Improvement of textural properties of yogurt made from reduced-fat carabao milk by whey protein content adjustment and heat treatment of the milk

Janice C. Mariano¹, John Hennessy M. Merced¹, Abbie June F. Miranda¹, and Philipina A. Marcelo¹,²*

¹Department of Chemical Engineering, ²Research Center for the Natural and Applied Sciences
University of Santo Tomas, España Boulevard, 1015 Manila, Philippines

If caseins and whey proteins were present in appropriate proportion in milk, and the milk was heat-treated at temperatures well above the usual milk pasteurization temperatures, these components have the ability to form firm gel of uniform porosity through heat-induced protein-protein interactions. In this study, the whey protein-to-casein ratio (by mass) in reduced-fat carabao milk was adjusted from its natural ratio to 0.25 and 0.35 by the addition of whey protein isolate (WPI). The milk samples were then heat-treated at 80°C for 25 min. The heat-treated milk samples were used in yogurt-making, and the viscosity evolution during acidification with starter culture inoculation was monitored to determine and compare gel setting times. Compression, puncture and syneresis tests were done on the yogurt samples to determine the effects of proteins ratio adjustment and heat treatment on the texture of the yogurt samples. It was found that the milk with 0.25 whey protein-to-casein ratio exhibited the most satisfactory results in all the tests. This suggests that the combination of proteins ratio adjustment and heat treatment used led to textural improvement of the yogurt, and may enable the production of set yogurt from reduced-fat carabao milk without using carbohydrates-based stabilizers and texture enhancers.

Keywords: yogurt, carabao milk, whey protein, whey protein isolate, casein, viscosity

INTRODUCTION

Protein enrichment and heat treatment are important in controlling the consistency and textural properties of yogurt [1]. Heat treatment is done to improve the keeping quality of milk by deactivating microorganisms. Subjecting milk to heat treatment primarily affects the behavior of milk proteins — caseins and serum proteins — in terms of conformational changes they undergo. During heat treatment, no noticeable heat induced effects can be observed on the structure of casein micelles in the temperature range of 70–100°C [2]. However, heating has a definite effect on the serum, or whey, protein fraction of milk, which is primarily
composed of β-Lactoglobulin (β-Lg) and α-Lactalbumin (α-La). During heating, the reactive thiol group is exposed in β-Lg due to conformational changes of the molecule [3]. This reactive thiol group can form links with other proteins having a reactive thiol group, or through thiol group-disulfide exchange. The reaction between thiol groups makes the denaturation process irreversible [4]. The process of denaturation and subsequent aggregation of β-Lg constitutes the polymerization process, in which the unfolding of the globular protein represents the initiation step [5]. β-Lactoglobulin also exhibits interactions with casein micelle thiol group-disulfide bridge exchange reaction through κ-caseins, which are located at the casein micelle surface. The initial step of this process is believed to be physical in nature, but the final interaction is covalent (i.e., disulfide linked) [6]. As a result, the “hairy strands” on the casein micelle surface contain casein associated with whey proteins after heating. Incorporating heat treatment in milk preparation, therefore, results in a complex mixture of native whey proteins, whey protein aggregates and casein micelle covered with whey protein [7].

Yogurt is made by the acidification of milk through lactic acid bacteria (LAB). During fermentation, LAB transforms milk sugar (lactose) into lactic acid. The lowering of pH due to milk acidification promotes the process of casein coagulation [8]. Casein micelle begins to destabilize at pH 5.3 where colloidal calcium phosphate (CCP), which supports the casein micelle structure by binding the casein submicelles together, leaches into the serum phase, setting a condition that favors micellar aggregation [9, 10]. Complete precipitation of casein occurs at its isoelectric point of pH 4.6. The precipitated casein complex constitutes the yogurt curd. The yogurt matrix formed was believed to exhibit much favorable consistency and textural properties if the matrix was primarily made up of κ-casein-β-Lg aggregate [11].

