



SENSE OF SMELL AND VOLATILE AROMA COMPOUNDS AND THEIR ROLE IN THE EVALUATION OF THE QUALITY OF PRODUCTS OF ANIMAL ORIGIN – A REVIEW*

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Abstract

The aim of this article is to examine how the sense of smell and aroma compounds influence the quality of food of animal origin, and to review gas chromatography-olfactometry methods of volatile substances analysis that can help to promote regional animal products. Smell and smell-inducing compounds play an important role in human life. People have made use of aromatic herbs and spices for ages. The classification of smells was developed by, among others, Aristotle, Linnaeus, Zwaardemaker, as well as Amoore, the creator of the stereochemical theory of olfaction. Smell is also of exceptional importance in a consumer's evaluation of food quality. Achievements in the area of chromatography, mass spectrometry and olfactometry have contributed to the development of tests for the content of volatile compounds in food materials, e.g. milk, cheese or meat. Analysis of these compounds is more and more often applied to regional products that are commonly characterised by different olfactory properties. The study's results of raw meat of different animal species show that nutria meat and mutton differ the most from others. Apart from genetic factors, meat composition depends on feeding method, quality and type of fodder, method of cutting, meat seasoning, pH and temperature of processing. Chromatographic and olfactometric tests on animal products, in conjunction with chemometrics, can contribute to the development of reliable characteristics and help to identify the products' origin. These tests are becoming an inseparable part of the policy of promotion of regional products with specific taste and olfactory properties.

Key words: aroma compounds, animal products, GCxGC-MS, olfactometry, chemometrics

The sense of smell, or olfaction, is one of two chemical senses (the other being taste). It is the ability to recognise stimuli coming from specific chemical compounds or mixtures thereof. On the other hand, smell is a property of chemical compounds and their mixtures, consisting in the stimulation of the sense of smell. A substance

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that induces the impression of smell is called an odourant, and may have a pleasant or an unpleasant smell. In the latter case, the smell is sometimes called a taint or off-flavour, as well as stench or stink.

Olfactory stimuli have played an important role in human life from the beginning of time. People have made use of perfumes, aromatic herbs, spices, resins and wood for ages. Currently these stimuli still constitute a part of our herbal tradition as well as the art of medicine and the preparation of various balms and extracts. The scents of various types of incenses, herbs, and resins are strongly associated with human cultural and spiritual life, as also evidenced by records from the distant past. In ancient Babylon, essential oils from cypress, cedar and sandalwood were applied in massages and baths. Perfumes were very precious gifts presented to kings and emperors. Evidence for this can be found in the Old Testament and ancient documents relating that the Queen of Sheba presented King Solomon with fragrant gifts. Moreover, these writings contain recipes for various fragrant mixtures. In ancient Egypt, ambergris was used as incense, and doctors applied it as a therapy for headache, cold and epilepsy. In the Middle Ages, Paracelsus used castoreum in the treatment of epileptic seizures, and in the eighteenth century it was commonly used, like ambergris, for headaches, hysteria and fever. These properties have not been verified by present-day science, but certainly the smell of incense has calming effects.

The review presents and discusses the role of the sense of smell and gas chromatography-olfactometry analyses of aroma compounds, in the evaluation of the quality of food of animal origin and promotion of regional animal products demonstrating specific taste and smell qualities.

Classifications of smells

Both smell and taste were mentioned as early as the fourth century BC by Aristotle in his treatise 'On the Soul'. He regarded the senses of smell and taste as related and believed that smells could be derived from tastes, since, as he wrote: 'There is an apparent similarity between the sense of smell and the sense of taste ... with the only exception that the sense of taste is more perfect in us'. Aristotle can be regarded as the creator of the first classification of impressions of taste and smell. He distinguished eight types of tastes and five types of smells. He considered the latter (sweet, oily, bitter, sharp and pungent) as derivatives of tastes, giving them the same names. In the eighteenth century, issues related to smell were studied by Carl Linnaeus, who distinguished aromatic, fragrant, ambrosial, garlic, caprine, stinking and nauseating smells. In the nineteenth century there were many more, for ten types of smells were defined by the Dutch inventor of the olfactometer, Hendrik Zwaardemaker. These included: aromatic, fragrant, ether, floral, ambrosial, burnt, garlic, caprine, offensive and nauseating smells. Later classifications were created by researchers in the twentieth century: the German psychologist Hans Henning and American psychologists E. C. Crocker and L. F. Henderson. The classification by Henning is in fact an extension of previous classifications. A different approach, based on only four smell impressions, was presented by Crocker and Henderson (1928), who assigned a 9-point intensity scale (from 0 to 8) to four

basic types of smells, floral, sour, caprine and burnt, thus enabling the distinction of 6,561 different smells.

However, the most famous classification of smells was created by the British biochemist John Earnest Amoore, author of the stereochemical theory of olfaction, the so-called 'lock and key' hypothesis (Amoore, 1952), the essence of which is the finding that a particle of an aroma substance of an appropriate structure (the key) attaches to a receptor with a complementary structure (the lock). Amoore thoroughly examined similarities in chemical structures of aroma-active compounds and distinguished more basic smells than Crocker and Henderson: camphor, musk, floral, minty, ether, pungent and putrid smells. He assigned seven shapes of olfactory receptors (Jacob, 2014) to them, based on the principle of complementarity, applied, among others, to a model similar to that explaining the attachment of enzyme to a substrate in enzymatic reactions.

No classification of smells created to date or the principles describing them are perfect; such classifications will always be simplified, due to the general rule of mental functioning called categorical perception. It is extremely hard to objectively define a number of smells, and the problem has not yet been finally solved. None of the known smell classifications is complete and therefore the statement in the Encyclopaedia Britannica of 1911 that 'it is impossible to create a satisfactory smell classification' unfortunately still seems valid.

Theories of smell

Amoore's stereochemical theory of smell was developed based on numerous observations that enabled him to conclude that the smell of low molecular weight alcohols and aldehydes clearly changes with the elongation of a particle chain; the smell of monocyclic compounds (benzene derivatives) clearly changes with changes in the position of a side substituent in the ring (*ortho*-, *meta*- and *para*- position); the smells of high molecular weight cyclic compounds are similar, irrespective of the arrangement of side substituents. Based on these observations, the British biochemist concluded that smell operates by means of the recognition of chemical compound spatial structures by receptors. The stereochemical theory of olfaction was criticised by another British researcher of smell, Moncrieff (1967), who found Amoore's suggestion (1952, 1963 a, 1963 b) that there were only seven classes of smells and receptors attributed to them overly simple and poorly documented.

Nowadays some papers have appeared providing arguments that support the new so-called vibrational theory, which attempts to explain the phenomenon of smell formation as the oscillation of intermolecular infrared frequency bonds. The foundation for this theory was created by Dyson (1938) and Wright (1961). In its most advanced form, smell is considered to be a spectroscopy, using the phenomenon of quantum electron tunnelling developed by Turin (1996) of the Fleming Biomedical Research Sciences Centre. Many scientists are sceptical about vibrational theory, due to two major disadvantages. Firstly, so far there is insufficient evidence that the enhancement of odourant particles with isotopes of specific atoms, affecting particle vibration frequency, causes a change in smell. Initially the smell induction hypothesis

based on the phenomenon of quantum electron tunnelling was supported by studies on fruit flies, but studies on humans did not support this theory, probably due to the insufficient sensitivity of human reactions to small changes in the vibration of particles of a given odourant. Secondly, based on vibrational theory, it is hard to explain differences in smell between enantiomers with identical rotation spectra. Therefore the studies conducted by Turin are inconclusive. It seems that the stereochemical and vibrational theories should not be regarded as competitive, but rather complementary, since each of them explains the phenomenon of reaction to smell only to some extent. However, there are more advocates of the stereochemical theory, which additionally explains the phenomenon of selective anosmia.

