Formation and Physical Properties of Yogurt

W. J. Lee[1] and J. A. Lucey[2],*

[1] Department of Animal Bioscience (Institute of Agriculture and Life Science),
Gyeongsang National University, Jinju 660-701, Korea

[2] Department of Food Science, University of Wisconsin-Madison,
1605 Linden Drive, Madison, WI 53706, USA

ABSTRACT : Yogurt gels are a type of soft solid, and these networks are relatively dynamic systems that are prone to structural rearrangements. The physical properties of yogurt gels can be qualitatively explained using a model for casein interactions that emphasizes a balance between attractive (e.g., hydrophobic attractions, casein cross-links contributed by calcium phosphate nanoclusters and covalent disulfide cross-links between caseins and denatured whey proteins) and repulsive (e.g., electrostatic or charge repulsions, mostly negative at the start of fermentation) forces. Various methods are discussed to investigate the physical and structural attributes of yogurts. Various processing variables are discussed which influence the textural properties of yogurts, such as total solids content, heat treatment, and incubation temperatures. A better understanding of factors contributing to the physical and structural attributes may allow manufacturers to improve the quality of yogurt. (**Key Words :** Yogurt, Rheology, Whey Separation, Microstructure)

INTRODUCTION

Yogurts are prepared by fermentation of milk with bacterial cultures consisting of a mixture of *Streptococcus* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. There are two major types; set and stirred yogurt. The main manufacturing procedures of these two types of yogurts are described in Figure 1. Set yogurt (which includes fruit-on-the bottom) is formed in retail pots as lactic acid bacteria ferment lactose into lactic acid giving a continuous gel structure in the consumer container. In stirred yogurt, the acid gel formed during incubation in large fermentation tanks is disrupted by agitation (stirring), and the stirred product is usually pumped through a screen which gives the product a smooth and viscous texture (Tamime and Robinson, 1999). The physical attributes of yogurts, including the lack of visual whey separation and perceived viscosity, are crucial aspects of the quality and overall sensory consumer acceptance of yogurts. An understanding of the mechanisms involved in the formation of texture in yogurts and the impact of processing conditions on texture development may help to improve the quality of yogurt. In this review, the yogurt manufacturing

process, techniques to investigate the physical and structural properties, and the major processing factors that influence the physical and structural attributes of yogurts are described.
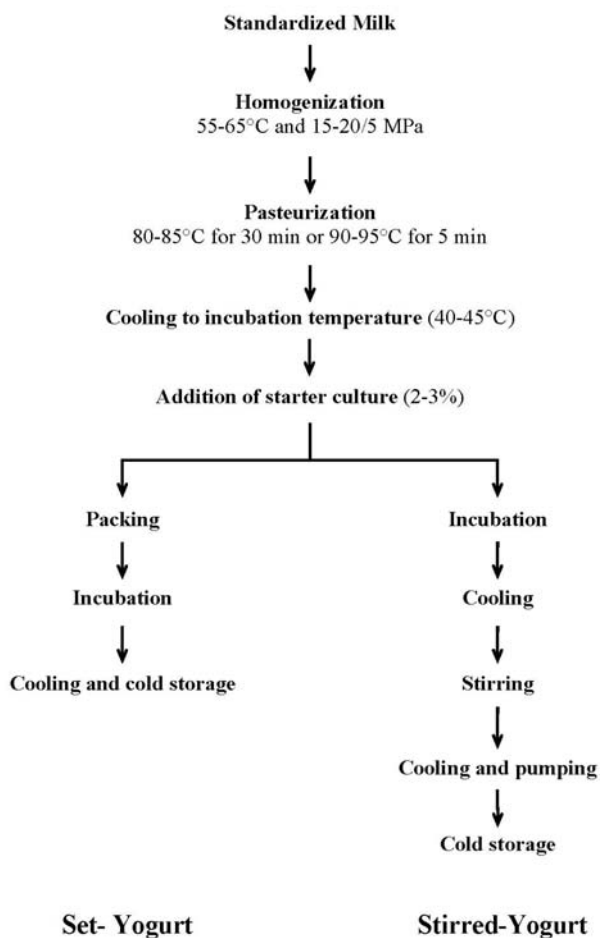
YOGURT MANUFACTURING PROCESS

The main processing steps involved in these two types of yogurt manufacture (Figure 1) include the standardization of milk (fat and protein content), homogenization, milk heat treatment, incubation/ fermentation, cooling, and storage.

