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CHARACTERISTICS OF THE COMPOSTION AND BIOACTIVE PROPERTIES OF MOUNTAIN MILK USED FOR EMMENTAL CHEESE MAKING - REVIEW

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Abstract

Wild mountain pastures are situated at high altitudes (800-1500 m), where they benefit from a rich and diversified flora, with terpenes and propionic bacteria that are found in salty soils, poor in iron and manganese, these conditions being crucial for the process of obtaining the Emmental cheese. The milk that comes from these ecological areas is characterised by a superior quality of the basic biochemical compounds and their ratio in addition to their rich content in biologically active substances and probiotic bacteria (especially lactic and propionic bacteria), all of these factors having a major impact on the process of obtaining and valorising the different types of Emmental cheese. The quality of the Emmental cheese is also ensured by the temperature-humidity regime that is specific to the mountainous region together with the addition of sodium chloride and the action of specific mechanical factors that help with the process of whey extraction. There are many types of Emmental cheese that differ based on the technological process, time of maturation and the specific of the region where it is made.

Key words*: biodiversity, cheese, Emmental cheese, milk bioactivity, mountain pastures.*

INTRODUCTION

The globalisation of food market has flourished in the last decades resulting in an important diversification and broadening of Emmental types of cheese. This situation has led to the exponential rise of commerce of food in the European community territory and thus, all over the world (Ssilvanikove et al., 2015). Globalisation represented a big challenge for milk processors that had to continuously raise the quality and security of the marketed products and also satisfy the diversified demands of the consumers (Svederberg et al., 2012). According to recent data, milk and dairy products represent a big proportion of the average Europeans' diet. Milk and dairy products constitute an important source of high quality nutrients (proteins, fats, carbohydrates) and biologically active molecules (enzymes, vitamins, minerals) (Visioli et al., 2014). Current technologies ensure the production of numerous cheese assortments that correspond to the organoleptic, physicochemical and microbiological characteristics needed in order to satisfy the needs and preferences of consumers. According to research conducted in the field, it is completely relevant to categorise dairy products into conventional products that have been prepared using conventional technologies and recipes, traditional products that have been prepared using recipes that are specific to some geographical areas and organic products, that have been obtained using milk that has been certified as organic (Ercolini et al., 2003). The same classification is applied to Emmental cheese. Different types of Emmental cheese (generic and traditional) are produced in large quantities in many European countries such as France, Austria, Germany, Finland and Ireland (Basig et al., 2010). Emmental cheese has its origin in the region with the same name situated in the Berna region of Switzerland, where it has been made ever since the XIIth century, its existence being firstly documented in 1542. In 2002, Emmental cheese acquired protected designation of origin certificate (Basig et al., 2010). In order to make Emmental cheese, raw milk from mountain pastures is required, where cow feed doesn't consist of silo and the milk is processed in the first 24 hours after milking (Berdague et al., 1990), in copper cauldrons (Rodrighez et al., 2011). Lately there has been a major interest for cheese that has been prepared with raw milk which comes from cows that graze mountain pastures, because it is considered by consumers to have more flavour (Martin et al., 2005). An important characteristic of Emmental cheese is considered to be its unique sensorial perception that consumers prefer, such as the taste, aroma, texture and colour (Spence et al., 2015). The production of raw milk cheese is on the rise because of the high demand of consumers, especially regarding traditional products (Laurenčík et al., 2008). This explains why the normal microflora of raw milk constitutes an essential factor in the production, fabrication and maturation of traditional cheese (Beresford et al., 2001).