Smell and the structure of particles

Groups of aroma compounds

The stereochemical theory of olfaction is based on a 'lock and key' principle and refers to an analogous hypothesis in enzyme kinetics. However, it fails to provide an explanation for all aspects of smell, especially since the situation is made more complex by the fact that not only one but several receptors can react to the presence of a specific compound (Grosch, 2001). Generally, however, Amoore's stereochemical theory associates smell with the shape of an odourant cell, as supported by various examples. This theory is also confirmed by previous observations by Henning (1916), who in many cases quite accurately associated certain groups of compounds with certain smells. Henning observed that acyclic hydrocarbons have a fruity smell, sometimes with a note of freshness that might be due to the presence of unsaturated bonds. On the basis of the Flavornet database (2014) and the authors' own experiences, aromatic hydrocarbons with substituents in *para*- position have a smell characterised as spicy and aromatic, while bicyclic compounds have a resinous smell, like pinene. On the other hand, substituted cyclic compounds, including those multi-substituted with a substituent in *ortho*- position, are generally characterised by a fruity smell (Figure 1, α -ionone). However, it is worth noting that chemical structure vary greatly (Figure 2, citronellol and 2-phenylethanol) within the group of floral smells. Moreover, compounds of the structure characteristic of substances with a floral smell can have a slightly different smell, additionally enhanced with a spicy note, such as cinnamal (Figure 3). It seems, based on the above-mentioned examples, that cyclic compounds with one or more substituents can be classified into one common group of substances responsible for the induction of spicy-floral smells. Besides, there are two more groups of substances: those with putrid smells due to organic sulphur compounds, and those with burnt smells due to flat heterocyclic compounds with nitrogen atoms in a ring. Typical compounds in these groups are butanethiol; mercaptane, with a very low detection threshold (e.g. the majority of volatile sulphur-containing compounds), characterised by an extremely unpleasant, garlic-nauseating smell, similar to that emitted by a skunk; and pyridine (Figure 4). Another group of chemical compounds observably correlated with smell consists of amines. These include mostly secondary and tertiary amines of fishy (at lower concentration) or ammonia-like (at higher concentration) smells and are formed as a result of plant and animal decomposition.

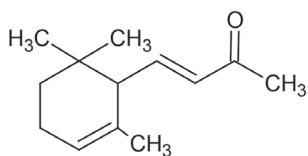
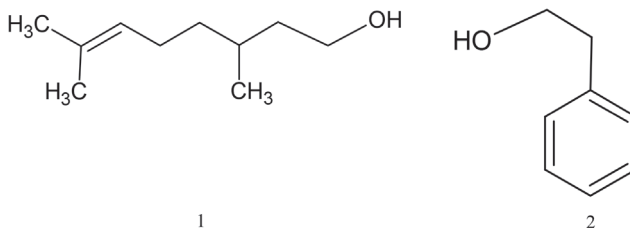
Figure 1. α -ionone

Figure 2. Various compounds: citronellol (1) and 2-phenylethanol (2-phenylethyl alcohol, 2) with similar smells (rosy)

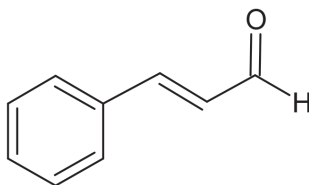


Figure 3. Cinnamal, with a floral, spicy smell

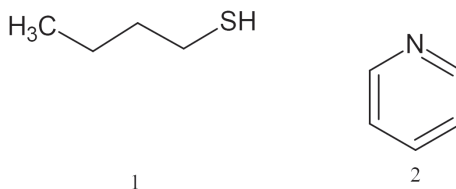


Figure 4. Compounds of putrid and burnt smells: butanethiol (1) and pyridine (2)

Particle stereochemistry and smell

The smells of aroma-active compounds are also affected by the spatial structures of their particles. Difference spatial arrangements of atoms can result in various smells. **It appears that particles of the same compound (isomers) do not necessarily induce the same smell, just as particles of different compounds can be responsible for similar smells.** The complexity of the question of smell detection is demonstrated in the following examples (Wińska et al., 2014, and own observations):

– Isomers of a compound can differ in smell

Two isomers of 4-isopropenyl-1-methyl-1-cyclohexene, occurring in the form of mutual mirror images as R(+)-limonene and S(-)-limonene (Figure 5), differ in smell. Both of them occur in citrus fruit peel, but the former gives off an orangy and slightly camphorous-minty smell, the latter a turpentine-lavender and citrus smell. Similarly, differences in smell detection can be observed for stereoisomers of other substances, such as those in carvone, found in caraway. Moreover, geraniol and nerol (Figure 6), geometric isomers of *trans*- and *cis*- 3,7-dimethyl-2,6-octadienol, respectively, have different smells. The *trans*- form, occurring in various species of lemon grass, lemon, geranium and lavender, has a waxy smell with a note of geranium, while the *cis*- form, found in substantial amounts in bitter orange and lavender, has a slightly camphor-lavender smell, also resembling the smell of rose petals. The above- mentioned essential oils, as well as others such as linalool and citronellal, can be classified in the group of citrus-rose smells and are commonly used in perfumery.

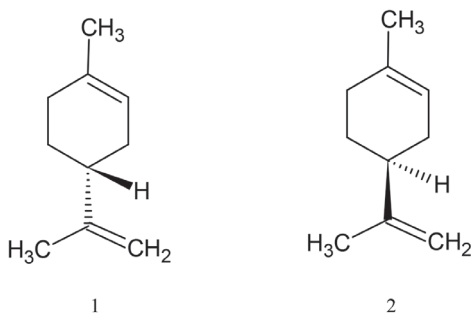


Figure 5. Enantiomers differing in smell: R(+)-limonene (1) and S(-)-limonene (2)

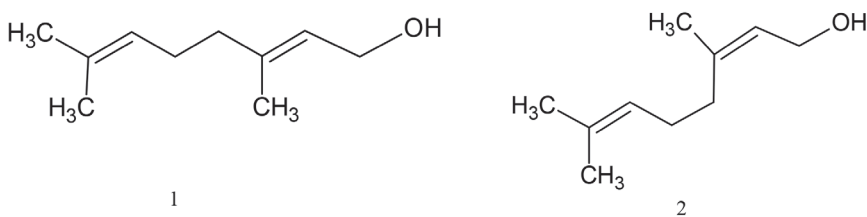


Figure 6. 3,7-dimethyl-2,6-octadienol isomers differing in smell: geraniol (*trans*-, 1) and nerol (*cis*-, 2)

– Different compounds can have similar smells

It appears that, just as different isomers of the same compound have different olfactory properties, different compounds that are not isomers can induce very similar olfactory impressions. This is true of citronellol and 2-phenylethanol (Figure 2). The former is monounsaturated acyclic alcohol, while the latter is alcohol with an aromatic ring in its particle. Despite their differing structures, both have a distinct rosy note.

