

Micro-organismos psicrotróficos em leite cru e a qualidade de queijos
Psychrotrophic microorganisms in raw milk and the cheese quality
Microorganismos psicrotróficos en la leche cruda y la calidad de los quesos

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Resumo

Os micro-organismos psicrotróficos, com destaque ao gênero *Pseudomonas* spp, compõem a microbiota do leite refrigerado pois são capazes de se multiplicar em temperaturas de refrigeração, independentemente de sua temperatura ótima de crescimento. O leite cru refrigerado com contagens de psicrotróficos entre 10^5 e 10^8 UFC/mL compromete a produção de queijos pois as enzimas termorresistentes sintetizadas prejudicam a qualidade nutricional, sensorial e a textura. No Brasil, dentro do setor de lácteos, o queijo representa o produto com a maior taxa de crescimento nos últimos anos, atendendo atualmente às tendências de alimentos nutritivos e práticos para o consumo. O objetivo desta revisão foi abordar a importância e a influência da microbiota psicrotrófica do leite cru na qualidade e nas características sensoriais dos queijos. As enzimas psicrotróficas promovem mudanças no sabor, tempo de coagulação indesejável de proteínas, aumento da concentração de ácidos gordurosos e aminoácidos livres, limitação da vida útil, bem como efeitos negativos sobre os rendimentos. As proteases psicrotróficas também estão associadas a problemas de fatiamento e perda progressiva da elasticidade dos queijos, gosto amargo e aumento do tempo de coagulação da massa dos queijos produzidos com leite pasteurizado. Já as lipases psicrotróficas conferem defeitos como sabor e odor de ranço, de sabão, metálico ou oxidado aos queijos, além do aumento do tempo de coagulação. O controle da população psicrotrófica do leite cru refrigerado contribui para um melhor rendimento na produção de queijos, textura e características sensoriais desejáveis, o que garante maior vida de prateleira dos queijos e o aceite pelo consumidor.

Palavras-chave: Refrigeração; Enzimas; Boas práticas.

Abstract

Psychrotrophic microorganisms, especially *Pseudomonas* spp., are present in the microbiota of refrigerated milk as they can grow at refrigeration temperatures irrespective of their optimal growth temperature. Psychrotrophic counts ranging from 10^5 to 10^8 CFU/mL in refrigerated raw milk affect cheese quality, since the synthesized thermoresistant enzymes affect the nutritional value, sensory properties, and texture. Cheese is the dairy product with the highest growth rate in the food industry in Brazil in recent years and meets the current consumption trends of nutritious and practical foods. The objective of this review was to address the importance and influence of the psychrotrophic raw milk microbiota on the quality and sensory properties of cheese. The enzymes produced by psychrotrophic microorganisms lead to taste changes, undesirable clotting times, increased concentrations of free fatty acids and free amino acids, and a reduced shelf-life, in addition to negatively affecting cheese yields. Proteases from psychrotrophs are also associated with slicing problems and progressive loss of the elasticity of cheese, a bitter taste, and increased clotting times of cheese produced with pasteurized milk. On the other hand, their lipases increase the clotting time and have a negative effect on the sensory properties by providing a rancid, soap, metallic, or oxidized smell and taste. The control of the psychrotrophic population found in refrigerated raw milk contributes to better cheese production yields and desirable texture and sensory properties, which extends the shelf-life of cheese and improves consumer acceptance.

Keywords: Refrigeration; Enzymes; Good practices.

