

Review Article

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Flavor Development in Various Forms of Cheese

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ABSTRACT

Though the cheese production through the curd milk is the oldest science, the fermentation technology unfurled the cheese production many thousand years ago and by now cheese is available in hundreds of different types and flavors from different climatic and environmental region. Availability of cheese in different flavors is mainly due to the origin/ source of milk and whether the milk is pasteurized and the butter content of the milk. The interplay of different microflora, physical and chemical conditions play role in production of various flavor types. Microorganisms growth is essential in development of flavor and true microflora growth is determined by the regulation of moisture and salt content, pH regulation, organic acid concentration, maintenance of ripening temperature, regulation of redox potential and also on nitrate production. Proteolysis and lipolysis also play an important role in flavor enhancement. If the ripening procedure goes haphazardly then there will be more chances of rancidity but our understanding of cheese ripening process and parameters will reduce the cost and time, energy and material consumption and also efficiently increase the production of variety of cheese. In this review, we had reviewed different parameters and their interplay for the successful cheese ripening and the better taste to provide.

Keywords

Cheese, Microflora,
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Introduction

Though food fermentation that is the oldest biotechnology, man can enjoy improved flavors, texture, shelf life and other health benefits of fermented products including sauerkraut, pickles, bread, beer, yogurt, sausages, cheese etc. by enzymatic and microbial processing (Giraffa, 2004). It's about the ancient era 8000 years ago, the dairy product, Cheese, making worldwide

with unique flavors and forms and originated through transportation of milk in bladders made of ruminants' stomachs due to their inherent supply of [rennet](#), which is the agent for the conversion of milk into curd (solid i.e. cheese) and whey (liquid). Different flavors are produced by different species of animals and it is thought that after cheese formation, the flavor becomes more intense day by day (Adda *et al.*, 1982) because of bacterial activity on the curd and it was not analyzed

till the 19th century. Gorgonzola was first taken into account in AD 879, Roquefort in 1070 and cheddar in 1500. Cheese (fig.1), a highly nutritious food, is a dairy fermented product constituting proteins and fats formed by coagulation of milk protein casein available in various flavors and textures throughout the world. Three parts of world milk production is used in cheese production, and over more than thousand cheese varieties are there in the world.

Fermentation is very important technology, unfurled the cheese production many thousand years ago. People enjoy various cheese types with different characteristics like texture, flavor, nutrition etc. To study these characteristics, one should focus on cheese microbiology. In cheese microbiology different aspects come in front of

microbiologists including microflora, carbonyl compounds, proline content, non-starter lactic acid bacteria, lipolysis etc. Greater expertise of biochemists and microbiologists regarding biochemistry and microbiology of cheese refinement technology is creating a diversity in cheese textures and flavors. The microbial, enzymatic and chemical transformation by the breakdown of milk fats, proteins, lactic and citric acid into a variety of volatile and non-volatile compounds during ripening, are the contributing factors to the cheese flavor and texture. (Adda *et al.*, 1986) Different varieties evolved involving the four integral components that are microorganisms, rennet, milk and salt. These are processed through following steps. (Beresford *et al.*, 2001)



In the blending process, variations in the ingredients along with subsequent processing causes the evolution in cheese variety. The processing parameters like curd handling and cook temperature affects the cheese type. Most importantly cheese microflora plays its role like backbone in developing unique characteristics in each kind of cheese.

Types of cheese

Swiss cheese

Flavor development characteristic of cheese dependent on the relatively lower amount of fatty acids. The lactate concentration of swiss cheese (Fig.2) is related to the lower free fatty

acids. Further after manufacture, the lactate content relates to the lactose amount. According to Langsrud and Reinbold the good quality cheese formed when there are propionic acid, acetic acid and low quantity of higher fatty acids and butyric acids. The flavor of swiss cheese become strong and rancid because of the higher fatty acids and bulk of butyric acid.