THE SPREAD AND PERSPECTIVE OF MOUNTAIN PASTURES

Mountain areas, especially the ones situated over 800 m altitude, present alongside the numerous advantages, several disadvantages. These are represented by the limited possibilities of proper utilisation of mountain grazes and terrains due to the difficult climate conditions and also because of the rough terrain that does not allow the use of farm equipment (Coppa et al., 2019). Therefore, wild pastures represent the only possibility of using agricultural terrains in high altitude regions. In the European Union, the limit of mountain regions start at minimum 600-700 m elevation over the sea levels. It is important to mention the fact that the levels of altitude can vary depending on the country, climate and the elevation of the geographical area (Coppa et al., 2019). Currently, it is estimated that the pastures area in the European Union represent 18.5% out of the usable agricultural land (Coppa et al., 2011). Alpine pastures make up 17.8% of the total usable agricultural land of the European Union and the cattle grazing on those pastures account for 10.5% of the total of dairy cattle in the EU (Santini și col., 2013). Production costs are higher in mountain areas because of the lower profitability of dairy cattle farms (Santini et al., 2013; Martin et al., 2014). This impediment can be overcome by the reduction of costs regarding the production and collection of the milk through commercial strategies to raise the prices of the dairy products that can be certified as products with protected designation of origin (Coppa et al., 2019). The advertisement of products with special sensorial characteristics has a major impact on the gratification of the consumers demand and preferences (Martin et al., 2005; Bentivoglio et al., 2019).

COMPOSITIONAL CHARACTERISTICS OF MOUNTAIN MILK

Milk is considered to be the "perfect food" because it contains all the essential nutrients and vitamins, enzymes and other biologically active molecules. The biochemical composition of milk is as follows: water (85.5- 89.5%), dry matter (10.5-14.5%), fat (2.5- 6.0%), protein (2.9-5.0%), lactose (3.6-5.5%), minerals (0.6-0.9%), vitamins (Asadullah et al., 2010). Water is found in the biggest proportion in whey (70-90%) and it helps stabilize the other constituents by assuring the dispersion of casein micelles, fat emulsification during the processing and bacterial growth (Guinee and O'Brien, 2010). Casein proteins represent approximately 80% of milk protein and they coexist amongst insoluble minerals like calcium phosphate and together with fat they make up the solid faction of the milk, known as rennet. Milk also has a liquid fraction represented by the whey, which is made up of water, lactose, lactic soluble proteins, citric acid, some minerals, enzymes, free amino acids and peptides (Guinee and O'Brien, 2010). During the process of cheese making, milk is partially dehydrated by the controlled expulsion of whey and partially of fat, casein and some minerals (Guinee and O'Brien, 2010). The processes of casein aggregation and dehydration ensure the quality of cheese as finite products (Guinee and O'Brien, 2010). There are four types of casein (α s1, α s2, β and k), that are found in different proportions in the total casein count (38%, 10%, 35%, 15%)

(Jerónimo et al., 2016). Through the process of casein combination, micelles are formed and they are part of the cheese structure. Casein micelles are colloidal particles that have a spherical shape and a diameter of approximately 40-300 nm (McMahon and Oommen, 2008).

Whey protein (0,6-0,7%) are represented by β lactoglobulines 54%), α lactalbumine (21%-metal protein that is bonded by Ca2+), immunoglobulins (14% - IgG1, IgG2, IgA, IgM) (Guinee et al., 2010) and lactoferrin (O'Callaghan et al., 2019). Lactic enzymes are very important as well, especially in the evaluation of the hygienic quality of milk. Milk has an important natural catalytic activity which is a useful indicator of a possible microbial contamination of the mammary gland and of milk itself (Maubois, 2018). Milk lipids can have significant variations (3-5%) depending on the breed, general health status, mammary glands and feed. Milk fat includes triglycerides (96-99%), phospholipids (0,8%), diglyceride (0,3%), sterols, some free fatty acids, carotenoids and fat-soluble vitamins (Huppertz et al., 2009). Milk fat is distributed as dispersed globules that are surrounded by a lipoprotein membrane (Wiking et al., 2004). The membrane has the role of protecting and stabilising the fat globules against turbulence and fusion, and also against microorganisms like *Pseudomonas spp*. (Ward și col., 2006). The deterioration of the membrane of fat globules during the manipulation of the milk leads to the accumulation of free fat in the milk, then in the cheese, that is responsible of undesirable taste (sour, metallic, soapy), which can be specific to hard and semi-hard cheese (Emmental, Gouda, Cheddar) (Deeth, 2006).

THE INFLUENCE OF MOUNTAIN PASTURES ON THE BIOLOGICALLY ACTIVE COMPONENTS OF MILK

Carotenoids (lutein and carotene) can be considered potential biomarkers and their concentrations must be monitored in milk and various dairy products (Nozière et al., 2006). Carotenoids are found in large quantities in cow milk and they have a big impact on the nutrition and general health status of humans. They work as natural antioxidants and precursors of vitamin A. The lactic concentration of carotenoids can present important variations, mainly due to season, sometimes making up to 50% (Agabriel et al., 2007). Carotenoids are liposoluble, so they easily transfer from milk to cheese (Nozière et al., 2006), their concentrations correlating with the yellow richness of cheese (Kilcawley, 2018).