Resumen

Los microorganismos psicrotróficos, especialmente el género *Pseudomonas* spp, conforman el microbiota de la leche refrigerada porque son capaces de multiplicarse a temperaturas de refrigeración, independientemente de su temperatura de crecimiento óptima. La leche cruda refrigerada con recuentos psicrotróficos entre 10^5 y 10^8 UFC/mL compromete la producción de queso porque las enzimas termorresistentes sintetizadas deterioran la calidad nutricional, sensorial y de textura. En Brasil, dentro del sector lácteo, el queso representa el producto con mayor tasa de crecimiento en los últimos años, actualmente cumpliendo con las tendencias de alimentos nutritivos y prácticos para el consumo. El objetivo de esta revisión fue abordar la importancia y la influencia del microbiota psicrotrófica de la leche cruda en la calidad y las características sensoriales de los quesos. Las enzimas psicrotróficas promueven cambios en el sabor, tiempo de coagulación indeseable de las proteínas, mayor concentración de ácidos grasos y aminoácidos libres, limitación de la vida útil, así como efectos negativos en los

rendimientos. Las proteasas psicrotróficas también se asocian con problemas de corte y pérdida progresiva de elasticidad del queso, sabor amargo y aumento del tiempo de coagulación de los quesos producidos con leche pasteurizada. Las lipasas psicrotróficas, por otro lado, confieren defectos como el sabor y el olor de rancio, jabón, metálico u oxidado a los quesos, además de aumentar el tiempo de coagulación. El control de la población psicrotrófica de la leche cruda refrigerada contribuye a un mejor rendimiento en la producción de quesos, textura y características sensoriales deseables, lo que asegura una mayor vida útil de los quesos y aceptado por el consumidor.

Palabras clave: Refrigeración; Enzimas; Buenas prácticas.

1. Introduction

Milk is a secretory product of mammary glands and a homogeneous aqueous mixture of compounds, such as proteins, carbohydrates, lipids, minerals, vitamins, and enzymes. Milk composition varies among animal species and breeds, but other factors can affect the chemical composition of milk, such as age, lactation stage, genetics, diet, and health (Ordóñez-Pereda et al., 2005; Walstra et al., 2006)

Milk is an excellent growth medium for microorganisms since it has a pH close to neutral, high water activity, and rich nutrient content. Therefore, the larger the population of microorganisms in raw milk, the lower the physical, chemical, microbiological, and sensory qualities of processed fluid milk and dairy products (Singh et al., 2011).

Considering the dairy industry in Brazil, the cheese market has shown the highest growth rate in recent years, with an emphasis on mozzarella, *Prato*, *Minas Frescal*, and curd cheese. The objective of cheesemaking is to extend the shelf-life of milk and conserve its nutritional components that meet the current consumption trends of nutritious and practical foods. In 2019, the production of cheese in Brazil was to amount to 770 thousand metric tons (Mendoza, 2020) and the average per capita consumption stands at 5.1 kg in 2020, including a wide variety of types, flavors, and sizes (Statista, 2020).

To improve the microbiological quality of raw milk and, consequently, its dairy products, the Ministry of Agriculture, Livestock, and Food Supply (MAPA) established, through Normative Instruction No. 769 (Brasil, 2018), that freshly retrieved milk should be refrigerated (4°C) in the dairy farm for a maximum period of three hours. The objective of this refrigeration is to control the multiplication of mesophilic bacteria that ferment lactose and

cause acidity. However, this practice promotes the multiplication of psychrotrophic microorganisms.

From a technological perspective, the control of psychrotrophic microorganisms is of great importance for the dairy industry, since, following the contamination of raw milk during or immediately after milking, psychrotrophs can grow at 7°C or lower, irrespective of their optimal growth temperature (Costa et al., 2002; Moyer & Morita, 2007; Jonghe et al., 2011). Psychrotrophic counts ranging from 10⁵ to 10⁸ CFU/mL in refrigerated raw milk significantly compromise cheese quality (Samarzija et al., 2012) since the thermoresistant enzymes synthesized by this deteriorating microbiota impair the nutritional, sensory, and texture qualities of dairy products (Chen et al., 2003). The enzymes hydrolyze proteins and lipids, thus, reducing the shelf-life of milk and its dairy products. Organoleptic changes, such as bitter and rancid taste, and lower cheese yields are also negative consequences associated with enzymatic activity (Sorhaug & Stepaniak, 1997; Molineri et al., 2012).

Given the above, the objective of this review was to address the importance and influence of psychrotrophic raw milk microbiota on the quality and sensory properties of cheese.