Databases of odour-impact compounds

Additional information on volatile aroma compounds can be found in databases accessible via Internet. One of the most popular is Flavornet (2014), created by Terry Acree and Heinrich Arn, containing a list of odourants classified by odour notes and chromatographic properties such as the Kovats Index, making it possible to assign a value to an odour-active substance in order to position it relative to n-paraffins and thus to facilitate its identification on a chromatogram. Another is Pherobase (2014), a database maintained by Ashraf M. El-Sayed, including more than 6,500 pheromones and semiochemicals. Yet another extraordinarily rich database containing retention indices (in linear and logarithmic form) for chemical compounds, is in the possession of the National Institute of Standardisation and Technology (NIST Chemistry Webbook, 2014). The retention indices, odour notes, and additionally, mass spectra, present in the databases, are the most useful for chromatographic and olfactometric studies on odour-active substances (Acree et al., 1984; Majcher and Jeleń, 2011; Nicolotti et al., 2013).

The astonishing world of smells

The world of smells and aroma substances is unusually interesting and can even be astonishing. Both plant and animal kingdoms are abundant in volatiles, a great number of which possess a smell detectable by animals or humans. However, it is worth noting that many of the same volatile chemical compounds occur in various species of living organisms and products derived from them, as evidenced by three examples concerning α -ionone, indol and skatole (Figure 7).

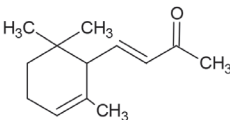
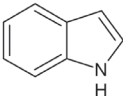
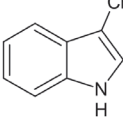
Substance	Occurrence
α -Ionone 	Violet (<i>Viola odorata</i>) Orchids (<i>Orchidaceae</i>) and Ambergis
Indole 	Jasmine (<i>Jasminum</i>) Narcissus (<i>Narcissus</i>) Orchids (<i>Orchidaceae</i>) Lemon (<i>Citrus limon</i>) and Faecal
Skatole 	Jasmine (<i>Jasminum</i>) Narcissus (<i>Narcissus</i>) Araceae (<i>Araceae</i>) and Faecal

Figure 7. The astonishing world of smells

Volatile compounds analysis by gas chromatography and sample preparation

If the compounds that induce smell sensations are so common in the plant and animal worlds, they must also be present in food. One-dimensional gas chromatography (GC), based on separation of components of a mixture on one column, is a basic chromatographic technique used to analyse volatile substances in food products (Majcher et al., 2011; Lv et al., 2014; Tu et al., 2014; Lv et al., 2015; Majcher et al., 2015). However, it is not always sufficient for analyses of complex mixtures and therefore for the last twenty five years two-dimensional gas chromatography, which is more efficient and shows greater resolution power, is under increasingly intensive development. When combined with a mass spectrometer (GCxGC-MS), it makes an even more sophisticated analytical tool. The issue of multi-dimensional chromatographic techniques was already known in the late 20th century (Liu and Phillips, 1991; Giddings, 1995), but considerable development thereof has taken place in recent years (Maikhunthod et al., 2010; Tranchida et al., 2012; Seeley and Seeley, 2013; Bueno et al., 2014). Improved resolution power is obtained by the combination of two chromatographic columns of different properties, usually polarity. A typical comprehensive two-dimensional gas chromatography system (comprehensive GCxGC) has a modulator that concentrates substances flowing out of the first dimensional (1D) column, and then it liberates them to the second dimensional (2D) column. Compared to one-dimensional GC, comprehensive GCxGC ensures greater sensitivity, which is useful especially when trace amounts of compounds to be analysed are present, and this technique is applied in tests of complex matrices, e.g. for food products. Currently the best solution for assaying compounds of very high volatility are liquid nitrogen cooled modulators, and for less volatile compounds – modulators with a CO₂ cooling medium. Two types of modulators in a multi-dimensional chromatography platform: longitudinally modulated cryogenic system (LMCS) and the CO₂ cryo-trap, were described by Maikhunthod et al. (2010). An interesting tunable GCxGC-MS system with three columns (1D – a non-polar column, 2D – a shorter polar column and a longer medium-polar column) was presented by Mommers et al. (2013). The system made it possible to change selectivity by sliding the 1D column through the modulator relative to the 2D column, and thereby altering its effective length. Chromatographic platforms with cryo-focusing modulators were also applied by other authors (Adahchour et al., 2003; Chin et al., 2011; Bueno et al., 2014). A less complicated solution than cryo-focusing modulation applied in comprehensive GCxGC is a simple flow modulation (Tranchida et al., 2011, 2013; Manzano et al., 2014). Another technique, which is a variation of two-dimensional chromatography, is heart-cutting multi-dimensional gas chromatography equipped with a Deans switch (H/C MDGC). The technique makes it possible to cut out co-eluting analytes flowing out of the 1D column and to transfer them to the 2D column where separation of previously non-separated compounds occurs. An efficient H/C MDGC-MS system consisting of two ovens with a special interface of low dead volume was developed by Shimadzu (Tranchida et al., 2012). Further interesting possibilities are presented by platforms combining GCxGC and MDGC techniques. An example may be a 3-column configuration, presented by Maikhunthod et al. (2010),

consisting of a long column (1D), as well as a short and long column (2D). It made it possible to carry out the following independent analyses: one-dimensional GC, comprehensive GCxGC and MDGC. Another interesting option is a Gerstel workstation with dedicated software and a mass spectrometer, consisting of two chromatographic lines: one-dimensional GC and MDGC. An important advantage of this solution is the fact that peaks originating from one- and two-dimensional analysis appear on the same chromatogram (Tranchida et al., 2012). An innovative system is proposed also by Cordero et al. (2008). The configuration contains one capillary column coated with two different stationary phases in series, which may be used for specific purposes. Both types of two-dimensional chromatography are being applied more and more extensively. Generally, H/C MDGC, apart from a slightly lower sensitivity than comprehensive GCxGC, proves to be much better in targeted analysis, since it shows greater separation power (Tranchida et al., 2012).

Sample preparation technique is one of the most important elements in the analysis of volatile compounds. Among numerous methods of sample preparation the following may be classified: liquid-liquid extraction (LLE, Komura, 2006; Inui et al., 2013), cold finger extraction (CFE, Cordero et al., 2015), solid phase extraction (SPE, Schmarr et al., 2010; Chin et al., 2011), focused ultrasound extraction (FUSE, Omar et al., 2012), steam distillation (SD), simultaneous distillation and extraction (SDE) and headspace co-distillation (HCD, Peng et al., 2004), as well as solvent assisted flavour evaporation (SAFE), developed by Engel et al. (1999). This last has found use in chromatography-olfactometry analyses (Majcher and Jeleń, 2011; Langos et al., 2013; Majcher et al., 2013). A disadvantage of the above-mentioned techniques is the difficult automation of the extraction process and integration with an analytical platform. Problems with integration with a chromatograph and automation were overcome by using high concentration capacity headspace techniques, such as stir bar sorptive extraction (SBSE), headspace sorptive extraction (HSSE), or solid phase microextraction (SPME). The latest has found the greatest appreciation, since it is fast, inexpensive and easy to automate (Cordero et al., 2015; Giri et al., 2015). It is based on sorption of substances found over the surface of a sample matrix to the extraction phase dispersed on a solid support – fibre (Pawliszyn, 2000). SPME performs well in rapid fingerprinting and has found numerous applications (Rochat et al., 2007; Leroy et al., 2009; Cordero et al., 2010; Majcher et al., 2011; Kiefl et al., 2012; Bordiga et al., 2013; Rivellino et al., 2013; Bordiga et al., 2014; Dugo et al., 2014; Lv et al., 2014; Tu et al., 2014; Lv et al., 2015). Less known are other microextraction techniques, such as single drop microextraction (SDME), hollow fibre liquid phase microextraction (HF-LPME), dispersive liquid-liquid microextraction (DLLME) and gas purge microsyringe extraction (GP-MSE, Yang et al., 2013).

