Aroma components of long ripened cheese and their origin, variation and contribution to flavour

– with focus on propionic acid

Smakämnen i lagrad ost och hur de varierar, bildas och bidrar till smak - med fokus på propionsyraostar

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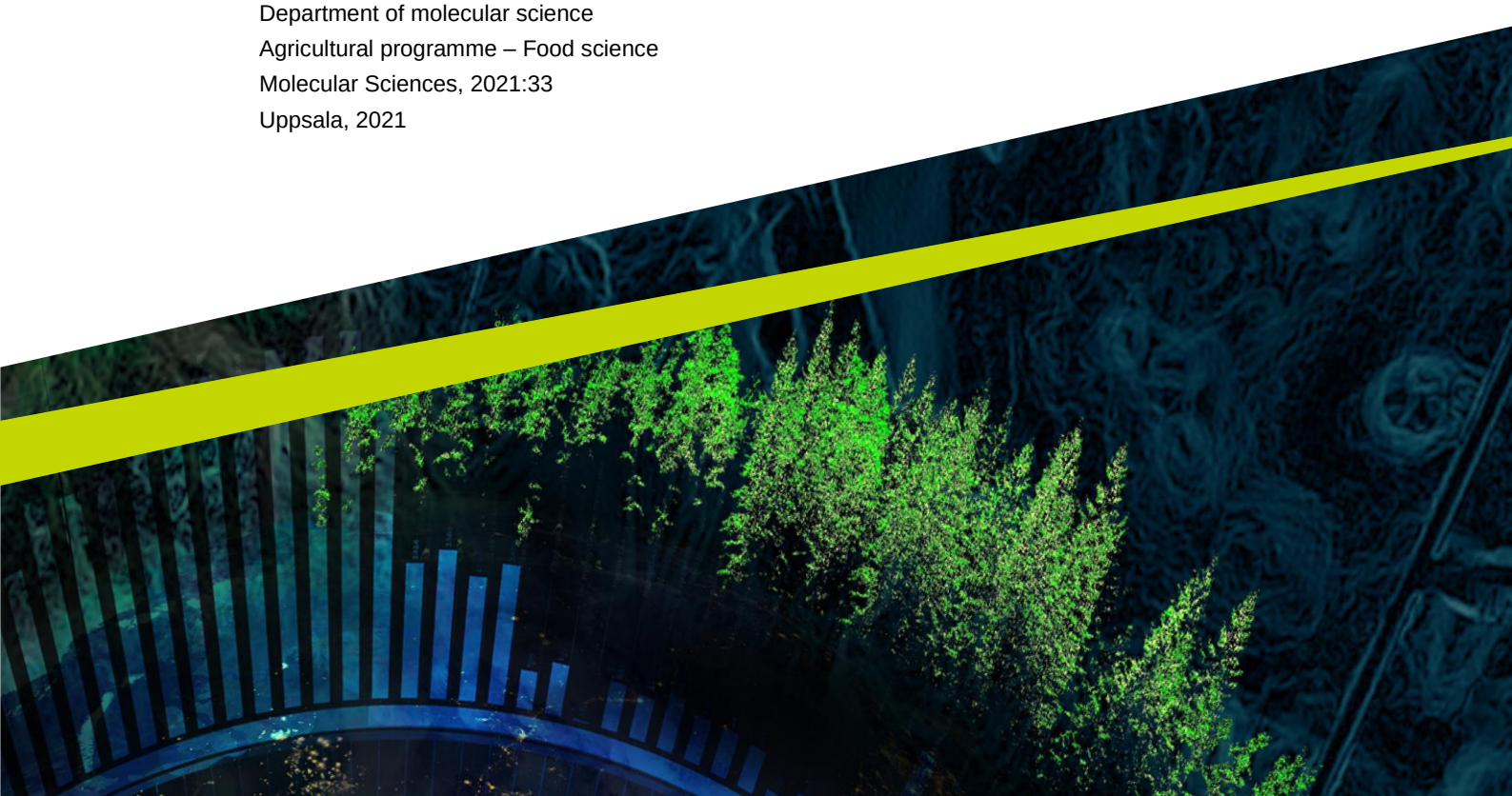

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Abstract

Flavour compounds in cheese are formed by metabolism of residual lactose, lactate and citrate, proteolysis and lipolysis through the activity of e.g., starter bacteria. Generally identified aroma components in cheese are fatty acids, esters, alcohols, ketones, lactones, aldehydes, and sulphur compounds. Various compounds contribute to specific flavours reviewed in this study. In propionic acid cheeses (e.g. Emmenthaler, Maasdamer, Grevé), lactate fermentation with products of propionic and acetic acids, and CO_2 is important for the characteristic sweet, nutty flavour and eye-formation of these cheeses. The variation of aroma components in cheese is influenced by many factors; type of starter culture and strain, milk quality and composition, pH, temperature, salt, moisture content and ripening conditions. To control the flavour and predetermined characteristic of a cheese variety, sensory evaluation is conducted. Methods for sensory analysis include discrimination methods, descriptive sensory method, affective consumer tests and grading methods. Depending on the objective of the analysis, methods are used to evaluate cheese quality and defects, in product development or after replacement of an ingredient, for research purposes, marketing, shelf-life evaluation and to investigate consumer acceptability.

The objectives of the literature review are to examine aroma compounds in cheese, with focus on propionic acid cheeses. The variation and origin of aroma components as well as their contribution to flavour in cheese are investigated. In addition, methods for sensory evaluation of cheese are reviewed.

