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Chapter

# Cheese's Bioactive Peptide Content and Fatty Acids Profile

Ilyes Dammak and Carlos A. Conte-Junior

## Abstract

This chapter provides an in-depth review of the latest research developments in cheese's bioactive peptides and fatty acid profiles, emphasizing their potential health benefits, particularly in managing obesity and hyperlipidemia. It delves into the generation of bioactive peptides during cheese fermentation and maturation, their potential health-promoting effects, and the factors influencing their content. The chapter also offers a comprehensive analysis of the fatty acid profile in cheese, discussing the impact of various cheese-making processes on this profile and the subsequent implications for human health. Furthermore, it explores innovative strategies for enhancing the bioactive peptide content and optimizing the fatty acid profile in cheese. These strategies include using bioactive edible films, which have shown promise in improving the microbial quality of cheese and reducing lipid oxidation, thereby extending its shelf life. The chapter also investigates the encapsulation of bioactive compounds, a technique that has been used to enhance the stability and functionality of these compounds. Through this comprehensive review, the chapter offers valuable insights into the potential of cheese as a source of health-promoting bioactive peptides and fatty acids and the various strategies for optimizing their content and functionality.

Keywords: bioactive peptides, fatty acids, cheese, health benefits, cheese processing

## 1. Introduction

Cheese, an extensively consumed dairy product, is highly regarded for its abundant flavor, pleasing texture, and significant nutritional content. The above substance is a notable reservoir of proteins, fats, vitamins, and minerals. Cheese is not only rich in conventional nutrients, but it also contains bioactive peptides and a distinctive composition of fatty acids. These components have garnered significant attention in scientific research due to their potential positive effects on human health [1, 2].

Bioactive peptides are specific segments of proteins that have advantageous effects on different physiological functions or states, potentially influencing overall wellbeing. The peptides are encoded within the primary structure of the parent protein and can potentially be released through various mechanisms, such as digestion or food processing. Peptide formation is a prevalent process observed in cheese production, primarily facilitated by fermentation and maturation. Recent studies have provided evidence suggesting that bioactive peptides can significantly impact health conditions such as obesity and hyperlipidemia [3, 4].

In contrast, the fatty acid composition in cheese is primarily determined by factors such as the origin of the milk, the feeding habits of dairy animals, and the methodologies utilized during the cheese manufacturing processes. The association of various health benefits with fatty acids, particularly unsaturated ones, has been documented [5, 6].

In addition to these naturally occurring compounds, innovative strategies are being developed to preserve cheese's bioactive peptide content and fatty acid profile. One such strategy is the use of bioactive edible films, which have shown promise in improving the microbial quality of cheese and reducing lipid oxidation, thereby extending its shelf life [7, 8]. Another promising approach is the encapsulation of bioactive compounds, a technique that has been used to improve the stability and functionality of these compounds [9].

The cheese's bioactive peptides are influenced by the cheese-making process and the type of milk it produces. An investigation on goat milk cheese revealed a higher concentration of bioactive peptides than cow milk cheese [10]. Moreover, certain probiotic strains in cheese-making have been shown to enhance the bioactive peptide content. A study on cheddar cheese showed that using *Lactobacillus casei* 300 resulted in the formation of peptides with antihypertensive activity [11]. Moreover, the utilization of specific enzymes, such as proteases, in the cheese production process has demonstrated the ability to augment the presence of bioactive peptides. The investigation conducted on Gouda cheese demonstrated that utilizing proteases derived from Lactobacillus helveticus led to the production of peptides exhibiting antihypertensive properties [12]. In addition, a study on blue cheese showed that the ripening process led to the formation of peptides with antioxidant activity [13]. Furthermore, a study on Camembert cheese showed that the ripening process led to the formation of peptides with antithrombotic activity [14]. Finally, a study on Roquefort cheese showed that the ripening process led to the formation of peptides with anti-inflammatory activity [15].

For cheese processing, reducing salt content in cheese through methods such as decreasing the brine soaking time has not impacted the bioactive peptide formation or fatty acid bioaccessibility in cheese [15]. The ripening process of cheese also plays a significant role in forming bioactive peptides. For instance, a study on Mexican goat cheese showed that the ripening process led to the formation of peptides with anti-oxidant activity [13]. Furthermore, an investigation conducted on Dutch-style cheese revealed that the presence of fatty acids and conjugated fatty acids not only plays a role in the overall nutritional profile of cheese but also, when combined with chemometric techniques, can serve as chemical biomarkers for evaluating the source and maturation status of cheeses, as well as verifying their authenticity [8]. Additionally, a research investigation on Parmigiano–Reggiano cheese revealed that the defatting procedure could retain the entire cheese's nutritional characteristics, encompassing bioactive peptides. This was accomplished through high-performance liquid chromatography (HPLC) [16].

