

Properties of acid-set Queso Blanco cheese made with
filtered milk products

by

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Abstract

As the demand for native whey protein ingredients continues to rise outside of the dairy industry, microfiltered milk products like Micellar Casein Concentrate (**MCC**) are becoming increasingly accessible for cheese production. This increase in microfiltered milk and milk products could cause potential issues within the acid-set cheese industry, as whey proteins often are incorporated within the cheese protein matrix. Thus, this research investigated the impact on functional properties of acid-set cheese produced with MCC using Queso Blanco as a model system. Three replications were completed where cheesemilk was standardized to a 1.2:1 casein-to-fat ratio using rehydrated Milk Protein Concentrate 80 (**MPC**), Milk Protein Isolate 90 (**MPI**) or MCC. Data were analyzed with univariate split-plot design methodology ($P < 0.05$) and significant means differentiated with Tukey's comparisons ($P < 0.05$). Proximate analysis results showed that the three cheeses contained similar moisture (51.18%) and fat (15.13%) contents, but the cheeses made from MPC and MPI standardized cheesemilk had greater protein (~55.2%) content compared with the cheese made from MCC standardized cheesemilk (52.6%) likely due to the lower whey protein content in MCC that carried through to the standardized cheesemilk and resultant cheese. Cheese texture was assessed under 50% strain with a standard double-bite texture profile analysis (**TPA**) at 10, 30, 50, 70 and 90 days of cheese age. TPA resilience, springiness and cohesiveness showed differences between the cheese made with cheesemilk standardized with MCC vs. the cheeses made with cheesemilk standardized with MPC and MPI, whereas chewiness differed between the cheeses made from cheesemilk standardized with MCC and MPI albeit both were similar to the cheese made with the MPC standardized cheesemilk. Hardness did not show differences among any cheeses. A trained descriptive panel assessed

cheeses at 30 and 70 days in a monadic sequential design on a standard 15-point line scale for texture and flavor attributes. No significant differences were detected in organoleptic texture or flavor properties at either day. When assessing for color, a significant difference for whiteness (L^*) was found between cheeses made from cheesemilk standardized with MCC and MPI, however, neither differed from the cheese made from cheesemilk standardized with MPC. Cheeses did not differ in red-green color (a^*), yellow-blue color (b^*), hue angle, or chroma. The change in cheese color was assessed from before to after heating under broiling conditions ($225^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 5 minutes) at 10, 30 50, 70 and 90 days. All cheeses exhibited an increase in the degree of color change from pre- to post-heating (ΔE) as the cheeses aged. These results suggest that removing whey proteins via microfiltration before standardizing cheesemilk for acid-set cheese production would not cause noticeable organoleptic or visual differences for consumers; however, there could be implications when converting the cheese to reduced formats like shreds or crumbles.

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Chapter 1 - Literature Review

1.1 Queso Blanco in the American Cheese Marketplace

Queso Blanco cheese is a fundamental component of Hispanic cuisine (Hill and Parnell-Clunies, 1986) and due to the growing Hispanic population in the United States, Queso Blanco is becoming an increasingly important part of the U.S. cheese industry (Guo et al., 2011; Tunick et al., 2008). Queso Blanco is an acid-set, firm, non-melting, white cheese with an expected shelf life of roughly three months, similar to the popular Hispanic cheese Queso Fresco (Tunick et al., 2008; Tunick et al., 2012a). These two cheeses differ in coagulation methods; Queso Fresco is typically made with rennet whereas Queso Blanco is traditionally made with simple acids like vinegar or lemon juice (Tunick et al., 2012a). Often, Queso Blanco is compared to acid-set cheeses from other regions of the world such as Chhana or Paneer in India, Queso del Pais in Puerto Rico, Olomouc in Moravia, or Kareish in Egypt (Parnell-Clunies et al., 1985b). Nevertheless, Queso Blanco was likely based on a European acid-set cheese but modified to fit Hispanic preferences and available cheese making practices (Van Hekken et al., 2007). As the cuisine in the United States continues to diversify, we need to understand more about these cornerstone ingredients of Hispanic cooking, including Queso Blanco.

Currently, U.S. consumers are seeking opportunities to improve health and have new, unique experiences. When considering the U.S. cheese marketplace, Queso Blanco may be able to meet both themes. Queso Blanco has greater protein and a lower fat-to-calorie ratio compared to most other cheeses, like Cheddar, and contains denatured whey proteins in the cheese matrix (Chandan et al., 1979; Torres and Chandan, 1981). These whey proteins provide essential amino

acids, have the highest biological value of food proteins (Wijayanti et al., 2014) and compared with casein proteins, have higher digestibility (97 to 98% versus 95%) (Barbé et al., 2014). These nutritional benefits are very appealing to the health-conscious U.S. consumers (Parnell-Clunies et al., 1985a). For U.S. consumers seeking to explore other cultures through food, Queso Blanco provides a more authentic and unique experience (Guo et al., 2011). The distinctive non-melt texture and simple acidic and salty tastes of Queso Blanco balance the other textures and flavors in Hispanic dishes, differentiating the food from other ethnic cuisines (Torres and Chandan, 1981). Delivering on both emerging consumer desires allows for the continued demand for Queso Blanco within the U.S. marketplace.

Queso Blanco provides considerable benefits for the U.S. cheese industry due to the short cheese production time, high cheese yield, and quick cheese maturation period (Hill and Parnell-Clunies, 1986; Siapantas, 1981; Torres and Chandan, 1981). Unlike Cheddar or other mainstream American cheeses, Queso Blanco is produced in a few hours with limited processes and inputs. The desired cheese characteristics are determined predominantly by the acid selection and curd pH achieved during cheese manufacture (Lucey et al., 2003). Further, the cheese functionality is established almost immediately once the final cheese pH is reached since limited proteolysis occurs as the cheese matures (Lucey et al., 2003). As the functionality of acid-set cheeses is determined during the cheese production, Queso Blanco can be consumed fresh, similar to what is expected in the process cheese industry (Everett, 2007; Hill and Parnell-Clunies, 1986; Lucey et al., 2003).

Obviously, the high production yield of Queso Blanco is a major advantage to the cheese producer (Hill and Parnell-Clunies, 1986). Unlike rennet-set cheeses where whey proteins are not retained in the cheese curd, Queso Blanco includes whey proteins in its cheese protein matrix

due to the high heat and direct acid inflicted upon the cheesemilk. This causes the whey proteins to denature and co-precipitate with the caseins (Hill and Parnell-Clunies, 1986). This flocculant aggregation of the milk proteins is an ideal mechanism to entrap fat molecules in the cheese protein matrix (Siapantas, 1981). Thus, the recovery of more fat and proteins, specifically denatured whey proteins, which increases water retention in the curd, results in increased cheese yield (Chandan et al., 1979; Hill and Parnell-Clunies, 1986; Lucey, 2020; Torres and Chandan, 1981). These two benefits – decreased production time and increased cheese yield – provide secured interest in Queso Blanco manufacture in the U.S. cheese industry.

However, a gap in scientific understanding exists in the United States regarding the production of Queso Blanco. To help bridge this gap, research conducted on Queso Fresco can aid understanding, even though the manufacture of these two cheeses is based on different casein coagulation mechanisms and production techniques (Tunick et al., 2012a). Yet, similarities do exist as both cheeses are produced without starter cultures and have crumbly, non-melting curd qualities (Siapantas, 1967; Tunick et al., 2012a). In general, Queso Fresco produced in the United States utilizes pasteurized milk with lower moisture targets (47 to 54%) resulting in a firmer, crumblier texture than Queso Fresco produced in Latin America, which utilizes raw milk with higher moisture targets (58 to 60% moisture) (Tunick et al., 2010). Cheese texture and microstructure remain constant over the relatively short eight to 12-week shelf life of Queso Fresco, as the expected degradation or proteolysis would be linked to spoilage bacteria (Tunick et al., 2012a; Tunick et al., 2012b).

In 2007, Everett outlined the basic microstructure principles for Chhana, Kariesh and Olomouc cheeses. Chhana contains fat globules embedded into the protein matrix as denaturation of β -lactoglobulin (β -lg) occurred when the curd was heated $> 90^{\circ}\text{C}$, causing β -lg to

complex with κ -casein on the surface of the casein micelle (**CM**) (Everett, 2007). Comparatively, the protein matrix of Kariesh exhibits a similar aggregation and combination of proteins as seen in microstructures of yogurt, whereas Olomouc cheese consisted of chains of aggregated casein particles, which fused as the cheese aged (Everett, 2007).

1.2 Chemistry of Acid-set Queso Blanco

The differences in cheese protein microstructure between rennet-set and acid-set cheeses are critical to cheese functionality. The main proteins affected during rennet-set cheese production are primarily the κ -caseins on the exterior of the CM. The κ -caseins are cleaved from the CM via the enzyme in rennet, chymosin, which allows the micelles to aggregate due to reduced repulsive charges between the micelles. The overall effect is that the whey proteins, unaffected by chymosin, are not recovered in the curd matrix of rennet-set cheese (Hill and Parnell-Clunies, 1986).

The main proteins affected during acid-set cheese production are primarily the β -lg, other whey proteins, and κ -caseins. Most acid-set cheese manufacturing processes include first heating the cheesemilk and then acidifying the cheesemilk shortly thereafter. This exposure to high temperatures denatures β -lg and promotes interactions with κ -caseins and other denatured whey proteins; thus, when cheese curd coagulation occurs, already aggregated proteins are involved (Lucey et al., 1997; Schorsch et al., 2001), resulting in large protein complexes. Additionally, the acidification step facilitates exclusion of colloidal calcium phosphate (**CCP**) from the CM, which in turn affects the stability of the CM (Singh et al., 1996; Lucey, 2002). Even though the integrity of the CM is compromised, the solubilization of CCP does not fully destroy the protein

matrix as other hydrophobic and electrostatic interactions remain (Lucey, 2002). When examining the microstructure of acid-set cheeses, descriptions include the evidence of fused CM creating a unique ‘core-and-lining’ structure instead of the individual, distinguishable casein proteins bonded together in chains and clusters observed in rennet-set cheese curd (Harwalkar and Kalab, 1981; Kalab and Modler, 1985). The resulting ‘core-and-lining’ structure of the CM develops from the combination of β -Lg and κ -casein forming filamentous appendages on the micelle surface (Harwalkar and Kalab, 1981). However, other important process parameters like pH, time, and heating temperature, as well as total protein concentration, will affect the interactions between κ -casein and β -lg (Lucey et al., 1998). Although these process parameters are equally important for rennet-set cheeses, the factor of heating temperature is particularly crucial to acid-set cheeses as this is the initial step towards controlling whey protein incorporation into the final cheese protein matrix.

When heating milk from 70 to 100°C, Vasbinder and de Kruif (2003) reported that α -lactalbumin (**α -La**) and β -Lg denature, while the CM maintains its structure. Thermal denaturation of whey proteins begins around 70°C with β -Lg unfolding and exposing interior amino acids that interact to aggregate proteins (Lucey, 2002; Lucey et al., 1998; Oldfield et al., 1998; Schorsch et al., 2001). Initially this denaturation can be reversible; but as more unfolding occurs, sulfide groups are exposed, allowing proteins to interact via disulfide bonds once the cheesemilk is acidified to the isoelectric point of the whey proteins (~pH 5.2), which is higher than that of the CM (Horne, 1998; Lucey et al., 1997; Lucey et al., 1998). Specifically, at pH < 6.7, the β -Lg proteins begin to aggregate with κ -caseins on the CM surface through irreversible bonds to stabilize the CM and reduce the dissociation of the κ -caseins from the CM (Anema, 1998; Oldfield et al., 2000; Singh, 1995; Vasbinder and de Kruif, 2003). Researchers have

theorized that these bonds are a mixture of calcium bridges, hydrophobic bonds, and intermolecular sulfhydryl-disulfide interactions (Anema and McKenna, 1996; Horne, 1998; Ji et al., 2016a, Oldfield et al., 1998). The unique connection of disulfide bonds between β -Lg and the CM is fostered by newly exposed hydrophobic groups on the proteins: exposed thiol groups of cysteine on the denatured β -Lg aggregate with the cystine residues on κ -casein due to the reduced electrostatic repulsive charge on the proteins (Lucey et al., 1997). Additionally, dependent upon cheesemilk temperature, denatured β -Lg can complex with other denatured whey proteins like α -La (Oldfield et al., 2000). These α -La- β -Lg complexes can then aggregate with the CM, increasing the overall bridging material in the cheese protein matrix (Oldfield et al., 2000); thus, increasing the strength of bond interactions (Lucey et al., 1997). Although some scientific debate continues regarding the exact mechanisms of the reactions (Wijayanti et al., 2014), the most accepted understanding of this aggregation process is the formation of covalent disulfide bonds between the κ -caseins at the CM surface and denatured whey proteins, primarily β -Lg. (Doi et al., 1983; Lucey et al., 1998; Singh et al., 1996; Cho et al., 2003). On the other hand, when the pH is > 6.7 , κ -caseins begin to dissociate from the CM aggregating with β -Lg separately in the serum phase, as well as stimulate more whey protein to whey protein aggregates (Oldfield et al., 2000; Vasbinder and de Kruif, 2003). As a result, at pH 6.7, only about 60% of β -Lg aggregates with CM, but at pH < 6.5 , more than 90% of β -Lg aggregates with CM (Oldfield et al., 2000). Nevertheless, the protein chemistry between κ -casein and β -Lg is critical to successful production of acid-set cheeses like Queso Blanco.

Although cheesemilk must be heated to a minimum of 70°C to initiate whey protein denaturation (Lucey, 2002; Lucey et al., 1998; Oldfield et al., 1998; Schorsch et al., 2001), the optimum temperature range to heat cheesemilk is 85 to 90°C. At these temperatures, it would be

anticipated that 10 to 20% of the denatured whey proteins will aggregate with the CM (Anema and Klostermeyer, 1997; Smits and Van Brouwershaven, 1980). When cheesemilk is maintained at 90°C for 20 minutes or more, over 80% of denatured β -Lg will aggregate with the CM (Oldfield et al., 1998; Singh, 1995). Qian et al. (2017) reported that milk held at 85°C for 10 minutes had only ~50% denatured whey proteins, whereas milk held at 95°C for 10 minutes had ~100% denatured whey proteins. Further, Qian and the research team presented a quadratic regression relationship between heating time and temperature, to whey protein denaturation within heated cheesemilk and to denatured whey proteins aggregation with CM (Qian et al., 2017). Ultimately, a complex mixture of proteins results due to heating and acidifying cheesemilk; CM-whey protein aggregates and whey protein-whey protein aggregates in the cheese protein matrix and native, non-denatured whey proteins in the serum (Vasbinder and de Kruif, 2003).

The aggregation of this complex protein mixture can be altered depending on the acid used in the acidification process (Chandan et al., 1979; Farkye et al., 1995; Hill and Parnell-Clunies, 1986; Lucey et al., 2003). Farkye et al. (1995) showed that compositionally, Queso Blanco made with lactic acid had a slightly higher moisture content than Queso Blanco made with citric acid or acetic acid. Functionally, Queso Blanco made with lactic acid had significantly less hardness than Queso Blanco made with citric acid, which in turn had significantly less hardness than Queso Blanco made with acetic acid (Farkye et al., 1995). These results suggested acetic and citric acids induced greater casein aggregation within the cheese protein matrix than lactic acid (Farkye et al., 1995). When presenting the sensory data, Queso Blanco made with acetic acid was significantly preferred for cheese body and texture vs. Queso Blanco made with citric acid or lactic acid (Farkye et al., 1995). This preference increased as the cheeses aged

(Farkye et al., 1995). However, the overall acceptability of the cheeses was equivalent for Queso Blanco made with acetic acid and citric acid while the Queso Blanco made with lactic acid was less liked ($P < 0.05$) (Farkye et al., 1995).

Citric acid, a strong chelator of calcium has been recommended for Queso Blanco production (Chandan et al., 1979). Compared with the other acids, citric acid is one of the most cost-effective acidulants and more importantly, does not cause off-flavors in the cheese (Chandan et al., 1979). However, when using citric acid in the production of Queso Blanco, cheese yield was reduced, likely due to the reduced moisture content in the cheese (Chandan et al., 1979).

1.3 Functionality of Queso Blanco

The functional characteristics inherent to Queso Blanco are a direct result of the acidification and its impact on protein interactions that occurs during cheese manufacture (Everett and Auty, 2008; Lucey et al., 2003). In general, the protein matrix in an acid-set cheese compared with a rennet-set cheese contains denser fibers due to the increased agglomeration of whey proteins into the cheese protein matrix, which in turn allows for a stronger protein microstructure (Everett, 2007; Kalab and Harwalkar, 1973; Schorsch et al., 2001, Vasbinder et al., 2004). This protein chemistry principle of an acid-set cheese differentiates the functionality from rennet-set cheeses in visual, textural, and sensorial aspects.

In most rennet-set cheeses, this acid is developed by the added starter cultures, which generate lactic acid from lactose in the milk. As the bacteria grow, various enzymes are released into the milk, using the milk components as substrates. These enzymes are glycolytic, lipolytic,

and proteolytic, and contribute to the overall flavor and texture characteristics as cheese ages. In an acid-set cheese, proteolytic activity is atypical. Hence, one of the most important aspects of acid-set cheese functionality is the relatively consistent texture during shelf life due to the lack of proteolytic enzymes in the cheese. Less proteolysis occurs in acid-set cheeses because the globular structure of the β -Lg integrated into the cheese protein matrix is more resistant to proteolysis than the flexible structure of caseins (Barbé et al., 2014). Additionally, the salt content in an acid-set cheese is typically greater than in a rennet-set cheese, and the salt concentration can delay spoilage bacteria from propagating, diminishing proteolytic activity in the cheese (Torres and Chandan, 1981).

Cheese rheology is often dictated by the cheese microstructure (Everett and Auty, 2008). The minimal proteolysis that occurs in acid-set cheeses impacts textural and rheological properties, including the cheese rigidity and viscoelasticity (Farkye et al., 1995; Lucey et al., 2003). Guerra-Martinez et al. (2012) showed that the moisture content had a greater impact on textural and rheological properties than proteolysis in Panela, a fresh cheese produced in Mexico. Chen et al. (1979) evaluated many types of cheeses ranging from Parmesan to process cheese and reported that moisture content is only a stronger contributor to cheese texture than pH and fat content, but protein content, and to some extent salt content, still are major determinants of cheese texture.

In acid-set cheeses, protein content, specifically denatured whey protein, is crucial to understanding cheese rheology (Perreault et al., 2017). Lucey et al. (1998) showed that small amounts of denatured whey proteins, bound to CM within the protein structure of acid gels made from heated milk, increased the G' of the gels. During cheese manufacture, when denatured whey proteins cross-link with CM, the pH at gelation increases but whey expulsion decreases as

the structural rearrangements of the proteins create a less permeable matrix (Everett and Auty, 2008; Lucey, 2002; Perreault et al., 2017). The gelation pH affects the CCP associated within CM since calcium phosphate solubilization and casein aggregation via hydrophobic bonds typically will not occur at > 5.8 pH (Everett and Auty, 2008; Guerra-Martinez et. al., 2012). In addition to water and calcium phosphate, the protein microstructure of an acid-set cheese can also trap fat globules, which can contribute, although to a lesser extent, to cheese texture (Everett and Auty, 2008).

Cheese texture can be analyzed via imitative texture profile analysis (**TPA**) methods, most notably the double-bite method which works well for comparing samples but is not always an accurate measure of rheological properties (Tunick and Van Hekken, 2002; Tunick and Van Hekken; 2010). The main factors assessed with TPA of cheese include hardness, cohesiveness, springiness, and chewiness (Tunick, 2000). General conclusions for TPA, since a multitude of acceptable test conditions exist, have shown that cheese hardness will increase with increases in protein content, but cheese hardness will decrease with increases in moisture and fat contents (Chen et al., 1979). When evaluating Queso Blanco using TPA, cheese hardness decreased if moisture content increased or cheese aged (Parnell-Clunies et al., 1985c).

Tunick et al. (2012a) reported that storage time, storage temperature, and salt content did not affect the hardness or cohesiveness of Queso Fresco, likely a result of reduced proteolysis. Cohesiveness is an important attribute for Queso Fresco and acid-set cheeses, due to the crumbly textures of the cheeses (Tunick et al., 2012b). Research on Oaxaca cheese, an acid-set pasta filata-style cheese, showed similar results; cohesiveness was constant, but hardness and springiness increased as protein content increased and fat content decreased (Caro et al., 2011).

Queso Blanco is expected to retain its texture during heating (Hwang and Gunasekaran, 2001). The restricted melt character of Queso Blanco distinguishes the cheese from other Hispanic-style cheeses and drives the topical usage of the cheese in Latin American dishes (Torres and Chandan, 1981). As with most cheeses, the protein matrix determines the melt character (Lucey et al., 2003). Lucey et al. (2003) describes cheese melt as two different, step-wise rheological functions: first, the loss of cheese elasticity (G') during heating followed by the flow of the protein matrix when the viscous modulus (G'') exceeds the elastic modulus (G'). The second step may not occur depending on the microstructure, primarily the type and strength of bonds within the protein matrix (Lucey et al., 2003). In an acid-set cheese, the protein matrix has an increased number of bonds, which directly affects the ability of the cheese to melt (Everett and Auty, 2008). Often, melt is prevented in acid-set cheeses due to the flocculent-style protein aggregation that occurs, which traps fat globules in the protein matrix (Siapantas, 1981). The fat globules have limited ability to flow when tightly trapped within the protein microstructure, which subsequently limits meltability when the cheese is heated (Tunick et al., 2012a).

When cheese is melted, the melting conditions affect the color properties of an acid-set cheese. Thus, heat can be applied for a) baking conditions (130°C for 30 minutes) or b) broiling conditions (232°C for 5 minutes) (Guo et al., 2011; Tunick et al., 2012a; Tunick et al., 2012b). When heating of Queso Fresco under baking conditions, Tunick et al. (2012b) reported that the cheese significantly decreased in whiteness (L^*) as well as color hue (ratio of b^* to a^*) due to a^* moving from a positive to a negative value (Tunick et al., 2012b). Guo et al. (2011) found that melting cheese under baking conditions caused ΔE (total change in color from before to after heat treatment) to be three times greater than if the cheese was melted under broiling conditions, even though broiling conditions often caused browning around the edges of the top surface of the

cheeses. Queso Fresco had strong color stability and did not exhibit color changes during eight weeks of storage or various salt contents up to 2.5% (Guo et al., 2011).

