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Innovative yoghurts: novel processing technologies for improving acid milk gel texture

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Key words

Acid milk gel, yoghurt, rheology, high pressure processing, high-pressure homogenization, transglutaminase cross linking, ultrasonic processing.

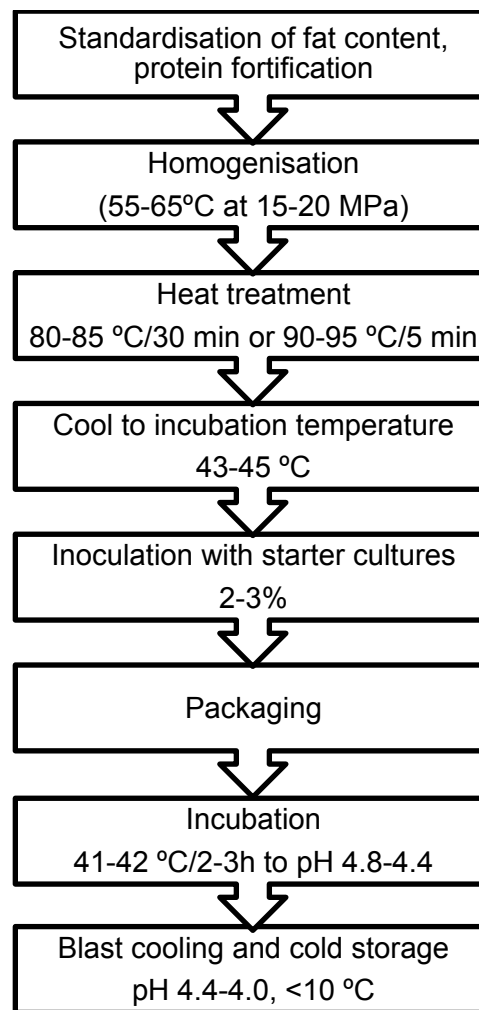
Abstract

Consumers are demanding low-fat yoghurts without hydrocolloid stabilisers, but they are unwilling to compromise on texture for the sake of a 'clean label'. Producing high quality low fat yoghurt without stabilisers is challenging, and there is a need for new processing technologies to address consumer demand. Here we examine four technologies that can potentially improve the texture of yoghurt: high-pressure processing (HPP), high-pressure homogenization (HPH), ultrasonic processing (USP) and protein crosslinking with the enzyme transglutaminase (TG). The benefits of HPH and USP depend on fat content, whilst HPP and TG work best in combination with other processes, and have strong potential for improving protein ingredients.

1. Introduction

Acid milk gels such as yoghurts are popular with a wide spectrum of consumers, mainly due to their nutritional benefits and convenience. The fundamental basis of yoghurt manufacture is the acidic destabilization of milk protein by thermophilic lactic acid bacteria together with optimum heat-induced protein denaturation, producing a three-dimensional gel network capable of holding a serum phase. The structure and properties of milk protein gels have been reviewed extensively in the last few years (Horne, 1999; Lucey, 2002; Sodini, Remeuf, Haddad, & Corrieu, 2004). However, producing a yoghurt with optimum firmness and stability remains a major challenge. The main unit operations involved in yoghurt manufacture are summarised in **Figure 1**.

Figure 1. The main processing steps in the manufacture of set yoghurt. Redrawn from Lucey & Singh (1997).



There are three main types of yoghurt products on the market: set yoghurts, stirred yoghurts and drinking yoghurts. Set yoghurts are usually fermented 'in pack', and have a firm gel texture and 'natural' flavour associated with their more traditional image. Stirred yoghurts are sheared after fermentation, which produces a semi-solid consistency, and stabilisers, fruit pieces and flavours are commonly added. Common stabilisers used in consumer yoghurts include pectins, alginates, carrageenan and celluloses. Drinking yoghurt is made by diluting a fermented yoghurt with water and/or fruit concentrate, and these products have similar characteristics to acidified milk drinks. Most of the scientific literature deals with set yoghurts,

but stirred yoghurts have also been examined in a few cases. Here we concentrate on ways to improve the texture of acid milk gels and reduce syneresis or 'wheying off', i.e. exudation of serum phase, which is often the limiting parameter that determines shelf life.

There is great interest in the nutritional value and health aspects associated with yoghurt, but texture of yoghurt still plays a decisive role in consumer acceptance. With this in mind, yoghurt research and development has focused on the control of gel texture, reduction of whey syneresis, improvement of firmness, and reduction of gelation time.

Yoghurt manufacturers have conventionally used hydrocolloids such as gelatin, pectin, carrageenan and starch to improve the texture of yoghurt and reduce syneresis. However, these stabilizers sometimes bring unwanted flavours and textural attributes, as well as detracting from the 'clean label' status of products. The types and amounts of stabilisers permitted in yoghurts vary between jurisdictions, which makes it problematic to export hydrocolloid-stabilized products.

Dairy ingredients such as skim milk powder (SMP), whey protein isolate (WPI), whey protein concentrate (WPC), sodium (Na)-caseinate or calcium (Ca)-caseinate are also used to increase the total solids content of the yoghurt mix and improve gel firmness. However, fortification with these expensive dairy commodities affects production costs.

Modified processing represents an attractive alternative to adding hydrocolloids or dairy proteins, both from a consumer's point of view, by enabling a 'clean label', and from the producer's point of view, by eliminating expensive ingredients. A number of processing variables can be manipulated, either alone or in combination, to improve the texture and stability of yogurts. The aim is to produce an acid milk gel with high gel strength, short gelation time, smooth texture and little or no syneresis.

We begin with a brief summary of yoghurt texture testing methods, provide a short overview of the roles that heating and acidification play in conventional yoghurt-making processes, then we survey the use of high pressure processing (HPP), high-pressure homogenization (HPH), ultrasonic processing (USP), and enzymatic crosslinking to improve the texture of set yoghurts.

2. Measuring texture and syneresis in acid milk gels

The texture of acid milk gels is typically measured with rotational shear rheometry and penetration tests. Small amplitude oscillatory shear (SAOS) rheometry measures the response of a material to a sinusoidal oscillating stress or strain with an amplitude small enough that deformation is linear, i.e. reversible and non-destructive. Strain/stress waveforms are mathematically processed to extract the elastic modulus, G' , which measures the ability of a material to store energy, the viscous modulus, G'' , which measures a material's ability to dissipate energy in viscous flow, and the phase angle difference between stress and strain, δ (Greek letter delta). The phase angle is often converted to a 'loss tangent', $\tan \delta$, which equals G''/G' , i.e. the ratio of viscous character to elastic character. The gelation time for acid milk gels is often defined as the time for $\tan \delta$ to fall below 1, or the time for G' to increase to an arbitrary limit such as 1 Pa. Other methods for measuring gelation of milk were summarised by Lucey (2002).

Rotational rheometry can also measure how a material fails when it is sheared beyond the linear region, and structure is irreversibly disrupted. Continuous rotational tests measure the fracture stress (units Pa) and fracture strain (no units) when a material is initially deformed

and fractures, then once steady state has been reached, viscosity (units Pa.s) can be measured.

Penetration tests involve pushing a probe into a sample at a constant, slow speed, and recording the force required to do so. A number of empirical parameters can be extracted from the force vs. time/distance curve, such as peak force at penetration (N), penetration depth at failure (mm), penetration work (N.mm) etc. Terms such as 'hardness', 'cohesiveness' and 'gumminess' have been attached to various parameters of compression or penetration curves in an empirical testing protocol called 'texture profile analysis' (Breene, 1975). Probe shapes and penetration speeds are not standardised, so reported penetration parameters from one study may not be comparable to the same parameters measured with a different test procedure.

The spontaneous appearance of free liquid on top of a gel is called 'syneresis' in a general context, or 'wheying off' for acid milk gels. It is a common defect in yoghurts, and reflects the release of serum phase from the protein network. Spontaneous syneresis can be expressed as the weight or volume of serum exuded per unit mass of yoghurt after a standard time at a constant temperature. The shape of the container is important, and Lucey, Munro and Singh (1998) developed simple empirical tests for spontaneous syneresis using 250 mL volumetric flask or large glass petri dishes. 'Forced syneresis' or 'water holding capacity' expresses the amount of liquid exuded after mild centrifugation or drainage through a screen. Forced syneresis is related to the rigidity of gels, which is not necessarily relevant to the spontaneous syneresis defect in set yoghurts (Lucey, 2001).