Milk Standardization

Milk is often mixed with skim milk and cream to standardize (or adjust) the fat content to the desired level. Milk powders, including nonfat dry milk, whey protein concentrates, or milk protein concentrate, can be blended with the milk using a powder dispersion unit. The milk solids content (including the fat content) for yogurt ranges from around 9% for skim milk yogurt to more than 20% for certain types of concentrated yogurt. Many commercial yogurt products have milk solids contents of 14-15% (Tamime and Robinson, 1999). The minimum milk solids-not-fat content required in standards or regulations in many countries ranges from 8.2 to 8.6% (Tamime and Robinson,

* Corresponding Author: J. A. Lucey. Tel: +1-608-265-1195, Fax: +1-608-262-6872, E-mail: jalucey@wisc.edu

Figure 1. Main processing steps in the manufacture of set and stirred yogurt.

1999). Codex regulations for yogurt indicate that the minimum milk protein content is 2.7% (except for concentrated yogurt where the minimum protein content is 5.6% after concentration) and the maximum fat content is 15% (Codex, 2008). The total solids content of milk can be increased by concentration processes, such as, evaporation under vacuum, and membrane processing (i.e., reverse osmosis and ultrafiltration).

Stabilizers, such as, pectin or gelatin, are often added to the milk base to enhance or maintain the appropriate yogurt properties including texture, mouthfeel, appearance, viscosity/consistency and to the prevention of whey separation (wheying-off) (Tamime and Robinson, 1999). The use of stabilizers may help in providing a more uniform consistency and lessen batch to batch variation. However, there can be textural defects related to the use of stabilizers, including over-stabilization and under-stabilization. Over-stabilization results in a "jello-like" springy body of yogurt while a weak "runny" body or whey separation can be produced due to under-stabilization (Vedamuthu, 1991). In some countries, such as, the Netherlands and France,

regulations do not allow the use of stabilizers for plain (unsweetened) yogurt (Tamime and Deeth, 1980). In fruit yogurts, stabilizers (e.g. pectin) are often added to the fruit preparation to help improve the yogurt texture.

Homogenization

Homogenization of the milk base is an important processing step for yogurts containing fat. Milk is typically homogenized using pressures of 10-20 and 5 MPa first and second stage pressures, respectively, and at a temperature range between 55 and 65°C. Homogenization results in milk fat globules being disrupted into smaller fat globules and the surface area of homogenized fat globules greatly increases. The use of homogenization prevents fat separation (creaming) during fermentation or storage, reduces whey separation, increases whiteness, and enhances consistency of yogurts (Vedamuthu, 1991). When milk is homogenized, caseins and whey proteins form the new surface layer of fat globules, which increases the number of possible structure-building components in yogurt made from homogenized milk (Walstra, 1998). Homogenized milk fat globules act like protein particles due to the presence of protein on the fat surface. Recently, ultra-high pressure homogenization at 200 or 300 MPa was investigated for the production of yogurt. Compared with a conventional homogenization at 15 MPa, the use of ultra-high pressure homogenization resulted in an increase in yogurt firmness and water-holding capacity (Serra et al., 2008, 2009). Ultra-high pressure causes whey protein denaturation as well as partial disruption of the casein micelles.

Heat treatment

Heating of milk is an important processing variable for the preparation of yogurt since it greatly influences the physical properties and microstructure of yogurt (Lucey et al., 1998a, b, c). In yogurt manufacture, milk is heated prior to culture addition. The temperature/time combinations for the batch heat treatments that are commonly used in the yogurt industry include 85°C for 30 min or 90-95°C for 5 min (Tamime and Robinson, 1999). However, very high temperature short time (100°C to 130°C for 4 to 16 s) or ultra-heat temperature (UHT) (140°C for 4 to 16 s) are also sometimes used (Sodini et al., 2004). The heat treatment of milk is also used to destroy unwanted microorganisms, which provides less competition for the starter culture. Yogurt starter cultures are sensitive to oxygen so heat treatment helps to remove dissolved oxygen assisting starter growth.

Fermentation process

After heat treatment, the milk base is cooled to the incubation temperature used for growth of the starter culture.

An optimum temperature of the thermophilic lactic acid bacteria, i.e., *Streptococcus* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, is around 40-45°C. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk. During acidification of milk, the pH decreases from 6.7 to ≤4.6. Gelation occurs at pH 5.2 to 5.4 for milk that was given a high heat treatment.

Cooling

When yogurts have reached the desired pH (e.g., ~4.6), yogurts are partially cooled (~20°C) before fruit or flavoring ingredients are added. Yogurt products are often blast chilled to <10°C (e.g., 5°C) in the refrigerated cold store to reduce further acid development (Tamime and Robinson, 1999). In the production of set yogurt, yogurts are directly transferred to a cold store or blast chilled in cooling tunnels. For stirred yogurts, cooling is first performed by agitation in the jacketed fermentation vat and the product is sheared and smoothened by devices like back-pressure values, high shear devices or sieves.