The organoleptic profile of Queso Blanco cheese is rather simple compared to most cheeses available in the U.S. cheese marketplace. Queso Blanco has a mild, salty, and milky taste, similar to many acid-set cheeses (Siapantas, 1981). These simple organoleptic attributes are important to the utilization of Queso Blanco in Hispanic cuisine because Queso Blanco is commonly consumed fresh and as a garnish on prepared dishes (Lucey, 2020). Queso Blanco can have off-flavors, attributes like cooked or bitter, which originate from the whey proteins agglomerated in the cheese protein matrix (Lemieux and Simard, 1991; Wijayanti et al., 2014). During denaturation, whey proteins can release small sulfur-containing compounds like hydrogen sulfide and methanethiol, which are responsible for these off flavors (Wijayanti et al., 2014). Moushumi et al. (2014) showed that regardless of the origin of peptides in cheese, their hydrophobic character can influence the overall cheese flavor. Since Queso Blanco contains β -Lg and other whey proteins, the degradation of these proteins will release peptides influencing the flavor profile. The simplicity of the flavor profile of Queso Blanco has allowed the cheese to proliferate in Hispanic cuisine and beyond.

1.4 Milk Protein Powders in Cheese Production

Milk proteins concentrates (**CMP**) have been used proficiently in the cheese industry to standardize cheesemilk to specific casein-to-fat ratios. Even though the general milk composition in the United States is less variable than in other regions of the world, cheesemilk standardization improves the overall economics of cheese production (Lucey, 2002). Two main factors that drive

the beneficial financial impact are a) increased cheese yield due to increased levels of available proteins and b) increased production speed due to reduced casein coagulation time and faster firming of the curd (Gaiani et al., 2009; Lucey, 2002). Similarly, by standardizing cheesemilk through different seasons and milk supplies, greater cheese consistency is achieved by reducing variability of cheese manufacture recipes, which also drives production efficiency (Fang et al., 2010). By contributing to a more consistent, faster-produced cheese, CMP are an important ingredient in the cheese industry.

Knowing that CMP are beneficial to the cheese industry, it is vital to understand how they are produced. The production of a CMP typically involves standard membrane separation technologies concentrating the desired protein fraction while reducing other components of milk like water, lactose, and minerals (Burrington, 2013). The resulting protein profile depends on the type of membrane technology employed. The two primary membrane technologies used in the dairy industry are ultrafiltration and microfiltration (Schokker et al., 2011). Microfiltration utilizes a larger pore size (100nm) within the membrane than ultrafiltration (1nm) (Burrington, 2013). Although both technologies separate water, minerals, and lactose into the permeate, microfiltration also separates whey proteins from the casein proteins, fat, and bacteria, which are maintained in the retentate (Burrington, 2013). Retentate, regardless of ultrafiltration or microfiltration membrane technology, is then typically spray dried to remove any remaining moisture (target final moisture of 3 to 4%), allowing for ease in storage and transportation (Schokker et al., 2011).

The protein chemistry that ensues during CMP production is important to understand when using CMP for further processing, like cheese production. Both membrane separation and spray drying processes impart strain on the milk proteins causing structural and functional

changes (Augustin et al., 2012). Sikand et al. (2011) showed that the partition of minerals during membrane separation processes can destabilize and dissociate individual caseins from CM due to the loss of CCP. These individual casein proteins are then available for interactions with other milk proteins during later process steps (Sikand et al., 2011). The application of heat, and thus further water removal, during the spray drying process drives additional changes to the proteins. Kalab (1979) observed that when drying retentates, spray drying preserved the integrity of casein better than roller drying, but modifications to proteins still did occur during spray drying. Spray drying most types of milk powders will denature whey proteins, causing insoluble aggregates to form once rehydrated and used in cheese manufacture (Omar and Buchheim, 1983; O'Sullivan, 1971). Furthermore, lactose can have a protective effect on proteins during dehydration because lactose will adhere to the surface of casein proteins via hydrogen bonds when water molecules are removed during evaporation, preventing protein interactions (Baldwin, 2010). Thus, when lactose is eliminated from the retentate during membrane filtration, that protective barrier is lost, allowing CM to agglomerate due to the possible increase in CCP, resulting in the overall increase in size of the CM during evaporation (Augustin et al., 2012; Singh, 2007). In addition to membrane filtration and spray drying, CMP production can include a pre-heat treatment step (Singh and Creamer, 1991). This step can improve the heat stability of the resulting retentate after membrane filtration, making pre-heating of the milk a common practice in the dairy industry, specifically for lower-protein ingredients like non-fat dry milk (Singh and Creamer, 1991). The heat applied to the milk can dissociate individual caseins from the CM, specifically κ -caseins, which contain the highest phosphoserine content, due to the dephosphorylation of the CM (Haque and Kinsella, 1988; Singh and Creamer, 1991). The κ -casein is available then to crosslink with denatured β -Lg via hydrophobic and disulfide bonds to form a stable protein

complex, just like in acid-set cheese manufacture (Haque and Kinsella, 1988). However, in the case of CMP production where protein concentrations are greater than that of non-fat dry milk, often, the pre-heating step is eliminated to avoid the denaturation of the whey proteins and subsequent agglomeration with κ -caseins (Singh, 2007). Consequently, the protein chemistry that occurs during the production of CMP affects the rehydration and subsequent coagulation properties of the proteins needed for cheese manufacture.

A common practice in the cheese industry is to purchase CMP in bulk; thus, CMP used to standardize cheesemilk is often stored prior to use. During storage, changes occur that are dependent upon the composition of the product and environmental storage conditions, which affect the rehydration as well as solubility of the CMP in cheese production. Gazi and Huppertz (2015) showed that solubility is almost virtually unaffected during CMP production; thus, diminished solubility must result from changes during storage. Although the most common reaction in a stored CMP is the crystallization of lactose, both Maillard and oxidation reactions can occur also (Thomas et al., 2004). These reactions can alter the functionality of CMP, specifically those with high-protein contents, referred to as high-protein milk protein concentrate (**HP-MPC**), typically greater than 80% (e.g., MPC-80). Factors that affect the rate of these reactions include storage time, storage temperature, and powder composition. Caron et al. (1997) reported that the shelf life of milk powders ranges from six months to two years, but the system is not static – chemical and physical changes occur during this time. Typically, if CMP are used within 60 days, minimal functional changes are expected (Gaiani et al., 2007a; Gazi and Huppertz, 2015). However, as storage duration extends, the physical and chemical properties of the CMP change due to bonding between components and the development of inter-particle structure (Fang et al., 2011). Haque et al. (2012) explained the reduction in CMP solubility in

relation to the glass transition curve over time where the powder remains in an amorphous glassy, thermodynamically, non-equilibrium state immediately after production. The powder would be expected to reach a more thermodynamically stable state upon aging due to a reduction of molecular mobility within the particle, also known as the relaxation enthalpy (Haque et al., 2012). It was suggested that age and storage conditions affect the relaxation enthalpy of dried dairy powders via changes in the macroscopic properties enabling the slow, non-reversible aggregation of CM during storage, hence, a loss of solubility (Haque et al., 2012; Schokker et al., 2011). Schokker et al. (2011) used small angle X-ray scattering to show that storage time does not result in major changes to the internal organization of CM, but rather the surface. It is most commonly believed that the CM slowly begin to create superficial, non-covalent bonds between α -s caseins and β -caseins over time due to attractive hydrophobic interactions and electrostatic forces (Fyfe et al., 2011; Havea, 2006; McKenna, 2000; Mimouni et al., 2009; Singh, 2007). Often, these fused CM are characterized within the insoluble material of rehydrated solutions of HP-MPC (Havea, 2006; McKenna, 2000). Further, Fyfe et al. (2011) suggested that the result is a monolayer of close-packed micelles at the surface of the powder particles, which increases the hydrophobicity of the particle and reduces the solubility. Anema et al. (2006) showed that HP-MPC stored for up to 60 days exhibited less defined protein bands when analyzed by SDS-PAGE, confirming the non-covalent crosslinking, probably hydrophobic and/or hydrogen bonding, between CM. Another factor affecting the hydrophobic nature of the particle surface in HP-MPC is the quick accumulation of lipids at the surface of the powder particle (Fyfe et al., 2011). This theory is supported by the fact that the temperature of spray drying is greater than the melting temperature of dairy lipids, allowing the migration of lipids towards the surface of the powder particles (Gaiani et al., 2009). At the same time, the partial denaturation of whey proteins

during CMP production exposes hydrophobic residues, which are available to crosslink via hydrophobic bonds (Havea, 2006; Lucey et al., 1998). Gazi and Huppertz (2015) provided an alternative mechanism of CM aggregation. Their work showed that CM aggregation was not the development of a hydrophobic crust layer, but rather resulted from the loss of steric stabilization of the κ -casein layer due to a combination of high calcium ion activity and the increased hydrophobicity of the environment during the CMP drying. This study aimed to prove the previous understanding of CM aggregation within HP-MPC incorrect; however, further studies would be needed to validate this theory. Regardless, these CM changes typically are accelerated by elevated storage temperatures recognized as between 35°C and 50°C (Anema et al., 2006; Fang et al., 2011; Fyfe et al., 2011; Havea, 2006). For example, storing powders at two different temperatures will exhibit functionality changes within a few weeks to many months (Fang et al., 2011). Thus, the recommendation is to store HP-MPC at 4°C to slow the rates of physical and chemical reactions in the powders (Mimouni et al., 2010b). Anema et al. (2006) reported that although the rate of soluble casein loss in HP-MPC increased at >30°C over 60 days, HP-MPC stored at 50°C did not exhibit loss in whey protein solubility. Data showed that roughly 20% protein solubility remained in the HP-MPC after storage, which matches the relative 20% whey protein composition.

Lactose composition in CMP affects Maillard reactions (Thomas et al., 2004), which need at least small amounts of carbohydrate substrate to commence. In the case of HP-MPC, Le et al. (2011) showed that Maillard reactions occur quicker with higher concentrations (addition of 2%, respectively) of milk sugars, especially in the case of the lactose-derivative, glucose. Many CMP produced via membrane filtration, however, contain small amounts of carbohydrate, reducing the impact of the Maillard reactions on the physical and chemical compositions.

The physical and chemical protein changes that occur during CMP storage will determine the ability and speed of rehydration. The rehydration kinetics of CMP are crucial to their use in the cheese industry and beyond, for CMP must rehydrate easily to maximize production efficiency (Crowley et al., 2016). The goal of any rehydration procedure is quick and complete hydration of the powder (Schokker et al., 2011). However, a wide variety of rehydration procedures exists as the chemical mechanisms of HP-MPC rehydration are not fully understood (Crowley et al., 2016). As previously mentioned, during storage of CMP, CM fuse via non-covalent bonds and once rehydrated, result mostly in the insoluble material while non-micellar caseins are found in solution (Gazi and Huppertz, 2015; Havea, 2006; McKenna, 2000). Researchers have suggested that the hydrophobic, non-covalent bonds prevent the dispersion of fused CM during rehydration (Hauser and Amamcharla, 2016; Le et al., 2011). During rehydration, the dispersion of CM from the powder particle is the rate-limiting step (Crowley et al., 2015; Haque et al., 2012; Mimouni et al., 2009; Mimouni et al., 2010b), not water penetration into the powder particles as reported by Anema et al. (2006). Even though minimal agreement exists among researchers and industry as to the appropriate solubility index for HP-MPC, the solubility index as a function of time generally results in an s-shaped curve representing initially slow, followed by quick acceleration and finally leveling off in solubility, respectively (Baldwin, 2010; Crowley et al., 2016; Sikand et al., 2011). Mimouni et al. (2009) and Hauser and Amamcharla (2016) showed during CMP rehydration, dissolution involves two different but simultaneous steps: 1. the breakdown of large-sized powder particles into smaller-sized particles and 2. the slow release of material within the powder particle into the surrounding water solvent, which has been identified as the primary rate-limiting mechanism in powder rehydration. Others suggested that three successive mechanisms occur during powder dissolution

when rehydrating CMP due to a shell on the exterior of the powder particles. First, the solvent wets and disperses the fragile powder particles, which results in a sudden decrease in mean size of the particles (Richard et al., 2013). Second, the solvent enters the external layer of the powder particle, resulting in a more gradual decrease in mean particle size as that external layer dissolves. Third, the cohesion of the CM is lost and becomes fragmented, thus completing the powder dissolution. Although research completed by Mimouni et al. (2010a) confirmed the theory brought forth by McKenna (2000) that the exterior of the powder particle has a crust-like surface, this research also showed a second, inner layer of the powder particle which closely resembled that of fresh powder (Mimouni et al., 2010a). As a result, when CMP were rehydrated, the CM crust dissociated in large pieces, which reduced the overall dissolution rate, suggesting that the powder particle surface contained strong CM interactions that were potentially irreversible but also likely aided in stabilizing the particle structure (Mimouni et al., 2010a; Mimouni et al., 2010b). Mimouni et al. (2010a) explained that the internal CM interactions from the second inner layer, as well as subsequent layers, could decrease dissolution rates in addition to the decrease in dissolution rate due to the initial rate-limiting step of bonded CM at the external surface of the powder particles. Although the kinetics of rehydration of CMP continues to be explored, the dissolution properties remain crucially important when using these powders within the food industry.

Research has focused on modifications of rehydration methods such as temperature, stirring speed, concentration, and powder composition related to rehydration time and efficiency. Fang et al. (2010) showed that the solubility of CMP decreased at rehydration temperatures $>50^{\circ}\text{C}$, likely due to further protein denaturation and aggregation. Thus, to rehydrate CMP, the recommended temperature of 40°C to 50°C has become common practice within the cheese

industry (Crowley et al., 2015; Fang et al., 2010; Havea, 2006). The effect of temperature alone on the rehydration efficiency of CMP does not have a strong correlation to increased solubility on an industrial scale because other factors impact CMP solubility (Crowley et al., 2015). For example, although temperature has a pronounced impact on rehydration kinetics, the effect of temperature increases when stirring speed increases (Hauser and Amamcharla, 2016; Jeantet et al., 2010). Commercially, rehydration occurs within mechanically agitated tanks, so stirring speed has an important role in rehydration efficiency for its relationship to total process time, cost, and energy input (Richard et al., 2013). In addition to rehydration temperature and stirring speed, powder solids concentration (w/w) can also impact the rehydration efficiency of CMP, albeit to a lesser extent than the other named rehydration parameters (Jeantet et al., 2010). Similarly, the composition of the powder solids, namely the protein content specifically, impacts rehydration efficiency. Crowley et al. (2015) observed the rehydration properties of CMP with different protein concentrations (from 35 to 90% protein) and reported that as protein content increased, the micellar casein crust on the surface of the powder particles increased, which in turn increased the quantity of undispersed powder particles. Bulk density decreased, likely due to the increase in interstitial air, and conductivity decreased, likely due to the depletion of minerals during membrane filtration (Crowley et al., 2015). As a result, these rehydration parameters should be considered during CMP rehydration to maximize production efficiency.

In addition to the general overall level of protein content in CMP, Mimouni et al. (2010b) showed that all individual powder components can be classified into one of two groups, based on speed of dissolution, by observing the composition of CMP and rehydrated CMP. The first group, fast-dissolving components, include lactose, whey protein, potassium, and sodium (Mimouni et al., 2010b). In the case of lactose, Richard et al. (2013) and Crowley et al. (2015)

suggested that the increased hydrophilicity of lactose likely increased the amount of water transfer into the CMP powder particle, enhancing the rate of rehydration. Hauser and Amamcharla (2016) showed that decreasing the protein content in CMP from 90 to 85%, while increasing the lactose content from roughly 3 to 5% and dissolution temperature from 40°C to 48°C, improved solubility. It was inferred that lactose likely prevented protein-protein interactions, which impede solubility because lactose interacted with the protein chains via hydrogen bonds (Baldwin, 2010). Other fast-dissolving components, like potassium and sodium, can be customized during production of CMP to improve overall rehydration efficiency (Baldwin, 2010; Sikand et al., 2011).

On the other hand, the slow-dissolving components include casein, colloidal calcium, colloidal phosphorus, and colloidal magnesium, which are generally associated with the CM structure (Mimouni et al. 2010b). These components affect the ionic strength of the dispersion during rehydration as the CM react and stabilize (Crowley et al., 2015). Specifically, CMP with > 60% protein stored at 50°C exhibited reduced casein solubility, but whey proteins maintained >75% solubility despite being stored at the same elevated temperature for 120 days (Gazi and Huppertz, 2015). The rehydration efficiency of these milk powder components is influenced by the solvent. For example, Gazi and Huppertz (2015) showed that the solubility of CMP increased when dissolved in milk or milk permeate compared with water. All of these rehydration factors influence the efficiency and effectiveness when CMP is used in the food industry.

1.5 Micellar Casein Concentrate in Cheese Production

Micellar casein concentrate (**MCC**), also known as native phosphocaseinate due to the native CM, has gained interest as an ingredient in the dairy foods industry due to its economical, nutritional, and functional values (Bong and Moraru, 2014; Richard et al., 2013). MCC has a roughly 90% total protein fraction with $\geq 95\%$ of the protein content being casein (Mistry, 2002). In general, proteins are the highest-value milk component (Mimouni et al., 2009). From a nutrition perspective, MCC provides all essential amino acids available in milk as well as a good level of calcium (Burrington, 2013). Compared with nonfat dry milk, MCC can be used to standardize cheesemilk and may actually increase cheese yield due to its lower lactose and whey protein contents (Burrington, 2013; Mistry, 2002). Early research on microfiltration in the dairy industry focused on the development of MCC for cheese manufacture (Caron et al., 1997; Saboya and Maubois, 2000). Papdatos et al. (2003) studied the economics of using cheesemilk standardized with microfiltered milk products for Cheddar and Mozzarella cheeses and reported a higher net revenue of \$0.40+/0.45 kg of cheese, based on 45 kg of standardized milk. Although production costs were greater when manufacturing cheese with MCC, the increased cheese yield and throughput were more profitable compared with the conventional cheesemilk standardization ingredients (Papdatos et al., 2003; Saboya and Maubois, 2000). These traditional ingredients are processed predominately via ultrafiltration and include products such as milk protein concentrate 80 (**MPC**) and milk protein isolate 90 (**MPI**).

The key reason, however, for using microfiltered milk products compared to the traditional ultrafiltered milk products, is the increased value of the permeate generated during the production of MCC. Removing whey proteins prior to cheese manufacture retains more of these

proteins in their native state, resulting in a more functional whey protein concentrate (**WPC**), e.g., increased foaming, emulsifying ability, solubility, and gel strength (Burrington, 2013; Li et al., 2020). During cheese production, the pasteurization and coagulation steps often denature whey proteins, which limits these functional attributes. Both MCC and WPC are desirable ingredients for the foods industry, especially, those within the nutrition-conscious category of products like meal replacement beverages and medical nutrition bars (Agarwal et al., 2015). The Hoogwegt Group (2019) reported that demand for native whey proteins specifically, to support these food products, has risen at a rate of 6-7% annually. Ultimately, MCC is a great fit for the cheese industry and the food industry as a whole.

In most MCC, the casein is in its native form because rapid-heat spray drying results in very slight casein denaturation (Burgain et al., 2016; Caron et al., 1997; Richard et al., 2013). The basic process to manufacture MCC starts with microfiltration of skimmed milk followed by diafiltration of the retentate (Burgain et al., 2016; Saboya and Maubois, 2000). The diafiltrate is often microfiltered a second time before spray drying (Saboya and Maubois, 2000). The difference between microfiltered products like MCC and ultrafiltered products like MPC or MPI is the degree of constituent removal. Although both microfiltration and ultrafiltration remove low molecular weight, water-soluble components like lactose and minerals, the resulting protein profile and content differ. With ultrafiltration, the milk protein remains in the original ratio of roughly 80% casein to 20% whey proteins. However, with microfiltration, whey proteins transcend the membrane into the permeate leaving $\leq 5\%$ whey proteins in the retentate with the casein (Mistry, 2002). The high concentration of CM in MCC impacts use and function when standardizing cheesemilk.

When milk powders are used to standardize cheesemilk, they must be hydrated first; thus, it is important to understand the rehydration differences between ultrafiltered HP-MPC and microfiltered MCC. As the microfiltration process removes the fast-dissolving compounds in milk, caseins produce a robust crust on the surface of the powder particles, thus MCC does not rehydrate well (Crowley et al., 2016; McKenna, 2000; Mimouni et al., 2010b). Five steps have been identified in the rehydration of MCC powder. First, the powder is wetted (Crowley et al., 2016; Gaiani et al., 2006b). Because the crust that forms on the surface of the MCC powder particle is generally hydrophobic, the wetting time is typically significant (Gaiani et al., 2005). In many instances, wetting time has been described as the rate-limiting step for many HP-MPC but this is not always the case for MCC (Gaiani et al., 2005; Ji et al., 2016b; Schuck et al., 2007). Second, the powder sinks into the water solvent (Crowley et al., 2016). Third, particles swell, which is typically the shortest period of the rehydration process (Crowley et al., 2016; Gaiani et al., 2005). Fourth, the proteins are dispersed and fifth, the proteins are dissolved into the solvent (Crowley et al., 2016). Typically, the slow dispersion of the powder particles is regarded as the rate-controlling step for MCC rehydration (Gaiani et al., 2005; Gaiani et al., 2007b; Ji et al., 2016b; Schuck et al., 2007).

