PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B,
Vol. 73 (2019), No. 6 (723), pp. 513–518.

DOI: 10.2478/prolas-2019-0078

CONTENT OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN FRESH APPLE, POMACE AND POMACE WATER EXTRACT — EFFECT OF CULTIVAR

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*The study aimed to evaluate the chemical composition (total phenol, flavonoid, and tannin content and the antioxidant activity) of 11 apple (*Malus domestica*) cultivars in fresh apples and their processing by-products — apple pomace and its water extract. In addition, the proportion of chemical compounds and antioxidant activity in fresh apples and pomace transferred to the resulting water extract was calculated. Three different season's cultivars (autumn–winter, winter and late winter) were selected in the research, and significant differences between chemical parameters within groups were found. Cultivars 'Zarja Alatau', 'Belorusskoje Malinovoje', 'Sinap Orlovskij' and 'Antej' are characterised by a higher content of determined compounds in fresh apples, pomace and pomace water extract. The results showed that the water extract in relation to fresh apples and apple pomace still contains an appropriate amount of total phenols, flavonoids and tannins in the range 2.5–9.6%. These results indicated that apple pomace water extracts could be recommended for use in food as well as in the pharmaceutical industry.*

Key words: Malus domestica*, by-products, hot water extraction, chemical composition.*

INTRODUCTION

Apples are one of the delicious, popular and widely used fruits in the world, which are consumed fresh or as processed products. According to statistics, the total production volume of apple juice in European Union from 2008 has increased and in 2017 reached roughly 2.05 billion litres (Schmid, 2018). In large-scale apple juice industry, about 70–75% of apples are utilised for juice and the remaining 25–30% is the by-product, apple pomace (AP), which is susceptible to biodegradation and is a problem for manufacturers (Grigoras *et al*., 2013). Wet apple pomace (AP) is most commonly used for animal feed or as fertiliser for soil agglomeration (Ajila *et al*., 2015). As AP is a material with high moisture and sugar content, its direct disposal in landfills can cause serious environmental pollution. It may also be a suitable material to aid composting, as this by-product application has two benefits: compost nitrogen conservation and fertiliser quality (Mao *et al.,* 2017). Fresh AP is perishable, and it must be preserved in order to be stored and used over a long period of time, including for its utilisation as animal feed (Shalini and Gupta, 2010; Maslovaric *et al*., 2015).

A lot of reviews and studies on the apple pomace chemical composition and its application appear in scientific literature (Bhushan *et al.,* 2008; Shalini and Gupta, 2010; Rabetafika *et al*., 2014). Many studies on the exploitation of apple pomace with biotransformation, for food products, ethanol production, pectin extraction, as a source of fibre, as well as for fuel purposes have been carried out. Apple pomace is a promising source of different functionally important bioactive compounds: carbohydrates, proteins, fatty acids, phenolic compounds, vitamins, minerals and others, with a wide range of food applications. Moreover, apple pomace can be widely used for obtaining several bioactive compounds (García *et al.,* 2009; Kalinowska *et al*., 2014; Rabetafika *et al.* 2014; Ajila *et al.,* 2015; Perussello *et al.* 2017; Waldbauer *et al*., 2017). Preclinical studies have found that apple pomace and its isolated extracts improved lipid metabolism, antioxidant status and gastrointestinal function, and had a positive effect on metabolic disorders (e.g., hyperglycemia, insulin resistance, etc.) (Bhushan *et al*., 2008; Fotschki *et al*., 2014).

The recovery of bioactive compounds from apple pomace is influenced by the solubility of these compounds in the solvent used for the extraction process. Furthermore, solvent polarity plays a key role in increasing the solubility (Grigoras *et al.*, 2013; Candrawinata *et al.,* 2015). Water extraction provides an opportunity to extract the antioxidants of apple pomace. The water extracts obtained contain high amounts of phenolic compounds with high antioxidant capacity (Cam and Aaby, 2010; Reis *et al.,* 2012). Phenolic compounds of AP, mainly phenolic acids and flavonoids that display antioxidant activity, are readily extracted with water and with food-compatible aqueous organic solutions. Water is environmentally friendly and cheap, making it an ideal solvent for the extraction of AP phenolic compounds (Reis *et al.,* 2012).

