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Aromatic compounds of cheese ripening in animal skin: An overview

Pregled istraživanja aromatskih spojeva sireva koji zriju u životinjskoj koži

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Abstract

The aroma of a cheese is one of the key quality parameters and signs of distinguishing certain cheese types by consumers. Therefore, the aim of this paper is review the existing knowledge of the aromatic profile of cheeses ripening in an animal skin. Cheeses that ripen in an animal skin and produced in similar technology in Croatia (mišina – cheese in a sack), in Bosnia and Herzegovina (Cheese in a sack (local name: mjeh), Montenegro (Cheese in a sack (local name: mjeh), Turkey (Tulum cheese), Lebanon (Darfiyeh cheese) and eastern Algeria (Bouhezza cheese). The specific characteristic of these cheeses is ripening in an animal skin, as in a sack (of ewe or goat). The aromatic profile of these cheeses has not been sufficiently investigated and has been conducted primarily on Tulum and to a smaller extent of Lebanese Darfiyeh cheese, and cheese in a sack. There is no data about other cheeses that ripen in an animal skin. Since very little research has been made on the aromatic profile of these cheeses, further research should be conducted.

Keywords: animal skin, aromatic compounds, cheese, ripening

Sažetak

Aroma sira jedan je od ključnih parametara kvalitete i znakova raspoznavanja pojedine vrste sira od strane potrošača. Stoga je cilj ovog rada pregled postojećeg znanja o aromatskom profilu sireva, koji se proizvode na tradicionalan način i zriju u životinjskoj koži. Sirevi, koji zriju u životinjskoj koži i imaju sličnu tehnologiju, proizvode se u Hrvatskoj (Sir iz mišine), na području Bosne i Hercegovine (Sir iz mijeha), Crne Gore (Sir iz mijeha), Turske (Tulum sir), Libanona (Darfiyeh) i istočnog

Alžira (Bouhezza sir). Specifičnost ovih sireva je zrenje u životinjskoj koži, odnosno mišini (ovčjoj, kozjoj). Aromatski profil navedenih sireva je slabo istražen, a istraživanja su najvećim dijelom provedena na Tulum siru i manjim dijelom na libanonskom Darfiyeh siru, siru iz mišine, dok za ostale sireve,koji zriju u životinjskoj koži nema nikakvih podataka. Obzirom da je vrlo malo istraživanja o aromatskom profilu ovih sireva do sada napravljeno, potrebno je provoditi daljnja istraživanja u ovom pravcu.

Ključne riječi: aromatski spojevi, sir, zrenje, životinjska koža

Introduction

According to many historians, cheese was first manufactured about 7,000-6,000 B.C. in the valley between the Tigris and the Euphrates rivers. Research has shown that cheese was made from ewe and goat milk and that sacks were the first storage vessels for milk of nomadic tribes. The first written evidence of the history of cheese making derives from Greece and Rome (Lukač Havranek, 1995). A sack can be described as a “bag” made of the entire skin of a lamb or goat (Tudor Kalit et al., 2010). Traditional cheese in a sack is an indigenous Croatian cheese produced mostly on family farms of Dalmatian Zagora (particularly in the Šibenik – Knin area), Velebit and a part of Lika (Figure 1). Cheese in a sack is known under various names and was originally manufactured from raw ewe’s milk obtained from sheep that were fed in the natural pastures of the sub-Mediterranean area (Milin, 1969). Today, this cheese is produced from ewe, cow or goat milk or a combination of them. Cheeses with the same technology that also ripen in an animal skin are produced in Bosnia and Herzegovina (Cheese in a sack (local name: mjeh), Montenegro (Cheese in a sack (local name: mjeh), Turkey (Tulum cheese), Lebanon (Darfiyeh cheese), where it is particularly appreciated by the local population (Grbavac, 2002; Bijeljac, 2004; Yilmaz et al*.*, 2005; Hayaloglu et al., 2007a; Cakmakci et al., 2008; Serhan et al., 2009, Hayaloglu et al., 2013a). Hayaloglu et al. (2007a) mention that in Turkey there are several types of cheese that ripen in an animal skin with a significant difference in the production technology. Thus, cheeses in Turkey that ripen in an animal skin are known by various names, like Izmir Brined Tulum, Divle, Karin kaymagi, Cimi, and Selcuklu. A cheese that is characterized by ripening in an animal skin (Figure 1) but by instead with different technology is also produced in eastern Algeria, the so-called *Bouhezza* cheese (Zitoun et al., 2011; Zitoun et al., 2012). The technology most similar to the production technology of the Croatian cheese in a sack is that of Tulum cheese. The aroma and the volatile composition profile of Tulum cheese has been examined the most. (Hayaloglu et al., 2007b; Hayaloglu et al., 2013a and b; Hayaloglu and Karabulut 2013a). In addition to Tulum cheese, the aromatic profiles of Lebanese Darfiyeh cheese (Serhan et al., 2010) and cheese in a sack (local name: Sir iz mišine) have also been examined or described, but only partially (Vrdoljak, 2016).

Figure 1. Ripening of cheese in a sack (Source: foto M. Vrdoljak)

Cheese aroma

Cheese aroma has been one of the key quality parameters and the characteristic used for distinguishing a certain cheese type by consumers (Hayaloglu and Karabulut, 2013a). But it is known that each type of cheese is distinguished not only by its physico-chemical properties but also by its taste, which depends on milk, added cultures, technological parameters and ripening conditions (Serhan et al., 2010). Cheese flavor and aroma are also influenced by content of water and the pH value of curd and later by cheese body, salt content, salting method, ripening temperature, the activity of primary and secondary microflora, feeding, milk type, etc. (Tratnik, 1998; Bugaud et al., 2001; Fernandez-Garcia et al., 2002). Goat or ewe milk cheeses are characterized by more expressed taste and smell, and a spicier aroma, than cow milk cheeses. The aroma can be defined as the combined perception of taste and smell in which the mouth and nose participates. The special properties of cheese aroma are not based on only a single specific compound but on a combination of various compounds that occur during ripening (Mikulec et al., 2010).