Not surprisingly, the parameters that affect rehydration efficiency of MCC include storage conditions, rehydration temperature, rehydration stirring speed, and MCC powder composition. Like other HP-MPC, fresh MCC rehydrates quicker than older MCC, especially if the storage temperatures or humidity are reduced as increases in these conditions promote component interactions, including lipid rearrangements (Burgain et al., 2016). Richard et al. (2013) found that rehydration time of MCC, when combined with whey protein isolate, was quickest when rehydrated at 35°C; however, decreasing the rehydration temperature to 25°C

increased rehydration time by roughly 150%, while increasing the rehydration temperature to 40°C increased rehydration time by more than 230%. It was predicted that when the rehydration temperature exceeded 37°C, caseins aggregation would occur, which caused the rehydration time to increase significantly (Richard et al., 2013). Like other HP-MPC, temperature affects rehydration time more than stirring speed for MCC (Richard et al., 2013). Stirring speed has an inversely proportional relationship to total rehydration time for MCC (Jeantet et al., 2010; Richard et al., 2013). The rehydration efficiency can be enhanced by adding fast-dissolving hydrophilic milk components, like lactose and minerals, to decrease the wetting time of MCC (Crowley et al., 2015; Gaiani et al., 2006a; Richard et al., 2013). These fast-dissolving milk components alter the micellar casein interactions at the surface of the powder particle (Gaiani et al., 2009). Schokker et al. (2011) showed that adding non-micellar casein components (serum caseins) during production of MCC can improve the total rehydration time of MCC. Ultimately, these parameters become critical to the effective use of MCC in the dairy industry and beyond.

1.6 Queso Blanco Made with Recombined Milk

Research on the use of microfiltered milk products like MCC in cheese production continues to evolve. Initial work has shown that MCC is an economical addition to cheesemilk by improving cheese yield without lessening cheese quality (Burrington, 2013). When standardizing cheesemilk, adding protein solids as opposed to removing cream (fat) increases the amount of solids within the cheese vat, thus increasing cheese yield (Hill and Parnell-Clunies, 1986). However, the choice of protein powder to use in the standardization process of cheesemilk is especially important for acid-set cheeses like Queso Blanco. This is because

denatured whey proteins are expected in the final cheese protein matrix due to the denaturation resulting from either the spray drying process during the production of the CMP or the high temperatures used during acid-set cheese manufacture (Omar and Buchheim, 1983).

Even though whey proteins have an indirect impact on an acid-set cheese protein matrix, whey proteins can influence final cheese functionality because of their water binding capacity and their effect on the final protein-to-fat ratio of the cheese (Everett, 2007; Mistry, 2002). For example, whey proteins, which are concentrated in ultrafiltered milk products, can contribute off flavors in a resulting cheese (Lemieux and Simard, 1991); this would suggest that cheeses made with microfiltered milk products like MCC, where whey proteins are depleted, should not exhibit as many off flavors. However, the entire system needs to be considered as Burrington (2013) noted that higher β -casein contents in MCC could actually contribute bitterness in cheese similar to that seen with whey proteins. Additionally, concentrating ultrafiltered milk products, where homogenization of the milk is required, has shown to increase the amount of protein that can cover milk fat globules, while the overall size of the fat globules decreases, further impacting the cheese protein matrix and resulting cheese functionality (Everett, 2007; Omar and Buchheim, 1983; Van Vliet and Dentener-Kikkert, 1982).

Specific whey protein impacts on a cheese protein matrix were demonstrated by Schorsch et al. (2001). These researchers showed that when whey proteins were double-heated (first heated to 80°C alone, then heated a second time to 20°C, 60°C, 80°C, and 90°C in the presence of casein), the disulfide bonds in whey protein aggregates were disrupted during the first heating, which allowed more interactions with κ -caseins on the CM during the second heating. When gels were made from these heated mixtures, gel strength significantly varied. These research results could be extrapolated to the spray drying and subsequent high-temperature cheese production

process used in acid-set cheese manufacture when ultrafiltered milk products are used to standardize cheesemilk. Since microfiltration removes nearly all whey proteins, a different casein-to-whey protein ratio is achieved when using MCC to standardize cheesemilk to a specific casein-to-fat ratio. Thus, a potentially different cheese protein matrix could be expected in an acid-set cheese where there is a reduced amount of denatured whey proteins available for interaction.

Ultimately, a large consensus exists that although using a product like MCC to standardize cheesemilk could cause adjustments in the cheese manufacturing process and the resulting cheese functionality, it will provide many benefits for the cheese industry (Burrington, 2013; Caron et al., 1997; Everett, 2007; Mistry and Pulgar, 1996; Saboya and Maubois, 2000). Thus, as the demand for whey proteins continues to grow in other capacities outside of the cheese industry (Hoogwegt Group, 2019), this research aims to investigate the role of whey proteins in the functionality of an acid-set cheese using Queso Blanco as a model system.

1.7 References

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Chapter 2 - Research Question

Do the presence and amount of whey proteins in cheesemilk used to manufacture an acid-set cheese, such as Queso Blanco, impact the functional properties of the resulting cheese so that noticeable differences are observed in the final cheese texture, flavor, and color?

Chapter 3 - Textural Properties of Acid-set Queso Blanco Cheese Made with Filtered Milk Products

3.1 Introduction

The demand for native whey proteins, often found in health-forward foods like protein shakes and bars, has risen annually at a rate of 6 to 7% (Hoogwegt Group, 2019). This is likely, in part, because whey proteins provide essential amino acids and have one of the highest biological values of all food proteins (Wijayanti et al., 2014). To produce desired dairy-based ingredients containing native whey proteins, microfiltration is used to separate and remove whey proteins from skim milk (Burrington, 2013). The resulting skim milk retentate, without whey proteins, can then be used to produce a common microfiltration product, Micellar Casein Concentrate (**MCC**). MCC contains ~90% total protein content with at least 95% of the protein content strictly comprised of casein proteins (Mistry, 2002) since the whey proteins flow into the permeate stream during the microfiltration process. This casein-to-whey protein ratio is what differentiates MCC from milk ingredients produced via ultrafiltration, like Milk Protein Concentrate 80 (**MPC**) and Milk Protein Isolate 90 (**MPI**). Ultrafiltration retains whey proteins in the retentate with the casein proteins due to smaller pore size filters (Burrington, 2013). Thus, the typical 80% casein to 20% whey protein ratio naturally found in milk is retained in these ultrafiltered milk products. In the case of MPC and MPI, the proteins are concentrated (typically to at least 80% and 90% protein on a dry basis, respectively) as some water, lactose, and minerals are removed during the ultrafiltration process (Burrington, 2013).

Standardizing cheesemilk with ultrafiltered milk ingredients like MPC and MPI is common in the cheese industry (Burrington, 2013). Standardization improves cheese quality and

consistency by reducing variability in seasonal milk supplies as well as increasing the economic gains for cheese producers by increasing yields and reducing production time (Lucey, 2002; Gaiani et al., 2009). Therefore, exploring the use of microfiltration technology to standardize cheesemilk in similar ways to that of ultrafiltration technology has been of interest since studies have shown the use of MCC in cheesemilk standardization can not only increase cheese yield and throughput in line with ultrafiltered milk powders but also improve the functionality of the resulting cheese (Mistry, 2002; Burrington, 2013). Specifically, standardizing cheesemilk with MCC for Cheddar and Mozzarella cheeses has shown to produce an increase in net revenue of \$0.40+ per 0.45 kg of cheese, based in 45 kg of standardized milk (Papadatos et al., 2003). Another economic gain also realized with microfiltration technology is the major co-product, the whey proteins. Manufacture of microfiltered milk products like MCC allows for whey proteins to be removed from milk in their more native state prior to cheese production. This is opposed to using ultrafiltered milk products like MPC or MPI, where the whey proteins are typically extracted from the cheese whey post-cheese production. These whey proteins have been subjected to the harsh conditions of cheese manufacture, and require purification techniques, often resulting in protein denaturation (Burrington, 2013). Native whey proteins exhibit increased foaming, emulsifying, and solubility properties compared with whey proteins extracted from whey post-cheese manufacture (Burrington, 2013), enhancing their ingredient functionality in health-forward foods which continues to drive the increased desire for the use of MCC in cheesemilk standardization.

Using MCC in acid-set cheese manufacture specifically, however, creates a particular dilemma, as whey proteins are critical to the structural protein matrix of the acid-set cheese. In acid-set cheeses, whey proteins like β -lactoglobulin are denatured during the heating ($>70^{\circ}\text{C}$) of

the cheesemilk (Oldfield et al., 1998). During denaturation, the whey proteins unfold, exposing interior amino acids that then bond to aggregate proteins further (Mulvihill and Donovan, 1987; de Wit, 1990). Aggregation between exposed hydrophobic groups on the denatured β -lactoglobulin and κ -casein proteins, specifically, has been suggested to result in filamentous appendages that develop on the micelle surface (Harwalkar and Kalab, 1981) driven by acidification during cheesemake to at least $<\text{pH } 6.7$ but preferably $<\text{pH } 6.5$ for maximum aggregation (Anema, 1998; Oldfield et al., 2000; Vasbinder and Kruif, 2003). Hence, covalent disulfide bonds between κ -casein and denatured β -lactoglobulin, as well as additional non-covalent hydrophobic interactions, are the primary mechanisms that support this aggregation (Doi, et al., 1983; Singh, 1995; Smits and van Brouwershaven, 1980; Cho et al., 2003). However, the dominant mechanism that drives the interaction between these two primary milk proteins has yet to be agreed upon within the scientific community (Wijayanti et al., 2014). In acid-set cheese, these bonds are reported to contribute to a unique ‘core-and-lining’ structure of fused casein micelles in contrast to enzyme-set cheeses, which typically exhibit a structure of distinguishable casein proteins bonded together in chains and clusters (Harwalkar and Kalab, 1981; Kalab and Modler, 1985).

The acid-set cheese, Queso Blanco, is a Hispanic-style cheese produced with simple acids like vinegar or lemon juice to coagulate cheese curd (Tunick et al., 2012a). Queso Blanco will have a dense protein microstructure due to the increased agglomeration of whey proteins with casein micelles, a chemical interaction driven by the heating and acidification processes experienced during acid-set Queso Blanco cheese production (Schorsch et al., 2001; Kalab and Harwalkar, 1973). Farkye et al. (1995) reported that certain acidifying agents used to make Queso Blanco, in particular acetic and citric acids, increased casein aggregation, which impacted

the protein microstructure, in turn affecting the textural and organoleptic functionalities of the resulting cheese. Citric acid, a strong chelator of calcium, is recommended for Queso Blanco cheese manufacture due to its low cost and more critically, its low impact on cheese flavor (Chandan et al., 1979). Because acid-set Queso Blanco cheese is made without starter cultures, it typically does not endure any significant proteolysis during storage, as seen in enzyme-set cheeses; this has a large impact on the functionality of the cheese, including cheese rigidity and viscoelasticity (Lucey et al., 2003), because functional properties are generally dictated by cheese protein microstructure (Everett and Auty, 2008). As a result, Queso Blanco is expected to achieve at least eight weeks of shelf life with minimal changes in cheese functionality and quality (Tunick et al., 2012b).

To assess the impacts of whey proteins in acid-set cheese production, a uniform comparison between full and reduced availability of whey proteins to agglomerate within the cheese protein matrix is needed. Using Queso Blanco cheese as a model system, this research aims to investigate the functional contribution of whey proteins on the texture of acid-set cheeses by comparing cheese produced with cheesemilk standardized with ultrafiltered milk products, MPC and MPI, and microfiltered milk product, MCC. All high-protein milk powders will undergo the same rehydration and standardization procedure; however, the primary difference in the resulting standardized cheesemilks will be the amount of available whey proteins that can ultimately contribute to the Queso Blanco cheese protein matrix.

3.2 Materials and Methods

Queso Blanco cheeses were produced with a uniform method based on a basic acid-set formulation. Three different high-protein milk powders: MPC (Milk Specialties Global, Eden Prairie, MN, USA), MPI (Milk Specialties Global) and MCC (Milk Specialties Global), were obtained, rehydrated, and incorporated into raw milk to standardize cheesemilk. For each replication, fresh milk powders (targeting <60 days from production date) were obtained and used for cheese manufacture to minimize the impact of storage on the rehydration ability of the powders (Gaiani et al., 2007; Gazi and Huppertz, 2015). Periodically over the course of 90 days of ripening, cheeses were assessed mechanically by texture profile analysis (**TPA**) and organoleptically by a trained descriptive analysis panel (**DAP**).

3.2.1 Cheesemilk Components: Estimations

Incoming raw milk was tested for protein and fat contents via a Milkoscan MarsTM FTIR spectrometer (FOSS Analytical A/S, Hillerød, Denmark). Raw milk results are in Appendix A. Total protein, casein, whey protein and fat contents of the rehydrated high-protein milk dispersions were calculated using data from the individual Certificate of Analysis that accompanied each powder and are referred to as estimates. Certificate of Analysis documents for each high-protein powder are in Appendix B. These calculations were used to estimate the composition of the standardized cheesemilk to a 1.2:1 casein-to-fat ratio (Table 3.1). pH of the cheesemilk was tested with a calibrated FiveEasyTM F20 pH meter (Mettler-Toledo GmbH, Greifensee, Switzerland) after pasteurization and prior to cheesemaking (Table 3.1).

Table 3.1. Chemical profiles of Queso Blanco cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Item ²	Milk Powder ¹			SEM
	MCC	MPC	MPI	
Total Solids, %	12.67	12.76	12.75	0.34
Protein, %	4.38	4.54	4.67	0.19
Casein, %	3.92	3.73	3.80	0.13
Whey Protein, %	0.46 ^b	0.81 ^a	0.87 ^a	0.06
Fat, %	3.34	3.24	3.28	0.08
pH ³	6.69	6.70	6.69	0.02

^{a-b}Means (n = 3) in the same row with different superscripts differ ($P < 0.05$)

¹High-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Estimations: calculated using results from the tested raw milk (total protein and fat) data and the calculated casein, whey protein and fat data from the COA for each of the high-protein milk powders

³Measured

3.2.2 Cheese Production

Over the course of one year, three independent production replications (trials) were completed to manufacture the Queso Blanco cheeses. Cheese production records are in Appendix C. The day prior to cheese production, each high-protein milk powder, MPC, MPI, and MCC, was rehydrated in a 38-Liter stainless steel milk can (Nelson Jameson, Marshfield, WI, USA) with a Rotomixx 100RX stainless steel mixer (Admix Inc., Londonderry, NH, USA) at 1740 rpm for 10 minutes using $50 \pm 3^\circ\text{C}$ softened water. The rehydrated high-protein milk powders will be referred to as **R-MPC**, **R-MPI** and **R-MCC**, respectively. Rehydration occurred at 15% powder concentration while maintaining a dispersion temperature of $50 \pm 5^\circ\text{C}$ (Havea, 2006; Fang et al., 2010) via an electric heating jacket (Global Industrial, Port Washington, NY, USA) placed on the outside of each milk can. Rehydrated dispersions were covered and stored at $4 \pm 1^\circ\text{C}$ for 15

to 18 hours. Two 22.7 kg batches were made for each high-protein milk powder, R-MPC, R-MPI and R-MCC, respectively.

After rehydration of powders, R-MPC, R-MPI and R-MCC was added to raw milk (Baker Cheese Inc., St Cloud, WI, USA) in a 2650-Liter stainless steel bulk tank (A&B Process Systems Corp., Stratford, WI, USA) to formulate the standardized cheesemilk. For each trial, the amount of rehydrated dispersion used to produce the standardized cheesemilk varied (Table 3.2), as the end cheesemilk was adjusted to obtain a 1.2:1 casein-to-fat ratio (Hill and Parnell-Clunies, 1986). Each standardized cheesemilk was pasteurized at 76°C for 16 seconds via a high-temperature, short-time pasteurizer (Electrol Specialties Company, South Beloit, IL, USA) and then collected in one of four 230-L open, double-O stainless steel vats (Kusel Equipment Co., Watertown, WI, USA). The cheesemilk was heated to 85°C via the steam-injected jackets within each vat and held at $85 \pm 2^\circ\text{C}$ for five minutes (Parnell-Clunies et al., 1985). The steam was turned off and approximately 3.89g of citric acid (Jungbunzlauer Canada Inc., Port Colborne, Ontario, Canada) (Chandan et al., 1979) per 1 L of cheesemilk was measured to be added to each vat, respectively, via a 25% citric acid solution. The amount of citric acid added to each cheesemilk was based on an estimation of the buffer capacity potential (Hill et al., 1982) and acidity (Siapantas and Kosikowski, 1973) of the standardized cheesemilk. The respective amount of citric acid solution was further diluted with 1 kg water prior to cheesemilk addition to prevent localized coagulation in the vat. After 35 minutes, the vat was stirred while the whey drained from the curd. The curd was removed from the vat and weighed in a tote on a large platform scale (Fairbanks Scales, Kansas City, MO, USA). Cheese yield was calculated for each vat based on the amount of cheesemilk used compared to the amount of cheese curd produced. Salt (Cargill Inc., Minneapolis, MN, USA) was added at a ratio of 1500 g:45 kg salt-to-curd and applied in

three equal portions in five-minute intervals while the curd was stirred continuously by hand. Five round, plastic moulds (Kusel Equipment Co.) designed for 4.5 kg finished cheese wheels were filled with 5.4 kg of curd in each mould and the 15 moulds (five from each vat) were pressed simultaneously with a vertical quad press (Kusel Equipment Co.) at 20 psi for four hours at $22 \pm 4^\circ\text{C}$. The Queso Blanco cheese wheels were removed from the moulds and cheeses were vacuum-sealed into clear, 51 X 76 cm Cryovac bags (material #100719776, Sealed Air, Charlotte, NC, USA). The Queso Blanco cheeses were stored at $4 \pm 1^\circ\text{C}$ until analysis, which occurred at 10, 30, 50, 70 and 90 days. Testing days were uniformly spread to include the entire expected shelf life of Queso Blanco cheese, which is typically 8-12 weeks (Torres and Chandan, 1981; Tunick et al., 2012b).

Table 3.2. Standardized cheesemilks: amounts (kg) of raw milk and rehydrated high-protein milk powders needed to achieve 1.2:1 casein-to-fat ratio

Replication	Milk Powder ¹	Rehydrated Dispersion	Raw Milk	Total Cheesemilk ²
1	MCC	34	185	219
	MPC	34	149	184
	MPI	41	174	216
2	MCC	30	188	222
	MPC	36	175	215
	MPI	35	185	226
3	MCC	34	212	246
	MPC	39	183	224
	MPI	37	185	222

¹High-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Includes water added, if needed, to clear infeed piping of raw milk and/or rehydrated dispersion

3.2.3 Cheese Composition Analysis

Queso Blanco cheeses made with R-MPC, R-MPI and R-MCC, referred to as **QB-MPC**, **QB-MPI** and **QB-MCC**, respectively, were analyzed for compositional attributes in triplicate between 20 to 25 days. Tests included moisture content via a standard forced air vacuum oven (Method 15.114) and pH (Method 15.022) using a standard combination electrode after calibration at pH 4 and 7 with standardized solutions (Oakton Traceable, Cole-Parmer, Vernon Hills, IL, USA) as described in Wehr and Frank (2004). Fat content was assessed via Babcock (Method B6.1) as described by Marth (1978). Total salt content was calculated from sodium analysis using flame emission spectroscopy (AOAC OMA 990.23). Protein content was completed by Kjeldahl (AOAC OMA 991.20) and the nitrogen content converted to total protein using 6.38 (Barbano et al., 1990).

Microbiological analyses were performed on each cheese within 24 hours of production to ensure product safety for sensory assessments. Microbiological analyses included: coliform (AOAC OMA 989.10), *E.coli* (AOAC OMA 991.14), *Staphylococcus aureus* (AOAC OMA 2003.07), *Listeria monocytogenes* (AOAC OMA 2016.08), *Salmonella* spp. (AOAC 2016.01) and yeast and mold (AOAC OMA 997.02).

3.2.4 Texture Profile Analysis

QB-MPC, QB-MPI and QB-MCC cheeses were analyzed with a TA.XTPlus Texture Analyzer (Texture Technologies Co., Hamilton, MA, USA) using a 40 mm compression platen (Texture Technologies Co.) based on an imitative double-bite method with a 50% strain and 100

mm/minute crosshead speed (Tunick and Van Hekken, 2002; Caro et al., 2011). A standard double-bite macro software (Texture Technologies Co.) was used to calculate chewiness, cohesiveness, hardness, resilience, and springiness (Tunick, 2000). Definitions of these terms are presented in Table 3.3.

Table 3.3. Texture Profile Analysis (TPA) attribute definitions

Attribute	Definition ¹
Chewiness	Product of cohesiveness, hardness, and springiness
Cohesiveness	Ratio between the positive force area of the second compression compared to the first compression
Hardness	Maximum force of the first compression
Resilience	Percentage of the energy needed to remove the platen divided by the energy needed to apply the platen of the first compression
Springiness	Percentage of cheese height recovery between the first and second compression

¹As defined by Texture Technologies Corp. and Stable Micro Systems, Ltd. 2020

Each cheese wheel was cut to obtain four cylindrical cheese plugs 30 (dia) X 15 (h) $\pm$ 2 mm using a 30 mm metal ring (Rambler Concepts, Boulder, CO, USA) at specific locations on each cheese wheel (Figure 3.1). Previous research has shown that structural orientation of Queso Fresco-type cheese did not affect TPA results (Hwang and Gunasekaran, 2001); therefore, all four cheese plugs were replicates ignoring cheese plug orientation. Once the cheese plugs were obtained, they were stored at $22 \pm 1^\circ\text{C}$ in a covered 90 (dia) X 18 (h) mm glass petri dish (Pyrex, Corning, NY, USA) until testing. Cheeses were assessed at 10, 30, 50, 70 and 90 days.

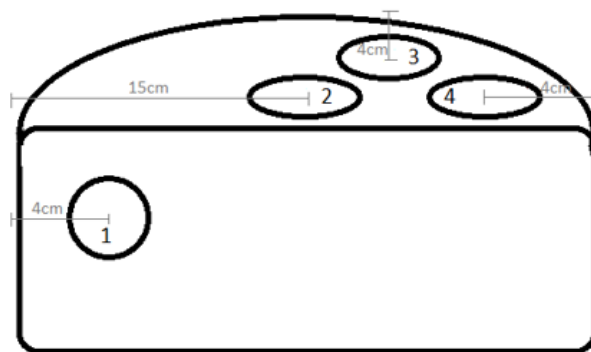


Figure 3.1. Sampling locations for plug extraction of Queso Blanco cheese wheels

3.2.5 Sensory Analysis

Organoleptic analysis with a trained descriptive panel ($n = 5$) was completed at 30 and 70 days for QB-MPC, QB-MPI and QB-MCC cheeses as described by Jimenez-Maroto et al. (2016). The panelists are part of an established panel, who have received training regularly for more than five years on the Spectrum method (Meilgaard et al., 2007) for quantitative descriptions of hand and mouth texture. Cheeses were cut into 2-cm^3 cubes two to four hours prior to evaluation. Five to six cheese cubes were placed in covered 113 g plastic containers (material #EPC400, Empress Products, Nesconset, NY, USA) labeled with a randomized three-digit code and stored at 4°C until evaluation. Each panelist received QB-MCC, QB-MPI and QB-MPC in a randomized order to assess once. Panelists used a standard, anchored, 15-point line scale (RedJade®, Martinez, CA, USA), to determine hand firmness, hand springiness, first bite firmness, adhesiveness, cohesiveness, rate of breakdown and smoothness (Table 3.4) in this sequence, respectively. The sensory ballot used for assessment is in Appendix D. Carrots and grapes were available to panelists during tasting to cleanse palates in between samples.