3. Heat Treatment

Heat treatment of milk prior to acidification is an integral part of yoghurt manufacture as shown in Figure 1. Aside from the role of pasteurization to destroy pathogenic microbes, heating of milk during yoghurt manufacture results in a number of physiochemical changes in the milk constituents (Lucey, Munro, & Singh, 1999). Significant changes occurring upon heating milk above 70°C include the denaturation and aggregation of whey proteins, mainly β -lactoglobulin (β -lg) and α -lactalbumin (α -lac) (Anema & McKenna, 1996; Oldfield, Singh, Taylor, & Pearce, 2000), interactions between denatured whey proteins and the casein micelles (particularly κ -casein) via hydrophobic interactions and disulphide bonds (Anema & Li, 2003; Corredig & Dalgleish, 1996), and the transfer of soluble calcium, magnesium and phosphate from the serum to colloidal casein micelles (Schreiber, 2001; Singh & Waungana, 2001). Heating milk at pH < 6.6 drives whey proteins to associate with casein micelles in preference to aggregating with other whey proteins (Lakemond & van Vliet, 2005). A significant proportion of thiol groups appear to be reactive even after cooling the milk (Vasbinder, Alting, Visschers, & De Kruif, 2003), enabling further disulphide bridging to occur at ambient temperature.

Donato et al. (2007) suggested that heat-induced soluble protein aggregates enabled the formation of an 'intermediate network' with micelle-associated whey proteins when protein mixtures were acidified. The intermediate network was detected ahead of gelation. Networking between micelle-associated whey proteins and soluble aggregates was thought to explain why heated milk protein mixtures gelled at higher pH than unheated mixtures.

As a result of the protein interactions described above, heat treatment of milk increases the storage modulus (G') of the acid gel, decreases the gelation time, reduces syneresis and

increases the pH at which gelation occurs (Anema, Lee, Lowe, & Klostermeyer, 2004; Lakemond & van Vliet, 2005; Lucey, Teo, Munro, & Singh, 1997).. Gels made from unheated milk have a 'tortuous' clustered network structure whereas gels made from heated milk consist of a highly 'branched' network with more interconnected aggregates (Lucey, et al., 1999). The denatured whey proteins appear to act as dense filamentous projections providing connecting bridges between the casein micelles.

4. Acidification

In industrial yoghurt production, milk is generally acidified by the metabolic action of bacterial cultures, which produce lactic acid from lactose. However, the most common method of preparing acid milk gels for research purposes is the use of glucono- δ -lactone (GDL), which yields gluconic acid on spontaneous hydrolysis of the internal ester bond. The rate of acidification can be controlled very reproducibly by varying the GDL concentration and incubation temperature (de Kruif, 1997; Lucey, Tamehana, Singh, & Munro, 1998), so GDL acidification avoids some of the variability associated with bacterial acidification. In this review, we use the term 'yoghurt' to indicate a bacterially acidified gel and 'acid milk gel' to indicate acidification with glucono- δ -lactone.

Lucey, Tamehana et al. (1998) examined structural, rheological and permeability differences between gels acidified with GDL and those acidified with a bacterial culture. They concluded that the different kinetic patterns of acidification produced differences in gel structure and physical properties, and they advised that model studies with GDL should be verified using bacterial cultures.

The effect of acidification on the forces within and between casein micelles can be delineated into three arbitrary pH zones, as summarized in Table 1 (Lucey, 2004). As milk is acidified, the charges on caseins decrease, which weakens the electrostatic forces holding micelles together, and weakens the steric stabilisation provided by charged κ -casein 'hairs' on the micelle surface. In the absence of steric stabilisation, there is a weak attractive force between micelles, which become 'adhesive' or 'sticky' and form a weak particle gel (Horne, 1999).

Shortly after incipient gelation there is a peak in $\tan \delta$. The peak is thought to indicate a loosening or increased bond mobility in the gel as calcium phosphate is solubilised, and there is a transition from a network dominated by the interactions of denatured whey proteins to a network in which casein-casein interactions become dominant (Lucey, 2008; Lucey, Tamehana, et al., 1998). This structural transition is more pronounced during acidification at higher temperature, which produces a coarse microstructure with large pores and high permeability (Lee & Lucey, 2004; Peng, Horne, & Lucey, 2010).

In acid milk gels, the incubation temperature at which acidification occurs influences gel structure, i.e. gels are coarser and more permeable when incubated at higher temperature (in the range 20-40 °C) (Lakemond & van Vliet, 2005). However an acid milk gel with a fine network structure will collapse when pasteurised at 80-90 °C, whereas a somewhat coarse gel resists pasteurisation because of thicker crosslinks (Lakemond & van Vliet, 2005).

5. Using novel processing technologies to modulate yoghurt texture

Novel processing technologies offer the potential to reduce costs and improve the quality of existing products, but in some cases they can produce new food structures and textures. Of

the novel food processing technologies that have emerged recently, the most promising for yoghurt manufacture are high pressure processing (HPP), high-pressure homogenization (HPH), enzymatic crosslinking by transglutaminase (TG), and ultrasonic processing (USP). The purpose of this section is to provide a focussed perspective of these emerging processing technologies, in which their mechanistic effects on milk components and potential for industrial use in yoghurt manufacture are evaluated.

5.1. High pressure processing (HPP)

The first study on use of HPP processing for food applications dates back to the late 19th century, but HPP processing is still considered a novel technology because industrial HPP processing equipment has only recently become available (Huppertz & de Kruif, 2006). HPP treatment has been applied for preserving a range of food products, notably for meat, vegetables, fruit juice, beverages and seafood products. Despite a number of well-documented studies into the effects of HPP processing on milk components and properties (see below), HPP-processed yoghurts have not yet reached the market.

HPP processing significantly influences the chemistry of milk proteins, and can improve their functional properties in dairy applications. Detailed information about impact of high-pressure processing on milk constituents can be found in recent reviews (Considine, Patel, Anema, Singh, & Creamer, 2007; Devi, Buckow, Hemar, & Kasapis, 2013; López-Fandiño, 2006). The changes relevant to yoghurt manufacture can be broadly summarized as 1) shrinking and/or disruption of casein micelles 2) denaturation of whey proteins, especially β -lg, and 3) alteration of the mineral balance, mainly by the release of colloidal calcium phosphate from casein micelles (Huppertz, Kelly, & Fox, 2002).

Casein micelles are initially destabilised by pressures above 250 MPa (2500 bar), resulting in a decrease in the amount of micellar casein and the size of micelles, a phenomenon that is more rapid and/or extensive at higher pressures (Huppertz, Kelly, & De Kruif, 2006; López-Fandiño, 2006) and lower temperatures (Anema, Lowe, & Stockmann, 2005; Orlie, Knudsen, Colon, & Skibsted, 2006). However, prolonged pressure treatment at 200-300 MPa results in a re-aggregation of caseins that is independent of the presence of whey proteins (Anema, Lowe, et al., 2005; Huppertz & de Kruif, 2006; Huppertz, et al., 2006; Orlie, et al., 2006). The initial disruption is attributed in part to increased solubility of colloidal calcium phosphate at high pressure (Anema, Lowe, et al., 2005; Huppertz, et al., 2006). Reassociation of pressure-dissociated caseins is thought to be driven by hydrophobic interactions, which are facilitated by charge-shielding by solubilised calcium (Anema, Lowe, et al., 2005; Orlie, et al., 2006). However, the effects of pressure on hydrophobic interactions are complex, and vary with the applied pressure (Huppertz, et al., 2006).