The unique cheese flavor is the result of the interaction of various complex volatile and non-volatile chemical compounds which derive from milk fat, proteins and carbohydrates released during cheese ripening (Delgado et al., 2010; Hayaloglu and Karabulut, 2013a). Cottage cheeses have a quite similar aroma (McSweeney and Sousa, 2000), while the characteristic smell and flavor of certain cheese types is formed during ripening (Mikulec et al., 2010). Mikulec (2010) mentions that the cheese flavor is concentrated in the water-soluble fraction (peptides, amino acids, organic acids and amines), while aroma is mostly concentrated in a volatile fraction (organic acids, aldehydes, amines and esters). The concentration and composition of volatile compounds directly determine aroma and also sensory properties of a cheese (Table 1). Flavor and aroma develop more quickly in the production of indigenous and traditional cheeses by using raw milk and the cheeses ripen significantly faster, i.e. ripening time is shortened (Fox and McSweeney, 1998). The reason for this is the presence of endogenous milk enzymes and primary microflora that are inactivated by thermal milk processing (Urbach, 1997; Delgado et al., 2011). According to Bugaud et al. (2001), the environment and a pasture with their parameters influence the composition of fatty acids, volatile ingredients and the proteolytic activity of micoorganisms in milk and some ingredients get directly into milk (terpenes). Hayaloglu et al. (2013b) mention that the cheese volatile profile changes depending on the addition of a starter culture. Enzymes originating from starter cultures (proteinases and peptidases) have the main role in forming small peptides and amino acids that serve as precursors for the compounds responsible for cheese aroma (Urbach, 1997).

Many authors (Hayaloglu et al., 2007b; Delgado et al., 2010**;** Hayaloglu and Karabulut, 2013a; Hayaloglu et al., 2013a; Vrdoljak, 2016) identified significant number of volatile compounds in cheeses that ripen in an animal skin, and among them are acids, esters, ketones, aldehydes, alcohols, compounds with sulphur and terpenes. The obtained results showed that each cheese type had a different profile of volatile compounds that most probably depends on the production technology of these cheeses and the conditions in a ripening chamber and the cheese ripening stage is crucial for those profiles.

Table 1a. The aromatic compounds that contribute to the characteristic cheese flavor

Aromatic compounds	Flavour notes
Acids	
Butanoic acid	Cheesy[1,2], rotten[1], sharp[1], rancid[2], putrid[2], sweaty[2]
Acetic acid	Vinegar sour[1,2], sharp[1], pungent[2]
Hexanoic acid (caproic)	Sharp-goaty[1,2], pungent[2], blue cheese[2]
Octanoic acid (caprylic)	Body odour[1,2], sweat[1], fatty[1], rancid[1], cheese[1], pungent[1], goaty[2], waxy[2], soapy[2]
2-Methylbutanoic acid	Fruity[1,2], sour[2], sweaty[1,2]
3-Methylbutanoic acid	Rotten cheesy[1], Swiss cheese[2], waxy[2], sweaty[2], old socks[2], fecal[2]
2-Methyl propanoic acid	Sweet[2], apple-like[2], rancid butter[2]
Decanoic acid	Warm[1], stale[1], butter[1], sour fruit[1], grassy[1], fatty[1], goat[1], rancid[2]
Pentanoic acid	Rain[1], wood[1], vegetable[1], spicy[1], nutty[1], grain[1], swiss cheese[1], cheesy-like[2], sweaty[2], rancid[2], waxy[2]
Propanoic acid	Vinegar[2], pungent[2]
Ketones	
2-Butanone	Acetone[1,2], etheric[2]
2,3-Butandione	Buttery[2]
2-Pentanone	Orange peel[1], fruity[2], acetone[2], sweet[2], etheral[2]
2-Heptanone	Musty[1], varnish[1], sweet[1], floral[2], fruity[2]
2-Nonanone	Floral[1,2], fruity[1,2], peachy[1], musty[2]
3-Hydroxy-2-butanone	Buttery[2]
6-Methyl-5-hepten-2-one	Woody-moss[1]
2-Hydroxy-3-pentanone	Truffle[1], earth-nut[1]

[1]Boltar et al. (2015); [2]Ferreira et al. (2009); [3]Weimer (2007); [4]Singh et al. (2003); [5]Smit et al (2005)

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Table 1b. The aromatic compounds that contribute to the characteristic cheese flavor

Aromatic compounds	Flavour notes
Alcohols	
Ethanol	Alcohol[2], mild[2]
3-Methyl-1-butanol	Harsh[1], dull[1], fruity[2], alcohol[2]
2-Methyl-1-propanol[2]	
2-Butanol	Alcoholic odor[1,2]
2-Pentanol	Fresh[1], mild green[2], fused oil[2]
1-Hexanol	Green[1]
Aldehydes	
Hexanal	Green[1]
Heptanal	Soapy[1], harbaceous[1]
Nonanal	Green[1], fatty[1], soapy[1]
Octanal	Fatty[1], green[1]
3-Methylbutanal	Green[1,2], malty[1,2], herbaceous[1], dark chocolate[2]
2-Methyl propanal	Malt[3,4] , banana[5], malty[5], chocolate-like[5]
2-Methyl butanal	Dark chocolate[3,4], malt[3,4]
Esters	
Ethyl acetate	Fruity[1,2], pinapple[1], juicy fruit gum[1], apples[1], solvent[2], pineapple[2]
Ethyl butanoate	Sweet[1,2], fruity[1], apple[1], green[1], pineapple[2], banana[2]
Ethyl hexanoate	Fruity[1], grape melon[1], pineapple[2], apple powerful[2]
Ethyl octanoate	Fruit[1], pear[1], banana[1], pineapple[1], wine[1], flowers[1,2], apricot[2]
Ethyl decanoate	Fruity[1]
3-Methylbutyl acetate	Fruity[1,2], banana[1,2], caramel[1], peanuts[1]
Terpenes	
Limonene	Mild[1], citrus[1], sweet[1], orange[1], lemon[1]
α-Pinene	Pinegreen[1]
p-Cymene	Weak[1], spicy harbecous[1], citrus-like[1], fresh[1]
Miscellaneous compounds	
Toluene	Sweet[1], pungent[1], caramel[1], ethereal[1], fruity[1], rubbery[1]
Dimethyl disulfide	Cabbage[3,4], strong onion[3,4], garlic-rotten