Due to proline content swiss cheese gives a specific sweet flavor, which is lesser in other cheese types. Low concentrations of carbonyl compounds are also important for flavor characteristics in cheese (Langsrud and Reinbold, 2016). Cheese used (NSLAB) to get their palatability which developed during processing.

Cheddar cheese

Bitterness in cheddar cheese (Fig.3) is due to peptides. (Broadbent *et al.*, 1998) *Prop. Shermanii* has slightly sweet and nutty flavor which is probably due to the high activity of peptidases at high level 107 cfu/mL. proteolytic activity is due to the rennet, milk and starter proteinases (visser *et al.*, 1977) There is large difference in the total and individual amino acids which is not the case with *Lactobacillus casei*, and it is due to the appropriate enzymatic release acting as limiting factor. The increase in amino acids is either due to autolysis or metabolism of viable cells. (El-Soda *et al.*, 1981) It is proposed that the flavor of cheddar cheese is also due to the fat degradation products as it has not exceptionally high specific free fatty acid contents as that of other Romano, Provolone, and Swiss cheese and some of the flavor is also due to the hydrogen sulphide. (Kristoffersen *et al.*, 1960) *M. freudenreichii* when added during manufacture disappears rapidly yet have an impact on flavor hastening in pasteurized milk but other strains are present in comparatively large amount (Alford *et al.*, 1950).

Iranian Lighvan cheese

Lighvan cheese (Fig.4) is semi-hard traditional Iranian cheese as Iranian Lighvan cheese raw ewes milk used to overcome the need of Iranian market. Isolated bacteria identified from the homogenized sample using microbiological analysis. Lactic flora produced from NSLAB in large proportion and then selection of colonies done. The characteristic to ferment carbohydrate is specific for isolates, due to which these classified into *lactobacillus*, *pediococcus* and *enterococcus* (46%, 12% and 42%). *E. faecium* through proteolysis activity on milk proteins and particularly on casein, along with high lipolytic activity, develop flavor

characteristics of cheese. (Abdi *et al.*, 2006)(abdi, Sheikh-Zeinoddin and Soleimanian-Zad, 2006)

Blue cheese

Methyl ketones play an integral part in the flavor of blue cheese. The constituents of blue cheese (fig.5) i.e.pentanone-2, heptanone-2 and nonanone-2 are obtained from fractional distillation of blue cheese. Acetone (identified as constituent of blue cheese by ether extracted steam distillate of blue cheese) has also the effect on cheese flavor. Beta-oxidation of fatty acids results in the formation of methyl ketones and due to these constituents' mold ripened blue cheese has a distinct flavor from other cheese types (Patton *et al.*, 1950)

Mozzarella

Mozzarella (Fig.6), made from buffalo milk, is a soft, un-ripened cheese of the *pasta filata* family. *Streptococcus thermophilus* is the starter bacteria for mozzarella cheese and the mozzarella cheese is composed of 23-25% fat and 50% moisture. (Jana *et al.*, 2011) The action of lactic acid on dicalcium-paracaseinate is the characteristic feature of mozzarella cheese. Branched short chain free fatty acids have an important role in its palatability and the bitterness is prevented by the reduced amount of rennet. (Micketts *et al.*, 1974) Mozzarella, rennet coagulated cheese undergoes a 3 weeks period of ripening.

Limburger cheese

Limburger cheese (Fig.7) has a strong complex flavor. It contains large amounts of C4 and C6 free fatty acids but there is no depiction in flavor and this is may be due to its 6.97 pH which due to lower vapor pressure of free fatty acid salts suppresses the flavor expression of free fatty acids. The presence of

peptides, amines, long chain ketones, free fatty acids play an integral role in extremely bitter flavor of Limburger cheese. (Woo *et al.*, 1984.)