This study aims to exploit the increased denaturation of whey proteins by elevating both the heat treatment temperature and the whey protein to casein ratio to improve yogurt texture. Carabao (Bubalus bubalis carabanesis) milk with reduced fat content was used in the study. The whey protein-to-casein ratio in the milk was adjusted from the natural ratio in milk samples to 0.25 and 0.35 by the addition of whey protein isolate (WPI). The milk samples were then heat-treated to a temperature moderately higher than the pasteurization temperature of 80°C, for 25 min. According to Dannenberg and Kessler [12] more extensive whey protein denaturation occurs at higher temperatures. However, cases of prolonged heating at high temperature and excessive protein addition in yogurts result in a grainy consistency of the product [1]. This kind of yogurt texture is a result of vigorous and uncontrolled protein-protein interactions where large protein aggregates that separate from the yogurt gel network are formed. The challenge, therefore, is to find the suitable proteins ratio and heat treatment conditions.

The results of this study could lead to textural properties improvement of yogurt made from carabao’s milk. Texture is one of the factors that consumers consider in buying food products, thus improvement of the texture of a nutritious product, such as yogurt, is a step in making the product a more attractive and commercially viable vehicle to deliver nutrients through the human diet. Buffalo milk, which was found to contain more caseins and minerals than cow’s milk is potentially a good raw material for this purpose [13]. The
utilization of carabao’s milk, which is the milk from Philippine buffalo, for dairy product development and manufacture will not only result in a wide range of nutritious choices for consumers but will also help in achieving high and sustained productivity in the local dairy industry.

**Experimental**

**Materials**

Pasteurized whole carabao milk (DVF Dairy Farm, Talavera, Nueva Ecija, Philippines) was purchased from a local retailer and the WPI was NZMP, Alacen 894, with 2% (w/w) moisture and 90% (w/w, dry basis) protein, which was a gift from Fonterra Philippines (Pasig City, Philippines). The starter culture was bought from Danasia Philippines (Makati, Philippines).

**Skimming of milk**

The pasteurized carabao milk was skimmed using the Ultra 8V-2 Centrifuge (LW Scientific, GA, U.S.A.) at 3300 rpm for 30 min, following the method developed by Hendrix and Reyes [14]. After centrifugation, the fat accumulated on the surface was removed using a small plastic spatula. For more effective skimming, the milk was centrifuged for another 10 min and the remaining fat skimmed off. Three batches of milk were skimmed to produce reduced-fat samples, which were used in the experiments. Representatives of the reduced-fat milk, together with the whole milk samples were sent to a private laboratory for chemical analyses.

**Compositional analysis of milk**

Compositional analyses, which included the total solids, fat, lactose, total nitrogen, non-protein and non-casein nitrogen contents, were determined for both the pasteurized whole and skimmed carabao milk samples by the First Analytical Services & Technical Cooperative (F.A.S.T) Laboratories (Cubao, Quezon City, Philippines).

**Preparation of milk samples**

Based on the compositional analysis of the reduced-fat carabao milk, the amount of WPI powder to be added that corresponded to the desired whey protein-casein ratio in the milk was computed. The following equations were used:

\[
WP_{\text{from milk}} = \text{weight of milk} \times (\text{NCN} - \text{NPN}) \times 6.38
\]

\[
\text{Whey protein to casein ratio} = \frac{(WP_{\text{from milk}} + WP_{\text{from WPI}})}{(\text{Weight of milk} \times (\text{TN} - \text{NCN})) \times 6.38)
\]

where:  
- WP = whey protein  
- TN = total nitrogen  
- CN = non-casein nitrogen  
- NPN = non-protein nitrogen  
- 6.38 = constant used in converting %nitrogen to %protein

The WPI powder was dispersed in the milk by gentle stirring for 90 min using PC-620 hotplate/magnetic stirrer (Corning Inc., NY, U.S.A.). The mixture was then allowed to equilibrate by storing overnight in the refrigerator at 4°C. The equilibrated milk solutions were used in the subsequent parts of the experiment.

**Standardization of milk pH**

The pH of the milk was measured before incubation and adjusted to 6.8 ± 0.02 as necessary for pH standardization. This ensured that the rate of pH decline due to the addition of LAB was approximately uniform in the milk samples.