[1]Boltar et al. (2015); [2]Ferreira et al. (2009); [3]Weimer (2007); [4]Singh et al. (2003); [5]Smit et al (2005)

Catabolic way of cheese aroma formation

The primary methods for forming flavor compounds in cheese are: lactose and lactate metabolism, lipolysis, and proteolysis (Urbach, 1997; McSweeney and Sousa, 2000; Smit, 2000; Marilley and Casey, 2004; Delgado et al., 2010). Lactose and citrate metabolism influence the accumulation of cheese compounds like acetaldehyde, acetate, ethanol, diacetyl, acetoin (McSweeney and Sousa, 2000). The breakdown of citrate leads to the extraction of diacetyl and acetoin that are linked to a butter aroma (Marilley and Casey, 2004). Fat hydrolysis, which is under the influence of esterases and lipases of bacterial origin, leads to fat acids (Hardi, 1987), which are precursors for the formation of ketones, primary and secondary alcohols, δ or γ-lactones, aldehydes and aromatic and aliphatic esters (McSweeney and Sousa, 2000; Collins et al., 2003). Tudor Kalit et al. (2014) mentioned that cheeses in which the ripening process takes place in an animal skin, lipolysis is a leading biochemical process responsible for cheese flavor and aroma and it leads to a higher accumulation of free fatty acids and aroma compounds (Figure 2).

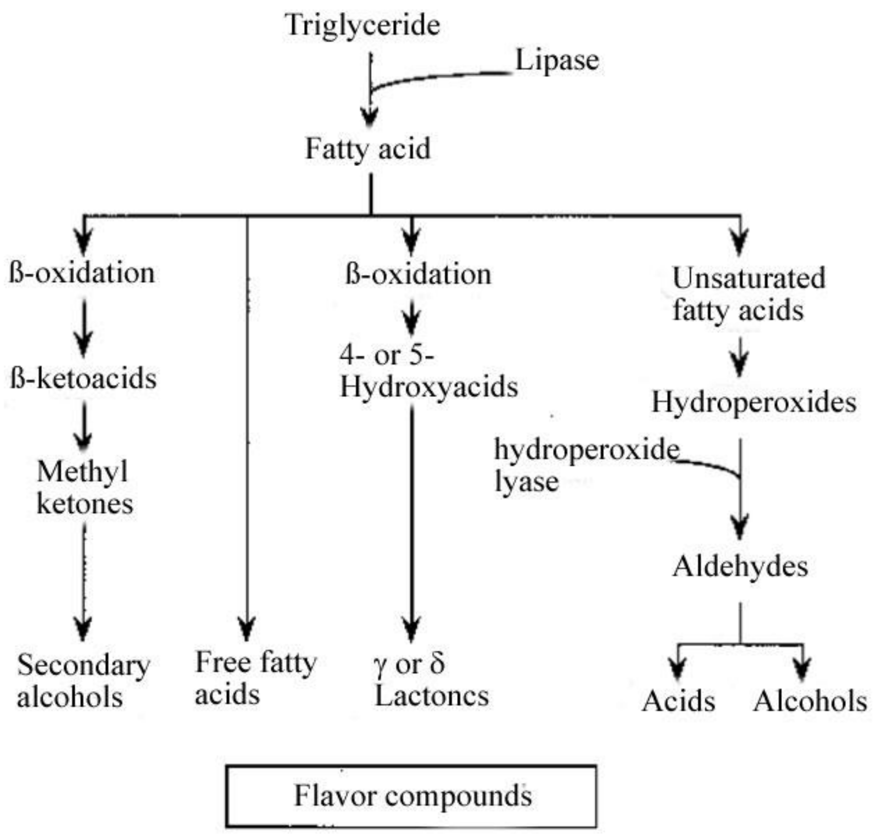

Figure 2. Pathways for the catabolism of free fatty acids in cheese (McSweeney and Sousa, 2000)