Gouda cheese

Major microflora during processing and ripening is lactic acid bacteria. Both rennet and proteases have an integral role for the bitterness in gouda cheese (Fig.8). Peptidases have an integral part in the formation of bitter peptides and also in the removal of bitter peptides once they are formed. Bitterness may also increase by increase in γ -caseins level and this is due to imbalance hydrolyzation of milk casein. (Habibi- Najafi *et al.*, 1996)

Camembert cheese

Camembert (Fig. 9) is a mold ripened cheese. Methyl ketones, alcohols, 2-phenylethanol and sulfur compounds are involved in aroma and flavor of the camembert cheese but it is still unknown that which compound is more important in its aroma and flavor. (Molimard *et al.*, 1996.)

Chevre cheese

More than 80 compounds are involved in composition of Chevre style goat cheese (Fig.no.10). Thirteen fatty acids have considerable importance due to their efficient rate of lipolysis and also due to their sweet flavor contribution. Specific goat flavor has been contributed by Octanoic, 4-methyloctanoic and 4-ethyloctanoic acids and are present in moderate concentrations and it has been characterized as sweet, rancid, fatty with medium intensity. (Sánchez-Palomo *et al.*, 2008) Sweet aroma and milk fat flavor has been also characterized by several gamma lactones. (Whetstine *et al.*, 2003) By decreasing sharpness imparted by fatty acids and bitterness imparted by amines, most

esters produce floral and fruity flavor. (Anderson *et al.*, 1966) Toasty flavor is imparted by furan compounds at low concentration (Sánchez-Palomo *et al.*, 2008)

Monterey jack cheese

Monterey jack (fig.no.11) is obtained from cow with 32.7% fat and 41.4% moisture. (Heisserer *et al.*, 1993). It has a milky flavor with slight sweet and slight bitter taste lacking fatty acid and ester flavor and contains very low volatile free fatty acids. (Ha *et al.*, 1991)

Determinants of growth of microorganisms in cheese

Moisture

As microorganisms tend to grow in presence of moisture, susceptibility to spoilage increases with increased moisture content and to reduce the susceptibility of spoilage moisture content is reduced either by dehydration or by the incorporation of water-soluble compounds. Water activity is a thermodynamic property, directly related to moisture content and inversely related to the concentration of salt and other low molecular weight compounds (Esteban *et al.*, 1989.) During the gel formation, a cheese manufacturing step, the water activity level is highest i.e. 0.99 to promote growth of microorganism and relatively decreases in the upcoming stages i.e. whey expulsion, salting and during ripening and are significantly lower (0.917-0.988) than the requirement of starter culture bacteria. (R. uegget *et al.*, 1981.) Water activity values of different bacteria are given in Table 1

Salt

Salt and moisture content are associated to each other as salts reduce moisture and hence inhibit starter and spoilage bacteria. But many

organisms are growing even at high salt concentration and low moisture level, which means they are capable to grow in such environments and it also suggests that there are many interacting factors to inhibit unnecessary growth of microorganism which would lead to spoilage and hence bad flavor and texture. The salt concentration relies on the nature of food, pH and its moisture contents. Corresponding to water activity values 0.97-0.99, the salt concentration ranges between 0.7 to 7g/100g.

pH and organic acids

Neutral pH is favorable for the growth of most typical microorganisms and at pH less than 5 the growth of most microorganisms is poor as there are more acid sensitive bacteria present in most of the environment. Due to the accumulation of organic acids, cheese curd has a pH between 4.5 and 5.3 and acid sensitive bacteria cannot survive these harsh conditions. Lactic acid, acetic acid and propionic acid are the most common acids found in cheese, depending on their pKa value the lactic acid is least inhibitor and propionic acid is the most effective inhibitor at the same pH value. pKa values of different organic acids are given in table no.2

Except swiss cheese in which Propionic acid is higher in content than lactic acid, all other varieties have higher content of lactic acid. (Steffen *et al.*, 1993.)

