PROPERTIES OF LOW-FAT YOGURT FROM ULTRAFILTERED
AND ULTRA-HIGH TEMPERATURE TREATED MILK

by

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Richard Alan Dargan
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Lack of correlation between mean level of whey protein denaturation and adjusted mean syneresis ($R^2 = .035$) of skim milk, *; ultrafiltered (UF) skim milk, o; and NDM fortified skim milk yogurts.
ABSTRACT

Properties of Low-fat Yogurt Made From Ultrafiltered and Ultra-high Temperature Treated Milk

by

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Yogurts were made from intermediate-high temperature (100, 110, 120, and 130°C for 4 or 16 s), ultra-high temperature (140°C for 4 or 16 s), and vat heat (82°C for 20 min) treatments of skim milk fortified to 5% protein by either ultrafiltration or the addition of nonfat dry milk (NDM). Whey protein denaturation in heated milks increased with temperature and holding time from indirect plate heating and was highest in vat-heated milks. Whey protein denaturation and yogurt water-holding capacity increased with protein levels in the fortified milks compared to skim milk. Penetrometer gel strength and stirred viscosity in 21 day-old yogurt made from heated ultrafiltered skim milk exceeded those of yogurts made from NDM-fortified skim milk, even though the NDM yogurts contained more solids (13.0 vs 11.4%). Maximum gel strength and viscosity, and least syneresis of yogurts from ultrafiltered and NDM fortified yogurts occurred following intermediate-high temperature treatments of 100°C for 16 s,
110°C for 4 or 16 s, and 120°C for 4 s. There was significantly lower whey protein denaturation at these intermediate-high temperatures compared to UHT or vat heating. Gel strength and viscosity were lower and syneresis greater in yogurts from ultrafiltered or NDM-fortified skim milk following UHT treatment compared to yogurts made with intermediate-high temperature treatments or vat heating. The water-holding capacity of yogurts from fortified milks treated at intermediate-high temperatures was comparable to that of yogurts from vat-heated milks. Fortification by ultrafiltration, to lower total solids (and without use of stabilizers) resulted in yogurt with higher gel strength and viscosity, and reduced syneresis compared to yogurt from NDM fortification. Yogurt prepared by intermediate-high temperature treatment had comparable or better gel strength and viscosity, and reduced syneresis compared to yogurt prepared by traditional vat heating.
PART 1. FORTIFICATION OF YOGURT TO IMPROVE PHYSICAL PROPERTIES - A REVIEW
INTRODUCTION

Yogurt Consumption Trends

Many different yogurt-type products are produced from cows’, sheep’s, and goats’ milk (31). In certain countries fermented foods are preferred over fresh for reasons of safety, better flavor and texture, and possible therapeutic effects. In developed countries yogurt is used as desserts, between meal snacks, complete lunches, and diet foods.

United States per capita sales of yogurt have increased almost 1500% in the last two decades (from .12 kg in 1960 to 1.9 kg per person in 1986) (40). Low fat (0.5 to 2.0%) traditional yogurt is currently the most popular form in the American market, comprising 66.3% of production (2).

Popularity of yogurt is because of increased consumption of traditional forms of yogurt as well as diversity of the types of yogurt products available. Dried and instant yogurt, drinkable yogurts, premium high-fat yogurts, and pasteurized or UHT/long-life yogurts have all entered the market place with variable degrees of success in different countries (3, 33, 38, 50).

Yogurt Quality Characteristics

The profitability of yogurt products continues to drive processors’ efforts to extend their shelf-life, to improve processing techniques, and to improve the quality characteristics of the yogurt product itself.

In set-style and stirred yogurt, consumers prefer a high viscosity product (35). The texture of yogurt should be such that it can be removed from the container and eaten with a spoon (39). Set-style yogurt should have sufficient
firmness, be free of lumps and granules, and resist syneresis with reasonable handling (39, 47).

Factors Affecting the Body and Texture of Yogurt

Factors controlling the body and texture of yogurt include: composition of the yogurt mix (total solids and protein content), heat treatment prior to inoculation with culture, homogenization, starter culture and incubation conditions, handling of ripened yogurt, the addition of stabilizers, and production of carbohydrate exopolymers by ropy strains of cultures (16, 39, 47).

Homogenization. When yogurt milk is homogenized it results in reduced creaming, enhanced water holding capacity (WHC) of the coagulum, increased hydrophilicity, "whiter" yogurt, and good mixing of dry ingredients (44). Homogenization also improves the consistency and viscosity of yogurt (41). However, the influence of homogenization is greater on full-fat or low-fat yogurts compared to nonfat yogurts.

Stabilizer Addition. Manufacturers add stabilizers as thickening agents to improve consistency and viscosity, increase firmness, and prevent syneresis in yogurt (21, 22, 26, 41). Although manufacturers may intentionally choose to manufacture yogurts with different viscosities, they all strive to minimize syneresis. Hence, stabilizers are added (21). Stabilizers are generally hydrocolloids of animal or plant origin. They include starch, gelatin, agar-agar, locust bean gum, guar gum, and pectin. Stabilizers are important to help
maintain good texture and visual properties during transport and storage (26). Relying on stabilizers has been criticized as "patchwork" (39); rather, more attention should be given to optimizing process parameters to obtain the desired product attributes. Some stabilizers, such as alginates, carrageenan, locust bean, guar gum, and carboxy-methyl cellulose, increase syneresis. They retard culture growth and cause whey separation (39). Additionally, flavor problems (34) with stabilizers and consumer pressure for "all natural" yogurt have motivated research into "non-additive" stabilization of yogurt.

**Purpose of Fortification**

From a manufacturer's point of view, fortification of milk determines the physical properties of yogurt (16, 44). The total solids in yogurt affect the physical and chemical properties of the product as well as its nutritional and dietetic properties. The solids (in particular, the protein) affect the organoleptic properties, color and flavor; the rheological properties, viscosity and consistency; and the kinesthetic properties, smoothness, mouthfeel, texture, lumpiness, and grittiness.

Fortification of solids in yogurt also minimizes the effect of the seasonal variation of milk composition (44, 49). Van Gennip (52) demonstrated the relationship between protein content of milk and consistency of yogurt. Periods of low protein content result in low viscosity yogurt. High protein content leads to high viscosity.

To enhance physical properties of yogurt, minimize seasonal variation,
and potentially reduce the cost of formulation, it is common to fortify solids-not-fat (SNF) in milk to 14 to 16% (23, 44). The purpose of part 1 is to report the effects of various methods of fortification on the physical properties of yogurt. The effects of heat treatment of the mix, also very important to yogurt properties, will be discussed in part 2.
FORTIFICATION PRACTICES AND LIMITATIONS

Methods of Fortification

Processors use concentrated milk fractions or powdered dairy ingredients to fortify milk for yogurt (50). Concentration methods include evaporation, reverse osmosis (RO), and ultrafiltration (UF). Although legal standards in various countries largely dictate the minimum percentage total solids in yogurts, the relatively low minima required (compared to what is necessary to achieve functional properties) allow processors to select the types and amounts of fortification based on the desired texture, aroma, and economics (49).

Fortification of yogurt may be on a total solids or protein basis. However, protein is not typically added as pure protein when dairy ingredients are added. Such is the case when nonfat dry milk (NDM), whey protein concentrate (WPC), or whey powder is used. Manufacturers must balance the effect of the added protein with the effect of other added solids, then consider the economics of the whole mix.

Legislation in many countries prohibits addition of any substance for yogurt processing, including milk solids (35). For this reason research has been encouraged into non-additive enhancements of yogurt texture (i.e., membrane filtration, heat treatment).

U.S. Standards of Identity
For Yogurt Products

In the United States, standards of identity exist for yogurt, lowfat yogurt,
and nonfat yogurt (10). The standard for nonfat yogurt specifies that yogurt must involve fermentation of cream, milk, partially skimmed milk, or skim milk used alone or in combination. Additionally, one or more other optional ingredients may be added to increase the nonfat solids of the food. These include concentrated skim milk, nonfat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins, or whey modified by partial or complete removal of lactose and minerals. The only limitation to the optional ingredients is that the ratio of protein to total nonfat solids of the food, and the protein efficiency ratio of all protein present shall not be decreased as a result of adding such ingredients. Nutritive sweeteners, flavoring ingredients, vitamins A and D, color additives, and stabilizers may also be added.

Nonfat yogurt, before the addition of bulky flavors, must contain less than .5% milkfat and not less than 8.25% solids-not-fat (SNF) (10). Lowfat yogurt and yogurt must contain not less than 9.25% SNF. However, yogurt must contain not less than 3.25% milk fat, and lowfat yogurt must contain not less than .5% nor more than 2% milkfat. Although the fat content is adjusted for market (and classified into yogurt, lowfat yogurt, or nonfat yogurt), the SNF are adjusted at the discretion of the processor within the broad range of optional dairy ingredients that may be added to yogurt. There is also considerable overlap between what is the milk to be fermented and what is an optional ingredient; between what is added to fortify the SNF, and what is added as a stabilizer (no limit specified). As a result, a wide range of yogurts can be found in the market (5, 32).
EFFECTS OF FORTIFICATION OF YOGURT WITH DRY INGREDIENTS

Fortification of Yogurt with Non-dairy Ingredients

Non-dairy protein fortification of yogurt is possible (29, 47, 48), though legal restrictions, supply, and economics limit the application of such protein sources. Kolar et al. (29) studied replacement of NDM with soy proteins for yogurt. Viscosity and gel strength were increased to a greater extent than when NDM or sodium caseinate was used on an equivalent protein basis. Schmidt and Morris (47) reported similar findings with blended soy protein/cow’s milk systems. Burgess and Cotton (7) reported a comparison of different types of protein available for yogurt fortification and their respective economics. Use of soy, peanut, or leaf protein by yogurt industries is very small. Greatest popularity is with protein types of dairy origin.

Fortification of Yogurt with Nonfat Dry Milk

Fortification with NDM is the traditional method for enhancing the physical properties of yogurt (31, 50, 53). This is particularly true in lowfat and nonfat yogurts. Addition of NDM is recommended in the range of 1 to 4%, because higher levels can result in a "powdery taste" (41, 50).

A range of physical properties of yogurt may be demonstrated through NDM fortification. Studies with NDM fortification of yogurt indicate that the consistency, firmness, and viscosity of yogurt are dependent on the dry matter
content or total solids (22, 43, 45), SNF (4, 21, 39, 41), and the protein content (36, 37, 44, 52) in the milk. Syneresis in yogurt decreases with increased total solids from NDM addition (4, 21, 22) or increased protein from NDM addition (36). Syneresis correlates generally with firmness and viscosity of yogurt (37).

Interpretation of results on fortification based on total solids, SNF, or protein enrichment must consider that total solids includes fat (which may be up to .5% in nonfat yogurt); SNF includes lactose and minerals; and protein fortification is generally not economical in a form that does not carry other solids. These considerations are important because NDM is the standard against which other fortification methods are judged.

Resubal et al. (43) showed that NDM fortification level affects the taste, smell, and general acceptability of yogurt. Yogurt from skim milk fortified with NDM to 20% total solids is not significantly different from yogurt made from whole milk fortified with NDM to 20% total solids. This suggests that to some extent the solids (perhaps the protein) can substitute for fat in fortified yogurt.

When yogurt is prepared entirely from reconstituted NDM, it has increased syneresis and decreased viscosity compared to yogurt prepared from fresh skim milk fortified to the same total solids with NDM (20). Experiments with yogurt manufacture from roller-dried versus freeze-dried NDM showed similar results (27). The freeze-dried NDM results in superior yogurt. Roller-dried NDM results in yogurt with poor flavor and texture, with a tendency to synerese. Apparently the harsh heat treatment given to evaporated, spray-dried, or roller-dried NDM results in some degree of irreversible denaturation of proteins as compared to
freeze-dried NDM of fresh pasteurized milk. The functional properties of yogurt may be affected by the previous denaturation from the process of drying skim milk. This may also apply to other dried dairy ingredients (e.g., WPC).

**Fortification of Yogurt with Caseinates or Coprecipitates**

Fortification with sodium or potassium caseinate results in higher yogurt viscosity compared to NDM fortification (41, 42, 52). The percent added protein to achieve the same viscosity is 1.2% for sodium caseinate versus 1.6% for NDM (52). Addition of caseinates or coprecipitates (casein and whey protein) prevents whey separation (41), but addition of greater than 2% results in undesirable thickening. Because some dairy ingredients, such as casein, act as stabilizers, the standard of identity for yogurt becomes more ambiguous.

Modler et al. (36, 37) examined the ratios of casein to non-casein protein in studies of three casein-based and three whey protein-based fortifications of yogurt. A ratio of 4.6 to 1.0 from sodium caseinate enrichment produces the firmest yogurt with the least syneresis. However, the texture is rough, coarse, and unsatisfactory compared to lower ratios from NDM or whey protein concentrate fortification.

**Fortification of Yogurt with Whey Derivatives**

Whey powder may be used to fortify yogurt. However, levels higher than 1 to 2% may result in undesirable "whey" flavor in the yogurt (44). In some cases the high lactose level added by whey powder addition may be
undesirable. Sweet whey powder generally contains 12.9% protein and 74.5% carbohydrate (lactose) (11). Table 1 presents composition data of yogurt and other dairy ingredients used in yogurt production.