Table 3.4. Attribute definitions and techniques for cheese used by the descriptive analysis panel (DAP)

Attribute	Definition ¹	Technique ¹
Adhesiveness	Degree to which the chewed sample sticks to the teeth and mouth surfaces	Chew the sample 15-20 times using your molars. Observe how the sample sticks to mouth surfaces
Cohesiveness	How well the sample holds together during manipulation	Work the sample between the thumb and first two fingers 5-7 times and observe how the sample breaks apart
Firmness, Hand	Force required to compress the sample	Using the thumb and index finger, press the sample and evaluate the strength required to compress the sample
Firmness, Mouth	Force needed to bite through the sample for the first time	Bite the sample using your molars. Observe how much strength is needed to bite through the cheese
Rate of Breakdown	How quickly the sample breaks apart while chewing	Chew the sample 20 times using your molars and observe how quickly the chewed mass disappears from your tongue
Smoothness	Degree of smoothness of the surface of the chewed mass	Chew the sample 20 times using your molars and observe how smooth the chewed mass is.
Springiness	Total amount of recovery of the sample after being compressed	After compressing the sample, observe how much it recovers its original shape

¹As defined by Jimenez-Maroto et al. (2016)

3.2.6 Statistical Analysis

Three cheeses, QB-MPC, QB-MPI and QB-MCC, were produced on the same day. This trial was replicated three times over the course of 12 months. Cheeses were assessed at various times during storage up to 90 days. Reported measurements were averages of at least triplicate test results and were used for analysis. Data were analyzed with SAS® VIYA® software (SAS Institute Inc., Cary, NC, USA) using a univariate split-plot design at $P < 0.05$ to determine the differences between samples. For DAP and TPA, cheese (QB-MPC, QB-MPI or QB-MCC) was the whole-plot while time (30 and 70 days or 10, 30, 50, 70 and 90 days, respectively) was the sub-plot. A Tukey's pairwise comparison was used for each assessment at a 95% confidence level to determine significant differences between individual means. The respective SAS® code used for analysis is in Appendix E.

3.3 Results and Discussion

3.3.1 Cheese Composition and Microbial Analysis

Queso Blanco cheese does not have a standard of identity within the United States. However, QB-MPC, QB-MPI and QB-MCC, aligned with previously published Queso Blanco compositional data (22 to 25% protein and 18 to 20% fat (Siapantas, 1981; Hill and Parnell-Clunies, 1986)) except for minimally elevated protein and reduced fat contents (26.5% and 15.1% on average, respectively). Mistry and Pulgar (1996) reported a 4% increase in protein and approximately 6% decrease in fat when Gouda cheese was produced with a high-protein (> 85%)

milk powder. As expected, the QB-MPC and QB-MPI had higher protein contents than did QB-MCC (Table 3.5) since the cheesemilk standardized with R-MCC had an estimated whey protein content approximately 50% less than the cheesemilk standardized with R-MPI or R-MPC (Table 3.1). There were also significant differences in fat, on a dry basis specifically, where QB-MCC (32.2%) was significantly higher in fat, on a dry basis, than QB-MPC (31.0%) which was significantly higher in fat, on a dry basis, than QB-MPI (29.7%). However, samples were not significantly different for moisture, fat, salt, or pH overall (Table 3.5). Raw data for composition analysis of Queso Blanco cheeses is in Appendix F. There were no differences in cheese yield ($18.4\% \pm 0.8\%$ on average) when using R-MPC, R-MPI or R-MCC to standardize the cheesemilk. Yield results for all Queso Blanco cheeses are in Appendix G.

Table 3.5. Proximate composition of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Item	Cheese ¹			SEM
	QB-MCC	QB-MPC	QB-MPI	
Moisture, %	51.5	51.5	50.5	1.04
Protein, %	25.5 ^b	26.8 ^a	27.3 ^a	0.22
Protein, Dry Basis, %	52.6 ^b	55.2 ^a	55.2 ^a	1.08
Fat, %	15.6	15.1	14.7	0.28
Fat, Dry Basis, %	32.2 ^a	31.0 ^b	29.7 ^c	0.22
pH	5.34	5.43	5.38	0.00
Salt, %	2.4	2.3	2.1	0.04

^{a-c}Means (n = 3) in the same row with different superscripts differ ($P < 0.10$)

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Microbiological results showed that QB-MPC, QB-MPI and QB-MCC had < 10 cfu/g for coliform, *E.coli*, *Staphylococcus aureus*, and yeast and mold, respectively. *Listeria monocytogenes* and *Salmonella* spp. were not detected in any of the cheeses.

3.3.2 Texture Profile Analysis

From the TPA results, Queso Blanco cheeses did not differ during storage for any of the textural attributes assessed. Yet, other studies have shown that the hardness of Queso Blanco cheese significantly increased 37% and 56% over 17 and 49 days, respectively (Parnell-Clunies et al., 1985; Farkye et al., 1995). Farkye et al. (1995) stated that this phenomenon should be explored as no reasonable explanation could be given for an increased hardness with age in acid-set cheese. Further, Farkye et al. (1995) reported that Queso Blanco chewiness and springiness, regardless of the acid used to coagulate the curd, were impacted, whereas cohesiveness was not significantly impacted, during seven weeks of storage. In Queso Fresco, the enzyme-set version of Queso Blanco, chewiness and springiness decreased over eight weeks of storage, but during that time, hardness and cohesiveness remained constant though SDS-PAGE analysis indicated proteolysis occurred in the cheese, likely due to spoilage organisms (Tunick et al., 2012a; Tunick et al., 2012b). When studying Panela cheese, a fresh Hispanic cheese that, similarly to Queso Blanco, does not use starter cultures to acidify the cheesemilk, Guerra-Martinez et al. (2012) reported similar trends for hardness, yet chewiness also remained constant during 15 days of storage, while cohesiveness and springiness fluctuated in non-consistent fashions, reporting ranges between 0.62 to 0.84N and 0.81 to 0.95N, respectively. Ultimately, minimal agreement exists as to the expected textural behavior of acid-set cheeses over storage with exception that most agree limited proteolysis and lipolysis are expected in acid-set cheeses, hence, drastic textural changes should not be observed over time (Farkye et al., 1995; Lucey et al., 2003).

When removing the effect of days, Queso Blanco cheeses did not differ in hardness (32.1kg of force on average) overall (Table 3.6). The lack of differentiation in cheese hardness

was unexpected as prior research has shown that differences in cheese protein contents (Table 3.5) could impact cheese texture, including cheese hardness. Specifically, Schorsch et al. (2001) observed that the presence of whey protein-casein micelle complexes (which would be expected in QB-MPI and QB-MPC but limited in QB-MCC) resulted in an increased gel strength compared with gels containing no whey protein and casein micelle aggregation because the milk proteins were not subjected to conditions, like heat, that would influence them to aggregate. When Chen et al. (1979) rated the factors responsible for cheese texture across a wide variety of cheeses, the most impactful factor was total protein content in the cheese, followed by salt content, water content, cheese pH and lastly, total fat content. In a slightly differing opinion, Guerra-Martinez et al. (2012) reported that the hardness and chewiness of Panela cheese specifically were most highly correlated with moisture content, not protein or salt contents. This could explain the similar hardness quality, as QB-MPC, QB-MPI and QB-MCC had equivalent moisture contents, even if significant differences in protein content were seen (Table 3.5).

Table 3.6. Mean scores for Texture Profile Analysis (TPA) attributes of Queso Blanco cheeses made from cheesemilk standardize with rehydrated high-protein milk powders, assessed over 90 days

Attribute	Cheese ¹			SEM
	QB-MCC	QB-MPC	QB-MPI	
Chewiness	16.86 ^b	20.48 ^{ab}	22.18 ^a	1.68
Cohesiveness	0.68 ^b	0.72 ^a	0.71 ^a	0.01
Hardness, kg	28.80	32.24	35.24	2.42
Resilience, %	26.92 ^b	30.38 ^a	30.09 ^a	0.54
Springiness, %	86.01 ^b	87.37 ^a	88.00 ^a	0.47

^{a-b}Means (n = 15) in the same row with different superscripts differ ($P < 0.05$)

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Although cheese hardness did not differ significantly on average, other textural attributes did. QB-MCC had significantly less cohesiveness, springiness and resilience compared to QB-MPC and QB-MPI (Table 3.6), suggesting that cheesemilk standardized with R-MCC may result in Queso Blanco cheese that is crumblier than cheese produced from cheesemilk standardized with R-MPC or R-MPI. This discrepancy in cheeses mimics the pattern of significant differences identified in the protein content of the respective cheeses (Table 3.5) and more specifically, the estimated whey protein contents of the respective cheeses (Table 3.1). Perreault et al. (2017) reported that denatured whey protein concentrate contributed to the complex modulus (G^*) and the loss tangent ($\tan \delta$) of semi-hard cheeses similarly as did casein proteins. Schorsch et al. (2001) showed through rheological and microscopic methods that double-heated whey proteins had disrupted the disulfide bonds within whey protein aggregates, increasing their availability to interact with κ -caseins on the casein micelles instead, contributing to the cheese protein matrix. The double heating of whey proteins can be extrapolated to the present study where whey proteins are first heated during spray drying to produce MPI and MPC, and then again during the high-heat processing in acid-set cheese production to manufacture QB-MPI and QB-MPC. This suggests that the increased amount of denatured whey proteins in the cheesemilks (Table 3.1) can increase the protein content of the cheese (Table 3.5) and ultimately impact the textural properties of the resulting Queso Blanco cheese. More specifically, the inclusion of whey proteins in a cheese protein matrix may contribute to less crumbly acid-set cheese. Conversely, Li et al. (2020) presented the findings that Cheddar cheese manufactured with MCC had greater springiness and cohesiveness compared with Cheddar cheese manufactured with skim milk; however, both Cheddar cheeses exhibited springiness similar to Cheddar cheese manufactured from low-heat skim milk powder. The greater springiness and cohesiveness of the Cheddar

cheese produced with MCC was attributed to the significantly higher pH (5.28 on average) compared to the Cheddar cheese manufactured with skim milk (5.08 on average) or low-heat skim milk powder (5.15 on average) (Li et al., 2020). Raw data for TPA results of Queso Blanco cheeses is in Appendix F.

Considering the last textural attribute assessed via TPA, chewiness (a highly correlating derivative of hardness (Guerra-Martinez et al., 2012), and the product of hardness, cohesiveness and springiness) differed among cheeses. QB-MCC was significantly less chewy than QB-MPI, but QB-MPC was not significantly different from QB-MCC or QB-MPI (Table 3.6). Considering the chemical composition of the Queso Blanco cheeses (Table 3.5), the combination of the protein content and fat content, on a dry basis (**FDB**), may provide insight into the discrepancies in chewiness. QB-MPI had a significantly greater protein content (27.3%) and significantly less FDB content (29.7%) compared with QB-MCC (25.5% and 32.2%, respectively) while QB-MPC was similar in protein content (26.8%) to QB-MPI but was significantly different from both QB-MPI and QB-MCC for FDB (31.0%). The discrepancies in protein content in combination with the significant differences for FDB may explain why chewiness for QB-MPC was not significantly different from either QB-MCC or QB-MPI, even though fat content is expected to have a minimal impact on cheese texture (Chen et al., 1979). Conversely, understanding chewiness is a product of hardness, cohesiveness and springiness, minor differences within each of these textural attributes can be intensified when they are multiplied together and therefore could be affecting the differences seen between cheeses, not cheese composition. Further investigation would be needed to fully understand the chewiness results as to why QB-MPC is similar to both other cheeses and yet QB-MCC and QB-MPI are significantly different from each other. Ultimately, more work is needed to understand the correlations between cheese

composition and cheese texture when producing cheese using standardized milk with increased protein content as was done with Cheddar (Oommen et al., 2000) and Oaxaca cheese (Caro et al., 2011).

3.3.3 Sensory Analysis

The Queso Blanco cheeses had similar sensory profiles for texture (Table 3.7). Raw data for sensory analysis of Queso Blanco cheeses are in Appendix F. Siapantas and Kosikowski (1967) reported that Queso Blanco cheeses produced with similar levels of acetic acid were hard and brittle in texture. These texture attributes are demonstrated by QB-MPC, QB-MPI and QB-MCC with high hand and mouth firmness values but low cohesiveness values, respectively (Table 3.7). It can be extrapolated from Farkye et al. (1995) that texture of Queso Blanco cheese made with citric acid may not change over time since acceptance tests showed that changes in flavor likely determined cheese acceptance during ripening. The lack of organoleptic differentiation between QB-MPC, QB-MPI and QB-MCC might be due to the limited lipolysis Queso Blanco cheese undergoes during aging as high temperatures during acid-set cheese manufacture inactivate lipase (Torres and Chandan, 1981) altering the texture and flavor of the cheese. As the acid-set process eliminates the need for starter cultures, less degradation of the protein network via proteolysis (Lucey et al., 2003; Tunick et al., 2012a) would be anticipated, further limiting potential texture and flavor changes in the cheese.

Table 3.7. Mean scores for sensory attributes of Queso Blanco cheese made from cheesemilk standardized with rehydrated high-protein milk powders, assessed at 30 and 70 days on an anchored 15-point line scale

Attribute	Cheese ^{1,2}			SEM
	QB-MCC	QB-MPC	QB-MPI	
Adhesiveness	6.6	5.9	6.6	0.8
Cohesiveness	4.3	3.8	4.1	0.8
Firmness, Hand	8.3	8.4	9.3	0.6
Firmness, Mouth	8.1	8.6	9.1	0.6
Rate of Breakdown	4.6	4.0	4.8	0.4
Smoothness	3.3	3.0	3.1	0.3
Springiness	8.1	7.7	8.2	0.4

¹No significant differences (n = 6) were identified between any treatments for any attribute at $P < 0.05$

²High-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

3.4 Conclusion

This research focused on comparing the resulting textural properties of acid-set cheese, using Queso Blanco cheese as a model system, when produced with ultrafiltered and microfiltered milk products. It was shown that cheese composition targets, like moisture, salt and pH can be achieved using either type of high-protein milk powder ingredient. This is a benefit for acid-set cheese producers who may need to resort to using whey-protein-depleted microfiltered milk products, like MCC, in the future as the demand for native whey proteins continues to rise. The increased protein values of QB-MCC, QB-MPI and QB-MPC showed, however, that process adjustments may be needed at cheese manufactures if cheese protein targets are to be maintained at current levels. Additionally, high cheese yields seen with ultrafiltered cheesemilk standardization can be maintained when producing cheese with microfiltered milk products.

The use of microfiltered milk products like MCC in Queso Blanco cheese production is not expected to impact consumer textural perceptions of the resulting cheese, which is another benefit for acid-set cheese producers who wish to maintain an established consumer base. The reported lack of change in organoleptic perceptions, including cheese flavor (Chapter 4), over time for Queso Blanco cheese produced with high-protein milk powders can provide a reassurance for acid-set cheese producers that may be concerned about diminished cheese shelf life. Exploration into potential microstructure changes between acid-set Queso Blanco cheese produced with ultrafiltered and microfiltered milk products could further support the use of microfiltered milk products in the standardization of cheesemilk for acid-set cheese production.

The mechanical texture differences noted with the TPA data, however, may require production modifications at cheese converters that would look to create reduced formats of acid-set Queso Blanco, like shreds or crumbles, common to the U.S. marketplace. This could have a potential negative cost impact for conversion operations which would warrant further understanding of acid-set cheese performance beyond initial textural assessments.

3.5 References

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Chapter 4 - Color and Flavor Properties of Acid-set Queso Blanco Cheese Made with Filtered Milk Products

4.1 Introduction

Chapter 4 provides color and flavor results of research conducted on the same Queso Blanco cheeses described in Chapter 3. **QB-MPC**, **QB-MPI** and **QB-MCC** refer to Queso Blanco cheese produced from standardized cheesemilk using rehydrated Milk Protein Concentrate 80 (**MPC**), Milk Protein Isolate 90 (**MPI**), or Micellar Casein Concentrate (**MCC**), respectively. For this chapter, the research question is expanded to determine the functional contribution of whey proteins on color and flavor properties of acid-set cheese using Queso Blanco as a model system. To do this, cheesemilk was standardized with rehydrated ultrafiltered milk products traditional to the cheese industry, MPC and MPI, and compared with cheesemilk standardized with rehydrated microfiltered MCC, a novel ingredient for cheese production.

4.2 Materials and Methods

The production, proximate analysis, and storage of Queso Blanco cheeses were described in Chapter 3. Cheeses were evaluated for color and flavor during 90 days of storage using mechanical and organoleptic assessments, respectively.

4.2.1 Color Analyses

Color analyses of QB-MPC, QB-MPI and QB-MCC were completed in triplicate using an IRIS Visual Analyzer 400 (Alpha MOS, Hanover, MD, USA) at 10, 30, 50, 70 and 90 days. The analyzer measures color via the CIELAB color space: L^* (whiteness), a^* (intensity of red to green), and b^* (intensity of yellow to blue). Standard calibration to ensure accurate exposure of the analyzer camera was completed each test day with an X-Rite ColorChecker® Passport Photo (X-Rite Inc., Grand Rapids, MI, USA). Data were used to calculate hue angle, h° (Equation 1) and relative saturation, chroma, C^* (Equation 2).

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (1)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (2)$$

For color analysis, three cylindrical 50 (dia) X 10 (h) $\pm$ 2mm cheese discs were cut from each cheese wheel using a 50 mm metal ring (Rambler Concepts, Boulder, CO, USA) (Figure 4.1). Individually, the cheese discs were placed in the center of a 90 X 18 mm glass petri dish (Pyrex, Corning, NY, USA), covered and stored at $22 \pm 1^\circ\text{C}$ until testing.

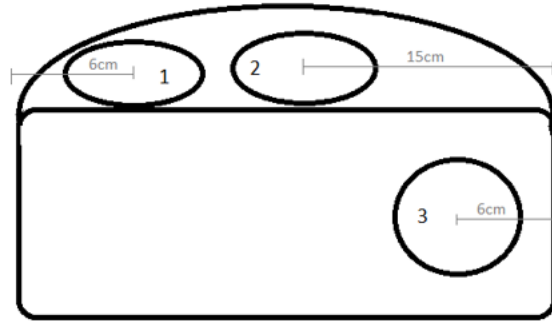


Figure 4.1. Sampling procedure to assess Queso Blanco cheese wheels for color analyses

For testing, each disc was removed from the petri dish with a metal spatula and placed into the visual analyzer to determine L^* , a^* and b^* . The cheese disc was returned to the petri dish, covered, and placed in a pre-heated $232 \pm 2^\circ\text{C}$ Shel Lab SMO5 oven (Sheldon Manufacturing Inc., Cornelius, OR, USA) for five minutes to mimic broiling conditions (Guo et al., 2011; Tunick et al. 2012a; Tunick et al., 2012b). The heated cheese disc was removed from the oven, cooled for ten minutes at $22 \pm 4^\circ\text{C}$, placed in the visual analyzer and assessed for color post-heating. Color change was defined as the total color difference, ΔE (Equation 3), from pre- to post-heating.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

4.2.2 Sensory Analysis

The same trained descriptive panel ($n = 5$) who evaluated QB-MPC, QB-MPI and QB-MCC texture (Chapter 3) assessed cheese flavor at 30 and 70 days. The participants are part of

an established descriptive analysis panel (**DAP**), who have received training regularly for more than 5 years on the Spectrum method (Meilgaard et al., 2007) for quantitative descriptions of the five basic tastes as well as other basic cheese flavors.

Cheeses were cut into 2-cm³ cubes two to four hours prior to evaluation. Five to six cheese cubes were placed in covered 113 g plastic containers (material #EPC400, Empress Products, Nesconset, NY, USA) labeled with a randomized three-digit code and stored at 4 °C until evaluation. Each panelist received QB-MPC, QB-MPI and QB-MCC in a randomized order to assess once. Panelists assessed texture first (Chapter 3), and then used a standard, anchored, 15-point line scale (RedJade®, Martinez, CA, USA), to rate the intensity of salty, sour, umami, sweet, bitter, and milky flavors (Table 4.1) in this sequence, respectively. Carrots and grapes were available to panelists during tasting to cleanse palates in between samples. The ballot used for organoleptic analysis can be found in Appendix D.

Table 4.1. Organoleptic attribute definitions used to assess Queso Blanco cheese

Attribute	Definition ¹
Bitter	Basic taste sensation elicited by caffeine or quinine
Milky	Aromatics associated with creamy, milky products
Salty	Basic taste sensation elicited by salt
Sour	Basic taste sensation elicited by acids
Sweet	Basic taste sensation elicited by sugars
Umami	Basic taste sensation generated by certain peptides or nucleotides

¹As defined by Jimenez-Maroto et al. (2016)

4.2.3 Statistical Analysis

Data were analyzed with SAS® VIYA® software (SAS Institute Inc., Cary, NC, USA) using a univariate split-plot design at $P < 0.05$ to determine the differences between samples and test days. Cheese (QB-MPC, QB-MPI or QB-MCC) was the whole-plot while time (30 or 70 days (flavor) and 10, 30, 50, 70 or 90 days (color)) was the sub-plot. A Tukey's pairwise comparison was used for each assessment at a 95% confidence level to determine significant differences between individual means. The respective SAS® code used for color and flavor analysis is in Appendix E.