Keywords: cheese ripening, aroma components, volatile compounds, metabolism of lactose, lactate and citrate, proteolysis, lipolysis, propionic acid bacteria, sensory evaluation

Sammanfattning

Smakämnen i ost bildas via metabolism av resterande laktos, laktat och citrat, proteolys och lipolys, med hjälp av bland annat starterkulturens bakterier. Fettsyror, estrar, alkoholer, ketoner, laktoner, aldehyder och svavelföreningar är generella grupper av ämnen som bidrar till ostens smak. Olika grupper av ämnen bidrar till specifika smaker som kommer att tas upp i rapporten. I propionsyraostar (exempelvis Emmentaler, Maasdamer och Grevé) är fermentering av laktat grundläggande eftersom det bidrar med ostarnas karaktäristiska hål och söta, nötaktiga smak från fermenteringsprodukterna propionsyra, ättiksyra, och CO_2. Variationen av smakämnen i ost beror på många faktorer; typ av starterkultur, mjölkkvalité, mjölkegenskaper och tillverkningsprocesser så som pH, temperatur, salt- och vätskehalt och lagringsförhållanden. En osts smak och karaktär utvärderas med hjälp av olika metoder för sensorisk analys, till exempel skillnadsmetoder, beskrivande metod av en tränad sensorisk panel och konsumenttester. Metoderna används för att utvärdera ostens kvalité vid produktutveckling, i forskningssyften, för marknadsföring, i studier av förändringar som sker under den förväntade hållbarhetstiden och för utvärdering av konsumenters acceptans.

Syftet med litteraturstudien är att undersöka smakämnen i ost, med fokus på propionsyraostar. Variationen i smakämnen i ost samt hur de har bildats, liksom deras bidrag till smak, kommer att analyseras. Dessutom ska olika metoder för sensorisk utvärdering av ostens smak undersökas.

Nyckelord: ostmognad, smakämnen, starterkultur, omsättning av laktos, laktat och citrat, proteolys, lipolys, propionsyrabakterier, sensorisk analys

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Abbreviations

FA	Fatty acids
FFA	Free fatty acids
GC-O	Gas chromatography-olfactometry
LAB	Lactic acid bacteria
LPL	Lipoprotein lipase
NSLAB	Non-starter lactic acid bacteria
PAB	Propionic acid bacteria

1. Introduction

1.1. Cheese manufacturing and ripening

Cheese is a fermented dairy product, and the manufacture of long ripened cheese involves complex interactions by milk, bacteria, and rennet. The production includes standardisation of the milk to achieve the desired fat and protein content and, depending on cheese variety, pasteurisation. The pH of the milk is initially decreased to desired level by starter lactic acid bacteria (LAB). Acidification is probably the most crucial step during cheese manufacturing as it prevents growth of spoilage and pathogens, affects the coagulant and promote syneresis as well as enzyme activity during ripening. By adding rennet, with the proteinase chymosin, the milk caseins will be destabilized forming a gel (McSweeney, 2007). By cutting the gel it will release whey, i.e. syneresis, and form a curd. The curd is pressed and shaped, salted and ripened two weeks to three years or even longer depending on cheese variety (Fox et al., 2017).

During ripening, the texture and characteristic flavour of the cheese will develop. Ripening involves complex biochemical changes including enzymes, divided into primary and secondary events. Primary changes involve the metabolism of lactose, proteolysis, and lipolysis. Further degradation occurs by secondary events, including amino acid catabolism, fatty acid catabolism and lactic acid catabolism, resulting in several aroma compounds. These compounds are important contributors to flavour and texture in cheese (Fox and McSweeney, 2017). Several agents catalyse the series of reactions. Microorganisms play an essential role by enzymes secreted or released after death and lysis of cells. Indigenous milk enzymes as well as added enzymes, such as rennet, also affect the reactions (Fox and Wallace, 1997). The ripening events are largely affected by the raw milk quality and starter cultures as well as the manufacturing process including pH, salt content, composition of moisture, fat and proteins in the curd and temperature (Bintsis and Papademas 2017)

During ripening microbiological changes occur as well. Nowadays, most cheeses are produced by pasteurised milk where almost all indigenous bacteria are

eliminated. The bacterial flora of raw milk can vary in a wide range, making it difficult to control the properties of the cheese during manufacturing (Engels and Düsterhöft, 2020). However, this also implies that cheese made from pasteurised milk lacks the full flavour potential present in traditional cheeses made from raw milk. Still, there are several traditional cheese varieties made from raw milk (Urbach, 1997). Thus, starter cultures are of great importance in cheesemaking and are divided into primary and secondary microflora. The role of primary starter cultures is mainly to carry out acidification of the cheese milk, but also contribute to the initial ripening. Predominant strains of mesophilic or thermophilic LAB are used as starters, such as *Lactococcis lactis, Leuconostoc* species or *Streptococcus thermophilus* (Parente et al. 2017). In addition, non-starter lactic acid bacteria (NSLAB), an adventitious microflora, will develop during ripening and these are important to almost all cheese varieties due to their contribution to flavour development (Cotter and Beresford, 2017). Besides, secondary starter cultures including moulds, yeast or bacteria are added to some cheese varieties and will develop during maturation. The secondary starter culture has no function in acid production but will contribute to biochemical changes during ripening and hence the characteristic flavour and texture of the cheese (Parente et al., 2017). NSLAB may be considered as a secondary starter culture.