**Preparation of yogurt**

A batch of pasteurized whole milk (blank) and reduced-fat carabao milk with whey protein-to-casein ratios adjusted to 0.25
and 0.35 were prepared as previously described. Following the method developed by Mesina et al. [15], the milk samples were then heated using a water bath at 80°C with constant stirring for 25 min. After heating, the milk samples were immediately cooled using a cold bath to the starter culture inoculation temperature of 45°C. The freeze-dried starter culture was then added to the milk with an approximate weight of 0.04 g per batch. The product was stirred gently for 10 min to ensure uniform distribution of the culture. The milk sample mixtures were distributed in 20 mL sterilized containers and placed in an incubator (Binder Inc., Tuttlingen, Germany) for about 12 h at a temperature of 45°C. After fermentation, the yogurts produced were stored in a cooler at about 4°C. They were then used in the subsequent parts of the experiments. Three batches of milk samples were studied.

**Yogurt characterization**

The ability of heat-denatured whey protein to improve yogurt texture was verified by comparing the viscosity evolution of milk samples as they set to yogurt gels, the strength and firmness of the yogurt gels that are related to sensory quality, and the percent syneresis from the yogurt at specific time intervals.

**Viscosity test.** The viscosity test was done to determine the time duration of the milk samples to set to yogurt gels, and to investigate the effects of heat treatment parameters and whey protein-to-casein ratio on the gel setting behavior of the milk. The whey proteins in the milk solution was polymerized and allowed to interact with the casein micelles by heat treatment and stored at 4°C for 12 h. LAB was then added at 45°C. The milk was set up in a water bath with the temperature maintained at 45°C. Continuous viscosity measurement after the addition of LAB bacteria was carried out using the Brookfield LVT viscometer (Brookfield, MA, U.S.A.) and the viscosity reading was recorded until a constant reading was achieved, which was deemed the onset of milk gelation.

**Compressibility test.** The compressibility test was done to estimate and compare the strength of the yogurt gel samples. A flat round disk was placed on top of the yogurt sample with the same cross-sectional area as the disk. Pressure was applied to compress the yogurt by adding weights in small increments on the disk until the yogurt collapsed. The total mass of the disk and the weight added were recorded, and the pressure computed by:

\[
p = \frac{F}{A}
\]

where:
- \(P\) = pressure applied on the yogurt sample, Pa
- \(F\) = (mass of the disk + mass of added weights) × acceleration due to gravity, N
- \(A\) = cross-sectional area of the disk or the yogurt sample, m²

The pressure necessary to cause the collapse of the yogurt network was recorded. The pressure was related to the firmness of the yogurt gel network as well as the porosity of the gel. Void spaces are present in a gel network and they are filled with water. During the compression test the void spaces, or pores, were compressed and water was expelled from the yogurt network. The test was performed on all samples in duplicate.

**Puncture test.** An improvised glass instrument, which consisted of a calibrated glass needle of measured mass, was used for the puncture test. The instrument was elevated to different heights which are 8, 10, and 12 cm, and was released directly on the yogurt surface and allowed to
puncture the yogurt within a fixed time interval. For each elevation the length of the glass imbedded in the yogurt was recorded at a fixed time interval. This test was done to measure and compare the cohesiveness of the yogurt samples.

This test also allowed the comparison of the water holding capacity of the samples. When the instrument was dropped at a certain height, the initial fraction of glass length submerged in the yogurt sample was recorded. The total glass length imbedded into the yogurt was noted after 30 min. The space that was occupied by the puncture instrument was the void space in the yogurt gel that held water. The water rose to the surface as the puncture went deeper, and more water was expelled. A large amount of water expelled would mean weak and less cohesive network. The variation of the drop height was done to see the impact of force applied to the samples.

Syneresis index

Time-dependent syneresis. The liquid expelled from each yogurt samples produced was recorded at the end of the first, fifth and seventh day of storage. It was done by carefully removing and weighing the liquid that separated from the yogurt gel. This corresponded to the initially entrapped liquid in the yogurt network and slowly separated due to diminishing water holding capacity of the yogurt gel during storage [16]. The percent liquid expelled was computed using the equation below [17].

\[
\text{%Syneresis} = \frac{\text{Amount of liquid removed}}{\text{Amount of water in the yogurt use}} \times 100
\]

Forced syneresis. After 7 days of storage, samples were centrifuged for 30 min at 3300 rpm. Empty test tubes were initially weighed, and then weighed once more after adding the samples. After centrifugation, the liquid expelled from the yogurt gel using pipette and the test tubes containing the samples were weighed again. The syneresis percentage was calculated by dividing the mass of the liquid separated from the yogurt after 7 days by the amount of liquid expelled after centrifugation and then multiplying by 100.