Terpenes are one of the bioactive components of milk that according to Agabriel et al. (2007), are found in higher concentrations in mountain milk than in milk that has other provenance. Other studies suggest that terpenes that are found in milk and cheese can reach high levels when the cows graze wild pastures, rich in plants from the *Apiaceous* family, thus explaining a more intense smell of the milk compared to the milk that comes from cattle, predominantly fed with *Gramineae* (Kilcawley et al., 2018). The terpene content of mountain pastures plants varies depending on the botanical family they belong to (Prache, 2009; Tornambé et al., 2006). Other researchers also found large quantities of sesquiterpenes and monoterpenes in the plants that are specific to mountain pastures (Engel et al., 2007). It is important to mention the findings of De Noni et al. (2008) regarding the terpene profile of mountain milk, which is dominated by monoterpenes, the same as in cheese made from this type of milk. It has been proven the fact that terpenes go through biotransformation in the rumen and large intestine without hepatic or renal excretion when their concentration exceed their normal levels (Poulopoulou et al., 2012). Even though data suggests there is a slight passing of terpenes found in plants in the milk and cheese, there is still a need for more research in order to confirm this hypothesis. Belviso et al. (2011) discovered other terpenes in dairy products that aren't found in the feed. The same authors highlighted the biosynthetic and metabolization potential of terpenes in the rumen. Lactic bacteria can modify and synthetize terpenes that reach the milk and consequently cheese, after ruminal metabolization.

Volatile compounds. Natural pastures, especially mountain pastures, give Emmental cheese a distinct aroma through the over 200 volatile compounds that are found in the greenery (Taylor et al., 2013). Volatile compounds are the result of proteolysis, lipolysis and metabolization of lactose, citrate and microorganisms during the maturation phase (Taylor et al., 2013). Volatile compounds make up several groups of non-terpenoid products (lactones, acids, esters, fenoles, aldehydes, alcohols, sulphide compounds) that are found in the pastures and they can contribute to the milk flavour (Kilcawley et al., 2018). The cheese flavour is more complex because of the activity of numerous microorganisms that are later added (lactic bacteria, yeasts and moulds). The concentrations of volatile compounds rise during the fermentation and maturation phases of the cheese (Kilcawley et al., 2018). As it was mentioned before, the high concentration of fatty acids and polyunsaturated fats in mountain milk can oxidase and generate lipid peroxides, aldehydes, ketones and alcohols (Havemose et al., 2006). The same author states that the sources of many aldehydes and ketones are extremely hard to identify because they come either directly from feed or from the maturation phase of the cheese. Stefanon et al. (2004) analysed cheese made from the milk of cattle which were fed with silage and mountain grass and they found a higher concentration of volatile alcohols than the concentration resulted from consumption of corn, corn silage or hay from lowland. It is important to also note the results obtained by Bugaud et al. (2011) that show the fact that cheese made from cattle mainly fed with hay contains larger quantities of 2-methyl-butanol and 3-methylbutane. Other compounds that contribute to the flavour of milk and cheese are simple carboxylic acids that result from lipolysis and the metabolism of amino acids and carbohydrates (Kilcawley et al., 2018). The highest concentrations of fatty acids are found in cheese made from mountain milk (Falchero et al., 2010). There are numerous studies regarding pastures, milk and cheese from mountain areas that emphasise the rich content of fatty acids depending on the assortment and the age of the cheese or on the transfer of fatty acids in the milk either by ingestion or inhalation. Sulphide volatile compounds are potentially aromatic compounds because of the smell and their intense flavour (Falchero et al., 2010). According to research studies, toluene can be a potential biomarker for milk that comes from cattle that graze natural pastures (O'Callaghan et al., 2016) because it is a product of ruminal degradation of carotene (Villeneuve et al., 2013). The highest levels of carotene can be found in pasture grass and other fresh feed (Coppa et al., 2011). Some authors state that toluene is largely present in cow milk, which explains its high concentrations in cheese made from milk that comes from cattle that graze natural pastures (Cornu et al., 2009). Toluene is considered to have numerous sensorial influences such as sweet, nutty, almond-like (O'Callaghan et al., 2017) pungent, etheric, fruity or rancid (Coppa et al., 2011).