Fortification with WPC results in softer, weaker yogurt with increased susceptibility to syneresis than when casein-based ingredients are used (37). However, WPC addition to skim milk at 1.0% and 1.5% of protein results in yogurt that is superior to casein-based yogurts in appearance and smoothness. Such WPC fortified yogurts had a ratio of 1.1 to 1.0 casein to non-casein protein in the finished product (36).

Guirguis et al. (21) sought to substitute WPC for NDM in fortification of yogurt milk as a means to reduce the cost of manufacture. Yogurt made with WPC replacing 25% of the NDM to 14%, 16%, and 19% total solids resulted in a firmer coagulum with higher viscosity and reduced susceptibility to syneresis compared to yogurts fortified to the same total solids with NDM alone. The authors suggest that WPC increases the water binding-capacity of yogurts, resulting in higher viscosity and reduced syneresis. However, no total protein measurements were reported. The WPC used contained 45% protein. Nonfat dry milk typically contains 35.5% protein (11). By substituting WPC on a pound-for-pound basis for NDM the total protein level was increased compared to yogurts fortified with NDM alone. This would be expected to reduce syneresis and increase gel strength and viscosity.

For economic improvement of yogurt mix, Whalen et al. (53) studied replacement of NDM with whey-caseinate blends in stabilized yogurts (0.5%
TABLE 1. Typical composition of yogurt and dairy ingredients used in the manufacture of yogurt (11).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Carbohydrate (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim Milk (fluid)</td>
<td>90.80</td>
<td>3.41</td>
<td>.18</td>
<td>4.85</td>
<td>.76</td>
</tr>
<tr>
<td>Whole Milk (dry)</td>
<td>2.47</td>
<td>26.32</td>
<td>26.71</td>
<td>38.42</td>
<td>6.08</td>
</tr>
<tr>
<td>Nonfat Dry Milk</td>
<td>3.16</td>
<td>36.16</td>
<td>.77</td>
<td>51.98</td>
<td>7.93</td>
</tr>
<tr>
<td>Nonfat Instantized Milk</td>
<td>3.96</td>
<td>35.10</td>
<td>.72</td>
<td>52.19</td>
<td>8.03</td>
</tr>
<tr>
<td>Buttermilk (dry)</td>
<td>2.97</td>
<td>34.30</td>
<td>5.78</td>
<td>49.00</td>
<td>7.95</td>
</tr>
<tr>
<td>Evaporated Whole Milk</td>
<td>74.04</td>
<td>6.81</td>
<td>7.56</td>
<td>10.04</td>
<td>1.55</td>
</tr>
<tr>
<td>Evaporated Skim Milk</td>
<td>79.40</td>
<td>7.55</td>
<td>.20</td>
<td>11.35</td>
<td>1.50</td>
</tr>
<tr>
<td>Sweet Whey (fluid)</td>
<td>93.12</td>
<td>0.85</td>
<td>.36</td>
<td>5.14</td>
<td>.53</td>
</tr>
<tr>
<td>Sweet Whey (dry)</td>
<td>3.19</td>
<td>12.93</td>
<td>1.07</td>
<td>74.46</td>
<td>8.35</td>
</tr>
<tr>
<td>Delactosed Whey (dry)</td>
<td>4.00</td>
<td>32.00</td>
<td>2.00</td>
<td>53.00</td>
<td>8.00</td>
</tr>
<tr>
<td>Whey Protein Concentrate (dry)</td>
<td>5.00</td>
<td>61.00</td>
<td>5.00</td>
<td>22.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Sodium Caseinate (dry)</td>
<td>5.00</td>
<td>89.00</td>
<td>1.20</td>
<td>.30</td>
<td>4.50</td>
</tr>
<tr>
<td>Lowfat Yogurt</td>
<td>85.07</td>
<td>5.25</td>
<td>1.55</td>
<td>7.04</td>
<td>1.09</td>
</tr>
<tr>
<td>Nonfat Yogurt</td>
<td>85.27</td>
<td>5.73</td>
<td>.18</td>
<td>7.68</td>
<td>1.18</td>
</tr>
</tbody>
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gelatin). At a 50% replacement, an experienced taste panel detected no flavor differences, and the yogurts had excellent body and texture.
EFFECTS OF FORTIFICATION OF YOGURT BY CONCENTRATION METHODS

Fortification of Yogurt by Evaporative Concentration

Yogurt has been made for many years by concentrating milk by boiling to two thirds the original volume (31, 44). This practice is still used today, especially in small rural areas around the world. The effect of such concentration is the removal of up to 35% of the water from milk, which is equivalent to increasing the total solids by 2% to 4.5%. Boiling might be compared to a single effect evaporator.

Evaporation is used to concentrate milk for yogurt, especially in countries where legislation prohibits addition of any substance to milk for yogurt (35). Evaporation has the advantage, compared to other methods of fortification, of removing undesirable flavors and odors from milk for yogurt, such as when making goats’ milk yogurt (34).

Increasing the SNF in milk by evaporation results in yogurt with superior organoleptic properties compared to NDM addition (41). Abrahamsen and Holmen (1) compared yogurt from whole milk concentrated by evaporation, UF, RO, or addition of NDM. Yogurt prepared by evaporation measured slightly lower in firmness, but ranked slightly higher in consistency and flavor compared to yogurts prepared by RO or addition of NDM. Yogurt from UF milk was firmer and more viscous than yogurts made from other methods.
Reverse osmosis (RO) may be used to concentrate milk for use in dairy products, including yogurt (6, 18, 20). This method of membrane filtration consists of very tight filtering, as 100% of solutes of greater than 200 dalton molecular weight are rejected by the membrane (6). The RO permeate contains water and very small amounts of small molecular weight organic compounds and inorganic ions. The high operating pressure of RO (30-100 kg/cm²) is necessary because the basis of this technique is to apply pressure to the fluid against a membrane in excess of the osmotic pressure of the solution. The temperature of milk in RO rarely exceeds 50°C; so negligible denaturation of proteins occurs during processing (6, 18, 20). This avoids the chemical damage and flavor changes caused by heating in other methods of concentration.

Concentration by RO results in yogurts with higher viscosity and reduced syneresis compared to yogurts fortified to the same level of total solids with NDM (14, 15, 20) though some authors found no difference (1). Dixon (15) found RO yogurt is preferred over NDM fortified yogurts or those made completely from NDM. The flavor is no different, but RO yogurts display enhanced physical properties. Guirguis et al. (20) postulated that the difference in RO versus NDM yogurts is due to limited heat treatment given to the RO milk compared to the irreversible protein denaturation occurring in NDM during evaporation and spray drying. The functional properties of NDM are affected by the manufacturing process. Addition of WPC to RO milk for yogurt does not
improve the viscosity of yogurt (20).

Fortification of Yogurt by Ultrafiltration

Ultrafiltration (UF) is a low pressure (1 to 10 kg/cm²), low temperature (<50°C) method of membrane concentration that can be used for dairy products (18, 28). As in RO, the proteins in UF receive negligible damage as a result of the low processing temperature (6, 18). Membranes of different pore sizes may be used for UF, the lowest retaining molecules greater than 1,000 dalton molecular weight. Permeate from UF contains water, lactose, small peptides, and very small amounts of other organic compounds and inorganic ions. The lactose content can be reduced by adding water and recirculating the concentrated milk (referred to as “diafiltration”). This feature is not applicable to RO concentration.

Ultrafiltration has been used to make acceptable full fat and lowfat yogurts (1, 4, 6, 8, 9, 12, 17, 19, 30, 42, 46, 51). Some of these yogurts were produced through UF with diafiltration, and equal or better firmness and viscosity were obtained as a result of lactose reduction (17, 19, 51). Kosikowski (30) prepared reduced lactose yogurts and milk beverages using UF. Rather than using diafiltration, these products were prepared from highly concentrated retentates (20% protein) rediluted with distilled water to 3.3% protein. A yogurt type prepared from such concentrate had 7.4% total solids, smooth viscous texture, and firm body with minimal syneresis. It had a clean, acid, slightly flat flavor and was lower in calories than commercial yogurt, yet comparable in pH and
filtratable acidity. This suggests that lactose may actually dilute the effect of the protein in the formation of the water holding yogurt gel.

Yogurt made from skim milk fortified using UF is superior to other methods of fortification in terms of gel strength, viscosity, and syneresis (1, 4, 9, 12, 13, 24, 42). Abrahamsen and Holmen (1) found UF yogurts with 2.3% higher total solids to be firmer and more viscous than yogurts with 2.5% to 2.7% higher solids from vacuum evaporation, RO, or addition of NDM. Renner (42) found no syneresis and best sensory appearance, odor, consistency, and taste from yogurt made from skim milk concentrated 1.2 to 1.4 times by UF. Becker and Puhan (4) attributed the highest viscosity and firmness in UF yogurts (compared to NDM fortification or evaporation) to the increased content of protein as a component of total solids. Dargan and Savello (12) reported similar findings. Yogurt fortified to a casein to non-casein protein ratio of 2.9 to 1.0 had optimum gel strength and syneresis (36). This was similarly produced by either UF concentration or NDM fortification, compared to other fortifications that would alter the casein to non-casein ratio (i.e., caseinates or WPC).
MICROSTRUCTURE OF FORTIFIED YOGURTS RELATED TO PHYSICAL PROPERTIES

Clues about the nature of the yogurt protein gel have been supplied using electron microscopy (25, 26, 27, 36). The dimensions of the casein matrix (average distances between micellar chains) in yogurts fortified with NDM depend on the level of fortification (25). These dimensions are reduced with increasing total solids (through NDM addition) from 10% to 20% (from NDM addition). In set yogurt, Kalab et al. (26) described the gel microstructure as consisting of casein micelles fused into chains and clusters, with large free spaces thought to be the site of immobilized water. Stirred yogurt had fewer chains and more clusters of micelles joined together by thin fibers. The authors suggest that it is probably very important to maintain the microstructure to preserve the physical structure of yogurt and thus prevent syneresis.

When Modler and Kalab (36) studied the microstructure of yogurt fortified with different milk proteins, they found the most extensive fusion of micelles and also the largest micelles in unfortified yogurts or those fortified with added casein. Yogurts with extensive micelle fusion in end-to-end links of micelle chains have lowest syneresis and highest gel strength. Yogurts with less fusion have increased syneresis. In contrast, yogurts made from skim milk fortified with WPC have individual micelles or micelles associated with flocculated protein in between. The floccules are mostly at the micelle surface and appear to participate in the linkages between micelles. This supports the findings that the physical properties of yogurt are dependent on the ratio of casein to non-casein
protein in milk. Casein enrichment must at least be in proportion to non-casein protein (as found in milk) to maintain or improve syneresis.
CONCLUSIONS

Fortification of yogurt is necessary to minimize the seasonal variation of milk composition and to bring the firmness, viscosity, consistency, and syneresis to the levels desired by the consumer. Physical properties of yogurt depend on the ratio of casein to non-casein protein, as a result of fortification method, and the extent to which the fortifying proteins have been denatured by previous heat exposure (such as during drying), rather than the total solids content. Excess caseinate fortification results in yogurt texture that is coarse and undesirable. Optimum textural properties are obtained when milk is fortified with both caseins and whey protein.

Reverse osmosis and ultrafiltration may be used to fortify yogurt by increasing the concentration of both caseins and whey proteins without denaturing them in the process. However, the RO process concentrates all the solids in milk, including lactose, while UF only concentrates protein and fat. Ultrafiltration has the added advantage that lactose is reduced while proteins are concentrated as a percent of total solids. Lactose has been shown to dilute the ability of the protein to form a firm water-retaining gel. Through diafiltration or redilution of concentrates with water, UF retentates may be adjusted to the desired lactose levels for purposes of optimizing gel strength, reducing calories, or adjusting of final yogurt pH. With UF it is possible to reduce the SNF required to achieve the same viscosity, without syneresis, compared to traditional NDM fortification. The end result can be a good tasting, high texture,
lower calorie, lowfat yogurt.
REFERENCES


PART 2. HEAT TREATMENT OF MILK TO IMPROVE YOGURT

PHYSICAL PROPERTIES - A REVIEW
INTRODUCTION

In yogurt, consumers desire firm body, high viscosity upon stirring, and minimal syneresis (2, 25, 32, 33). The physical characteristics of the curd are important to the quality, texture, and body of the finished cultured product (26, 41). Factors that affect the chemical or physical milk composition may affect the strength of the curd formed upon coagulation. As opposed to cheesemaking, where curds are cut to induce shrinkage and expulsion of whey (58), yogurt receives minimal handling during fermentation to encourage immobilization of the water phase within the gel (20, 25). The ability of the gel to hold water is closely related to the firmness and viscosity of the product (20, 23, 37).

The Yogurt Gel

Yogurt gel is formed through a combination of heat-, calcium-, and acid-induced reactions of proteins (50). Lactic acid fermentation results in decreased pH, increased soluble calcium, and enhanced interaction between proteins. Gelation occurs at or above the isoelectric point of casein (pH 4.6).

Microstructurally, yogurt gel exists in unfortified yogurt as a three-dimensional network of casein micelles fused together and limited in long chains, seldom in clusters (24). Large free spaces exist that are believed to be the sites of immobilization of water.