The incorporation of plant-based diets, such as flaxseed, in dairy animals has been demonstrated to enhance the nutritional profile of cheese [10, 11]. Flaxseed is recognized as a substantial reservoir of  $\alpha$ -linolenic acid, classified as an omega-3 fatty acid, and widely acknowledged for its advantageous impact on cardiovascular well-being. Research has indicated that the inclusion of flaxseed in the diet of dairy animals results in an elevated presence of omega-3 fatty acids in their milk. Consequently,

this dietary modification contributes to the resulting cheese's enhanced fatty acid composition [10, 11].

Similarly, certain forages in dairy animals' diets can enhance cheese's bioactive peptide content. A notable example is sulla, a leguminous forage, which has been shown to increase the bioactive peptide content in cheese [17]. Bioactive peptides refer to fragments of proteins that can positively impact bodily functions or conditions, potentially influencing overall health. Moreover, the germination of certain seeds, such as black soybeans, has been found to enhance their anti-Alzheimer activity [14]. This suggests a potential application in cheese production, where these germinated seeds could be used as an ingredient or supplement. The resultant cheese could have enhanced neuroprotective properties, offering a novel approach to preventing or managing neurodegenerative diseases like Alzheimer's. Later, dairy animals' diet and specific ingredients or supplements can significantly influence cheese's nutritional and bioactive properties. This opens up exciting possibilities for the production of functionally enhanced cheese products, contributing to the field of functional foods.

This chapter aims to comprehensively review recent research developments on cheese's bioactive peptides and fatty acid profiles. It will delve into their healthpromoting potential, the factors influencing their content, and the innovative strategies for enhancing their presence and functionality in cheese. The chapter is designed to be accessible to readers from various fields, not just those who are dairy science or nutrition experts.

### 2. Bioactive peptides in cheese

In the realm of functional foods, cheese holds a unique position due to its rich content of bioactive peptides and specific fatty acid profiles. This section delves into the intricate processes that lead to the generation of these bioactive peptides during cheese production and the subsequent health benefits they confer.

#### 2.1 Generation of bioactive peptides in cheese

Producing bioactive peptides in cheese is a complex procedure that necessitates a comprehensive comprehension of the biochemical and microbiological mechanisms at play for its thorough analysis. Hence, acquiring a thorough comprehension of these factors can facilitate the optimization of the proteolysis process and augment the production of bioactive peptides. These peptides are primarily produced due to gastrointestinal digestion, a multifaceted physiological process characterized by the enzymatic degradation of proteins into smaller peptides and amino acids. Simultaneously, the process of milk processing, which involves pasteurization and homogenization, has the potential to generate these bioactive peptides. Moreover, enzymatic hydrolysis, which involves the enzymatic degradation of proteins into smaller peptides, constitutes another noteworthy factor. Indeed, many digestive enzymes, including pepsin, trypsin, and chymotrypsin, are responsible for the cleavage of proteins at specific locations. Microbial fermentation, a biological process in which microorganisms such as bacteria and yeast metabolize organic substances, frequently produces peptides. The various processes, both independently and in combination, play a significant role in producing bioactive peptides. These peptides have garnered considerable attention due to their potential to promote health (Figure 1).



Figure 1. Production mechanisms of bioactive peptides [18].

The enzymatic degradation of proteins by starter cultures plays a crucial role in this process [19]. Nevertheless, it is crucial to consider the involvement of nonstarter cultures, as they can play a significant role in the proteolytic activity throughout the ripening phase and influence the cheese's ultimate peptide composition. Within the given framework, nonstarter cultures, called secondary or adjunct cultures, encompass bacteria deliberately introduced into the milk used for cheese production alongside the primary starter cultures. Nevertheless, their contribution to the acidification of the cheese curd, the primary function of starter cultures, is not substantial. In contrast, cultures that do not initiate fermentation play a significant role in shaping cheese's flavor, texture, and various attributes as it undergoes the ripening phase. Nonstarter cultures encompass various microorganisms, including lactobacilli, propionibacteria, and specific fungal species. These organisms can synthesize enzymes that catalyze the hydrolysis of proteins, fats, and carbohydrates in cheese, forming flavor compounds and other substances, such as bioactive peptides [20].

The research conducted by Kurbanova et al. offers significant insights into the contribution of distinct starter cultures in producing bioactive peptides [21]. However, it would be interesting to investigate further the potential influence of the interaction between various starter and nonstarter cultures on the peptide profile. For example, what impact would the inclusion of additional strains of lactobacilli or other bacterial species have on the production of bioactive peptides? Furthermore, it would be advantageous to investigate the impact of fermentation conditions, including temperature and duration, on the efficacy of these cultures and the consequent production of peptides.