Both β -lg and α -lac denature when heated above 70 °C, and minor whey proteins are even more heat-sensitive, but β -lg denatures above 150 MPa, whereas α -lac and BSA are quite stable up to higher pressures (Considine, et al., 2007; Huppertz, Fox, & Kelly, 2004; López-Fandiño, 2006). The extent of α -lac and β -lg denaturation during HPP treatment increases with increasing temperature, milk pH, pressure and time (Anema, Stockmann, & Lowe, 2005; Huppertz, et al., 2004; López-Fandiño, 2006; Walsh-O'Grady, O'Kennedy, Fitzgerald, & Lane, 2001). Anema, Stockmann and Lowe (2005) carried out a detailed kinetic analysis of pressure-induced β -lg denaturation, and suggested that the rate-limiting step for loss of native β -lg changed from aggregation reactions below 300 MPa to monomer unfolding above 300 MPa. A subsequent study examined the effect of the total solids content on the kinetics of pressure-induced β -lg denaturation in skim milk (Anema, 2012). Denatured β -lg associates

extensively with caseins during pressure treatment, but is also present as monomers or small aggregates (Huppertz, et al., 2004).

There is no real consensus about the effect of HPP on the mineral balance in milk. Equivocal results may stem in part from the variability in processes and substrates, as well as different ways of measuring calcium in milk (Huppertz, et al., 2006).

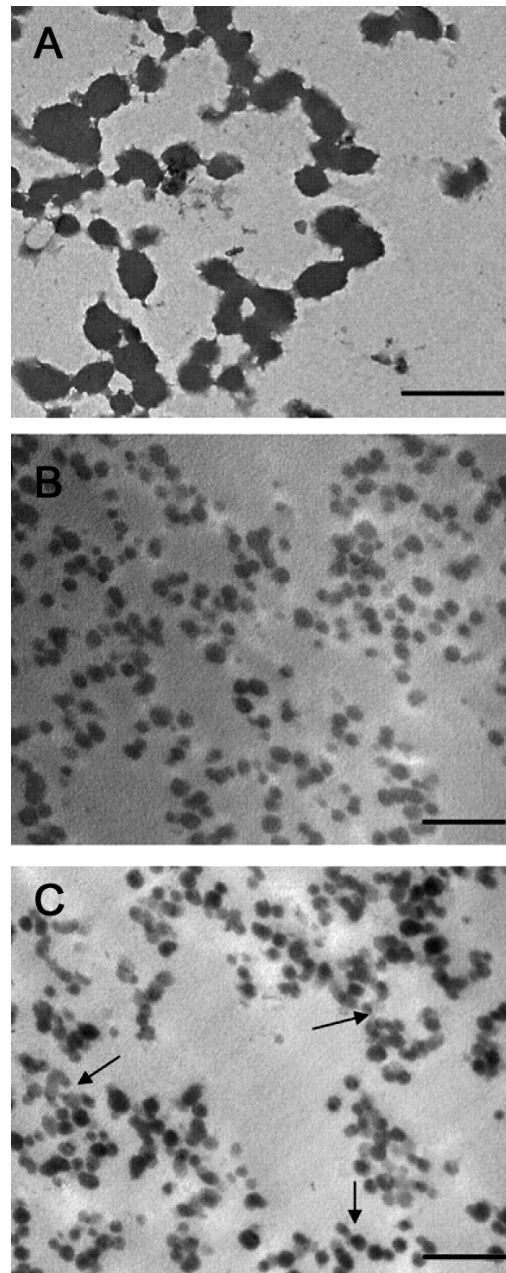
The changes in protein chemistry and interactions that result from HPP treatment of milk generally improve the properties of resulting acid gels. HPP-treated milk can produce acid gels with increased rigidity, breaking strength and decreased syneresis as compared with gels from untreated milk (Considine, et al., 2007; Harte, Amonte, Luedecke, Swanson, & Barbosa-Cánovas, 2002; Harte, Luedecke, Swanson, & Barbosa-Cánovas, 2003). Anema (2010) noted that HPP treatment in the absence of heating dramatically increased the gelation pH, even when a shear step was included. Comparisons between HPP- and heat-treatment are more complicated because there is no physical basis for selecting comparable conditions. Although 'typical' industrial heat treatments are used in comparisons, e.g. 80 °C for 30 min, no analogous 'typical' combinations of pressure and time exist for HPP, and treatment parameters vary widely.

Needs et al. (2000) compared fermented set yoghurts made from WPC-fortified skim milk that was HPP-treated (600 MPa for 5 min) or heat treated (85 °C for 20 min). Both treatments resulted in > 90% denaturation of β -lg, but denatured β -lg was associated with only the surface of casein micelles after the heat treatment, whereas denatured β -lg in HPP-treated milks interacted more intimately and uniformly with micelle fragments as a result of pressure-induced micelle dissociation. This may have been responsible for the higher G' and G'' at low deformation, but large-deformation tests showed that fracture stress and strain were lower in HPP gels, which the authors attributed to the lack of long-range β -lg homopolymer bridges at micelle surfaces (Needs, et al., 2000). Similar microstructural differences were reported in other studies of set or stirred fermented yoghurts made from heat- or HPP-treated milks. In those studies, yield stresses were lower in both stirred (Penna, Subbarao, & Barbosa-Canovas, 2007) and set (Harte, et al., 2003) HPP yoghurts, although yield stress results varied with measurement method and milk composition (Harte, et al., 2002; Harte, et al., 2003). **Figure 2** shows an example of how heat and HPP affect the microstructure of fermented yoghurt.

Walsh-O'Grady et al. (2001) examined whether HPP-treatment of whey proteins alone (i.e. as WPI prior to mixing with caseins) could enhance the properties of acid milk gels. HPP of WPI solutions (700 MPa for 20 min) did improve gel properties relative to the untreated control, but HPP in the presence of casein gave a superior result, and this in turn was surpassed by heat treatment of WPI (78 °C for 30 min) or the WPI-casein mixture (90 °C for 10 min).

For stirred acid milk gels, Knudsen et al. (2006) reported that treating skim milk at 600 MPa for 20 min produced gels with similar rheological properties (G' , G'' , fracture stress) to those derived from milk that was heat-treated at 90 °C for 30 min. Gels from milk treated at 400 MPa for 20 min had inferior rheological properties to those treated at 600 MPa for the same time. However another study found that longer HPP pre-treatments at 400 MPa (60 or 120 min) produced set acid milk gels with comparable properties to those from heat-treated milks (90 °C for 15 min) (Anema, Lauber, Lee, Henle, & Klostermeyer, 2005). Both HPP and heat treatment denatured virtually all β -lg, but heat treatment produced four times the amount of α -lac denaturation of HPP. Interestingly, HPP-treatment for 60 min produced stronger gels than HPP-treatment for 120 min (Anema, Lauber, et al., 2005).

Figure 2. Microstructure of fermented yogurt made using milks that were pre-treated with (A) heat: 85 °C for 30 min, (B) HPP: 676 MPa for 5 min, or (C) HPP then heat. Arrows indicate filamentous bridges between micelles. Scale bars are 1000 nm. From Penna et al. (2007).