PHYSICO-CHEMICAL MECHANISMS INVOLVED IN THE FORMATION OF YOGURT GELS

Acidification of milk leads to the disruption of the internal structural properties of casein micelles due to the solubilization of CCP (Dalgleish and Law, 1989). As caseins approach their isoelectric point (pH 4.6), the net negative charge on casein is reduced, which decreases electrostatic repulsion between charged groups, including the phosphoserine residues that are exposed when the CCP is solubilized. Electrostatic attraction increases and protein-protein attraction also increases through enhanced hydrophobic interactions (Lucey, 2004). Physico-chemical mechanisms for the formation of acid milk gels can be discussed for three pH regions (Lucey, 2004).

pH 6.7 to 6.0

When the pH of milk decreases from 6.6 to 6.0, the net negative charge on the casein micelles decreases, which results in a decrease in electrostatic repulsion. Since only a small amount of CCP is solubilized at pH >6.0, the size of the casein micelles is largely unchanged.

pH 6.0 to 5.0

As the pH of milk decreases further from pH 6.0 to 5.0, the net negative charge on casein micelles greatly decreases and the charged "hairs" of κ-casein may shrink (or curl up). This results in a decrease in electrostatic repulsion and steric stabilization, which are both responsible from the stability of casein micelles in the original milk. At pH ≤6.0 the rate of solubilization of CCP increases, which weakens the internal structure of casein micelles and increases

electrostatic repulsion between the exposed phosphoserine residues. In milk, CCP is completely solubilized in casein micelles by pH ~5.0. However, in rennet-coagulated cheese, a significant amount of CCP is not solubilized at this pH, probably because of a protective role on CCP solubility from the higher solids content of curd compared with milk. Dalgleish and Law (1988) reported that the amounts and proportions of caseins dissociated from the micelles were both temperature- and pH-dependent. More caseins are dissociated from micelles into the serum as temperature decreases from 30 to 4°C. The pH at which maximum dissociation occurs is between pH 5.6 and ~5.1 (Dalgleish and Law, 1988), which may be attributed to a partial loosening of bonds within and between caseins due to loss of CCP (Lucey, 2004). At low temperatures, hydrophobic interactions involved in casein association are very weak.

pH ≤5.0

When the pH of milk becomes close to the isoelectric point of casein (pH 4.6), there is a decrease in the net negative charge on casein, which leads to a decrease in electrostatic repulsion between casein molecules. On the other hand, casein-casein attractions increase due to increased hydrophobic and plus-minus (electrostatic) charge interactions (Horne, 1998). The acidification process results in the formation of three-dimensional network consisting of clusters and chains of caseins (Mulvihill and Grufferty, 1995).

RHEOLOGICAL AND PHYSICAL PROPERTIES OF YOGURT

Food rheology is the study of the deformation and flow of food materials (Rao, 1999). Yogurt can be classified as pseudoplastic material (contains a yield stress that has to be exceeded for flow to be initiated) that can be either a viscoelastic fluid if we are dealing with stirred or drinking yogurt or a viscoelastic solid if we are dealing with set yogurt. Viscoelastic indicates the material has some of the elastic properties of an ideal solid and some of the flow properties of an ideal (viscous) liquid. Yogurt also exhibits time-dependent shear thinning behavior but yogurt is not a true thixotropic material since structural breakdown due to shear is not completely reversible once the shear stops.

Small amplitude oscillatory rheology

Small amplitude oscillatory rheology (SAOR) has been used to characterize the rheological properties of yogurt during the gel formation process (fermentation) without damaging the weak gel network. Small deformation is defined as a small relative deformation (strain or change in dimension) (e.g. ≤1%), which when applied does not disrupt the development of the network structure, i.e.,

within the linear viscoelastic region. In this "linear" region, the dynamic moduli are independent of the applied stress or strain. SAOR testing involves applying an oscillatory (sinusoidal) stress or strain and measuring the strain or stress responses. There are several rheological parameters determined in a SAOR test. Elastic or storage modulus (G′) expresses the measure of energy stored per deformation cycle and indicates the solid-like properties. Viscous or loss modulus (G″) indicates the magnitude of energy lost as viscous dissipation per cycle of deformation and reflects the liquid-like properties. Loss tangent (LT) is defined as ratio of loss modulus to storage modulus (G″/G′) and indicates the type of viscoelastic properties in a material. A high LT value (i.e., G″ >G′) means that the material has liquid-like behavior (Rao, 1999).