Manipulation of the mix composition and processing parameters for yogurt have been studied and are used by processors to enhance the properties of the yogurt gel. Particularly, the total solids and total protein
content of milk fortified for yogurt has a predominant effect on the gel characteristics (2, 20, 38, 41, 45). The effect of mix composition may overshadow the effects of all other processing parameters as it is easily demonstrated that fortified yogurts exhibit greater firmness and viscosity with less syneresis than unfortified yogurts (2, 20, 21, 57). Fortification of milk for yogurt is thus widely used to enhance yogurt physical properties throughout the world and has been reviewed in another paper (6). Heat treatment can be combined with fortification to further enhance the physical properties of yogurt.

Once the composition of the milk to be used for yogurt is established, the processing parameters play a significant role in maximizing the yogurt gel properties from the mix (33, 38, 41, 50). The role of acid production by the starter culture (25, 50, 55), incubation conditions (25, 33), homogenization conditions (38, 43, 50), use of ropy strains that produce carbohydrate exopolymers (43, 54, 56), stabilizer condition (24, 25, 38, 43), cooling procedures (32, 33, 38), handling (25, 32, 38), and particularly heat treatment of the mix (41, 42, 47, 51) affect the physical properties of the yogurt gel. Optimization of processing parameters (such as heat treatment) has been suggested as enabling the use of lower total solids in the milk or reduced stabilizer, which may lower the overall cost of the formula (4, 33, 38).

**Stabilizer Addition Versus "Nonadditive" Enhancement of Yogurt Physical Properties**

Where regulations permit, stabilizers are routinely used to improve the consistency, viscosity, and water-holding capacity (WPC) of yogurt (12, 24, 25,
Manufacturers may vary the target viscosity of their products, but because they must prevent syneresis under all fortification conditions, stabilizers are added to the mix (19). Stabilizers are generally hydrocolloids of animal or plant origin. Their use has been reviewed by Robinson and Tamime (46). Milk solids-not-fat may also function as stabilizers (33, 35), but economics influence processors' choice of this approach. Use of non-dairy stabilizers has been criticized as "taking the easy way out" (33), and "patchwork" (38). Rather, appropriate technologies (43) or increased attention to process parameters (38) is recommended.

Manufacturers in many European countries are not allowed to add stabilizers or, in some cases, any other ingredients to milk for yogurt (33). Likewise, consumer pressure in the United States and elsewhere, for "all-natural" yogurt, has encouraged research into "non-additive" means of optimizing yogurt texture. These may include concentration of milk solids by evaporation or membrane filtration, and manipulation of heat treatment of the milk for yogurt (33, 42).
HEATING OF MILK FOR YOGURT

Purpose of Heat Treatment

Heat treatment is universally used in yogurt manufacture (4, 36, 50). In the United States, the standard of identity for yogurt dictates that milk must be pasteurized or ultrapasteurized (3). However, heat treatments given to milk during yogurt manufacture are usually far in excess of the minimum required to insure food safety and provide other beneficial effects (36, 45, 54). Besides destroying pathogens, heating eliminates other microorganisms that would compete for nutrients with the starter culture or produce flavor or gas defects in the finished product (43, 45). Chemical changes that are stimulatory to the growth of the starter culture, including expulsion of oxygen from the milk and release of free amino acids, occur as a result of heating (38, 45). Heating improves the keeping quality of yogurt by inactivating lipases and other enzymes. Heat treatment also lowers the pH of milk (56) and converts ionic and soluble calcium and magnesium phosphates to colloidal phosphates (36). Furthermore, it causes major changes in the physicochemical structure of the proteins (45, 56) by denaturing whey proteins. The subsequent interaction between denatured whey proteins and casein is believed to increase the hydrophilic properties of the casein (17, 56). Heat sensitizes the whey proteins to calcium ions which may also enhance coagulation (45).
General Effects of Heating Milk for Yogurt

The type and extent of heating has a major effect on the physical and sensory properties of yogurt (21, 29, 39, 41, 42, 49). This effect is believed to be a result of the heat treatment of the milk proteins (17, 39). When milk is heated, an initial increase in viscosity occurs (believed to be due to micelle aggregation and interaction between whey proteins and casein) (50, 56) which upon further heating is followed by coagulation and then flocculation or separation (21). In consideration of various heat treatments of milk for yogurt, one must put this continuum in perspective relative to the heat treatment given.

In studies using traditional vat-heated versus unheated milk for yogurt, heating results in improved consistency (17) and firmer, more viscous yogurt (9, 20, 23, 40, 42) with less syneresis (9, 17, 20, 23). Kalab et al. (23) found yogurts made from unheated milk are 50% weaker than those made from heated milk. Harwalker and Kalab (20) fortified milk to 10, 12.5, and 15% total solids and found uniformly firmer yogurts with less syneresis from heated milks at all total solid levels. Yogurts from heated milk with 12.5% total solids have less syneresis (as measured by centrifugation) than from milk with 15% total solids that are not heated. Unheated gel networks are weak and unable to immobilize water compared to heated milks. Firmness is inversely related to syneresis (20), which agrees with the findings of other authors (4, 35) and substantiates the findings that immobilization of water gives strength to the gel and viscosity upon stirring. Water-holding capacity, expressed as percentage pellet weight of sample weight
after centrifugation, increases as a result of heat treatment of milk (23, 40, 42). Gelation is more rapid in heated milks for yogurt (9, 17, 23) compared to unheated milk.

Schmidt et al. (51) demonstrated that in addition to weak-bodied yogurt being produced as a result of insufficient heating, excessive heating also can result in lower gel strength with increased tendency to syneresis. Dolezalek et al. (13) showed that extended heating at 95°C for 30 min reduced yogurt firmness. However, Meiklejohn (33) reported that a commercial yogurt plant in Denmark used 95°C for 30 min, claiming that more or less heat treatment hurt viscosity. Though the type of heating system and the time and temperature parameters are generally agreed to affect the body and texture of yogurt (29, 41, 42), the optimum heat treatment remains unclear. Nonetheless, some form of heating is necessary to promote good yogurt physical properties.

**Previous Heat Treatment of Ingredients in the Yogurt Mix**

In considering the effects of heat treatment of fortified milk for yogurt, one must consider the previous heat history of ingredients that go into the mix. For example, 30 to 50% of the whey proteins in powdered whey protein concentrate (WPC) are denatured (36). It is difficult to determine if further heat treatment after WPC incorporation into a yogurt mix damages or enhances the whey protein contribution to yogurt physical properties.

When yogurt was prepared from fully reconstituted nonfat dry milk (NDM), it displayed increased syneresis and reduced viscosity compared to yogurt
made from fresh milk with added NDM to the same total solids level (18). The severity of the heat treatment of NDM was examined related to yogurt physical properties (23) in a study of yogurts made from spray-dried, roller-dried, and freeze-dried NDM. Yogurts from reconstituted, roller-dried NDM resulted in the weakest yogurt, with poor flavor and texture, and a tendency to synerese. Yogurt from reconstituted freeze-dried NDM had superior physical properties. Heat treatment of reconstituted yogurt mixes improved properties, but not to comparable properties from heated fresh liquid milk. The heat treatments given to NDM in the various processes of its manufacture affect the functional properties of the proteins when used in yogurt manufacture (18, 23).

Glover (16) described the use of ultrafiltration and reverse osmosis to concentrate milk for dairy products as having the advantage of low operating temperatures resulting in minimal chemical damage to the proteins, compared to the harsh heating in other concentration methods. Concentration by reverse osmosis results in yogurts with higher viscosity and reduced syneresis compared to yogurts fortified with NDM to the same total solid levels (10, 12, 18). Reverse osmosis processing concentrates milk without changing the proportion of solids in relation to each other, and only negligible quantities of solids pass through the membrane into the permeate (16). This suggests that the primary difference between yogurts from milk concentrated by reverse osmosis and yogurt from NDM-fortified milk is the previous heat treatment and irreversible protein changes NDM receives during manufacture. Guirguis et al. (18) concluded that the physical properties of yogurt are improved when the
casein component receives minimal heat exposure prior to heat treatment for yogurt. The previous heat history of ingredients added to milk for yogurt must be considered when trying to establish optimum heat treatment for yogurt.
COMPARISON OF METHODS FOR HEAT TREATMENT OF MILK FOR YOGURT

Conventional Vat Heating

Vat heating of milk for yogurt is the conventional standard to which other heating methods are compared (26, 39, 49). Much of the research demonstrating the benefits of the heating of milk for yogurt involved vat heating methods (17, 20, 23, 40, 51). However, recommendations for time and temperature of vat heating vary considerably. Most commonly reported heat treatments include 80 to 85°C for 20 to 30 min and 90 to 95°C for 5 to 10 min. Lower temperatures or shorter holding times than these ranges produce less than optimum yogurt properties from vat heating (14, 17, 23, 26). Kalab et al. (23) found yogurts produced from temperatures less than 75°C to be similar to each other in physical characteristics while those produced from temperatures greater than 85°C have increased firmness (compared to those from lower than 75°C heating) and are free of syneresis. As part of a study of various heat treatments, Labropoulos et al. (26) showed higher apparent viscosity and gel firmness as well as lower fluidity and spreadability in yogurts from milk heated at 82°C for 30 min compared to yogurts from milk minimally pasteurized at 63°C for 30 min. The authors suggest the highest curd tension and viscosity received from the 82°C for a 30-min treatment is possibly due to increased water-binding. Schmidt et al. (51) produced yogurts with increased penetration force and stirred viscosity as temperature increased from 70 to 90°C with a 30-min holding time.

Conversely, excess vat heating also results in detrimental effects on
yogurt physical properties (4, 13, 17, 33, 42, 51). Parnell-Clunies et al. (42) saw no improvement of viscosity or firmness by extending holding time at 85°C from 10 to 20, 30, or 40 min. Although these yogurts are firmer than yogurts prepared from high-temperature-short-time (HTST) or ultra-high temperature (UHT) treatments, they display considerable syneresis. They are described as grainy and lumpy, increasing with increased holding time. The greater firmness and lower water-holding capacity in vat-heated yogurts are speculated to be due to greater molecular aggregation between whey proteins and casein occurring during the heating of milk, leading to loss of surface area and water-binding sites with increased heat intensity. Heating at 95°C for 30 min reduces yogurt firmness (13). Yet, at a Dutch producer and a factory in Denmark, 95°C for 30 min was reported to be optimum, with a higher or lower temperature resulting in lower viscosity (33). Grigorov (17) minimized syneresis with a heat treatment of 85°C for 30 min, compared to higher or lower heat treatments. For Schmidt et al. (51), excess vat heating results in lower gel strength and grainy texture with a tendency to synerese.

Vat heating generally produces yogurts of higher firmness than other heat treatments (HTST or UHT) (26, 37, 41, 48, 49). However, reduced WHC and increased syneresis are also found coincidentally with vat heating compared to other heat treatments (39, 49). Vat heating is also energy-expensive and less suitable for large-scale operations (14).
High-Temperature-Short-Time Heating

Heating of milk for yogurt by HTST methods has been adopted by some processors because it provides a continuous, enclosed processing system, and saves time and energy (14). Physical properties of yogurts made from milk that has been processed by HTST methods are generally intermediate between those found by vat processing and those from UHT processing (39, 41, 42). When yogurts are prepared from milk heated at 98°C for holding times between .5 and 1.87 min, the firmness and viscosity increases with increased holding time (41). Although firmness and viscosity are weaker than vat-heated yogurt, the 1.87-min HTST yogurt was preferred by the taste panel because it was smooth and free of the graininess found in the vat-heated yogurt. Highest WHC was also found in HTST yogurt compared to vat-heated or UHT-treated yogurt. Heating by HTST was suggested as a viable alternative to traditional vat heating.

Ultra-high Temperature Heating

The processing of milk for yogurt by UHT treatment has been studied because, like HTST processing, UHT offers a continuous enclosed system and potential energy and time savings compared to conventional heating (14, 49). More closely standardized process control, improved sanitation, and potentially stimulatory effects on starter cultures are also advantages UHT processing may offer.

In studies of yogurt from skim milk fortified with 3% NDM, UHT treatment of 138°C for 3 or 6 s produces weaker gels with lower viscosity than vat-heated
controls (49). Increasing the holding time of UHT processing from 3 to 6 s increases yogurt firmness and consistency. Increasing the temperature to 149°C, however, results in weak-bodied yogurt. Similar rheological findings were produced by other laboratories (14, 26, 28, 29, 37, 39, 41, 48).

Labropoulos et al. (26, 27) found firmest yogurts from UHT (149°C) treatment of milk for 3.3 s, compared to 5.2, 8.2, or 13.7 s. Sedimentation occurs in the 8.2 and 13.7 s treatment, indicating a severe level of protein denaturation. Yogurts from UHT treatments are higher in spreadability, fluidity, and shear stress, but lower in gel firmness and apparent viscosity than vat-heated yogurts. Taste panel scores support objective measurements, with significantly lower scores for body in UHT yogurts, compared to vat-heated yogurts. No difference is noted for flavor and appearance of UHT versus vat-heated yogurts (28). UHT yogurts are even lower in apparent viscosity than minimally pasteurized (63°C 30 min), vat-heated yogurt (29). Curiously, UHT-treated milk (prior to culturing) has higher viscosity than vat-heated milk, which is higher than the raw (unheated) milk. The increase in viscosity is possibly due to increased aggregation of protein. If so, the specific aggregation is a hindrance to good texture formation in yogurt from UHT-treated milk. The UHT yogurt is described as resembling the texture of buttermilk.