1.2. Propionic acid cheeses

The extent of this review limits the possibility to include all cheese varieties. For that reason, the review will focus on ripening and aroma compounds formed in cheeses made with propionic acid fermentation. Many popular cheeses are propionic acid cheeses produced by dairy *Propionibacterium* as secondary starter culture. Commonly, such cheeses are referred to as Swiss-type cheeses, including e.g. Emmenthaler, Maasdamer, Swedish Grevé, and Norwegian Jarlsberg among many others. Propionic acid bacteria (PAB) are essential for propionic acid fermentation which contributes to the slightly sweet and nutty flavour, playing a key role for eye-formation characteristic in Swiss-type cheeses (Thierry et al., 2011). Additionally, PAB is to some extent involved in proteolysis and lipolysis during ripening (Fröhlich-Wyder et al., 2017).

Cheeses with propionic acid fermentation are produced either by pasteurised or raw milk. Hard Swiss-type cheeses are made by thermophilic LAB, whereas in semi-hard cheeses, such as Grevé and Jarlsberg, mesophilic starter is used. Because optimal growth of PAB is at a relative high pH, water is added to the milk to reduce lactose concentration and consequently lactic acid production. The cooking temperature in the production of Swiss-type cheeses is commonly 40-50 °C causing a complete inactivation of chymosin (Fröhlich-Wyder et al., 2017). There are large

variations in the ripening procedure among Swiss-type cheeses. Generally, the ripening involves a drying period at 4-12 °C for ~3 weeks, a warm-room period at 20 °C for some further weeks and a final cooling period. PAB will start to develop during the warm-room period (Fröhlich-Wyder et al., 2017; Ji et al., 2004). Due to a relatively high pH and maturation at high temperature for several weeks, propionic acid cheeses are sensitive to cheese defects by clostridia. The most common defects are "late blowing" caused by butyric acid fermentation by *C. tyrobutyricum* (Fröhlich-Wyder et al., 2017).

There are six species of dairy PAB, but in cheesemaking, selected strains of *P. freudenreichii* are preferably used. *P. freudenreichii* is found in cheese, milk, and silage. It is a mesophilic bacterium with optimal growth at ~30°C and pH 6-7. The growth is significantly decreased at pH <5.5 (Thierry et al., 2011).

1.3. Aim/objectives

The purpose of the literature review is to examine the aroma components in cheese, with focus on propionic acid cheeses. The variation and origin of aroma compounds as well as their contribution to flavour in cheese are investigated. Also, methods for sensory evaluation of cheese are reviewed.

1.4. Methods

The review is based on information from scientific articles in journals and books and reports obtained by following databases: *Scopus*, *FSTA* (Food Science Technology Abstract), *Web of science*, *PubMed*, and *Google scholar*. Also, *Primo* from the library of SLU have been used. The searches were made by combinations of following search terms: *cheese ripe*, volatile*, proteolysis, lipolysis, glycolysis, amino acid catabolism, fatty acid catabolism, metabolism of lactose, metabolism of lactate, flavour compound*, Propionibacteri*, Swiss-type cheese, sensory evaluation*. The books "Cheese: chemistry, physics and microbiology" (2017) and "Encyclopedia of dairy science" (2011) have been very helpful.

2. Literature review

2.1. Origin of aroma components

Lactose, milk proteins and milk fat are metabolised by combined biochemical and microbial activities in cheese manufacturing and ripening. Series of reactions leads to a range of flavour compounds (McSweeney, 2004). Figure 1 shows the biochemistry of cheese ripening and aroma components produced (McSweeney, 2017).