Typical gelation profiles during the fermentation of yogurt gels are shown in Figure 2. During gel formation, G′ values increase due to the formation of additional bonds between protein particles, rearrangements in the protein network and possible attachment of dangling gel strands to the network. There is a maximum in LT in yogurt gel made from heated milk (Figure 2). An initial increase in LT (i.e., up to the maximum) may result from a partial loosening of gel network due to the solubilization of CCP while a decrease in LT (i.e., after the maximum) can be due to the decreased electrostatic repulsion and increased casein-casein interactions as caseins approach their isoelectric point (Lucey, 2004).
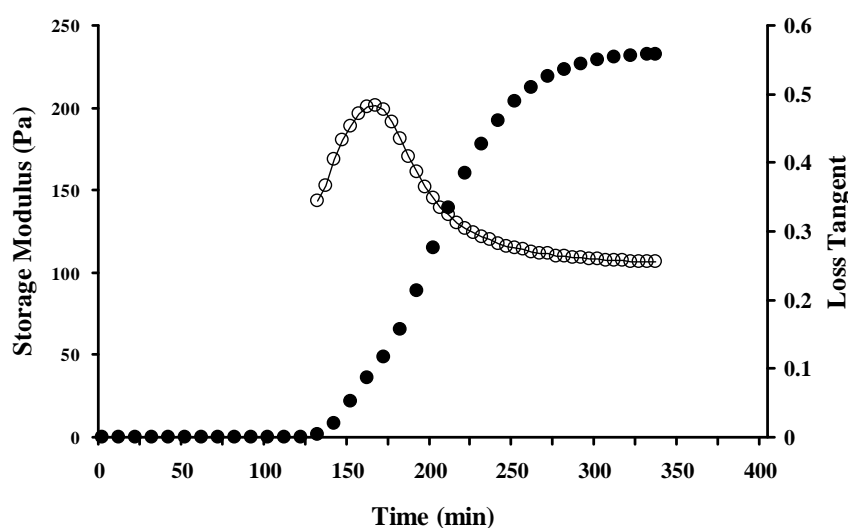
Large deformation rheology

The large deformation rheological properties of yogurt are also important since most products are the stirred-type where the initial gels are sheared and stirred. Large-deformation characteristics of food gels are related to functional properties including shaping, cutting/slicing and eating characteristics (van Vliet and Walstra, 1995). Therefore, sensory textural attributes are often correlated with the results from large deformation instrumental tests. One type of large deformation test is a stress overshoot experiment or constant shear rate test, which is shown in Figure 3. Rheological parameters that can be obtained from this type of test include yield stress (σ_{yield}) and yield strain (γ_{yield}), which are defined as the point when the shear stress begins to decrease (Lucey et al., 1997). A low σ_{yield} value implies that the yogurt gel has weak network, while a low value of γ_{yield} implies that it is a brittle or short textured gel (Lucey, 2001). The strength of protein-protein bonds, the number of bonds per cross-section of the strand, relaxation times for the network bonds, and the orientation of the strands in the matrix all contribute to the yield properties of gels (van Vliet et al., 1991). The rheological parameters obtained from an overshoot test depend on the applied shear rate, the use of higher shear rate result in higher σ_{yield} values due to less time for bond relaxation during the shearing process.

Viscosity and flow properties of stirred yogurt

There have been many studies on the viscosity and flow properties of stirred yogurts (Skriver et al., 1993; Skriver, 1995; van Marle et al., 1999; Afonso and Maia, 2000; Haque et al., 2001; Lee and Lucey, 2006). In most studies, stirred yogurts were tested on a viscometer or rheometer to determine the flow properties after the original set gels were empirically agitated using a spoon or a high-speed mixer (Skriver et al., 1993; van Marle et al., 1999). During the mixing or loading steps there are structural changes in