In studies of yogurt prepared by heating milk with industrial UHT systems, direct UHT treatment (steam injection) results in lower yogurt hardness than indirect UHT (37). Both systems produce yogurts that are lower in hardness, viscosity, and shear resistance compared to yogurts from milk heated by a vat.
at 90°C for 10 min. The results suggest that the weakness of UHT yogurts is due to low intensity of heating.

Parnell-Clunies et al. (42) studied batch versus continuous heating systems related to physical and sensory properties of yogurt. Treatment of milk by UHT was 140°C for 2, 4, 6, or 8 s. Yogurts from UHT-treated milk are lowest in firmness and viscosity compared to HTST (95°C for .5, .95, 1.42, and 1.87 s) or vat-heating (85°C for 10, 20, 30, or 40 min). Viscosities of yogurts from unheated milk are similar to those received from UHT yogurts. UHT yogurts has intermediate WHC between HTST (highest) and vat (lowest) yogurts. Yogurts from UHT treatments of 2 and 4 s are weaker than 6- and 8-s treatments. Similarly, HTST treatments below 1.87 min are weaker than the 1.87-min treatment. Increasing the residence time at these temperatures must have had an effect on the proteins that positively contributes to yogurt texture. However, the work (discussed earlier) of Schmidt et al. (49) and Labropoulos et al. (27) suggests that a break point occurs between 140 and 149°C based on temperature, because 149°C produced weaker yogurts than 138°C (49). Extending the holding time at 149°C resulted in weaker, denatured (visible sedimentation) yogurt (27).

In response to finding lower viscosity in yogurt from UHT milk (compared to vat heating), Driessen (14) suggested the selection of a ropy culture to produce more viscous character in UHT yogurts. Preliminary tests found the yogurt susceptible to structural damage. The author suggested the addition of NDM to milk to be processed by UHT treatment in order to resist structural
damage and match the properties of yogurt from vat-heated milk without NDM.

A two-stage mechanism was proposed to explain changes in yogurt consistency based on heating of proteins (39). In the first stage, denaturation of protein exposes hydrophilic groups which increases the WHC of yogurts produced from such milks (i.e., HTST and UHT treatments). In the second stage, continued heating forms hydrophobic aggregates, resulting in exclusion of water from the protein surface. This would result in yogurts with generally firmer character but decreased water holding capacity (i.e., vat-heated yogurts). This theory fails to address the correlation of the firmest yogurts with those of lowest syneresis, reported by several authors (20, 23, 37).

Morr (36) suggested that weaker gels from UHT-processed milk result from aggregation of casein micelles into large particles that do not participate adequately in the formation of the yogurt gel matrix. This agrees with the higher viscosity found in UHT-treated milks (29).

Questions remain about whether UHT treatment of milk produces weaker yogurt because the treatment is too brief or too severe. Several investigators suggest that UHT processing results in weaker yogurt because the heat treatment is of too little intensity (37, 43). However, the properties resulting from temperatures higher than 138°C (49) and longer holding times at 149°C (27) prompted researchers to reconsider because these conditions may have been too severe. Examination of whey protein denaturation and microstructural studies may provide additional clues.
Physicochemistry of Whey Protein Denaturation

To understand the relationship of whey protein denaturation to yogurt properties, a closer examination of the events of whey protein denaturation must be made. Although there is considerable controversy around the definition of denaturation (11), it is generally believed to involve dissociation of subunits (if applicable) and unfolding of the motive, compact globular structure into a less-organized structure. Unfolding is followed (with continued heating) by protein aggregation and then precipitation (11, 45). Denaturation manifests itself through a loss of solubility of the protein.

Casein micelles are subject to dissociation and re-association upon heating of milk (36). Kappa-casein contains a single disulfide group that may react with β-lactoglobulin (β-LG) and possibly other whey proteins upon heating. Whey proteins contain significant amounts of sulfhydryl and disulfide groups that render them sensitive to denaturation and intermolecular reaction during heating (30, 36, 50). Kappa-casein, β-LG, and α-lactalbumin (α-LA) are involved in complex formation when milk is heated (15).

The thermolability of whey proteins is due to the marginal stability of their compact three-dimensional globular structure. By comparison, casein has relatively little organized tertiary structure which contributes to its high heat stability (11). Upon heating milk, whey proteins unfold, activating sulfhydryl
groups which interact with sulfhydryls on other β-LG, α-LA, or caseins (36).

Approximately 1,540 whey protein molecules may be found per casein micelle in unheated milk. However, the casein micelles are theoretically capable of interacting with all the whey protein molecules during heating because each micelle contains approximately 18,000 κ-casein molecules (36).

Two distinct heat effects on whey proteins were demonstrated through the use of differential scanning calorimetry (11). Using this technique the unfolding of protein structure, which is highly endothermic, may be visualized with respect to temperature. The first effect occurs near 70°C and is referred to as "denaturation heat." Near 130°C the unfolding of the residual protein is believed to occur. The pasteurization of β-LG at pH 6.9 and 72°C for 16 s results in less than 3% irreversible protein unfolding (first-stage denaturation). However, UHT processing at 140°C for 4 s results in 40% irreversible denaturation due to the second stage of denaturation (aggregation).

Rates of denaturation of different whey proteins are not equal, and there is considerable temperature dependence (30, 52). Processing factors controlling the interaction, polymerization, and aggregation of milk proteins include protein content and composition, time and temperature of heating, pH, total solids, ionic composition, viscosity, and agitation (36, 56).

**Measurement of Whey Protein Denaturation**

Ramos (44) demonstrated whey protein denaturation (WPD) and complexation with casein through measurement of the percentage change in the
nitrogen fractions of milk upon heating. The casein fraction increased, whereas
the noncasein fraction decreased in percentage as well as soluble protein
content at pH 4.6. Loss of solubility of whey protein is routinely used as a
measure of whey protein denaturation in studies related to yogurt properties (4,
23, 27, 37, 41, 42). With this method, values varied depending on whether or
not nonprotein nitrogen (27, 31, 37, 42) and the proteose-peptone fraction (27,
42) were subtracted from the noncasein nitrogen (soluble nitrogen) fraction.
Highest WPD values were obtained when both were subtracted from noncasein
nitrogen. Alternatively, measurement of available sulfhydryl groups has also
been used (19, 44). Additional methods for measurement of whey protein
denaturation in processed milks were recently compared by Manji and Kakuda
(31). Percentage WPD (loss of solubility method) ranged from 20.6 to 30.4 for
vat-pasteurized milk (63°C for 30 min) which is much higher than the 2.3 to 3%
reported by other investigators (11, 44). For HTST (80°C for 30 s), percentage
WPD ranged from 36.5 to 42.9. The UHT-treated milks resulted in 64.6 to 68.4%
WPD for indirect (145°C for 3 s) and 51.1 to 54.1% for direct (142 for 3 s).
Reasonable agreement was found among fast protein liquid chromatography,
Kjeldahl nitrogen, and whey protein nitrogen (turbidimetric) methods.
Denaturation in milk involves a number of complicated reactions and depends
on environmental conditions. It is often difficult to compare the results of
different investigators because of different environmental conditions and different
methodology used to measure whey protein denaturation.
Relationship of Whey Protein Denaturation to Yogurt Physical Properties

Denaturation of whey proteins has continually been implicated as being a determinant for yogurt physical properties (4, 14, 21, 23, 36, 38, 39, 40, 41, 42, 43, 47, 49, 50), though some authors found no relation (7, 8, 19, 27, 37), or restricted the relation to certain temperature ranges or holding times (4). Denatured whey protein-casein micelle complexes from highly heated milk products are believed to imbibe water resulting in increased viscosity, gel strength, and reduced syneresis in yogurt (36, 38, 56).

Recommendations of WPD of milk for yogurt have varied partially due to differences in methodology. Driessen (14) suggested that greater than 80% WPD of milk provides best yogurt properties, with the water binding capacity increasing by a factor of three compared to milk with native proteins. Likewise Rohm (47) found greatest firmness in yogurts from heat treatments that resulted in 2% residual undenatured \( \beta \)-LG B. Puhan (43) described the level of WPD needed as dependent on the particular product. Plain yogurt with minimal total solids (9.5 to 12%) required higher WPD than fermented milks with high total solids (greater than 14%). He recommended a range of 70 to 95% denaturation to promote the best yogurt physical properties. An upper limit of WPD effect on yogurt physical properties has been suggested to be 88 ± 5% WPD (42) or 99% denaturation of \( \beta \)-LG (4). Heat treatment in excess of these levels of denaturation provides no improvement in yogurt physical properties. Nielsen (38) also suggested that whey proteins may be protected by high total solids.
and that optimum viscosity may be obtained from yogurt mixes with high total solids without complete denaturation of whey proteins. De Wit (11) concurred that sugars may protect against loss of solubility of protein during heat treatment.

Guirguis et al. (19) found greater WPD when milk was fortified with whey protein concentrate than with NDM. This may have been due to an increased level of protein as a percentage of solids because of the difference in composition of the two additives. A higher protein content would increase the opportunity for molecular collision and potential disulfide interchange (1). The whey protein concentrate would also contribute to WPD measurement from the WPD received during its manufacture (perhaps as much as 30 to 50%) (36). When yogurt premixes were heated from 15 to 60 min at 80°C, viscosity increased and syneresis decreased independent of WPD (19). This suggests that protein reactions beyond simply WPD (i.e., complexing with casein) are involved in determining yogurt properties.

Lower WPD in UHT-treated milk for yogurt was suggested as the reason yogurts from UHT-treated milk typically have lower firmness and viscosity than vat-heated yogurts (43, 49, 53). However, other investigators report high levels of WPD in UHT-treated milks for yogurt and yet low relative values of gel strength and viscosity (27). Labropoulos et al. (27) found lower curd firmness and shear stress in yogurts from UHT-treated milk (70% WPD) than in yogurt from minimally pasteurized (63°C for 30 min) vat-heated milk (10% WPD). Vat heating of milk at 82°C for 30 min produced firmest yogurts. The authors
concluded that in vat processes (at 63°C or 82°C), specific protein changes occur that do not happen in the brief UHT process (149°C for less than 10 s). This does not explain the lower firmness received by these authors upon extending the holding time of UHT processing of milk for yogurt.

Parnell-Clunies et al. (40, 42) correlated yogurt firmness and viscosity to WPD (.83 and .89), though little effect on yogurt properties is seen until WPD is at least 50%. No effect on yogurt firmness is seen after 85 ± 5% WPD. However, WHC decreases slightly with increased WPD from 70 to 93%. Lower WHC occurs in the highly denatured (88 to 100%) vat-heated yogurts, and higher WHC occurs in milks that had much lower WPD. Increased WHC is correlated with increased water association with the protein as measured through a protein hydration index (freeze drying of pellets following centrifugation). The authors concluded that whey protein denaturation is not a necessary precursor to improved WHC. Rather, increased WHC may be related to unfolding of protein, exposure of changed groups, and increased surface area. Whey protein denaturation effects manifest themselves through covalently linked protein complexes which, by their nature, do not improve WHC. Increased WPD results in increased surface hydrophobicity and reduced sulfhydryl content--implicating aggregation by disulfide bridging and hydrophobic interaction. Firmness and viscosity correlates to the extent of hydrophobic aggregation. Parnell-Clunies et al. (42) suggested that possibly more than one type of physiochemical transition occurs during heating of milk.
Dannenberg and Kessler (4, 5) demonstrated that the properties of the yogurt gel are not only dependent on whey protein denaturation, but more specifically on the extent of denaturation of \( \beta \)-LG and subsequent reaction with \( \kappa \)-casein. Denaturation is indicated by loss of solubility at pH 4.6. Measurement of the individual whey protein fractions was by densitometric evaluation of bands from isoelectric focusing on polyacrylamide gels. Gel firmness increases and whey separation decreases uniformly with increased denaturation of \( \beta \)-LG independent of temperature. At 99% denaturation of \( \beta \)-LG, WHC improves but gel firmness decreases slightly. When holding times at various temperatures are increased to 2.5 times that necessary to result in 99% denaturation, there is no further improvement in firmness. Traditional heat treatment at 90 to 95°C for 10 to 30 min is far more severe than necessary to denature 99% of the \( \beta \)-LG. However, yogurt from milk preheated to 120°C (for holding times to result in a high level of denaturation of \( \beta \)-LG) has weaker structure than yogurt from milk with the same level of denaturation from heat treatment at lower temperatures. Very long holding times (i.e., 1.5 h) at elevated temperatures also result in poor correlation between degree of denaturation of \( \beta \)-LG and yogurt consistency. Exclusion of temperatures greater than 120°C means that the relationship does not hold for UHT treatment. This finding agrees with those of other authors (7, 8, 27). Dannenberg and Kessler (4) concluded that optimum firmness and little syneresis could be obtained from heat treatments below 120°C that result in 90 to 99% denaturation of \( \beta \)-LG. They suggest that it may be possible to reduce total solids or stabilizer content through optimization of heat treatment.
Through the use of immunogold labelling techniques, Mottar et al. (37) showed that α-LA also plays a significant role in yogurt physical properties. Both β-LG and α-LA were bound to the casein micelle, depending on the heating process and intensity. Heat treatments (including direct and indirect UHT and vat heating) are characterized by the degree of WPD (loss of pH 4.6 protein solubility). Hardness and viscosity are not directly correlated to WPD. Yogurts from UHT-treated milk with comparable WPD to yogurt from vat-heated milk have lower gel firmness and viscosity. The ratio of β-LG to α-LA associated with the casein micelle seemed to be the determinant for the textural properties in yogurt. The ratio also affects the hydrophobic-hydrophilic properties of the gel. A lower ratio results in increased WHC and decreased surface hydrophobicity. Higher heat intensities such as UHT (indirect) or 10 min at 90°C increase the concentration of α-LA on the micelle. The denaturation and association of α-LA with the casein micelle is a determinant for the hydrophilic properties of casein after fermentation.