PRINCIPLES OF PROCESSING ADAPTED TO THE SPECIFIC OF CHEESE MADE IN MOUNTAIN AREAS

Cheese production includes the jellification of milk and the dehydration of the gel in order to form the cheese. The cheese making process is based on the destabilisation of the micelle structure of casein through the alteration of the physico-chemical properties of the solution (O'Callaghan et al., 2019). After the primary processing (stirring, texturization, pouring and pressing), cheese is poured in different shapes (that range from small quantities to 100-120 kg). After the preparation of the cheese, it can be consumed fresh in the first week after it was made or it can be matured for longer periods of time (starting from 2 weeks up to 3-6 months, even up to a year or two) like Emmental cheese (Guinee et al., 2010). In order to make most cheese it is mandatory to add rennet that contains chymosin, an enzyme capable to hydrolyse the k casein (Fagan et al., 2017). This process will determine the destabilisation of the casein micelles and it will lead to the coagulation of milk under the form of a gel that represents the actual cheese (Fagan și col 2017).

The coagulation process represents an important step in the fabrication of cheese because it marks the passing of milk from the liquid state to solid state (Gassi et al., 2017). There are two procedures used for milk coagulation namely the lactic way (acidification) and the enzymatic pathway through the addition of ferments in the milk.

The enzymatic pathway consists in the addition of rennet in the mildly acidified milk and it is specific to pressed cheese including Emmental cheese (Gassi et al., 2017). The two procedures are often combined in cheese making technologies. The acidity is due to the lactic bacteria that transform lactic acid. This process is essential for milk coagulation and it requires a minimal temperature threshold and it varies according to the cheese assortment (Fox and McSweeney, 2017). Acidophilic bacteria from milk can be either mesophilic or thermophilic. Mesophilic rod-shaped bacteria are used in order to obtain raw milk cheese (processed at temperatures below 40°C) and for the formation of the rind of the cheese. In contrast, thermophilic bacteria are used in high temperature processes (over 50°C) to obtain boiled cheese like Emmental (Fox and McSweeney, 2017). Mesophilic bacteria grow at temperatures between 25-40°C (Fröhlich-Wyder et al., 2017) and they consist of *Lactococcus* spp. and *Leuconostoc* spp. (Hayaloglu, 2016). Various bacteria are used in the cheese making process like *Lactococcus lactis, Lactococcus lactis* subsp. *cremoris, Lactobacillus paracasei, Lactobacillus rhamnosus, Leuconostoc* spp. and *Staphylococcus* spp. (Cotter et al., 2017). *Lactococcus* spp. are essential because they transform lactic acid in glucose and so they are considered to be homofermentative (Fröhlich-Wyder et al., 2017). *Leuconostoc* spp also have essential properties because they gather heterofermentative bacteria involved in the metabolization of lactose in lactate, ethanol and CO_2, which are responsible for the holes in the Emmental cheese (Fröhlich-Wyder et al., 2017). It is important to mention the fact that these bacteria withstand low pH levels, which is an advantage at the end of the acidifying process. Among thermophile bacteria, there are *Streptococus termophilus, Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. *lactis* (Fröhlich-Wyder et al., 2017). The rennet is a mixture of animal enzymes used to make cheese (Andrén, 2011). After 1980, the recombinant DNA technology allowed the creation of chymosin through gene cloning of a protein making gene in *Escherichia coli* (Uniacke-Lowe and Fox, 2017). Rennet has been used in cheese production for thousands of years; its traditional form was a mixture of chymosin and pepsin enzymes secreted in the abomasum (Uniacke-Lowe and Fox, 2017). These two enzymes have a proteolytic activity that is the basis of the coagulation process, jellification being the selective hydrolysis of casein in phenylalanine-methionine. This peptide bond is caused by the addition of the rennet and the acidifying to 20-40°C and a pH around 4.6 (Andrén, 2011). The calcium ions play an important part in milk coagulation by inducing casein micelle aggregation and the coagulation of the casein (Guinee and O'Callaghan, 2013). Milk ionized calcium is in an equilibrium with the casein calcium with a direct influence over the aggregation and jellification of the milk (Guinee and O'Callaghan, 2013). Also by the addition of calcium chloride in the milk, a soft, elastic texture is produced, which is essential for the formation of holes that are specific to Emmental cheese (Fröhlich-Wyder et al., 2017). The milk clot contracts and removes the whey in the presence of the rennet, proper acidity and optimal temperature (Gassi et al., 2017). The resulted gel is ten times more concentrated in casein, fat and calcium phosphate than milk. The resulted product is processed in order to help remove the whey by cutting it into cubes (of approximately 0.5-1.5 cm in diameter), stirring, rising the temperature and reducing the pH levels in order to ferment lactose in lactic acid (Lucey et al., 2003). After the whey is drained, small particles of cheese are formed and they make up a coagulated mass that is put in the desired mould, it is pressed and then the salt is added, depending on the type of cheese that is produced (Lucey et al., 2003). Traditionally Emmental cheese is made in copper cauldrons. The propionic fermentation in the Emmental production is a unique phenomenon. It is well-known that propionic bacteria that can be found in raw milk can cause problems during the maturation phase. Copper slows down the explosive fermentation of propionic acid and inhibits spores growth like *Clostridium tyrobutyricum*, thus reducing the risk of the cheese blowing during the later stage of maturation (Rodriguez and Alatossava, 2010). Copper forms bonds with the sulphide compounds resulted from the metabolism of amino acids which leads to the nutty sweet taste of the cheese.