Ripening temperature

Ripening temperature is the balance between the requisite to enhance ripening reactions, the consideration of desirable secondary flora and the inhibition of spoilage due to over ripening, as higher temperatures increase ripening (Folkertsma *et al.*, 1996) but at the same time are detrimental due to over growth of spoilage bacteria. Mesophiles are most

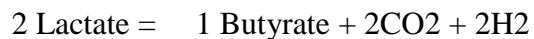
commonly involved in cheese ripening working optimally at 32⁰C and thermophiles working optimally at 42⁰C.

Reduction-oxidation potential

The oxidation-reduction potential (Eh) of milk is +150mV while that of cheese is -250mV. It may be mostly due to the reduction of trace amounts of O₂ and also due to fermentation of lactic acid by the starter culture during growth. Eh value may span from about +300mV for aerobes which resides within the cheese to less than -400mV for anaerobes which resides on the surface of cheese for different microbial cultures and hence Eh also suggest the existence of different microbial flora either within the cheese or on the surface of cheese

Nitrate

In the production of Dutch-type cheeses, to hinder the growth of *clostridium tyrobutyricum*, which ferment lactate to butyrate, hydrogen and carbon dioxide, nitrate is added as sodium nitrate (NaNO₃) or potassium nitrate (KNO₃).



In the Dutch-type cheeses, the rate of NaCl shift is very low and moisture content is also very high and for this reason nitrate is added to prevent large hole formation as large holes are created by H₂ and CO₂ and also to prevent off-flavor formation by butyrate. The xanthine oxidase present in the curd, reduce the nitrate into nitrite, the actual growth inhibitor. Nitrite inhibit Propionibacterium; thus, nitrate is not used in cheese where Propionibacterium is needed for growth. However, nitrite does not inhibit lactic acid bacteria. Most of the nitrate is detected within the whey and brine and by the time the cheese is ready for consumption, the nitrate level is

exceptionally beneath the permissible levels i.e. 50mg/kg (Beresford *et al.*, 2001)

Microorganism effect on cheese flavor

Growth of microflora after cheese production

Taking intervals after maturation for identification of isolates, after 7 days of manufacturing cheese both lactic acid bacteria and Enterobacter increases while after 18 days lactic acid bacteria increases but Enterobacteriaceae may stop. This work gives the confirmation that, during ripening of cheese the evolutionary microflora is quantitatively and qualitatively similar in various types. (Malcata, 1998).

All these components involved in the flavor development of various cheeses. Here we are considering 4 articles of different authors of different times which all discussed the production of lactic acid flora through different means. Malcata 1998 and Abdi, Sheikh-Zeinoddin

Starter culture bacteria

A controlled fermentation is used when the manufacturing's yield is unstable and the chance of pathogenic microbes' growth is present. In this fermentation bacteria are first selected and isolated for the manufacturing and maintained for the use and referred to as starter culture (Giraffa, 2004).

The production of acid is the basic function of the starter culture during fermentation as well as starter culture is also involved in cheese ripening process. Enzymes of the starter bacteria basically convert amino acids into flavor compounds and also contribute in proteolysis process (Fox *et al.*, 1997). In the milk isolates starter bacteria are used which are tend to reduce the pH of milk. Starter

bacteria could be added at initial stages of cheese manufacturing process or these may be found in the milk as natural contaminants as in many cheese varieties that are manufactured using raw milk. within the starting hours of manufacturing the starter culture grow and densities of 10⁸ cfu/g is maintained. May be thermophilic or mesophilic starter bacteria could be used that depend on the variety of cheese being produced. In Cheddar, Camembert and Blue cheese mesophilic cultures are used while for Parmesan and Emmental cheese thermophilic culture are used.

