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Invited review: Bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption

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ABSTRACT

Traditionally, cheese is manufactured by converting fluid milk to a semisolid mass through the use of a coagulating agent, such as rennet, acid, heat plus acid, or a combination thereof. Cheese can vary widely in its characteristics, including color, aroma, texture, flavor, and firmness, which can generally be attributed to the production technology, source of the milk, moisture content, and length of aging, in addition to the presence of specific molds, yeast, and bacteria. Among the most important bacteria, lactic acid bacteria (LAB) play a critical role during the cheese-making process. In general, LAB contain cell-envelope proteinases that contribute to the proteolysis of cheese proteins, breaking them down into oligopeptides that can be subsequently taken up by cells via specific peptide transport systems or further degraded into shorter peptides and amino acids through the collaborative action of various intracellular peptidases. Such peptides, amino acids, and their derivatives contribute to the development of texture and flavor in the final cheese. In vitro and in vivo assays have demonstrated that specific sequences of released peptides exhibit biological properties including antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and analgesic/opioid activity, in addition to angiotensin-converting enzyme inhibition and antiproliferative activity. Some LAB also produce functional lipids (e.g., conjugated linoleic acid) with anti-inflammatory and anticarcinogenic activity, synthesize vitamins and antimicrobial peptides (bacteriocins), or release γ-aminobutyric acid, a nonprotein amino acid that participates in physiological functions, such as neurotransmission and hypotension induction, with diuretic effects. This review provides an overview of the main bioactive components present or released during the ripening process of different types of cheese.
Key words: cheese, bioactive compound, multifunctional activities, health effect

INTRODUCTION

Cheese, defined as the fresh or matured product obtained from the coagulation of milk, is easily digestible and rich in nutritional components, thus constituting an important source of proteins, short-chain fatty acids, vitamins, and minerals. It is therefore an important source of a wide variety of biologically active substances (Walther et al., 2008; Diana et al., 2014). Cheese can be classified based on the type of milk used, manufacturing process, fat content, type of fermentation, and its microbiota (Walther et al., 2008).

Several compounds in cheese are derived from the metabolism of lactic acid bacteria (**LAB**), which play an important role in the cheese-making process and contribute to the development of the texture and flavor of the final product. Lactic acid bacteria hydrolyze lactose during fermentation and produce high concentrations of lactic acid and other organic acids (Settanni and Moschetti, 2010). Several milk enzymes, such as rennet and enzymes from LAB, participate in the ripening process, resulting in the subsequent transformations that target the diverse constituents of curds (Leroy and De Vuyst, 2004; McSweeney, 2004).

The following bioactive compounds are found in cheese: peptides, exopolysaccharides, fatty acids, organic acids, vitamins, γ-aminobutyric acid (**GABA**), and CLA. All of these have biological activities. In vitro and in vivo studies have demonstrated that these compounds inhibit angiotensin-converting enzyme (**ACE**) and exhibit antioxidant, antimicrobial, and antiproliferative activities (Faure et al., 2006; Sprong et al., 2010; Geurts et al., 2012). The above bioactivities lead to health-protective effects associated with a reduced incidence of cardiovascular disease risk factors, such as

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obesity, dyslipidemia, and type 2 diabetes (Sullivan et al., 2001), as well as reduced incidence of metabolic syndrome (**MetS**; Bonthuis et al., 2010; Sonestedt et al., 2011). Mexican cheeses have potential benefits because of their native microflora, the type of milk used, and artisanal techniques applied during their production. A previous study (Torres-Llanez et al., 2011) demonstrated the ACE-inhibitory action of peptides derived from fresh cheese and a model cheese (made at the laboratory scale). Studies in Mexico on bioactive compounds present in artisanal cheeses are limited, and those available focus primarily on the antioxidant and ACE activity of water-soluble extracts (**WSE**) obtained from different types of artisanal cheese (e.g., Crema de Chiapas, Cocido, and Fresco of Sonora) from different storage conditions (Aguilar-Toalá et al., 2013, 2015; Santos-Espinosa et al., 2013).