The significance of proteolysis in milk conversion into cheese, as emphasized by Lepilkina and Grigorieva [22], is undeniably pivotal. Nevertheless, it is important to acknowledge that proteolysis is a multifaceted phenomenon that can be affected by various factors, such as the specific enzymes utilized, the properties of the milk proteins, and the parameters of the cheese production procedure. Hence, acquiring a thorough comprehension of these factors can facilitate the optimization of the proteolysis process and augment the production of bioactive peptides.

The research conducted by Helal et al. [23] offers a noteworthy viewpoint on the temporal aspects of proteolysis and the production of bioactive peptides in whey fermentation. However, further investigation into optimizing the fermentation regime is warranted to maximize the production of specific bioactive peptides. For example, could an extended duration of fermentation lead to an increased concentration of specific peptides? Alternatively, could it result in the deterioration of these peptides and the formation of additional compounds?

The study conducted by Araújo-Rodrigues et al. highlights the potential of utilizing autochthonous starter cultures to improve cheese's sensory attributes and safety characteristics [24]. Nevertheless, it is crucial to consider the potential obstacles linked to this methodology, including the fluctuation in microbial populations within unpasteurized milk and the possible impact of environmental factors on the functionality of these microorganisms.

Furthermore, it is crucial to emphasize the research conducted by Sturova et al. [25], which involved the development of a distinctive approach to cheese maturation. This method involved the utilization of a noble mold derived from a combination of whole milk and secondary protein-carbohydrate raw materials. Their research findings indicated that the proteolysis and lipolysis processes exhibited higher-intensity levels in the experimental cheeses manufactured using a noble mold. Consequently, the final product displayed enhanced organoleptic qualities. Utilizing this methodology can potentially augment the production of bioactive peptides within the context of cheese manufacturing.

In addition, Samelis et al. conducted a study to assess the efficacy of a blended thermophilic and mesophilic starter culture comprising *Streptococcus thermophilus* ST1 and the Greek autochthonous nisin-A-producing *Lactococcus lactis* in the production of the traditional Galotyri Protected Designation of Origin (PDO) cheese [26]. According to their study, it has been indicated that the distinctive characteristics of cheese, including its fermentation techniques and the type of milk employed, have the potential to affect the behavior of bacteria and the subsequent production of bioactive peptides.

In addition, a research study conducted by Moiseenko et al. examined the capacity of *Lacticaseibacillus paracasei* strains, which were obtained from the traditional South African fermented beverage mahewu and kefir grains, to generate bioactive peptides possessing antioxidant and angiotensin I-converting enzyme inhibitory (ACE-I) characteristics during the process of milk fermentation [27]. The current research study provides valuable insights into the exploration of unconventional isolation sources for synthesizing bioactive peptides in the domain of cheese production. To summarize, the generation of bioactive peptides in cheese is a complex occurrence that is influenced by various factors, including the use of diverse starter and nonstarter cultures, the intricacies of the fermentation process, and the unique characteristics specific to the type of cheese being produced. In order to optimize the generation of bioactive peptides and enhance the health-promoting properties of cheese, future investigations should prioritize a thorough analysis of these factors.

In summary, the generation of bioactive peptides in cheese is a complex process influenced by various factors, including the use of diverse starter and nonstarter cultures, the intricacies of the fermentation process, and the unique characteristics specific to the type of cheese being produced. Understanding these factors can help optimize the proteolysis process and enhance the production of bioactive peptides.

Transitioning to the health benefits of these peptides, it is important to note that the bioactive peptides produced in cheese have been associated with various healthpromoting effects.

### 2.2 Health-promoting effects of bioactive peptides in cheese

Bioactive peptides in cheese have been associated with various health benefits. These peptides are thought to exert their beneficial effects through various mechanisms, including inhibiting enzymes involved in disease processes, modulation of the immune system, and scavenging free radicals (**Table 1**). These studies present supplementary evidence to substantiate that cheese can produce bioactive peptides that confer various health benefits, including antithrombotic and antidiabetic properties.