Various investigators have shown that similar WPD levels, achieved by different heat treatments, may result in different yogurt physical properties (4, 8, 37). In some cases the results are almost opposite to that expected through the traditional view of increased yogurt gel strength, etc., with increased WPD (19, 27, 28, 42). Other investigators have discarded treatments or narrowed the range of temperatures and holding times to allow the WPD data to be predictive of yogurt properties (4, 26, 49). Collectively, the research supports the complexity of WPD, subsequent complexing between β-LG and κ-casein, and the
potential for different heat treatments to result in different rates of denaturation of individual proteins and different forms of casein-whey protein complexes. Measurements beyond simple loss of whey protein solubility must be pursued in order to better understand the effect of heat treatments on yogurt physical properties.
Microstructure of Yogurts Made With Heated and Unheated Milk

The microstructure of yogurt is influenced by the heat treatment given to the milk prior to fermentation (9, 22, 23, 37, 40). The effects may best be demonstrated through comparison of unheated versus heated milks that have been made into yogurt (23, 40). Yogurt from unheated milk exhibits a coarse protein network of large casein particles in large clusters or aggregates. Void spaces are generally 20 \( \mu \text{m} \) across. Milk heated by various heat treatments (vat, HTST, UHT) similarly display a finer, more continuous protein network composed of smaller casein particles linked in a three-dimensional network via chains. Void spaces are 3 to 10 \( \mu \text{m} \) across.

Microstructure and Physical Properties of Yogurts from Heated Milk with Added Milk Proteins

When considering the effects of heat treatment on the microstructure of yogurt, the composition of the mix must not be ignored. Tamime et al. (55) compared microstructures of yogurts prepared from the same heat treatment of milks fortified with NDM or sodium caseinate, or concentrated by evaporation (EV), ultrafiltration (UF), or reverse osmosis (RO) to similar protein contents (5.0 to 5.5\%). All the yogurts had microstructures consisting of casein micelle chains and clusters. However, yogurts from NDM, EV, or RO had short simple chains
with small clusters. Yogurt from milk fortified with sodium caseinate was firmest. Its microstructure consisted of a coarse, open matrix of robust casein particle chains and large clusters.

Comparing the microstructure of three casein-based and whey-based fortified yogurts, most extensive micelle fusion and the largest micelles are found in unfortified yogurts or those with added casein (34). Yogurts made from skim milk fortified with whey protein concentrate (WPC) have individual micelle or micelles associated with flocculated protein in between. The floccules appear to participate in the linkages between micelles. Such yogurts have very weak body and display severe syneresis. Compositional biasing with casein or whey protein can aid in interpretation of heat effects on yogurt microstructure.

**Protein Composition of Yogurt Microstructure from Heated Milk**

Heat treatment of milk results in the formation of filamentous appendages around casein micelles (4, 9, 37). Davies et al. (9) concluded that the appendages were β-LG. Appendages form when casein micelles (separated by ultracentrifugation) are heated with whey powder, milk supernatant, or pure β-LG. Appendages are not present and micelles are smooth in raw milk and after heating of casein micelles with milk dialysate or pure α-LA. Sulfhydryl bonding has been implicated through reduced appendage formation with the use of sulfhydryl blocking agents. The authors concluded that during heating of milk for yogurt, denaturation of β-LG and subsequent association with casein micelles are important determinants of micelle fusion. Similar conclusions have
been drawn by other researchers (4, 22, 37, 39).

Through the use of immunogold labeling with electron microscopy, the involvement of α-LA, in addition to β-LG, was substantiated in heated milk complexes with casein (37). Both β-LG and α-LA are bound to casein depending on the heating process and intensity. The filamentous appendages described by several investigators (4, 9, 22, 37) are similar in nature to the flocculated protein found surrounding casein micelles in yogurts made from milk fortified with whey protein concentrate (34).

Yogurt Microstructure Formation and Relationship to Yogurt Physical Properties

Although the terms "coalescence" and "fusion" are not always used consistently by different investigators, some conclusions may be drawn from the interpretation of the microstructural effects of heating on yogurt texture. The adsorption of whey proteins onto casein micelles is important to the chemical and physical properties of the micelle and the resultant yogurt texture (9, 37). Upon sufficient heating the filamentous appendages of β-LG and β-LG-κ-casein are formed around the casein micelles which result in reduced coalescence. Instead of coalescing, the micelles fuse during fermentation into a network of micelle chains producing yogurt firmness and immobilizing water (4, 9, 20, 22, 40). Insufficient heating (or no heating) of milk results in yogurt with micelles that have coalesced into large clusters with reduced hydrophilic properties (9, 20, 23, 40). Yogurts with this type of microstructure are soft and suffer severe syneresis (23). The clusters of micelles form a three-dimensional network that is more
open than that from heated milk. Davies et al. (9) found a relationship between yogurt firmness, water-holding capacity, and degree of micelle coalescence. Weak bodied yogurts are composed of more coalesced, less hydrated micelles than firmer yogurt.

Mottar et al. (37) took the fusion/coalescence theories one step further. By identifying the proteins present in appendages of casein micelles, they were able to show that low intensity heat treatments result in filaments of β-LG on the micelle surface. These filaments are not only a barrier to coalescence (such as in unheated yogurt) but are also a barrier to micelle fusion, necessary for formation of the strong gel network. At higher heat intensities, α-LA also precipitates on the micelle, filling in the gaps from β-LG, enabling micelle fusion into chains during fermentation. Mottar et al. (37) concluded that α-LA deposition onto casein micelles (in addition to β-LG), plays an important role in the fusion and hydration of micelles, and the rheological properties of yogurt.

The presence of appendages around micelles of weak-bodied yogurts as a result of heat treatment is similar to that seen microstructurally as a result of fortification of milk for yogurt with WPC (34). High levels of flocculated protein surrounding the micelles, which interfered with micelle fusion, are found in the WPC-fortified yogurt. Parallel to the predominant micelle fusion in casein-fortified yogurts (34) is the higher gel strength and reduced syneresis found in sufficiently heated milks, which display smooth, fused micelles.
Little information can be derived from microstructural studies of yogurts prepared with different types of heat treatments. Davies et al. (9) found similar filamentous appendages around micelles heated 95°C for 10 min or autoclaved at 121.7°C for 15 min. Parnell-Clunies (40) found yogurts prepared by vat, HTST, or UHT heating of milk not too dissimilar in microstructural detail. Physical properties of such yogurts (discussed earlier) were quite different. No observable microstructural difference was seen due to increased residence time. Yogurt from UHT-treated milk was described as consisting of a casein-particle network connected by particle-to-particle attachment rather than micelle fusion (as seen in HTST- and vat-heated yogurts). "Particles" were referred to rather than micelles due to increased size (suggesting clusters of micelles).

Similarly, the yogurt produced from UHT-treated milk by Mottar et al. (37) also contained floccules formed by particle-to-particle attachment rather than micelle fusion. Conventional vat-heated milk resulted in yogurt with extensive micelle fusion. Milk heated by UHT, with comparable WPD to vat-heated milk, had a higher level of superficial filaments around micelles. The microstructural differences were suggested to explain the reduced firmness in the yogurt from UHT-treated milk. The spatial or chemical hindrance to micelle fusion, caused by the appendages, interfered with texture formation.

The microstructure of yogurt reflects gross differences in physical properties due to heat treatment or fortification methods. Microstructure appears
to be related to yogurt firmness, syneresis, and water-holding capacity. To optimize yogurt physical properties heat treatment must be sufficient to inhibit micelle coalescence during fermentation. Heat treatment should not be so severe that the deposition of whey proteins on the micelle surface interferes with micelle fusion into a chained network upon fermentation. Whether inhibition of micelle fusion is the result of too little intensity or too much intensity of heat treatment remains subject to debate.
CONCLUSIONS

Heat treatment of milk prior to fermentation into yogurt is necessary to promote optimum firmness and minimal syneresis. Heat treatment affects the chemical and physical nature of the yogurt gel, determines its ability to hold water, and is closely related to the firmness and viscosity of the product. The previous heat history of ingredients used to fortify milk for yogurt must be considered relative to assessing the adequacy of heat treatment. Undenatured forms of these ingredients tend to result in better yogurt physical properties upon receiving the heat treatment for yogurt.

Vat heating of milk at 80 to 85°C for 20 to 30 min or 90 to 95°C for 5 to 10 min appears to result in optimum firmness and minimal syneresis compared to other vat heat treatments. Continuous heating methods, such as HTST or UHT, result in greater WHC and potentially lower syneresis in yogurts, but gel strength is lower than that received from vat heating. Insufficient or excess heating results in weak-bodied yogurt with a tendency to synerese. If HTST heating methods are used, 98°C for at least 1.87 min should be used. Good organoleptic properties have been produced with this type of treatment. No recommendation for UHT processing of milk for yogurt is given as the physical properties produced from UHT-treated milks were inadequate compared to other heating methods. The question as to whether UHT treatment is insufficient or too severe remains unclear. Better properties are obtained by using temperatures less than or equal to 140°C. Reducing holding times at 149°C...
suggests that high-end UHT may be too severe, rather than too brief as suggested by some investigators.

Whey protein denaturation appears to be predictive of yogurt physical properties only within limited temperature ranges, which do not include UHT treatments. Differences in findings among investigators may be due to the unique temperature profile parameters of each heating system and the methodologies used to determine WPD. Most promising information includes measurements of denaturation of individual whey proteins as well as complexing ratios with casein. The evidence suggests that perhaps multiple denaturation-complexation events with dependence on temperature and composition occur.

The microstructure of yogurt yields much information about the effects of various heat treatments on milk prior to fermentation. Yogurts with fused casein micelles in a compact uniform matrix are firm, with minimal syneresis. Coarse, coalesced micelle matrices or those consisting of chains of micelles linked together by particle-to-particle attachment (with whey proteins in between) display weak gels with a tendency to synerese. Comparisons can be made between the microstructural effects of different heat treatments and the microstructural effects of casein- or whey-based fortifications of yogurt.

Optimization of the heat treatment of milk for yogurt represents a valuable "nonadditive" approach to enhancement of yogurt texture. Such optimization could enable reduced dependence on added expensive dairy solids or stabilizers.
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PART 3. IMPROVED YOGURT PHYSICAL PROPERTIES WITH ULTRAFILTRATION AND INTERMEDIATE-HIGH TEMPERATURE HEATING
ABSTRACT

I compared the physical properties of yogurts made from intermediate-high temperature, ultra-high temperature (UHT), and vat heat treatments of skim milk fortified to 5% protein by either ultrafiltration or addition of nonfat dry milk (NDM). Penetrometer gel strength and stirred viscosity at 21 d of yogurts made from ultrafiltered skim milk exceeded those of yogurts made from NDM-fortified skim milk, even though the NDM yogurts contained more solids (13.0 vs 11.4%). The maximum gel strength and viscosity of ultrafiltered and NDM yogurts occurred following intermediate-high temperature treatments of 100°C for 16 s, 110°C for 4 or 16 s, and 120°C for 4 s. The gel strength and viscosity of yogurts from ultrafiltered or NDM fortified skim milk following UHT treatment (140°C for 4 or 16 s) were inferior to those of yogurts made following intermediate-high temperature treatments or vat heating. Fortification by ultrafiltration, to fewer total solids (and without the use of stabilizers), resulted in yogurt with higher gel strength and viscosity than yogurt from NDM fortification. Yogurt prepared by intermediate-high temperature treatment had comparable or better gel strength and viscosity than yogurt prepared by traditional (vat) heating methods.
INTRODUCTION

Increasing the total solids in yogurt milk and proper heat treatment before fermentation are common to yogurt manufacture around the world (12, 17, 28, 35, 36). These practices optimize yogurt physical properties, minimize the seasonal variations in milk composition, and reduce the formula cost (29, 35).

Although legal standards usually dictate the minimum percentage total solids in yogurts, processors may choose the types and amount of fortification used to achieve a desired consistency, flavor, and aroma (35). Processors can concentrate milk fractions or add powdered dairy ingredients to fortify milk for yogurt (36). Concentration methods include evaporation, reverse osmosis, and ultrafiltration (UF). Dry fortification methods include addition of powdered whole milk, skim milk, buttermilk, caseinate, and whey or its derivatives, or combinations of the above (36).