Volatile aroma compounds analysis by gas chromatography-olfactometry

Discoveries within the technology of the production of chromatographic capillary columns, mass spectrometry and olfactometry have contributed to the development of food product testing for the presence of volatile compounds (Machiels et al., 2003, 2004; Moon et al., 2006; Ba et al., 2010). The gas chromatography method, especially with the use of an additional olfactometry port (GC-O) is a basic and

continuously developing analytical technique used for the detection of volatile, and sometimes odour-impact, substances. In this case, a carrier gas that contains analytes is split off into two flows after exiting the column: one flowing to a chromatographic detector, and the second to an olfactometer. By using this technique, one may evaluate the smell of odour-active compounds flowing out of the column and determine aroma profiles, which may be additionally associated with the sensory evaluation of a test product (Chin et al., 2011; Robinson et al., 2011 a, b; Kiefl and Schieberle, 2013). The recent set-ups of these types of analytical techniques include multidimensional gas chromatography integrated with an olfactometer and mass spectrometer. An example may be a system with two cryo-focusing modulators, with an olfactometer attached to a long (by effluent splitter) and short (by Y-type device) 2D column (Chin et al., 2012). The configuration has two circuits (with one olfactometer) placed in one chromatographic oven: MDGC-MS/Olfactometry (with two long columns, one polar and the other non-polar) and GCxGC-FID/Olfactometry (with a long polar column, as in the first circuit, and a short non-polar column).

Chromatographic systems with one or more olfactometry ports may also be found in other papers (Bueno et al., 2014; Giri et al., 2015). To evaluate the intensity of smell, dilution techniques are applied, which are useful in gas chromatography-olfactometry analyses to determine aroma compounds that play the most important role in the mixture being analysed. These techniques are based on dilution to odour detection threshold principles, such as: combined hedonic aroma response measurement, (CHARM, Acree et al., 1984; Acree, 1997) and aroma extraction dilution analysis (AEDA, Grosch, 1994). Both methods are similar, while the CHARM method, as compared to AEDA, additionally includes the duration of olfactory perception. However, the most commonly used is the AEDA method (Song and Cadwallader, 2008; Majcher et al., 2013; Takakura et al., 2014; Usami et al., 2014; Hausch et al., 2015). Olfactory concentration may be expressed in various units, usually related to each other, mostly as odour-active values (OAV), CHARM values or flavour dilution (FD) factor. Other methods used in gas chromatography-olfactometry analyses include aroma extract concentration analysis (AECA; the extract is concentrated, and not diluted as in the AEDA method) gas chromatography-olfactometry of static headspace (GCOH, lower and lower volumes of an odorant-containing headspace are analysed), olfactometry global analysis (OGA, intensities are defined by assessors based on the frequency of detection) and Osme (intensities of smells are evaluated by assessors based on Stevens' psychophysical power law). Moreover, attention should be paid to omission experiments, consisting in deduction from a given aroma model of subsequent odour-active compounds and testing the effect of this procedure on the odour of the whole composition. Omission experiments demonstrated that odorants of higher concentrations have the most effective influence on the odour of composition of aroma compounds, however exceptions to this rule may also be found (Grosch, 2001).

Gas chromatography, often together with olfactometry, is probably most often applied to volatile aroma compounds analysis in multiple products of plant origin, e.g. in nuts (Cordero et al., 2010; Kiefl et al., 2012; Kiefl and Schieberle, 2013), olive and oils (Purcaro et al., 2014; Tu et al., 2014), coffee (Majcher et al., 2013),

cocoa (Nicolotti et al., 2013), strawberries (Samykanno et al., 2013), mushrooms (Grosshauser and Schieberle, 2013; Usami et al., 2014), teas and herbs (Baba and Kumazawa, 2014; Lv et al., 2014, 2015), as well as in beverages (Butkhuip et al., 2011; Avellone et al., 2013; Inui et al., 2013; Langos et al., 2013). However, this method is also used for analysis of volatile aroma substances in products of animal origin, such as: honey (Stanimirova et al., 2010; Dymerski et al., 2013; Rivellino et al., 2013), milk (Contarini and Pavolo, 2002; Vazquez-Landaverde et al., 2006), cheeses (Frank et al., 2004; Ziino et al., 2005; Majcher et al., 2015) and meat (Greenberg, 1981; Arnold and Senter, 1998; Brunton et al., 2002; Hodgen, 2006; Moon et al., 2006; Yuan et al., 2006; Calkins and Hodgen, 2007; Soncin et al., 2007; Zviely, 2009; Øverland et al., 2011; Bueno et al., 2014).

Smell – evaluation of food quality by consumers

The importance of smell and volatile compounds in the evaluation of food quality is emphasised by Susan Schiffman of the University of North Carolina and Alan Hirsch of the Smell and Taste Treatment and Research Foundation, who believe that majority of sensations (80–90%) that accompany human consumption of various food products include smell sensations.

Studies on odour-active chemical compounds in food products consumed by humans were already being conducted in the twentieth century. A turning point in this type of study was the discovery of the so-called Maillard compounds (Maillard, 1912) formed in reactions between proteins and carbohydrates occurring in food. Taste and smell (i.e. flavours) precursors include sugars, nucleotides, amino acids, peptides, thiamine and fats. Another turning point in the studies on precursors was the finding that heating cysteine and ribose results in the formation of compounds that smell like meat (Morton et al., 1960).

Studies on volatile aroma compounds in meat coming from local animal breeds

Meat, an important component of the human diet, has a specific odour determined by volatile substances characteristic both for the gender, species, and breed of an animal (Elmore et al., 2000; Gorraiz et al., 2002; Insausti et al., 2005; Lu et al., 2008). Moreover, it should be considered that, apart from genetic factors, the composition of volatile compounds is also associated with factors such as feeding, method of maintenance, quality and type of fodder (Mandell et al., 1997; Elmore et al., 2000; Nuernberg et al., 2005; Scollan et al., 2006), ripening of meat in a freezer (Gorraiz et al., 2002; Yancey et al., 2006), and, for meat and cured meats, the type of carcass from which a product is manufactured (Lu et al., 2008). In cured meat, alcohols, phenols, aldehydes, ketones, esters, furans, pyrazines, and hydrocarbons were detectable, as well as carboxylic acids and odouriferous sulfur compounds (Shahidi et al., 1986; Ramarathnam et al., 1993). In American country ham, Song and Cadwallader (2008) found odour-active substances, such as: 1-octen-3-one, 2-acetyl-1-pyrroline, 1-nonen-3-one, decanal, and (E)-2-nonenal, together with (at lower concentrations): 3-methylbutanal, 1-hexen-3-one, octanal, acetic acid, phenylacetaldehyde, and furaneol. Also sotolon, a very powerful odourant (with an

aroma of maple or spice), was found in squid broth (Carrascon et al., 2014), although this compound is also present in food products other than meat (Majcher and Jeleń, 2005; Chen et al., 2013). Other odour-impact compounds, such as cresol or guaiacol are responsible for the smoked odour in smoked salmon (Varlet et al., 2006). Meat processed in other ways is also abundant in aroma-active substances. Seventy six volatile aroma compounds identified in roasted duck meat marinated in orange juice, an Asian speciality (Baruth and Ternes, 2011), included two “transfer substances” (originating from roasted orange juice): limonene (with an odour of citrus fruits) and β -myrcene (with a mouldy aroma), and several “reaction substances” (absent in the aroma of plain roasted duck meat and that of orange juice, but present in the roasted duck meat marinated in orange juice), e.g. furfural (roasted aroma), phenylacetaldehyde (flowery aroma), (Z)-limonene oxide and (E)-limonene oxide (both with less desirable odours).