Studies on physical properties and chemical composition of different apple cultivars fruits are widely available in scientific literature, but there is a lack of information on the chemical compounds of cultivars available in different seasons (autumn–winter, winter and late winter) in the complex view: apples > pomace > water extract. Therefore, the aim of this study was to evaluate chemical composition (total phenols, flavonoids, tannins) and antioxidant activity of 11 apple cultivars grown in Latvia in fresh apples, apple pomace and apple pomace water extracts. In addition, an aim was to evaluate the proportion of chemical compounds and antioxidant activity of fresh apples and pomace transferred to the resulting water extract.

MATERIALS AND METHODS

Research objects and experimental design. The research was carried out at the Institute of Horticulture, Latvia, in 2017–2018. Eleven apple (*Malus domestica* Borkh.) cultivars were included, according to three seasonal groups: autumn–winter (AW): 'Auksis', 'Dace', 'Saltanat'; winter (W): 'Alesja', 'Edite', 'Gloster', 'Rubin' (Kazakh cv.), 'Zarja Alatau' and late winter (LW): 'Antej', 'Belorusskoje Malinovoje', 'Sinap Orlovskij'. The apples were harvested in Dobele in the Institute of Horticulture orchards, Latvia, accordingly to each cultivar's appropriate readiness for fresh consumption. All samples until analyses were stored in a cooling chamber (3 ± 0.5 °C temperature, relative humidity (RH) 85 ± 3%). According to the seasonality of fruits, AW apples were analysed in the second half of November, W apples in the second half of December, and LW apples in the end of January or beginning of February. The objects of the research were: fresh apples, apple pomace and apple pomace water extract.

Apple pomace was obtained after juice pressing from fresh apples using a basket press with a shredder (Basket press 60K, Voran, Austria). Fresh pomace was used for analyses and water extract preparation.

Chemical analyses: content of total phenols, flavonoids, tannin content and the antioxidant activity (AOA), using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was determined in fresh apples, apple pomace and apple pomace water extract. The total research scheme is shown in Figure 1.

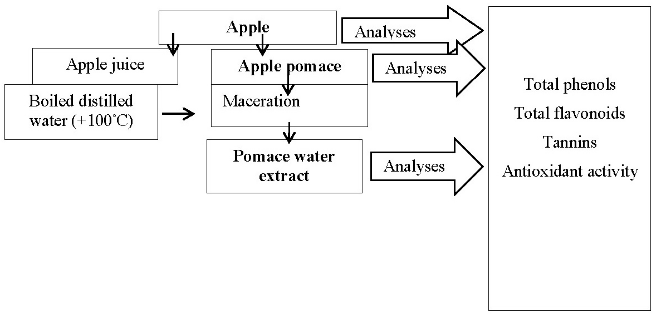


Fig. 1. Preparation scheme of apple pomace water extract.

Water extract preparation. In our experiment the apple pomace water extract was prepared by maceration in the following ratio: 200 g of fresh pomace was mixed with 800 ml distilled boiled hot water (95 ± 2 ^{o}C temperature), intensively stirred, followed by 10 hours of maceration, then decanted, and apple pomace water extract was obtained. Extraction proportions and times were selected by order of a private company, who commissioned this study.

Sample preparation for chemical analyses. Fresh apples and apple pomace were crushed with a kitchen blender to a homogeneous mass (Philips, Germany). For extraction by ultrasound, a definite amount of apple and apple pomace (1.5–2.5 g, according to structure) was mixed with 30 ml of 70% ethyl alcohol and the extraction was carried out in ultrasonic water baths at room temperature for 30 min (Bandelin, Sonorex). Then extract samples were centrifuged, and the supernatant fraction decanted for analysis.

Total phenols (TP) content was determined by the photometric method using Folin-Ciocalteau reagent by Singleton *et al.* (1999). The total phenol content in the apples, apple pomace and its water extracts were calculated as gallic acid equivalents (mg GAE·100 g^{-1}).

Total flavonoids (TF) content was determined by the photometric method using aluminium chloride complex forming assay by Shirazi *et al.* (2014). Total flavonoids content was calculated in the apples, apple pomace and its water extract as catechin equivalents (CAE mg·100 g^{-1}).

