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Rita Székelyhidi[1], Erika Hanczné Lakatos[1], Zsolt Ajtony[1]

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Application of the SPME technique for determinate the quality indicators of dairy products

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1. SUMMARY

Solid Phase Microextraction (SPME) is a relatively new extraction technique that combines taking samples from a mixture with a simpler desorption of the components to be analyzed of the unenriched sample in its original state in the analytical instrument. Based on a large number of publications in the literature, the authors describe the possibilities of using the SPME technique in the case of dairy products. The main groups of components that can be detected by the SPME sampling procedure, such as fatty acids, aldehydes and ketones, esters, alcohols, sulfur-containing organic compounds, furans, phenols and terpenoids are presented, through examples in the literature. Due to the limited scope of the manuscript, the authors report only on the possibilities of sample preparation using the SPME technique. Instrumental analytical methods following solid state microextraction, mainly using gas or liquid chromatography, are not described. Details of instrumental analyses are available in the cited literature sources.

2. Introduction

The methods that can be used for the analysis of volatile components are selected on the basis of the amount and nature of the active aroma components, and this can be even more complicated in the case of food samples because of the matrix effect. The success of the analysis depends mainly on how the volatile components, which are often present in negligible amounts, react with the different food ingredients, e.g., fats or carbohydrates.

The analysis of the aroma components in dairy products is a complex task due to the complexity of the sample matrix and the complicated nature of the most commonly used sample preparation methods, such as vacuum distillation or liquid-liquid extraction **[20, 38, 40, 59]**.

Extraction analyses are constantly being updated and new techniques are being developed in order to reduce the extraction time, its solvent and equipment requirements, and to increase the sensitivity of the measurement and ensure wide applicability **[49]**.

The groups of chemical compounds that can be determined by SPME sample preparation play a role in the development of the taste and smell of the products, and act as indicators if there is a quality change in the product (microbiological deterioration, chemical decomposition, contamination).

2. Major groups of compounds that can be detected in dairy products by the SPME method

2.1. Fatty acids

The hydrolysis of natural fats produces aliphatic monocarboxylic acids, which can be grouped according to their carbon chain length, the number, position and configuration of the double bonds and the functional groups **[19]**. Fatty acids are composed of an alkyl chain and a carboxylic acid functional group connected to it **[50]**. Fatty acids with carbon numbers between 4 and 12 can be extracted using the SPME technique **[2, 16, 35, 42, 73]**.

Fatty acids and triglicerides are the main energy components of milk. Triglicerides consist of three

[1] Széchenyi István University, Faculty of Agricultural and Food Sciences, Department of Food Science

fatty acid molecules covalently linked to a glycerol molecule **[44]**. It should be noted that fatty acids are often the precursor compounds of esters, ketones and aldehydes found in foods **[47, 48, 72]**.

Fatty acids are biomarkers of microbiological activity. They are formed during the bacterial degradation of lactose, amino acids and casein, and the transformation of amino acids **[3, 69]**, and contribute significantly to the formation of the volatile aromas in cheeses **[21, 28, 71]**. Fatty acids with 12 carbon atoms have been detected by the HS-SPME method in cheeses **[8]** and whey proteins **[36]**.

The SPME technique can also be used to determine the fatty acid profile of different foods. Cheeses and yogurts can be characterized by the relative concentration of fatty acids **[4, 25]**.

According to other authors, measuring the amount and relative ratio of fatty acids is suitable for distinguishing between got cheese and cheese made from cow's milk, by identifying hexanoic acid which gives goat cheese its characteristic taste and smell **[46, 58]**. Since the predominant flavor in low-fat cheeses is provided by fatty acids with 4 to 8 carbon atoms and acetic acid approximately in constant concentration ratio **[13]**, the analysis of the fatty acid spectrum is also suitable for the detection of cheese counterfeiting **[41]**.

Fatty acid analysis is also used to determine the effectiveness of treatments used to increase the shelf life of dairy products **[73]**, however, other compounds present in the sample my interfere with the analysis, so defatting of the sample is advised when analyzing other components **[43]**.