4.3 Results and Discussion

4.3.1 Color Analyses

QB-MPC, QB-MPI and QB-MCC were similar in the degree of red-green (a^*) and yellow-blue (b^*) color, as well as hue (h°) and chroma (C^*), but the cheeses differed in whiteness (L^*) (Table 4.2). QB-MCC was more white than QB-MPI, with QB-MPC being similar in whiteness to QB-MCC and QB-MPI. Raw color data are in Appendix F. Li et al. (2020) reported that Cheddar cheese made from reconstituted MCC, without the addition of a colorant (e.g., annatto), exhibited less whiteness (L^*) than Cheddar cheese made with skim milk or whole milk. On the other hand, Caro et al. (2011) reported that when skim milk or MPC was used to standardize cheesemilk for Oaxaca cheese, no differences in Oaxaca cheese color was observed when compared to the Oaxaca cheese made with whole milk.

Table 4.2. Mean scores for color analysis (CIELAB color space) attributes of Queso Blanco cheese made from cheesemilks standardized with rehydrated high-protein milk powders, assessed over 90 days

Item	Cheese ¹			SEM
	QB-MCC	QB-MPC	QB-MPI	
<i>L</i> *	88.38 ^a	87.60 ^{ab}	87.46 ^b	1.23
<i>a</i> *	0.52	0.62	0.11	0.69
<i>b</i> *	21.46	22.40	22.01	0.81
Hue angle, <i>h</i> °	0.36	0.08	0.10	0.89
Chroma, <i>C</i> *	21.53	22.50	22.08	0.81
Total color difference, ΔE	5.20	4.76	5.01	0.50

^{a-b}Means (n = 15) in the same row with different superscripts differ ($P < 0.05$)

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Cheese color can be influenced by acid development during cheese manufacture. Johnson (1999) reported that the pH affects the density of the caseins in the cheese protein matrix by altering the hydrophobic interactions between casein micelles, which in turn, affects the amount of water absorbed within the cheese protein matrix. These associations impact the reflected light and ultimately, cheese color (including whiteness). In Queso Fresco, limited acid is developed, which allows casein micelles to form tightly bound clusters and reflect the most amount of light, resulting in a bright white appearance (Johnson, 1999). Additionally, Johnson (1999) reported that fat globules also can reflect large amounts of light contributing to whiter cheese, albeit substantial amounts of fat can reflect more yellow color than white color. This chemistry seems to explain the *L** results, as QB-MPC, QB-MPI and QB-MCC skewed to the bright white end of the *L** spectrum (*L** >80), and all cheese resulted with a pH >5.3 which is a higher pH than expected for rennet-set cheeses where more acid is developed due to the addition of starter cultures.

However, it is important to consider the combination of protein and fat contents in these cheeses, specifically the estimated whey protein contents, as it concerns the discrepancies

identified for cheese whiteness (L^*) (Table 4.2). The reduced estimated whey protein content in QB-MCC (Table 3.1) would cause increased density of casein micelles within the cheese protein matrix, increasing the whiteness of the cheese. Conversely, the presence of more whey proteins expected in the QB-MPI and QB-MPC cheeses (Table 3.1) may cause less casein micelle bonding, reducing the total amount of light reflected, and resulting in a lower level of whiteness (L^*). As QB-MPC exhibited similar whiteness (L^*) to QB-MCC and QB-MPI however, it may be necessary to also consider the fat content in these cheeses (Table 3.5). The fat content, on a dry basis (**FDB**), of QB-MPC (31.0%) was significantly less than the FDB of QB-MCC (32.2%) but was significantly more than the FDB of QB-MPI (29.7%), which when coupled with the protein content, could explain why QB-MPC reflected similar amounts of light as did QB-MCC and QB-MPI. Ultimately, more research into the Queso Blanco cheese protein matrix may elucidate this structure-color relationship.

Comparing the color change attributed to broiling (ΔE), QB-MPC, QB-MPI and QB-MCC did not exhibit differences (Table 4.2). However, differences were observed for cheeses as they aged (Table 4.3). The cheeses displayed the same general trend where, on average, ΔE increased significantly by 1.5X from 30 to 90 days and 3.5X from 10 to 90 days. QB-MPC and QB-MPI exhibited significant differences from day 10 compared with days 50, 70 and 90 for L^* and b^* , becoming darker and more yellow after heating with age, respectively. Post-heating CIELAB color space results of cheeses are in Appendix H. Statistically, the individual readings for a^* were not affected by day for any of the cheeses. Interestingly, QB-MCC also did not show any significant changes during storage for L^* nor b^* either, which makes the gradual ΔE during storage (Table 4.3) all the more perplexing.

Table 4.3. Total color difference (ΔE)¹ of Queso Blanco cheeses made from cheesemilks standardized with rehydrated high-protein milk powders, from before to after broiling², assessed at 10, 30, 50, 70 and 90 days

Cheese ³	Days					SEM
	10	30	50	70	90	
QB-MCC	3.18 ^c	4.05 ^{bc}	5.11 ^{abc}	6.68 ^{ab}	6.98 ^a	0.71
QB-MPC	1.40 ^b	4.35 ^a	4.85 ^a	6.18 ^a	7.01 ^a	0.71
QB-MPI	0.91 ^b	4.58 ^a	5.79 ^a	7.11 ^a	6.66 ^a	0.71

^{a-c}Means (n = 3) in the same row with different superscripts differ (P < 0.05)

¹Defined in Equation 3

²Broiling conditions defined as 225 ± 2°C for 5 minutes

³Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Kuehni and Marcus (1979) have reported that experienced color matchers can detect $\Delta E = 1$ within the CIELAB color space. However, assessing color similar to the typical off-white tones of Queso Blanco cheese, Seghi et al. (1989) determined that dental professionals could always decipher differences in translucent porcelain disks with $\Delta E \geq \sim 2$. Therefore, with this understanding, the color change as a function of broiling would be detected visually in QB-MPC and QB-MPI at 30 days whereas in QB-MCC the color change would be detected visually as early as 10 days. This discrepancy could relate to the reduced protein content in QB-MCC (Table 3.5). With increased temperatures, hydrophobic interactions become stronger within the cheese protein matrix, which increases the density of the cheese; and as the density of the cheese protein matrix increases, water is expelled which affects cheese color (Johnson, 1999). With QB-MCC having less protein compared with QB-MPC and QB-MPI (Table 3.5), there is a greater opportunity for a larger change in the density of the cheese protein structure and therefore, a greater change in cheese color when broiled.

Cheeses produced without starter cultures, like Queso Blanco, typically undergo limited proteolysis over ripening (Lucey et al., 2003; Tunick et al., 2012a). The effect of age on ΔE from

pre- to post-heating was not seen in Queso Fresco when heated under the same conditions during eight weeks of storage (Guo et al., 2011; Tunick et al., 2012a; Tunick et al., 2012b) as ΔE ranged from 5.7 to 6.5 (Tunick et al., 2012a). Oaxaca cheese produced with rehydrated MPC also was reported to have no significant change in color as a function of heating between 125°C to 200°C, compared with Oaxaca cheese made with whole milk (Caro et al., 2011). Thus, these results suggest that more research is necessary to completely understand the relationship of storage time, color change (as a function of broiling) and cheese composition.

4.3.2 Sensory Analysis

Queso Blanco cheeses did not exhibit differences in flavor attributes (Table 4.4). The high salty flavor (average = 10.4) and low bitter, sour, sweet and umami scores (< 2.0), agree with Siapantas (1981) and Siapantas and Kosikowski (1967), who reported that Queso Blanco cheeses produced with acetic acid had a salty, acidic flavor profile when stored at $<10^{\circ}\text{C}$.

Table 4.4. Mean scores for sensory attributes of Queso Blanco cheese made from cheesemilks standardized with rehydrated high-protein milk powders assessed at 30 and 70 days on an anchored 15-point line scale

Attribute	Cheese ^{1,2}			SEM
	QB-MCC	QB-MPC	QB-MPI	
Bitter	0.2	0.1	0.0	0.1
Milky	3.4	3.0	3.5	0.5
Salty	10.6	10.5	10.1	0.5
Sour	1.4	1.7	1.4	0.3
Sweet	0.5	0.4	0.5	0.2
Umami	0.4	0.2	0.2	0.1

¹No significant differences ($n = 6$) were identified between any treatments for any attributes at $P < 0.05$

²Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

These results are surprising as QB-MPI and QB-MPC contain greater amounts of protein (Table 3.5), and theoretically whey proteins (Table 3.1) contribute to undesirable cheese flavors, specifically bitterness (Lemieux and Simard, 1991; Papadatos et al., 2003). Likewise, Keller et al. (1974) reported Mozzarella cheese made from cheesemilk that was acidified to pH 5.2 with citric acid, exhibited increased bitterness compared with Mozzarella cheese made from cheesemilk acidified with other types of acid (i.e., malic, acetic, phosphoric and hydrochloric acid) and at pH 5.4 and 5.6. Flavor defects due to use of concentrated proteins to standardize cheesemilk could explain, in part, why Mistry and Pulgar (1996) reported Gouda cheese produced with a high-protein (> 85% protein) milk powder, was less liked than Gouda cheese produced from cheesemilk without high-protein milk powders, yet none of the Gouda cheese had any detectable bitterness over 22 weeks.

The flavor profile of the cheeses was not expected to change considerably over time due to the limited lipolysis and proteolysis in the cheese as result of the high cooking temperatures and lack of starter culture addition, respectively, during the Queso Blanco cheese manufacture (Torres and Chandan, 1981, Lucey et al., 2003; Tunick et al., 2012a). Farkye et al. (1995) showed that consumers identified Queso Blanco cheese as “bland” at three weeks of age, however the overall acceptance of the cheese increased at seven weeks, implying that some flavor might develop during ripening. Torres and Chandan (1981) also reported that Queso Blanco cheese produced without any flavor enhancers such as cultures or lipolytic enzymes, had the greatest consumer appeal over six weeks, indicating that low levels of flavor are expected for this type of cheese.

4.4 Conclusion

Producing Queso Blanco cheese with cheesemilk standardized with rehydrated high-protein milk powders, did not have strong impacts on cheese flavor or color. It should be anticipated that the color change from pre- to post-heating of the cheese could increase as the cheese ages; however, this was not a factor of the rehydrated high-protein milk powder used to standardize the cheesemilk. More work to understand the structural components of the respective cheese protein matrices would help clarify any discrepancies seen between the samples over time.

4.5 References

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Chapter 5 - Research Summary and Conclusion

5.1 Research Summary

When making acid-set cheese, whey proteins are an expected part of the cheese protein matrix due to the high heat and direct acidification processes employed during manufacture. The heat ($> 70^{\circ}\text{C}$) denatures whey proteins while the acidification ($\text{pH} < 6.7$) drives agglomeration with other cheese proteins including κ -caseins on the casein micelle surface. It was hypothesized that these whey proteins play a functional role in the functional attributes of acid-set cheese due to their incorporation in the cheese protein matrix. Thus, the objective of the study was to understand if whey proteins are depleted in the cheesemilk via standardization with a microfiltered milk product like Micellar Casein Concentrate (**MCC**), would differences in functionality be seen in the resulting acid-set cheese over shelf life. This was accomplished by comparing cheese produced with cheesemilk standardized with MCC to that of cheese produced with cheesemilk standardized with ultrafiltered milk products, Milk Protein Concentrate 80 (**MPC**) and Milk Protein Isolate 90 (**MPI**), which would maintain the expected level of whey proteins in the cheesemilk. Queso Blanco, an emerging acid-set cheese in the United States marketplace, was chosen as a model system to explore such research over a standard 90-day shelf life assessment.

Ultimately, limited differences were discovered between cheeses produced with cheesemilk standardized with MPC and MPI compared to cheese produced with cheesemilk standardized with MCC. No significant differences were found for organoleptic attributes, including texture and flavor properties, between any of the cheeses over shelf life. When

assessing cheeses with standard Texture Profile Analysis (**TPA**) methods, differences were seen for resilience, springiness, and cohesiveness between cheese made from cheesemilk standardized with MCC vs. cheeses made from cheesemilk standardized with MPC and MPI. Hardness when determined by TPA was similar among all these cheeses, but chewiness (a product of cohesiveness, hardness, and springiness) showed differences between cheese made from MCC standardized cheesemilk and cheese made from MPI standardized cheesemilk, but neither were different from cheese made from cheesemilk standardized with MPC. For color analysis via CIELAB color space assessments, similar to TPA chewiness, a significant difference for whiteness (L^*) was found between cheese made with cheesemilk standardized with MCC and cheese made from cheesemilk standardized with MPI, however, neither were different from cheese made from cheesemilk standardized with MPC. No differences were found between cheeses for red-green color (a^*), yellow-blue color (b^*), hue angle, or chroma. When cheeses were assessed for the degree of color change from pre- to post-heating (ΔE) under broiling conditions on average, no differences in cheeses were found. However, when observing ΔE over shelf life, all cheeses showed an increase in ΔE as the cheeses aged. This change seemed to stem from whiteness (L^*) and yellow-blue color (b^*) changes post-heating over cheese age for cheeses made from cheesemilk standardized with MPC and MPI but there is still a lack of understanding as to why cheese made from cheesemilk standardized with MCC also saw an increase in ΔE over age.

These results suggest that standardizing cheesemilk with MCC compared to the more traditional ultrafiltered MPC and MPI would cause limited differences in the resulting acid-set cheese. As a trained panel did not detect noticeable organoleptic differences, it is predicted that consumers will not either. However, there may be some issues for conversion of cheese, if these

cheeses were processed in reduced formats like shreds or crumbles, of which modifications to the cheese making process may be needed.

5.2 Conclusion

With the demand for native whey proteins continuing to rise, further exploration into using microfiltered milk products to support cheese manufacture will continue to expand. This change in approach to cheese manufacture could impact acid-set cheese functionality because traditionally these cheeses have whey proteins incorporated in the cheese protein matrix. This research has shown that minimal changes in cheese processing and functionality should be expected when using microfiltered milk products to standardize cheesemilk for acid-set cheese manufacture. Final cheese composition targets may change slightly, namely protein content, but overall yield should not. Additionally, no differences in organoleptic properties of cheese made with microfiltered milk products means unnoticed changes for an established acid-set cheese consumer base. There could be slight differences, however, as to how the cheese changes color during cooking applications or how the cheese converts into reduced formats like shreds or crumbles. Ultimately, this study has shown that microfiltered milk products can be used effectively in the acid-set cheese industry, as needed.

5.3 Future Studies

Although this research laid valuable groundwork to explore the use of microfiltered milk products in acid-set cheese manufacture, more research is still warranted. Some opportunities

include diving deeper into the microstructure of an acid-set cheese produced with microfiltered milk products. This could be accomplished either visually through Scanning Electron Microscopy or Confocal Microscopy analysis, or through chemical means via SDS-PAGE analysis. Additionally, understanding the use of microfiltered milk products in acid-set cheese production on a larger commercial scale could identify if there are any blind spots that are missed when conducted in a smaller pilot-plant setting. This could include cheese conversion trials to understand if mechanical texture differences, identified in this study, could translate to conversion modifications on a production scale. Finally, exploring the use of microfiltered milk solely to produce acid-set cheese, compared to using a microfiltered milk products to just standardize cheesemilk, would provide a greater depth of understanding as to the degree of impact whey proteins have on a cheese protein matrix. The work in this study could then be repeated with such cheese to provide insight into the most extreme difference between ultrafiltered and microfiltered milk, which is the presence and absence, respectively, of whey proteins and how that impacts acid-set cheese production and functionality.

Appendix A - Raw Milk Composition Data

Table A.1. Compositional analysis of raw milk to support Queso Blanco cheesemake trials

Item ¹	Trial (Replication)		
	Trial 1	Trial 2	Trial 3
Protein, %	3.08	3.04	2.89
Fat, %	3.85	3.66	3.62
Lactose, %	4.90	4.70	4.41
Solids-not-Fat, %	8.65	8.46	8.11
Total Solids, %	12.65	12.29	11.88

¹Raw milk tested via FOSS Milkoscan MarsTM FTIR spectrometer

Appendix B - High-Protein Powder Certificates of Analysis

Figure B.1. Trial 1 Certificate of Analysis: Milk Protein Concentrate 80



MILK SPECIALTIES

GLOBAL

CERTIFICATE OF ANALYSIS

Product Name: 100382 **MPC80**

Lot/Batch Number: VS9239R1

Customer P.O. #: 4500086854

Customer Bill of Lading: 0630716

Certificate Print Date: 10/12/2019

Customer Name: Elkhart Lake Innovation Center

Quantity: 1 20KG BAGS

Pallet Numbers: 9

Material: **5502363**

PP PWD MILK PROTEIN CONCENTRATE 44 LB

Make Date: 08/27/2019

Expiration Date: 08/16/2021

Bin Location: CMC-NGR1



Batch: 000204212

Date: 10/22/2019

Analysis: **BF0000204212**

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis Protein	86.1	%	80.0 min.	IDF 185 2002
As Is Protein	81.3	%		IDF 185 2002 Dumas
Mojonnier Fat Analysis	1.2	%	3.0 max.	AOAC 939.05
Ash	6.9	%	8.0 max.	AOAC 939.39
Moisture	5.4	%	6.5 max.	AOAC 927.05
pH	6.7		6.0 to 7.5	SM 16mFd 4520H
Scorched Particle	7.5	mg/25g	15.0 max	ADPI
Aerobic Plate Count	<2500	cfu/g	30000 max	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 20.03.06
Salmonella 375 gram	Negative	1/375g x2	Negative	AOAC 2013.02

Additional Comments:

Production Date: 8/27/2019

Expiration Date: 8/27/2021

Allergen Information: MILK

Made in the USA

Expires in 24 months

Results Verified by: Luis Arreola

Signature: 

Title: Lab Tech II

Company: Milk Specialties Global

CoA Number: 116554

Milk Specialties Global • 7500 Flying Cloud Drive • Suite 300 • Eden Prairie, MN 55344 U.S.A.

Toll-free: 800.325.4271 • Phone: 952.942.7310 • Fax: 952.942.7011 • MilkSpecialtiesGlobal.com

Figure B.2. Trial 1 Certificate of Analysis: Milk Protein Isolate 90



MILKSPECIALTIES

CERTIFICATE OF ANALYSIS

Product Name: 100368 **MPISO**

Lot/Batch Number: VS9272R1

Customer P.O. #: 4500086854

Customer Bill of Lading: 0630716

Certificate Print Date: 10/12/2019

Customer Name: Elkhan Lake Innovation Center

Quantity: 1 20KG BAGS

Pallet Numbers: 23

Material: 5502364

PP PWD MILK PROTEIN ISOLATE 44 LB

Make Date: 09/29/2019

Expiration Date: 09/18/2021

Bin Location: CMC-INGR1



Batch: 0002042113 Date: 10/22/2019

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	Sy9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	
Dry Basis Protein	91.2	%	90.0 min.	IDF-185 2002
As is Protein	86.8	%	84.6 min.	IDF-185 2002
Mojonnier Fat Analysis	1.2	%	3.0 max.	AOAC 989.05
Ash	6.6	%	8.0 max.	AOAC 930.30
Moisture	4.9	%	6.5 max.	AOAC 927.05
pH	6.9		6.0 to 7.5	SM 19th Ed. 4500H
Scorched Particle	7.5	mg/25g	15.0 max.	ADPI
Aerobic Plate Count	<2500	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	/ 375g x2	Negative	AOAC 2013.02

Additional Comments:

Production Date: 9/29/2019

Expiration Date: 9/29/2021

Allergen Information: MILK

Made in the USA

Expires in 24 months.

