaging for purchase as a frequently used sweet. The majority of consumers (586) considered that they would like to choose marshmallow for a gift or social evening (234).

Subsequent questions on the product were aimed to evaluate respondent opinion about the packaging style as well as on how the packaging design influences the choice of product in the market. Most respondents admitted that both style and the manner how the product is offered to the customer in the retail sector are important (Fig. 4).

An important consumer opinion was on the amount of marshmallow in one package (Fig. 5). It is determined that the optimal amount for most of people (35.1% to 45.4%) was two to four pieces in one package.

A sensory evaluation was performed to determine the optimal bee pollen addition to marshmallow raw material mass, (Fig. 6). ANOVA indicated significant difference between concentrations of bee pollen used ($P > 0.05$). The Tukey's test was used to test for differences between preference of the marshmallow samples. The analysis showed significant difference ($P > 0.05$) in the degree of liking between the control sample and samples with pollen A (1.0%) and C (2.5%). Consumers recognised the marshmallow sample with 2.5% bee pollen as having a too typical taste, sharp af-

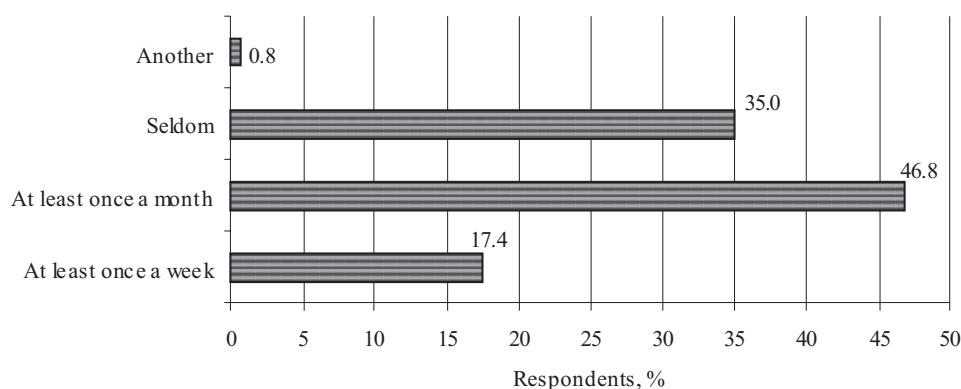


Fig. 2. Purchasing frequency of marshmallow, %.

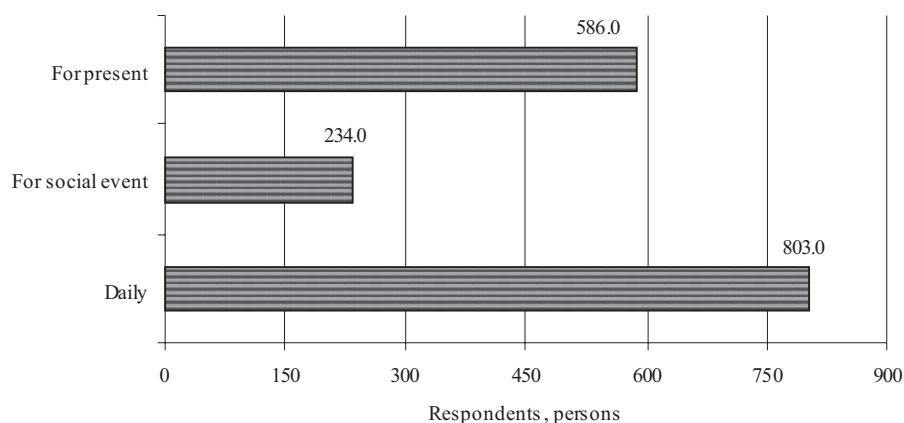


Fig. 3. Purchase intent of marshmallow.

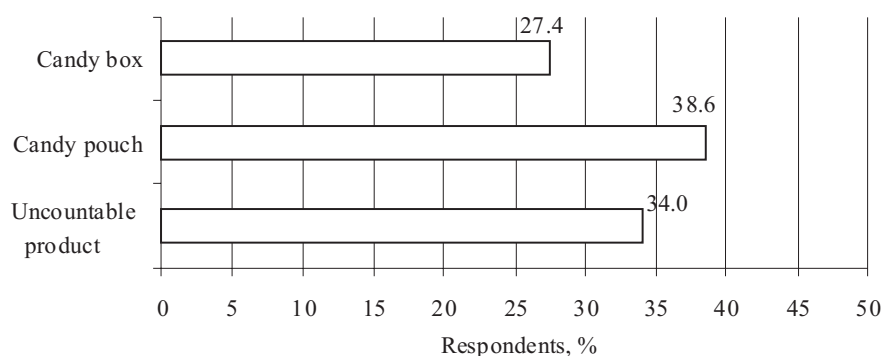


Fig. 4. The choice of marshmallow purchasing style, %.

