Approaches to minimise yoghurt syneresis in simulated tzatziki sauce preparation

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The phenomenon of syneresis becomes more profound in increased moisture yoghurt-based products. Such a product is the traditional Greek appetizer tzatziki that contains cucumber, a vegetable with a high moisture content. During the manufacture of tzatziki, the addition of cucumber causes the protein network to break up, provoking an increase in syneresis. The aim of this study was to investigate a tzatziki manufacturing procedure that will lead to significantly decreased syneresis.

Two different manufacturing procedures were compared: the extra moisture coming from cucumber was introduced before or after fermentation. The effect of adding whey protein concentrate (WPC), albumin, sodium caseinate or a mixture of these was also studied. The results show that the addition of extra moisture before yoghurt fermentation leads to a significantly lower syneresis (7.5%) and higher consistency (2000 cp) than those obtained in the case of addition after fermentation (25% and 1500 cp, respectively). The use of albumin, WPC or a mixture of albumin, WPC and sodium caseinate further decreased the phenomenon of syneresis to below 5%, without altering the colour of the product (ΔΕ* < 2.3).

Keywords Syneresis, Stirred yoghurt, Tzatziki, Yoghurt, Casein, Whey protein concentrate.

INTRODUCTION

Yoghurt is defined as a fermented milk product produced with thermophilic lactic bacteria, usually Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus (Mottar et al. 1989; Lucey et al. 1999; Ginovart et al. 2002).

All yoghurt-manufacturing procedures are basically the same. The milk is clarified and separated into cream and skim milk and then standardised to achieve the desired fat and protein content. The mixture is homogenised using high pressures (10–20 MPa) at temperatures of 55–70 °C (Tamime and Robinson 1999). The milk is pasteurised by heating for 30 min at 85 °C or 10 min at 95 °C. Next, the milk is cooled to approximately 43 °C, which is an optimum growth temperature for the yoghurt starter culture that is added to the milk in a fermentation tank. A temperature of 43 °C is maintained for 4–6 h. The acid development of yoghurt is carefully monitored until the pH reaches 4.0–4.7; then, the fermentation is stopped by rapid cooling (Connolly 1978).

During milk fermentation, the casein becomes unstable and coagulates to form a firm gel, composed of strands of casein micelles, with whey entrapped within this matrix, which is interlocked via hydrogen bonds, forming a protein matrix. Yoghurt structure is the result of disulphide bonding between k-casein and denatured whey proteins and by aggregation of casein as the pH drops to the isoelectric point of the casein proteins during fermentation (Damin et al. 2009).

An important aspect of a milk gel is whey separation, which refers to the appearance of a liquid on the surface of milk gel. It is a common defect in fermented milk products such as yoghurt (Lucey et al. 1998). Syneresis is defined...
as the shrinkage of gel, and this occurs concomitantly with expulsion of liquid or whey separation and is related to instability of the gel network resulting in the loss of the ability to entrap all the serum phase (Walstra 1993). According to Lucey et al. (1998), some possible causes of wheying-off in acid gels are very high incubation temperatures, excessive treatment of the mix, low total solids content (protein and/or fat) of the mix, movement or agitation during or just after gel formation and very low acid production (pH > 4.8) (Magenis et al. 2006; Donato and Guyomarc’h 2009). Magenis et al. (2006) reported that factors influencing yoghurt texture and syneresis include total solids content, milk composition (proteins, salts), homogenisation, type of culture, acidity resulting from the growth of bacterial cultures and heat pretreatment of milk.

Traditionally, the solid content of milk is increased for yoghurt production. The three main systems available nowadays are good options to achieve desired protein and solids contents: (i) addition of protein ingredient powders (skimmed milk, whey protein concentrates, caseinates); (ii) evaporation of water from milk under vacuum; or (iii) removal of water by membrane filtration (Tamime et al. 2001). Fortification with skim milk powder (SMP) is the common practice to increase the solid content in conventional yoghurt manufacture, but when enriching the protein content is the main target, the amount of SMP that can be added to provide extra protein content becomes limited, since too high levels of SMP can lead to a powdery taste and high lactose content, which ultimately results in a highly acidic product (Abd El-Mahdi and El-Khair 2009; Supavititpatana et al. 2009; Marafon et al. 2011a,b). An alternative approach of fortification of the milk for yoghurt manufacture is by ultrafiltration. Protein concentrates produced by ultrafiltration have better nutritional value than that produced by traditional methods. Another advantage of UF milk is that it contains a higher level of protein with a lower level of lactose than normal milk (Karlssson et al. 2005). However, UF concentration of milk for yoghurt manufacture leads to an excessively firm coagulum and a more viscous product than conventional SMP fortification (Schkoda et al. 2001).

The phenomenon of syneresis becomes more profound in increased-moisture yoghurt-based products. Such a product is the traditional Greek appetizer tzatziki. Tzatziki is made of strained yoghurt mixed with cucumbers (in a ratio of 4:1), garlic, salt, olive oil and sometimes lemon juice, and dill or mint or parsley. Strained or Greek-style yoghurt is traditionally made by straining fermented yoghurt curd in a cloth bag to reach the desired solids level by removing acid whey. This step is achieved by mechanically separating the whey from the curd using either a centrifugal separator or membrane filtration (Nsabimana et al. 2005) or by fortification of milk with milk protein concentrates (Bong and Moraru 2014). Cucumber is a vegetable with a high moisture content (~96% w/w), which practically increases the moisture content of tzatziki. During the manufacture of tzatziki, migration of water from the high moisture content ingredient (cucumber) to the low moisture content ingredient (yoghurt) occurs. Thus, the gel matrix of the yoghurt is broken and cannot hold the extra water, resulting in increased syneresis.