Results Verified by: Luis Arreola

Signature: 

Title: Lab Tech II

Company: Milk Specialties Global

CoA Number: 116553

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Toll-free: 800.323.4274 • Phone: 952.942.7510 • Fax: 952.942.7611 • MilkSpecialtiesGlobal.com

Figure B.3. Trial 1 Certificate of Analysis: Micellar Casein Concentrate



MILK SPECIALTIES

CERTIFICATE OF ANALYSIS

Product Name: 100917 Micellar Casein

Lot/Batch Number: VS9270R1

Customer P.O. #: 4500066854

Customer Bill of Lading: 0630716

Certificate Print Date: 10/12/2019

Customer Name: Elkhart Lake Innovation Center

Quantity: 1 20KG BAGS

Pallet Numbers: 26

Material: 5502365

PP PWD MICELLAR CASEIN 44 LB

Make Date: 09/27/2019

Expiration Date: 09/16/2021

Bin Location: CMC-INGR1

Batch: 000204217 Date: 10/22/2019

Analysis:

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Minnelli Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis LECO Protein	90.81	%	88.0 min.	IDF 185 2002
Protein As Is	86.74	%	84.0 min.	IDF 185 2002
Mojonnier Fat Analysis	1.1	%	3.0 max.	AOAC 989.05
Ash	6.6	%	8.0 max.	AOAC 930.30
Moisture	4.8	%	6.0 max.	AOAC 927.05
pH	7.0		6.0 to 7.5	SM 188Ed. 4503H
Scorched Particle	7.5	mg/25g	15.0 max.	ADPI
Aerobic Plate Count	3000	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.07
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	/ 375g x2	NEGATIVE	AOAC 2013.02

Additional Comments:

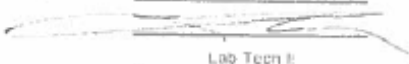
Production Date: 9/27/2019

Expiration Date: 9/27/2021

Allergen Information: MILK

Made in the USA

Results Verified by: Luis Arreola

Signature: 

Title: Lab Tech II

Company: Milk Specialties Global

CoA Number: 116552

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Figure B.4. Trial 2 Certificate of Analysis: Milk Protein Concentrate 80



CERTIFICATE OF ANALYSIS

Product Name 100382 **MPC80**
 Lot/Batch Number VS9312R1 Pallet Numbers: 042
 Customer P.O. # 4500117154
 Customer Bill of Lading: 0652050
 Certificate Print Date: 03/05/2020
 Customer Name: Sargento
 Quantity: 1 20Kg bag

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis Protein	86.5	%	80.0 min.	IDF 185:2002
As Is Protein	81.5	%	75.2 min.	IDF 185:2002
Mojonnier Fat Analysis	1.3	%	3.0 max.	AOAC 969.05
Ash	6.9	%	8.0 max.	AOAC 930.30
Moisture	5.3	%	6.5 max.	AOAC 927.06
pH	6.9		6.0 to 7.5	SMEDP 18th Ed.
Scorched Particle	7.5	mg/25g	15.0 max.	ADPI
Aerobic Plate Count	8800	cfu/g	30000 max.	AOAC 980.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	1/375g x2	Negative	AOAC 2013.02

Additional Comments:

Production Date: 11/8/2019
 Expiration Date: 11/8/2021 Expires in 24 months.
 Allergen Information: MILK
 Made in the USA

Results Verified by: Thai Xiong
 Signature: *John Pong*
 Title: QA Specialist
 Company: Milk Specialties Global

CoA Number: 125333

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Figure B.5. Trial 2 Certificate of Analysis: Milk Protein Isolate 90



CERTIFICATE OF ANALYSIS

Product Name 100368 MPI90
 Lot/Batch Number VS0041R1 Pallet Numbers: 030
 Customer P.O. # 4500117154
 Customer Bill of Lading: 0852050
 Certificate Print Date: 03/05/2020
 Customer Name: Sargento
 Quantity: 1 20Kg bag

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis Protein	91.7	%	90.0 min.	IDF 185:2002
As Is Protein	87.5	%	84.6 min.	IDF 185:2002
Mojonnier Fat Analysis	1.3	%	3.0 max.	AOAC 989.05
Ash	6.5	%	8.0 max.	AOAC 930.30
Moisture	4.7	%	6.5 max.	AOAC 927.05
pH	6.9		6.0 to 7.5	SMEDP 18th Ed.
Scorched Particle	7.5	mg/25g	15.0 max.	ADPI
Aerobic Plate Count	<2500	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	1/375g x2	Negative	AOAC 2013.02

Additional Comments:

Production Date: 2/10/2020
 Expiration Date: 2/10/2022 Expires in 24 months.
 Allergen Information: MILK
 Made in the USA

Results Verified by: Thai Xiong
 Signature:
 Title: QA Specialist
 Company: Milk Specialties Global

CoA Number: 125339

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Figure B.6. Trial 2 Certificate of Analysis: Micellar Casein Concentrate



CERTIFICATE OF ANALYSIS

Product Name	100917 Micellar Casein	Pallet Numbers: 031
Lot/Batch Number	VS0017R1	
Customer P.O. #	4500117154	
Customer Bill of Lading:	0652050	
Certificate Print Date:	03/05/2020	
Customer Name:	Sargento	
Quantity:	1 20Kg bag	

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis LECO Protein	91.4	%	88.0 min.	IDF 185:2002
Protein As Is	88.5	%	84.0 min.	IDF 185:2002
Mojonnier Fat Analysis	1.3	%	3.0 max.	AOAC 989.05
Ash	6.6	%	8.0 max.	AOAC 930.30
Moisture	5.4	%	6.0 max.	AOAC 927.05
pH	6.8		6.0 to 7.5	SMEDP 18thEd
Scorched Particle	7.5	mg/25g	15.0 max.	ADPI
Aerobic Plate Count	<2500	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	/ 375g x2	NEGATIVE	AOAC 2013.02

Additional Comments:

Production Date:	1/17/2020	
Expiration Date:	1/17/2022	Expires in 24 months.
Allergen Information:	MILK	
	Made in the USA	

Results Verified by:	Thai Xiong
Signature:	
Title:	QA Specialist
Company:	Milk Specialties Global

CoA Number: 125336

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Figure B.7. Trial 3 Certificate of Analysis: Milk Protein Concentrate 80



CERTIFICATE OF ANALYSIS

Product Name: 100382 MPC80
 Lot/Batch Number: VS0289C1
 Customer P.O. #: 4500159394
 Customer Bill of Lading: 0685267
 Certificate Print Date: 10/21/2020
 Customer Name: Sargento
 Quantity: 1 20Kg bag
 Pallet Numbers: 012

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis Protein	87.3	%	80.0 min.	IDF 185:2002
As Is Protein	82.4	%	75.2 min.	IDF 185:2002
Mojonnier Fat Analysis	1.0	%	3.0 max.	AOAC 989.05
Ash	6.9	%	8.0 max.	AOAC 930.30
Moisture	5.5	%	6.0 max.	AOAC 927.05
pH	6.8		6.0 to 7.5	SMEDP 18th Ed.
Scorched Particle	7.5	mg/15g	15.0 max.	ADPi mod
Aerobic Plate Count	<2500	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	/ 375g x2	Negative	AOAC 2013.02

Additional Comments:

Production Date: 10/15/2020
 Expiration Date: 10/15/2022
 Allergen Information: MILK
 Made in the USA
 Expires in 24 months.

Results Verified by: Thai Xiong
 Signature: 
 Title: QA Specialist
 Company: Milk Specialties Global

CoA Number: 139697

Production Plant Address: 715 North Divisadero
 Visalia, CA 93291
 (559) 732-1220

Figure B.8. Trial 3 Certificate of Analysis: Milk Protein Isolate 90



CERTIFICATE OF ANALYSIS

Product Name: 100368 MPi90
 Lot/Batch Number: VS0249C1
 Customer P.O. #: 4500159394
 Customer Bill of Lading: 0885287
 Certificate Print Date: 10/21/2020
 Customer Name: Sargento
 Quantity: 1 20Kg bag
 Pallet Numbers: 036

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis Protein	91.0	%	90.0 min.	IDF 185:2002
As Is Protein	88.5	%	84.6 min.	IDF 185:2002
Mojonnier Fat Analysis	1.2	%	3.0 max.	AOAC 989.05
Ash	6.7	%	8.0 max.	AOAC 930.30
Moisture	5.0	%	6.5 max.	AOAC 927.05
pH	6.8		6.0 to 7.3	SMEDP 18med
Scorched Particle	7.5	mg/15g	15.0 max.	ADPI mod
Aerobic Plate Count	4800	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	/375g x2	Negative	AOAC 2013.02

Additional Comments:

Production Date: 9/5/2020
 Expiration Date: 9/5/2022
 Allergen Information: MILK
 Made in the USA
 Expires in 24 months.

Results Verified by: Thai Xiong
 Signature:
 Title: QA Specialist
 Company: Milk Specialties Global

CoA Number: 139695

Production Plant Address: 715 North Divisadero
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Figure B.9. Trial 3 Certificate of Analysis: Micellar Casein Concentrate



CERTIFICATE OF ANALYSIS

Product Name: 100917 Micellar Casein
 Lot/Batch Number: VS0279U1
 Customer P.O. #: 4500159394
 Customer Bill of Lading: 0685267
 Certificate Print Date: 10/21/2020
 Customer Name: Sargento
 Quantity: 1 20Kg bag

Pallet Numbers: 028

Analysis

Test Name	Results	Reporting Units	Acceptance Criteria	Reference to the Test Method
Color	0.5	5y9.25	0 to 2.5	Munsell Charts
Flavor	Pass		PASS	Organoleptic
Dry Basis LECO Protein	91.97	%	88.0 min.	IDF 185:2002
Protein As Is	87.18	%	84.0 min.	IDF 185:2002
Mojonnier Fat Analysis	1.1	%	3.0 max.	AOAC 989.05
Ash	6.6	%	8.0 max.	AOAC 930.30
Moisture	5.3	%	6.0 max.	AOAC 927.05
pH	6.9		6.0 to 7.5	SMEDP 16th Ed.
Scorched Particle	7.5	mg/15g	15.0 max.	ADPI mod
Aerobic Plate Count	<2500	cfu/g	30000 max.	AOAC 990.12
Coliforms	<10	cfu/g	<10	AOAC 991.14
Yeast	<10	cfu/g	100 max.	AOAC 997.02
Mold	<10	cfu/g	100 max.	AOAC 997.02
Escherichia coli	<10	cfu/g	<10	AOAC 991.14
Staphylococcus aureus	<10	cfu/g	<10	AOAC 2003.08
Salmonella 375 gram	Negative	/ 375g x2	NEGATIVE	AOAC 2013.02

Additional Comments:

Production Date: 10/5/2020
 Expiration Date: 10/5/2022
 Allergen Information: MILK
 Made in the USA

Expires in 24 months.

Results Verified by: Thai Xiong
 Signature: [Signature]
 Title: QA Specialist
 Company: Milk Specialties Global

CuA Number: 136656

Appendix C - Cheese Production Records

Figure C.1. Trial 1 Cheese Production Record: Milk Protein Concentrate 80

SARGENTO Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Leah Iocco
Date: 10/29/2019

VAT # 4

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 1

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	10:34	88.7		Quantity: 42.6 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	10:45	70.2	6.67	Steam Jacket: 185F RPM: 10
Reach Temperature	11:17	175.2		Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 202917 b#2025875
Add Citric	11:22	174.4	6.37	Quantity: 2019g + 100mL H ₂ O RPM: 10
End Stir	11:27	170.7	5.11 / 5.34	Stir Time: 5 minutes
Drain	11:57	160.5	5.25 / 5.33	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	12:16	128.7	5.31	Quantity: 328.8g Lot #: 676747
Salt 2**	12:21	129.7		Quantity: 328.8g Lot #: see above
Salt 3**	12:26			Quantity: 328.8g Lot #: see above
Hoop	12:28	111.4	5.28	Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)
Press***	12:35		N/A	PSI: 20 Hours: 4
				** note 4 composite samples of fill rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFDM MPC80 MPI MCC Standardization Order = 4 Standardization Tank = (1) 2 Pasteurization Order = 4 Pasteurization Codes = 0			
Cheesemaker Signature _____ Date _____				

Comments: *Std calculations completed with total vs true protein on percent

*Incoming Milk: True Protein = 3.08 Fat = 2.85 Lactose = 4.70 SnF = 8.65 TS = 12.65
C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1801 Calculated Reconstituted Casein = 10.0032

**5min increments between salt steps (1500g total per 100# cheese -> 15 X 65.76lbs cheese curd = 986.4 grams salt)

Theory: 100# standardized cheesemilk = 80.72 # milk + 19.28 # reconstituted powder (18.95 #cheese from 123.89 #cheesemilk)

Actual: 400 # standardized cheesemilk = 328 # milk + 76 # reconstituted powder + 0 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
1	42.54	7.52	= 15.022
2	42.54	7.50	= 15.988

15.205%

Figure C.2. Trial 1 Cheese Production Record: Milk Protein Isolate 90

SARGENTO. Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Leah Iocco
Date: 10/29/2019

VAT # 2

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 1

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments				
Initial Milk*	9:31	91.1	6.69	Quantity: 50 gallons RPM: 6 (collect 4 vials of milk sample)				
Heat Milk	9:43	88.8		Steam Jacket: 185F RPM: 10				
Reach Temperature	10:17	175.0		Target Temperature: 175F for 5 minutes				
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 202917 lot# 2025875				
Add Citric	10:22	175.4	5.08 *post add	Quantity: 2885g + 1000mL H ₂ O RPM: 10				
End Stir	10:27	175.0	1	Stir Time: 5 minutes				
Drain	10:57		5.10 15.07	Drain Time: 30 minutes after stir RPM: 6				
Salt 1**	11:18		5.28	Quantity: 387g Lot #: 676747				
Salt 2**	11:23	124.6		Quantity: 387g Lot #: see above				
Salt 3**	11:28		5.17	Quantity: 387g Lot #: see above				
Hoop	11:31	127.4		Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)				
Press***	11:42		N/A	PSI: 20 Hours: 4				
				***create 4 composite samples of 1lb rounds for micro/pathogen testing				
Standardization Notes	Ingredient = NFDM MPC80 (MPI) MCC Standardization Order = 2 Standardization Tank = 1 Pasteurization Order = 2 Pasteurization Codes = 3							
Cheesemaker Signature _____ Date _____								
Comments: *std calculations completed with total vs true protein on acetent								
*Incoming Milk: True Protein = 3.08 Fat = 3.85 Lactose = 4.90 SnF = 8.05 TS = 12.65								
C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1808 Calculated Reconstituted Casein = 10.728								
**5min increments between salt steps (1500g total per 100# cheese --> 15 X 77.4 lbs cheese curd = 1161 grams salt)								
Theory: 100# standardized cheesemilk = 81.80# milk + 18.20# reconstituted powder (18.74# cheese from 122.5# cheesemilk)								
Actual: 476# standardized cheesemilk = 384# milk + 92# reconstituted powder + 0# water								
Milk Can #	Water (lbs)	Powder (lbs)	% Solids					
1	42.50	7.54	= 15.0679					
2	42.52	7.54	= 15.0619	> 15.0649%				

transferred due to program issues

Figure C.3. Trial 1 Cheese Production Record: Micellar Casein Concentrate

SARGENTO Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Leah Iocco
Date: 10/29/2019

VAT # 1

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 1

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	8:22	88.1	6.67	Quantity: 60 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	8:39	92.8		Steam Jacket: 185F RPM: 10
Reach Temperature	9:13	175.7		Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 208917 bF: 2025875
Add Citric	9:18	175.4	6.37	Quantity: 34.62 oz + 1000 mL H ₂ O RPM: 10
End Stir	9:23	172.3	1	Stir Time: 5 minutes
Drain	9:54	164.8	5.13 / 5.02	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	10:17	131.5	5.13	Quantity: 457.8g Lot #: 676747
Salt 2**	10:22	126.3		Quantity: 457.8g Lot #: see above
Salt 3**	10:27			Quantity: 457.8g Lot #: see above
Hoop	10:31		5.08	Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)
Press***	10:39		N/A	PSI: 20 Hours: 4
				***create 4 composite samples of 1lb rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFD M MPC80 MPI MCC Standardization Order = 1 Standardization Tank = 12 Pasteurization Order = 1 Pasteurization Codes = 0			
Cheesemaker Signature _____ Date _____				

Comments: *std calculations completed with total vs true protein on accurate

*Incoming Milk: True Protein = 3.08 Fat = 3.85 Lactose = 4.90 SnF = 8.65 TS = 12.65

C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.11653 Calculated Reconstituted Casein = 13.0379

**5min increments between salt steps (1500g total per 100# cheese --> 15 x 91.56 lbs cheese curd = 1373.4 grams salt)

Theory: 100# standardized cheesemilk = 84.60 # milk + 15.40 # reconstituted powder (18.89 #cheese from 118.21 #cheesemilk)

Actual: 482 # standardized cheesemilk = 408 # milk + 74 # reconstituted powder + 0 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
1	42.52	7.52	15.04
2	42.50	7.52	15.04

15.04%

Figure C.4. Trial 2 Cheese Production Record: Milk Protein Concentrate 80

SARGENTO Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Leah Iocco
Date: 3/24/2020

VAT # 1

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 2

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	8:04	90.3	6.71	Quantity: 51.1 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	8:17			Steam Jacket: 185F RPM: 10
Reach Temperature	8:48	175.3	6.39	Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 2025875
Add Citric	8:53	176.1	6.39	Quantity: 2948g + 1000mL H ₂ O RPM: 10
End Stir	8:58	172.4	1	Stir Time: 5 minutes
Drain	9:32	164.1	15.06	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	9:53			Quantity: 392.5g Lot #: 2019593
Salt 2**	9:58			Quantity: 392.5g Lot #: see above
Salt 3**	10:03		5.07	Quantity: 392.5g Lot #: see above
Hoop	10:12			Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)
Press***	10:18		N/A	PSI: 20 Hours: 4
				***create 4 composite samples of 1lb rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFDM MPC80 MPI MCC Standardization Order = 1 Standardization Tank = 2 Pasteurization Order = 1 Pasteurization Codes = 0			
Cheesemaker Signature _____ Date _____				

Comments:

*Incoming Milk: True Protein = 3.04 Fat = 3.10 Lactose = 4.70 SNF = 8.46 TS = 12.29

C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1949 Calculated Reconstituted Casein = 10.0547

**5min increments between salt steps (1500g total per 100# cheese -> 15 X 78.5 lbs cheese curd = 1177.5 grams salt)

Theory: 100# standardized cheesemilk = 82.93 # milk + 17.07 # reconstituted powder (18.01 # cheese from 120.58# cheesemilk)

Actual: 474 # standardized cheesemilk = 386 # milk + 88 # reconstituted powder + 8 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
------------	-------------	--------------	----------

1	42.50	7.50	= 15.00
2	42.50	7.50	= 14.76

> 14.99%

Figure C.5. Trial 2 Cheese Production Record: Milk Protein Isolate 90

SARGENTO. Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Leah Iocco
Date: 3/24/2020

VAT # 4

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020

TRIAL# 2

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	9:26	89.4	6.67	Quantity: 48.0 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	9:38	90.2		Steam Jacket: 185F RPM: 10
Reach Temperature	10:07	175.0	6.38	Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 2025B75
Add Citric	10:13	172.3	6.43	Quantity: 2769g + 1000mL H ₂ O RPM: 10
End Stir	10:18		1	Stir Time: 5 minutes
Drain	10:48	165.3	5.06	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	11:14		5.20	Quantity: 345.1g Lot #: 2019593
Salt 2**	11:19			Quantity: 345.1g Lot #: see above
Salt 3**	11:24		5.07	Quantity: 345.1g Lot #: see above
Hoop	11:30		5.11	Quantity: 5 forms Weight/Form: 10N round (fill ~12lbs)
Press***	11:42		N/A	PSI: 20 Hours: 4
Standardization Notes	Ingredient = NFDM MPC80 MPI MCC Standardization Order = 4 Pasteurization Order = 4 Pasteurization Codes = 0 Standardization Tank = 202			

Cheesemaker Signature _____ Date _____

Comments: _____

*Incoming Milk: True Protein = 3.04 Fat = 3.66 Lactose = 4.70 SNF = 8.46 TS = 12.29
C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1951 Calculated Reconstituted Casein = 10.77

**5min increments between salt steps (1500g total per 100# cheese --> 15 X 69.02 lbs cheese curd = 1035.3 grams salt)

Theory: 100# standardized cheesemilk = 83.71 # milk + 16.09 # reconstituted powder (18.0 #cheese from 119.18 #cheesemilk)

Actual: 498 # standardized cheesemilk = 408 # milk + 78 # reconstituted powder + 12 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
1	42.50	7.50	15.00
2	42.50	7.52	15.02

15.01%

Figure C.6. Trial 2 Cheese Production Record: Micellar Casein Concentrate

SARGENTO. Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Leah Iocco
Date: 3/24/2020

VAT # 2

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 2

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	9:07	91.0	6.69	Quantity: 51.4 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	9:20			Steam Jacket: 185F RPM: 10
Reach Temperature	9:49	175.2	6.35	Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 2025075
Add Citric	9:56	174.2		Quantity: 2905g + 1000 mL H ₂ O RPM: 10
End Stir	10:01	172.6	1	Stir Time: 5 minutes
Drain	10:31	163.1	14.98	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	10:49			Quantity: 365.8g Lot #: 2019593
Salt 2**	10:54			Quantity: 365.8g Lot #: see above
Salt 3**	10:59		5.01	Quantity: 365.8g Lot #: see above
Hoop	11:10			Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)
Press***	11:25		N/A	PSI: 20 Hours: 4
				***create 4 composite samples of 1lb rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFDM MPC80 MPI (MCC) Standardization Order = 3 Standardization Tank = 1 Pasteurization Order = 3 Pasteurization Codes = 0			
Cheesemaker Signature _____ Date _____				

Comments:

*Incoming Milk: True Protein = 3.04 Fat = 3.46 Lactose = 4.70 SnF = 8.41 TS = 12.29

C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1955 Calculated Reconstituted Casein = 13.01

**5min increments between salt steps (1500g total per 100# cheese → 15 X 76.16 lbs cheese curd = 1097.4 grams salt)

Theory: 100# standardized cheesemilk = 86.34 # milk + 13.66 # reconstituted powder (17.97 #cheese from 115.87#cheesemilk)

Actual: 490 # standardized cheesemilk = 414 # milk + 106 # reconstituted powder + 10 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
1	42.50	7.50	15.00
2	42.50	7.54	15.07

15.04%

Figure C.7. Trial 3 Cheese Production Record: Milk Protein Concentrate 80

SARGENTO. Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Danny Clayton
Date: 11/3/2020

VAT # 2

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 3

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	8:32		6.72	Quantity: 51.0 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	8:46	89.6		Steam Jacket: 185F RPM: 10
Reach Temperature	9:17	175.0	6.39	Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 2066981
Add Citric	9:22	173.9	6.41	Quantity: 2943g + 1000mL H ₂ O RPM: 10
End Stir	9:27		1	Stir Time: 5 minutes
Drain	9:57	172.4	5.12 / 5.08	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	10:34			Quantity: 365.5g Lot #: 2069834
Salt 2**	10:39			Quantity: 365.5g Lot #: see above
Salt 3**	10:44			Quantity: 365.5g Lot #: see above
Hoop	10:53		5.17	Quantity: 5 forms Weight/Form: 10# round (fill ~12lbs)
Press***	11:00		N/A	PSI: 20 Hours: 4
				***create 4 composite samples of 1lb rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFDM MPC80 MPI MCC Standardization Order = 2 Standardization Tank #02 Pasteurization Order = 2 Pasteurization Codes = 0			
Cheesemaker Signature _____ Date _____				

Comments:

*Incoming Milk: True Protein = 2.89 Fat = 3.62 Lactose = 4.41 SnF = 8.11 TS = 11.88

C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1503 Calculated Reconstituted Casein = 10.16

**5min increments between salt steps (1500g total per 100# cheese -> 15 X 73.1 lbs cheese curd = 1096.5 grams salt)

Theory: 100# standardized cheesemilk = 82.69 # milk + 17.31 # reconstituted powder (17.78 #cheese from 120.94 #cheesemilk)

Actual: 494 # standardized cheesemilk = 404 # milk + 85 # reconstituted powder + 5 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
------------	-------------	--------------	----------

1	42.50	7.52	= 15.03
2	42.50	7.52	= 15.03

15.03%

Figure C.8. Trial 3 Cheese Production Record: Milk Protein Isolate 90

SARGENTO Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Danny Clayton
Date: 11/3/2020

VAT # 1

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020

TRIAL# 3

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	9:12		6.70	Quantity: 48.7 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	9:30	89.3		Steam Jacket: 185F RPM: 10
Reach Temperature	9:58	175.1	6.39	Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 2066981
Add Citric	10:03		6.41	Quantity: 2810g + 1000 mL H ₂ O RPM: 10
End Stir	10:08	172.6	1	Stir Time: 5 minutes
Drain	10:39	164.0	5.07 / 5.08	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	11:05			Quantity: 357.6g Lot #: 2066984
Salt 2**	11:10			Quantity: 357.6g Lot #: see above
Salt 3**	11:15			Quantity: 357.6g Lot #: see above
Hoop	11:20		5.09	Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)
Press***	11:31		N/A	PSI: 20 Hours: 4
				***create 4 composite samples of 1lb rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFDM MPC80 (MPL) MCC Standardization Order = 3 Pasteurization Order = 3 Pasteurization Codes = 0			
Cheesemaker Signature _____ Date _____				