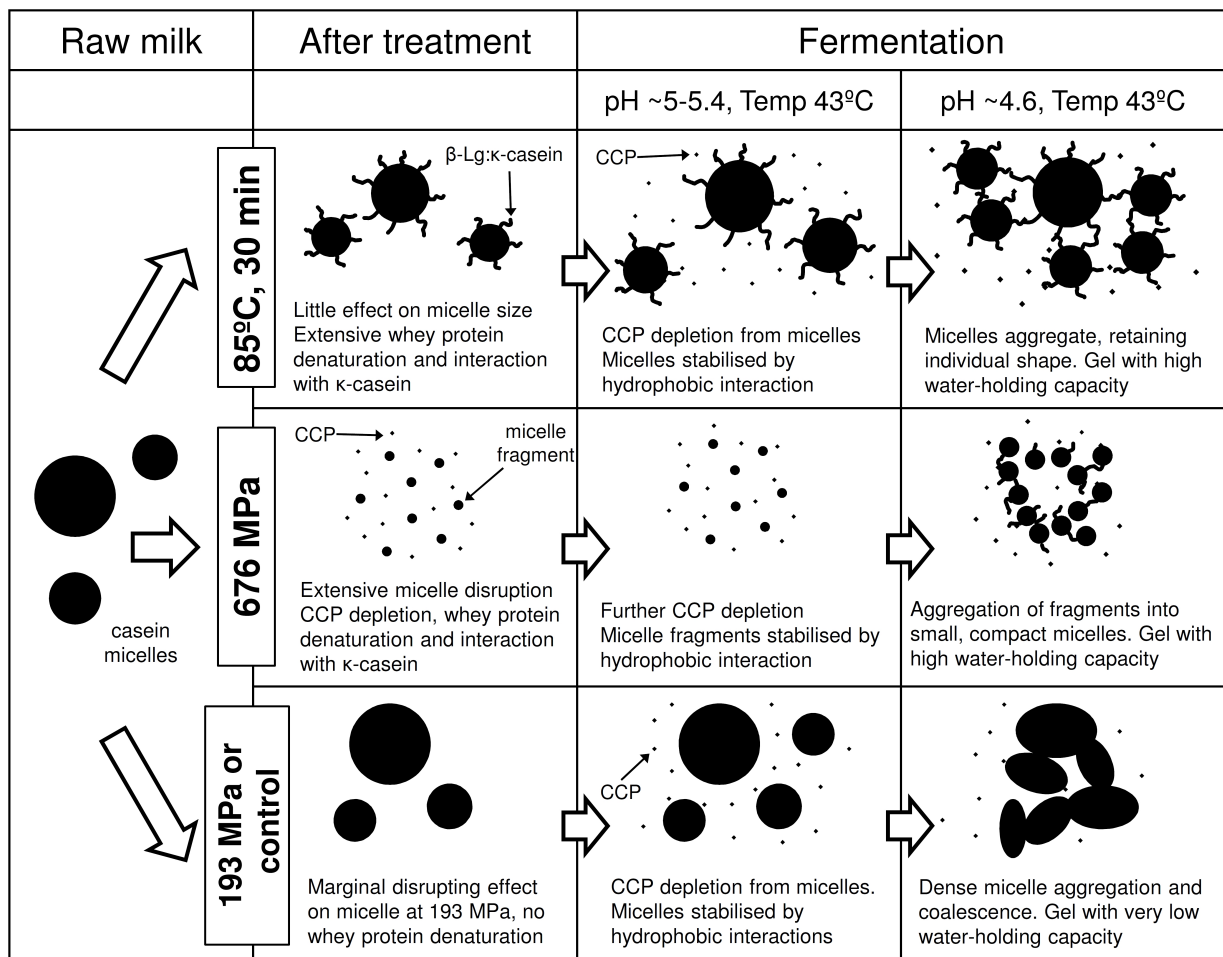
Harte et al. (2003) trialed combined heat and HPP treatments in a set yoghurt process involving fermentation with commercial starter cultures. A combined HPP treatment (300 to 676 MPa, 5 min) and heating (85°C, 30 min) prior to inoculation produced higher G' , viscosity and yield stress, and reduced syneresis in comparison with yogurts made from thermally treated milk alone or raw milk. Yield stress, G' and viscosity were insensitive to the pressure level, but syneresis decreased with increasing pressure, and better gels were formed when the heat treatment preceded HPP treatment than the other way around (Harte, et al., 2003). In a similar study with stirred fermented yoghurt (Penna, et al., 2007), heating before HPP produced an unappealing colour change, but HPP (676 MPa for 5 min) then heating (85 °C for 30 min) produced a more uniform microstructure and reduced syneresis compared with heating alone. Yoghurts produced with the combination treatment had comparable

rheological properties to those produced with heat alone, except that yield stress was higher or lower, depending on the starter strain (Penna, et al., 2007).

Mild HPP can be applied to yoghurts after fermentation to kill spoilage organisms and inhibit further acidification during shelf life. However lactic acid bacteria are killed by HPP at pressures >300 MPa, and HPP can alter yoghurt texture under some conditions (Jankowska, Grzekiewicz, Winiewska, & Reys, 2012).

To conclude, HPP produces some of the same changes in milk protein chemistry as heating, e.g. whey protein denaturation, but other effects are different due to pressure-induced disruption of casein micelles. It is difficult to generalise further than that, because HPP treatment time and pressure have complex non-linear effects on the integrity of casein micelles and the kinetics of whey protein denaturation reactions. The contrasting effects of HPP and heat are summarised schematically in **Figure 3**.

Figure 3. Effect of selected milk treatments and fermentation on casein micelles and gel structure. CCP = Colloidal Calcium Phosphate. Redrawn from Harte et al. (2002).



The more uniform association between denatured β-Ig and micelle fragments that occurs during HPP appears to be responsible for reduced syneresis and improvements in small-deformation rheological parameters in HPP set yoghurts. However large-deformation properties are not always improved, and stirred yoghurts show less textural improvement than set yoghurts. The literature is inconclusive with regards to the effect of combined heat and HPP treatments in yoghurt manufacture, and more research is needed in this area.

5.2. High-pressure homogenization (HPH)

Homogenization is a process in which milk is subjected to high shear, typically by pumping through a small orifice, in order to break up milk fat globules into much smaller droplets that are slower to rise to the surface and coalesce into a cream layer. Homogenization has been a standard process in the dairy industry since the early 20th century, and general principles and practices are reviewed elsewhere (Walstra, Wouters, & Geurts, 2005). Conventional homogenization involves pressures of up to 30 MPa, but high-pressure homogenization (HPH), in which pressures of up to 400 MPa (4000 bar) are used, has recently emerged as a process for achieving more uniform fat globule size and partially denaturing whey proteins, as well as inactivating enzymes and microorganisms (Huppertz, 2011). HPH is sometimes termed 'ultra high-pressure homogenization', or 'dynamic high-pressure treatment, and 'microfluidisation' refers to a specific type of HPH in which two fluid streams are forced together. HPH is usually done with valve homogenizers (Huppertz, 2011), but other designs are also used, and a given homogenization pressure may produce different effects with different equipment designs.

Homogenization is an important step in the manufacture of yoghurts containing fat, because as well as reducing fat globule size dramatically, it leads to the adsorption of casein and some whey proteins at the globule surface, which allows fat droplets to mingle and interact with the protein network that forms on acidification (Titapiccolo, Corredig, & Alexander, 2011). HPH can produce a modest reduction in the size of casein micelles, which coincides with increased solubility of calcium and some caseins, but little change in micelle morphology (Roach & Harte, 2008; Sandra & Dalgleish, 2005). Higher pressures and more passes through the homogenizer give larger reductions in micelle size, and heat treatment before or after homogenization slightly mitigates micelle shrinkage (Sandra & Dalgleish, 2005).

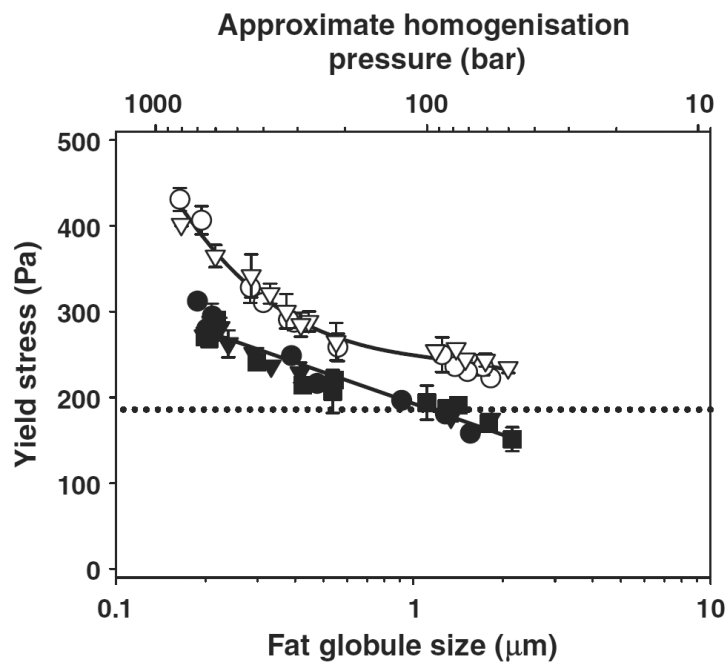
Serra, Trujillo, Jaramillo, Guamis, and Ferragut (2008) reported that β -lg denaturation was much lower in HPH-treated milks compared with heat-treated milks, and yoghurts from HPH milks had lower firmness and higher forced syneresis than controls. The acidification rate was virtually unchanged in HPH yoghurts, but they had higher optical density than the control yoghurts (Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007). Other studies by this group showed that HPH treatment had no significant adverse impact on the sensory properties of yoghurt, and the survival rate of bacterial cultures in yoghurt was largely unaffected (Serra, Trujillo, Guamis, & Ferragut, 2009; Serra, Trujillo, Pereda, Guamis, & Ferragut, 2008).

