Intl. J. Food. Ferment. 5(1): 1-9, June 2016

DOI: 10.5958/2321-712X.2016.00001.6

# Clean Labels: The New Wave Sweeping the Fermented Milks Industry

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**Abstract**

Functional ingredients from naturally occurring food biopolymers are a trend catching up amongst food and beverage developers. This is driven by the demand of consumers for clean label ingredient listings on products. The clean label trend is extending into the yogurt market (acid gel) with consumers demanding hydrocolloids, stabilizers or thickeners are purged from ingredient listings. Substituting hydrocolloids in food systems especially yogurts could mean compromising on rheological properties and water holding capacities. However, structure functionality relationship of naturally occurring biopolymer caseins in milk can be exploited to overcome this foreseen loss of product characteristics. The following review is an attempt to delineate clean label solutions for the manufacture of acid milk gels.

**Keywords:** Biopolymers, consumers, acid gel, hydrocolloids, milk

## Consumer insights

Consumer surveys are the crux of marketing driven new product development activity in any food company (Childs, 1997). An important component of these investigations includes consumer perception of products and choices. Once such strong and sure footed perception in the minds of consumers is that of "clean label ingredient listing". Consumers' consider food products containing greater than five ingredients to be a non-healthy choice and avoid the products that display ingredient listings they cannot comprehend. Therefore, the dairy industry is moving towards products that have a cleaned up or shortened ingredient listing on their products.

This transition does pose challenges in product formulations, but a number of brands are committing themselves towards products with a cleaner label for improved consumer goodwill and an enhanced brand image. A dairy brand that is a market leader in the segment of the clean label is Daisy Brand (Dallas, Texas, USA) with its cottage cheese and sour cream devoid of any hydrocolloids or stabilizing agents. A growing number of multinational food companies are committing towards adopting a clean label. Consumer research findings reveal that the clean label perception amongst consumers varies.

Various ingredients and additives are added to food to confer certain functional benefits or traits that consumers demand. Consumers prefer yogurts to be firm and viscous. To cater to this choice; manufacturers of yogurts add hydrocolloids to bind water and interact with proteins to stabilize the structure that lead to reduced syneresis and increased hardness (Tamime & Robinson, 1999).

Similarly, gelatin is added to yogurts for improved water binding and texture properties (Fiszman & Salvador, 1999), while xanthan gum, κ- carrageenan and locust bean gum are reported to increase the consistency coefficient values in stirred yogurts (Keogh & O'Kennedy, 1998; Baeza, Carp, Perez & Pilosof, 2002).

Surprisingly controversy surrounds the use of these additives in foods with consumers relating their consumption with food safety risks (Aoki, Shen, & Saijo, 2010) even though no concrete evidence exists that establishes the link between consumption of such additives and perceived risks. When consumers come across a positive information (no food safety risk associated with the presence of additives) or negative information (additives lead to food safety hazards), the negative information dominates the positive information in the minds of a consumer leading to a situation where the willingness to pay for a product containing these additives decrease (Fox, Hayes & Shorgen, 2002; Hayes, Fox & Shorgen, 2002). Moreover, esoteric names of food additives on ingredient labels lead to consumers associating these food additives to food safety risks (Song & Schwarz, 2009). Studies on consumer's preference towards natural foods have classified food additives, pesticides and hormones in the same category (Roosen, Thiele, & Hansen, 2005). Since the success of any food product is correlated with consumer acceptance, the best possible solution would be to eliminate additives from foods those invoke an iota of doubt in the minds of the consumers.