RESULTS AND DISCUSSION

Compositional analysis of the milk

The compositional analyses of the milk samples used in the experiments are shown in Table 1. It is interesting to note that while the protein contents are about the same, the whole carabao milk contained more than double of cow’s milk fat content, which is 3.1% (w/w) on average. This is consistent with the findings of Ahmad et al. [13] for raw buffalo milk from the Murrah breed of B. bubalis. However, for this study, it was

<table>
<thead>
<tr>
<th>Parameters, % (w/w)</th>
<th>Whole Carabao Milk</th>
<th>Reduced-fat Carabao Milk</th>
<th>Test Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Solids</td>
<td>18.2</td>
<td>12.6</td>
<td>AOAC 990.20, Air Oven</td>
</tr>
<tr>
<td>Fat</td>
<td>8.0</td>
<td>0.31</td>
<td>Mojonnier Extraction</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.7</td>
<td>4.8</td>
<td>AOAC 930.28, Gravimetry</td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td>0.5</td>
<td>0.3</td>
<td>Kjeldahl</td>
</tr>
<tr>
<td>Non-Protein Nitrogen</td>
<td>0.03</td>
<td>0.03</td>
<td>Kjeldahl</td>
</tr>
<tr>
<td>Non-Casein Nitrogen</td>
<td>0.04</td>
<td>0.06</td>
<td>Kjeldahl</td>
</tr>
</tbody>
</table>
necessary to reduce the fat content of the carabao milk to ascertain that textural characteristics were due mainly to the adjusted protein ratio and heat treatment. Therefore, the fat content of the milk samples were reduced by skimming up to the limit of available means.

**Textural characterization tests pH of milk.** The pH of the different milk samples with and without adjusted whey protein-to-casein ratios are shown in Table 2. The variation in pH prior to heat treatment can affect the mechanism of denaturation because the molecular conformation of the whey proteins is pH-dependent [18]. At higher pH values, heat treatment will result in the formation of more whey protein aggregates while lower pH will result in more association of whey proteins with casein micelle [7, 19, 20]. These emphasize the importance of keeping the pH of milk samples the same prior to heat treatment. It was to ascertain that the variation in yogurt characteristics was due to the heat treatment and the adjustment of whey protein-to-casein ratio.

**Viscosity.** Heat treatment denatures the globular whey proteins, and high-heat treatment leads to high degree of whey protein denaturation and polymerization of the denatured proteins [21]. Aside from being more effective water binder compared with those in their native form, the denatured proteins interact with the casein micelles through the κ-caseins on the casein micelle surface more effectively, thereby causing an increase in viscosity of the milk samples continuously as acidification ensues until they set to yogurt gels [22]. This behavior was quantified by monitoring how the apparent viscosities of the milk samples evolved with time.

The milk samples slowly thickened until about 30 min. The viscosity reading then started to increase more considerably, and then rapidly, until they reached the limit of the viscometer, which indicate that the samples then started to set into gels.

Figure 1 shows the viscosity curves for the different milk samples considered in this study. The milk sample of 0.35 whey protein-to-casein ratio exhibited a rapid increase in viscosity after 40 min. This indicates high protein aggregation rate and extensive interaction between caseins and whey proteins within a short period of incubation. Excess amount of whey protein present in the milk sample must have led to interaction between whey proteins other than just κ-casein-β-Lg interactions (κ-Cβ-L) [23]. On the other hand, the blank showed a considerable increase in viscosity only after 100 min, which was slower compared with both milk samples with 0.25 and 0.35 whey protein-to-casein ratios. This slow

<table>
<thead>
<tr>
<th>Test Samples</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>6.80 ± 0.00</td>
</tr>
<tr>
<td>0.25 Ratio</td>
<td>6.79 ± 0.01</td>
</tr>
<tr>
<td>0.35 Ratio</td>
<td>6.78 ± 0.02</td>
</tr>
</tbody>
</table>

Figure 1. Change in the apparent viscosity with time of the blank and the milk samples with whey protein-to-casein ratios adjusted to 0.25 and 0.35.
viscosity increase was a result of limited interactions between whey proteins and caseins. The milk sample with 0.25 whey protein-to-casein ratio exhibited a moderate viscosity build-up, which started to increase considerably after about 75 min.