The tannin (TAN) content in apple, apple pomace and water extract samples was estimated by the method of Price and Butler (Paaver *et al.*, 2010). Then 2–3 g of apples and apple pomace were transferred to a 100 ml flask, 50 ml water was added and boiled for 30 min. After filtration through a cotton filter, the solution was further transferred to a 100 ml flask and water was added to the 100 ml mark. Accordingly, 2 ml of apple pomace water extract was transferred to a 50 ml flask, 30 ml water added and flasks put in ultrasound water baths at room temperature for 30 min. After filtration through a Watman filter the solution was used for testing. For analysis 0.5 ml aliquots were finally transferred to vials, 1 ml 1% K3Fe (CN) 6 and 1 ml 1% FeCl3 were added, and water was added to 10 ml volume. The tannin content was expressed as µg tannin acid equivalents (TAE µg·100 g^{-1}).

Antioxidant activity (AOA) was measured by the radical scavenging activity method using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH assay). The analysis was performed according to Brand-Williams *et al.* (1995) with minor modifications. Briefly, the test sample (100 µl) was reacted with 2.9 mL of $DPPH^{-}$ solution (0.0039 g $DPPH^{-}$ in 100 mL ethanol). Absorbance of the test sample (apple, apple pomace, water extracts) was measured at 515 nm using a spectrophotometer (UV probe 1800, Shimadzu). The absorbance results were converted using a calibration curve of the standard, and expressed as mmol Trolox equivalent antioxidant activity per 100 g^{-1} of the apple, apple pomace and water extract.

The moisture content of apples and apple pomace was determined according to the Standard ISO 6496:1999.

The results of the apples and apple pomace chemical analyses were expressed on a dry-matter basis (DW), but for water extracts they were expressed as fresh sample weight and for determination of the proportion of chemical compounds reaching the extract, calculation on a dry-matter basis was done.

The statistical analysis. Significant differences between cultivars for apple, pomace and extracts were determined by one-way analysis of variance (ANOVA) with replication and by the Independent Samples *t* Test using SPSS 17 software package. The mean arithmetic value, percentage and standard deviation were calculated for the obtained results. Pearson correlation analysis was used to determine significant relationships between parameters. The Tukey's multiple range test was used to determine significant differences ($p < 0.050$) among the studied samples.

Table 1

MOISTURE CONTENT OF THE APPLES AND APPLE POMACE (%)

	Cultivar	Fresh apple, mean ± SD	Apple pomace, mean ± SD
AW	Auksis	86.87 ± 0.30^{cd}	83.05 ± 0.87^{cdef}
	Dace	86.40 ± 0.30^{cd}	83.46 ± 0.19^{def}
	Saltanat	83.28 ± 0.09^{ab}	79.68 ± 0.58^{ab}
W	Alesja	85.10 ± 0.18^{bc}	81.74 ± 0.99^{cd}
	Edite	84.98 ± 0.16^{bc}	83.05 ± 0.16^{cdef}
	Gloster	85.87 ± 0.40^{cd}	78.40 ± 1.51^{a}
	Rubin (Kazakh cv.)	85.33 ± 0.03^{cd}	82.72 ± 0.33^{cde}
	Zarja Alatau	85.94 ± 2.15^{cd}	84.22 ± 0.32^{ef}
LW	Antej	87.19 ± 0.18^{d}	84.37 ± 0.11^{ef}
	Belorusskoje Malinovoje	86.32 ± 0.11^{cd}	84.76 ± 0.36^{f}
	Sinap Orlovskij	82.73 ± 0.25^{a}	81.33 ± 0.05^{bc}

The mean data are indicated by standard deviations (n = 4).

* values, marked with the same superscript letters in a column, are not significantly different ($p > 0.05$).

AW, autumn-winter; W, winter; LW, late winter.

RESULTS

The moisture content in tested samples of 11 apple cultivars and apple pomace is shown in Table 1. The results indicated that moisture content is significantly different among cultivars for fresh apple samples ($p = 0.012$), but no significance was found between apple pomace samples ($p = 0.102$). The difference in moisture content among the examined apples was related to apple size, genotype, rootstocks and biological factors of cultivars.