## Food hydrocolloids/stabilizers

Food hydrocolloids may be defined as high molecular weight biopolymers that are obtained from a number of sources that include plants, seaweeds, and microbes. Hydrocolloids are polymers of polysaccharides. Proteins are not classified under this category even though they share some functional attributes with the classically defined hydrocolloids. The one protein candidate that has been classified under this family of food hydrocolloids is gelatin that owes it functional properties to a unique amino acid composition and hydrophilicity (Dickinson, 2003). They are added to foods for their use as stabilizers. The European Food Safety Authority (2012) defines stabilizers as "substances that make it possible to maintain the physicochemical state of food". Thickeners or gelling agents belong to the functional class of stabilizers. Food hydrocolloids may have additional functional attributes like food emulsification properties, inhibiting sugar and ice crystal formations, controlled release of flavors, etc. (Williams & Phillips, 2014).

**Table 1: Sources and examples of hydrocolloids**

| Source | Examples |
|---|---|
| Trees | cellulose |
| Tree gum exudates | gum arabic, gum karaya, gum ghatti, gum tragacanth |
| Plants | starch, pectin, cellulose |
| Seeds | guar gum, locust bean gum, tara gum |
| Algal | agar, carrageenan, alginate |
| Microbial | xanthan gum, curdlan, dextran, gellan gum |
| Animal | gelatin, chitosan |

**Adapted from Williams and Phillips (2014)**

A key consideration of the use of a particular hydrocolloid in dairy systems apart from its functionality is pricing. That makes starch a common ingredient in dairy systems which apart from its competitive pricing imparts a host of functional properties. The use of a hydrocolloid in dairy systems is governed by a number of factors which includes the critical concentration (C*) of hydrocolloids in the system. The C* influences shear behavior that is regulated by the hydrodynamic size (which in turn is influenced by its molecular structure, charge on the polymer, ionic strength, and pH) which may vary in dairy systems (Dickinson, 2003).

## Caseins in milk

Holt and Sawyer (1993) termed caseins as rheomorphic structures owing to their open, flexible structure with amino acid proline held to be responsible for the inability of caseins to form stable structures. It is known that approximately 25% of the casein side groups are hydrophobic, but the lack of secondary and tertiary structures makes caseins have avery high surface hydrophobicity. Owing to their highly uneven distribution of charges, the caseins have a very distinctive amphipathic structure. The unequal distribution of the hydrophobic and hydrophilic residues leads to this uneven distribution of charges. This distribution pattern makes caseins a good choice for certain functional properties like emulsification and foaming (O'Mahony & Fox, 2013). Caseins have a tendency to self-aggregate mainly through hydrophobic interactions and owing to the presence of phosphoserine residues on some of the casein gene products, divalent cations are bound strongly by these. The protective effect against $Ca^{2+}$ aggregation of caseins is conferred by κ-casein that can stabilize ~ 10 times its mass by the formation of colloidal micellar aggregates. κ-casein are on the surface of the micelles, and the $Ca^{2+}$ sensitive caseins are inside the core. Casein micelles also carry within them inorganic minerals like Ca and P, necessary for the growth of the calves. It is often argued that the primary function of caseins in the nutrition of calves it to supply Ca and P rather than as a source of amino acids (Horne, 2002).

**Whey proteins**

Whey proteins, a class of serum proteins, contribute to around 20% of the proteins in milk and is obtained by the isoelectric precipitation of caseins at pH 4.6 and 20°C from skim milk (Farell *et al.*, 2004). β-LG is of significance during the heat treatment and processing of milk due to the presence of a free sulfhydryl group on the Cys 121 in the native form of the protein. Of the five Cysteine residues, four are involved in disulfide linkages that are between residues 66 and 160 and between residues 106 and 119 (Edwards, Creamer, & Jameson, 2009). At neutral pH and ionic strength, the heat denaturation of β-LG possibly involves mechanisms related to hydrophobic aggregation and disulfide bond interchange reactions ( Havea, Carr & Creamer, 2004). This hydrophobic bond interchange reaction has consequences when it occurs in dairy systems meant for conversion into acid gels. It has been proposed that on heat exposure of β-LG there is an interchange of disulfide bonds and the free Cys at 121 forms an S-S with Cys 106 leading to a free Cys at 119. Cys at 119 reacts with Cys 66 forming an S-S bond leaving the Cys 160 residue free. The free Cys 160 has the potential to react with other free Cys groups if present in the medium like that on κ-casein (Considine, Patel, Anema, Singh, & Creamer, 2007). The $\alpha$-LA monomer has four disulfide bonds and exists in the Halo form ( Ca-bound form). It is reported that one of the disulfide bonds (Cys6-Cys120) is heat susceptible (Kuwajima, Ikeguchi, Sugawara, Hiraoka, & Sugai, 1990). It is also generally accepted that $\alpha$-LA does not undergo any disulfide-linked interchange reactions because of the absence of a free thiol group when heated above 70°C (Calvo, Leaver, & Banks, 1993).