Hernandez and Harte (2008) investigated how combinations of HPH and heat treatment affected the properties of acid skim milk gels. HPH treatment alone did not improve the gelation time or G' of gels. Combining a heat treatment of 90 °C for 5 min with HPH did not affect gelation time or whey holding capacity, but it increased the G' of gels when pressures of >200 MPa were used, and it made little difference whether HPH was before heat treatment or after (Hernández & Harte, 2008). By contrast Ji, Lee and Anema (2011) found that yield stress and yield strain of acid whole milk gels were higher when HPH occurred before heat treatment. HPH markedly improved rheological properties, particularly at pressures >200 MPa. Results are summarised in **Figure 4**. The different results in these two studies can be reconciled by considering that fat content was very different.

The influence of fat content on the HPH effect was evident in recent work by Ciron et al. (2010). They reported that the texture of non-fat stirred yoghurts prepared from milk that was heated then homogenized was detrimentally affected by HPH (compared to a conventionally-homogenized control), as measured with an empirical large-deformation test. However, low-fat yoghurts (1.5% w/w fat) made with HPH-treated milk had very similar

texture profiles to control yoghurts, despite having much smaller fat globules, which were much more closely associated with the protein network, as shown in Figure 5 (Ciron, et al., 2010).

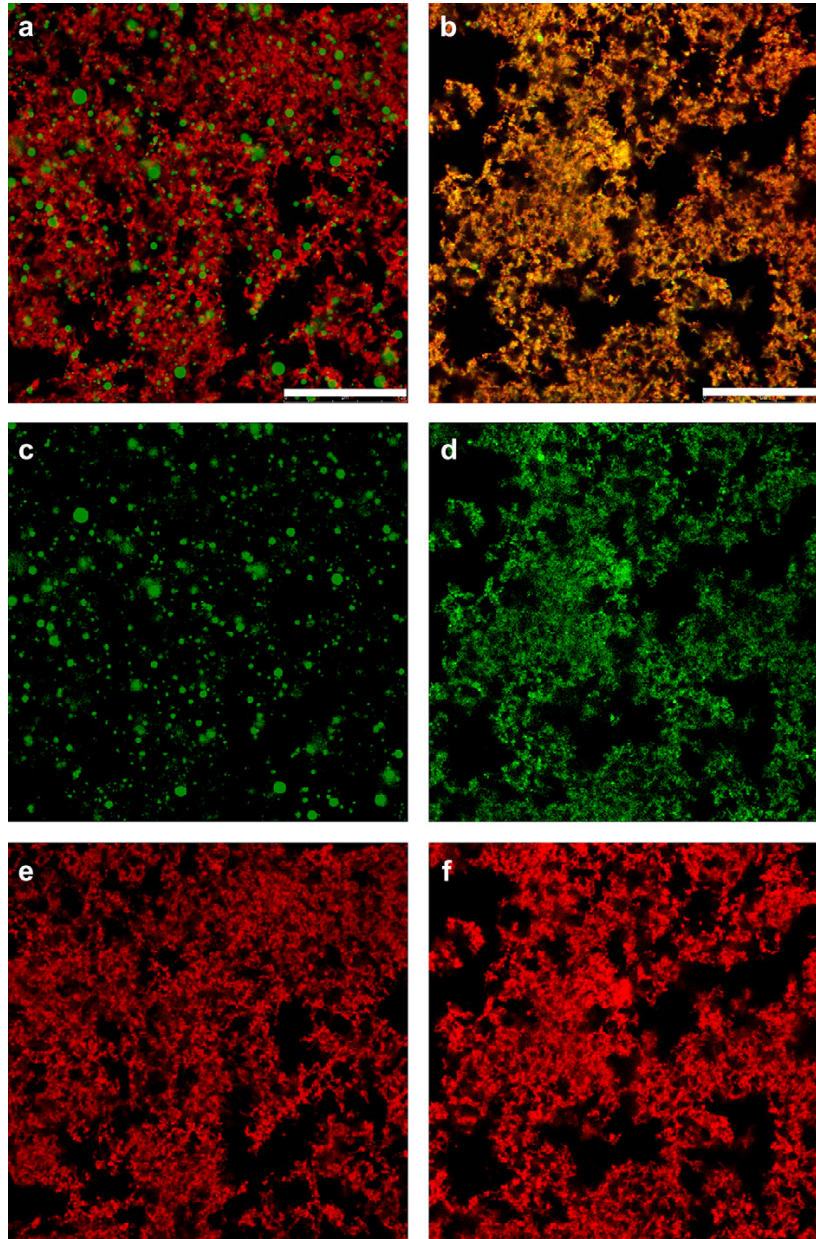
Figure 4. Yield stress as a function of fat globule size for acid gels from recombined milks that were prepared by either homogenising unheated skim milk and anhydrous milk fat then heating the samples at 80 °C /30 min (open symbols), or by heating skim milk at 80 °C /30 min and then homogenising with anhydrous milk fat (filled symbols). Different symbols show the results from repeated experiments, and error bars are the standard deviations from repeated measurements of the same sample. The dotted line indicates the yield stress for the control acid gel prepared from heated skim milk. From Ji et al. (2011).



Subsequent work using more fundamental rheological measurements (Ciron, Gee, Kelly, & Auty, 2011), showed that HPH stirred yoghurts (both non-fat and low-fat) had similar characteristics to controls in small-amplitude oscillatory shear measurements, but much higher yield stress and viscosity in continuous shear tests. The hysteresis loop area was greater with HPH, i.e. less ability to recover structure after disruption by shearing (Ciron, et al., 2011), which is consistent with stronger interactions between fat droplets and the protein matrix (Ciron, et al., 2010). Rheological differences were reflected in sensory characteristics: both desirable and undesirable characteristics were accentuated in non-fat yoghurt, whereas only desirable characteristics such as creaminess, smoothness and thickness were accentuated in low-fat yoghurts (Ciron, et al., 2011).

Further work by this group (Ciron, Gee, Kelly, & Auty, 2012) examined whether HPH treatment (25-150 MPa) before heating could improve the properties of low-fat stirred yoghurts. The homogenization pressure had a significant effect in the range 25 – 50 MPa, and at pressures \geq 50 MPa the rheological parameters of HPH low-fat yoghurts (1.5% w/w fat) and conventional full-fat yoghurts (3.5% w/w fat) were statistically indistinguishable, despite differences in microstructure. HPH at \geq 50 MPa produced low-fat yoghurts with sensory attributes similar to, or better than, full-fat conventional yoghurts (Ciron, et al., 2012).

Figure 5. Confocal micrographs of low-fat stirred yoghurts as affected by homogenization condition: conventional homogenisation (a, c, e) and high pressure microfluidisation (b, d, f). Green (c and d) and red (e and f) channels are also presented separately to show fat and protein phases, respectively. The protein matrix is red while the fat globules are green; yellow indicates intermixing of red and green channels due to co-localization of pixels, scale bar = 25 μm . From Ciron et al. (2010)



Sensory differences were less evident in set yoghurts produced from heated milks (90 °C for 20 min) with or without HPH treatment (60 MPa), even though HPH improved texture profile results (Patrignani, et al., 2009). Earlier work with a similar set yoghurt system identified a complex nonlinear relationship between viscosity, milk solids percentage, and homogenization pressure (Lanciotti, Vannini, Pittia, & Guerzoni, 2004). These studies identified that HPH favoured the growth of *Streptococcus thermophilus* over that of *Lactobacillus delbrueckii* subsp. *Bulgaricus* (Lanciotti, et al., 2004; Patrignani, et al., 2009). After 35 days' storage at 4 °C, the loading of both cultures was generally higher in yoghurts made with HPH-treated milk than in those made with heat-treated milk (Patrignani, et al., 2009).