The content of total phenol, total flavonoid, and tannin content and antioxidant activity in 11 analysed apple cultivars is given in Table 2. The highest TP, TF and AOA was found in cultivar 'Belorusskoje Malinovoje', while the tannin content was higher in 'Antej', and this cultivar had similar antioxidant activity as 'Belorusskoje Malinovoje'. Low TP content was found in 'Auksis', while TF content was low in 'Saltanat'. The lowest tannin content was for three cultivars: 'Gloster', 'Dace' and 'Edite', and between them 'Dace' was characterized by the lowest antioxidant activity. Correlation was found between fresh apple chemical parameters: between total phenol and total flavonoid content there was a tight correlation, while between total phenol and tannin content an average correlation existed (Table 3). The antioxidant activity was tightly correlated with total phenol and total flavonoid content, but was medium tight with tannin content.

The results of chemical composition and antioxidant activity of analysed fresh apple samples by the Independent Samples t Test showed differences between autumn–winter, winter and late winter cultivars. Comparing AW and W apple cultivars, we found that the total phenol content and antioxidant activity were significantly different (accordingly, $p = 0$ and $p = 0.003$), while no differences were found for total flavonoid ($p = 0.080$) and tannin content ($p = 0.218$). There were significant differences between groups AW and LW in all tested indicators: total phenol ($p = 0$), total flavonoid ($p = 0.001$), and tannin ($p = 0.007$) content and antioxidant activity ($p = 0$). When results were compared between groups W and LW, significant differences were found for total phenol ($p = 0.034$) and flavonoid ($p = 0$) content, but no significant differences for tannin ($p = 0.549$) content and antioxidant activity ($p = 0.091$). Significant differences was found in the content of polyphenols in fresh fruits: in LW apple cultivars it was two times higher than in AW apples, and in W apples it was 1.5 times higher than in AW apple cultivars.

The apple pomace moisture content was from 84.76% to 78.40% (Table 1). The total phenol, total flavonoid, and tannin content and antioxidant activity in the apple pomace samples are given in Table 2. TP differed among all tested cultivars apple pomace samples. The highest TP, TF, TAN content and AOA were found in 'Zarja Alatau' apple pomace, while the lowest in 'Saltanat' pomace. There was tight correlation of TP content with TF content and AOA, but medium tight with tannin content (Table 3). TF content

Table 2

CHEMICAL COMPOSITION OF FRESH APPLES, APPLE POMACE, WATER EXTRACTS

	Cultivar	Total phenols, mg GAE 100 g^{-1} DW			Total flavonoids, mg CAE 100 g^{-1} DW			Tannins, mg TAE 100 g^{-1} DW			AOA, mmol TE 100 g^{-1} DW		
		apple	pomace	water extracts	apple	pomace	water extracts	apple	pomace	water extracts	apple	pomace	water extracts
AW	Auksis	219.57^{a}	652.70 ^{f}	21.16^{d}	342.97^{g}	437.60 ^{e}	32.73^{g}	36.43^{b}	43.79^{e}	2.71^{cd}	1758 ^{d}	2172^{e}	24.05^{gh}
	Dace	325.77^{b}	451.47^{c}	14.04^{a}	167.30^{b}	308.70 ^{b}	22.00^{e}	25.62^{a}	35.90^{bc}	2.46^{bc}	1137^{b}	1499^{c}	18.05^{bcd}
	Saltanat	322.50^{b}	377.10 ^{a}	14.43^{a}	145.30^{a}	239.63^{a}	16.97^{b}	42,79^{d}	29,11^{a}	1.74^{a}	1270^{c}	1172^{a}	16.25^{b}
W	Alesja	333.17^{b}	472.53 ^{c}	14.93^{a}	231.40^{d}	493.63 ^{g}	14.70^{a}	42.22^{cd}	37.67 ^{c}	1.71^{a}	1871^{e}	2006^{d}	13.56^{a}
	Edite	402.47^{c}	517.90 ^{d}	15.64^{ab}	189.23^{c}	487.70^{g}	18.43^{cd}	27.41^{a}	53.97^{f}	2.11^{ab}	1275^{c}	2203^{e}	18.90^{cd}
	Gloster	590.67^{e}	637.50 ^{e}	18.57^{c}	260.20^{e}	473.83^{f}	29.20^{f}	25.53^{a}	37.11^{bc}	3.36^{efg}	2397^{h}	2168^{e}	21.51^{ef}
	Rubin (Kazakh cv.)	493.30^{d}	545.90 ^{d}	16.95^{bc}	329.27^{f}	394.83^{d}	19.40^{d}	46.07^{e}	41.31^{d}	2.55^{bcd}	1843^{e}	2243^{f}	17.24^{bc}
	Zarja Alatau	641.97^{e}	740.00 ^{i}	18.38^{c}	433.40^{i}	685.93 ^{i}	17.80^{bc}	40.19^{c}	73.40^{g}	3.50^{g}	2059^{g}	2741^{i}	19.78^{de}
LW	Antej	628.17e	716.07^{g}	24.05^{e}	480.63^{j}	595.50^{h}	33.43^{g}	55.81^{g}	55.16^{f}	3.42^{fg}	2513^{i}	2664^{g}	26.02^{h}
	Belorusskoje Malinovoje	679.93^{f}	739.03^{h}	24.23^{e}	559.53^{k}	603.40 ^{h}	32.80^{g}	48.68^{f}	44.45^{e}	2.96^{def}	2539^{i}	2701^{h}	24.75^{h}
	Sinap Orlovskij	516.07^{d}	584.77^{e}	27.97^{f}	391.30^{h}	439.50 ^{e}	34.90^{h}	35.97^{b}	35.44^{b}	2.90^{cde}	1903f	2002^{d}	22.30^{fg}