2. Methodology

This paper is a literature review research about psychrotrophic raw milk microbiota and the impact of these microorganisms on physical and sensory characteristics of cheese. It is a qualitative research and were conducted in online databases (Medline, Scielo, PubMed, Lilacs, CAPES Journals), that provided original indexed scientific articles, and in physical consultation sources (scientific articles and books). Were included the articles most relevant to the subject, written in English, Portuguese or Spanish, between 2000 and 2020, as well as classical literatures (scientific articles and books) published before this period (Pereira et al., 2018).

3. Results and Discussion

Milk

The global milk production in 2019 was estimated at 852 million tons, which represents an increase of 1.4% compared to 2019, mainly resulting from production increases

in India, Pakistan, Brazil, the European Union, the Russian Federation and the United States of America (FAO, 2020) In South America, milk production is estimated at 61.8 million tones in 2019, with output expansions in Brazil and Peru.

Milk is a complete food that provides essential nutrients for the human diet and is an important source of protein, fat, calcium, magnesium, selenium, riboflavin, cyanocobalamin, and pantothenic acid (FAO, 2019). However, milk is vulnerable to microbial contamination due to its intrinsic properties, such as high-water activity, pH close to neutral (6.6 to 6.8), and rich nutrient content (Singh et al., 2011).

Milk is sterile following synthesis and secretion in the alveoli of mammary glands (Tolle 1980) but can be contaminated by microorganisms found in the mammary gland, external surface of the udder and teats, and surface of milking equipment and utensils and expansion tanks (Bramley & McKinnon, 1980). Therefore, to guarantee the quality of milk and its dairy products, it is important to collect milk from healthy animals in adequate sanitary conditions, and to immediately refrigerate the milk to 4°C (Arcuri et al., 2006). The health status of mammary glands, sanitary conditions during milking, environment, and cleaning procedures for milking equipment are factors that are directly associated with the contamination of raw milk. In addition to these, other important factors are the temperature and milk storage times, which are related to the multiplication of microorganisms (Bramley & McKinnon, 1980).

The most important deteriorating microorganisms in milk belong to two large groups, the mesophiles and psychrotrophs (Becker et al., 2010). Mesophiles are mostly found in situations of poor sanitary conditions during milking, and in the absence of milk refrigeration grow well at 20°C and 40°C, and can ferment lactose, which is the main sugar in milk, to produce lactic acid, which increases the acidity of milk and causes clotting of casein, thus, separating the milk into whey and curd and compromising the industrial processing and quality of the final products (Mcauley et al., 2016). Psychrotrophs are part of the refrigerated milk microbiota since they can grow at refrigeration temperatures (7°C or below), irrespective of their optimal growth temperature (Frank & Yousef, 2004).

Psychrotrophic microorganisms

Psychrotrophic microorganisms are the main agents causing deterioration of refrigerated raw milk and can multiply through enzymatic activation, which allows cell membranes to maintain structural and functional integrity at low temperatures (Frank &

Yousef, 2004; Samarzija et al., 2012; Oliveira et al., 2015). These bacteria are cocci, bacilli, or vibrio, aerobic or anaerobic, and spore-forming or not (Nornberg et al., 2009). Psychrotrophic microorganisms predominantly have a lipoproteolytic metabolism at temperatures below 10°C by producing thermoresistant extracellular enzymes that influence the shelf-life and quality of milk and its dairy products (Arslan et al., 2011). Therefore, the storage of milk in refrigeration tanks (4°C) and the collection of raw material every 48 hours (Brasil, 2018) promote the multiplication of psychrotrophic bacteria and the synthesis of enzymes (Nornberg et al., 2009).