In conclusion, HPH has the potential to improve texture and fat perception in low-fat yoghurts, but has detrimental effects on non-fat yoghurts. Homogenization pressure needs to be above a critical limit, but the literature is equivocal about whether further increases in pressure lead to improvements in texture. The benefits of HPH are highest when combined with a heating step, and the few studies of combined processes indicate that HPH should precede heating.

5.3. Ultrasonic processing (USP)

Ultrasonication or ultrasonic processing (USP) can generate significant change in physical and chemical properties in liquid foods using high-intensity ($10\text{-}1000\text{ W}\cdot\text{cm}^{-2}$) sound waves at frequencies above those audible to the human ear ($>18\text{ kHz}$). High power ultrasound processing at frequencies in the kilohertz range should be distinguished from low power, high-frequency ultrasound analytical techniques. High-powered sound waves create cavitation in liquids, leading to transient local extremes of pressure, temperature and shear. These conditions are useful in food processing for destroying microorganisms and disrupting structures, particles or droplets in solution. Ultrasonic processing (USP) is a relatively novel technology, which has potential as an alternative to conventional pasteurization (Bermúdez-Aguirre, Mawson, Versteeg, & Barbosa-Cánovas, 2009) or even combined pasteurization and homogenization in a single unit operation .

The principles of USP and its applications in food processing have been the subject of recent reviews (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Chandrapala, Oliver, Kentish, & Ashokkumar, 2012; Esclapez, García-Pérez, Mulet, & Cárcel, 2011), and the use of USP in dairy processing is discussed by Deeth and Datta (2011) and Villamiel, van Hamersveld and de Jong (1999).

One by-products of USP is the production of free radicals, and care must be taken to avoid oxidative damage to lipids and labile nutrients during USP (Ashokkumar, et al., 2008). Another by-product of USP is the concomitant heating of the solution, which can be very significant under typical USP processing conditions. The heating effect is beneficial for yoghurt processes, in which whey protein denaturation is desirable. Villamiel and de Jong (2000) tested a continuous flow USP process in which milk reached $75.5\text{ }^{\circ}\text{C}$, and compared the effects on milk chemistry with those of a heat treatment of the same duration at the same temperature, or USP with the equipment submerged in an ice/water bath to eliminate thermal effects. Cold USP partially denatured $\beta\text{-lg}$ and $\alpha\text{-lac}$ (denaturation was measured as casein-associated protein), and USP-associated heating synergistically enhanced denaturation. The effect on whey proteins was much larger in whole milk than in skim milk, and the denaturation of milk enzymes followed the same pattern. In another study using the same equipment operated at higher pressure and lower temperature, USP-treated milk produced firmer yoghurts, but gelation took longer (Vercet, Oria, Marquina, Crelier, & Lopez-Buesa, 2002).

Nguyen and Anema (2010) examined how thermal and non-thermal effects of USP affected the properties of USP-treated skim milk and acid gels produced from it. In the absence of temperature control, USP at 22.5 kHz and 50 W increased the temperature of skim milk to $95\text{ }^{\circ}\text{C}$ within 15 min, which completely denatured whey proteins. Resulting acid gels had slightly higher G' than with heated milks ($80\text{ }^{\circ}\text{C}$ for 30 min). USP decreased the size of casein micelles at low temperature and increased it at high temperature, as well as solubilising $\kappa\text{-casein}$, which was thought to form soluble aggregates with denatured whey proteins. Nguyen and Anema (Nguyen & Anema, 2010) concluded that most of the benefit from USP derived from

the heat that it generated, and non-thermal effects produced only small improvements over conventional heating.

An earlier study of USP processing in a full-fat yoghurt process (Wu, Hulbert, & Mount, 2001) reported that USP could produce higher water holding capacity, higher viscosity (empirical measure), and lower syneresis. USP was carried out in a water bath at 15 °C to reduce thermal effects, and all milks were heat-treated (95 °C for 10 min) prior to USP. At the highest power level used, USP produced much smaller fat droplets than the conventional homogenization control treatment (12.4 MPa at 60 °C), which may account for some of the improvement in texture. Interestingly, inoculating with starter cultures before USP produced faster acidification, improved water holding and higher viscosity.

Riener et al. (2009) shed light on how fat content (0.1%, 1.5% or 3.5% w/w) influences the results of USP processing for set yoghurts. They compared a 10 min USP treatment, in which the temperature reached 72 °C after 6 min, with a heat treatment at 90 °C for 10 min. USP-processed milks gave higher final G' , water holding capacity and gelation pH than heated controls, and the effects became larger as fat content increased. The microstructure of yoghurt from USP-treated milk was more open, and had smaller pores, compared with a heated control. A subsequent study by the same authors (Riener, Noci, Cronin, Morgan, & Lyng, 2010) confirmed effects of both USP and fat content using penetration tests, continuous flow rheometry and measures of spontaneous and forced syneresis.

In summary, the effects of USP on milk chemistry are complex, and strongly dependent on temperature and fat content. The main effects are related to moderate whey protein denaturation and a large reduction in fat globule size. USP has shown promise for improving the texture of low-fat and full-fat yoghurts at laboratory scale, but this has yet to be translated to benefits at production scale.

5.4. Transglutaminase (TG) Crosslinking

The enzyme transglutaminase (TG) catalyses the formation of a covalent bond between a primary amine such as in lysine, and the carboxamide group of a glutamine residue. The full name of TG is glutaminyl-peptide:amine γ -glutamyltransferase (EC 2.3.2.13), and the covalent bond it produces is called an ϵ -(γ -glutamyl)lysine isopeptide bond. TG also catalyses a side reaction that converts glutamine residues to glutamate, and this may have significant textural and/or nutritional effects in some food systems (Gerrard, et al., 2012).

The use of TG crosslinking as a tool to manipulate food texture has become feasible only since a microbial source of TG was commercialised in the late 1980s. TG is classed as a processing aid by the European Union (EU regulation 1332/2008) and has 'generally recognised as safe (GRAS)' status in the USP. The characteristics of TG and its use in foods are described in several general reviews (de Jong & Koppelman, 2002; Kuraishi, Yamazaki, & Susa, 2001), and the use of TG in dairy products is covered in another review (Jaros, Partschefeld, Henle, & Rohm, 2006).

The caseins are a good substrate for TG in milk (de Jong & Koppelman, 2002; de Jong, Wijngaards, Boumans, Koppelman, & Hessing, 2001), a fact that is attributed to their open, flexible structure, in which lysine and glutamine residues are easily accessible to the enzyme (Jaros & Rohm, 2011). When casein is in a micellar form, TG cross-links κ -casein faster than the other caseins, apparently due to its location at the surface of micelles (Sharma, Lorenzen, & Qvist, 2001; Smiddy, Martin, Kelly, de Kruif, & Huppertz, 2006). Cross-linking of casein micelles seems to be exclusively intra-micellar, since TG treatment of micellar casein does not

increase particle size, as measured by light scattering (Bönisch, Lauber, & Kulozik, 2007; Ercili-Cura, et al., 2013; Vasbinder, Rollema, Bot, & de Kruif, 2003). The susceptibility of individual caseins to TG crosslinking varies with temperature, pH and calcium molarity, for example increasing temperature or CaCl_2 promotes κ -casein cross-linking by TG, but the rate of α_{s1} -casein crosslinking is unaffected by either (Hinz, Huppertz, & Kelly, 2012).

Sodium caseinate, in which the micellar structure has been destroyed, is crosslinked much more extensively by TG than is micellar casein (Bönisch, Lauber, et al., 2007). In contrast to caseins, the whey proteins have more compact, folded structures stabilised by disulphide bonds, and are generally poor substrates for TG unless reduced or denatured (de Jong, et al., 2001).