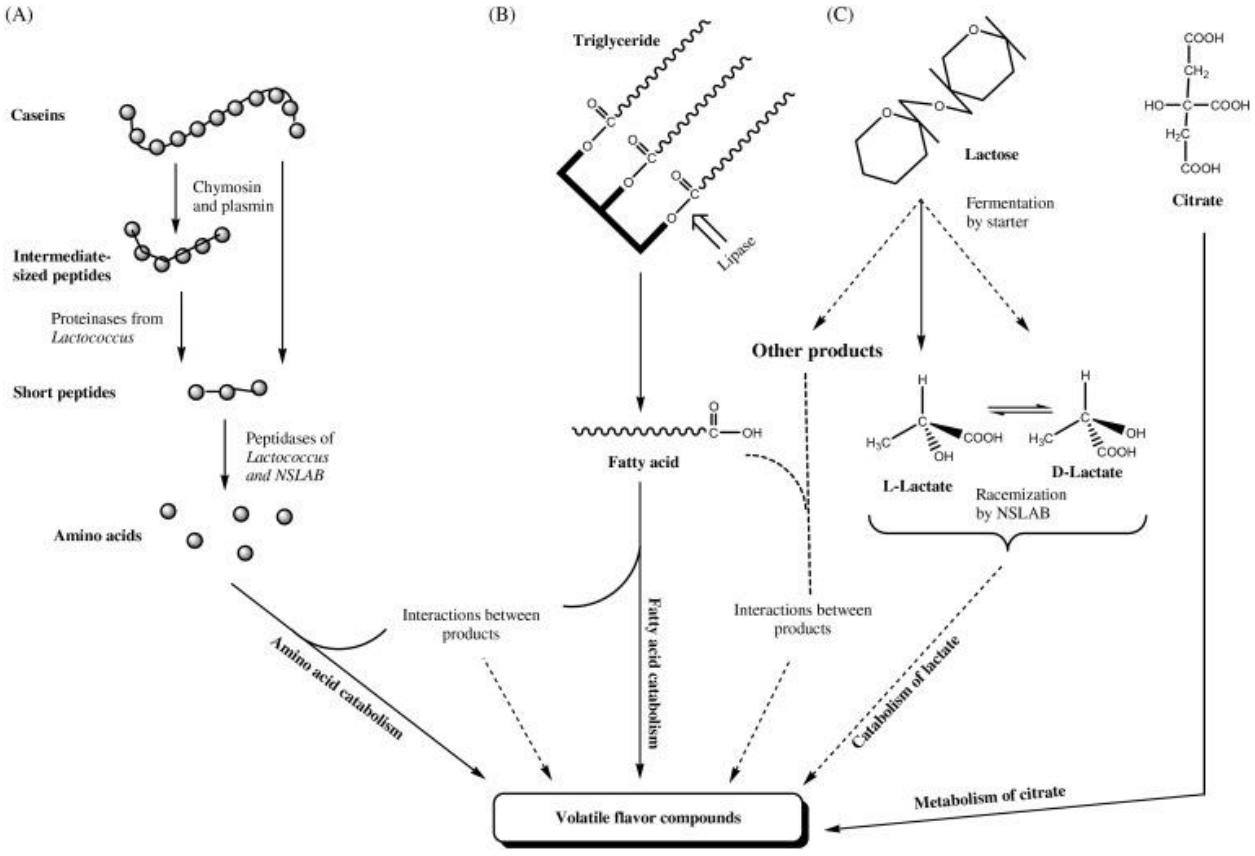

Figure 1. General biochemical pathways during cheese ripening. (A) proteolysis, (B) lipolysis, (C) lactose, lactate and citrate metabolism. Volatile flavour compounds formed include e.g. short-chain fatty acids, esters, volatile alcohols, ketones, lactones, aldehydes, and volatile sulphur compounds (adapted from McSweeney 2017).

2.1.1. Metabolism of residual lactose, lactate and citrate

As cheese is a dairy fermented product, the initial step, i.e. formation of lactose to lactate by LAB, is a key feature in cheesemaking for all varieties (McSweeney, 2004). Most lactose is lost in the whey and residual is rapidly converted to lactate by starter LAB. A rapid conversion of lactose to lactate is crucial to produce good quality cheeses because residual fermentable carbohydrates can cause undesired properties (McSweeney et al. 2017). In Swiss-type cheeses there are interactions between LAB and PAB in utilization of carbon sources. LAB converts lactose to lactate subsequently used by PAB as an energy source (Smid and Lacroix, 2013). The growth of PAB is affected by the speed of lactic acid production from lactose. PAB is favoured by slow acid production while fast acidification can inhibit PAB (Fröhlich-Wyder et al. 2017).

Metabolism of lactate

Lactate originating from lactose is an important substrate for different reactions, depending on cheese variety. Lactate is racemized from L-lactate to D-lactate in most cheeses by NSLAB. In surface mould-ripened cheeses, lactate is catabolized to H_2O and CO_2 by *Penicillium camemberti* essential for texture development in Camembert and Brie. By *Clostridium,* lactate can be utilized anaerobically causing "late gas blowing" defects in cheese, especially in Swiss-type cheeses. Lactate can also be oxidized by NSLAB to produce acetate and formate (McSweeney et al., 2017).

In cheeses with propionic acid fermentation lactate is metabolised by PAB as an energy source and acetate as an electron acceptor. The main compounds produced by fermentation include propionic acid, acetic acid, succinic acid and CO_2. The latter is essential for the characteristic eye formation. Via glycolysis or the pentose phosphate pathway lactate is oxidized to pyruvate generating ATP and co-enzymes (Thierry et al., 2011). Further, pyruvate is catabolized via different metabolic pathways as follows; pyruvate is oxidized to CO_2 and acetate or reduced to propionate by a pathway called Wood-Werkman. Also, when aspartate is present, the lactate metabolism is connected with aspartate metabolism forming succinate via fumarate (Fröhlich-Wyder et al., 2017). These reactions are shown in figure 2.

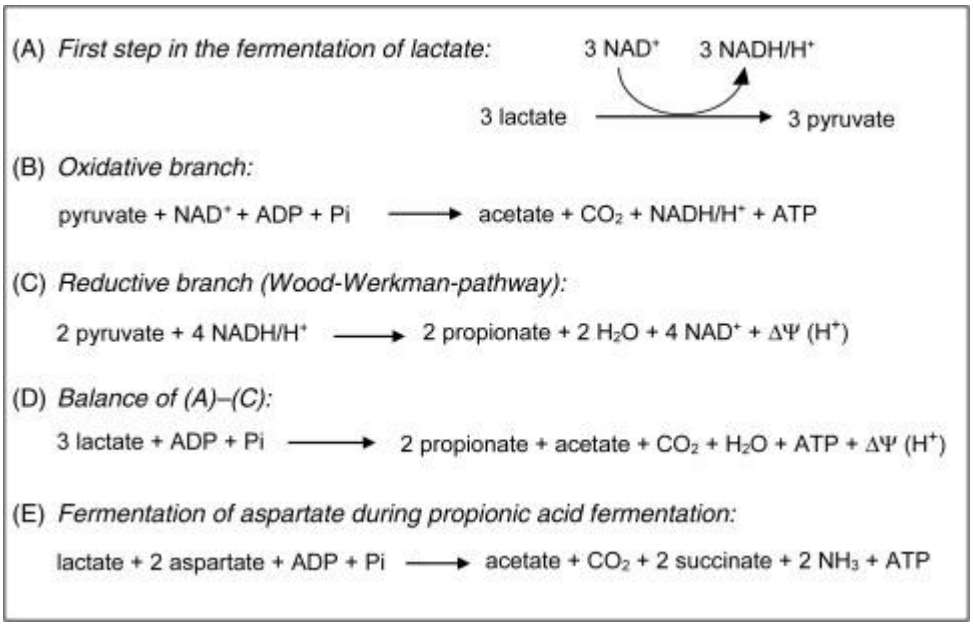

Figure 2. The metabolic pathways of lactate metabolism by propionic acid fermentation (Adapted from Fröhlich-Wyder et al. 2017)