In a recent study conducted by Helal et al. [23], the researchers examined six distinct types of cheese to assess the concentrations of bioactive peptides and their corresponding physiological effects. The cheeses examined in this study consisted of three distinct types of Egyptian cheese, namely Karish, Domiati, and Ras, and three internationally popular cheese varieties, including Feta-type, Gouda, and Edam. The study's results revealed that a significant portion of the bioactive peptides identified exhibited inhibitory effects on crucial enzymes associated with the advancement of cardiovascular diseases, specifically angiotensin-converting enzyme (ACE), as well as diabetes, particularly dipeptidyl peptidase-IV (DPP-IV). It is worth mentioning that Gouda cheese exhibited the most significant ACE inhibitory and DPP-IV-inhibitory activities, in addition to displaying the highest level of antioxidant activity. Further investigation has clarified the involvement of bioactive peptides in the composition of cheese and other edible substances. The study conducted by Helal et al. [28] contributes additional knowledge to the ongoing scholarly conversation regarding synthesizing bioactive peptides in cheese. The primary objective of this study was to investigate the effects of spontaneous fermentation and the inclusion of natural whey starters on the peptidomics profile and biological activities of cheese whey. The study revealed spontaneous fermentation incorporating natural whey starters considerably deteriorated whey proteins. The breakdown process facilitated the subsequent release of peptides with biological activity and enhanced the digestibility of the protein content. The researchers conducted an investigation involving identifying more than four hundred peptides. The peptides being examined were primarily derived from  $\beta$ -casein,  $\kappa$ -casein, and  $\alpha$ -lactalbumin. Out of the identified peptides, a collective count of 49 exhibited bioactive characteristics, with 21 of these peptides demonstrating inhibitory effects on the angiotensin-converting enzyme (ACE).

 Cheese type	Bioactive peptides	Bioactivities	References	
Cheddar	$\alpha_{S1}\text{-}$ and $\beta\text{-}CN$ fragments	Angiotensin-converting enzyme (ACE) inhibitory Antioxidative	[21]	
Mozzarella, Crescenza, Italico, Gouda —	β-CN f(58–72)	ACE inhibitory	[22]	
	α <sub>S1</sub> -CN f(1–9)	ACE inhibitory Cytomodulatory	[23]	
-	β-CN f(60–66)		[24]	
Festivo	α <sub>S1</sub> -CN f(1–9), f(1–7), f(1–6)	ACE inhibitory	[25]	
Emmental	Peptides not identified	ACE inhibitory	[18]	
Manchego	Ovine $\alpha_{S1}$ -, $\alpha_{S2}$ - and $\beta$ -CN	ACE inhibitory	[26]	

#### Table 1.

Biologically active peptides isolated from several cheeses.

Moreover, the study additionally revealed that the lactotripeptide isoleucineproline-proline (IPP) exhibited higher levels compared to valine-proline-proline (VPP), with the most significant concentration observed during the period of spontaneous fermentation after 24 hours. The results of this study indicate that the fermentation process, regardless of whether it occurs spontaneously or is initiated using natural whey starters, plays a significant role in producing bioactive peptides in cheese. Rehman et al. [29] conducted an independent investigation to evaluate the therapeutic efficacy of water-soluble peptides (WSPs) derived from probiotic cheddar cheese produced from buffalo milk. The study focused specifically on the antithrombotic properties of these peptides [29]. The study's results demonstrate a significant increase in antithrombotic activity as the ripening period progressed for both the control and probiotic cheddar cheese samples.

Incorporating a probiotic adjunct in cheddar cheese production led to a significant improvement in its antithrombotic activity. Pontonio et al. conducted an independent study to increase the worth of whey obtained from ricotta cheese. Their approach involved the development of a biotechnological method for generating bioactive peptides that demonstrate inhibitory properties against angiotensin-Iconverting enzyme (ACE) [30]. The study utilized a methodology that incorporated the combination of membrane filtration and fermentation techniques. The fermented R-UF demonstrated a notable anti-angiotensin-converting enzyme (ACE) activity. The purified active fractions of the fermented R-UF displayed sequences that partially or fully coincided with  $\kappa$ -case antihypertensive fragments that have been previously reported. The ricotta cheese, which was enriched with a fortification level of 5%, demonstrated a concentration of approximately 30 mg of bioactive peptides. The investigation conducted by Vázquez-García et al. involved the assessment of peptide fractions found in Mexican goat cheeses during different ripening periods [13]. An examination was conducted to explore the correlation between the peptide fractions and the antioxidative properties demonstrated by the cheeses [13]. The study findings indicate that the observed DPPH radical scavenging activity in ripened Mexican goat cheese can be attributed to the peptides that naturally occur in the milk or are produced as a result of the action of starter cultures during the cheese ripening process.

In addition, Martini et al. undertook a research investigation aimed at assessing the impact of different stages of ripening in Parmigiano-Reggiano (PR) cheese peptide fractions on the enzymatic activity of  $\alpha$ -glucosidase,  $\alpha$ -amylase, and dipeptidyl peptidase-IV (DPP-IV), as well as the formation of fluorescent advanced glycation end-products (fAGEs) [31]. The PR peptide fractions exhibited inhibitory properties against the specific enzymes and the formation of fAGEs. These studies present supplementary evidence to substantiate that cheese can produce bioactive peptides that confer various health benefits, including antithrombotic and antidiabetic properties. Bioactive peptides demonstrate diverse pharmacological characteristics, encompassing opioid activity, anti-tumor effects, anti-lipidemic properties, and immunomodulatory activities. As depicted by Shafique et al. [32], cheddar cheese-derived bioactive peptides have the potential as functional foods, affecting chronic diseases like obesity, cardiovascular, and diabetes. These peptides have multifunctional therapeutic potentials, including antimicrobial, immunomodulatory, antioxidant, enzyme inhibitory, antithrombotic, and phytopathological effects. They regulate immune, gastrointestinal, hormonal, and neurological responses, which are crucial in disease prevention and treatment. Figure 2 illustrates the depiction of the action mechanism of bioactive peptides.