The content of volatile compounds is also affected by pH value and the method of preparation and thermal processing (Meynier and Mottram, 1995; Mottram, 1998; Ames et al., 2001). Studies demonstrate among others that longer cooking or baking meat at lower temperatures may result in the formation of odours that are less acceptable to some consumers (Ba et al., 2012), as may excessively long storage of meat in a freezer, for more than two (Spanier et al., 1997) or three weeks (Ba et al., 2012). The low stability of meat and its products, in conjunction with consumers’ increasing demand for food products free of artificial preservatives, has even led to the investigation of “natural” preservation technologies, e.g. modified-atmosphere-packed (MAP) foods (Konieczny and Kijowski, 2005; Latou et al., 2014; Stoops et al., 2015). It was also found that high levels of polyunsaturated fatty acid contents cause undesirable aroma flavours in meat due to the lowering of the formation of some Maillard products (Ba et al., 2012).

As it can be seen, the issue is complex, therefore chromatographic and olfactometric tests on animal products are undergoing constant development. As for tests of aroma substances in meat, especially from breeds prevalent in Poland, it is difficult to find any data concerning this topic. Similar questions rarely appear in papers by foreign authors (Machiels et al., 2003, 2004; Yuan et al., 2006; Baruth and Ternes, 2011), and there are no such publications in Poland. Therefore, tests for the content of volatile, and sometimes odour-impact, compounds in animal products of domestic breeds, including Polish local breeds, should be of special interest.

Volatile aroma compounds in raw meat – authors’ studies

Meat contains volatile compounds, that can be detected using gas chromatography-mass spectrometry (GC-MS). In the Central Laboratory of the National Research Institute of Animal Production, initial studies were carried out, incorporating qualitative and semi-qualitative analyses and resulting in the identification of individual volatile substances in five types of meat, i.e. pork, beef, mutton, chicken broiler breasts and nutria meat. To supplement GC-quadrupole MS analyses, the initial determination of the scent of several compounds was also carried out using an olfactometer. A sample was prepared using the SPME (solid-phase microextraction) method on DVB/CAR/PDMS fibre. Chromatographic analysis was carried out on

a polar WAX-type column, using a temperature program (from 45°C to 240°C). Each type of meat, except the chicken breasts, was a mixture of various low-fat parts of carcasses. Forty-seven compounds were identified (Table 1), which could be classified as aldehydes, ketones, alcohols, hydrocarbons, fatty acids, furanes, sulphides and esters. Essential substances whose content in test samples was markedly higher than others included: hexanal, 1-octen-3-ol, nonanal, 2,3-octanedione, heptanal, pentanol, hexanol. Bar graphs presenting area % profiles, i.e. relative percentage of surface areas in the sum of the areas of all of the identified compounds, indicated the differences between individual meat types (Figure 8). More detailed statistical analyses based on analysis of variance proved these differences for several compounds. The greatest differences were observed between mutton and nutria meat, which differed in the content of 23 volatile compounds. Slightly less significant differences were observed between beef and nutria meat (18 volatile compounds), between pork and nutria meat, and between beef and chicken (16 volatile compounds). Based on these studies, it can be stated that nutria meat and mutton differ the most from others. Identified among the compounds that distinguish nutria meat from the others were 3-octen-2-one and 2-octenal (data not published).

Table 1. Volatile chemical compounds identified in raw pork, beef, mutton, chicken and nutria meat

No.	Retention time RT, ZB-Wax	Volatile compound	Identification method: S, MS(1), KI(2) ¹	Evidence in the literature
1	2	3	4	5
1	1.69	Carbon disulphide	1,2	Soncin et al., 2007
2	1.85	Octane	S,1,2	Hansen et al., 1987
3	1.93	Acetone	1,2	Øverland et al., 2011; Moon et al., 2006
4	2.01	Octene	1,2	Hansen et al., 1987
5	2.22	Butanal	1,2	Øverland et al., 2011; Moon et al., 2006
6	2.37	2-Butanone	1,2	Ba et al., 2012; Øverland et al., 2011; Moon et al., 2006
7	2.51	3-Methylbutanal	1,2	Ba et al., 2010, 2012; Baruth and Ternes, 2011; Calkins and Hodgen, 2007; Moon et al., 2006; Arnold and Senter, 1998
8	2.85	2-Ethylfuran	1,2	Calkins and Hodgen, 2007; Moon et al., 2006
9	3.19	Pentanal	1,2	Øverland et al., 2011; Calkins and Hodgen, 2007; Moon et al., 2006
10	3.71	Trichloromethane	S,1,2	Hodgen, 2006
11	4.76	2,3-Pentanedione	1,2	Zviely, 2009; Yuan et al., 2006
12	5.26	Hexanal	1,2	Øverland et al., 2011; Soncin et al., 2007; Moon et al., 2006
13	5.48	Undecane	1,2	Soncin et al., 2007; Yuan et al., 2006
14	6.18	2-Butylfuran	1,2	Brunton et al., 2002

Table 1 – contd.