Figure 2. Typical gelation profiles of yogurt gels. Yogurt gels were made from milk pre-heated at 90°C for 30 min and incubated at 40°C. Solid (●) and open (○) symbols are storage modulus (G′) and loss tangent (LT), respectively.
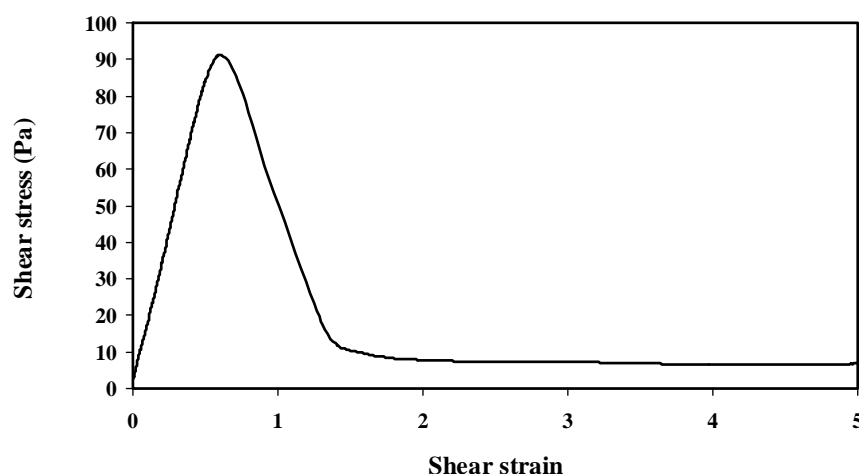
Figure 3. Large deformation properties of a yogurt gel tested at pH 4.6 and made from milk treated with 83°C heating temperature and with an incubation temperature of 46°C. Gels were subjected to a low constant shear rate test.

yogurt, which affect the flow properties. For stirred yogurt products it should be recognized that steps, such as, mixing result in a reduction in viscosity that is only partially restored after shearing is stopped. Recovery of structure is called "rebodying" and is a time-dependent phenomenon. Structural recovery also affects the apparent viscosity of yogurts. Arshad et al. (1993) reported that glucono-δ-lactone (GDL)-induced gels had only 30% recovery of the original value of the dynamic moduli even after allowing 20 h for recovery after shearing.

Lee and Lucey (2006) investigated the structural breakdown of the original (intact) yogurt gels that were prepared *in situ* in a rheometer, as well as, the rheological properties of stirred yogurts made from these gels. Lee and Lucey (2006) found that the rheological properties of stirred yogurts were greatly influenced by the physical properties of the original intact (set) yogurt gels.

Rotational viscometers, such as the Brookfield viscometer, are often used to characterize the flow behavior of stirred yogurts. However, these methods have several drawbacks. For example, since stirred yogurts exhibit non-Newtonian behavior, viscosity is dependent on shear rate. The Brookfield viscometer only measures an "apparent" viscosity at one spindle speed that is empirically selected as "consistent" reading after some shearing period. Thus, only limited information on the fundamental flow properties of stirred yogurts can be obtained.

The flow behavior of stirred yogurt has been modeled from shear rate sweep tests (Ramaswamy and Basak, 1991; De Lorenzi et al., 1995; Afonso and Maia, 2000; Lee and Lucey, 2006). Data from flow curves were fitted to power law (Parnell-Clunies et al., 1986; Keogh and O'Kennedy, 1998; Geraghty and Butler, 1999), Hershel-Bulkley (Ramaswamy and Basak, 1991), or Casson equations (Parnell-Clunies et al., 1986; Skriver et al., 1993; Lee and

Lucey, 2006). The parameters obtained from these flow curve models are useful in comparing different yogurt samples but these models are essentially empirical or mathematical models. It should be noted that the power law model does not have a yield stress term while all stirred yogurts have yield stress unless they have been sheared first and no recovery time allowed to rebuild some structures. The equations for these models are (Rao, 1999):

$$\text{Power law model: } \sigma = K\,(\dot{\gamma})^n \qquad (1)$$

$$\text{Herschel-Bulkley model: } \sigma = \sigma_o + K\,(\dot{\gamma})^n \qquad (2)$$

$$\text{Casson model: } \sigma^{1/2} = \sigma_0^{1/2} + \eta_a\,(\dot{\gamma})^{1/2} \qquad (3)$$

Where σ is the shear stress, σ_0 is the yield stress, η_a is the apparent viscosity, $\dot{\gamma}$ is the shear rate, K is the consistency index, *n* is the flow behavior index.