Comments:

*Incoming Milk: True Protein = 2.89 Fat = 3.10 Lactose = 4.41 SnF = 8.11 TS = 11.88
C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1804 Calculated Reconstituted Casein = 10.66
**5min increments between salt steps (1500g total per 100# cheese --> 15 x 71.52 lbs cheese curd = 1072.8 grams salt)
Theory: 100# standardized cheesemilk = 67.33 # milk + 16.67 # reconstituted powder (17.120# cheese from 120.0 # cheesemilk)
Actual: 490 # standardized cheesemilk = 408 # milk + 82 # reconstituted powder + 0 # water
Milk Can # Water (lbs) Powder (lbs) % Solids

1 42.50 + 7.52 = 15.03
2 42.50 + 7.52 = 15.03 > 15.03%

Figure C.9. Trial 3 Cheese Production Record: Micellar Casein Concentrate

SARGENTO Cheese Production Record

Cheese Type: Queso Blanco
Cheesemaker: Karley Blake/Danny Clayton
Date: 11/3/2020

VAT # 3

Sargento Foods, Inc.
Elkhart Lake Technical Center
305 Pine Street
Elkhart Lake, WI 53020
TRIAL# 3

Operation	Time	Temperature (F)	pH Cheese / Whey	Comments
Initial Milk*	9:54	89.9	6.70	Quantity: 55.2 gallons RPM: 6 (collect 4 vials of milk sample)
Heat Milk	10:10			Steam Jacket: 185F RPM: 10
Reach Temperature	10:41	175.0	6.38	Target Temperature: 175F for 5 minutes
(Dilute Citric)		N/A		Dilution: 4:1 (25g citric to 100g water) Lot #: 20060981
Add Citric	10:46		6.40	Quantity: 3185g + 1000ml H ₂ O RPM: 10
End Stir	10:52	172.4	1	Stir Time: 5 minutes
Drain	11:22	165.2	5.08 / 5.04	Drain Time: 30 minutes after stir RPM: 6
Salt 1**	11:42			Quantity: 393.5g Lot #: 2009024
Salt 2**	11:47			Quantity: 393.5g Lot #: see above
Salt 3**	11:52			Quantity: 393.5g Lot #: see above
Hoop	11:57		5.19	Quantity: 5 forms Weight/Form: 10# round (fill = ~12lbs)
Press***	12:00		N/A	PSI: 20 Hours: 4
				***create 4 composite samples of 1lb rounds for micro/pathogen testing
Standardization Notes	Ingredient = NFDN MPC80 MPC MCE Standardization Order = 4 Standardization Tank = 12 Pasteurization Order = 4 Pasteurization Codes = 1			
Cheesemaker Signature _____ Date _____				

Comments:

*Incoming Milk: True Protein = 2.89 Fat = 3.62 Lactose = 4.41 SnF = 8.11 TS = 11.88

C/F Ratio = 1.2 Calculated Reconstituted Fat = 0.1653 Calculated Reconstituted Casein = 13.10

**5min increments between salt steps (1500g total per 100# cheese --> 15 X 78.7 lbs cheese curd = 1180.5 grams salt)

Theory: 100# standardized cheesemilk = 86.06 # milk + 13.94 # reconstituted powder (17.76 # cheese from 116.20 # cheesemilk)

Actual: 536 # standardized cheesemilk = 439 # milk + 76 # reconstituted powder + 0 # water

Milk Can #	Water (lbs)	Powder (lbs)	% Solids
1	42.54	7.52	15.02
2	42.50	7.52	15.03

15.03%

Appendix D - Descriptive Analysis Panel Ballot

Figure D.1. RedJade® Descriptive Analysis Panel (DAP) ballot used for assessing Queso Blanco cheese samples



Survey Created Using RedJade Software

Questionnaire Page 1

☐ Only answer this question if your current position is one of the following: "1"

1 Please enter your FULL NAME below.

Today you will assess a total of 3 samples of Queso Blanco, one at a time, monadic sequentially. You will be assessing each of

the samples individually. No discussion will be allowed during testing.

Please proceed to assess each Queso Blanco sample for Aroma, Hand Texture, Mouth Texture, Taste, Flavor and Aftertaste.

Use references available to you as needed.

The scale you will use is the 0-15 scale. If you think you cannot detect some of the attributes listed in the questionnaire,

please go ahead and mark the attribute(s) as ZERO in the scale.

If you have any questions, please do not hesitate to contact the test administrator.

Thanks for your participation.

2 Please comment on sample {{sample_code}}'s APPEARANCE

Please, proceed to rate this sample {{sample_code}} for the following **AROMA**.

3 Please comment on sample {{sample_code}} Aroma Attributes as needed. If no additional attributes detected, just enter N/A

Please, proceed to rate this sample {{sample_code}} for the following **HAND TEXTURE** Sensory Attributes



Please, proceed to rate this sample {{sample_code}} for the following **MOUTH TEXTURE** Sensory Attributes



Please, proceed to rate this sample {{sample_code}} for the following **TASTE** Sensory Attributes



Please, proceed to rate this sample {{sample_code}} for the **AFTERTASTE**.

Questionnaire Page 6

- 17 Please comment on sample {{sample_code}} **AFTERTASTE** Attributes as needed. If no additional attributes detected, just enter N/A

Appendix E - SAS CODE

Figure E.1. SAS® code used to assess proximate analysis of Queso Blanco cheeses

```
proc glm data=input;  
  where day=10;  
  class Replication Protein;  
  model Moisture Fat FDB pH Salt Prot PDB = Replication Protein;  
  lsmeans Protein/pdiff adjust=tukey;  
run;
```

Figure E.2. SAS® code used to assess texture, flavor and color attributes of Queso Blanco cheeses

```
proc mixed data=input;  
  class Replication Protein Day;  
  model Attribute = Protein Day Protein*Day/ddfm = sat;  
  random Replication Replication*Protein;  
  lsmeans Protein/pdiff adjust=tukey adjdfe=row;  
  lsmeans Day Protein*Day/pdiff adjust=tukey adjdfe=row;  
run;
```

Appendix F - Raw Data

Table F.1. The raw composition data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Compositional Attribute, %						pH ²
			Moisture	Protein	Protein _{dry basis}	Fat	Fat _{dry basis}	Salt	
1	QB- MPC	1	50.50	26.99	54.53	15.50	31.31	2.45	5.51
		2	50.90	27.23	55.46	16.00	32.59	2.45	5.52
		3	51.10	27.10	55.42	16.00	32.72	2.35	5.49
	QB-MPI	1	50.00	28.35	56.70	15.00	30.00	1.80	5.41
		2	49.70	28.20	56.06	15.50	30.82	1.77	5.42
		3	49.90	28.19	56.27	15.50	30.94	1.92	5.41
	QB-MCC	1	51.80	25.46	52.82	16.00	33.20	2.48	5.37
		2	51.60	25.44	52.56	15.50	32.02	2.40	5.35
		3	51.20	25.42	52.09	16.00	32.79	2.48	5.35
2	QB-MPC	1	53.70	25.79	55.70	14.00	30.24	2.03	5.37
		2	53.00	25.74	54.77	14.00	29.79	1.98	5.45
		3	53.50	25.74	55.35	13.50	29.03	2.03	5.49
	QB-MPI	1	49.90	26.31	52.51	14.00	27.94	1.92	5.47
		2	50.80	26.09	53.03	14.50	29.47	2.10	5.44
		3	50.70	26.41	53.57	15.00	30.43	2.37	5.42
	QB-MCC	1	52.40	25.19	52.92	15.00	31.51	2.28	5.32
		2	52.50	24.91	52.44	15.50	32.63	2.15	5.35
		3	52.30	24.75	51.89	14.50	30.40	2.22	5.34
3	QB-MPC	1	50.70	27.84	56.47	15.50	31.44	2.50	5.38
		2	50.30	27.15	54.63	15.00	30.18	2.40	5.31
		3	49.80	27.57	54.92	16.00	31.87	2.28	5.32
	QB-MPI	1	50.60	27.58	55.83	14.00	28.34	2.30	5.31
		2	51.40	27.31	56.19	14.00	28.81	2.25	5.27
		3	51.50	27.35	56.39	15.00	30.93	2.33	5.29
	QB-MCC	1	50.20	25.77	51.75	16.50	33.13	2.35	5.29
		2	50.50	26.20	52.93	16.00	32.32	2.67	5.38
		3	51.40	26.31	54.14	15.50	31.89	2.55	5.32

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²pH is the only unitless compositional attribute

Table F.2. The raw Texture Profile Analysis (TPA) chewiness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day				
			10 ²	30	50	70	90
1	QB- MPC	1	14.36	17.46	17.97	17.72	23.57
		2	21.55	13.51	22.68	21.15	25.34
		3	19.59	20.17	20.54	19.01	18.25
		4	16.18	21.49	24.45	28.56	20.04
	QB-MPI	1	16.40	19.11	19.47	22.36	19.00
		2	26.22	20.53	23.84	25.81	24.88
		3	29.59	23.16	23.39	30.15	25.32
		4	20.63	24.48	22.10	23.57	25.86
	QB-MCC	1	11.69	12.75	14.70	16.02	17.57
		2	20.10	15.59	17.85	18.49	18.53
		3	16.47	17.78	16.60	18.71	18.50
		4	16.07	15.52	14.93	21.56	18.91
	QB-MPC	1	12.40	12.99	14.21	15.58	14.73
		2	-	16.30	15.98	16.68	16.21
		3	20.87	18.50	16.47	17.62	18.37
		4	15.08	20.67	14.47	19.31	15.66
2	QB-MPI	1	15.18	16.71	16.81	17.89	18.25
		2	-	21.86	22.77	22.68	23.11
		3	18.97	18.73	23.17	25.15	18.71
		4	14.25	20.61	21.41	19.71	22.39
	QB-MCC	1	12.29	13.77	13.20	14.40	15.53
		2	-	15.18	20.39	15.51	15.49
		3	13.72	14.03	16.57	16.62	18.37
		4	10.74	15.40	16.85	16.96	14.85
	QB-MPC	1	25.02	21.75	24.11	22.16	23.26
		2	26.52	25.97	26.74	24.24	25.94
		3	23.13	23.02	25.93	22.27	27.23
		4	29.61	22.00	26.29	23.35	28.33
3	QB-MPI	1	16.98	23.05	21.73	25.14	19.82
		2	21.35	21.14	26.10	22.49	24.33
		3	20.18	26.79	27.71	33.44	25.04
		4	17.12	23.54	31.59	21.82	21.31
	QB-MCC	1	17.38	18.72	16.26	16.18	18.56
		2	16.56	18.09	23.23	18.60	17.34
		3	15.76	18.85	21.06	17.76	18.55
		4	21.57	21.15	17.30	19.37	18.77

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of equipment errors on springiness

Table F.3. The raw Texture Profile Analysis (TPA) cohesiveness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day				
			10	30	50	70	90
1	QB- MPC	1	0.67	0.74	0.70	0.69	0.72
		2	0.72	0.74	0.74	0.75	0.75
		3	0.73	0.77	0.75	0.71	0.73
		4	0.66	0.78	0.74	0.75	0.74
	QB-MPI	1	0.71	0.72	0.71	0.68	0.71
		2	0.73	0.75	0.72	0.73	0.71
		3	0.75	0.76	0.72	0.75	0.73
		4	0.68	0.76	0.75	0.72	0.70
	QB-MCC	1	0.62	0.70	0.69	0.65	0.67
		2	0.70	0.72	0.70	0.70	0.70
		3	0.66	0.73	0.69	0.69	0.72
		4	0.66	0.73	0.66	0.70	0.67
2	QB-MPC	1	0.72	0.67	0.69	0.70	0.70
		2	0.52	0.70	0.69	0.69	0.70
		3	0.82	0.71	0.70	0.70	0.72
		4	0.74	0.73	0.72	0.73	0.71
	QB-MPI	1	0.73	0.67	0.68	0.70	0.72
		2	0.52	0.71	0.71	0.75	0.72
		3	0.73	0.67	0.74	0.73	0.75
		4	0.53	0.70	0.72	0.69	0.74
	QB-MCC	1	0.71	0.64	0.64	0.63	0.68
		2	0.74	0.64	0.71	0.67	0.68
		3	0.72	0.61	0.69	0.72	0.70
		4	0.53	0.67	0.69	0.70	0.68
3	QB-MPC	1	0.69	0.71	0.73	0.69	0.78
		2	0.71	0.71	0.74	0.73	0.73
		3	0.73	0.72	0.75	0.73	0.74
		4	0.72	0.72	0.73	0.73	0.73
	QB-MPI	1	0.66	0.71	0.69	0.73	0.70
		2	0.68	0.72	0.72	0.71	0.72
		3	0.70	0.70	0.75	0.75	0.75
		4	0.66	0.69	0.76	0.72	0.72
	QB-MCC	1	0.61	0.67	0.69	0.70	0.70
		2	0.65	0.65	0.71	0.68	0.68
		3	0.69	0.69	0.73	0.71	0.70
		4	0.66	0.66	0.69	0.70	0.71

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Table F.4. The raw Texture Profile Analysis (TPA) hardness (kg) data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day				
			10	30	50	70	90
1	QB-MPC	1	25.67	27.08	29.79	29.40	35.98
		2	35.80	21.65	34.51	31.97	38.00
		3	30.63	29.16	31.05	30.60	28.46
		4	29.38	30.24	37.46	41.93	30.11
	QB-MPI	1	27.01	30.29	31.68	36.94	30.51
		2	41.31	30.41	38.12	40.09	39.75
		3	44.01	34.20	36.33	45.18	38.55
		4	34.95	34.96	33.19	36.58	40.78
	QB-MCC	1	23.11	21.44	25.42	27.65	30.22
		2	33.21	24.38	29.78	30.90	30.37
		3	29.11	26.95	28.59	31.14	29.54
		4	28.03	23.93	26.99	34.39	32.01
	QB-MPC	1	19.75	23.58	24.17	26.46	24.44
		2	22.91	27.46	26.63	28.02	26.54
		3	29.22	29.63	27.31	28.73	28.87
		4	23.91	32.57	23.70	30.09	25.46
2	QB-MPI	1	24.00	28.95	28.88	29.23	29.50
		2	30.48	35.96	35.68	34.36	36.14
		3	29.87	32.73	36.50	38.40	28.39
		4	30.49	34.06	33.86	31.79	34.82
	QB-MCC	1	20.43	25.87	24.63	25.97	26.32
		2	22.76	27.66	32.30	27.22	26.78
		3	21.69	27.18	28.50	27.42	29.68
		4	23.05	27.15	29.25	28.00	26.00
	QB-MPC	1	43.13	36.02	37.33	37.31	33.18
		2	43.08	41.18	40.41	36.58	39.46
		3	36.70	35.93	38.62	34.58	40.79
		4	46.30	34.63	40.29	37.62	42.67
3	QB-MPI	1	30.97	36.22	36.29	37.39	32.73
		2	35.99	34.11	40.93	36.36	38.01
		3	33.27	42.23	41.81	49.30	38.11
		4	30.28	38.87	45.42	33.40	34.06
	QB-MCC	1	33.90	32.84	27.88	26.91	30.18
		2	30.12	32.57	36.26	31.96	29.42
		3	28.18	33.15	32.18	29.48	30.83
		4	39.08	37.46	29.39	31.75	29.35

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Table F.5. The raw Texture Profile Analysis (TPA) resilience (%) data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day				
			10	30	50	70	90
1	QB- MPC	1	24.59	30.68	27.33	26.62	29.50
		2	30.97	31.43	32.55	32.09	32.92
		3	31.99	34.03	32.59	29.17	29.92
		4	25.77	34.25	32.39	33.20	30.22
	QB-MPI	1	27.37	29.31	28.43	28.13	28.47
		2	32.21	32.54	31.64	31.06	30.27
		3	35.29	33.82	30.13	34.20	30.68
		4	27.69	33.65	32.64	30.66	30.05
	QB-MCC	1	20.16	26.02	25.97	22.62	25.40
		2	29.15	28.90	28.56	28.32	28.75
		3	24.52	29.98	26.93	27.77	29.38
		4	24.11	28.99	25.56	28.50	27.40
2	QB-MPC	1	29.17	25.62	25.54	27.97	26.29
		2	30.64	27.96	28.33	27.90	28.77
		3	38.80	29.20	27.80	28.75	29.47
		4	32.33	30.21	29.32	30.51	28.66
	QB-MPI	1	29.98	23.64	25.80	25.99	28.43
		2	31.07	28.79	29.47	30.34	30.80
		3	30.23	26.36	31.00	31.09	32.11
		4	32.98	28.10	28.68	28.08	32.25
	QB-MCC	1	27.27	23.09	22.68	22.71	25.64
		2	31.90	23.77	29.14	26.96	27.61
		3	28.11	23.21	26.89	29.65	27.78
		4	27.80	25.42	26.81	28.51	26.68
3	QB-MPC	1	28.26	28.20	30.96	29.05	29.48
		2	32.34	31.21	33.47	32.16	32.62
		3	32.51	29.30	34.32	31.86	32.49
		4	32.07	30.92	32.58	31.26	32.09
	QB-MPI	1	26.81	30.67	27.73	28.48	26.61
		2	28.47	31.05	31.18	29.82	30.86
		3	29.46	29.96	32.48	34.86	32.96
		4	26.78	29.20	35.01	29.99	29.28
	QB-MCC	1	22.41	25.24	27.33	25.75	28.99
		2	25.10	26.36	29.74	27.82	27.83
		3	27.14	27.88	29.64	27.91	27.70
		4	27.75	26.98	28.03	28.47	28.77

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Table F.6. The raw Texture Profile Analysis (TPA) springiness (%) data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day				
			10 ²	30	50	70	90
1	QB- MPC	1	83.63	87.64	86.19	87.49	90.45
		2	84.09	84.01	88.40	88.63	89.35
		3	87.12	89.63	88.10	87.26	87.63
		4	83.62	90.97	88.68	90.45	90.18
	QB-MPI	1	85.98	87.51	86.40	88.62	88.15
		2	86.99	90.08	86.50	88.58	88.43
		3	89.26	88.96	89.11	89.04	89.84
		4	86.59	91.74	88.97	89.12	90.10
	QB-MCC	1	80.97	85.02	83.42	89.29	86.37
		2	86.51	88.73	85.90	85.56	87.46
		3	85.51	89.86	84.38	86.96	87.44
		4	86.50	89.31	83.80	89.98	87.80
2	QB-MPC	1	86.77	82.27	85.14	84.33	86.33
		2	-	85.24	86.67	85.85	87.13
		3	86.73	87.90	86.01	88.10	87.91
		4	84.82	86.57	84.60	87.54	86.41
	QB-MPI	1	87.16	86.81	85.73	87.89	86.39
		2	-	86.20	89.39	88.10	88.59
		3	87.22	84.93	86.27	89.85	87.47
		4	87.62	86.08	87.26	89.28	87.04
	QB-MCC	1	84.76	83.00	83.81	87.41	86.27
		2	-	86.41	88.56	84.65	84.74
		3	88.29	85.26	84.31	84.07	88.10
		4	88.34	84.44	83.13	86.83	84.12
3	QB-MPC	1	84.46	85.53	88.72	85.74	90.33
		2	86.16	88.63	89.47	90.60	89.68
		3	86.32	89.10	89.09	88.41	90.61
		4	89.18	88.00	89.80	85.14	91.34
	QB-MPI	1	82.86	89.19	86.60	92.39	87.02
		2	86.77	86.10	88.76	87.47	88.90
		3	86.61	90.27	88.30	90.84	87.88
		4	85.64	87.83	92.10	90.75	87.19
	QB-MCC	1	84.48	85.35	84.47	86.06	87.45
		2	84.32	84.82	90.09	85.77	86.86
		3	81.31	82.18	89.84	85.14	86.43
		4	83.96	85.97	85.24	86.91	89.57

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of equipment errors

Table F.7. Raw sensory adhesiveness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	2.0	5.0
		2	4.5	4.5
		3	7.0	9.0
		4	5.0	3.0
		5	-	6.0
	QB-MPI	1	3.0	6.0
		2	9.0	2.0
		3	7.0	9.0
		4	4.0	7.0
		5	-	8.0
	QB-MCC	1	1.5	5.5
		2	7.0	4.0
		3	7.0	9.0
		4	4.0	6.0
		5	-	8.0
2	QB-MPC	1	11.0	10.0
		2	3.0	3.0
		3	8.5	9.0
		4	5.5	9.5
		5	3.0	5.0
	QB-MPI	1	11.0	10.0
		2	4.1	6.0
		3	4.0	9.0
		4	6.0	10.0
		5	8.0	3.0
	QB-MCC	1	11.0	10.0
		2	5.0	6.0
		3	8.0	9.0
		4	7.0	8.0
		5	6.0	6.0
3	QB-MPC	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
	QB-MPI	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
	QB-MCC	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges (Trial 1) and ballot errors (Trial 3)

Table F.8. Raw sensory cohesiveness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	0.5	1.0
		2	5.0	3.5
		3	2.5	2.0
		4	3.5	3.0
		5	-	3.0
	QB-MPI	1	0.5	0.5
		2	3.5	3.0
		3	4.0	1.5
		4	3.0	1.0
		5	-	8.5
	QB-MCC	1	0.5	5.0
		2	4.0	3.0
		3	5.0	3.0
		4	3.0	1.0
		5	-	5.0
2	QB-MPC	1	10.0	9.5
		2	4.0	4.0
		3	4.0	4.0
		4	4.5	5.5
		5	3.5	3.5
	QB-MPI	1	9.0	10.0
		2	3.0	3.0
		3	4.0	4.0
		4	4.5	6.0
		5	7.5	4.0
	QB-MCC	1	8.0	7.5
		2	2.5	3.0
		3	4.0	5.0
		4	6.0	8.0
		5	7.5	5.0
3	QB-MPC	1	4.0	5.5
		2	2.0	3.5
		3	1.0	4.5
		4	3.0	3.0
		5	-	7.0
	QB-MPI	1	7.0	7.0
		2	2.5	3.0
		3	5.5	5.0
		4	2.0	3.0
		5	-	0.5
	QB-MCC	1	8.0	4.0
		2	2.0	3.0
		3	7.5	2.0
		4	2.0	3.5
		5	-	2.5