Free fatty acids are obtained by fatty acid lipolysis and directly contribute to the cheese aroma. Methyl-ketones are responsible for mushroom cheese aroma and are extremely significant products of fatty acid catabolism (Collins et al., 2003). Hayaloglu and Karabulut (2013b) mentioned that proteolysis is very important biochemical process that influences cheese flavor and aroma. Proteolytic processes assist in the formation of cheese texture that releases flavor compounds during consumption (Fox et al., 2000). It also assists in aroma development by breaking down casein into peptides and amino acids. Short polypeptides that are the result of proteolysis and aromatic compounds that are the result of amino acid catabolism are very important in forming cheese flavor and aroma (McSweeny and Sousa, 2000). Amino acids are substrates for transamination, dehydrogenation, decarboxylation and reduction, producing a wide spectrum of flavor compounds like phenyl-acetic acid, p-cresol, methanethiol, 3-methylbutanal, 3-methylbutane-1-ol etc. (Marilley and Casey, 2004). Amino acids are transformed by various enzymes into aldehydes, alcohols, carboxyl acids, hydroxyl acids, indoles, sulphur compounds, ammoniac, α-keto acids, volatile amines and amides and phenol compounds (McSweeney and Sousa, 2000; Hayaloglu et al., 2013a). According to Marilley and Casey (2004), the main amino acids that serve as precursors in forming cheese aroma are branch-chained amino acids: valine (Val), isoleucine (Ile), leucin (Leu), aromatic amino acids: tryptophan (Trp), tyrosine (Tyr), phenylalanine (Phe), and amino acids that contain sulphur: cysteine (Cys), methionine (Met). The most important factor is their quantity and type in cheese and the enzymes that decompose it (McSweeney and Sousa, 2000; Smit et al., 2000; Mikulec et al., 2010). Hayaloglu et al. (2007b) examined the profile of the volatile compounds of Turkish Tulum cheese in various ripening conditions (one in a goat skin and the other in a plastic barrel). They identified a total of 100 compounds including acids, esters, methyl ketones, aldehydes, alcohols, sulphur compounds, terpenes and other compounds and they proved that both cheeses had similar aromatic profiles, while the concentration of certain compounds was different. Serhan et al. (2010), researching the Lebanese Darfiyeh cheese during its ripening in a goat skin (20, 40 and 60 days), detected volatile compounds that mostly included alcohols, then ketones and to a smaller extent aldehydes. Examining 11 types of Turkish cheeses Hayaloglu and Karabulut (2013a) proved that the compounds most present were aldehydes, ketones and alcohols in Divle Tulum cheese that also ripens in a goat skin. Researching cheese in a sack (ewe skin) during its 45 day period of ripening, Vrdoljak (2016) identified the following compounds: acids, alcohols, aldehydes, esters, ketones, terpenes and other compounds.

Carboxyl acids

Carboxyl acids do not just bring aroma to cheese. They also act as precursors of other aromatic compounds, like methyl ketones, alcohols, aldehydes, lactones and esters (Collins et al., 2003). During cheese ripening, carboxyl acids can occur in three primary biochemical ways: lipolysis, proteolysis and lactose fermentation (McSweeney and Sousa, 2000; Delgado et al., 2010; Hayaloglu et al., 2013c). According to the results of research of the aromatic profile of 11 Turkish cheeses, Hayaloglu and Karabulut (2013a) mention butyric acid, acetic acid and caproic acid as the most present, both in Tulum cheese that ripens in an animal skin and in other Turkish cheeses, which is in accordance with the research results of Vrdoljak (2016) on ewe cheese in a sack. In addition to the above-mentioned acids, Hayaloglu and Karabulut (2013a) noted caprylic acid, 3-methylbutanoic and 2-methylbutanoic acid. Hayaloglu et al. (2013c) determined that the use of various starter cultures can influence acid share during cheese ripening. The same authors also reported that the use of starter cultures causes significant changes in acetic acid concentrations. Acetic acid, which is known to decrease sensory properties in larger concentrations, develops by lactose fermentation and amino acid catabolism and has a sharp (sour) smell. Caproic acid is detected in many cheeses and also in cheeses that ripen in an animal skin as the main fraction of free fatty acids (Hayaloglu et al., 2007b; Delgado et al., 2011). It is also responsible for the stingy (hot) flavor (Collins et al., 2003). Caprylic and butanoic acid are probably the result of lipolysis or fermentation of lactose or lactic acid (McSweeney and Sousa 2000; Hayaloglu and Karabulut, 2013a), while 3-methyl butanoic acid occurs by isoleucine metabolism and gives an unpleasant sweaty odor (Hayaloglu et al., 2013a). Butanoic acid imparts a rancid taste, while caprylic acid can give a waxy, soapy, rancid and fruity flavor (Collins et al., 2003). Short-chained fatty acids were more present in Tulum cheese than middle-chained acids and significant differences in 2-methyl propanoic, 2-methyl butanoic and 3-methyl butanoic acids were detected depending on the ripening medium (Hayaloglu et al., 2007b).

Alcohols

Alcohols are considered to be an important volatile compounds that impart to cheese the flavor of alcohol, wine, sweetness, and fruit, and they give a sharp aroma to cheeses (Hayaloglu and Karabulut, 2013a). They can be produced extremely quickly from aldehyde under the strong reduction conditions present in some cheeses, or they occur in other metabolic ways, like lactose metabolism and amino acid catabolism (Molimard and Spinnler, 1996). Many authors (Hayaloglu et al., 2007b; Delgado et al., 2010; Serhan et al., 2010; Hayaloglu and Karabulut, 2013a; Hayaloglu et al., 2013a; Vrdoljak, 2016) identified various alcohols as the most present volatile compounds in cheeses that ripen in an animal skin. Hayaloglu et al. (2013c) proved that the use of various starter cultures can influence alcohol share during cheese ripening. Ethanol is mentioned as the most common alcohol in both Tulum cheese that ripens in an animal skin (Hayaloglu et al., 2007b), in cheese in a sack (Vrdoljak, 2016), and in other types of cheeses, such as feta cheeses (Bintsis and Robinson, 2004), Halloumi (Kaminarides et al., 2007), and Pirot kačkavalj cheese (Milosavljević et al., 2010), etc.

Ethanol is usually the result of lactose fermentation and alanine catabolism and plays an important role in ester formation (Hayaloglu et al., 2013c). In addition to ethanol, both Tulum cheese and cheese in a sack contain 3 methyl-butanol, which is the result of leucine metabolism (Marilley and Casey, 2004) and 2-methyl-propanol, 2-methyl-butanol, 2-butanol, 2-pentanol, 1-hexanol (Hayaloglu et al., 2007b; Vrdoljak, 2016). Serhan et al. (2010) studied the profile of volatile compounds of Lebanese Darfiyeh goat cheese, which ripens in an animal skin (20, 40, and 60 days) and identified that the alcohols are the most present in all examined samples including: phenyl ethanol, octanol, 3-methyl-butanol, 1-propanol and 3-methyl-butanol. 1-hexanol, which gives fruit aroma to cheeses, and 2-pentanol, which oscillates during ripening due to various metabolic actions, was noted by Hayaloglu et al. (2013a). The concentration of some alcohols is either increased or decreased during cheese ripening in an animal skin (Hayaloglu et al., 2007b; Serhan et al., 2010; Hayaloglu i Karabulut, 2013a; Vrdoljak, 2016). Thus, Delgado et al. (2010) reported that the alcohol quantity is increased in the first month of ripening, but usually decreases after 30 days of ripening. This decrease probably occurs due to ester increase as a result of acid and alcohol esterification. Hayaloglu et al. (2007b) described that Tulum cheese contains more 2-methyl-1-butanol and 2-methyl-1-propanol, which is probably the result of various metabolic actions since primary alcohols originate from adequate aldehydes obtained from fatty acids and amino acid metabolism (Barbieri et al., 1994; McSweeney and Sousa, 2000).