Permeability

In biological systems, the flux rate of solutes (diffusing material) through membranes is controlled by the permeability of the membrane, which in turn is dictated by the size of the pores and is given a unit of measure called the permeability coefficient. The permeability coefficient (*B*) of a gel matrix is determined by measuring the flow of the continuous liquid phase (serum) through a gel caused by a known (small) pressure gradient (van Vliet et al., 1991). The *B* of a gel is an indication of the size of the pores in the network (van Dijk and Walstra, 1986). A high *B* indicates that there are large pores in a gel, which facilitates more rapid flow of serum through the matrix. In acid casein gels, the pore size is a reflection of the type of gel microstructure formed during the acidification process. Rearrangements in

acid gel networks can cause ongoing fusion of casein particles and breakage of casein strands making up the network. Both of these processes could lead to slightly increased B and pore size (van Vliet et al., 1997; van Vliet, 2000). Once yogurt gels reach pH 4.6 the B of the gel network is determined with little further change likely during the incubation period, in contrast, the B of rennet gels increases with time due to microsyneresis. Cooling of yogurt gels results in a reduction in B due to swelling of the casein gel matrix. The B of casein gels can be determined with the following equation (van Dijk and Walstra, 1986):

$$B = -\left[\ln \frac{(h_\infty - h_{t2})}{(h_\infty - h_{t1})} \right] \eta H / [\rho g(t_2 - t_1)] \qquad (4)$$

Where B is the permeability coefficient, h_∞ is the height of whey in the reference tube, h_{t1} is the height of whey in tube at time t1, h_{t2} is the height of whey in tube at time t2, η is the viscosity of whey, h is the length of gel, ρ is the density of whey and g is acceleration due to gravity.

Whey separation

Whey separation (wheying-off) is defined as the expulsion of whey from the network which then becomes visible as surface whey. Wheying-off negatively affects consumer perception of yogurt as consumers think there is something microbiologically wrong with the product. Yogurt manufacturers use stabilizers, such as, pectin, gelatin and starch, to try to prevent wheying-off. Another approach is to increase the total solids content of yogurt milk, especially the protein content, to reduce wheying-off. Spontaneous syneresis, which is contraction of gel without the application of any external force (e.g., centrifugation), is the usual cause of whey separation (Lucey et al., 1998a).

Spontaneous whey separation is related to an unstable network, which can be due to an increase in the rearrangements of the gel matrix or it can be induced by damage to the weak gel network (e.g., by vibration or cutting) (Lucey et al., 1998a). The extent of rearrangement that occurs is related to the dynamics (average life-time) and relaxation of the protein-protein bonds as expressed in terms of the LT and to the resistance to yielding of the casein strands (van Vliet et al., 1997; Lucey, 2001). Mellema et al. (2002) classified the main types of rearrangements in rennet-induced gels: i) sub-particle or intra-particle rearrangements (size in casein gels <~0.2 μm), ii) inter-particle rearrangement (size in casein gels ~0.2-1 μm), iii) inter-cluster rearrangements (size in casein gels ~1-40 μm), and iv) syneresis (macroscopic).

Many researchers have measured whey expulsion from yogurts through the use of high-speed centrifugation or the drainage of whey from stirred yogurt through a screen or mesh (Harwalkar and Kalab, 1983, 1986; Guirguis et al., 1984; Dannenberg and Kessler, 1988). However, these methods are not directly relevant for set yogurt products and the spontaneous whey separation defect. The centrifugation method is a measure of the water-holding capacity as a result of a high external force, i.e., resistance of the gel to compaction. The drainage method is useful in products that have a serum separation step through screen, such as, traditionally manufactured concentrated yogurt. A simple test using gels formed in glass volumetric flasks has been used to quantify spontaneous whey separation in acid milk gels (Lucey et al., 1998a). In this test, surface whey that is expelled from acid milk gels is gently poured off and quantified. This test has been used to evaluate whey separation in set-type yogurt gels (Lee and Lucey, 2006).

Microstructure

It is well recognized that the structure of foods greatly affects their various properties including texture, functionality and appearance. Microstructure has a major impact on the texture and other physical properties of acid milk gels. The microstructure of acid milk gels, such as GDL-induced gels and yogurt gels, have been observed using electron microscopy (EM) and confocal scanning laser microscopy (CSLM) (Harwalkar and Kalab, 1980; Lucey et al., 1998b). EM and CSLM studies on acid gels have shown that these gels consist of a coarse particulate network of casein particles linked together in clusters, chains and strands (Harwalkar and Kalab, 1980; Kalab et al., 1983). The network has pores or void spaces in which the aqueous phase is confined. In fat-containing products, the presence of (large) fat globules obscures the finer details of pores and strands. The diameter of these pores varies considerably, with larger pores in gels made at a high gelation temperature (usually <30 μm). Harwalkar and Kalab (1980) reported that acid gels made from unheated milk had larger protein clusters (coarse structure) than gels made from heated milk, which they described as highly branched (fine structure). Lee and Lucey (2003) reported that yogurt gels made from milk heated at high temperature (>80°C) had a more cross-linked and branched protein structure with small pores compared with milk heated at low temperature. Stirred yogurt has very large clusters of caseins presumably created by the collisions and shearing during the mixing process (Lee and Lucey, 2006). The characteristic three-dimensional gel matrix of set yogurt is no longer visible in stirred products. Stirred yogurt is a weak gel system and although "particle size" is sometimes reported for stirred yogurt it should be recognized that there are no individual particles rather there are weakly associated clusters of proteins that make up the network. Stirring and dilution during the particle size measurement process disrupt the weak network and creates "particles".