Recent Trends on Cheese as Functional Food with Great Nutritive and Health Benefits



Figure 2. Action mechanism of bioactive peptides [32].

In summary, bioactive peptides in cheese have been linked to a range of health benefits, including antithrombotic and antidiabetic properties. These peptides have multifunctional therapeutic potentials, including antimicrobial, immunomodulatory, antioxidant, enzyme inhibitory, antithrombotic, and phytopathological effects. They regulate immune, gastrointestinal, hormonal, and neurological responses, which are crucial in disease prevention and treatment.

In the next section, we will delve into the fatty acid profile of cheese and how it contributes to the overall nutritional value and health benefits of this popular dairy product.

## 3. Fatty acid profile in cheese and its health implications

The fatty acid composition of cheese is primarily influenced by the origin of the milk, as well as the feeding practices of dairy animals and the methods employed during the cheese manufacturing process. The consumption of unsaturated fatty acids has been linked to numerous health advantages. Gas chromatography (GC) is a widely recognized analytical method that provides high precision and accuracy in the quantification of fatty acid composition in cheese. The findings are commonly reported in milligrams of fatty acids per gram of cheese (mg FA/g cheese), as illustrated in **Table 2**. The obtained data comprehensively analyze the fatty acid composition of the cheese, encompassing saturated, monounsaturated, and polyunsaturated fatty acids. The provision of this information is of utmost importance in comprehending the nutritional characteristics of cheese, as distinct fatty acids exert varying impacts on human well-being. For instance, it is widely acknowledged in the academic literature

Cheese's Bioactive Peptide Content and Fatty Acids Profile	е
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	Samples of cheese
C8:0	4.49 ± 1.55
C9:0	0.04 ± 0.07
C10:0	14.97 ± 4.97
C11:0	0.13 ± 0.05
C12:0	8.04 ± 1.90
C13:0	0.18 ± 0.08
C14:1	0.96 ± 0.56
C14:0	23.07 ± 4.69
C15:0	1.35 ± 0.61
C16:1	2.50 ± 0.67
C16:0	73.75 ± 19.02
C17:1	0.50 ± 0.21
C17:0	0.96 ± 0.33
C18:2	6.15 ± 1.71
C18:1 cis	38.62 ± 7.93
C18:1 trans	5.57 ± 3.95
C18:1 total	50.40 ± 11.29
C18:0	27.36 ± 7.38
C19:0	0.06 ± 0.08
C20:0	0.29 ± 0.14
∑SFA	154.69
$\sum$ UFA	54.35

#### Table 2.

Cheese's fatty acid profiles (mg FA/g cheese) as determined by gas chromatography [33].

that monounsaturated and polyunsaturated fatty acids are generally regarded as advantageous for maintaining cardiovascular health.

The fatty acid composition of cheese exhibited notable variations throughout the production season, potentially influenced by many factors, including the grazing patterns of cows, moisture content in their feed, ambient temperature, lactation stage, and the overall health condition of the animals [34].

Recent studies have investigated dietary factors' influence on dairy products' fatty acid composition. For example, a research investigation on Kivircik ewes revealed that the incorporation of 3% palm oil into their dietary regimen resulted in a notable elevation in the concentration of palmitic acid in their milk, escalating from 28% to 36% [35]. The observed rise in palmitic acid concentration in the milk corresponds to a 29% increase, assuming that the palm oil utilized contained 80% palmitic acid. The research study additionally demonstrated that the incorporation of palm oil into the dietary regimen resulted in elevated levels of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), primarily attributable to heightened concentrations of palmitic acid (C16:0) and oleic acid (C18:1), respectively.

The ramifications for human health resulting from alterations in fatty acid composition are multifaceted and intricate. Based on a specific perspective, there exists evidence indicating a potential association between certain saturated fatty acids, namely lauric (C12:0) and myristic (C14:0) acids, and a decreased risk of developing coronary heart disease [36]. On the other hand, incorporating palm oil into one's dietary intake leads to an elevation in the collective levels of saturated fatty acids, which have been established to have adverse effects on human well-being [37].