TG itself is heat-sensitive, and its catalytic activity is also eliminated by thiol-blocking agents such as N-ethylmaleimide and zinc (Jacob, Nöbel, Jaros, & Rohm, 2011; Jaros & Rohm, 2011; Lu, Zhou, Tian, Li, & Chen, 2003). Milk serum is thought to contain a small heat-sensitive TG inhibitor, which must be thermally deactivated (Bönisch, Tolkach, & Kulozik, 2006; de Jong, Wijngaards, & Koppelman, 2003) or chemically blocked (Bönisch, Lauber, et al., 2007) before TG will be effective. The inhibitor affects TG crosslinking of micellar casein but not sodium caseinate (Bönisch, Lauber, et al., 2007), which suggests that the affinity between the inhibitor and the TG binding site is higher than that of micellar casein and lower than that of sodium caseinate. Some studies have reported that TG is still active in raw skim milk (Jacob, et al., 2011; Jaros, Jacob, Otto, & Rohm, 2010), and these results have yet to be reconciled with other findings.

In TG-crosslinked milks, the $\tan \delta$ peak seen during acidification of untreated milks (see section 4) is absent (Ercili-Cura, et al., 2013; Mounsey, O'Kennedy, & Kelly, 2005), even though colloidal calcium phosphate is still solubilised (Mounsey, et al., 2005). Intramicellar crosslinks formed by TG inhibit the structural rearrangement that otherwise occurs shortly after gelation, and for that reason, TG-treated milks form acid gels with a more homogeneous microstructure that has smaller pores (Ercili-Cura, et al., 2013).

TG treatment generally does not affect the rate of pH decline during acidification (Anema, Lauber, et al., 2005; Bönisch, Huss, Weitzl, & Kulozik, 2007; Ercili-Cura, et al., 2013; Jacob, et al., 2011). The effect on gelation time varies between studies, with some showing that gelation occurs slightly later in TG-treated milks (Ercili-Cura, et al., 2013; Jaros, Pätzold, Schwarzenbolz, & Rohm, 2006; Mounsey, et al., 2005), indicating gelation slightly earlier (Anema, Lauber, et al., 2005; Jacob, et al., 2011), but differences are small in all cases.

The use of TG in acid milk gel processes was comprehensively reviewed several years ago by Jaros, Partschfeld, et al. (2006), and here we focus on work published since that review. TG may be used in several ways in yoghurt manufacture: 1) TG treatment of milk followed by deactivation of TG then acidification/fermentation, or 2) addition of TG to milk at the same time as the starter culture, i.e. crosslinking during acidification.

5.4.1. Crosslinking prior to acidification

Using TG-treated milk in which TG has been deactivated prevents further crosslinking reactions during subsequent processing. In this scenario, TG is usually deactivated by heating, and any influence of TG on acid milk gel texture must be distinguished from the positive influence of the heat treatment.

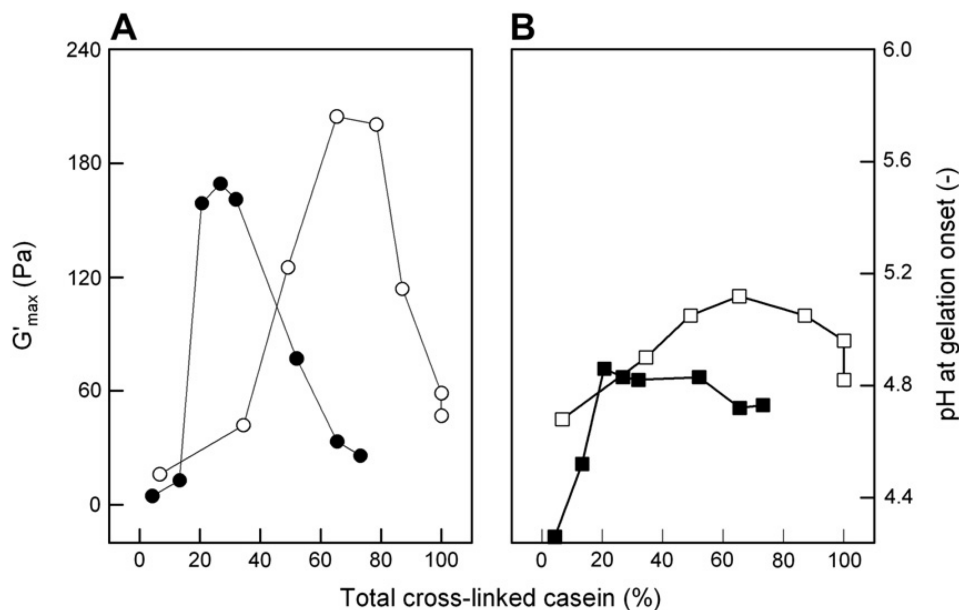
Jaros, Pätzold, et al. (2006) addressed this distinction in a 2 x 2 factorial experiment, in which UHT milk was TG treated or untreated, and subsequently heated or unheated before

acidification with GDL. TG treatment increased G' during acidification, and produced gels with higher force at fracture (penetration test) and larger fracture strain (large amplitude oscillation). Heat treating the milk (85 °C for 10 min) produced stronger gels in the absence of a TG treatment, but slightly weaker gels with TG-treated milk. The authors believed that covalent isopeptide bonds introduced by TG may have restricted the formation of further physical bonds (e.g. hydrophobic interactions) that would otherwise be produced during the heat treatment. They also suggested that residual TG activity in unheated milks may have produced further crosslinks during acidification.

Several groups have examined how the amount of TG and/or the treatment time affect the amount of crosslinking and the texture of acid milk gels (Bönisch, Lauber, et al., 2007; Jaros, et al., 2010; Jaros, Pätzold, et al., 2006). In experiments testing the effect of treatment time, these studies used the same TG product (Activa[®] MP from Ajinomoto Foods) applied at the same level of 3 enzyme units per gram of milk protein ($\text{U}\cdot\text{g}^{-1}$), although Jaros, Pätzold, et al. (2006) measured an activity of 77.5 U per gram of powder, whereas Bönisch, Lauber, et al. (2007) used the declared activity of 100 U per gram of powder in their calculations.

Bönisch, Lauber, et al. (2007) reported that the degree of crosslinking in TG-treated micellar casein and sodium caseinate increased at a diminishing rate with treatment time, and crosslinking plateaued after 90 min of treatment. Jaros, Pätzold, et al. (2006) found that G'_{max} of acid gels during *in situ* acidification was constant with 0-60 min of TG treatment, then declined with longer treatment. The effect on penetration test results depended on whether TG treatment was followed by heat treatment: in unheated TG-treated milks, gels became stronger (higher force at fracture) and more elastic (larger penetration depth at fracture) with increasing treatment time up to 90 min, but when TG was inactivated with a heat treatment, best results were obtained with 60 min of TG treatment, and longer treatments produced weaker gels. A subsequent study by the same group (Jaros, et al., 2010) showed that 20-30% crosslinking of casein produced the highest G'_{max} in acid milk gels, and a similar pattern was seen for acid casein, with the optimum at a higher crosslinking level (**Figure 6**).

Figure 6. (A) Maximum gel stiffness and (B) pH at gelation onset ($G' > 1 \text{ Pa}$) of acidified gels from cross-linked $27 \text{ g}\cdot\text{kg}^{-1}$ casein solution (open symbols) and raw skim milk (closed symbols) as a function of total cross-linked casein. Enzyme treatment was with $3 \text{ U TG}\cdot\text{g}^{-1}$ protein, and inactivation was with $1 \text{ g}\cdot\text{L}^{-1}$ N-Ethylmaleimide. Gelation was induced by $40 \text{ g}\cdot\text{L}^{-1}$ D-glucono- δ -lactone at $30 \text{ }^\circ\text{C}$. From Jaros et al. (2010).



The study of Jaros et al. (2010) is the only one we are aware of that reported moisture sorption isotherms for untreated and TG-crosslinked caseins. There was no difference between the two isotherms, which indicated that effects of TG crosslinking on water holding or syneresis were derived from improved retention of water physically entrapped within fine capillaries in the gel network, with no change to the amount of water interacting strongly with proteins at the molecular level.