The mean data are indicated by standard deviations (n = 4).

* values, marked with the same superscript letters in a column, are not significantly different ($p > 0.05$).

Table 3

CORRELATION COEFFICIENTS

	Apple			Pomace			Extracts		
	TP	TF	TAN	TP	TF	TAN	TP	TF	TAN
TF	0.695**			0.860**			0.868**		
TAN	0.461**	0.621**		0.528**	0.571**		0.591**	0.603**	
AOA	0.787**	0.817**	0.440*	0.914**	0.939**	0.674**	0.788**	0.900**	0.736**

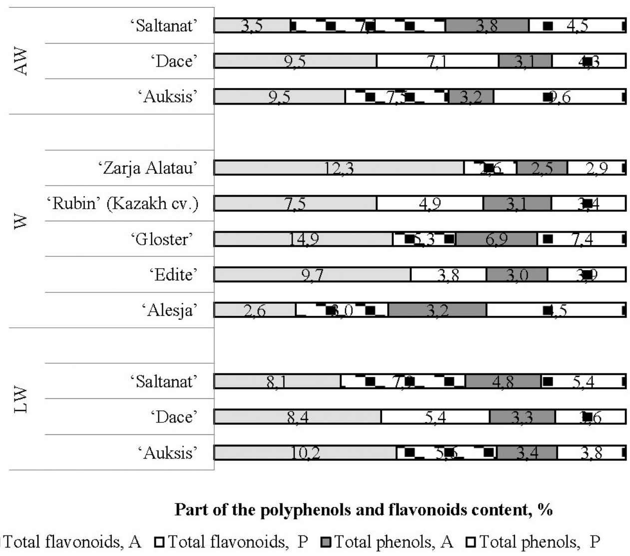
* Correlation is significant at 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

TP, total phenol; TF, total flavonoids; TAN, tannin; AOA, antioxidant activity; A, apple; P, pomace

was tightly correlated with AOA and tannin content, medium tightly with AOA.

The content of total phenols in analysed apple pomace water extracts varied significantly between cultivars (Table 2). The highest content of TP and TF was found in ‘Sinap Orlovskij’ pomace water extract, the lowest TP content in ‘Dace’, and the lowest TF content in ‘Alesja’ pomace water extract. The highest tannin content was observed in ’Zarja Alatau’ pomace water extract. The antioxidant activity of apple pomace water extracts is presented in Table 2. The results indicate that the late winter varieties had positive effect on the antioxidant activity of the extract, being higher (on average 24.36 mmol TE 100 g^{-1}) compared to the autumn–winter (on average 19.45 mmol TE 100 g^{-1}) and winter varieties (on average 18.20 mmol TE 100 g^{-1}). AOA was tightly correlates with total flavonoid and tannin content, and but medium tightly with total phenol content (Table 3).