Their fatty acid profile determines the nutritional quality of dairy products, and various indices are employed to evaluate the diet's nutritional value and its impact on consumer health. The research presented empirical findings that the inclusion of 3% rumen-protected palm oil in the dietary regimen enhanced ewe milk's health characteristics, particularly in relation to the optimal levels of desirable fatty acids (DFA) for the n-6/n-3 ratio. Despite the lack of observed improvement in the atherogenicity index (AI), thrombogenicity index (TI), health-promoting index (HPI), and h/H (hypocholesterolemic/hypercholesterolemic acids) indices, the values obtained in this study align with those documented in prior scholarly works. This discovery suggests that the milk examined in the current investigation does not harm consumers' health [35].

The research conducted by Zajác et al. examined the fatty acid composition of traditional Slovak cow's lump cheese, as depicted in **Figure 3** [38]. The cheese, which a nearby local farmer manufactures, exhibited diverse levels of fatty acids. The primary fatty acids identified in cow's lump cheese were palmitic acid (C16:0) with a concentration range of 29–37 g/100 g, oleic acid (C18:1) (n-9) with a concentration range of 19–26 g/100 g, myristic acid (C14:0) with a concentration range of 10–13 g/100 g, and stearic acid (C18:0) with a concentration range of 8–10 g/100 g. The investigation recorded noteworthy alterations in the concentrations of particular fatty acids throughout the production duration. The research determined that the fat content of cow's lump cheese was 23%, with a standard deviation of ±1.5%. The lowest observed fat content was 21%, whereas the highest recorded fat content was 27%. The cheese



#### Figure 3.

Cow's lump cheese combines saturated, monounsaturated, and polyunsaturated fatty acids [38].

sample exhibited a composition of 70 g/100 g for saturated fatty acids, 26 g/100 g for monounsaturated fatty acids, and 3 g/100 g for polyunsaturated fatty acids.

The sensory attributes of cheese, including color, texture, flavor, and nutritional value, are notably impacted by the composition of fatty acids in the cheese. Various types of fatty acids have the potential to impact human health, with certain ones offering benefits while others may pose potential risks. The fatty acid composition can also function as a discriminative characteristic for different types of cheese, depending on the milk utilized during their manufacturing process. In their investigation, González-Martín et al. utilized Near-Infrared Spectroscopy (NIRS) technology to determine the presence of 19 fatty acids in cheese, ranging from C8:0 to C20:0. The evaluation involved the analysis of both the aggregate amount of saturated fatty acids ( $\Sigma$ UFA) and the aggregate amount of unsaturated fatty acids ( $\Sigma$ UFA). The research study substantiated the feasibility of Near-Infrared Spectroscopy (NIRS) as a prompt, dependable, and effective technique for obtaining data on the lipid composition of cheese samples [33].

Furthermore, it is important to exercise caution when interpreting the use efficiencies of diet ingredients in production systems that employ varying ingredient inclusion rates. The organic production systems demonstrated higher efficiency in nongrazing and concentrated utilization, underscoring the significance of incorporating pasture in cows' diets. This approach reduces the reliance on costly ingredients that compete with human food sources and presents opportunities to enhance profitability and sustainability within the system by promoting greater pasture intake. The existing data do not provide sufficient evidence to support the notion that incorporating higher levels of concentrate or nonpasture feeds in the diets of organic cows is justified. This is because the data does not demonstrate that organic herds possess a greater capacity to utilize concentrate or nonpasture ingredients more effectively than conventional herds.

The current study's findings suggest that organic herds demonstrated a reduced occurrence of mastitis cases, expressed as a proportion of the overall herd, compared to conventional herds. This observation is consistent with the findings documented by Ellis et al. in their study [39]. In contrast, Stergiadis et al. [40] observed no significant variation in mastitis occurrences across herds with varying levels of production intensity, ranging from organic to highly intensive. Nevertheless, in terms of numerical data, it was observed that the higher-intensity systems exhibited a greater incidence of mastitis cases, even in the presence of preventive antibiotic measures. Nevertheless, the Redundancy Analysis (RDA), as determined in the present study, aligns with the findings of Stergiadis et al., as it reveals the presence of adverse correlations between grazing practices and incidences of mastitis [40]. Hence, the elevated levels of pasture consumption within organic systems may have limited significance for mastitis. In their study, Ellis et al. [39] and Ward et al. observed a correlation between enhanced cow cleanliness on farms and decreased mastitis cases and somatic cell count (SCC). However, the authors reported no significant disparity in cow cleanliness during outdoor grazing between organic and conventional systems [41]. The study provides no documentation regarding the cleaning and milking strategies employed.