1	2	3	4	5
15	7.61	1-Penten-3-ol	1,2	Øverland et al., 2011; Moon et al., 2006
16	7.72	Heptanal	1,2	Øverland et al., 2011; Moon et al., 2006
17	8.30	Dodecane	1,2	Ba et al., 2010; Moon et al., 2006
18	8.64	2-Pentylfuran	1,2	Soncin et al., 2007; Hodgen, 2006; Moon et al., 2006
19	9.12	6-Methyl-2-heptanone	1,2	Ba et al., 2012; Calkins and Hodgen, 2007
20	9.00	4-Heptenal	1,2	Calkins and Hodgen, 2007
21	9.47	3-Octanone or 2-octanone	1,2	Ba et al., 2010, 2012; Baruth and Ternes, 2011; Moon et al., 2006
22	9.58	Pentanol	1,2	Soncin et al., 2007; Moon et al., 2006
23	10.19	Octanal	1,2	Moon et al., 2006
24	10.23	1-Octen-3-one	1,2	Calkins and Hodgen, 2007; Moon et al., 2006
25	10.50	Tridecane	S,1,2	Moon et al., 2006; Yuan et al., 2006
26	10.88	2-Heptenal	1,2	Calkins and Hodgen, 2007
27	10.84	2,3-Octanedione	1,2	Moon et al., 2006; Yuan et al., 2006
28	11.75	Hexanol	1,2	Soncin et al., 2007; Moon et al., 2006
29	12.13	Nonanal	1,2	Øverland et al., 2011; Soncin et al., 2007; Moon et al., 2006
30	12.41	3-Octen-2-one	1,2	Calkins and Hodgen, 2007; Greenberg, 1981
31	12.53	3-Ethyl-2-methyl-1,3-hexadiene	1,2	Hodgen, 2006
32	12.73	2-Octenal	1,2	Moon et al., 2006
33	13.17	1-Octen-3-ol	1,2	Ba et al., 2012; Baruth and Ternes, 2011; Soncin et al., 2007; Moon et al., 2006; Yuan et al., 2006
34	13.39	Heptanol	1,2	Soncin et al., 2007; Moon et al., 2006; Yuan et al., 2006
35	13.96	Decanal	1,2	Moon et al., 2006
36	14.32	Benzaldehyde	1,2	Soncin et al., 2007; Moon et al., 2006
37	14.57	2-Nonenal	1,2	Moon et al., 2006
38	15.21	Octanol	S,1,2	Soncin et al., 2007; Moon et al., 2006; Arnold and Senter, 1998
39	15.96	2-Octen-1-ol	1,2	Calkins and Hodgen, 2007
40	16.45	Decenal	1,2	Øverland et al., 2011; Calkins and Hodgen, 2007; Moon et al., 2006
41	16.57	Caproic acid vinyl ester or 2-methyl-3-octanone	1,2	Calkins and Hodgen, 2007
42	17.26	2,4-Nonadienal	1,2	Moon et al., 2006; Yuan et al., 2006
43	17.52	Pentanoic (valeric) acid	S,1,2	Ba et al., 2012
44	17.93	2-Undecenal	1,2	Moon et al., 2006

Table 1 – contd.

1	2	3	4	5
45	18.95	Hexanoic (caproic) acid	S,1,2	Soncin et al., 2007; Moon et al., 2006; Yuan et al., 2006
46	20.16	Heptanoic (enanthic) acid	1,2	Calkins and Hodgen, 2007
47	21.59	Octanoic (caprylic) acid	S,1,2	Calkins and Hodgen, 2007

¹S – substance reference standard; MS – mass spectrometer; KI – Kovats Index showing the position of a volatile compound on the chromatogram in relation to reference hydrocarbons.

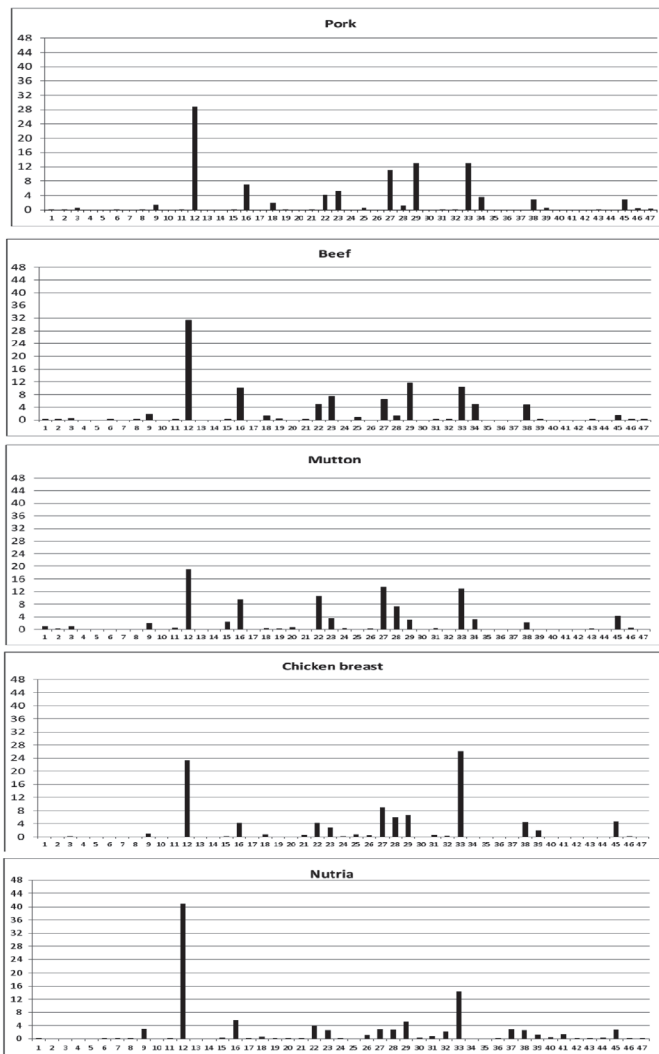


Figure 8. Profiles (area %) of volatile compounds in raw pork, beef, mutton, chicken and nutria meat. Y axis – area % of peaks due to volatile compounds. X axis – order number of a volatile compound (no.) according to the Table 1. Data obtained based on SPME-GC-MS analysis, column ZB Wax Plus, 30 m, 0.25 mm, 0.25 μ m

It should be noted that the differences between various types of meat are not necessarily always the same, since the composition of volatile compounds is also affected by the degree of meat maturity (Campbell et al., 2001; Stetzer et al., 2008), as well as a number of other genetic and non-genetic factors (Tai and Ho, 1998; Ames et al., 2001; Campbell et al., 2001; Insausti et al., 2005; Calkins and Hodgen, 2007; Cerny and Briffod, 2007). Moreover, the importance of reproducibility of chromatographic analysis, including column and SPME fibre wear, for the reliability of results should be emphasised (own observations).

Volatile aroma compounds in thermally processed meat

Much more abundant in volatile products are items subjected to ripening processes or thermal processing (Mottram, 1998; Elmore et al., 1999). Some substances formed in cooked or baked meat cannot be found in fresh meat. These include: 2-furfurylthiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methyl-3-furanthiol, bis(2-methyl-3-furyl) disulphide, 2,3-diethyl-5-methylpyrazine, 2-ethyl-3,6-dimethylpyrazine and 2-isopentyl-3,6-dimethylpyrazine. These substances are basic odourants in meat subjected to thermal processing, bearing a smell of baked and cooked meat, as well as caramel and earthy smells (Gasser and Grosch, 1988; Cerny and Grosch, 1992; Specht and Baltes, 1994; Kerscher and Grosch, 1997; Rochat and Chaintreau, 2005; Calkins and Hodgen, 2007).

In meat in which ripening processes have occurred or which was cooked, baked or fried, three groups of odour-active substances are formed: first group – compounds formed from lipid decomposition, second group – compounds formed in Maillard reactions, and third group – compounds formed as a result of interactions between lipids and compounds formed in Maillard reactions.

First group

In oxidative-reductive reactions from fatty acids, among others, unsaturated aldehydes are formed (Figure 9).

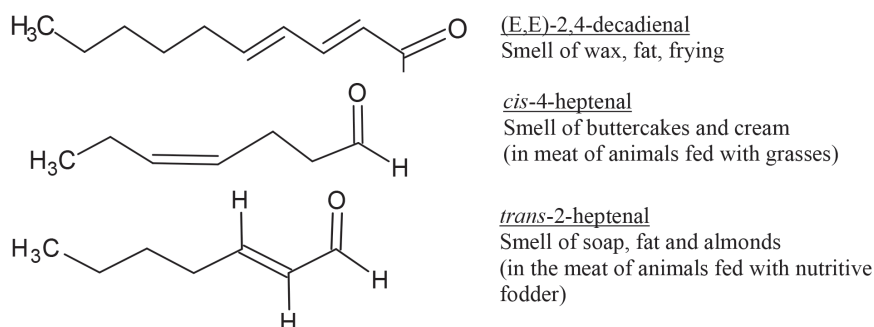


Figure 9. Volatile compounds formed in the decomposition of lipids

Second group

An example is 2-methyl-3-furanthiol, formed from 2-methyl-3-furanone and hydrogen sulphide, which is further condensed to bis (2-methyl-3-furyl) disulphide. Furanes with thiol and sulphide group in position 3 of the furan ring are responsible for the meat smell (Figure 10).