**Acid gelation of milk**

Different methods exist for acidification of milk and include the addition of lactic acid bacteria (LAB) and acidulant like Glucono-delta-lactone (GDL). LAB utilizes milk sugar lactose to convert it into lactic acid that brings down the pH of milk to the isoelectric pH of caseins. Some studies have reported the acid gelation properties of milk using GDL as an acidulant. The acidification kinetics of the formation of an acid gel using GDL differs from that formed by the action of LAB. However, the use of GDL as a preferred mode of acidification remains popular while studying protein interactions during acid gelation. The rate of GDL addition to attain the desired rate of acidification depends on the temperature of incubation and the buffering properties of the medium into which it is added (Singh, McCarthy, & Lucey, 1997). It is reported that acid milk gels made from GDL and LAB may differ in physical properties especially when acid gels are formed at higher incubation temperatures (Lucey & Singh, 1997).

Casein in milk undergoes various physicochemical changes during the acidification of milk. The effect of pH on phosphocaseins at different stages of acidification was classified into five phases by Famelart, Lepesant, Gaucheron, Graet, & Schuck, 1996. In the first phase wherein the pH falls from 7 to 6.1 in a suspension of native phosphocaseinate (NPCS) in water or NaCl solution, a decrease in solvation and size of the casein micelle accompanied by a fall in the viscosity of the suspensions was reported. This was hypothesized to be brought about by ionization of the negatively charged groups on amino acids resulting in a reduction of repulsive forces between caseins. Moreover, a reduction in the levels of non-micellar caseins was also reported.

These findings were also corroborated by Dalgleish and Law (1988). In the second phase, as the pH of NCPS was lowered to ~ 5.5, an increase in viscosity and casein solvation was reported. This was hypothesized to be due to a reduction in electrostatic interactions between caseins and an increase the casein-water interactions. An alternate theory proposed was a reduction in hydrophobic interactions due to thermodynamic considerations resulting in a solvated micelle (McGann and Fox, 1974). Phase III involved a lowering in pH to 5.3 which corresponded to a decrease in casein dissociation and an increase in solvation of casein micelles. Furthermore, a decrease in pH to ~ 4.5 resulted in adecrease in solvation of the caseins attributed to the precipitation of caseins. In Phase V, a reduction in pH below 4.5 was reported to increase in water holding properties of the caseins and was attributed to the development of positive charges on the caseins. Alternatively, Roefs, Walstra, Dalgleish, and Horne (1985) observed little change to the hydrodynamic diameter of casein micelles during acidification of unheated milk. Heat treatment of milk brings about changes in milk which include denaturation of whey proteins and its interaction with caseins either at the colloidal phase or the serum phase.

These interactions of proteins on the heat treatment of milk can cause changes in the acid gelation properties of the resultant acid gels. The changes outlined earlier in the section relate to solubilization of CCP on acidification in NCPS. The physico chemical properties of casein micelles in the presence of whey proteins in heated milk and subjected to acid gelation could differ due to changes in the ionic environment, pH or $Ca^{2+}$ ion activities.Elucidating these changes and tailoring protein interactions could potentially improve rheological properties in acid gels. These approaches are tools that could help in potentially replacing hydrocolloids used in the manufacture of acid gels.