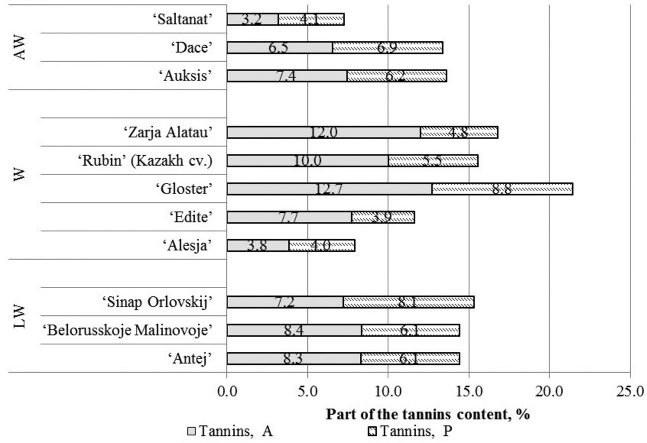
Figures 2–4 show the proportion of components of fresh apples transferred to the water extract: total phenols 2.5–6.9%, flavonoids 2.6–14.9% and tannins 3.2–12.0%. For apple pomace the respective proportions were total phenols



A, apple; P, pomace; AW, autumn-winter; W, winter; LW, late winter.

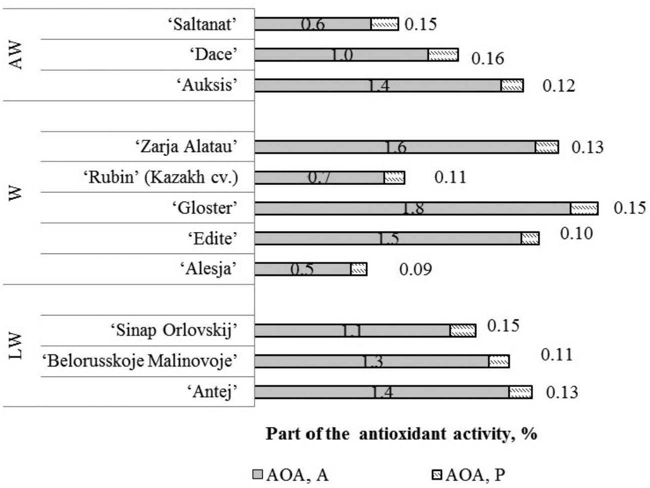
Fig. 2. Calculated proportion of total phenols and flavonoids in water extracts in relation to fresh apples and apple pomace.

2.9–9.6%, flavonoids 2.6–7.95 and tannins 3.9–8.1%. The antioxidant activity of the water extract in relation to fresh



A, apple; P, pomace; AW, autumn-winter; W, winter; LW, late winter.

Fig. 3. Calculated proportion of tannins in water extracts in relation to fresh apples and apple pomace.



AOA, antioxidant activity; A, apple; P, pomace; AW, autumn-winter; W, winter; LW, late winter.

Fig. 4. Calculated proportion of antioxidant activity in water extracts in relation to fresh apples and apple pomace.

apples was 0.5–1.6%, and 0.09–0.16% in relation to the apple pomace.

DISCUSSION

Phenolic compounds are a large group of organic substances that create fruit taste and aroma. Flavonoids and phenolic acids contribute to the quality aspects of apples. These compounds are involved in the quality characteristics of fresh fruits and their processing products (Soares *et al.*, 2008), and their content in different cultivar apples depends on their genetic diversity, as well as from the geographical growth location (Kaack and Pedersen, 2010; Begi *et al.,* 2011). In more severe climatic conditions, phenol content in apples is higher (Viškelis, 2018).

During the production of apple juice, which is associated with mechanical shredding, in the presence of air oxygen and light, as well as heating, the stability of polyphenols in the final products is affected. Apple pomace contains fruit skin, which can explain the elevated content of polyphenols (Persic *et al.*, 2017). Composition and concentration of phenolic compounds differ between the peel and flesh of the fruits (Jakobek *et al.,* 2013; Cebulj *et al.,* 2017), and are higher in the peel compared to the flesh (Vieira *et al.*, 2011). The epicarp (peel) has two to four times higher content of phenolic compounds than the mesocarp (pulp), depending on the cultivar (Zardo *et al.*, 2015).