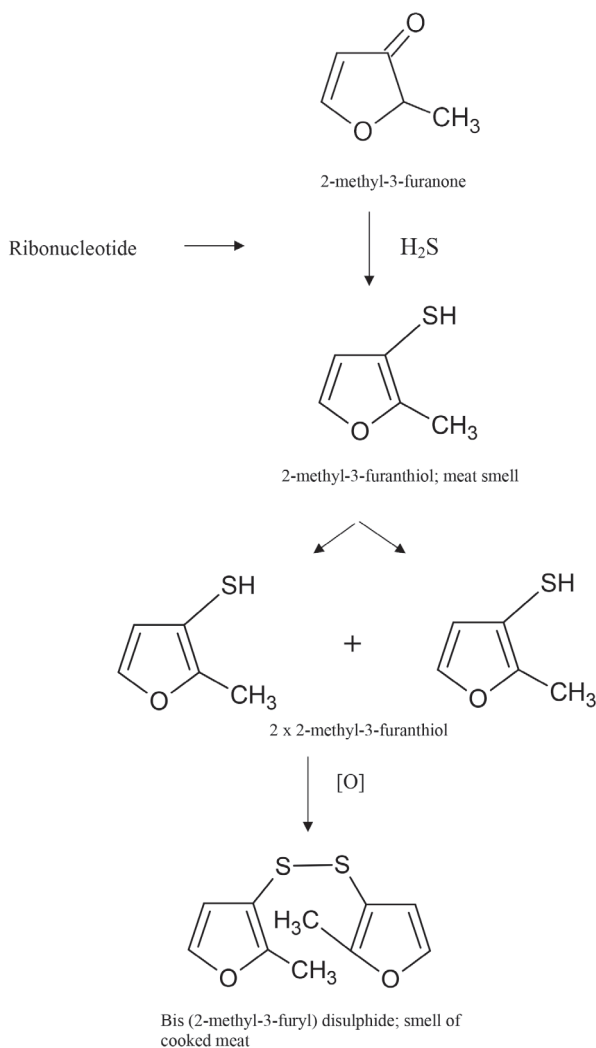


Figure 10. Volatile compounds formed in Maillard reactions

Third group

An example is 2-pentylpyridine formed from 2,4-decadienal and ammonia formed in Maillard reactions (Figure 11).

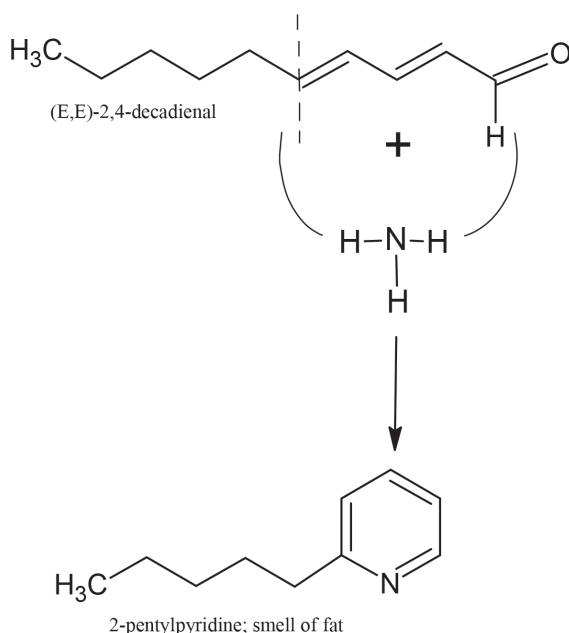


Figure 11. 2-pentylpyridine formed from interactions between lipids and ammonia obtained in Maillard reactions

Food products authentication

The studies relating to the determination of volatile compounds also apply to products other than meat. Analysis of these substances is increasingly being applied to regional and local products, which are often characterised by distinct taste and smell properties. Along with the determinations of other food components, this type of analysis enables demonstration of the specificity of these products in a scientific and objective manner and can be a very useful tool for the promotion of healthy and tasty food.

Volatile aroma substances as indicators to prove the origin of milk

Cornu et al. (2002) determined the terpene profiles in milk of cows fed in six different ways. Feeding was carried out: 1) in mountain pastures, 2) using hay from mountain region grasses, 3) using rye grass hay, 4) using rye grass silage, 5) using corn silage, or 6) using nutritive fodder. Significant differences in terpene content were observed between the milk coming from cows fed in mountain pastures and coming from cows fed predominantly with other types of fodder. This means that volatile compounds – terpenes contained in plants – pass easily from fodders to milk fat. Therefore differences in terpene profiles can be used to determine the region from which a given fodder comes.

Volatile aroma substances as indicators to prove the origin of cheeses

In volatile fractions in cheeses, as ripening products, fatty acids often predominate (both more and less volatile), but other volatile substances are also present.

In these products the following substances are typically identified: acetic, butyric, valeric, isovaleric, capronic, heptanoic, and caprinic acids; ethanol, propanol, butanol, pentanol, dimethyl disulphide, dimethyl sulphide, carbon disulphide, methional, 1-octen-3-on, 2-nonanon, heptanal, benzaldehyde, 2,4-decadienal, 2-nonenal, nonanal, ethyl butyrate, ethyl capronate (Thierry et al., 1999; Carpino et al., 2004; Frank et al., 2004).

Cheese type	Cheese	Smell
Cheeses from a <u>mountain shelter</u> made from unpasteurised milk	Original <i>oscypek</i> cheese	Stinging, milky, briny, buttery
	<i>Oscypek</i> cheese (cow's milk)	Smoky, scent of butyric acid
Industrial cheeses made from pasteurised milk	Industrial sheep's milk cheese	Toast
	Industrial cow's milk cheese	-

Figure 12. Characterisation of smells in original *oscypek* cheese and *oscypek*-type cheeses