Aldehyde

Aldehydes are transient compounds and are not accumulated in cheese because they very quickly transform into alcohols and corresponding acids (McSweeney and Sousa, 2000; Hayaloglu et al., 2007b; Delgado et al., 2010). Branch-chained and non-branch-chained aldehydes probably develop through the microbiological breakdown of amino acids (transamination accompanied by decarboxylation) or by Strecker's breakdown (McSweeney and Sousa, 2000; Hayaloglu et al., 2007b; Hayaloglu and Karabulut, 2013a). Hayaloglu et al. (2007b) noted that aldehydes can significantly contribute to the flavor of Tulum cheese due to a low perception threshold. The most frequent aldehydes identified in cheese in a sack during all ripening stages from the 45th day are: hexanal, heptanal and nonanal (Vrdoljak, 2016). Hayaloglu et al. (2013c), represented that aldehyde concentration (particularly of heptanal, hexanal and nonanal) significantly depend on ripening, the milk originating from various breeds, and without using various starter cultures in cheese production. These aldehydes are the products of lipid oxidation, some of which can significantly influence the formation of desirable cheese aroma due to their extremely mild aroma. A smaller share of aldehydes in cheeses that ripen in an animal skin was also identified by Serhan et al. (2010) and Hayaloglu and Karabulut (2013a). Seven aldehydes were identified in Tulum cheese including 2-propanal, 2 methylpropanal and 3-methylbutanal (Hayaloglu et al., 2007b). Apart from 3-methylbutanal (which imparts an oil and butter aroma), octanal, which imparts a green plant aroma, was detected in Darfiyeh cheese which ripens in an animal skin (Serhan et al., 2010) and was also identified in cheese in a sack (Vrdoljak, 2016). Also, a low share of aldehydes was noted in other cheeses like Pirot kačkavalj (Milosavljević et al., 2010) and beaten cheese from various geographic areas of Macedonia (Sulejmani et al., 2013).

Ketones

Ketones have a unique aroma and significantly contribute to cheese flavor due to a low perception threshold. Methyl ketones are responsible for mushroom cheese odor and are extremely important products of fatty acid catabolism (Collins et al., 2003). They are the main compounds in blue cheeses and contribute to the typical flavor of cheeses with molds (McSweeney and Sousa, 2000; Hayaloglu et al., 2007b). Serhan et al. (2010) determined that 2-heptanon, 2-nonanon and 2-undecanone are present in all ripening stages of Darfiyeh cheese that ripens in an animal skin and that their content increases during ripening. It is known that methyl ketones occur from fatty acid oxidation (McSweeney and Sousa, 2000), which explains their increase.

Furthermore, the same authors mentioned the presence of other ketones in certain ripening stages of Darfiyeh cheese like 2,3–butanedione, which gives a buttery aroma, 2-undecanone (iris and rose) and 2-pentanone (flowers and ether). Hayaloglu and Karabulut (2013a) who examined 11 types of Turkish cheeses, reported that 3-hydroxy-2-butanone was the most present ketone in the Turkish Mihalic cheese, which corresponds to the results of research by Vrdoljak (2016) on cheese in a sack. They also mentioned that 2-heptanone, 2-pentanone and 2-nonanone were detected in Divle Tulum cheese that ripens in an animal skin, while 6-methyl-5-hepten-2-one (rarely identified in other cheeses), was detected in Civil Turkish cheese and in a cheese in a sack during certain ripening stages (Vrdoljak, 2016). Their influence on forming the mentioned ketones is possible by the use of various cultures (Hayaloglu et al., 2013c). Serhan et al. (2010) also determined that 2,3-butandione was present in all ripening stages of Darfiyeh cheese that ripens in an animal skin and gives a buttery aroma to cheeses. 3-hydroxy-2-butanone, 2,3 butanediol and 2-butanone can also occur through further bacterial activity (McSweeney and Sousa, 2000).

Esters

Esters are the most abundant chemical compounds identified in the unstable cheese fraction and are responsible for the fruity flavor in cheese (Hayaloglu and Karabulut, 2013a). They occur by esterification reaction between short and middle chains of fatty acid with alcohols obtained by lactose fermentation or amino acid catabolism (Marilley and Casey, 2004). The microorganisms involved in ester formation are mostly yeasts, as well as some lactic acid bacteria (Delgado et al., 2010). Some esters, like ethyl ethanoate, ethyl-butanoate, ethyl lactate, propyl ethanoate, and 3-methylbutil ethanoate represent the main volatile compounds of Tulum cheese that ripens in an animal skin (Hayaloglu et al., 2007b). In addition to Tulum cheese, ester ethyls are the most common esters in the cheese in a sack (Vrdoljak, 2016) and other cheese types like Minas cheese (Corrëa Lelles Nogueira et al., 2005), Pirot kačkavalj (Milosavljević et al., 2010), Torta del Casar (Delgado et al., 2010), Ezine and Van Otlu Turkish cheeses (Hayaloglu and Karabulut, 2013a), Nanos cheese (Boltar et al., 2015), while methyl esters are dominant in the Turkish Civil cheese (Hayaloglu and Karabulut, 2013a). Vrdoljak (2016) mentions the following dominant esters during all ripening stages of the cheese in a sack during a 45-day ripening: ethyl-acetate, ethyl-butanoate, ethyl-caproate and 3-methylbutyl-ethanoate. According to Ferreira et al. (2009), the following aromatic properties are attributed to characteristic aroma of cheese: ethyl-butanoate contributes to the formation of pineapple, sweet and banana flavors and aromas, ethyl caprylate (apricot and flowers), ethyl caproate (pineapple, apple), ethyl acetate (pineapple and fruit), 3-methylbutyl ethanoate (fruit and banana). Hayaloglu et al. (2007b) defined aromatic properties of Tulum cheese during its ripening in a goat skin and a plastic barrel and reported that a ripening medium can significantly contribute to the ester share (e.g. ethyl butanoate share, ethyl caproate, 3-methylbutyl ethanoate and propyl ethanoate) during cheese ripening. Specifically, the mentioned esters were significantly present in Tulum cheeses that ripen in an animal skin compared to cheeses that ripen in plastic barrels.