A number of scientists have paid attention to the study of polyphenols and antioxidant activity in apple pomace. TP and TF content differs among cultivar apples, and a tight correlation exists among phenols, flavonoids and AOA (Ćetković *et al.*, 2008; Maragò *et al.*, 2015). The total phenolic content and antioxidant activity were observed to be significantly correlated in both flesh and peel (Vieira *et al.*, 2011).

The chemical composition of apple pomace extracts depends not only on the cultivar, juice squeezing technology, and water content in pomace, but also on extraction agents, their concentration, temperature, the type of extractor (Suárez *et al.,* 2010; Candrawinata *et al.,* 2014; Perussello *et al.,* 2017), and the different drying techniques (Rana *et al.*, 2015).

Researchers have found that extraction time, water temperature and ratio of pomace to water are the main significant parameters affecting TPC and antioxidant activity in the extract. The optimal temperature for the water extraction process is from 85 to 95 °C (Candrawinata *et al*. 2014). Waldbauer in a review noted that water extraction using a combination of 100 °C water temperature, 37 min extraction time, and 100 ml/g solvent to solid ratio provided an opportunity to extract the antioxidants of apple pomace by limiting the formation of 5-hydroxymethylfurfural (Waldbauer *et al.*, 2017). Under hot water extraction conditions, the cellular structure of plant tissues can be disrupted, releasing compounds of interest, which then may dissolve in the liquid water (Plaza *et al.*, 2013).

Our research increased knowledge on the total phenol, flavonoid and tannin content as well as antioxidant activity in fruits of Latvian grown apple cultivars, and their processing by-products — apple pomace and apple pomace water extract. Winter and late winter season apple cultivars were characterised by a higher amount of these compounds in fresh apples, pomace and pomace water extracts. It can be concluded that apple pomace hot water extracts contain a certain amount of phenolic compounds and could be recommended for use in food as well as pharmaceutical industry, particularly by choosing apple varieties with higher biochemical parameters.

ACKNOWLEDGEMENTS

Research and publication were done in the framework of Project "Latvian Food Industry Competence Center" No. 1.2.1.1/16/A/004.

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Received 20 November 2018
Accepted in the final form 23 October 2019

FENOLU SAVIENOJUMU DAUDZUMS UN ANTIOKSIDATĪVĀ AKTIVITĀTE SVAIGOS ĀBOLOS, SPIEDPALIEKĀS UN SPIEDPALIEKU ŪDENS EKSTRAKTĀ — ŠĶIRNES IETEKME

Šī pētījuma mērķis bija novērtēt ķīmisko sastāvu: kopējo fenolu, flavonoīdu, tanīnu saturu un antioksidantu aktivitāti 11 šķirņu svaigos ābolos, to pārstrādes blakusproduktos — spiedpaliekās un no tām pagatavotā ūdens ekstraktā. Papildus izvērtēts, kāda ķīmisko savienojumu un antioksidantu aktivitātes procentuālā daļa no svaigiem āboliem un spiedpaliekām pāriet iegūtajos ūdens ekstraktos. Pētījumā tika atlasītas trīs dažādu sezonu augļu šķirnes: rudens–ziemas, ziemas un vēlas ziemas. Noteikta būtiska atšķirība starp ķīmiskajiem rādītājiem grupās. 'Zarja Alatau', 'Belorusskoje Malinovoje', 'Sinap Orlovskij' un 'Antej' augļiem ir raksturīgs lielāks analizēto savienojumu daudzums svaigos ābolos, spiedpaliekās un spiedpalieku ūdens ekstraktos. Tika aprēķināts, ka ūdens ekstraktā attiecībā pret svaigiem āboliem un ābolu spiedpaliekām satur no 2,5 līdz 9,6% kopējo fenolu, flavonoīdu un tanīnu. Pētījuma rezultāti liecina, ka ābolu spiedpalieku ūdens ekstraktus var ieteikt lietošanai pārtikā, kā arī farmācijas nozarē.