**Processing technologies to alter protein interactions that could influence rheological properties of acid milk gels**

***High-pressure treatment of milk***

High-pressure processing (HPP) HPP could influence gelation behavior in acid gels by altering the disaggregation/aggregation behavior of the caseins (Anema, Lowe & Stockman, 2005), denaturation of whey proteins (Considine *et al.,* 2007), and interaction between whey proteins and caseins. HPP is also reported to influence mineral equilibria in milk by increasing the diffusible Ca in milk exposed to pressures >400 MPa (Huppertz, Kelly & Fox, 2002). Shifts in mineral equilibria can potentially alter acid gelation behavior of milk proteins. On HPP treatment of milk above 100MP a,β-LG is reported to be denatured with 70-80% of denaturation occurring at pressures > 400 MPa (Lopez-Fadino, Carrascosa, & Olano, 1996). HPP of milk above 450 MPa has an influence on casein micelle integrity and is influenced by the temperature of processing. Gaucheron, Famelart, Mariette, Raulot, Michel, & LeGraet (1997) reported an increase in micellar size on HP treatment of milk >40$^{o}$C.

This was related to interactions of dissociated caseins with the denatured whey proteins, in a fashion similar when caseins and whey proteins are exposed to heat treatment. Anema (2010) observed that milk subjected to HPP(400 MPa at 40$^{o}$C) had acid gelation properties similar to that observed in acid gels from milk that was heat treated at 80$^{o}$C for 2 minutes. WPC fortified milk subjected to HPP at 600 MPa for 5 minutes had higher G' values as compared to WPC fortified milk subjected to heat treatment (85$^{o}$C for 20 minutes). Moreover, as reported for heated milk, HPP of whey proteins in the absence of caseins did not increase the G' of acid milk gels when compared to HPP of whey proteins in the presence of caseins (Walsh-O'Grady, O'Kennedy, Fitzgerald & Lane, 2001). HPP of milk (600 MPa for 20 min) in the manufacture of stirred yogurts resulted in a G' similar to the yogurts obtained from heat-treatedmilk (90°C for 30 min). Hence, it can be concluded the HPP of milk can bring about changes to rheological properties of acid gels similar to what is obtained from milk subjected to heat treatment.

**High-pressure homogenization and Ultrasonication**

High-pressure homogenization (HPH) and Ultrasonication (US) aim to compromise the micellar integrity of caseins by the generation of high shear forces. Although both these technologies use different principles to achieve the desired shear forces acting on the micelle, their aim is to bring about structural changes to the proteins of milk. HPH like conventional homogenization results in the inclusion of fat into the protein gel network on acid gelation (Roach & Harte, 2008). The literature cites that HPH alone does not cause substantial increases in G' of acid gels. However, when used in conjunction with heat treatment brings about increases in the final G' (Hernandez & Harte, 2008). Ciron, Gee, Kelly, and Auty (2011) reported greater hysteresis area in yogurts containing fat and subjected to HPH. The increase in hysteresis was correlated to the inclusion of fat into the protein matrix. For acid gels from milk subjected to US, Nguyen *et al.* (2010) reported that increases in the G' of acid gels were caused by the heat generated during the US of skim milk and its subsequent consequences on protein structure rather than effects of US alone. Riener, Noci, Cronin, Morgan, and Lyng (2009) studied the effect of fat content in milk subjected to US and its impact on the G' of yogurts. The authors reported that compared to heated controls, a 10 min US treatment at 72°C yielded yogurts with a significantly higher G'.