2.2. Aldehydes and ketones

Aldehydes and ketones have a carbonyl group to which an alkyl chain and a hydrogen atom are attached in the case of aldehydes and two alkyl chains are attached in the case of ketones **[28]**.

Aldehydes are formed from primary alcohols by mild oxidation and have the general structure R–CHO. They are among the most reactive compounds. Further oxidation of aldehydes produces carboxylic acids. Typical reactions of aldehydes are addition, polymerization and condensation. In foods, aldehydes may form during the biosynthesis of amino acids, the enzymatic deamination or transamination of amino acids, in the reaction between amino acids and carbonyl compounds, during the autooxidation of unsaturated fatty acids, the enzymatic degradation of linoleic and linolenic acids and the transformation of carotenoids. Due to their characteristic odor, they are components of the aroma ingredients of various foods and play a role in the non-enzymatic tanning processes of foods. Their reactions of ketones are similar to those of aldehydes, but they are more difficult to oxidize, resulting in chain breakage. Most of them are compounds with a characteristic but not unpleasant odor **[19]**.

The presence of aldehydes and ketones in foods generally indicates the oxidation of fatty acids or amino acids, or the formation of metabolites in the course of bacterial activity **[28]**, but may also be added to the products during the manufacture of dairy products **[70]**.

It is also possible to analyze food quality indicators by determining the amount of certain aldehydes and ketones. The determination of the amount of diacetyl is used to check the quality of butter **[15],** but it can also be used to characterize the degree of ripeness of yogurts **[25]**. 3-Hydroxybutanone is a ketone that can be used to determine the degree of ripeness of cheeses **[5]**.

Methyl ketones are ketones in which a carbonyl functional group is located on the penultimate carbon atom of the alkyl chain. These compounds are often extracted from foods using the SPME technique. Methyl ketones may form in some cheeses due to microbiological activity **[41, 71]**. The methyl ketone 2-nonanone is a compound commonly found in most cheeses and whey proteins and has been identified using SPME **[28, 32, 46, 54]**. Analysis of other cheeses by the SPME technique has shown that 2-undecanone contributes greatly to the plant ("green") aroma of cheeses **[32, 69]**.

The analysis of methyl ketones is also suitable for the control of production processes, as they are formed from fatty acids during the heat treatment of milk **[73]**. DVB-CAR (Divinylbenzene-Carboxen) SPME fiber is used in the production of UHT milk to check thermal exposure **[65]**. It has been found that up to a fifty-two-fold increase in 2-heptanone and 2-nonanone concentrations can be observed in pasteurized milk compared to raw milk. A decrease in the concentration of the methyl ketone 2-butanone indicates when the milk has reached the end of its minimum durability or use-by date. Decreases in 2-butanone concentrations have also been recorded during the analysis of yogurt fermentation using SPME **[60]**, and in cheese production **[66]**.

Aldehydes are formed from acids of different classes by oxidation. Lower aldehydes, such as 2-methylpropanal and 3-methylbutanal are formed during the thermal decomposition of amino acids **[65]**.

Aldehydes can both improve or degrade food quality in the diary industry. Sample preparation with a CAR-PDMS SPME fiber can be used to identify components that indicate milk spoilage **[42]**. An increase in the concentration of aldehydes shows how close the milk is to the end of its shelf life or, in the case of UHT milk, its minimum durability date. An increase

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in the concentration of aldehydes is undesirable, because these compounds have a strong aroma, so their presence results in an unpleasant taste **[28]**. On the other hand, low molecular weight aldehydes (ca. ≤100 g/mol) produce a pleasant organoleptic effect in fermented dairy products. SPME sample preparation has also been used to determine the concentrations of benzaldehyde, acetaldehyde and various acetones **[66, 69]**. Acetaldehyde plays a basic role in forming the aroma of yoghurt [60] and several cheese, which is the most concentrate aldehyde in that types of cheeses **[8, 13, 41]**.

2.3. Esters

Esters are generally formed by condensation processes between acids or aldehydes and alcohols **[30]**. They make up one of the groups of food aromas that are present in cheeses and fruits, among other things **[36, 64]**.