Increasing the total solids in milk for yogurt increases the firmness and viscosity of yogurts (24, 26, 29, 30, 35, 36, 38). The viscosity associated with the various fortification methods depends on the protein content of the fortified milk (16, 35, 38).

Modler et al. (22) found that the gel firmness of yogurt stabilized with casein-based or whey-based proteins depends on the ratio of casein to non-casein protein in the yogurt milk. An unsatisfactory rough, coarse texture resulted when ratios of casein to non-casein protein are too high. The best enrichment involves an increase in both casein and whey protein.
Ultrafiltration of skim milk for yogurt increased not only the SNF, but also the protein content (casein and whey protein) as a percentage of milk SNF. Becker and Puhan (2) demonstrated that UF resulted in firmer nonfat yogurts with higher viscosity than those prepared to the same SNF content by either evaporation or addition of nonfat dry milk (NDM). The effect was more dramatic in samples with higher SNF.

Good quality yogurts may be produced from UF milks (1, 3, 4, 18, 20, 27, 31, 37) and this technique is commonly used to manufacture yogurt in Europe (13, 21). Ultrafiltration may make it possible to manufacture yogurt with the same viscosity and firmness as traditional fortification methods, but using milk that contains fewer total solids (1, 2, 13, 18). UF yogurts may also provide low lactose and lower calorie alternatives to traditional yogurt (18).

Heat treatment of the milk prior to fermentation also improves the viscosity and consistency of yogurt (24, 26). Legislation in many countries prohibiting addition of stabilizers and nonfat solids has encouraged research into “non-additive” methods such as heat treatment to optimize yogurt texture (21).

Plate exchange heating of milk for yogurt potentiates process improvements including standardized process control, improved sanitation, and energy and time savings (33). However ultra-high temperature (UHT) treatment of milk prior to fermentation generally results in yogurt that is less firm and less viscous than yogurt manufactured by conventional (vat) heating methods (19, 23, 25, 33).

Insufficient heating of milk for yogurt results in a weak bodied, thin
product, with a tendency to synerese (10, 15, 32, 33) as does excessive heating (6, 9, 11, 32, 33). Schmidt et al. (33) significantly improved yogurt firmness and viscosity by increasing holding time at 138°C (from 3 to 6 s), to the point that it was rated acceptable by taste panel. However, UHT treatment of milk at 149°C produces weak bodied yogurt. Labropoulos et al. (19) found that at 149°C, gels produced after a holding time of 3.3 s were firmer than those produced at 5.2, 8.2, and 13.7 s.

Within defined temperature ranges, adequacy of heat treatment related to firmness and viscosity of yogurt correlates with the extent of β-lactoglobulin denaturation (6, 25, 26, 30). This relationship has been used to explain why yogurt made from UHT treated milk generally has a weaker body (6, 10). Interactions between casein and denatured β-lactoglobulin may be responsible for the texture of yogurt (6). Dannenberg and Kessler (6) demonstrated that yogurt firmness generally increases with the extent of denaturation of β-lactoglobulin; however, firmness decreases slightly when denaturation is 99%. Increasing the heat treatment holding time to 2.5 times that required to denature 99% of the β-lactoglobulin results in reduced firmness in the yogurt gel. Dannenberg and Kessler (6) found that heat treatment of milk at temperatures above 120°C also reduced yogurt firmness.

Recently, Mottar et al. (23) demonstrated through immunochemical electron microscopy that depending on the heating process for yogurt, a portion of the β-lactoglobulin and α-lactalbumin became bound to the casein. The extent of WPD varies between direct and indirect industrial UHT treatment. Denaturation
from indirect heating approached the level of denaturation found in the control (vat-heated 90°C for 10 min) milk. However, UHT yogurts were less viscous and had lower hardness compared to the control. Hardness and viscosity are not simply related to the extent of WPD. Mottar et al. (23) found that at lower heat intensities β-LG denatures into appendage-like structures on the micellar surface. These structures may impede micelle fusion, resulting in inferior texture. At higher heat intensities α-LA also precipitates, filling in the gaps, to create a smooth micellar structure that enabled particle fusion rather than particle-to-particle attachment. The enhanced particle fusion may promote hydration and enhance yogurt texture.

UF concentration of milk for yogurt results in a firmer coagulum with fewer total solids needed to achieve the same textural properties as conventional (NDM) fortification (8). UHT treatment of milk for yogurt results in a weaker coagulum, but with less syneresis than with conventional (vat) heat treatment (7). Heat treatments lower than UHT but higher than with HTST produces higher yogurt gel strength and viscosity in skim milk, 1% fat milk, and UF yogurts (12.5 and 15% total solids) compared to UHT yogurts from the same milks (7, 8). This study investigated whether UF and UHT technologies could be combined to enhance gel strength and viscosity without deleteriously affecting syneresis of yogurts standardized to 5% protein. Several studies have emphasized either high-end UHT (138 to 149°C) or high-temperature-short-time (HTST 90 to 98°C) treatments. This study evaluated the effects of intermediate-high temperatures (100 to 130°C)(IHT) which are between UHT and HTST ranges.
MATERIALS AND METHODS

Milk Processing and Yogurt Preparation

Raw milk from the Utah State University dairy farm was collected and separated at 4°C. Skim milk was fortified to 5% protein by adding NDM and was vat pasteurized at 63°C for 30 min. Skim milk was also vat pasteurized and then fortified to 5% protein by UF via a spiral wound (5,000 mw cutoff) membrane system (Osmonics, Inc., Minnetonka MN). A separate batch of skim milk (not fortified) was vat pasteurized at 63°C for 30 min to serve as a control.

The skim milk, UF skim milk, and NDM-fortified skim milk were processed at IHT and UHT temperatures in preparation for yogurt manufacture. Milk was heated with an indirect plate exchange system (Alfa-Laval Sterilab®, Prairie View WI). Milk was pre-heated from 77 to 80°C prior to final heating at 100, 110, 120, 130, or 140°C for 4 or 16 s. Different holding tubes were used to obtain different residence times. Milk was homogenized following cooling to 55°C, via an in-line homogenizer at 145 kg/cm² first stage, 36 kg/cm² second stage pressures. After final cooling, samples were collected at 15 to 20°C.

To compare effects of these heat treatments to those of conventional (vat) heating methods, separate quantities of pasteurized skim milk, UF skim milk, and NDM-fortified skim milk were homogenized at 55°C and vat-heated in milk cans at 82°C for 20 min (come-up and come-down times were approximately 15 min).

Commercial yogurt culture (CH-1 from Chr. Hansen Laboratory, Inc., Milwaukee WI) was used at a 1% (w/w) level to inoculate aliquots of each type
of heat-treated milk. Each aliquot was mixed thoroughly and then approximately 100 ml was added to five sterile sample cups. One of the cups from each treatment was used to monitor pH over time. The yogurt cups were incubated quiescently at 37°C to pH 4.9, then transferred to 4°C storage for 21 d.

Physical Properties Measurements

Relative gel strength was measured by a penetrometer manufactured to permit descent of different probes at various speeds into yogurt samples (14). An analytical balance served as the strain gauge for the instrument (Figure 1). The 100 ml yogurt samples in open cups at 4°C were tared on the analytical balance. An electric motor simultaneously turned threaded shafts to lower the probe platform. A 15.5 mm diameter cylindrical probe was lowered at 10 mm per min into the yogurt samples. Gel strength was defined as the first point at which the balance reading decreased following a steady increase in readings. For almost all samples, this reading was taken between 20 and 40 s following probe contact with the sample surface. In the rare case that no break was observed, a reading was taken at 60 s following probe contact. Four samples from each treatment were measured after 21 d storage.

To examine profiles of gel strength as the probe force was applied, a video camcorder was used to capture balance readings every s for 60 s.

Following gel strength measurement, the stirred viscosity was measured on the same sample according to the method of Schmidt et al.(33). A Brookfield
Figure 1. Schematic diagram of penetrometer. 1, threaded shafts; 2, probe platform; 3, probe; 4, sample; 5, analytical balance; 6, speed control; 7, direction (up/down) switch; 8, motor and chain drive.
(Stoughton MA) synchrolectric viscometer Model LVT was used with a helipath stand. T-bar spindles traveled at 3 rpm within a range of 19 mm, 13 mm from the top and bottom of the sample in the cup. Samples were stirred for 30 s with a spatula (19 mm wide) before measuring viscosity. Readings were taken every 30 s. An average of four readings was calculated for each sample. Four samples were measured for each treatment. All samples were measured at 10 ±1°C. Total solids were measured by microwave method (CEM Corporation).

Statistical Analysis

Experiments were replicated twice. A split plot design was used in which skim milk lot, based on each type of fortification method, was the whole plot. Because it was not possible to achieve exactly the same pH across all 264 sample cups, analysis of covariance procedures were applied to the data with pH as the covariate. Using analysis of covariance, the response variable mean was adjusted to reflect a common level of covariate to enable valid comparisons between treatment means. The applicable linear model with a single covariate was:

\[ Y_{ijk} = \mu + \rho X_{ijk} + R_i + M_j + \epsilon_{ij} + T_k + MT_{jk} + \delta_{ijk} \]

where:

- \( \mu \) = overall mean
- \( X_{ijk} \) = covariate (pH)
- \( \rho \) = slope of the covariate
- \( R_i \) = \( i^{th} \) replicate
A second analysis of covariance was performed in which the effect of heat treatment ($T_k$) was divided into the effect of temperature, the effect of residence time, and the interaction between temperature and residence time. Data from vat treatments were removed from the data set for this comparison, to create a balanced factorial of temperature by time.

All reported means were adjusted for covariance by Minitab (Minitab Statistical Software, State College PA). Treatment means were separated using Fisher's protected LSD (5), adjusted for use with covariance (34).
RESULTS AND DISCUSSION

Typical gel strength profiles are illustrated by several representative samples in Figure 2. Gel strength usually reached a maximum (recorded value) before rupturing between 20 and 40 s after the probe contacted the yogurt sample. Although some samples showed a second peak within the 60 s of measurement, the peak usually only approached that received in the first rupture of the gel.

Adjusted mean gel strength and viscosity were greater (for all heat treatments) for yogurts made from ultrafiltered skim milk than those from skim milk fortified with NDM (Figure 3). All UF and NDM yogurts had greater gel strength and viscosity than the skim milk yogurts from corresponding vat, IHT, and UHT treatments. The overall adjusted mean gel strengths were 48.4, 29.1, and 16.4 g for UF, NDM, and skim milk yogurts. Adjusted overall mean viscosities were $2.04 \times 10^4$, $9.36 \times 10^3$, and $3.98 \times 10^3$ cp for UF, NDM, and skim milk yogurts. The greater gel strength and viscosity of UF yogurts occurred despite the higher average total solids in the NDM yogurts (13.0% vs 11.4%). Skim milk yogurts contained 9.2% average total solids.

Both UF and NDM-fortified skim milk yogurts had maximum adjusted mean gel strength among yogurt samples from the IHT treatments 100°C for 16 s; 110°C for 4 or 16 s; and 120°C for 4 s. Adjusted mean gel strength of yogurts from 100, 110, and 120°C IHT treatments of UF skim milk were significantly greater ($P < .05$) than those of UHT treatments (140°C for 4 or 16
Figure 2. Typical gel strength profiles of skim milk, UF skim milk, and NDM fortified skim milk yogurts prepared by various heat treatments of the yogurt milk. A. UF skim milk heated 110°C for 16 s. B. UF skim milk heated 130°C for 16 s. C. NDM fortified skim milk vat heated 82°C for 20 min. D. NDM fortified skim milk heated 110°C for 16 s. E. NDM fortified skim milk heated 130°C for 4 s. F. Skim milk vat heated 82°C for 20 min. G. Skim milk heated 130°C for 16 s. A 15.5 mm diameter probe was run at 10 mm per min into cold (4°C) samples.
Figure 3. Adjusted gel strength means of skim milk, UF skim milk, and NDM fortified skim milk yogurts with respect to heat treatment. Plate exchange heat treatment was for 4 or 16 s (pooled, n=16), vat heat treatment was for 20 min at 82°C (n=8). Adjusted means (bars) only within the same fortified milk (row) labeled with the same lower-case letter are not significantly different (P < .5).
s). IHT processing (100, 110, 120, and 130°C) of UF skim milk resulted in yogurt gel strength that was not significantly different from that received from traditional vat heat treatment. The gel strength of yogurts from NDM-fortified skim milk heated to 100 and 110°C (for 4 or 16 s) was significantly greater ($P < .05$) than that of UHT treated NDM yogurts. Except for 110°C treatment, the gel strength means of NDM-fortified yogurts were significantly lower ($P < .05$) than for vat-heated NDM fortified yogurt. Gel strength increased with holding time at 100°C, but increasing holding time of UHT treatment had a deleterious effect, though difference in gel strength due to residence time was not statistically significant overall. Because residence time did not significantly affect gel strength, both residence times were pooled within temperatures in Figures 3 and 4. The gel strengths of IHT and UHT skim milk yogurts were generally lower than that for vat-heated skim milk yogurt. UHT treatment (140°C for 4 or 16 s) reduced skim milk yogurt gel strength compared to all other skim milk treatments, though this difference was not statistically significant at $\alpha = .05$.