Metabolism of citrate

The metabolism of citrate is most important for the formation of aroma compounds in cheeses produced with mesophilic starter cultures. Citrate-positive strains of e.g. *Lactococcus, Leuconostoc* and *Lactobacillus*, have a plasmid for citrate transport, that metabolizes citrate. The products of their metabolism include diacetyl, acetate, acetoin, and CO_2. CO_2 is responsible for the formation of small eyes in cheeses such as Gouda and Edamer. In Swiss-type cheeses citrate metabolism is of minor importance (McSweeney et al., 2017).

2.1.2. Proteolysis

Proteolysis in cheese ripening is initiated by enzymatic hydrolysis of the milk protein caseins into large and intermediate-sized peptides. Proteinases involved here mainly originate from residual rennet or indigenous milk enzymes such as plasmin (Ardö et al., 2017). The activity of the coagulant will increase with low pH and low cooking temperature. Consequently, the enzymes differ depending on cheese variety. Chymosin, a heat-labile protease, plays a minor role in Swiss-type cheeses as they are produced at high-cooking temperatures and relatively high pH. Instead, the heat-stable indigenous protease in milk, plasmin, is of importance to initiate proteolysis in these cheeses (Ardö et al., 2017; Larsson et al., 2006).

The initial peptides formed by proteolysis are further hydrolysed to short peptides by a cell envelope-associated proteinase (CEP) secreted from starter LAB, i.e., lactocepin. In the absence of lactose, LAB require amino acids for growth (Morishita et al., 1981). In most cheese varieties, starter LAB as well as NSLAB, constitute the main sources of peptidases responsible for hydrolysing small peptides and liberation of amino acids. In cheeses produced with a secondary starter culture, proteinases and peptidases from these bacteria are also involved (Ardö et al., 2017).

PAB are not significant contributors to primary proteolysis but are slightly peptidolytic. Peptides containing proline and phenylalanine are released by an endopeptidase originating from PAB (Ardö et al., 2017).

Amino acid catabolism

Each cheese variety has a complex characteristic amino acid pattern. Amino acids are the main contributors to the development of flavour compounds in cheese and precursors of aldehydes, ketones and volatile sulphur compounds (Ganesan and Weimer, 2007). Metabolism of amino acids occurs by series of events of transamination or elimination (Adda et al., 1982). The elimination pathway occurs in aromatic amino acids and methionine and involves cleavage of the side chain of the amino acid by amino acid lyases. Products produced are methanethiol (a volatile sulphur compound), phenol and indole. The second pathway in amino acid catabolism is transamination by amino acid aminotransferases to α-keto acids. This metabolism has been observed in branched-chain amino acids, aromatic amino acids, and methionine. The α-keto acids are in further steps metabolized to alcohols and aldehydes (Yvon and Rijnen, 2001).

In Swiss-type cheeses, PAB is converting branched-chain amino acids to the branched-chain fatty acids 3-methylbutanoic (isovaleric acid) and 2-methylbutanoic acids. These products are present at 3-10 times higher concentrations in Swiss-type cheeses than in cheeses where PAB are absent. The compounds originate from leucine and isoleucine degradation by transamination to ketoacids and oxidation reactions (Thierry et al., 2011; Thierry et al., 2004).

2.1.3. Lipolysis

The main lipids in milk are triacylglycerides. Enzymatic hydrolysis of triacylglycerides by lipases and esterases produces glycerol and free fatty acids (FFA), mono- or diacylglycerides. Liberated short- and medium-chain fatty acids (FA) of primary lipolysis directly affect the cheese flavour, and mono- or diacylglycerides are catabolised in further reactions (Collins et al., 2003). Chemical degradation by oxidation of lipids can also occur, but levels of lipid oxidation in

cheese is low due to low redox potential and natural antioxidants in cheese (Fox and Wallace 1997).

The sources of lipases in cheese include lipoprotein lipase (LPL), found indigenous in milk, and enzymes secreted by microorganisms. The extent of lipolysis in cheese is largely related to the lipolytic activity of the secondary starter culture and in general, LAB and NSLAB possess weak lipolytic activity. However, in cheese types that lack lipolytic secondary starter culture, e.g., Cheddar and Gouda, LAB will be present in high numbers and responsible for significant release of FFA (Collins et al., 2003). Mould-ripened varieties, such as blue cheese, display a very high lipolytic activity by producing two extracellular lipases (McSweeney, 2007). In a study conducted by Chamba and Perreard (2002), it was shown that PAB is the major agent of lipolysis in Swiss-type cheese. Due to the high cooking temperature LPL is inactivated during manufacturing (Chamba and Perreard 2002). The lipolytic potential of PAB is significantly higher than that of LAB and shows esterase activities on esters of FA up to C10:0. The shorter the FA, the higher the esterase activity by PAB (Dupuis and Boyaval, 1993).