Terpenes

Terpens in milk derives from the plants, compound feed or pastures (Hayaloglu et al., 2007b; Hayaloglu and Karabulut, 2013a), and directly contribute to cheese aroma. They most frequently appear as monoterpenes that occur from geranyl pyrophosphates and have a basic skeleton with ten carbon atoms. Limonene, which gives a citric aroma, was also detected in cheese that ripens in an animal skin: Tulum cheese (Hayaloglu et al., 2007b), Hellim, Divle Tulum (Hayaloglu and Karabulut, 2013a), cheese in a sack (Vrdoljak, 2016) and other cheeses like Parmigiano Reggiano (Barbieri et al., 1994), Minas cheese (Corrëa Lelles Nogueira et al., 2005), Pirot kačkavalj (Milosavljević et al., 2010). Hayaloglu et al. (2007b) also identified α-pinene and p-cymene, carane in Tulum cheese apart from limonene. Hayaloglu and Karabulut (2013a) also detected copaene and β-cymene in addition to limonene in Divle Tulum cheese that ripens in an animal skin. Besides the mentioned terpenes like *β*-cymene, *p*-cymene, *α*-pinene, α-phellandrene was also detected in a cheese in a sack (Vrdoljak, 2016).

Sulphuric compounds

Sulphuric compounds develop by the catabolism of sulphuric amino acids, like dimethyl sulphide (DMS), dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS), which greatly contribute to cheese aromatic profile. Each biosynthetic path that leads to the development of compounds with sulphur probably includes cysteine or methionine as starting compounds. Methional and methanethiol are important aromatic compounds that occur from methionine (Jerković, 2011). Many factors influence the breakdown of methionine, such as cell lysis, redox potential, precursor transport, and intercellular position of catabolic enzymes, all of which influence the formation of specific aromatic and flavor compositions of the finished product (Mikulec et al., 2010). Dimethyl disulphide and dimethyl sulfone are the most common sulfuric compounds in Tulum cheese (Hayaloglu et al., 2007b), while methanethiol was found only in a sample of Civil Turkish cheeses (Hayaloglu and Karabulut, 2013).

Other compounds

Carbohydrates are products of lipid oxidation and precursors for the formation of aromatic compounds. Among carbohydrates (in cheeses that ripen in an animal skin), the most frequently occurring are hexane, pentane, octane, nonane, 3-octane, 2,2,4,6,6 - pentamethyl heptane, 3,7-dimethyl-2-octane, 3,7-dimethyl - 1,6 – octadiene (Hayaloglu et al., 2007b; Hayaloglu and Karabulut, 2013; Vrdoljak, 2016).

Toluene, which can be found in Tulum cheese (Hayaloglu et al., 2007b; Hayaloglu and Karabulut, 2013a) and in cheese in a sack (Vrdoljak, 2016), could be the result of the breakdown of carotene in milk or the solvent used for an analysis (Molimard and Spinnler, 1996). Toluene was also identified in Feta cheese (Bintsis and Robinson, 2004) and Nanos cheese, which has a characteristic nutty flavor (Boltar et al., 2015).

Styrene, which smells of plastic, is a usual compound in cheeses that ripen in an animal skin (Hayaloglu et al., 2007b; Hayaloglu and Karabulut, 2013a; Vrdoljak, 2016). It is likely the result of polystyrene from the plastic bags used for storing samples before carrying out the analysis. This compound can also be found in other cheese types (Bintis and Robinson, 2004; Hayaloglu et al., 2008; Milosavljević et al., 2010; Delgado et al., 2011). These compounds can originate from the environment or develop through contamination during storage or the analysis itself.

Among other compounds, diethyl ether, chloroform, brome and carbon dioxide were detected in Tulum cheese (Hayaloglu et al., 2007b). Carbon dioxide, which was detected in most cheeses and also in a cheese in a sack (Vrdoljak, 2016) is a product of the fermentation of lactate, citrate and fatty acids in these cheeses (Hayaloglu and Karabulut, 2013).

The most frequently used methods for isolating volatile compounds in cheeses that ripen in an animal skin

There are many laboratory methods for isolating volatile compounds that can be divided into methods of solvent extraction, distillation methods, the headspace technique and sorption techniques. The most frequently used method of isolation for cheeses that ripen in an animal skin is headspace solid phase micro extraction, HS-SPME. SPME is used in combination with gas chromatography (GC). This technique uses silicone fiber covered with a polymer film that collects volatile compounds from the sample being analyzed. Fibers are placed in a cylindrically shaped container and can be built into an injection put on SPME holder for sampling and desorption. A sample is put into SPME vessel and closed by septa. A fiber is introduced through a needle into headspace above a sample. After certain time when volatile compounds absorbed on the fiber, the fiber is pulled in and desorbed by direct insertion into GC or GC-MS injector (Jerković, 2011). The main advantages of this technique are: simple and fast performance, low cost, using small quantities of samples, no organic solvents are used, simple transportation, it can be used for a huge number of samples, good technique for fast sample comparison (Delgado et al., 2010). The flaws of this technique of aromatic compound isolation are: aromatic profile of volatile compounds depends on the type, thickness and length of the used fiber and the length of the used fiber and the temperature and time of sampling, the best comparison results are obtained by using the same fiber on the all samples (Jerković, 2011).