The effect of IHT treatment on the viscosity of UF and NDM yogurts was similar to that of gel strength. UHT treatment (140°C for 4 and particularly 16 s) significantly reduced ($P < .05$) viscosity of UF and NDM yogurts compared to IHT treatments. Viscosities in UF/IHT yogurts were not significantly different from that of the vat-heated UF yogurt. The viscosities of NDM/IHT yogurts were not significantly different from those of vat-heated NDM controls.
Gel strength and viscosity were highly correlated in this study ($R^2 = .96$) as indicated in Figure 5. The equation describing the relationship was:

\[
\text{viscosity} = -4009.9 + 487.78 \text{ (gel strength)} \\
-4.01 \times 10^3 + 488 \text{ (gel strength)}
\]
Figure 4. Adjusted viscosity means of skim milk, UF skim milk, and NDM fortified skim milk yogurts with respect to heat treatment. Plate exchange heat treatment was for 4 or 16 s (pooled, n = 16), vat heat treatment was for 20 min at 82°C (n=8). Adjusted means (bars) only within the same fortified milk (row) labeled with the same lower-case letter are not significantly different ($P < .5$).
Figure 5. Correlation of adjusted viscosity means to adjusted gel strength means ($R^2 = .96$) for skim milk, •; NDM fortified skim milk, ▲; and UF skim milk yogurts, ○.
CONCLUSIONS

The reduction of gel strength and viscosity of yogurts associated with UHT treatment of milk (compared to vat heating) is consistent with other reports (7, 8, 19, 25, 33). This was the first study to examine IHT treatment of milk (standardized to 5% protein) for yogurt. The increased gel strength and viscosity associated with IHT treatment are in agreement with the results of previous studies in this laboratory related to IHT treatment of skim milk, 1% fat milk, and skim milk ultrafiltered to 12.5 or 15% total solids (7, 8). I conclude that IHT treatment can result in yogurt of adequate gel strength and viscosity, and may have potential for use when yogurt manufacturers desire continuous heating methods. Combining UF of skim milk with IHT treatment provides the processor with yogurt physical properties that compete favorably with traditional methods (NDM fortification and vat heating). With these techniques greater quality and economic advantage can be achieved using milk containing lower total solids and without the use of stabilizers. IHT and UF technologies may also provide nutritional (low calorie) advantages over traditional methods of yogurt manufacture.
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PART 4. REDUCED YOGURT SYNERESIS WITH ULTRAFILTRATION AND INTERMEDIATE-HIGH TEMPERATURE HEATING
ABSTRACT

I compared syneresis and water-holding capacity of yogurts prepared from skim milks that had been fortified to 5% protein by either ultrafiltration or the addition of nonfat dry milk, and exposed to intermediate-high temperature (100, 110, 120, and 130°C for 4 or 16 sec), ultra-high temperature (140°C for 4 or 16 sec), or vat heating (82°C for 20 min). Whey protein denaturation increased with the temperature and residence time by indirect plate heat exchange and was highest in vat-heated milks. Whey protein denaturation and yogurt water holding capacity increased with protein levels in the fortified milks compared to skim milk. The water-holding capacity of yogurts from fortified milks treated at intermediate-high temperatures was comparable to that of yogurts from vat-heated milks. In ultrafiltered skim milk yogurts, there was significantly less syneresis associated with intermediate-high temperature treatments (100, 110, 120, and 130°C) than in milk heated to 140°C or vat-heated milk (82°C for 20 min) even though there was significantly lower whey protein denaturation in the former treatments. Syneresis was reduced to less than .2% and water-holding capacity was comparable to that associated with vat heating in yogurts from milk containing lower total solids and no stabilizer, following ultrafiltration and intermediate-high temperature heating.
INTRODUCTION

Syneresis is commonly regarded as a defect which negatively affects consumer perception of the appearance of set-style yogurt (35). A desirable yogurt gel structure is firm with minimal syneresis (32). Syneresis has been related to the factors (listed below) which are critical to proper formation of the yogurt gel (12). Casein micelles are linked in chains and clusters (14) through a combination of heat-, calcium-, and acid-induced reactions (32); the resulting three-dimensional matrix forms the yogurt gel. The extent of immobilization of the liquid phase within the yogurt gel matrix largely depends on the total solids content (3, 11, 12, 35), the concentration of calcium and fat (12), homogenization (28, 30), and the preheat treatment (12, 14, 21, 25, 33).

Manufacturers routinely add stabilizers and thickening agents such as pregelatinized starch, alginates, gelatin, etc. to facilitate immobilization of the liquid (15, 20, 28). However, flavor problems associated with stabilizers (20) and consumer pressure for "all natural" yogurt have prompted continued research into "non-additive" stabilization (19).

Research concerning the reduction of syneresis has involved various methods of fortification of milk for yogurt (1, 3, 11, 12, 20), with particular attention to protein levels (30). Milk for yogurt has traditionally been fortified with nonfat dry milk (NDM) to reduce syneresis and improve the textural properties of the cultured product. But when greater than 2% NDM is added, saltiness, powder flavor, and coarseness in the end product may result (30). Yogurt made
with reconstituted roller-dried NDM (severe heat treatment) has poor flavor and texture, and the yogurt gel is more susceptible to syneresis than yogurt made with freeze-dried (undenatured) NDM (14). Addition of more than 1% whey powder may result in a "whey" flavor. Yogurt made from skim milk with added whey protein concentrate produces softer, weaker gels that are more susceptible to syneresis than yogurts made from casein-based ingredients (20). Sodium caseinate is highly effective in reducing syneresis but produces a yogurt with a coarser texture than gelatin-stabilized yogurt. Fortification with higher levels of milk protein concentrate and skim milk powder also results in undesirable texture. A casein to non-casein ratio of about 3.3 to 1.0 (as with NDM fortification, evaporative concentration, or concentration by ultrafiltration) produces good yogurt (19). The ideal system of enrichment may involve increasing both casein and whey proteins.

Ultrafiltration (UF) to enrich yogurt milk protein content increases the concentrations of both the caseins and whey proteins and does not subject solids to previous harsh (denaturing) heat treatment (such as with powders) (3, 30). UF has been used to fortify yogurt (1, 3, 4, 5, 9, 10) with qualities, particularly syneresis, as good as or superior to commercial yogurts (16, 29). UF may make it possible to manufacture high viscosity yogurt with lower total solids, yet without a tendency to synerese (13).

Many researchers have sought to optimize heat treatment to minimize syneresis and enhance yogurt physical properties (9, 25, 31). Ultra-high temperature (UHT) treatment of milk for yogurt has been studied due to the
advantages it may offer in process control, sanitation, energy usage, and time savings compared to conventional batch heating (31). Conventional vat-heated and HTST yogurts have higher firmness and viscosity but lower water-holding capacity (WHC) and are more prone to syneresis than UHT yogurts (25, 27, 31).

Heat-induced reduction of syneresis and other rheological properties of yogurt have been attributed to whey protein denaturation (WPD) (6, 7, 22, 26, 28) though other studies have not found any relationship (11, 17). Dannenberg and Kessler (6) correlated denaturation of β-lactoglobulin (β-LG) and syneresis, but syneresis was independent of temperature. Through immunogold labeling studies, Mottar et al. (22) concluded that denaturation of not only β-LG, but also α-lactalbumin (α-LA) onto the micelle surface is critical to the rheological properties of yogurt. They suggested that different heat treatments (i.e., vat versus UHT) might result in different behavior of the whey proteins. Puhan (28) suggested that lower WPD in UHT-treated milks might explain the lower consistency and viscosity of yogurt made from such milk. However, this does not explain the reduced syneresis of UHT yogurts by Schmidt et al. (31). In general, less WPD may be required to achieve satisfactory properties in higher solids yogurt (28), and higher levels of solids may actually confer protection from WPD (18, 23). Optimum viscosity can be achieved in high solids mixes without complete WPD.

In previous reports from this laboratory (8, 9, 10) intermediate-high temperature (IHT) treatment (temperatures between HTST and UHT) unexpectedly resulted in yogurt with gel strength and viscosity matching that of
yogurt from vat-heated standardized milks. UHT treatment resulted in yogurt with inferior gel strength and viscosity compared to yogurt made from milk subjected to intermediate-high temperature (IHT) treatment or vat heating. This report concerns the effects of IHT treatment on yogurt syneresis, especially when applied in combination with ultrafiltration to fortify milk. The ability to concentrate both casein and whey protein, in the ratio normally found in milk, without denaturation, is afforded by UF. Lactose concentration is not increased by UF; thus protein increases as a percentage of total solids. These features, plus the desirable rheological properties from UF yogurts, may make UF complementary to IHT treatment in terms of potentially improving yogurt physical properties and reducing syneresis.
MATERIALS AND METHODS

Milk Processing and Yogurt Preparation

Raw milk from the Utah State University dairy farm was collected and separated at 4°C. Skim milk was fortified to 5% protein by adding NDM and was vat pasteurized at 63°C for 30 min. Skim milk was also vat pasteurized and then fortified to 5% protein by UF via a spiral wound (10,000 mol wt cutoff) membrane system (Osmonics, Inc., Minnetonka MN). A separate batch of skim milk (not fortified) was vat pasteurized at 63°C for 30 min to serve as a control.

Skim milk, UF skim milk, and NDM-fortified skim milk were processed at IHT and UHT temperatures. Milk was heated with an indirect plate-exchange system (Alfa-Laval Sterilab®, Prairie View WI). Milk was pre-heated to 77 to 80°C prior to final heating at 100, 110, 120, 130, or 140°C for 4 or 16 s. Different holding tubes were used to obtain different residence times. Milk was homogenized following cooling to 55°C, via an in-line homogenizer at 145 kg/cm² for the first stage, 36 kg/cm² for the second stage pressures. After final cooling, samples were collected at 15 to 20°C.

For comparison of the effects of these heat treatments to those of conventional (vat) heating methods, separate quantities of pasteurized skim milk, UF skim milk, and NDM-fortified skim milk were homogenized at 55°C and vat-heated in milk cans at 82°C for 20 min.

Commercial yogurt culture (CH-1 from Chr. Hansen Laboratory, Inc., Milwaukee WI) was used at a 1% (w/w) level to inoculate aliquots of each type
of heat-treated milk. Each aliquot was mixed thoroughly and approximately 100 ml of each type of heated milk was added to five sterile sample cups. One cup from each treatment was used to monitor pH over time. The yogurt cups were incubated quiescently at 37°C to pH 4.9, then transferred to 4°C for 21 d of storage.

**Whey Protein Denaturation**

Samples of the heated milks from all temperatures and holding times were diluted 1:4 and brought to pH 4.60 ± .05 with .1 N HCl. The precipitated casein was removed by filtration with #42 Whatman filter paper. The filtrate was tested for nitrogen content by the semi-micro Kjeldahl method (2). Soluble nitrogen at pH 4.6 was subtracted from calculated total soluble pH 4.6 nitrogen of raw (unheated) milks and the difference was expressed as a percent of unheated soluble pH 4.6 nitrogen. Loss of solubility at pH 4.6 was used as a relative index of WPD (17). Two samples were measured for each treatment of each replicate. Four samples were measured for vat heat treatment.

**Physical Properties Measurements**

Syneresis of yogurts was measured at 21 d by the method of Schmidt et al. (31). Cold (4°C) samples were inclined 90° and whey was collected by aspiration. The weight of whey was expressed as a percent of sample weight to yield percent syneresis. Four samples were measured for each heat treatment of each fortified milk for each of two replications.

Water-holding capacity was determined on yogurt samples at 21 d by the
method of Parnell-Clunies et al. (27). Approximately 40 g of yogurt was weighed into centrifuge tubes. Samples were centrifuged at 13,500 x g for 30 min at 10°C. Supernatants were immediately decanted, and pellets were inverted and allowed to drain for 10 min. The resultant pellet weight was expressed as a percent of sample weight as an index of WHC. Two samples were measured for each heat treatment of each fortified milk for each of two replications.

Statistical Analysis

Experiments were replicated twice. In the split plot design, skim milk from each type of fortification method was the whole plot. Because it was not possible to achieve exactly the same pH across all 264 sample cups, an analysis of covariance was applied to the data with pH as the covariate (24). Using analysis of covariance, the response variable mean was adjusted to reflect a common level of covariate so that valid comparisons could be made among treatment means. The applicable linear model with a single covariate was:

\[
Y_{ijk} = \mu + \rho X_{ijk} + R_i + M_j + \epsilon_{ij} + T_k + M_T_{jk} + \delta_{ijk}
\]

where:

\( \mu \) = overall mean

\( X_{ijk} \) = covariate (pH)

\( \rho \) = slope of the covariate

\( R_i \) = i\textsuperscript{th} replicate

\( M_j \) = j\textsuperscript{th} milk fortification method

\( \epsilon_{ij} \) = experimental wholeplot error
A second analysis of covariance was performed whereby the effect of heat treatment ($T_k$) was subdivided into the effect of temperature, the effect of residence time, and the interaction between temperature and residence time. Vat-heated data were removed from the data set to enable a balanced factorial of temperature by time.

Parallel analysis of variance (with and without vat heating data) was conducted on the whey protein denaturation data. Covariance was not included because pH did not have an effect until after heat treatment (and fermentation).