Most psychrotrophs are Gram-negative microorganisms, such as *Pseudomonas*, *Achromobacter*, *Aeromonas*, *Serratia*, *Alcaligenes*, *Chromobacterium*, and *Flavobacterium*. Gram-positive bacteria, such as *Bacillus*, *Clostridium*, *Corynebacterium*, *Streptococcus*, *Lactobacillus*, and *Microbacterium*, are also present but to a lesser extent (Mcphee & Griffiths, 2011). As these microorganisms originate from the milking environment and equipment (Fagundes et al., 2006; Molineri et al., 2012), good practices can minimize the growth of psychrotrophic agents and the action of enzymes, thus, extending the shelf-life of milk and its dairy products by maintaining the integrity of nutritional components and, consequently, improving industrial performance (Ribeiro Júnior et al., 2018).

Among psychrotrophic bacteria, *Pseudomonas* spp. predominate in refrigerated raw milk since they have great genetic diversity, metabolic versatility, and can efficiently adapt to cold temperatures due to the large amount of unsaturated lipids present in the cell membranes (Cleto et al., 2012; Samarzija et al., 2012; Decimo et al., 2014; Hammad et al, 2015; Neubeck et al., 2015; Oliveira et al, 2015; Al-Rodhan & Nasear, 2016; Xin et al., 2017 a). The enzymes synthesized by *Pseudomonas* spp. in raw milk have 100% hydrolytic activity and can maintain this activity after pasteurization and sterilization, respectively (Sorhaug & Stepaniak, 1997; Bagliniere et al., 2013). The *Pseudomonas* spp. most isolated from milk, the milking environment, and industrial plants are *P. fluorescens*, *P. putida*, *P. fragi*, and *P. aeruginosa* (Wiedmann et al., 2000; Dogan & Boor, 2003; Decimo et al., 2014; Scatamburlo et al., 2015). Some strains of *Pseudomonas* spp. can simultaneously synthesize three types of hydrolytic enzymes (proteases, lipases, and phospholipases), while others only have proteolytic or lipolytic activity (Wiedmann et al, 2000; Arcuri et al., 2008; Decimo et al., 2014). Were related that of the 173 isolates confirmed as *Pseudomonas* spp., 91.38% (159) possessed proteolytic and lipolytic capacities, indicative of their high deterioration abilities (Aguar et al., 2019).

Brazilian legislation does not establish a standard of identity and quality for the enumeration of psychrotrophic microorganisms (Brasil, 2018). However, the synthesis of enzymes that cause changes in milk and its dairy products is associated with a high cell density with psychrotrophic bacterial populations ranging from 10^5 to 10^7 CFU/mL (Mahieu, 1991; Muir, 1996; Walstra et al., 2006). It is important to note that differences in extracellular enzymatic activities of different strains are associated with the genetic characteristics of each group (Haryani et al., 2003). In addition to good milking practices, genetic studies that evaluate the strains of *Pseudomonas* spp. present in the milking environment and the origin of these isolates should be considered as tools for better quality and shelf-life of dairy products (Ercolini et al., 2009). Researchers detected an increase in proteolysis after the storage of milk at 2°C, 4°C, and 8°C, even with low counts of psychrotrophic bacteria (Haryani et al., 2003; Wiking et al., 2002). According to Ribeiro et al. (2018), reducing the temperature and storage times of milk with a high initial count of *Pseudomonas* spp. does not affect this population of psychrotrophs over time.

Extracellular enzymes from psychrotrophic microorganisms and cheese quality

The production of proteases, lipases, and phospholipases depends on the temperature, growth phase of the microorganism, availability of oxygen, and composition of the medium, while the enzymatic activity depends on the temperature, pH, and substrate concentration (Nuñez & Nuñez, 1983). The production of these enzymes at refrigeration temperatures occurs mainly at the end of the log phase and during the stationary phase of the bacterial growth curve, and greater amounts are synthesized at a temperature below the optimal growth temperature (Mahieu, 1991). Kumaresan et al. (2007) observed that milk storage at 2 °C resulted in lower bacterial growth and lower proteolytic and lipolytic activity.