Catabolism of free fatty acids

FFA produced by lipolysis can act as precursor compounds for a series of catabolic pathways. The products include aroma components e.g., esters, thioesters, lactones, methyl ketones, secondary alcohols, and alkanes. FFA that react with an alcohol, originating from amino acid catabolism or fermentation of lactose, form esters. Esters are important in many cheese varieties and ethyl esters are the most common ones due to ethanol production from lactate fermentation. The reaction of a thiol and a fatty acid leads to formation of a thioester. Methyl ketones are volatile compounds produced extensively in mould-ripened cheeses such as blue cheese. Further, the enzymatic reduction of methyl ketones produces secondary alcohols. Lactones are formed by the esterification of hydroxy FA (Collins et al., 2003; McSweeney, 2007).

As mentioned in the amino acid catabolism section, short branched-chain FA (3-methylbutanoic and 2-methylbutanoic acids) are produced by catabolism of branched-chain amino acids. The production of these compounds is fundamental in PAB (Thierry et al., 2011).

2.2. Flavour development

Many hundreds of flavour compounds have been detected in cheese, whereas each has a unique flavour character. Consequently, the diversity of cheese flavour is large (Drake and Delahunty, 2017). Every cheese variety is associated to a group of flavour compounds characteristic for the specific cheese type.

2.2.1. General flavour contribution

Long-chain FFA, consisting of more than 12 carbons, are not significantly important to flavour development. However, short-chain FFA are greatly influencing cheese flavour and originate from both lipolysis, amino acid catabolism and carbohydrate metabolism. Butyric acid ($C_{4:0}$), caproic acid ($C_{6:0}$), and caprylic acid ($C_{8:0}$) are hydrolysed during lipolysis and contribute to cheesy aroma and sweaty, goaty and rancid flavours. By carbohydrate metabolism formic acid ($C_{1:0}$), acetic acid ($C_{2:0}$), and propionic acid ($C_{3:0}$) are produced contributing to umami and sweet flavours (Kilcawley and O'Sullivan, 2017). Citrate metabolism generates diacetyl with buttery flavour (Ardö, 2006). Esters derived from the various short-chain FFA are common in all cheeses. Aroma variations exist among the different esters but these provide in general fruity flavours (Liu et al., 2004). Methyl ketones produced by FFA catabolism are important flavour compounds in blue cheeses with descriptions of musty, fruity, blue cheese, tea-like aromas. Secondary alcohols formed by the reduction of methyl ketones contribute to flavours such as fresh, fruity and green. Lactones contribute to buttery flavours in cheese and are important in Gouda-cheese, and these compound also provide an overall flavour of cheese (Ganesan et al., 2007).

Primary products of proteolysis contribute to bitter tastes, mainly short hydrophobic peptides. Bitterness is associated with flavour defects in dairy products. Thus, amino acid catabolism is essential to achieve favourable aroma development (Ganesan and Weimer, 2017; McSweeney, 2007). Amino acid catabolism generates many FFA, aldehydes and volatile sulphur compounds. Aldehydes contribute to malty or grass-like flavours. Volatile sulphur compounds, such as methanethiol, are significant flavour contributors in cheddar cheese with an aroma description of cooked cabbage (Kilcawley and O'Sullivan, 2017).

The concentration of each aroma compound and its aroma activity have impacts on sensory perception. Molecules associated with negative aromas can in low concentrations be beneficial for flavour, for example molecules originating from amino acids with sulphur (Kilcawley and O'Sullivan, 2017). FFAs can contribute to savour cheese flavour, but also to rancidity in high concentration and in specific varieties. FFAs are most important for positive flavour development in cheeses with low pH (Thierry et al., 2017).

2.2.2. Flavour development in propionic acid cheeses

The typical aroma of Swiss-type cheeses is determined by compounds arising from lactate fermentation by PAB including propionic acid, acetic acid, succinic acid, and diacetyl. Drake et al (2007) showed that succinic acid, together with the amino acid glutamic acid and propionic acid, are responsible for umami taste in propionic acid cheeses (Drake et al., 2007). PAB also show a high capacity to convert isoleucine to isovaleric acid. Characteristic flavours contributed by isovaleric acid are associated with old cheese; putrid, sweat, rancid, fruity and sweet. (Ganesan et al., 2007; Thierry and Maillard, 2002). Ethyl esters of propionic acid are present in Swiss-type cheese and contribute to a fruity, pineapple aroma (Ganesan et al., 2007). Swiss-type cheeses are particularly associated with a sweet and nutty taste. The amino acid proline released in proteolysis by PAB contributes to sweet flavour as well as propionic acid (Ardö et al., 2017).

2.3. Variation of aroma components in cheese

2.3.1. Milk quality and milk properties

The milk composition and the biochemical and microbiological quality of the milk influence the cheese yield and flavour pattern. Higher amounts of proteins and lipids in the milk, depending on the breed and diet of the milk-producing animal, will increase the rate of proteolysis and lipolysis and thereby the flavour development (Urbach, 1993). Cheeses produced by raw milk consist of a greater variety of microorganism and enzymes. Hence, those cheeses mature more quickly and show stronger flavour development compared to cheeses produced by pasteurised milk (McSweeney, 2007). Traditionally made Swiss Emmenthaler with protected designation of origin (PDO) is produced by spontaneous propionic acid fermentation of raw milk. However, nowadays Emmentaler as well as Grevé and Jarlsberg are usually produced by pasteurised milk. By spontaneous propionic acid fermentation, the size and number of eyes vary a lot. A selected culture of PAB used in cheeses with pasteurised milk results in a more controlled fermentation with regular eyes (Fröhlich-Wyder et al. 2017). Enzymes indigenous in milk, such as LPL, or chymosin in rennet play significant roles in lipolysis and proteolysis in cheeses made with raw milk. However, in cheese produced with pasteurised milk those enzymes are partly inactivated and thus play a minor role (Fox and Wallace, 1997).