For a constant TG treatment time of 120 min, Bönisch, Lauber, et al. (2007) found a large increase in crosslinking as the TG level was increased up to 5 U.g⁻¹, and little increase with higher concentrations. For a treatment time of 60 min, Jaros, Pätzold, et al. (2006) reported constant maximum G' with increasing TG level up to 3 U.g⁻¹, beyond which G' decreased. Heat treatment again had a limiting effect on penetration test results, in that gel strength and elasticity increased with increasing enzyme level up to 6 U.g⁻¹ in unheated milks, but TG levels beyond 2 U.g⁻¹ produced weaker gels.

In a different study on sheared yoghurts, Bönisch, Huss, Weitzl, & Kulozik, (2007) reported that a TG level of 3 U.g⁻¹ was required for any real increase in yoghurt viscosity at 500 s⁻¹, and this corresponded to approximately 10% crosslinking. Much greater increases in viscosity and degree of crosslinking at lower enzyme concentrations were obtained using a TG product containing yeast extract, which is a source of glutathione and peptides. Glutathione blocks the endogenous TG inhibitor in milk (Bönisch, Lauber, et al., 2007), and yeast extract stimulates lactic acid bacteria, resulting in slightly faster acidification (Bönisch, Huss, et al., 2007).

The effect of TG treatment on acid gel texture is influenced by the rate of acidification, which may be varied by changing the amount of GDL at constant temperature. Jacob et al. (2011) reported the effect of GDL level (3-7%) on acid milk gels made from skim milk that was TG-treated, heated to inactivate TG then spray-dried. Faster acidification using more GDL produced gels with lower G' , lower penetration modulus and unchanged forced syneresis level, which is in agreement with related work on both microbially-acidified yoghurts (Lee & Lucey, 2004; Peng, et al., 2010). TG treatment made G'_{\max} more sensitive to GDL level, while forced syneresis was much less sensitive to % GDL. The authors believed that TG crosslinking inhibited the structural reorganising of casein micelles that otherwise occurs during acidification of milk (Peng, et al., 2010), thereby exacerbating the effect of acidification rate on G'_{\max} (Jacob, et al., 2011).

Guyot and Kulozik (2011) used a similar method to that of Jacob et al. (2011) to produce TG-treated skim milk powders (SMPs). These were used to fortify milk in the production of sheared yoghurt. TG-treated SMPs produced greater increases in viscosity than untreated SMP, and the amount of viscosity enhancement was directly related to the degree of polymerisation produced by TG treatment. The viscosity of yoghurts made with TG-treated SMP was stable during storage, whereas yoghurts made by direct addition of TG at the time of inoculation showed increasing viscosity during storage.

5.4.2. Simultaneous crosslinking and acidification

When TG is not deactivated prior to acidification, crosslinking reactions continue during acidification and storage, but the activity of TG is maximal at pH 5-7, and diminishes as the pH drops below 5 (Lu, et al., 2003). Continued crosslinking may be desirable in some processes. Şanlı et al. (2011) found that TG treatment before pasteurising produced no improvement in yoghurt viscosity, whereas post-pasteurisation TG treatment or addition of TG with the

starter culture significantly improved viscosity. Yüksel and Erdem (2010) found that yoghurts made with TG-treated milks had higher initial hardness when TG was active during acidification, and hardness continued to increase during storage in those samples containing active TG.

Ercili-Cura et al. (2013) investigated how incubation temperature (20-40 °C) affected gel properties when TG was added at the same time as GDL. Higher temperatures resulted in faster acidification (Ercili-Cura, et al., 2013), but will have also increased the rate of TG crosslinking, which varies four-fold over the temperature range selected (Lu, et al., 2003). Incubation at higher temperature gave higher final G' and higher force at rupture in penetration tests, with lower water holding capacity. Gels formed at higher temperature had coarse networks of thick strands, which may explain stronger gels.

Extensive TG crosslinking or continued TG activity during storage can lead to the development of texture defects such as coarseness or grittiness (Bönisch, Huss, et al., 2007; Guyot & Kulozik, 2011; Jaros, Partschfeld, et al., 2006). For that reason, yoghurts containing active TG are likely to have a relatively short shelf life.

5.4.3. Summary and future prospects

TG treatment shows great promise as a means to achieve acceptable yoghurt texture at reduced solids content and/or reduced fat. It is effective when treated milks are dried and reconstituted, and the most likely format for large scale industrial application of TG in yoghurt-making is *via* the use of TG-treated milk or protein powders.

Future applications of TG in yoghurt manufacture may well combine TG treatment with other processes. For example, Tsevdou et al. (2013) reported large improvements in the firmness of set yoghurts when milk was treated with HPP then TG. Anema et al. (2005) found that milk treated with TG during HPP (i.e. while pressurised) produced acid gels with rheological properties much better than milks treatment with TG or HPP alone. Since that work, there have been a few studies using HPP-TG combinations with isolated caseins or whey proteins (Huppertz & de Kruif, 2007; Menéndez, Schwarzenbolz, Partschfeld, & Henle, 2009; Smiddy, et al., 2006), but the apparent synergy of HPP and TG in improving acid milk gel texture (Anema, Lauber, et al., 2005) has yet to be exploited.

Caution should be exercised in light of emerging research on the nutritional effects of TG crosslinking in other food systems. TG treatment can reduce the digestibility of protein from soy beans (Tang, Li, & Yang, 2006) and other beans (Mariniello, Giosafatto, Di Pierro, Sorrentino, & Porta, 2007). The consumption of TG-treated wheat proteins may trigger or aggravate celiac disease in genetically predisposed individuals (Cabrera-Chávez & Calderón de la Barca, 2008; Gerrard & Sutton, 2005). However TG crosslinking of lysine residues prevents them from participating in Maillard reactions, and may thereby reduce Maillard-associated lysine losses in some food processes (Gerrard, et al., 2012).

6. Conclusions

Yoghurt is seen as a natural, nutritious snack, and consumers increasingly expect a 'clean label' listing a small number of 'natural' ingredients as well as a low fat content. However they are unwilling to compromise on texture and other sensory properties for the sake of a clean label. Hence there is an increasing need for manufacturing technologies that can produce high-quality yoghurts with less fat and no stabilisers. Manufacturers are also under pressure to cut production costs, and novel processing operations may prove an economical

substitute for expensive ingredients such as texture-modifying hydrocolloids and proteins as well as simplifying regulatory compliance. Here we have surveyed the scientific literature regarding four novel processing technologies that could contribute to these goals.

Most of the published research on these technologies involved bench-scale experiments, but the few pilot-scale studies available suggest that bench-scale benefits translate well to pilot scale. Both set and stirred yoghurts benefit from these technologies. USP and HPH improve the texture of full-fat and low-fat yoghurts, but have little or no benefit for non-fat formulations. HPP and TG treatment are batch or semi-batch processes, whereas HPH is a continuous operation and USP can be applied in continuous or batch formats. Batch processes can sometimes be introduced into a continuous operation by using ingredients that have been pre-treated in liquid form then dried. This approach has proven successful for TG treatment and may be applicable for HPP. However, USP pre-treatment is unlikely to be suitable for manufacturing dry ingredients, because USP acts primarily on fat droplet size, which is difficult to control throughout drying and rehydration.

Some of the technologies surveyed have shown synergistic effects when combined with other processes. For example, HPP, HPH or TG treatment work well when combined with heating, and simultaneous HPP and TG treatment has shown promise.

The industrial potential of the technologies surveyed here depends in part on the cost, scale and reliability of processing equipment on the market. These concerns are beyond the scope of this review, but we note that some technologies addressed here have already been implemented in other sectors of the food industry. Large-scale HPP equipment has been used for several years to pasteurise juice, semisolid products and seafood. TG is used in processed meat manufacture and in the bakery industry. Homogenization has long been a standard part of milk processing, and HPH is a logical extension of existing technology.

A mechanistic understanding of how novel processing technologies affect the constituents of milk is needed in order to optimise their implementation and integration with other unit operations in yoghurt manufacture. Building this understanding requires well-designed experiments, high-quality standardised measurement techniques and an awareness of existing knowledge. Rigorous scientific practice should be matched with an awareness of industrial practice so that findings have relevance for the ultimate end users. Emerging technologies present exciting opportunities for creating new and better foods, and continued innovation will benefit both food manufacturers and consumers.

7. References

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