Enzymes from psychrotrophs lead to taste changes, undesirable clotting of milk proteins, increased concentrations of free fatty acids and free amino acids, reduced shelf-life of dairy products, and negative effects on yield (Cempírková et al., 2009). The activity of psychrotrophs in raw milk has an impact on the dairy industry, aroma, quality, and curd yield (Chambers, 2002). Sensory defects, such as rancidity, soap flavor, undesirable bitter flavors, fish odor, and fruity and putrid flavors have been reported in dairy products and are associated with enzymes produced by psychrotrophs (Cousin, 1982; Arcuri et al., 2008; Jonghe et al., 2011).

Milk proteins and the action of proteases from psychrotrophs

Milk proteins are composed of 19 different types of amino acids, have high nutritional value, are found in milk in a colloidal dispersion state, and, on average, account for 3.5% of total milk solids. The proteins can be classified into two distinct groups: casein that represents approximately 80% of total proteins and whey proteins that represent 20% (Robinson, 1990; Goursaud, 1991; Fox & Mcsweeney, 1998; Walstra et al., 2006) Casein is the main protein used for cheese production (De Kruif & Holt, 2003).

Casein is present in milk in a micellar form, is associated with calcium and phosphorus, and is formed by several submicelles, namely α_{S1} -, α_{S2} -, β -, and κ -casein. They are held together by hydrophobic interactions and salt bridges and show different behaviors in the presence of calcium. The α_{S1} , α_{S2} , and β -caseins are more sensitive to high concentrations of calcium salts, while κ -casein, which is located on the surface of the micelle, is stable in the presence of calcium ions and is fundamental for the stability of the micelles (Robinson, 1990; Fox & Mcsweeney, 1998; Walstra, 1999). Enzymatic hydrolysis that occurs during heat treatments at elevated temperatures or cheese processing causes the removal or dissociation of κ -casein, thus, affecting the electrostatic and steric stabilization of the micellar surface and promoting the aggregation of micelles, which leads to clot formation (Fox & McSweeney, 2004).

Milk proteolysis can occur through the action of a natural enzyme, plasmin, or through enzymes produced by psychrotrophs. Plasmin is the main proteolytic enzyme found naturally in fresh milk (Nielsen, 2002). It mainly hydrolyzes β - and α_{S2} -casein and, to a lesser extent, α_{S1} -casein, while κ -casein is resistant to hydrolysis by this enzyme (Recio, Garcia-Risco, Ramos & López-Fandiño, 2000).

Bacterial proteases are predominantly metalloproteinases encoded by the *aprX* gene that act by cleaving the internal peptide bonds of protein chains and preferentially hydrolyzing κ -casein, followed by β - and α_{S1} -casein (Cousin, 1989; Decimo et al., 2014). The optimum pH for the activity of proteases is 7.8 and these enzymes exhibit maximum activity at temperatures ranging from 40°C to 45°C, although they are still active at lower temperatures and pH (Mahieu, 1991). The action of bacterial proteases, especially from *Pseudomonas* spp., is similar to that of chymosin, which is an enzyme used for enzymatic coagulation of milk during cheese production that acts on κ -casein and cleaves the Phe105-Met106 bond, causing destabilization and denaturation of casein micelles (Fox & Mcsweeney, 2004).

The proteases from psychrotrophs are associated with sensory defects and technological problems during cheese production, such as changes in clotting times, reduced yields, slicing problems, and progressive loss of elasticity (Kindstedt & Fox, 1993; Samarzija et al., 2012). The first change caused by the presence of psychrotrophs is the destabilization of the milk plasmin system, and this stimulates the release of plasmin and plasminogen from the casein micelle, which are lost in the serum. Subsequently, this affects the development of cheese flavor and texture since plasmin is an important enzyme in these processes (Samarzija et al., 2012).

The clotting time is reduced in cheese produced from raw milk but the use of pasteurized milk increases the time of this manufacturing step. Clotting time is reduced due to higher concentrations of free amino acids resulting from enzymatic action, which stimulates the growth of starter cultures (Champagne et al., 1994; Samarzija et al., 2012). The clotting time can increase due to protein changes in raw milk by the action of proteases, which makes this component more sensitive to heat treatment. For example, the formation of different complexes, such as β -lactoglobulin and casein, increase the clotting time of milk (Cousin, 1982). Problems with clotting times are also associated with the partial solubilization of β -casein in raw milk, which subsequently tends to leave the casein micelle. Consequently, the casein diameter decreases and the hydration capacity increases, which promotes a more fragile and less compact curd (Samarzija et al., 2012).