It was deemed that the shorter the time it took for the milk sample to set to yogurt gel, the stronger the gel network formed by the milk sample, and that the result indicated that as the whey protein-to-casein ratio increased in the heat-treated milk samples, the shorter the time for the onset of gelation. This is consistent with the findings of Beaulieu et al. [24]. However, it was unclear how this supposed gel strength could be related with textural properties relevant to sensory quality. This was further investigated in the subsequent tests.

**Compression test.** In the compression test, compressive pressure was applied on the gels and the corresponding change in their volume measured against the applied pressure until the gel collapsed. The compressive pressures at which the gels collapsed were recorded as indication of their strength. These were then compared and analyzed.

The yogurt gel produced from the blank required the lowest compressive pressure of only about 220 Pa before a change of 0.2 cm became apparent, followed by its subsequent collapse (Fig. 2). Moreover, water was expelled as the yogurt gel was compressed and the pores of the gel network where water was held collapsed upon compression. This behavior must be due to weak gel network brought about by insufficient protein interactions that hold the gel network together [25].

The yogurt gel made from the milk sample with 0.25 whey protein-to-casein ratio required greater pressure of about 1400 Pa before structural collapse became apparent. The yogurt gel also expelled water as compression ensued, which means that the formed yogurt was also porous and had a satisfactory water holding capacity, but the gel network was stronger compared with that produced from the blank. According to Tamime and Robinson [26], an increased protein interaction and protein-protein bond increases the elastic character of the gel, making yogurt less susceptible to rupture. It was deemed that the addition of whey protein lead to a more extensive whey protein-casein interaction, which strengthened the gel network, while maintaining a porosity that allowed satisfactory water holding capacity. A strong gel network with satisfactory water holding capacity is desirable because the water held in the network acts as a plasticizer [27]. This will render the yogurt to be firm but not rigid. Also, this will allow the yogurt to retain water-soluble nutrients, such as minerals and the remaining native whey proteins, making the yogurt more nutritious.

The yogurt gel made from the milk sample with 0.35 whey protein-to-casein ratio required the largest pressure of more than 5000 Pa for apparent collapse, and expelled very little liquid compared to the other two samples (Fig. 2). It was deemed that at a
whey protein-to-casein ratio of 0.35, the amount of whey protein must have been excessive so that a massive protein-protein interactions as well as casein cross-linking via denatured whey proteins took place during heating and acidification, making a strong network with small pores [28]. This made the yogurt gel very dense and, therefore, rigid and with small water holding capacity.

**Puncture test.** The experimental results (Fig. 3) show that the yogurt gel samples from the blank and the milk with 0.25 whey protein-to-casein ratio allowed the same length of the puncture glass to be imbedded in them within the predetermined time interval. However, close observation indicated that the yogurt gel from the blank milk sample expelled more liquid. This observation is in agreement with the compression test results based on the water expelled upon puncture.

It is interesting to note that, while the yogurt from the milk with 0.25 whey protein-to-casein ratio exhibited greater network strength in the compression test, its response to the puncture test was similar to that of the less strong yogurt from the blank. This indicates that the addition of whey protein strengthened the gel network without making the gel rigid and dense. This is relevant to sensory and overall quality related to mouthfeel and spooning the yogurt.

The yogurt gel from the milk sample with 0.35 whey protein-to-casein ratio allowed a shorter length of the puncture glass to be imbedded in it. This must be due to the dense and highly cohesive texture of the yogurt, which must have been rendered by a more massive protein-protein interaction and casein cross-linking due to excessive amount of whey proteins [28]. Once again, this is consistent with the results of the compression test.

The combined results of the compression and puncture tests indicate that the addition of whey proteins improved the strength and stability of the yogurt gel network, which lead to the improvement of water holding capacity of the gel. However, an excessive amount of whey proteins may also render the gel to be rigid, dense and with reduced water holding capacity, which are characteristics of overly stabilized gel and are, generally, not desirable [1]. While samples made from the blank and 0.25 whey protein-to-casein milk exhibited similar behavior during puncture test, the latter exhibited greater
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gel strength in the compression test. However, whether this translates to greater stability against syneresis during storage is unclear. Therefore, syneresis tests were subsequently performed.