CSLM can be used to dynamically evaluate the development of microstructure in acid milk gels (Auty et al., 2001). Although the EM technique has a higher resolution than CSLM, EM has considerable sample preparation steps including dehydration, fixation, and sectioning, which may increase the likelihood of microstructural artifacts that could affect the native structure of gels (Schmidt, 1982). Unlike EM, CSLM has minimal sample preparation steps due to its optical sectioning capabilities, which can enable the microstructure of yogurt gels to be monitored without disturbing the gel structure or causing artifacts. Specific components of gels, such as, protein, fat, and (live or dead) bacteria, can be identified using specific fluorescent probes, such as, acridine orange, fast green FCF, and fluorescein isothiocyanate.

FACTORS AFFECTING THE PHYSICAL AND SENSORY PROPERTIES OF YOGURTS

Dry matter fortification

The physical and sensory properties of yogurt gels are greatly influenced by the total solids content of the yogurt milk, especially the protein content. The G′ values of yogurt increases with an increase in the total solids content obtained by the addition of skim milk powder or by ultrafiltration (Biliaderis et al., 1992). Increased yogurt viscosity is observed when the total solids content of milk is increased (Guirguis et al., 1984; Becker and Puhan, 1989; Wacher-Rodarte et al., 1993). The oral viscosity of yogurt or perceived thickness also increases with an increase in total solids content of milk (Skriver et al., 1999; Sodini et al., 2004). Peng et al. (2009) compared the impact of different types of milk proteins used for fortification on the textural properties of yogurt. The G′ values at pH 4.6 of fortified yogurts increased in the order: skim milk powder = micellar casein<milk protein isolate<sodium caseinate (Peng et al., 2009). Addition of whey protein concentrates (WPC) to milk followed by high heat treatment led to increased G′ values and decreased gelation time in yogurt (Lucey et al., 1999). One issue with the popular use of WPC to fortify yogurt milks is the possible coagulation of whey proteins during the high heat treatment process. The susceptibility to heat coagulation is related to the calcium content of the WPC, with high Ca levels, such as, the levels found in acid whey WPC, making the solutions very unstable. In practice, there is an upper limit (before an increased risk of heat coagulation) of around 4% additional whey protein (from WPC) to give a total milk protein level of about 7%. Improper hydration of powders can result in a number of defects in yogurt including lumpiness, chalkiness, and powdery off-flavors. The increased solids content in yogurt milk as a result of fortification also creates increased buffering that requires additional acid development by the starter cultures to achieve a similar pH target.

Most yogurt products are sweetened (not plain). The use of sucrose increases the total solids of the mix and strengthens the gel network. A range of sweeteners are used commercially, especially for low calorie products. Another option is to use β-galactosidase to hydrolyse lactose as the products are glucose and galactose, which are much sweeter than lactose.

Heat treatment

Native whey proteins from unheated milk are inert fillers in yogurt (Lucey et al., 1999). When milk is heated at >70°C, the major whey proteins, such as, β-lactoglobulin, are denatured. During denaturation β-lactoglobulin interacts with the κ-casein on the casein micelle surface (and any soluble κ-casein molecules, i.e. κ-casein that dissociates from the micelle at high temperatures) by disulfide bridging, which results in increased gel firmness and viscosity of yogurt (Dannenberg and Kessler, 1988; Lucey et al., 1997). Denatured whey proteins that has become attached to the surface of casein micelles are a critical factor involved in the increased stiffness of yogurt gels made from heated milk (Lucey et al., 1998c). Soluble complexes of denatured whey proteins with κ-casein also associate with the micelles during the acidification process. Heat treatment of milk for 15 min at ≥80°C results in significantly increased denaturation of β-lactoglobulin compared with milk heated at 75°C for a similar time (Lucey et al., 1997). The extent of denaturation of whey proteins during the heat treatment of milk affects the firmness and viscosity of acid milk gels (Dannenberg and Kessler, 1988). High heat treatment of milk causes a shift in gelation pH towards higher pH values, Lucey et al. (1998c) suggested that this shift was due to the higher isoelectric pH (~5.3) of β-lactoglobulin, which is the main whey protein.