The cheese yield can decrease due to the action of bacterial proteases that partially degrade β - and κ -casein and, thus, promote the release of soluble components, such as polypeptides and amino acids, which are lost in the serum (Mcphee & Griffiths, 2011). A bitter taste is an important sensorial defect and occurs due to the presence of low molecular weight peptides (Fox & Mcsweeney, 1998), more pronounced in aged cheeses. Researchers evaluate the influence of the milk's psychrotrophic population in raw milk on smoked provolone cheese and related that the greatest psychrotrophic population of raw milk induced a 3% reduction in cheese yield (Gasparini et al., 2020). The texture is an important characteristic regarding cheese quality and, consequently, consumer acceptance and is directly associated with the intact casein-to-moisture ratio and the pH (Lawrence, 1987). Bacterial proteases reduce cheese elasticity (rate at which the product returns to its original shape when it is compressed between teeth) and hardness (force necessary to compress a solid food between teeth (Szczeniak, 2002). According to Gasparini et al. (2020) an increase in hardness and chewiness parameters in smoked provolone cheese produced with raw milk containing psychrotrophic populations of 7 log cfu/mL presented. These technological defects

are associated with a reduction in the amount of intact α_{s1} -casein, which no longer contributes to the formation of the cheese protein matrix following hydrolysis by bacterial proteases (Ayyash & Shah, 2011).

Milk lipids and the action of bacterial lipases

On average, milk contains approximately 33 g of fat per liter and triacylglycerols account for 95% of this lipid fraction. The other lipids in milk are diacylglycerols (2%), cholesterol (up to 0.5%), phospholipids (about 1%), and free fatty acids (up to 1%) (Walstra et al., 2006; Jensen & Newburg, 1995).

Triglycerides are composed of three fatty acids covalently linked to a glycerol molecule by ester bonds. This fat is secreted by mammary gland cells, is found in the form of globules that are surrounded by a lipid bilayer that promotes stabilization, and forms an emulsion in the milk aqueous phase (Walstra et al., 2006) The membrane that maintains the milk fat globule structure also prevents the lipolysis of triglycerides (Cousin, 1982; Sorhaug & Stepaniak, 1997). Mechanical processes, such as homogenization, high-speed pumping, and agitation, can disrupt the membranes of the milk fat globules. In addition, the synergistic action of enzymes can damage this membrane through the hydrolysis of its proteins and phospholipids. When the membrane of the milk fat globule is damaged, the triglycerides are exposed and subject to the activity of hydrolases, such as lipases (Muir, 1996).

Lipolysis of milk fat globules can be defined as a process of hydrolytic breakdown of these lipids, which is catalyzed by enzymes and results in the production of free fatty acids and partial glycerides. These enzymes are usually called lipases and can be either endogenous, wherein most are associated with the casein micelle structures, or exogenous, which are secreted as a result of bacterial metabolic action (Deeth & Fitz-Gerald, 2006; Ray et al., 2013).

Milk contains a potent endogenous lipase, lipoprotein lipase, which contributes to the metabolism of bovine plasma triglycerides (Olivecrona et al., 2003) and is associated with casein. Endogenous lipases exhibit maximum activity at 37°C and pH 8 (Ordonez-Pereda et al., 2005), are thermosensitive at pasteurization temperatures, and do not cause any damage to the fat fraction of processed and properly handled milk (Aehle, 2007). The sensory defects in raw milk are not associated with endogenous lipases since these enzymes are associated with casein molecules (Fox et al., 2004) in addition to having the fat globule membrane as a barrier that prevents their action.