Conclusion

The unique cheese flavor is the result of balance between various complex volatile and non-volatile chemical compounds which derive from lactic acid, protein and carbohydrates released during cheese ripening. Cheeses which ripen in an animal skin are characterized by a unique aroma due to the medium in which they ripe. Volatile components or compounds which give characteristic aroma to these cheeses are: acids, esters, alcohols, aldehydes, ketones, terpenes and carbohydrates. A low concentration of aldehyde and the high concentration of ester and terpene, especially limonene was determined in cheeses which ripen in an animal skin. In order to define the aromatic profile of the Croatian cheese in a sack, it is important to stimulate further research. Defining of the aromatic profile of the cheese in a sack can contribute to the standardization and protection of the mentioned cheese by which its market value would increase.

References

Barbieri, G., Bolzoni, L., Careri, M., Mangia, A., Parolari, G., Spagnoli, S., Virgili, R. (1994) Study of the volatile fraction of Parmesan cheese. Journal of Agricultural and Food Chemistry, 42, 1170–1176.

Bijeljac, S. (2004) Sir iz mijeha. Mostar: Udruga „Pramenka“.

Bintsis, T., Robinson, R.K. (2004) A study of the effects of adjunct cultures on the aroma compounds of Feta-type cheese. Food Chemistry, 88, 435–441.

Boltar, I., Čantek Majhenić, A., Jarni, K., Jug, T., Bavcon Kralj, M. (2015) Volatile compounds in Nanos cheese: their formation during ripening and seasonal variation. Journal of Food Science and Technology, 52, 608-623.

Bugaud, C., Buchin, S., Hauwuy, A., Coulon, J.B. (2001) Relationships between flavour and chemical composition of Abondance cheese derived from different types of pastures. Lait, 81, 757-773.

Cakmakci, S., Dagdemir, E., Hayaloglu, A.A., Gurses, M., Gundogdu, E. (2008) Influence of ripening container on the lactic acid bacteria population in Tulum cheese. World Journal of Microbiology and Biotechnology, 24, 293–299.

Collins, Y.F., Mc Sweeney, P.L.H., Wilkinson, M.G. (2003) Lipolysis and free fatty acid catabolism in cheese: A review of current knowledge. International Dairy Journal, 13, 841-866.

Corrëa Lelles Nogueira, M., Lubachevsky, G., Rankin, S.A. (2005) A study of the volatile composition of Minas cheese. Food Science and Technology, 38, 555–563.

Delgado, F.J., Crespa, J.G., Cava, R., Garcia-Parra, J., Ramirez, R. (2010) Characterisation by SPME–GC–MS of the volatile profile of a Spanish soft cheese P.D.O. Torta del Casar during ripening. Food Chemistry, 118, 182-189.

Delgado, F.J., Crespa, J.G., Cava, R., Ramirez, R. (2011) Formation of the aroma of a raw goat milk cheese during maturation analysed by SPME–GC–MS. Food Chemistry, 129, 1156-1163.

Fernandez-Garcia, E., Carbonell, M., Nunez, M. (2002) Volatile fraction and sensory characteristics of Manchego cheese. 1. Comparison of raw and pasteurized milk cheese. Journal of Dairy Research, 69, 579–593.

Ferreira, I.M.P.L.V.O., Pinho, O., Paulo Sampaio, P. (2009) Volatile fraction of DOP Castelo Branco cheese: Influence of breed. Food Chemistry, 112, 1053–1059.

Fox, P.F., McSweeney, P.L.H. (1998) Enzymology of milk and milk products. In: Fox, P.F., McSweeney P.L.H., eds. Dairy chemistry and biochemistry. London: Blackie Academic and Professional, 317-346.

Fox, P.F., Guinee, T.P., Cogan, T.M., McSweeney, P.L.H. (2000) Cheese flavour. In: Colilla, J., ed. Fundamentals of cheese science. Gaithersburg, Maryland: Aspen Publishers, 282-303.

Grbavac, J. (2002) Istraživanje proizvodnje i kakvoće sira iz mješine pod nazivom Ovčji planinski sir iz Zapadne Hercegovine. Magistarski rad. Zagreb: Sveučilište u Zagrebu, Veterinarski fakultet.

Hardi, J. (1987) Optimiranje sastava supstrata za dobivanje arome sira trapista. Mljekarstvo, 37, 233-241.

Hayaloglu, A.A., Fox, P.F., Guven, M., Cakmakci, S. (2007a) Cheeses of Turkey: 1. Varieties ripened in goat-skin bags. Lait, 87, 79-95.

Hayaloglu, A.A., Cakmakci, S., Brechany, E.Y., Deegan, K.C., McSweeney, P.L.H. (2007b) Microbiology, biochemistry, and volatile composition of Tulum cheese ripened in goat‘s skin or plastic bags. Journal of Dairy Science, 90, 1102-1121.

Hayaloglu, A.A., Brechany, E.Y., Deegan, K.C., McSweeney, P.L.H. (2008) Characterization of the chemistry, biochemistry and volatile profile of Kuflu cheese, A mould-ripened variety. LWT-Food Science and Technology, 41, 1323-1334.

Hayaloglu, A.A., Karabulut, I. (2013a) SPME/GC-MS characterization and comparison of volatiles of eleven varieties of Turkish cheeses. International Journal of Food Properties, 16, 1630-1653.

Hayaloglu, A.A., Karabulut, I. (2013b) Primary and secondary proteolysis in eleven Turkish cheese varieties. International Journal of Food Properties, 16, 1663-1675.