Consequently, it is not feasible to make any assertions regarding the potential influence of these strategies on mastitis cases. Nevertheless, previous research has established a correlation between genetic selection for a high milk yield, particularly observed in the Holstein breed, which was more prevalently utilized in the conventional herds examined in this current study, and somatic cell count (SCC) levels,

consequently leading to mastitis [42]. The present study observed that the organic herds exhibited reduced milk yields and somatic cell counts (SCC), alongside a greater proportion of lower-yielding breeds such as Ayrshire and Shorthorn. This particular breed composition could potentially account for the decreased incidence of mastitis.

Previous research has demonstrated that low-input and organic milk display reduced levels of saturated fatty acids (SFA) compared to high-intensity and conventional milk [43, 44]. In contrast to prior research, the current study reveals divergent outcomes. Prior studies have demonstrated a reduced concentration of saturated fatty acids (SFAs) in organic milk compared to milk derived from intensively managed herds [45]. The observed disparity has been ascribed to the elevated intake of fresh herbage within organic farming systems. However, no notable differences were observed between the organic and conventional systems regarding pasture intake. The variation in intake was minimal, measuring 123 g/kg dry matter (DM) [46]. The present study reveals a comparable pattern, wherein the disparity in the proportion of total forage and pasture between the conventional and organic herds was found to be less than 131 g/kg and 166 g/kg DM per day, respectively. The results derived from the RDA demonstrate a significant association between the consumption of whole crops and grass silage and the saturated fatty acid (SFA) content of milk compared to grazing. As mentioned earlier, the observation is supported by the research conducted by Ellis et al., wherein their investigation revealed that incorporating whole crops and grass silage into the diet increased saturated fatty acids (SFA) levels in milk [47].

Furthermore, Ormston et al. [48] discovered a positive correlation between saturated fatty acid (SFA) consumption and grass and maize silage intake. The observed disparity in organic diets, with average values of 778 g/kg DM and 920 g/kg DM for whole crop and grass silage, respectively, compared to conventional diets, may have played a significant role in the elevated levels of saturated fatty acids (SFA) found in organic milk during the present investigation. Furthermore, substantial evidence supports that breed is crucial in determining the fatty acid (FA) profile. This is evident from studies that have observed lower concentrations of saturated fatty acids (SFA) in Holstein-Friesian cows' milk than in other breeds [48, 49]. The present study's findings also demonstrated a positive association between certain individual fatty acids (C6:0, C8:0, C10:0, C12:0, and C14:0) and non-Holstein breeds, as indicated by the results obtained from RDA. However, the disparities in the breed systems were not found to be statistically significant. Conventional herds exhibited a slight numerical advantage of approximately 14 Holstein cows per 100 cows compared to crossbreeds and organic herds. The observed variation in the composition of the herd may have played a role in the observed rise in the levels of specific saturated fatty acids (SFAs) in the milk, including C6:0, C8:0, and C14:0. It is worth mentioning that the somatic cell count (SCC) in organic milk exhibited a higher value exclusively during September to December, a period characterized by minimal or zero pasture intake in both agricultural systems. This finding is consistent with the research conducted by Butler et al. [43], which observed a higher level of saturated fatty acids (SFA) in milk from organic farms compared to nonorganic low-input farms during the indoor period in August and October.

In summary, the fatty acid composition of cheese has complex and multifaceted implications for human health. While certain saturated fatty acids have been linked to an increased risk of cardiovascular disease, other fatty acids, such as monounsaturated and polyunsaturated fatty acids are generally regarded as beneficial for cardiovascular health. The fatty acid profile of cheese can also be influenced by

various factors, including the grazing patterns of cows, the moisture content in their feed, ambient temperature, lactation stage, and the overall health condition of the animals. Therefore, understanding these factors and their impact on the fatty acid profile of cheese is crucial for optimizing the health benefits of cheese consumption.

In the following section, we will delve into the innovative strategies aimed at enhancing the health benefits of cheese consumption. Specifically, we will focus on methods designed to increase the production of bioactive peptides and optimize the fatty acid profile in cheese, as these components play a crucial role in promoting health and preventing disease.

## 4. Innovative strategies for enhancing bioactive peptides and optimizing fatty acid profile in cheese

In conjunction with naturally existing compounds, novel methodologies are being devised to augment the bioactive peptide composition and optimize the fatty acid configuration in cheese. One strategy that has been explored is the utilization of bioactive edible films. These films have demonstrated the potential to enhance the microbial characteristics of cheese and mitigate lipid oxidation, consequently prolonging its shelf life [50, 51]. In cheese preservation, Aloe-based bioactive edible films have been observed to enhance the cheese's lipid stability and microbial quality (**Figure 4**). *Aloe vera*, a botanical species widely recognized for its therapeutic attributes, encompasses diverse bioactive constituents, such as polysaccharides, vitamins, enzymes, and antioxidants. Including these bioactive compounds in an edible film can provide advantages to the packaged food item [7]. The Aloe film has been found to contain bioactive compounds that can inhibit lipid oxidation, which significantly contributes to the degradation of cheese quality [7].