In the course of the analysis of dairy products, CAR-PDMS-SPME fibers are used to determine the increase in the concentrations of ethyl acetate and methyl esters, as these compounds are indicators of the age of milk **[42]**. One of the significant sources of esters in dairy products is the esterification of fatty acids during manufacturing processes **[31, 39, 70]**. In dairy products, especially cheeses, fatty acid esters are mainly formed by enzymatic processes **[8, 21, 22, 71]**, but can also be added to dairy products and yogurts during production processes either by direct addition or by inducing artificial enzymatic activity **[25, 28, 41, 60, 66, 69, 70]**.

Esters can also appear in foods as contaminants. For example, phthalates supposedly released from plastic tubes used in the manufacturing technology have also been identified in milk using SPME sample preparation **[26, 73]**. The presence of phthalates in foods poses a food safety risk because they have endocrine disrupting effects **[62]**. Phthalates have also been detected in cow's milk, also from the technological environment. Of these, the presence of bis(2-ethylhexyl) phthalate was most common, when PVC pipes were used in the plant **[26]**.

2.4. Alcohols

Alcohols are among the most commonly identified compounds in fruits and vegetables, with hydroxyl groups attached to carbon atoms, and they can also be detected in dairy products, including unprocessed milk **[73]**.

Alcohols are present in most dairy products. Certain straight chain and methylated alcohols can even be detected in unprocessed milk using DVB-CAR-PDMS SPME fibers **[73]**. In yogurts **[69]** and Italian „Fossa" cheese **[28]**, alcohols have been identified as the second most common group of compounds after esters. The alcohol content of cheeses is often determined by SPME sample preparation **[5]**. Ethanol is usually the most abundant alcohol in cheeses **[41, 69, 71]**, although 1-phenylethanol **[58]** and 2-butanol are present in the largest amounts on goat cheeses **[22]**. Ethanol is also used as an indicator component of the success of fermentation of yogurts and soft cheeses **[25, 60]**. The presence of other types of alcohols is usually due to the presence of various bacterial strains used for the ripening of the cheeses **[8, 32, 71]**. Complex alcohols are also formed during the enzymatic conversion of fatty acids and amino acids. The alcohol content of cheeses decreases over time as alcohols are converted to esters **[21]**.

2.5. Sulfur-containing organic compounds

Volatile sulfur-containing organic compounds in dairy products are generally small molecules with an unpleasant odor and/or taste and their presence is therefore undesirable **[45]**. They may appear as degradation products of amino acids or vitamins **[23]**, however, certain sulfur-containing organic compounds may also have beneficial effects in foods, such as cheeses **[34]**.

In dairy products, sulfur-containing organic compounds are formed during heat treatment. Sulfur compounds in milk, such as dimethyl sulfide, come from the thermal decomposition of sulfur-containing amino acids **[42]**. Similarly, in another study, sulfur-containing organic compounds, formed during protein degradation, were detected during the analysis of whey proteins using SPME **[36]**. On the other hand, in the case of cheeses, sulfur-containing compounds are formed primarily by microbial activity and not due to heat treatment **[34]**. Analysis of sulfur-containing organic compounds using SPME allowed the detection of several significant volatile components, between the the anethiol, which is responsible for the characteristic garlic like odor of Camembert cheese **[32]** and goat cheese **[22]**.

2.6. Furans

Furans are oxygen-containing aromatic heterocyclic compounds that contain a five-membered ring. Furans have been the focus of research ever since they were presumed to form carcinogenic compounds during the heat treatment of foods **[37]**.

Among dairy products, large amounts of furan compounds can be detected in smoked **[41]** and deep-fried **[13]** cheeses using the PDMS-CAR-DVB SPME sample preparation method. In one case, a furan compound not characteristic of dairy products, 2-n-octylfuran was identified in whey protein. This compound is typically found in meat and bones **[36]**. 2(5H)-Furanone was identified by researchers in non-heat-treated consumer milk treated with a pulsating electric field using DVB-CAR-PDMS SPME fibers **[73]**.

2.7. Phenols

Phenols are aromatic compounds in which an alcoholic hydroxyl group is directly attached to a benzene ring. Headspace analysis of phenols is a complex process because most phenol derivatives are not volatile due to the strong intermolecular interaction with the sample matrix **[55]**.