One of the main conditions for propionic acid to form and to later stimulate CO_2 to create the specific holes, is to add water to the milk or into the cheese. For Emmental cheese, 12-20% water is added in order to reduce the lactose concentration and to rise the pH (up to 5.2-5.35) and accelerate the formation of propionic acid (Fröhlich-Wyder et al., 2017). Studies suggested that the formation of the holes during maturation is due to some microparticles that through their capillary structures are found in plant tissue and they are most likely the natural precursors of some nuclei that generate the holes during propionic acid fermentation (Guggisberg et al., 2015). Another particularity of the formation of the holes is the fact that during maturation, the propionic bacteria consume the lactic acid and it releases CO_2 and the resulted gas makes the specific holes in Emmental cheese. This type of cheese is industrially produced with pasteurized milk in countries all over the world and the process is controlled by enzymes and perfected lactic cultures in order to obtain similar qualities with the original. The addition of salt is a very important step in the cheese making process. It has both a conservational role and it also gives it flavour (Guinee and Fox, 2017). Salting can be accomplished either through dipping the cheese in brine for a certain period of time or by searing the cheese with fine or coarse salt or both, all of this depending on the type of cheese (Guinee and Fox, 2017). The success of the maturation phase depends on following the guidelines of temperature and humidity in the storage room, their values depending on the type of cheese being made.

CONCLUSIONS

The gathered data argue the effects of mountain areas on the composition of milk and cheese, especially Emmental cheese, that are differentiated by the higher quality assured by the usage of milk from cattle raised in mountain conditions. The flora of mountain pastures is characterised by the bioactive compounds rich in terpenes, carotenoids, propionic bacteria and volatile compounds that can be found in the raw milk and dairy products. All of these mostly assure the nutritious, bioactive and sensorial qualities of Emmental cheese that is considered to be the "king" of all assortments of cheese. The fermentation process is also potentiated by the enzymatic content of the mountain milk that is biodiverse because of the mountain pastures and also because of the quality of the rennet and added mesophilic lactic bacteria. An important part in the processing of milk that is used to make Emmental cheese is the use of copper cauldrons that neutralise pathogen microorganisms and slow down the explosive fermentation of propionic bacteria thus preventing cheese defects. Propionic bacteria will stimulate the normal fermentation that will release CO_2 that is essential in order for the specific holes to form. The water that is added during the process reduces the lactose concentration and helps the pH levels rise consequently assuring the optimal conditions for propionic fermentation and release of CO_2. In order for the Emmental cheese making process to be success, it is important to strictly obey the temperature regulations during the coagulation phase and also during the pressing, salting and maturation phases. The specific conditions that need to be followed during the maturation phase are the temperature and humidity, the process lasting from a few weeks up to 12-24 months depending on the tradition of the region. Generally mountain cheese and other dairy products present attractive sensorial profiles compared to lowland products.

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