In mesophilic and thermophilic starter cultures there are of two categories one is mixed or undefined culture and other is known as defined culture. Citrate metabolizing strains of *Lactobacillus lactis sp. cremoris* and *Lactobacillus lactis sp. lactis* could be present as undefined culture of mesophilic culture, for the production of flavor (Lodics *et al.*, 1993). Mesophilic cultures are used worldwide for Cheddar cheese manufacturing. Thermophilic starters are composed of strains of *Lactobacillus delbrueckii sp. delbrueckii*, *Lactobacillus delbrueckii sp. lactis*, *Lactobacillus delbrueckii sp. bulgaricus*, or *Lactobacillus helveticus*. Thermophilic undefined cultures are used for manufacturing of Emmental cheese at small scale in Italy and France.

Non-starter lactic acid bacteria

Mesophilic *pediococci* and *lactobacilli* microbial flora may be present as non-starter lactic acid bacteria during ripening process of various cheese. These NSLAB are not component of the starter flora because their growth is not good in milk and they do not take part in the acid production in manufacturing of cheese (Cogan *et al.*, 1997). Isolated mesophilic *lactobacilli* species from cheese include *lactobacillus casei* or

Lactobacillus paracasei, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus curvatus* (Jordan *et al.*, 1993; Coppola *et al.*, 1997; Fitzsimons *et al.*, 1999). While *Pediococcus acidilactici* and *Pentosaceus* are more frequent in cheese.

It is obvious that strain selection is very important in the quality of cheese as high quality of cheddar cheese is produced by some strains of *Lactobacillus casei sp. casei* and *Lactobacillus casei sp. pseudoplantarum*. On the other hand, bitter flavor acid in cheese is due to other strains of these species (Lawrence *et al.*, 1987). During the production of cheddar cheese gas level that is due to inoculated heterofermentative is reduced when heterofermentative *Lactobacillus* is added (Laleye *et al.*, 1990). In cheese NSLAB comes from post-pasteurization contamination or strains that survive after pasteurization (Turner *et al.*, 1986; Martley *et al.*, 1993).

Propionic acid bacteria (PAB)

During cheese ripening of various cheese types propionic acid bacteria grow. The characteristic microflora of most of the cheeses such as Emmental propionic acid bacteria include short rod-shaped gram-positive bacteria and they are involved in lactate metabolism (Steffen, 1973). Five species of PAB are recently recognized that includes *Propionibacterium jensenii*, *Propionibacterium freudenreichii*, *P. acidipropionic*, *Propionibacterium thoenii*, and *Propionibacterium cyclohexanicum* and one *Propionibacterium coccoides* proposed (Vorobjeva, 1999). Wild type PAB was present in sufficient amount during manufacturing of cheese from raw milk.

The characteristic flavors of these Swiss-type cheeses is developed from post manufacture propionic acid fermentation. While eye

formation in cheese is due to carbon dioxide production. After the enough eyes formation in cheese it is stored at lower temperatures to further cease the metabolism and growth of PAB (Baer, 1995). Infection during the growth in swiss type cheese from bacteriophage *P. freudenreichii* is recently recognized, these infections cause *propionic acid bacteria* lysis during the cheese ripening process (Gautier *et al.*, 1995). Interactions of other bacteria with *propionic acid bacteria* during cheese ripening is also important. These stimulatory and inhibitory interaction of *propionic acid bacteria* with other cheese bacteria impose an important impact on cheese quality and need to be considered in detail.

Yeast

Yeasts are found in many varieties of cheeses but their workout plan in ripening is unclear. (Fleet *et al.*, 1990) *D.hansenii* is most commonly found yeast in most variety of cheeses such as weinkase, Roquefort, carables etc. but there are also other important species such as *K.lactis*, *Yarrowia lipolytica* and *Trichospora beigeli* which are also found in different variety of cheeses. Yeast grow optimally under the conditions of high salt levels, low temperature and pH and low moisture content. At high salt concentrations yeast metabolize the lactate and it grows during the early days of ripening and it will help in deacidification. (Eliskases-Lechner *et al.*, 1995) Yeast play an important role in the development of texture and flavor. Flavor is also enhanced due to lipolysis and deacidification by yeast.