Using advanced chromatographic techniques, the specificity of an individual type of cheese, depending on its origin and production method, can also be determined. An example may be Polish *oscypek* cheese, belonging to a group of 37 Polish regional products currently registered in the European Union, labelled with Traditional Speciality Guaranteed (TSG), Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO) marking. Along with *Bryndza Podhalańska* (rennet, non-smoked cheese), it is the second Polish product registered by the European Union (Commission Regulation (EC), 2007). Till now it is the only Polish regional product registered in the European Union (Commission Regulation (EC), 2008), which has been characterized in detail as concerns the content of volatile compounds. Key comparative studies, based on volatile compounds, of smoked cheeses, including *oscypek* cheese, were carried out by Majcher et al. (2010), by using the SPME method and chromatography/time-of-flight mass spectrometry (SPME-GC/TOF-MS). Four types of ripening cheeses in two groups were compared (Figure 12): two cheeses from a mountain shelter, i.e. original *oscypek* cheese (sheep's milk cheese) and *oscy-*

pek-type cheese (cow's milk cheese), as well as two industrial *oscypek*-type cheeses (sheep's and cow's milk cheese). 51 volatile compounds were identified; these could be classified as aldehydes, ketones, alcohols, sulphur compounds, free fatty acids, furanes, furanones, esters, phenols and terpenes. Non-pasteurised milk cheeses had a richer profile of volatile compounds; original *oscypek* cheese was characterised by the greatest number of identified volatile compounds and by a richer bouquet of aromas. Moreover, as with milk (Cornu et al., 2002), with cheeses there are some differences in the profiles of volatile compounds between original *oscypek* cheese and *oscypek*-type cheeses. Therefore, Majcher et al. (2010) concluded that volatiles and aroma-active compounds can be utilised as indices to find adulteration in PDO labelled *oscypek* cheese. Studies concerning the content of aroma compounds in smoked cheeses were extended in the paper by Majcher and Jeleń (2011), by performing additional quantitative analyses of 13 selected compounds using stable isotope dilution assays (SIDA) and in the paper by Majcher et al. (2011), where microbiological tests were also conducted.

Gas chromatography and chemometrics used in authentication

To demonstrate authenticity, semi-quantitative and quantitative targeted (Majcher et al., 2010; Nicolotti et al., 2013), or qualitative and quantitative non-targeted analyses (Majcher et al., 2015; Cordero et al., 2010; Kiefl et al., 2012) are applied. Comparison of samples based on chemical analyses may cover individual compounds, groups of compounds or all of the substances being determined (Reichenbach et al., 2012). It is also worth noting that to determine differences between test product samples, both one-dimensional (Lv et al., 2014, 2015; Majcher et al., 2015) and two-dimensional chromatography (Cordero et al., 2008, 2014; Giri et al., 2015) are applied.

With targeted techniques, the accuracy of quantitative or at least semi-quantitative assay is important to profile volatile compounds, since the greater the accuracy, the easier is discriminant analysis. Accuracy is ensured by the use of standards. Chromatographic quantitation is usually carried out by SIDA (Majcher and Jeleń, 2011), by standard addition (SA), or by the external standard approach. Attention should be paid to the multiple headspace extraction (MHE) technique (Nicolotti et al., 2013). Four consecutive extractions applied in this technique occur at the validation step only, in order to draw a calibration line against an external standard, while routine sample analysis is carried out using single extraction only. MHE is promising, since the relative standard deviation for this technique is lower than with the SA method.

With non-targeted techniques, to determine differences and similarities between samples mass spectra that contain a high number of data on the type and intensity of m/z fragment ions are used predominantly. Fragmentation pattern interpretation is difficult, but makes an important part of the fingerprinting procedure based on chemometric techniques. To determine similarities between data sets being compared, various algorithms are used, e.g. based on retention time and peak size criterion (i.e. chromatographic fingerprinting, Lv et al., 2015), or on the criterion of retention time and similarity of mass spectra of corresponding peaks (i.e. comprehensive template matching, Cordero et al., 2010; Kiefl et al., 2012). For this purpose, correlation and

Tucker's congruence coefficients may be used, which also serve as useful statistical parameters helpful in discriminant analysis (Lv et al., 2015).

Chromatographic analyses of complex biological matrices and food provide a very large number of data of high variability. The variability may be associated with the analytical technique applied or with the nature of the sample itself. To reduce this variability and to improve data interpretation capability, pre-processing data procedures are used. A primary method to reduce data variability is logarithmisation (Lehallier et al., 2012; Majcher et al., 2015). The variables that are the best to determine the differences between samples are selected. One variable selection method is variable importance in projection (VIP). An interesting approach towards data variability was presented by Lehallier et al. (2012), who applied the systematic ratio normalization (SRN) method, based on logarithm transformation of ratios of quantities of substances subject to chromatographic assay. In the SRN method no internal standard is required and it very efficiently emphasizes the factor-of-interest while diminishing the relative effect of variability due to experimental conditions. It was also found that it is more effective as compared to another method of data pre-processing, comprehensive combinatory standard correction (CCSC), and it may be used *a posteriori* on data sets.

Following pre-processing, data are subject to an applicable differentiation process. For this purpose it is necessary to use advanced multivariate statistical techniques. The most frequently used statistical models include: principal component analysis (PCA), linear discriminant analysis (LDA), soft independent modelling by class analogy (SIMCA), and support vector machine (SVM, Majcher et al., 2015), cluster analysis (CA) and PCA (Guerreiro et al., 2013; Tu et al., 2014; Lv et al., 2014, 2015), discriminant partial least squares (DPLS) and SVM (Stanimirova et al., 2010), PCA (Purcaro et al., 2014), as well as partial least squares regression (PLSR, Bueno et al., 2014). Application of data pre-processing procedures along with statistical models enhances the efficiency of sample differentiation based on chemical composition. An example may be the use of a VIP index along with a PLS model (Majcher et al., 2015), which is characterised by good classification accuracy of more than 90%. In this last paper multidimensional statistical techniques were combined with the SPME-GC-MS method. In the study quadrupole mass detection was applied instead of time-consuming and expensive TOF-MS. Moreover, a capillary silicon tube without stationary phase was used instead of an analytical column. The result of the analysis without chromatographic separation was a single peak representing a mixture of compounds, with characteristic fragment ions, the intensities of which were then used as variables for chemometric calculations.

Conclusions

Aroma compounds have always played an important role in human life. Their importance in the evaluation of food quality by consumers is constantly growing. Therefore, studies aiming at the recognition of the mechanisms of formation of volatile flavour substances and the determination of their influence on the taste and smell properties of food products are of great importance. These properties seem to be strongly related to the method of production of a given product, including param-

eters such as temperature and pH, as well as the breed, gender and method of feeding and maintenance of animals from which consumable products are produced. One important component of the human diet is meat, whose chemical composition determines its qualities. Preference for specific tastes and smells is mostly an individual matter for each consumer; however, it should be noted that significant amounts of unsaturated fatty acids in meat and meat products, used for health reasons, can have negative influences on its aroma. Temperatures used in product processing also affect the formation of volatile compounds. Meat processing at lower temperatures is detrimental to the taste and flavour, as is the ripening of meat for too long in a freezer.

Repeatability of conditions in chromatographic analysis, especially in the comparison of long-term studies, plays a crucial role in obtaining reliable results. First of all, the type and wear of a chromatographic column and SPME fibre should be taken into consideration. Therefore the validation and improvement of methods of determination of volatile flavour compounds by a gas chromatography-mass spectrometry method, using proper reference standards and analytical techniques (e.g. multiple headspace extraction) in order to ensure sufficient reproducibility of analyses, is an extremely important task.

Chromatographic and olfactometric studies on animal products are of great scientific and cognitive value and may contribute to the development of reliable characteristics of animal origin products. They are becoming an inseparable part of the policy of promotion of regional products demonstrating specific taste and smell qualities. It can also be expected that, apart from their high cognitive value, they will enhance the evaluation of animal products through assessment of culinary quality, contribute to improvements in this quality, and enhance the effectiveness of controls. These studies, especially when used in conjunction with multidimensional gas chromatography-mass spectrometry (GCxGC-MS or H/C MDGC-MS) and multivariate statistical techniques, will also help to identify the origin of unknown products and to prove their authenticity.

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