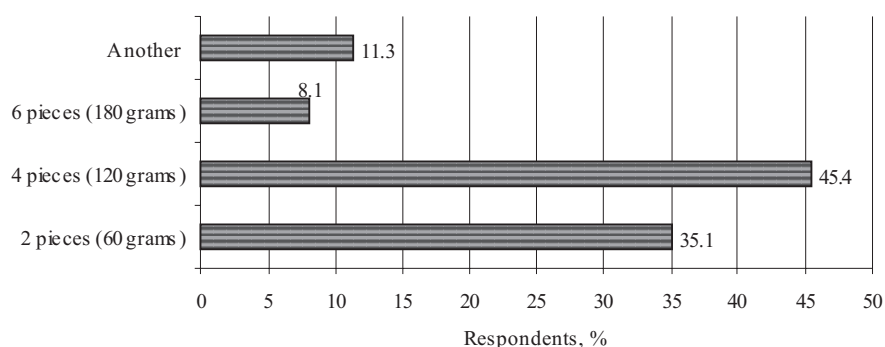


Fig. 5. Consumer view on the optimum marshmallow quantity (pieces) in one package.

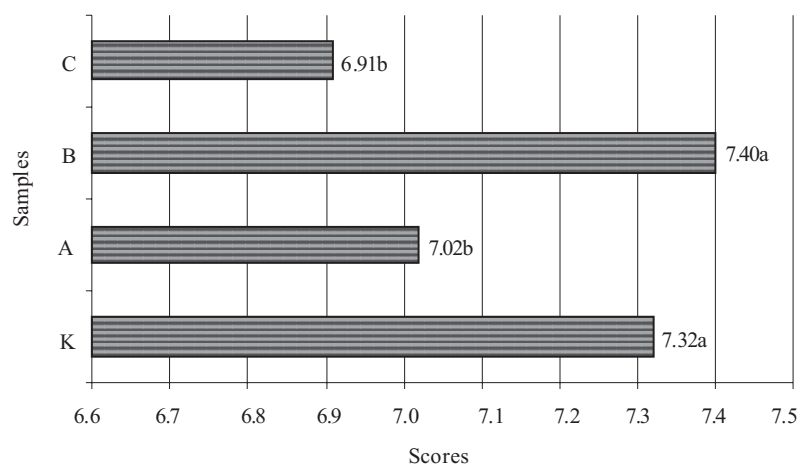


Fig. 6. Evaluation results of marshmallow with bee pollen using 9-point hedonic scale.

K – marshmallow without pollen (control); **A** – marshmallow with 1.0% pollen addition; **B** – marshmallow with 1.5% pollen addition; **C** – marshmallow with 2.5% pollen addition.

*Values, marked with the same letters, are not significantly different ($P > 0.05$).

tertaste, and a not so airy structure. Significant differences also were found between B and A, and between B and C ($P > 0.05$), in liking degree. Results of the hedonic scores showed that the panellists most liked samples K (control)

and B (with 1.5% bee pollen), and there was no significant difference between these ($P > 0.05$). Thus, the consumer preferred both the classical marshmallow with vanilla, and with bee pollen (1.5%) enriched marshmallow.

DISCUSSION

The European confectionery market grew at a fairly steady rate during the period 2005–2009, as a result of steady sales growth in the chocolate category. Although the overall market growth is expected to decelerate slightly in the forecast period, the annual rate of growth is set to rise from a low of 2.5% in 2009 to a high of 2.9% in 2014. Chocolate in 2009 is the largest segment of the confectionery market in Europe, accounting for 51.7% of the market's total value. The sugar confectionery segment accounts for a further 34.6%, gum 11.1% and cereals bars 2.6% of the market (Anonymous, 2010).

In Europe, the main distribution channels for the confectionery market are supermarkets and hypermarkets (30.4%), independent retailers (26.5%), convenience stores (21.4%) and other of the market value (21.7%) (Anonymous, 2010). Supermarket chains are often able to negotiate very strongly on price with confectionery producers; this boosts their buyer power significantly. An increasing number of product lines have been developed to create healthier alternatives to their existing lines, with substitute versions offering no artificial colours, low sugar, as well as the inclusion of additives such as ingredients with high nutrition value.

The leading players in the Eastern European confectionery market include Mars, Inc. (14.3%), The United Confectionary Manufacturers management company and Nestle S.A. (8.9%), Cadbury plc (8.6%) and others – 68.3%. In Latvia the leading players are GmbH Laima, Ltd Skriveru saldumi, Ltd Pure Chocolate and Ltd Bona Dea. The largest marshmallow manufacturer in Latvia is GmbH Laima, however, other manufacturers, like Ltd. Bona Dea, Ltd. Mikas M, as well as importers from Russia and Lithuania are coming into the market.

The Latvian consumer survey results showed that the amount of choice sweets in a package strongly affect whether they are purchased daily. Often in proposed packagings the amount of sweets is too large. This suggests that a purchasing manner could be goods sold by weight, but in this case the quality of product often does not conform to consumer wishes. Marshmallows packed in boxes usually are chosen as gifts, since they are too expensive for daily consumption. Consumers normally purchase marshmallows in simple polypropylene (PP) pouch packaging. The respondents suggested that they would like to buy marshmallows packed as one piece in a pouch, as this could provide a good solution for a replacement of small chocolate bars or candy packs. This kind of supply on the market could present good opportunities for sales concerning coffee breaks during conferences and other public activities.