**Enzymatic cross-linking**

Rheological properties of acid milk gels can be altered by the application of various physical processes like heat treatment, high-pressure homogenization, ultra-high pressure homogenization, sonication,etc. These processes bring about a change to the casein micelle structure thereby increasing interactions between caseins and whey proteins. Covalent disulfide bonds formed between free SH groups of κ-caseins and β-lactoglobulins are primarily held to be responsible for the observed phenomenon. Apart from the introduction of S-S linkages, the introduction of additional covalent bonds through transglutaminase (TG) treatment of milk is reported to increase G' of acid milk gels. TG is a transferase thatcross-links glutamine and lysineresidues to form γ-glutamyl-lysine peptide linkage (Griffin, Casadio, & Bergamini, 2002). TG is reported to increase both inter and intra-micellar crosslinking.

This crosslinking of casein micelles stabilizes it against Intra micellar casein destabilization when complete solubilization of CCP occurs during acidification (Hupeprtz & De Kruif, 2008). Literature (Bonisch, Manfred, Lauber, & Kulozik, 2007) suggests greater Intra micellar crosslinking of caseins with κ-caseins crosslinked to a greater extent than caseins found in the core of the micelles. Ercilli-Cura *et al.* (2013) reported the absence of a tan $\delta_{max}$ in acid gels from TG treated milk. The lack of a tan $\delta_{max}$ in TG-treated acid gels is an indication of resistance to Intra micellar destabilization of the casein micelles at the point of maximum CCP solubilization that could potentially destabilize the micelle. It is also widely reported in literature that TG treatment of milk before and during the process of acid gelation results in an increase in penetration depth at fracture and an increase in G' of acid gels (Jaros, Patzgold, Schwarzenbolz, & Rohm, 2006; Jaros, Jacob, Otto, & Rohm, 2010).

**Hydrodynamic cavitation**

Application of shear forces that compromise the integrity of casein micelles is known to improve rheological properties of the resulting acid gels. Acoustic cavitation (US) has been applied to improve the rheological properties of acid milk gels. Hydrodynamic cavitation is an alternate technology that can generate shear forces similar to US (Shlenskaya *et al.*, 2011) with the added advantage of industrial scale up. In hydrodynamic cavitation, the formation of bubblesis due to pressure fluctuations that a fluid undergoes during its passage through specially designed rotors and stators (Milly, Toledo, Harrison & Armstead, 2007). The kinetic energy of the fluid flowing through the geometry is proportional to the cavitation events generated in the medium (Promtov & Monastirsky, 2000).

During the flow of any liquid variable, pressure zones are created in the rotary hydrodynamic cavitator essentially due to the design of the rotor and the velocity of the fluid inside the rotor. Vapor bubbles are created in low-pressure zones that collapse as it enters high-pressure zones inside the cavitator releasing avast amount of energy that can influence protein interactions. In many of the industrially designed hydrodynamic cavitator, fluid is forced through a rotor and stator assembly that leads to the formation of eddy currents. Particulates, debris or biological entities could provide a surface for the bubble nuclei to attach, expand and collapse. No studies have reported the effect of hydrodynamic cavitation on the rheological properties of acid milk gels. An important application of this process could be in the manufacture of Greek yogurt and formulated Greek style yogurts where high protein concentration could lead to excessive build up of viscosity. The application of this process could reduce the protein interactions thereby improving mouthfeel and texture of the Greek yogurts.

**Fortification of yogurts with milk solids**

Fortification techniques involve the addition of high protein ingredients into milk and include the addition of nonfat dry milk (NFDM), milk protein concentrates (MPC), whey protein concentrates (WPC). The addition or fortification of these high protein ingredients either changes the casein to whey protein ratios or protein to total solids ratio in the formulations. The use of MPC in the manufacture of yogurts can alter the ionic and non-ionic environment in the dispersions. The use of ultrafiltration (UF) in the manufacture of MPC powders brings about changes to the ionic and non-ionic environment brought about by a reduction in minerals and lactose. The extent of reduction varies with the protein content of the MPC powders. This removal of minerals and lactose employing UF and diafiltration (DF) can change influence protein interactions when these powders are used as ingredients in the manufacture of acid gels. Casein and whey proteins exist in a dynamic equilibrium with its surrounding medium, and the properties of the medium are influenced by its ionic strength and carbohydrate composition. Any changes to this medium can have changes to observed protein interactions that could potentially influence rheological properties. However, UF and DF followed by drying of MPC do not influence casein micelle structure (Shuck, 2009).