The means for syneresis and WHC were adjusted for covariance by Minitab (Minitab Statistical Software, State College PA). Treatment means were separated using Fisher's protected LSD adjusted for use with covariance (34).
RESULTS AND DISCUSSION

Whey protein denaturation (Figure 6) increased with temperature for skim milk, UF skim milk, and NDM fortified skim milk ($P < .05$). Vat heating resulted in the highest level of WPD for all milks.

In UF and NDM-fortified skim milks, WPD was significantly ($P < .05$) lower from IHT treatments at 100, 110, and 120°C compared to 130, 140°C and vat heating. Skim milk showed significantly lower ($P < .05$) WPD from IHT treatments 100, 110, 120, and 130°C compared to UHT (140°C) or vat heating. In all cases, WPD from UHT treatment was not different from WPD from vat heating.

Increasing the holding time from 4 to 16 s at 100, 110, 120, 130, and 140°C increased whey protein denaturation ($P < .05$). Data for different holding times in Figure 6 are pooled within temperatures to compare physical properties. (Holding time did not significantly affect these characteristics.)

There was less WPD in NDM-fortified skim milk at all temperatures than in UF skim milk, though this difference could not be statistically analysed with this experimental design because of confounding of replicates with milk fortification. The split plot sacrifices precision in the wholeplot (skim milk lot of a particular fortification method) in order to elucidate differences at the subplot level (heat treatments within milks). The lower WPD in NDM-fortified skim milk might be due to a slight protective effect of the extra lactose from the NDM (23). Total solids content was 13.0% in the NDM-fortified skim milk, 11.4% in the UF
Figure 6. Whey protein denaturation of skim milk, UF skim milk, and NDM fortified skim milk with respect to heat treatment. Plate exchange heat treatment was for 4 or 16 s (pooled, n=8). Vat heat treatment was for 20 min at 82°C (n=8). Means of WPD (vertical bars) only within the same fortified milk (row) labeled with the same lower-case letter are not significantly different (P < .05).
skim milk, and 9.2% in the skim milk. Whey protein denaturation increased with the increased protein content in the NDM-fortified and UF skim milks compared to unfortified skim milk. This agrees with the findings of of Yousif (36).

At all heat treatments, the adjusted mean WHC of NDM-fortified and UF skim milk yogurts exceeded that of skim milk yogurts (Figure 7). The WHC of NDM-fortified skim milk yogurts was comparable to that of UF yogurt, even though NDM-fortified skim milk yogurts contained more total solids. The yogurt from NDM-fortified skim milk heated at 120°C had significantly ($P < .05$) greater adjusted mean WHC than that from 100 or 140°C treatments and no difference from vat heating. UF yogurts had greater WHC in 120 and 130°C treatments compared to 100, 110, or 140°C though this was not statistically significant at this level of replication.

Syneresis values (Figure 8) were higher for skim milk yogurts with 100, 110, 120, 130, and 140°C heat treatments compared to UF skim milk or NDM-fortified skim milk yogurts. Syneresis of yogurts manufactured from skim milk heated at 100, 110, or 120°C was significantly lower ($P < .05$) than that yogurt from UHT (140°C) treatment. Vat-heated skim milk yogurts had adjusted mean syneresis of .58% with "ab" significance letters (hidden on graph).

In UF skim milk yogurts, adjusted mean syneresis was significantly lower following treatment at 100, 110, 120, and 130°C than at 140°C or following vat heating. The reduced syneresis associated with fortified yogurts from IHT treatments (100, 110, and 120°C) occurred despite significantly lower ($P < .05$) WPD which suggests that WPD may not be the best index of yogurt properties.
Figure 7. Adjusted water holding capacity (WHC) means of skim milk, UF skim milk, and NDM fortified skim milk yogurts with respect to heat treatment. Plate exchange heat treatment was for 4 or 16 s (pooled, n=8). Vat heat treatment was for 20 min at 82°C (n=4). Adjusted means (vertical bars) only within the same fortified milk (row) labeled with the same lower-case letter are not significantly different (P < .05).
Figure 8. Adjusted syneresis means of skim milk, UF skim milk, and NDM fortified skim milk yogurts with respect to heat treatment. Plate exchange heat treatment was for 4 or 16 s (pooled, n=16). Vat heat treatment was for 20 min at 82°C (n=8). Adjusted means (vertical bars) only within the same fortified milk (row) labeled with the same lower-case letter are not significantly different (P < .05). Vat-heated skim milk yogurt had adjusted mean syneresis of .58% with "ab" significance letters (hidden).
Figure 9. Lack of correlation between mean level of whey protein denaturation and adjusted mean syneresis ($R^2 = .035$) of skim milk, •; ultrafiltered (UF) skim milk, ○; and NDM fortified skim milk yogurts, ▲.
Figure 9 shows that the adjusted mean syneresis was not correlated with whey protein denaturation ($R^2 = .035$). Although some whey protein denaturation is necessary to promote good textural properties in yogurt, simply increasing WPD (by UHT treatment, for example) did not reduce syneresis or increase WHC.

Gel strength and viscosity were greater following IHT treatments (100°C for 16 sec, 110°C for 4 or 16 sec, 120°C for 4 sec) than following UHT treatment (140°C) or treatment at other temperatures to either side of this range (8, 9, 10). IHT treatments resulted in yogurt gel strength and viscosity that were equal to or better than those of vat heating, especially when UF was used to fortify yogurt milks. The same treatments that resulted in highest gel strength and viscosity in UF yogurts also resulted in the lowest syneresis and with WHC comparable to yogurts with higher total solids (NDM fortified). IHT treatment and UF fortification of skim milk resulted in yogurt with optimum physical properties.
CONCLUSIONS

Percent whey protein denaturation did not determine the physical properties of yogurt. Highest denaturation, such as with UHT or vat heating, did reduce syneresis which was generally higher than in yogurts produced from IHT treatment. IHT, UHT, and vat heating apparently have different effects on whey proteins, resulting in yogurts with different physical properties at the same "measured" level of whey protein denaturation. Water-holding capacity was correlated with gross differences in syneresis of fortified (UF or NDM) versus unfortified skim milk yogurts; however, it did not explain differences in syneresis between IHT treatment, UHT treatment, or vat heating. Syneresis and WHC in yogurt made from milk ultrafiltered to 5% protein was similar to yogurt from milk fortified to percent protein with NDM that contained more total solids. I previously used ultrafiltration to produce yogurts with higher gel strength and viscosity than NDM-fortified yogurts (10). The physical properties of UF-fortified yogurts were as good or better than yogurt fortified with NDM. UF-fortified yogurt contained fewer total solids, thus potentially reducing costs and calorie content. Yogurt from IHT-treated milk had gel strength and viscosity that were equal to or better than that of yogurt from vat-heated milk. In addition, syneresis was improved without stabilizers. Combining IHT and UF technologies can optimize the physical properties of yogurt through non-additive means.
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GENERAL SUMMARY

Yogurt milks were prepared from skim milk fortified to 5% protein by UF or addition of NDM, or no fortification. Aliquots of each of the yogurt milks were heated at 100, 110, 120, 130, or 140°C for 4 or 16 s. Separate aliquots of each of the yogurt milks were vat-heated for comparison to those heated by indirect plate heat exchange. The heated milks were made into yogurts, and the yogurts were evaluated for physical properties at 21 d. The heated milks were analyzed for whey protein denaturation.

A split plot design was chosen because it was impractical (from a fortification and heating standpoint) to perform the experiments as a randomized block design. Expected mean squares for the split plot design with two factors (fortification method and heat treatment) are shown in Table 2 (Appendix).

Covariance procedures were additionally applied to the physical properties data (gel strength, viscosity, WHC, and syneresis) because there was significant variation in pH between repetitions (Table 3, Appendix). Use of this procedure required that there be no treatment effect on pH, which is evident from the Appendix.

Analysis of covariance for gel strength, viscosity, WHC, and syneresis can be seen in Tables 4, 5, 6, and 7 (Appendix). Significant heat treatment effects can be seen for gel strength, viscosity, and syneresis. Plate heat exchange temperature and holding time effects were delineated by a second analysis of covariance on the data set with vat-heated data excluded to enable a balanced
factorial (Tables 8, 9, 10, and 11, Appendix). Temperature had significant effects on gel strength, viscosity, and syneresis. Holding time had no significant effect on yogurt physical properties.

Analysis of variance for whey protein denaturation was performed in the split plot design (Table 12, Appendix). Covariance was not used because the effect of pH occurred after heat treatment (and fermentation). Heat treatment had a highly significant effect \( (P < .001) \) on whey protein denaturation. As was done for physical properties, a second analysis of variance was performed without the vat-heated data to determine the effect of plate heat exchange temperature and holding time in a balanced factorial (Table 13, Appendix). Both temperature and holding time produced highly significant effects \( (P < .001) \) on whey protein denaturation.

The fortification and heat treatment conditions that produced the highest gel strength also produced the highest viscosity and lowest syneresis. Measurements of WHC did not correlate with gel strength, viscosity, or syneresis. Whey protein denaturation, though clearly affected by heat treatment, was not predictive of yogurt physical properties. I would recommend use of IHT treatment (particularly 110°C for 16 s) in combination with fortification by ultrafiltration, to make yogurt. The temperature ranges defined as IHT in these studies (100 to 130°C) are close to high-end HTST ranges (95 to 98°C) referred to in the literature. Future studies should include extended holding times within HTST and IHT ranges.

Although not part of the original variables to be studied, the shelf life of
yogurts prepared by IHT treatment of milk was greatly enhanced. Samples that were stored at 4°C for greater than nine months showed no whey separation, flavor, or texture defects. This potential shelf life benefit of IHT treatment, in combination with routine pasteurization, merits further study.
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TABLE 2. Expected mean squares for covariance analysis of a two-factor experiment (milk and heat treatment).

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<td></td>
<td></td>
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<tr>
<td>Replications (Rep)</td>
<td>r-1</td>
<td>$\sigma_{\epsilon}^2 + at\sigma_{\beta}^2$</td>
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<tr>
<td>Milks</td>
<td>m-1</td>
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<td>$\sigma_{\epsilon}^2 + \sigma_{\alpha \beta}^2$</td>
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<td>Heat treatments</td>
<td>t-1</td>
<td>$\sigma_{\epsilon}^2 + ab\theta_T$</td>
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<td>Milk x Heat treatment</td>
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TABLE 3. Analysis of variance for pH with respect to repetition, milk fortification, and heat treatment.

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<td>.1833</td>
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*Elements of subplot error
TABLE 4. Analysis of covariance for gel strength with respect to repetition, milk fortification, and heat treatment, with pH as the covariate.

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*Elements of subplot error
TABLE 5. Analysis of covariance for viscosity with respect to repetition, milk fortification, and heat treatment, with pH as the covariate.

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*Elements of subplot error
TABLE 6. Analysis of covariance for water-holding capacity with respect to repetition, milk fortification, and heat treatment, with pH as the covariate.

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*Elements of subplot error
TABLE 7. Analysis of covariance for syneresis with respect to repetition, milk fortification, and heat treatment, with pH as the covariate.

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*Elements of subplot error
### TABLE 8. Analysis of covariance for gel strength with respect to repetition, milk fortification, plate heat exchange temperature, and holding time, with pH as the covariate. Data from yogurts made from vat-heated milks have been excluded to enable a balanced factorial.

<table>
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*Elements of subplot error
TABLE 9. Analysis of covariance for viscosity with respect to repetition, milk fortification, plate heat exchange temperature, and holding time, with pH as the covariate. Data from yogurts made from vat-heated milks have been excluded to enable a balanced factorial.

<table>
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*Elements of subplot error
TABLE 10. Analysis of covariance for water-holding capacity with respect to repetition, milk fortification, plate heat exchange temperature, and holding time, with pH as the covariate. Data from yogurts made from vat-heated milks have been excluded to enable a balanced factorial.

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*Elements of subplot error
TABLE 11. Analysis of covariance for syneresis with respect to repetition, milk fortification, plate heat exchange temperature, and holding time, with pH as the covariate. Data from yogurts made from vat-heated milk have been excluded to enable a balanced factorial.

<table>
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<td>4</td>
<td>2.15</td>
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<td></td>
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<tr>
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<td>15.68</td>
<td></td>
<td></td>
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<tr>
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*Elements of subplot error

<table>
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<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P</th>
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<tr>
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<td>8.10</td>
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<td>13.45</td>
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<td>25.97</td>
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*Elements of subplot error
**One degree of freedom was removed from each of these categories for two estimated values
TABLE 13. Analysis of covariance for whey protein denaturation with respect to repetition, milk fortification, plate heat exchange temperature, and holding time, with pH as the covariate. Data from yogurts made from vat-heated milk have been excluded to enable a balanced factorial.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
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<td>(Rep x Milk)</td>
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<td>1.19</td>
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<tr>
<td>Temp x Time</td>
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<td>Rep x Time*</td>
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<td>Rep x Temp x Time*</td>
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</table>

*Elements of subplot error

**One degree of freedom each of these categories for two estimated values
VITA

Richard Alan Dargan

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Properties of Low-fat Yogurt from Ultrafiltered and Ultra-high Temperature Treated Milk

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Ph.D. Utah State University, Nutrition and Food Sciences, 1992

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Abstracts and Publications:


Personal Data:

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