This review article provides an overview of the main bioactive components present or released during the ripening process of different cheese types from different countries.

INFLUENCE OF CHEESE PROCESSING TECHNOLOGY ON THE DEVELOPMENT OF BIOACTIVE COMPOUNDS

Cheese is a biologically and biochemically dynamic product, in which a series of sequential changes take place during the cheese-making process. Some technological procedures such as heat treatment, homogenization, pressure application, and milk coagulation can affect the structure of the milk constituents and promote the development or the release of bioactive compounds (Kumar et al., 2006; Cruz et al., 2011).

Some studies have reported that heat treatments alter the final milk and cheese composition and thereby determine cheese quality (Albenzio et al., 2001; McSweeney, 2004). Meanwhile, other authors have hypothesized that pasteurization could influence the amount of protein and PUFA in milk or cheese; however, the results of a study showed that heat treatment did not affect the amount of protein or the fatty acid profile of cheese (Cuchillo-Hilario et al., 2010). Albenzio et al. (2001) reported that heat affected the content of water-soluble N and total N, which were higher in cheese made with raw milk than in cheese made with pasteurized or raw milk (heat treatment of curds at 80°C for 30 s in hot whey). Thus, temperature conditions can affect the amount and specific sequence of bioactive peptides. Changes to proteins are generally related to their functional properties and are favored by the action of enzymes specific to milk. Additionally, the microorganisms present or added during cheese-making may release bioactive compounds (Claeys et al., 2012).

Proteases and peptidases present in milk (e.g., plasmin, cathepsin D) lead to formation of large and intermediate-sized peptides; subsequently, these peptides are further hydrolyzed by residual coagulant retained in the curd and finally, by enzymes from the cheese starter and the nonstarter microbiota. This set of reactions is known as primary proteolysis. Of the enzymes present in milk, the enzyme that mainly contributes to proteolysis is plasmin. Plasmin is extremely thermostable, and its activity increases after heat treatment, either by inactivation of natural plasmin inhibitors or the activation of plasminogen in milk during heating (Chavan et al., 2011). Thus, pasteurization or milk heating during cheese manufacture can enhance the formation of peptides. Moatsou et al. (2008) found that the activity of plasmin decreased directly related to increased temperature and pressure during the cheese-making process. The combination of both factors (temperature and pressure) on reduction of plasmin activity is desirable because of the effect on cheese yield, proteolysis, and quality milk during storage. In contrast, the activity of another proteolytic enzyme, cathepsin D, is mostly suppressed following pasteurization and whey drainage (McSweeney, 2004).

Paul et al. (2012) found that the high pressure and temperature applied during the packaging of fresh cheese affected the antioxidant activity [evaluated by the oxygen radical absorbance capacity (ORAC) method] of water-soluble proteins containing bioactive peptides. The lowest antioxidant activity [10.2 Trolox equivalents (**TE**)/g of cheese) was for cheese processed at 600 MPa for 20 min at 22°C; meanwhile, for cheese processed at 400 MPa for 10 min at 22°C, the antioxidant activity was 67.6 TE/g. In this sense, the length of processing time and pressure used can affect the antioxidant activity of fresh cheese.

Another study showed that the application of high hydrostatic pressure to Garrotxa cheese (400 MPa, 5 min, 14°C) affected lipolysis and generated a cheese with lower amounts of free fatty acids. This can be attributed to the resulting decrease of microorganisms or the inactivation of lipolytic enzymes of the secondary microbiota (Saldo et al., 2003).

ROLE OF RIPENING AND LAB ON THE PRODUCTION OF BIOACTIVE COMPOUNDS

In the complex microbial niche of cheese, LAB, yeast, and some molds are present. These microbes play an important role in the development of the sensory characteristics of cheese and in the technological aspects of cheese production (Irlinger and Mounier, 2009).

During the cheese-making process, LAB can be added as a starter culture, contributing to the coagulation