Lecithinase and other phospholipases are important lipases from psychrotrophs that promote enzymatic hydrolysis of milk lipids, which releases fatty acid and glycerol molecules, increases the amount of short-chain (C-4 to C-8) fatty acids (butyric, caproic, caprylic, and capric), and provides a rancid flavor and odor to dairy products. Most bacterial lipases have specificity for positions sn-1 and 3 of triacylglycerols, and some hydrolyze monoacylglycerols and diacylglycerols faster than triacylglycerols (Sorhaug & Stepaniak, 1997).

Greater amounts of microbial lipases are synthesized at 20°C to 21°C, and these enzymes can maintain 50% of their enzymatic activity at 0°C (Mahieu 1991). The maximum activity occurs at an alkaline pH (7 to 8) and temperatures of 40°C to 50°C (Ordonez-Pereda et al., 2005). Thermal treatments of milk, such as pasteurization and ultrapasteurization, reduce lipolytic activity. According to Xin et al. (2017 b), the crude lipases from *Pseudomonas* spp retained 25% to 87% of their initial activity after heating at 65°C for 30 min and even retained 6% to 71% after a heat treatment at 110°C for 5 min.

Although the lipolytic degradation of milk is not as intense as proteolytic degradation, defects resulting from the actions of lipases are the first noticeable sensory changes (Deeth, 2006). Most defects caused by lipolysis in cheese are associated with flavor and aroma changes (Deeth, 2011). Hydrolysis of triglycerides by lipases leads to the production of free fatty acids, such as butyric and caproic. These acids, even in small amounts, reduce consumer acceptance of the product since they are volatile and associated with a strange taste and odor (Downey, 1980; Shah, 1994). The soap flavor and odor are produced by the hydrolysis of fatty acids with higher molecular weights (C-10 to C-12), while a metallic or oxidized flavor result from the oxidation of unsaturated fatty acids to ketones and aldehydes (Chen et al., 2003). *P. fragi* may be responsible for the fruity odor in milk and cheese caused by the formation of volatile esters following the hydrolysis of fatty acids by esterases (Champagne, 1994). The reduction in storage time and milk temperature results in a decrease in lipid lysis and a lower index of free fatty acids (FFA) by *P. putida* and *P. fluorescens* (Pereira et al., 2019). The reduction in storage time and milk temperature resulted in a decrease in lipid lysis and a lower index of FFA by *P. putida* and *P. fluorescens*, with *P. fluorescens* showing a higher lipolytic capacity. In addition to defects associated with sensory changes, lipases can cause other problems, such as inhibition of the starter culture during cheese production and slow clotting (Cousin, 1982; Shah, 1994). An increase in clotting time is associated with higher concentrations of free fatty acids resulting from bacterial lipolysis, which inhibits the multiplication of starter cultures (Samarzija et al., 2012).

In addition to the lipolytic and proteolytic enzymes involved in the deterioration of milk, phospholipase C is of note, since it can degrade the membrane of fat globules and facilitate the action of lipases (Cousin, 1980; Shah, 1994). In contrast to the natural lipase in milk, most microbial lipases can attack the milk fat globule even if it is protected by the phospholipid membrane (Deeth, 2011).

This review emphasized the relationship between the synthesis of thermoresistant enzymes by psychrotrophic microorganisms and the decrease in cheese yield, texture changes, and sensory defects. These researches are consistent with the reality of the dairy industry and point out to the control of the psychrotrophic population in raw milk as the best way to minimize the defects in dairy products. Therefore, efficient quality programs implemented in the milk production chain, especially with rural milk producers, are essential to reduce the impact of psychrotrophic microorganisms in the quality and shelf life of the cheeses.

4. Final Considerations

The quality of the raw material used for cheese production is crucial for the development of the physical, chemical, and sensory characteristics of cheese. Control of the psychrotrophic population found in refrigerated raw milk contributes to good cheese production yields and desirable texture and sensory characteristics, which improves the likelihood of consumer acceptance, in addition to extending the shelf-life of cheese.

Therefore, to dairy products, especially in the cheese market, is important more researches to understand the impact of psychrotrophic microbiota on the reality of the dairy industry and the better way to control these microorganisms.

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