Consequently, lipids' stability is improved, maintaining the cheese's sensory attributes and nutritional composition throughout its shelf life. In addition, the antimicrobial properties of *Aloe vera* have been found to contribute to the preservation of the microbial quality of cheese [7]. The bioactive compounds present in the Aloe film exhibit inhibitory effects on spoilage microorganisms and pathogens, thereby effectively prolonging the cheese's shelf life and ensuring its safety for consumption.

Encapsulation of bioactive compounds is a technique employed to improve the stability and functionality of these compounds [52], presenting itself as a promising method. Pop et al. [53] thoroughly examined the encapsulation process for *Moringa oleifera* bioactive compounds in their study. The authors emphasized the significant potential of encapsulation techniques in facilitating the incorporation of bioactive molecules into various food products.



Figure 4.

A bioactive edible film derived from Aloe improved the lipid stability and microbial quality of cheese [7].

Furthermore, the utilization of whey proteins in cheese manufacturing is gaining traction owing to their advantageous effects on human health. Whey proteins are a significant provider of essential amino acids, including leucine, isoleucine, and valine, classified as branched-chain amino acids [50]. Rapidly digestible proteins offer older individuals enhanced nutritional advantages compared to casein and other protein sources. As a result, their extensive integration into clinical nutritional products has been observed [50].

Nevertheless, the general reception of whey protein-fortified products among consumers tends to be low owing to unfavorable flavor and aroma characteristics and negative mouth-feel attributes. These include the accumulation of mouthdrying, mouth-coating, chalky, metallic, and filming sensations associated with repeated consumption, as indicated by previous research [50]. Hence, further investigation is necessary to improve the sensory characteristics of these products, thereby augmenting consumer acceptance and appropriateness. Concerning the composition of fatty acids, the utilization of distinct starter cultures and adjuncts has the potential to yield cheese products that exhibit an elevated content of conjugated linoleic acid (CLA) and omega-3 fatty acids, which are known to possess health-promoting properties [45].

Furthermore, it should be noted that specific processing techniques, such as highpressure processing (HPP), have the potential to modify the fatty acid composition of cheese. The study conducted by Inácio et al. [54] proved that subjecting cheddar cheese to high-pressure processing (HPP) resulted in an elevation of unsaturated fatty acid levels, particularly oleic acid.

In summary, pursuing novel approaches to augment the bioactive peptide composition and optimize the fatty acid makeup in cheese represents a vibrant and auspicious realm of scholarly investigation. The abovementioned strategies are designed to enhance cheese's nutritional composition, prolong its shelf life, and enhance its sensory characteristics.

## 5. Conclusions

Exploring cheese as a source of bioactive peptides and fatty acids has opened new avenues in functional foods. This chapter has provided a comprehensive overview of the current knowledge and recent advancements in this area. Bioactive peptides generated during cheese fermentation and maturation have been associated with various health benefits, including managing obesity and hyperlipidemia. They exert their beneficial effects through various mechanisms, such as inhibiting enzymes involved in disease processes, modulating the immune system, and scavenging free radicals. The content of these peptides in cheese is influenced by several factors, including the type of milk used, the cheese-making process, and the specific strains of bacteria involved in fermentation.

The fatty acid profile of cheese, particularly the unsaturated fatty acids, also plays a crucial role in human health. The type of milk used, the diet of the dairy animals, and the cheese-making processes largely determine this profile. Recent research has shown that specific starter cultures and adjuncts can produce cheese with a higher content of health-promoting fatty acids, such as conjugated linoleic acid (CLA) and omega-3 fatty acids.

Innovative strategies are being developed to enhance the bioactive peptide content and optimize the fatty acid profile in cheese. These include the use of bioactive edible

films and the encapsulation of bioactive compounds, which have shown promise in improving the microbial quality of cheese, reducing lipid oxidation, extending its shelf life, and improving the stability and functionality of these compounds.

In conclusion, cheese, a staple food item consumed worldwide, holds significant potential as a source of health-promoting bioactive peptides and fatty acids. The ongoing research and development in this field are expected to produce cheese varieties with enhanced health benefits, thereby contributing to the growing functional foods market. However, further research is needed to fully understand these bioactive compounds' mechanisms of action and optimize the cheese-making processes for their production. The potential of these findings extends beyond the cheese industry, offering insights that could be applied to the broader field of food science and nutrition.

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## **Conflict of interest**

The authors declare no conflict of interest.



Ilyes Dammak<sup>1\*</sup> and Carlos A. Conte-Junior<sup>2</sup>

1 University of Kairouan, Tunisia

2 Federal University of Rio de Janeiro, Brazil

\*Address all correspondence to: dammakilyes@hotmail.fr

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