Hayaloglu, A.A., Yasar, K., Tolu, C., Sahingil, D. (2013a) Characterizing volatile compounds and proteolysis in Gokceada artisanal goat cheese. Small Ruminant Research, 113, 187-194.

Hayaloglu, A.A., Tolu, C., Yasar, K. (2013b) Influence of goat breeds and starter culture systems on gross composition and proteolysis in Gokceada goat cheese during ripening. Small Ruminant Research, 113, 231-238.

Hayaloglu, A.A., Tolu, C., Yasar, K., Sahingil, D. (2013c) Volatiles and sensory evaluation of goat milk cheese Gokceada as affected by goat breeds (Gokceada and Turkish Saanen) and starter culture systems during ripening. Journal of Dairy Science, 96, 2765–2780.

Jerković, I. (2011) Kemija aroma. Interna skripta. Split: Sveučilište u Splitu, Kemijsko-tehnološki fakultet.

Kaminarides, S., Stamou, P., Massouras, T. (2007) Changes of organic acids, volatile aroma compounds and sensory characteristics of Halloumi cheese kept in brine. Food Chemistry, 100, 219–225.

Lukač Havranek, J. (1995) Autohtoni sirevi Hrvatske. Mljekarstvo, 45, 19-37.

Marilley, L., Casey, M.G. (2004) Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. International Journal of Food Microbiology, 90, 139-159.

McSweeney, P.L.H., Sousa, M.J. (2000) Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Lait, 80, 293–324.

Mikulec, N. (2010) Promjene sadržaja topljivih peptida i aminokiselina tijekom zrenja Krčkog sira. Disertacija. Zagreb: Sveučilište u Zagrebu, Agronomski fakultet.

Mikulec, N., Habuš, I., Antunac, N., Vitale, Lj., Havranek, J. (2010) Utjecaj peptida i aminokiselina na formiranje arome sira. Mljekarstvo, 60, 219-227.

Milin, M. (1969) Prilog poznavanju proizvodnje i kvalitete mješnog sira u okolici Knina. Magistarski rad. Zagreb: Sveučilište u Zagrebu, Veterinarski fakultet.

Molimard, P., Spinnler, H.E. (1996) Review: Compounds inovolved in the flavour of surface mold-ripened cheeses: Origins and properties. Journal of Dairy Science, 79, 169-184

Milosavljević, N., Joković, N., Radulović, N., Blagojević, P., Savić, D. (2010) Isparljiva jedinjenja pirotskog kačkavalja od ovčijeg mleka. Prehrambena industrija – Mleko i mlečni proizvodi, 1-2, 122-126.

Serhan, M., Cailliez-Grimal, C., Borges, F., Revol-Junelles, A., Hosri, C. i Fanni, J. (2009) Bacterial diversity of Darfyeh, a Labanese artisinal raw goat`s milk cheese. Food Microbiology, 26, 645-652.

Serhan, M., Linder, M., Hosri, C., Fanni, J. (2010) Changes in proteolysis and volatile fraction during ripening of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. Small Ruminant Research, 90, 75-82.

Singh, T.K., Drake, M.A., Cadwallader, K.R. (2003) Flavor of Cheddar cheese: A chemical and sensory perspective. Comprehensive Reviews in Food Science and Food Safety, 2 (4), 166–189.

Smit, G., Verheul, A., Van Kranenburg, R., Ayad, E., Siezen, R., Engels, W. (2000) Cheese flavour development by enzymatic conversions of peptides and amino acids. Food Research International, 33, 153-160.

Sulejmani, E., Rafajlovska, V., Guneser, O. (2013) Characterization of volatiles in beaten cheeses (Bieno Sirenje) by SPME/GC-MS: Influence of geographical origin. Journal of the Serbian Chemical Society, 78, 1-16.

Tratnik, LJ. (1998) Grušanje mlijeka djelovanjem enzima. In: Volarić, V., ed. Mlijeko - tehnologija, biokemija i mikrobiologija. Zagreb: Hrvatska mljekarska udruga, 207-216.

Tudor Kalit, M., Kalit, S., Havranek, J. (2010) An overview of researches on cheeses ripening in animal skin. Mljekarstvo, 60, 149-155.

Tudor Kalit, M., Kalit, S., Delaš, I., Kelava, N., Karolyi, D., Kaić, D., Vrdoljak, M., Havranek, J. (2014) Changes in the composition and sensory properties of Croatian cheese in a lamb skin sack (Sir iz mišine) during ripening. International Journal of Dairy Technology, 67, 255-264.

Urbach, G. (1997) The chemical and biochemical basis of cheese and milk aroma. In: Law B.A., ed. Microbiology and biochemistry of cheese and fermented milk. London, UK, 253–298.

Vrdoljak, M. (2016) Probiotičke kulture *Lactobacillus plantarum* B i *Lactococcus lactis* subsp. *lactis* S1 u poboljšanju funkcionalnih svojstava sira iz mišine. Disertacija. Zagreb: Sveučilište u Zagrebu, Agronomski fakultet.

Weimer, B.C. (2007) Improving the flavour of cheese. Cambridge, UK: CRC Press.

Yilmaz, G., Ahmet, A., Akin, N. (2005) The effect of microbial lipase on the lipolysis during the ripening of Tulum cheese. Journal of Food Engineering, 69, 269-274.

Zitoun, A.O., Benatallah, L., Ghennam, H.E., Zidoune, N.M. (2011) Manufacture and characteristics of the traditional Algerian ripened Bouhezza cheese. Journal of Food, Agriculture and Environment, 9, 96-100.

Zitoun, A.O., Pediliggieri, C., Benatallah, L., Lortal, S., Licitra, G., Zidoune, N.M., Carpino, S. (2012) Bouhezza, a traditional Algerian raw milk cheese, made and ripened in goatskin bags. Journal of Food, Agriculture and Environment, 10, 289-295.