Syneresis. Syneresis is the extraction or expulsion of liquid from a gel [29]. Figure 4 shows the percent liquid expelled from yogurt made from blank and milk samples with 0.25 and 0.35 whey protein-casein ratios. The data shows that yogurt samples from milk with no added whey protein had the highest percentage of expelled water compared with yogurts from the milk samples with added whey proteins.

The blank exhibited high percentage of liquid expulsion because of weak protein interactions due to limited amount of whey proteins, which lead to weak gel network. Samples with added whey proteins exhibited relatively smaller amount of liquid expelled compared with the yogurt from blank. This can be attributed to sufficient amounts of whey proteins for k-casein-b-Lg interactions [30]. In addition, cross-linking of caseins by polymerized whey proteins enabled the forming of a stable gel network to hold more water within a prolonged period. On the other hand, excessive amount of whey protein may lead to the formation of whey protein aggregates in addition to whey protein-linked casein aggregates, which result in pore tightening and cause lowering of water holding capacity [1].

Time dependent syneresis. The effect of the increased whey protein-to-casein ratio was studied by Lorenzen et al. [31] where it was found that cross-linking of protein chains can stabilize the three-dimensional network of yogurt gel and prevent liquid expulsion as a result of a decrease in gel porosity, thus reducing syneresis.

The combined results of the compression and puncture tests indicate that, compared with the other two samples, the yogurt gel prepared from milk with 0.35 whey protein-to-casein ratio was denser and, therefore, must have been less porous [32]. Therefore, its water holding capacity can be expected to be smaller compared with the other samples. Results of the syneresis test (Fig. 4), showing the 0.35 sample to have the lowest % liquid expelled confirmed this hypothesis.

Forced syneresis. Syneresis during prolonged storage was simulated by subjecting the samples to centrifugation. The %liquid expelled from the different yogurt samples made from milk samples of different whey protein-to-casein ratios were tested (Fig. 5). The experimental results can verify the water holding capacity and, therefore, the porosity of the yogurt samples as well. The amount of liquid separated from the sample affirmed that the yogurt with more whey proteins was more resistant against syneresis.

Based on the results (Fig. 5), the 0.35 whey protein-to-casein ratio had the smallest amount of water expelled, which is consistent with the results gathered from the time-dependent syneresis. This
indicates that this sample had the smallest water holding capacity while the blank had the highest water holding capacity. This is indicative of the lesser porosity of the 0.35 yogurt sample, which must have been brought about by the highly stabilized gel network from the excessive amount of whey proteins added [22].

Figure 6 shows the %syneresis based on the total liquid expelled on the seventh day of test and the liquid expelled by centrifugation. While the yogurt sample made from the blank had the highest water holding capacity, Fig. 6 indicates that it also gave the highest %syneresis while the yogurt from the milk with 0.35 whey protein to casein ratio had the smallest %syneresis. The yogurt sample from the milk with 0.25 whey protein-to-casein ratio had a water holding capacity close to that of the blank (Fig. 5), but showed an average %syneresis that was more than twice lower than that of the blank. This is consistent with the results of the compression and puncture test, indicating that these samples had similar porosity, which was confirmed by their similar water holding capacities as per Fig. 5, but the sample made from the blank had a weaker gel network that resulted in a higher %syneresis (Fig. 6).

The overall results of the syneresis tests are consistent with the results of the previous tests, which indicated that the yogurt gel made from milk sample with 0.25 whey protein-to-casein ratio had the most satisfactory gel strength and water holding capacity. This may potentially lead to satisfactory sensory quality.

CONCLUSION

The adjustment of whey protein-to-casein ratio in reduced-fat carabao milk to 0.25, and the heat treatment of this milk at a temperature higher than pasteurization temperature of 80°C for 25 min, resulted in significant improvements of yogurt texture. The yogurt gel exhibited relatively high water holding capacity and low %syneresis, moderate gel setting time as shown by the moderate increase in viscosity during acidification, and moderate gel strength compared with those samples made from the milk with whey protein-to-casein ratio adjusted to 0.35 and that with unadjusted ratio. These characteristics are deemed ideal in producing reduced-fat, high-protein, set yogurt without using carbohydrates-based stabilizers and texture enhancers.

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