Only a few reports were found on the study of phenols with SPME sample preparation in the case of dairy products. In the course of the analysis of cheeses it was found that phenol concentration increases during a 90-day maturation **[21]**. In another study, seven different ethyl- and methylphenols were identified in cheeses made from sheep's and cow's milk **[41]**.

2.8. Terpenoids

Terpenoids are derivatives of compounds consisting of isoprene units. SPME sample preparation analysis of this compound family can be used to determine the nature of the feeding animals received and the regions the animals fed had come from **[1, 13, 25, 27, 41]**. Poulopoulou et al. **[52, 53]** tested sheep's and goat's milk and dairy products made from them using SPME sample preparation. It was found in the study that the terpenoids detected may appear in milk as biomarkers of the feed consumed. Although terpenes are compounds of critical importance, their detection in milk samples using SPME is difficult. The reason for this is the matrix effect of milk fat and the significantly different vapor pressure of the individual terpenes **[1]**.

Terpenoids can be transferred to dairy products primarily from the food of plant origin consumed. Mono- and sesquiterpene compounds can be transferred from plants to milk in two ways: through the digestive tract or through the respiratory tract. In the first case, the molecules are transferred from the plants to the rumen, where the terpenes occasionally undergo chemical transformation. All of these molecules are transferred from the plants to the rumen, then they are absorbed from the rumen and enter the blood, from where they are excreted into the milk. Terpenes of plant origin, as well as those produced in the rumen, are easy to detect **[57]**. In the second case, the components spread through the air and enter the animal's lungs and, from there, the blood **[68]**. As volatile components enter milk, its biological and chemical characteristics, especially its microbiological state and aroma substance content, can change **[9, 11, 56, 68]**.

According to the data available in the literature, milks from different production areas (highlands, lowlands) and seasons (winter, summer) differ in their composition and organoleptic properties **[9, 11, 56]**.

Feeding of dairy animals is an important factor in shaping the character of cheeses, as it typically affects the fat and protein content of milk, its taste and microflora, as well as the functioning of lactic acid bacteria, which play a significant role in cheese making **[17, 29]**. It has been mentioned by several researchers that the milk of animals grazed on pastures of dicotyledonous plants contains significantly higher amounts of aroma components than the milk of their counterparts fed with hay, fodder or monocotyledonous plants **[24, 67]**. This difference is due to the fact that green (fresh) dicotyledonous plants contain higher amounts of terpene compounds, especially mono- and sesquiterpenes, than hay or other feedstuffs **[40]**.

Several research groups have found correlations between the organoleptic and physicochemical properties of cheeses and their place of production, the feeding of the animals and the compounds derived from the feed **[10, 11, 12, 18, 33, 40, 56, 67, 68]**.

Dumont and Adda **[24]** found that sesquiterpene compounds appeared only in Beaufort cheeses made in the summer, when the dairy herd grazed on mountain pastures. Mariaca et al. **[40]** identified 42 terpene compounds, such as β-pinene, β-mircene, linalool, limonene, α-phellandrene, α-terpinene, δ-3-carene, *p*-cymol, α-copaene and α-humulene, which are also present in the 13, mainly dicotyledonous plants found in the pastures, in Gruyere and Etivaz cheeses made in the mountains. Bugaud et al. **[12]** investigated terpene components in cheeses made from the milk of cows grazed in different areas. The results showed that the terpene profile of the cheeses studies was related to the botanical composition of the pastures and that these compounds were present in significantly larger amounts in mountain pastures with larger amounts of dicotyledonous plants than in the lowlands, where typically monocotyledonous plants grow.

Aroma component studies have been performed on several types of cheese, the best known and most popular of which are Emmental, Cheddar, Camembert and Parmigiano **[6, 7, 51, 61, 63]**. A wide range of volatile components is responsible for the aroma of the cheeses. These components are similar compounds in the different types of cheese, but their proportions may differ **[74]**. No sufficient data was found in the literature to clearly demonstrate the effect of mono- and sesquiterpene compounds on the taste of cheeses, with the exception of limonene, which has been proven to impart a citrus aroma to many cheeses **[14]**.

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