A study by Hickey et al (2006) showed that the aroma profile of Cheddar cheese was significantly influenced by the stage of lactation of milk. The extent of proteolysis during maturation increased by the use of milk from late lactation, as well as levels of FFA because levels of FFAs are higher in late-lactation milk (Hickey et al., 2006). However, Swiss-type cheeses produced by milk from late-lactation were more prone to split defects. The reasons for the texture defects are not completely determined, but the higher rate of proteolysis is one probable factor (Panthi et al. 2017).

2.3.2. Manufacturing impacts

The moisture content of the cheese has a great impact on the activity of the primary and secondary starters and their enzymes that further affect flavour development. Also, pH and temperature are important parameters for microbe and enzyme activity. The salt concentration in cheese affects the starter culture and pH as well as the rate of fermentation and proteolysis, and hence the formation of flavour compounds (Bintsis and Papademas, 2017). The ripening conditions also affect the aroma profile in cheeses. In general, the longer the ripening, the higher the intensity of flavour, aroma and aftertaste (Drake & Delahunty 2017).

2.3.3. Strain dependence of starter cultures

The addition of different strains of lactobacilli has been reported to enhance flavour development by affecting proteolysis to release more amino acids that impact the flavour positively (Hannon et al., 2003). Similarly, a study conducted by Thierry and Maillard (2002) suggested that the potential of *P.freudenreichii* to convert amino acids and FFA to flavour molecules is highly strain-dependent. The levels of fermentation products have a great impact on cheese ripening. Strains with high potential to ferment aspartate produce higher proportions of PAB fermented products; propionic, acetic, succinic acid and CO_2 and thus higher flavour contributions to the cheese (Thierry et al., 2011). However, high production of CO_2 results in more numerous and larger eyes and increase the risk of split defects. Thus, strains of PAB with high aspartase activity are more prone to secondary fermentation (Fröhlich-Wyder et al., 2017).

2.4. Sensory evaluation of cheese

Cheese quality is determined by sensory characteristics. Components that affect perception are texture, flavour and appearance. Sensory perception is an interaction between mouthfeel, vision, sense of smell, touch and gustatory (salty, sweet, sour and bitter tastes) by human sensory ability and sensory attribute of foods can be measured by humans, instrumental or in combination (Drake and Delahunty, 2017).

2.4.1. Determination of key-flavour compounds

In cheese, which is a very complex food with many flavour components, the balance of flavours is an important factor to decide if the cheese is liked or disliked. By predetermining the key-flavour compounds that contribute to the overall quality the liking of the cheese can be controlled (Smit et al., 2005). As the aroma profile of cheese includes many compounds whereof a few are directly significant in flavour contributions, those compounds are called key-flavour compounds (Grosch, 1993). The key-flavour compounds can be identified by a combination of gas chromatography (GC) and olfactometry, i.e. analytical examinations by human sensory systems, called GC-O. The compounds detected in the highest amount by GC are considered as the key-flavour compound. To involve the sensory perception, the aroma intensity by nasal threshold is accounted in a ratio between the concentration by GC and aroma intensity (Grosch, 1993; Smit et al., 2005).

2.4.2. Methods for sensory evaluation

There are several methods for sensory evaluation, depending on the aim of the assessment. Some objectives are for product development or replacement of an ingredient, evaluation of cheese quality and defects, research purposes, marketing of the cheese, testing consumer acceptability and investigation of product changes during storing.

Descriptive sensory analysis

Descriptive sensory analysis is the most effective and functional evaluation of cheese flavour (Kilcawley and O'Sullivan, 2017). The method is intended for product development, research aims and investigations of product changes during shelf-life and effects of packaging (Murray et al., 2001). In descriptive analyses all sensory attributes with a complete sensory characterisation are described. The strength of this method is the ability to determine the association of descriptive sensory analysis with instrumental and/or consumer tests. The method is complex and require a trained panel, with knowledge of the desired composition of the cheese, in opposite to the other methods. A sensory panel in a company develops a

sensory vocabulary to describe the products accurately and comprehensively. This language is a basis for panel members to differentiate products in a similar way (Stone and Sidel, 1985). Although descriptive sensory method is very effective, the method is time consuming and expensive as it requires a trained panel. Within this method there are different practices to profile the cheese including flavour profile, texture profile, quantitative analysis and spectrum profiling (evaluation by using reference material) (Kilcawley and O'Sullivan, 2017).

Discrimination test

Another method used in sensory evaluation is sensory discrimination test that aims to measure differences among cheeses. This method is carried out to evaluate a product after replacement of an ingredient or product development to ensure an improvement of the product. The method is conducted to determine sensory differences between cheese samples by blind tests to compare the new product to the previous (Rousseau, 2015). Drawbacks of discrimination tests are difficulties to find out consumers acceptability and sensory descriptions of e.g. flavour and texture. If that is necessary, a supplementary sensory test can be performed beyond (Drake, 2007).