Further questions were given to determine respondent reaction to the wide assortment of marshmallows in the market. There are several marshmallow forms currently in retail:

traditional (with vanilla), covered with chocolate layer or glazed, as well as marshmallow refined with various additions, like strawberries, apricots, condensed milk, or cappuccino. Most respondents (97%) had noticed the high diversity of marshmallow in the market, but 3% of respondents did not pay attention to new products in the retail. 69% of respondents preferred marshmallows with additions, while 31.4% preferred traditional vanilla marshmallow (Fig. 5). Marshmallow flavour needs to follow the market demand and trends.

Much research has been carried out that has revealed high nutritional value of bee pollen. Considering pollen nutritional composition and according to non-scientific studies, dried bee pollen used as food in human diets provides a sense of well-being and contributes to functional and harmonious balance of the body. Confectionery products are usually selected for consumption based upon flavour and textural properties and are often considered as sources of fats and carbohydrates (Grembecka and Szefer, 2012). By enriching sugar confectionery with pollen, it would be possible to supplement the diet with biologically active substances. Increased competition and new opportunities stimulated by progressively vanishing trade barriers and expanding world markets have greatly accelerated the food industry's world-wide requirement for new products, quality improvements and extended shelf-life (Sidel and Stone, 1993). The survey data and consumer attitude towards bee products (marshmallow with 1.0, 1.5 and 2.5% bee pollen) can be used to determine preferences. Traditional marshmallow without pollen was used as a control. The marshmallow enriched with 1.5% bee pollen had an agreeable bee pollen taste, aftertaste and aroma. This indicated that the optimal bee pollen concentration for further experiments should be 1.5%. Regarding willingness to buy, most of the respondents (91.9%) after testing considered that they would buy this product as interesting. However, 8.1% of respondents had a dislike to the new product, because they did not like honey and bee pollen taste, and they would not choose any product with this taste. Since about 92% of the respondents considered marshmallow with 1.5% bee pollen as tasty and with a pleasant pollen taste and aftertaste, it can be expected that the new product would take a certain niche of the market.

The conclusions are:

- Latvian consumers recognise marshmallow, sugar-candy with foam-like structure. It is purchased every day and in social events. The data showed that consumer choice is affected by the type of packaging and the variety of flavours. For everyday use, consumers would prefer smaller package size. Consumers admitted that they would like to see a new product with pollen rich marshmallow on the market.
- The results of sensory evaluation demonstrated that there was no significant difference between the control sample (marshmallow without bee pollen) and the marshmallow sample with 1.5% bee pollen ($P > 0.05$).

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ZEFĪRU SENSORĀ VĒRTĒŠANA UN PATĒRĒTĀJU ATTIEKSME

Sensorai vērtēšanai un tirgus izpētei ir liela nozīme jaunu produktu izstrādē. Lai arī zefīrs Latvijas tirgū ir pieejams sen, tomēr arvien ir novērojamas tendences šī produkta bagātināšanai ar dažādām piedevām. Zinātniskajā literatūrā atspoguļoti pētījumi, kas parāda ziedputekšņu augsto uzturvērtību, līdz ar to ir saprotama cilvēku interese par to sastāvu un izmantošanas iespējām uztura papildināšanai un pilnveidošanai. Pētījuma mērķis ir noskaidrot situāciju Latvijas tirgū, izvērtēt patērētāju informētību par zefīru, tā pieprasījumu, optimālo iepakojuma daudzumu un tā noformējumu, kā arī veikt sensoro vērtēšanu ar ziedputekšņiem bagātinātam zefīram. Anketēšana veikta interneta vietnē www.visidati.lv. Kopumā aptaujāti 900 respondenti (28% vīrieši un 72% sievietes). Lai noskaidrotu potenciālo patērētāju viedokli, ar ziedputekšņiem bagātināts zefīrs vērtēts Starptautiskajā pārtikas izstādē „Riga Food 2011”. Vērtēšanā piedalījās 130 izstādes apmeklētāji. Sensori pēc hedoniskās skalas novērtēts zefīrs ar dažādu ziedputekšņu koncentrāciju (1, 2 un 2.5%). Lai noteiktu paraugu patikšanas pakāpi (ISO 4121:2003), izmantota 9 punktu hedoniskā skala (9 – ārkārtīgi patīk, 1 – ārkārtīgi nepatīk). Pēc sensorās vērtēšanas rezultātiem var secināt, ka patērētājiem vislabāk patīk kontroles paraugs (klasiskais zefīrs) un zefīrs ar 1,5% ziedputekšņiem, starp tiem patikšanas ziņā nepastāv būtiska atšķirība ($P < 0,05$). Tas liecina, ka patērētājiem vienlīdz labi patīk gan klasiskais zefīrs, gan ar ziedputekšņiem bagātināts (1,5%) zefīrs.