Smear bacteria

Smear bacteria cheeses are characterized by the presence of smear on the surface of cheese and these cheeses are found in many European countries in large amounts. During

early phase of ripening, *staphylococci* are most commonly found but in later stages, they are replaced by *Corynebacterium*. They are generally in soft or semi-soft variety but there

are also few hard cheeses such as Comte. Amino acids and fatty acids produced from the proteinases, peptidase and lipases play role in the flavor of in these types of cheeses.

Table.1 Water activity values of different bacteria(Weber *et al.*,1987)

Bacteria	Water activity values
<i>Lactococcus lactis</i>	0.93
<i>Streptococcus thermophilus</i>	0.98
<i>Lactobacillus helveticus</i>	0.96
<i>Propionibacterium freudenreichii</i>	0.96

Table.2 pKa values of different organic acids involved in cheese manufacture

p	pKa value
Lactic acid	3.08
Acetic acid	4.75
Propionic acid	4.85

Table.3 Component effect on growth of microflora

Authors	Fatty acid microflora	Proline content	Carbonyl compounds	NSLAB	Lipolysis
(Langsrud and Reinbold, 2016)	√	√	√	X	X
(Abdi, Sheikh-Zeinoddin and Soleimanian-Zad, 2006)	√	X	X	√	√
(Arnold, Shahani and Dwivedi, 2010)	√	X	X	X	√
(Malcata, 1998)	√	X	X	√	X

Fig.1 Various forms of cheese



Fig.2 Swiss cheese



Fig.3 Cheddar cheese



Fig.4 Iranian lighvan cheese



Fig.5 Blue cheese



Fig.6 Mozzarella cheese



Fig.7 Limburger cheese



Fig.8 Gouda cheese



Fig.9 Camembert cheese



Fig.10 Chevre cheese



Fig.11 Monterey jack cheese



Component effect on cheese flavor

Proline and carbonyl compounds

Langsrud and Reinbold, 2016 lighten up the proline content and carbonyl compound while discussing production of swiss cheese. But other three articles don't bother about these two compounds. The table shows that these two components of flavor may not get its importance yet and the microbiologists should focus on these two components to enhance the flavor characteristics of cheese. The swiss cheese possessed sweet flavor due to this proline, important is how its structure interact in swiss cheese production and which part of its structure play role here.

Flavor of swiss cheese may enhanced by using low concentrations of carbonyl compounds. Since cheese flavor development may get advancement if these two components become the hottest topic for microbiologists.

Lipolysis

Lipolysis is process in which free fatty acids are produced through the hydrolysis of triglycerides that is enzyme catalyzed. It is due to lipase and microbial lipases that found in dairy products as well as in milk and causing the formation of flavor, may rancid defects. (Arnold *et al.*, 2010)

Malacata, 1998 and (Langsrud and Reinbold, 2016); didn't discuss lipolysis which is the hydrolytic cleavage of triglycerides but remaining two articles investigate this property. Due to combination of lipolytic enzymes free fatty acid produced, help in increase of flavor. Lipolysis may produce rancid defect.

In conclusion, cheese involves a very diverse microflora and selection of suitable strains is required for the development of required flavor. There are a lot of variety of cheeses and every cheese is different from other in flavor, texture and aroma only by the up and down of one or more composition elements and by regulating some factors influencing the components growth. Every cheese is characterized by different series of interaction between different components therefore we should have an understanding of the way these components will behave efficiently by producing high yield in less time and energy consumption. By the understanding of different microbiological techniques, there will be improvement and facilitation in the detection, isolation and identification of essential flavor compounds in specific cheese type and by this we can also enhance the controlled production of variety of cheeses

There is a cheese for every taste preference and a taste preference for every cheese (Olson *et al.*, 1990).

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