Affective consumer test

Quantitative consumer test has the objective to explore consumer preferences and liking levels of a product by scoring. Generally, these tests are a final confirmation of quality, e.g. in product development before launching the product (Kilcawley and O'Sullivan, 2017). Due to high variation in preferences among individuals (age, product attitudes, advertising and so on), consumer tests are conducted with large number of consumers. The objective of the method is to test the product by individuals who are not trained in the area. Furthermore, there is a qualitative affective consumer test, with same objective, i.e. to evaluate consumers liking, but with a focus group of 8-10 members. In contrast to quantitative consumer tests, qualitative tests are performed in an early stage of product development (Drake, 2007).

Grading test

The traditional method of sensory evaluation of cheese is quality judging or scoring. The cheese is evaluated visually and judged by colour, adhesiveness and appearance and by inhaling the aroma and tasting of the cheese (Kilcawley and O'Sullivan, 2017). The scoring system typically consists of grades, where identified defects, designated by a list, results in reduced points. Today, quality judging and scoring are used to identify problems in product quality and to educate students. By

identifying the defects and their potential source, corrective measures can be taken. However, because the cheese sensory profile is not fully described by this method, it is excluded from product development as well as evaluation of consumers liking (Drake, 2007).

3. Discussion and conclusions

The objective of this study was to examine the origin and variation of aroma components in long ripened cheese and how they contribute to flavour, with focus on propionic acid cheeses. Methods and objectives of sensory evaluation of cheese flavour was also investigated.

The origin of aroma components of long ripened cheese is lactose, proteins and fats, which are enzymatically hydrolysed to lactic acid, amino acids, and fatty acids, and further metabolized to compounds that to some extent react with each other forming aroma compounds (Figure 1. General biochemical pathways during cheese ripening. (A) proteolysis, (B) lipolysis, (C) lactose, lactate and citrate metabolism. Volatile flavour compounds formed include e.g. short-chain fatty acids, esters, volatile alcohols, ketones, lactones, aldehydes, and volatile sulphur compounds (adapted from McSweeney 2017). Degradation of the components to aroma compounds is very complex. As many hundreds of various aroma compounds have been detected in cheese (Drake and Delahunty, 2017), only the most important groups of compounds can be referred to within the limit of this study.

Lactate produced in lactose metabolism is utilized differently depending on the secondary starter culture, but in propionic acid cheeses acetic acid, propionic acid and CO_2 are produced contributing to sweet and umami flavours. Amino acid catabolism involves series of events producing alcohols, aldehydes, ketones, and volatile sulphur compounds. FFA released in lipolysis can either directly affect flavour or be catabolized to esters, thioesters, lactones, methyl ketones, secondary alcohols, and alkanes. Many of the compounds produced by amino acid- and fatty acid metabolism form new compounds with each other such as esters and thioesters. Esters contribute to fruity flavours, methyl ketones with blue-cheese and musty flavours, secondary alcohols with fresh and green flavours and lactones to buttery flavours. Aldehydes contribute to malty or grass-like flavours and methanethiol aromas of cooked cabbage. The main flavour contributor in propionic acid cheeses are propionic acid, acetic acid and succinic acid originating from lactose metabolism, with flavours of umami, sweet and nutty. Also, the amino acid proline provides a sweet taste. Isovaleric acid originates from isoleucine and leucine giving

rise to flavours of sweaty, putridity, fruity and rancidity. Esters in Swiss-type cheeses contribute to fruity and pineapple aromas.

The variation of aroma components in cheese are highly affected by the manufacturing process and used starter cultures. The use of raw milk contributes to stronger flavour development, but the fermentation and ripening events can vary greatly depending on milk quality. Milk composition also impacts flavour development as higher amounts of fats and proteins in general leads to more flavour compounds. Earlier studies have reported that late-lactation milk contributes to stronger proteolysis and lipolysis. To control the appearance and flavour of a cheese variety, standardisation and pasteurisation are effective tools to control quality and composition of the milk. Therefore, in cheesemaking by raw milk, high milk quality is of great importance. Additionally, the starter cultures are essential in cheese making and ripening. Their activity has shown to be affected by pH, temperature, salt and moisture content. This implies that the aroma compounds formed during ripening are strongly influenced nor only by the milk used, but very much by the manufacturing conditions. Also, the study shows that flavour development is strain-dependent. In conclusion, the diversity of aroma components in cheese is affected by many factors, indicating that cheese ripening is a very complex topic, and that cheese quality is influenced by many parameters.

Cheese quality is measured by sensory evaluation and depending on objectives of the evaluation, e.g. cheese quality control, product development, research purposes, control of changes of cheeses during shelf-life and consumer preferences, different methods are used. The most effective method is descriptive sensory analysis as a complete sensory characterisation is described including all sensory attributes. However, the different methods are beneficial depending on purposes. For instance, a trained panel is usually more aware of specific attributes and critical than typical consumers (Drake, 2007) and therefore a consumer test can complement other methods to provide a more extensive evaluation. Furthermore, the main compounds that contribute to the overall liking of a cheese can be determined by measure key-flavour compounds by GC-O. Although the diversity of flavour compounds is large, this indicates that only a small number are central for the perception and liking of the cheese.

3.1. Conclusions

The origin of aroma components in cheese is lactose, proteins and lipids and their catabolism products. Every group of compounds contribute to specific flavour development. The variation of aroma compounds depends on milk quality, starter culture and the specific strains used, the manufacturing process e.g. milk, pH,

temperature, salt and moisture content, and the ripening conditions. The diversity of cheesemaking processes and the complex processes cheese undergo during ripening, lead to a wide diversity of cheeses.

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