However reconstituted MPC dispersions from MPC powders that vary in protein content can have different physicochemical properties. Recently MPC powders were manufactured by the injection of carbon dioxide ($CO_2$), before and during the process of UF resulting in the partial removal of calcium and minerals (Marella, Salunke, Biswas, Kommineni, & Metzger, 2015). These MPC powders were reported to have modified functional characteristics and enhanced solubility. Shah (2012) studied the effect of different MPC powders and their storage on the viscosity profiles of yogurts. The author observed a decrease in the viscosity of yogurts when high protein MPC powders were used for the manufacture of yogurts. The observed changes were related to fused casein aggregates in high protein MPC powders. However differing ionic and non-ionic environment in MPC dispersions of varying protein content can be tailored to influence protein interactions and thereby rheological properties of acid milk gels.

**Protein fortification in the manufacture of Greek-style yogurts**

Greek yogurts are manufactured by employing a dewheying step after the fermentation of milk. The dewheying step in modern processing plantsis accomplished by mechanical separators like Quarg separators (Kulkarni, Belsare & Lele, 2006). One of the major drawbacks of the straining process is the generation of large quantities of acid whey that’s hard to dispose of as effluent waste due to the stringent statutory waste disposal norms. A popular approach that is gaining ground is the elimination of dewheying step by manufacturing fortified Greek style yogurts (GSY) that have the desired protein content before fermentation. Some of the common dairy ingredients that are used to achieve the target protein content prior to fermentation are MPC's and Micellar casein concentrates (Bong & Moraru, 2014). Various grades of MPC's varying in protein content, minerals, lactose and protein to total solids ratios due to the process of UF and DF employed in its manufacture are available to the processor. However, the influence of reconstituted MPC powders varyingin protein content on the rheological properties and microstructure of high protein acid gels have not been reported in the literature. Moreover, the challenges faced in the manufacture of GSY include body and texture defects that include the formation of grains and higher acidity.

The defect of graininess is particularly pronounced in yogurts with a high protein content (8.5% protein and above). It is hypothesized to be a defect arising due to casein whey protein interactions in a system that have high protein concentration when compared to regular yogurts. The gelation of whey proteins is reported to occur at pH of ~ 5.3 which is followed by the gelation of caseins at pH of ~ 4.8. The high protein concentration and differences in gelation pH of the caseins and whey proteins may cause local rearrangement and fractures in the gel matrix leading to graininess (Lucey & Singh, 1997). However, there is a gap in knowledge on the application of processes that could potentially influence protein interactions in GSY and yield a product that has comparable rheological characteristics and microstructure of a strained Greek yogurt.

## Conclusion

An increase in consumer demand of clean label yogurts has led to the application of innovative unit processes in its manufacture. These processes include the application of physical forces and chemical modification that are targeted to modify the structure of caseins in milk. Moreover, the development of membrane separation processes can potentially change the dynamic equilibrium of milk proteins with its surrounding medium and could influence their interactions during processing into milk and milk products. Functional ingredients from naturally occurring food biopolymers are a trend catching up amongst food and beverage developers. This is driven by the demand of consumers for clean label ingredient listings on products. The clean label trend is extending into the yogurt market (acid gel) with consumers demanding hydrocolloids, stabilizers or thickeners are purged from ingredient listings. Substituting hydrocolloids in food systems especially yogurts could mean compromising on rheological properties and water holding capacities. However, structure functionality relationship of naturally occurring biopolymer caseins in milk can be exploited to overcome this foreseen loss of product characteristics.

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