Compared with gels made from heated milk, lower dynamic moduli were observed in acid gels made from unheated milk (Lucey et al., 1997). The higher G′ value of yogurts made with milk heat treated at ≥78°C for 30 min was largely due the increased covalent cross-linking of proteins by the denatured whey proteins associated with the casein micelles (Lucey et al., 1997; Lee and Lucey, 2003).

A maximum in the LT (LT_{max}) value at pH ~5.1 was observed in yogurts and GDL-induced gels made from heated milk (Biliaderis et al., 1992; Ronnegard and Dejmek, 1993; Lucey et al., 1997). However, no LT_{max} was observed in GDL-induced gels prepared from unheated milk (Lucey et al., 1998c). A decrease in heating temperature from 93 to 72°C resulted in an increase in LT_{max} and *B* of yogurt gels (Lee and Lucey, 2003). High LT_{max} and high *B* values indicates that relaxation of bonds in yogurt gel networks are more likely, which may enhance rearrangements of the gel

network resulting in the formation of larger pores (van Vliet et al., 1991; Lee and Lucey, 2003).

In stirred yogurts, Cayot et al. (2003) reported that the consistency index of stirred acid gels, calculated from the Ostwald model, increased as milk heating temperature increased from 70 to 100°C. An increase in milk heating temperature resulted in an increase in apparent viscosity of stirred yogurts (Lee and Lucey, 2006). An increase in heat treatment resulted in an increase in oral viscosity and perceived mouth coating attributes, as well as, a decrease in the chalkiness attribute of stirred yogurt (Skriver et al., 1999; Lee and Lucey, 2006).

Incubation temperature

Physical properties and microstructure of yogurt are influenced by incubation temperature. The use of high incubation temperature resulted in a decrease in gelation time and G′ values at pH 4.6, and an increase in LT_{max}, B, and whey separation compared with yogurt gels incubated at low temperature (Lee and Lucey, 2003, 2004). This result indicates that gels formed at high temperature are weak and have a coarse gel network due to extensive rearrangement resulting in the formation of large pores and greater whey separation (Lucey, 2004). During the formation of yogurt gels at a low incubation temperature, slow protein aggregation occurs resulting in the formation of a large number of protein-protein bonds and less rearrangement of the particles/clusters. A highly cross-linked and branched protein network that had small pores was observed in micrographs of yogurt gels incubated at low temperature (Lee and Lucey, 2003, 2004). At lower incubation temperature, there is an increase in the voluminosity of casein particles, which results in an increase in the area of the junctions between aggregated casein particles. Increased contact area between casein particles could contribute to the increased stiffness of gels observed at low temperature (Walstra, 1998).

Higher viscosity was observed in stirred yogurts that had been incubated at lower temperatures (e.g. <40°C) compared to gels incubated at high temperature (e.g. >40°C) (Beal et al., 1999; Martin et al., 1999; Sodini et al., 2004; Lee and Lucey, 2006). As incubation temperature increased, there was a decrease in the sensory attributes, such as, mouth coating and smoothness of stirred yogurts (Cho-Ah-Ying et al., 1990; Martin et al., 1999).

Recently, a novel two-stage incubation temperature method was proposed. Peng et al. (2010) reported that if incubation temperature was changed after gelation, the textural properties of yogurt became similar to those of yogurts made at that new temperature for the entire fermentation process. It may be possible to use high incubation temperature for the initial stage of fermentation to facilitate rapid growth of the starter cultures and then slowly reduce the incubation temperature at some stage to achieve better textural properties.

In this review, physico-chemical mechanisms for the formation of yogurt gels were discussed. The effects of processing variables on the physical and structural properties of yogurts, including whey separation, were reviewed. The physical properties of yogurt gels including gel stiffness and permeability, the rearrangement of protein particles in gel network, and the structure breakdown of stirred-type yogurts are important factors influencing the physical and structural properties of yogurts.

Enzymatic cross-linking of caseins

Transglutaminase (TGase; EC 2.3.2.13) catalyses covalent intermolecular protein cross-linking through an acyl-transfer reaction, between the γ-carboxyamide group of a peptide-bound glutamine residue (acyl donor) and the primary amino group of an amine (acyl acceptor). The application of TGase in various types of dairy products has been reviewed (Jaros et al., 2006). Acid-induced gelation of TGase-cross-linked casein resulted in increased gel firmness, lower permeability, finer protein networks and improved whey drainage (Færgemand and Qvist, 1997; Færgemand et al., 1999; Schorsch et al., 2000). However, during yogurt storage slow ongoing action of TGase (unless it is inactivated) can cause textural problems.

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