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.9. Raw sensory hand firmness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	8.5	9.0
		2	9.0	10.0
		3	9.0	8.0
		4	5.0	7.0
		5	-	9.0
	QB-MPI	1	8.0	10.0
		2	7.0	12.0
		3	6.0	10.0
		4	9.0	9.0
		5	-	11.0
	QB-MCC	1	8.5	10.0
		2	6.5	10.5
		3	7.0	9.0
		4	7.0	9.0
		5	-	11.0
2	QB-MPC	1	8.0	7.5
		2	10.0	5.0
		3	5.5	6.0
		4	10.5	10.0
		5	7.0	6.0
	QB-MPI	1	11.0	8.0
		2	10.0	7.0
		3	6.0	7.0
		4	11.5	10.0
		5	10.0	7.0
	QB-MCC	1	9.0	8.0
		2	8.0	5.5
		3	5.0	7.0
		4	11.0	9.5
		5	7.0	6.0
3	QB-MPC	1	11.0	6.0
		2	9.0	7.0
		3	13.0	9.5
		4	11.0	7.0
		5	-	10.5
	QB-MPI	1	10.0	6.5
		2	12.0	8.0
		3	11.5	11.0
		4	10.5	8.0
		5	-	12.0
	QB-MCC	1	10.5	6.0
		2	9.0	5.0
		3	9.0	7.5
		4	11.0	7.0
		5	-	12.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.10. Raw sensory mouth firmness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	8.5	8.5
		2	6.0	10.0
		3	9.0	9.0
		4	4.0	7.0
		5	-	11.0
	QB-MPI	1	8.5	8.5
		2	8.0	10.0
		3	8.0	10.0
		4	8.5	8.0
		5	-	11.0
	QB-MCC	1	9.5	8.0
		2	7.0	10.5
		3	6.0	8.0
		4	6.0	8.0
		5	-	9.0
2	QB-MPC	1	9.5	8.0
		2	8.5	6.0
		3	7.0	8.0
		4	10.0	9.5
		5	7.0	6.0
	QB-MPI	1	10.5	7.0
		2	10.0	6.5
		3	8.0	8.5
		4	11.0	9.0
		5	8.0	7.0
	QB-MCC	1	8.5	10.5
		2	8.5	5.5
		3	6.0	8.0
		4	10.5	9.5
		5	7.5	4.5
3	QB-MPC	1	11.0	8.0
		2	12.0	8.5
		3	12.0	9.0
		4	11.0	7.0
		5	-	8.0
	QB-MPI	1	11.0	7.5
		2	12.0	8.0
		3	10.5	11.0
		4	9.5	7.0
		5	-	10.0
	QB-MCC	1	10.0	8.0
		2	12.0	5.0
		3	9.0	7.0
		4	9.0	6.0
		5	-	8.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.11. Raw sensory rate of breakdown data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	3.5	3.5
		2	5.0	4.5
		3	3.0	3.0
		4	6.0	8.0
		5	-	2.0
	QB-MPI	1	5.0	7.5
		2	4.0	3.0
		3	3.0	1.5
		4	10.0	7.0
		5	-	2.0
	QB-MCC	1	3.5	5.5
		2	4.0	4.0
		3	4.0	3.0
		4	6.5	6.0
		5	-	3.0
2	QB-MPC	1	2.0	1.0
		2	9.0	5.0
		3	3.0	3.0
		4	5.0	7.0
		5	5.0	8.0
	QB-MPI	1	2.0	1.0
		2	7.5	8.0
		3	3.0	3.0
		4	5.5	6.5
		5	3.0	7.0
	QB-MCC	1	2.0	2.0
		2	6.0	8.0
		3	3.0	3.0
		4	6.0	5.5
		5	8.0	8.0
3	QB-MPC	1	1.5	1.0
		2	2.0	4.0
		3	2.0	3.5
		4	3.0	4.0
		5	-	6.0
	QB-MPI	1	2.0	1.0
		2	2.0	4.0
		3	3.0	4.0
		4	10.0	9.0
		5	-	9.0
	QB-MCC	1	4.0	1.0
		2	2.0	3.0
		3	5.0	2.0
		4	10.0	3.0
		5	-	8.5

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.12. Raw sensory smoothness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	1.0	3.5
		2	4.0	3.5
		3	3.5	2.5
		4	3.0	3.0
		5	-	2.0
	QB-MPI	1	1.0	6.0
		2	4.5	3.5
		3	3.5	1.0
		4	2.0	3.0
		5	-	3.0
	QB-MCC	1	2.0	4.0
		2	3.5	4.5
		3	3.5	3.0
		4	3.5	3.0
		5	-	3.0
2	QB-MPC	1	3.5	4.0
		2	4.5	4.0
		3	3.0	2.5
		4	5.0	5.5
		5	3.0	4.0
	QB-MPI	1	3.5	4.0
		2	3.0	3.5
		3	3.5	2.5
		4	3.5	5.0
		5	2.9	3.0
	QB-MCC	1	2.0	4.0
		2	3.0	6.5
		3	3.0	2.5
		4	4.0	4.5
		5	3.0	2.5
3	QB-MPC	1	2.5	3.0
		2	2.0	4.0
		3	0.5	0.0
		4	2.0	3.0
		5	-	3.5
	QB-MPI	1	2.5	2.5
		2	2.0	1.0
		3	2.0	6.0
		4	3.0	3.0
		5	-	3.5
	QB-MCC	1	3.5	5.0
		2	2.0	3.5
		3	3.5	0.0
		4	3.0	3.0
		5	-	3.5

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.13. Raw sensory springiness data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	10.0	8.5
		2	7.0	6.5
		3	10.0	11.0
		4	7.0	11.0
		5	-	5.5
	QB-MPI	1	9.0	8.5
		2	7.0	7.0
		3	10.0	11.0
		4	8.5	10.0
		5	-	9.0
	QB-MCC	1	11.0	10.0
		2	6.0	7.0
		3	10.0	11.0
		4	7.0	8.0
		5	-	6.0
2	QB-MPC	1	6.0	10.0
		2	7.0	5.0
		3	7.0	7.0
		4	7.0	7.0
		5	7.0	7.0
	QB-MPI	1	8.0	10.0
		2	1.0	7.0
		3	7.0	7.0
		4	7.0	7.0
		5	8.0	11.0
	QB-MCC	1	7.0	9.0
		2	8.0	6.0
		3	7.0	7.0
		4	7.0	7.0
		5	11.0	6.0
3	QB-MPC	1	6.0	7.0
		2	7.0	7.0
		3	7.0	7.0
		4	12.0	11.0
		5	-	5.0
	QB-MPI	1	7.0	7.0
		2	7.0	7.0
		3	7.0	7.0
		4	11.0	11.0
		5	-	11.0
	QB-MCC	1	7.0	7.0
		2	7.0	7.0
		3	7.0	7.0
		4	11.0	11.0
		5	-	11.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.14. Raw sensory bitter data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		4	0.0	0.0
		5	-	0.0
	QB-MPI	1	0.0	0.0
		2	0.0	0.3
		3	0.0	0.0
		4	0.0	0.0
		5	-	0.0
	QB-MCC	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		4	0.0	0.0
		5	-	0.0
2	QB-MPC	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		4	0.5	0.5
		5	0.0	0.5
	QB-MPI	1	0.5	0.0
		2	0.0	0.0
		3	0.0	0.0
		4	0.5	0.0
		5	0.0	0.0
	QB-MCC	1	3.0	0.5
		2	0.0	0.0
		3	0.0	0.0
		4	0.5	0.0
		5	0.0	0.0
3	QB-MPC	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		4	0.0	0.0
		5	-	0.0
	QB-MPI	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.0
		4	0.0	0.0
		5	-	0.0
	QB-MCC	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.5
		4	0.0	0.0
		5	-	0.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.15. Raw sensory milky data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
	QB-MPI	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
	QB-MCC	1	-	-
		2	-	-
		3	-	-
		4	-	-
		5	-	-
2	QB-MPC	1	1.0	1.0
		2	4.5	5.5
		3	4.0	6.0
		4	1.5	1.0
		5	0.0	5.0
	QB-MPI	1	2.0	1.0
		2	6.0	6.0
		3	5.0	8.0
		4	1.5	1.0
		5	6.0	6.0
	QB-MCC	1	1.5	1.0
		2	5.5	6.0
		3	3.0	5.0
		4	1.5	0.5
		5	5.5	6.0
3	QB-MPC	1	0.0	1.0
		2	4.0	6.0
		3	0.5	0.0
		4	6.0	6.0
		5	-	5.0
	QB-MPI	1	0.0	0.5
		2	0.0	6.0
		3	1.0	0.0
		4	6.0	5.0
		5	-	6.5
	QB-MCC	1	0.0	0.0
		2	3.0	6.0
		3	1.5	0.0
		4	7.0	6.0
		5	-	5.5

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges (Trial 3) and ballot errors (Trial 1)

Table F.16. Raw sensory salty data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	8.5	9.0
		2	10.5	10.5
		3	9.0	11.0
		4	11.0	10.5
		5	-	7.5
	QB-MPI	1	6.5	9.0
		2	11.5	11.0
		3	10.0	11.0
		4	10.0	11.0
		5	-	11.0
	QB-MCC	1	5.5	8.5
		2	11.0	11.0
		3	11.0	12.0
		4	10.5	11.0
		5	-	10.0
2	QB-MPC	1	12.0	12.0
		2	11.0	10.0
		3	11.0	12.5
		4	12.0	11.5
		5	12.0	12.0
	QB-MPI	1	11.0	9.5
		2	10.0	9.5
		3	11.0	9.5
		4	10.5	10.5
		5	12.0	12.0
	QB-MCC	1	10.5	11.0
		2	10.0	9.0
		3	11.0	13.0
		4	12.0	11.0
		5	11.0	11.0
3	QB-MPC	1	6.5	6.5
		2	11.5	11.5
		3	10.0	12.5
		4	11.0	11.0
		5	-	10.0
	QB-MPI	1	12.0	6.5
		2	13.0	1.0
		3	11.0	10.0
		4	11.0	11.0
		5	-	11.0
	QB-MCC	1	11.0	7.0
		2	12.0	12.0
		3	11.0	11.0
		4	11.0	12.0
		5	-	9.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.17. Raw sensory sour data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	0.5	1.0
		2	0.5	1.0
		3	1.5	2.0
		4	1.5	1.0
		5	-	1.5
	QB-MPI	1	1.0	1.5
		2	0.5	2.0
		3	2.0	2.0
		4	0.5	1.0
		5	-	0.0
	QB-MCC	1	0.5	0.5
		2	1.0	0.5
		3	2.0	4.5
		4	1.0	0.3
		5	-	0.5
2	QB-MPC	1	0.5	0.0
		2	0.5	1.0
		3	2.0	1.0
		4	2.0	1.5
		5	2.0	4.0
	QB-MPI	1	1.5	0.0
		2	1.5	1.0
		3	1.5	1.0
		4	2.0	0.5
		5	1.0	1.5
	QB-MCC	1	1.0	0.5
		2	0.5	0.5
		3	3.0	1.0
		4	2.5	1.0
		5	4.0	1.5
3	QB-MPC	1	1.5	0.5
		2	1.0	1.0
		3	1.0	2.5
		4	2.0	1.5
		5	-	0.0
	QB-MPI	1	0.5	0.0
		2	5.0	3.0
		3	2.0	2.0
		4	0.5	1.5
		5	-	1.0
	QB-MCC	1	1.5	0.5
		2	3.0	1.5
		3	1.5	1.5
		4	0.5	1.5
		5	-	0.5

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.18. Raw sensory sweet data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	0.5	0.0
		2	0.5	0.5
		3	0.0	0.0
		4	1.0	1.0
		5	-	0.0
	QB-MPI	1	1.0	0.0
		2	0.5	0.5
		3	0.0	0.0
		4	1.0	0.0
		5	-	0.0
	QB-MCC	1	0.5	0.5
		2	0.5	0.5
		3	0.0	0.0
		4	2.5	0.5
		5	-	0.0
2	QB-MPC	1	0.0	0.0
		2	0.5	0.5
		3	0.5	0.5
		4	0.5	1.0
		5	0.5	0.5
	QB-MPI	1	0.0	0.0
		2	6.5	0.0
		3	0.5	0.0
		4	0.5	1.0
		5	1.0	0.5
	QB-MCC	1	0.0	0.0
		2	0.0	0.5
		3	0.5	0.5
		4	1.0	0.5
		5	1.0	1.0
3	QB-MPC	1	0.0	0.0
		2	0.0	0.0
		3	1.0	0.5
		4	0.5	1.0
		5	-	0.5
	QB-MPI	1	0.0	0.0
		2	0.0	0.5
		3	0.5	0.5
		4	0.0	0.5
		5	-	0.0
	QB-MCC	1	0.0	0.0
		2	0.0	0.5
		3	1.0	0.5
		4	0.5	1.5
		5	-	0.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.19. Raw sensory umami data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day ²	
			30	70
1	QB- MPC	1	0.5	0.5
		2	0.0	0.5
		3	0.0	0.5
		4	0.5	0.0
		5	-	0.0
	QB-MPI	1	0.5	0.0
		2	0.0	0.8
		3	0.0	0.5
		4	0.0	0.0
		5	-	0.0
	QB-MCC	1	0.5	0.5
		2	0.0	0.5
		3	0.5	0.5
		4	0.5	1.0
		5	-	0.0
2	QB-MPC	1	0.0	0.0
		2	0.5	0.5
		3	0.0	0.0
		4	0.5	0.5
		5	0.0	0.5
	QB-MPI	1	0.0	0.0
		2	0.5	0.0
		3	0.5	0.0
		4	0.5	0.5
		5	0.0	0.5
	QB-MCC	1	0.0	0.0
		2	0.0	1.0
		3	0.0	0.5
		4	0.5	0.5
		5	1.0	2.0
3	QB-MPC	1	0.0	0.0
		2	0.0	0.0
		3	0.5	0.0
		4	0.0	0.0
		5	-	0.0
	QB-MPI	1	0.0	0.0
		2	0.0	0.0
		3	0.0	0.5
		4	0.5	0.5
		5	-	0.5
	QB-MCC	1	0.0	0.0
		2	0.0	0.0
		3	0.5	0.0
		4	0.0	0.0
		5	-	1.0

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

²Blank spaces are a result of absence of judges

Table F.20. The raw CIELAB *L data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders**

Trial	Cheese ¹	Replication	Day				
			10	30	50	70	90
1	QB- MPC	1	85.75	85.97	86.51	89.33	88.53
		2	86.38	86.21	85.99	89.70	88.78
		3	85.90	85.92	86.36	88.37	88.10
	QB-MPI	1	85.57	85.88	86.62	90.00	89.88
		2	85.52	86.05	86.72	89.42	89.92
		3	85.65	86.31	86.49	89.48	89.61
	QB-MCC	1	87.63	86.74	88.38	90.60	90.52
		2	86.69	87.86	89.19	90.17	90.66
		3	87.47	86.92	87.54	91.23	90.24
2	QB-MPC	1	90.14	89.77	90.38	89.65	90.27
		2	88.87	90.36	89.97	89.60	90.47
		3	90.02	90.02	90.44	90.28	90.29
	QB-MPI	1	89.24	88.33	88.98	90.05	88.52
		2	89.49	89.34	89.60	89.97	89.35
		3	88.74	89.85	89.27	89.70	88.89
	QB-MCC	1	90.23	90.42	90.54	90.24	90.63
		2	89.96	90.21	90.56	90.55	90.52
		3	90.15	90.62	90.23	90.68	90.43
3	QB-MPC	1	85.57	85.69	85.61	85.34	85.68
		2	85.85	85.58	85.77	85.77	85.71
		3	85.32	85.65	85.58	85.31	85.41
	QB-MPI	1	85.54	85.65	85.26	85.52	85.40
		2	85.89	85.69	85.63	85.67	85.71
		3	85.73	85.53	85.44	85.09	85.43
	QB-MCC	1	86.17	85.97	85.80	85.92	86.14
		2	86.02	85.90	86.42	85.96	85.99
		3	85.79	85.65	85.96	85.89	85.86

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Table F.21. The raw CIELAB a^* data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders

Trial	Cheese ¹	Replication	Day				
			10	30	50	70	90
1	QB- MPC	1	1.28	2.04	2.61	-0.9	-0.63
		2	1.57	2.49	1.34	-0.72	-0.43
		3	1.15	1.71	1.84	-0.80	-0.37
	QB-MPI	1	1.26	1.59	2.83	-0.68	-0.38
		2	1.03	2.29	2.79	-1.26	-0.67
		3	1.00	1.78	2.18	-1.56	-1.26
	QB-MCC	1	1.69	2.90	2.68	0.12	-0.42
		2	1.56	2.93	2.29	2.48	-8.00
		3	2.10	3.47	3.30	1.94	-0.39
2	QB-MPC	1	-0.53	-0.71	-0.52	-1.01	-0.68
		2	-1.73	-0.62	-0.93	-0.82	-0.43
		3	-0.37	-0.68	-0.45	-0.55	-0.63
	QB-MPI	1	-1.58	-1.65	-1.25	-0.93	-1.25
		2	-1.58	-1.38	-1.13	-1.04	-1.08
		3	-1.45	-0.83	-1.62	-1.48	-1.04
	QB-MCC	1	-0.73	-0.17	-0.34	-0.30	-0.32
		2	-1.07	-0.95	-0.37	-0.33	-0.22
		3	-1.09	-0.13	-0.71	0.16	-0.11
3	QB-MPC	1	0.16	0.43	0.06	0.23	7.55
		2	0.52	0.12	0.51	0.35	7.08
		3	0.28	0.30	0.19	8.32	0.15
	QB-MPI	1	0.44	0.28	0.21	7.04	1.76
		2	0.76	0.42	0.41	0.39	0.20
		3	0.36	0.17	0.33	0.25	0.18
	QB-MCC	1	0.95	0.75	1.04	0.91	0.53
		2	0.93	0.80	0.83	0.74	0.74
		3	0.60	0.53	0.84	0.74	0.42

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Table F.22. The raw CIELAB *b data of Queso Blanco cheeses made from cheesemilk standardized to 1.2:1 casein-to-fat ratio using rehydrated high-protein milk powders**

Trial	Cheese ¹	Replication	Day				
			10	30	50	70	90
1	QB- MPC	1	20.14	18.22	18.94	21.85	22.61
		2	19.43	18.05	20.75	22.21	22.74
		3	20.97	20.14	18.38	20.99	22.51
	QB-MPI	1	19.85	19.42	18.03	22.94	23.20
		2	21.11	17.73	18.06	22.14	23.02
		3	21.58	18.53	17.84	21.85	22.53
	QB-MCC	1	19.72	18.28	19.84	21.82	23.21
		2	18.86	19.78	20.40	22.46	22.98
		3	19.36	18.88	19.13	22.13	23.28
2	QB-MPC	1	22.90	23.09	23.20	22.47	22.79
		2	21.35	23.14	22.94	22.67	23.10
		3	22.82	22.71	23.34	22.79	22.84
	QB-MPI	1	22.07	21.47	21.87	22.86	20.85
		2	22.41	22.37	22.35	22.55	22.00
		3	21.44	22.87	21.73	22.38	21.4
	QB-MCC	1	22.70	22.92	22.90	21.98	22.07
		2	22.75	22.67	22.85	22.51	22.22
		3	22.69	22.44	22.28	20.92	22.06
3	QB-MPC	1	24.17	23.38	24.40	23.66	24.50
		2	22.73	24.39	22.92	23.57	24.46
		3	23.57	23.37	24.16	24.40	24.10
	QB-MPI	1	22.60	23.47	23.79	24.35	24.29
		2	21.80	23.17	23.21	23.42	23.87
		3	23.02	24.10	23.35	23.79	23.99
	QB-MCC	1	20.14	21.72	20.36	20.96	22.10
		2	20.94	21.45	19.62	21.68	20.82
		3	21.47	22.87	21.28	21.59	22.41

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Appendix G - Queso Blanco Cheese Yield Data

Table G.1. Cheese yield results (%) of Queso Blanco cheese made with rehydrated high-protein milk powders

Cheese ¹	Trial (Replication)		
	Trial 1	Trial 2	Trial 3
QB-MPC	19.06	18.97	17.70
QB-MPI	19.11	17.75	18.13
QB-MCC	18.84	18.29	17.60

¹Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90

Appendix H - Color Analysis Post-Heating Results

Table H.1. Color analysis (CIELAB color space) attributes, after broiling¹, of Queso Blanco cheeses made from cheesemilks standardized with rehydrated high-protein milk powders

Cheese ²	Item	Days					SEM
		10	30	50	70	90	
QB-MPC	<i>L</i> *	86.52 ^b	83.72 ^{ab}	83.09 ^a	82.85 ^a	82.27 ^a	1.39
	<i>a</i> *	0.12	-0.09	0.39	0.19	-0.48	0.65
	<i>b</i> *	22.23 ^a	23.55 ^{ab}	23.49 ^{ab}	23.84 ^{ab}	25.24 ^b	0.88
QB-MPI	<i>L</i> *	86.77 ^b	83.60 ^{ab}	82.55 ^a	82.18 ^a	81.77 ^a	1.39
	<i>a</i> *	0.05	-0.10	-0.22	-1.40	-0.74	0.65
	<i>b</i> *	21.71 ^a	23.62 ^{ab}	24.01 ^b	24.34 ^b	24.74 ^b	0.88
QB-MCC	<i>L</i> *	85.87	84.90	84.36	83.70	82.90	1.39
	<i>a</i> *	-0.13	-0.19	-0.20	-0.89	-0.33	0.65
	<i>b</i> *	22.63	23.05	22.71	23.09	24.13	0.88

^{a-b}Means (n = 15) in the same row with different superscripts differ ($P < 0.05$)

¹Broiling conditions defined as $225 \pm 2^\circ\text{C}$ for 5 minutes

²Cheese produced from cheesemilk standardized with high-protein milk powder: MCC = Micellar Casein Concentrate; MPC = Milk Protein Concentrate 80; MPI = Milk Protein Isolate 90