The aim of this study was to investigate a tzatziki manufacturing procedure that will lead to significantly decreased syneresis. For experimental purposes, a simulated tzatziki sauce preparation was used. This preparation was strained yoghurt with added moisture where, instead of adding whole cucumber, garlic and dill, only the extra moisture coming from cucumber was added. As stirring of yoghurt breaks up the protein network leading to an increased syneresis, we tested the hypothesis that extra moisture can be better retained by introducing it before the formation of yoghurt. To this end, two different ways of manufacturing were compared: the extra water coming from cucumber was added (a) before and (b) after fermentation and compared to tzatziki made from a commercial readymade strained yoghurt. The effect of adding whey protein concentrate, albumin, sodium caseinate or mixtures of these protein ingredients on syneresis was also studied.

MATERIALS AND METHODS

Materials

Homogenised, pasteurised fresh cow’s milk (72 °C for 15 s) (Meygal S.A., Thessaloniki, Greece) was used as a stock of raw material throughout the experimental work to avoid variation in milk composition.

Globulal 70 N whey protein concentrate (70% protein content) and Emulac sodium caseinate (50% protein content) were purchased from Meggle AG (Wasserburg, Germany), whereas hen egg albumin powder (78% protein content) was obtained from Kallbergs Industri AB (Töreboda, Sweden).

Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus were obtained from Aristomenis D. Phikas & Co (Thessaloniki, Greece). The cultures were stored at −20 °C in a concentrated form before use.

Soft potable water was used for tzatziki manufacture.

Yoghurt–Tzatziki manufacture

Tzatziki is made of strained yoghurt mixed with cucumbers in a ratio of 4:1. The moisture contents of strained yoghurt and cucumber are about 82 and 96% w/w, respectively (Trichopoulou and Georgia 2004). For experimental purposes, a simulated tzatziki sauce preparation was used. This preparation was strained yoghurt with added moisture, where instead of adding whole cucumber, garlic and dill, only the extra moisture coming from cucumber was added. Thus, during tzatziki manufacture, 1.2 kg of water was added per 5 kg of yoghurt/yoghurt formulation.
Two different yoghurt-manufacturing procedures were followed as depicted in Figure 1. In the first case (Figure 1a), water was introduced after the incubation of condensed milk with the micro-organisms, whereas in the second one (Figure 1b), the water was added before the incubation. In both cases, different mixes of protein ingredients were added as agents for improving texture and reducing syneresis. The protein ingredients used were (w/w) whey protein concentrate 1% and 5%, sodium caseinate 1% and 5%, and albumin 1% and 5%, as well as a protein mixture 5% consisting of 2% albumin, 2% whey protein concentrate and 1% sodium caseinate. The percentages were based on preliminary experiments. Both preparations were compared to a control sample, which was manufactured using a commercially ready strained yoghurt, with the same total solids and fat contents.

The milk was concentrated from an initial solid content of about 11.2% to a final concentration of approximately 21.5% in a rotary vacuum evaporator (Model R 114, Buchi Laboratoriums-Technik, Flawil, Switzerland) at 45 °C. A quantity (500 mL) of the milk samples was prewarmed to 45–50 °C in a microwave oven and heated in a water-boiling bath to 80 °C. When this temperature was reached, the samples were kept for 30 min in the bath and protein ingredients were added. In the case presented in Figure 1b, where the water was added before the incubation, soft potable water in a quantity that accounts for the extra moisture content of the tzatziki was also added. After cooling to

![Figure 1 Tzatziki manufacturing procedure (a) water added after fermentation and (b) water added before fermentation.](image-url)
42 °C, the mixes were inoculated with the active starter (9 g/53.5 kg), divided into three equal portions in plastic cups and incubated at 42 °C in a fermentation store until a 4.7 pH was reached in about 5 h. The yoghurt was then cooled to room temperature for 1.5 h by placing the cups in a bath of cold water and stored at 4 °C in the cold refrigerator. In the case presented in Figure 1a, where the water was added after the incubation, the quantity of water that accounts for the extra moisture content of the tzatziki was added during storage. All samples were prepared in triplicates. Formulations used for tzatziki manufacture are listed in Table 1.

Tzatziki samples were drawn at different cold storage intervals (2, 9, 14, 16, 18, 22, and 25 days) and characterised. Formulations used for tzatziki manufacture are listed in Table 1.

Physicochemical characteristics

Solubility
Milled protein ingredient powders were dispersed (1% w/w) in deionised water and the pH adjusted with 2 N NaOH to 7.0. Dispersions were centrifuged at 2000 g for 15 min. Protein concentrations were determined according to Lowry et al. (1951). Solubility was obtained from the concentration ratio of the supernatant and the dispersion before centrifugation.

Syneresis
Syneresis was measured according to Keogh and O’Kennedy (1998). Briefly, yoghurt (30–40 g) was centrifuged (T 52.1, VEB MLW Zentrifugenwerk Engelsdorf, Germany) at 222 g for 10 min at 4 °C. The clear supernatant was poured off, weighed and recorded as syneresis (%).

Viscosity
Viscosity measurements were obtained using a Brookfield viscometer (Model DV-II+; Brookfield Engineering Labs, Inc., Middleboro, MA, USA) with a No 4 spindle rotating at 100 rpm. The sample temperature was 4 °C. For a relative comparison between treatments, viscosity reading was taken at the point of the 30th s and torque was maintained at all times between 10% and 100%.

Moisture content
Moisture was determined by an Ohaus moisture analyzer (Model MB35 Halogen, Ohaus Co., Pine Brook, NJ, USA).

Colour
Colour was determined using a Hunter Lab colorimeter. a* and b* values were determined, while the total difference in colour (ΔE) was calculated using the following equation:

\[ ΔE = \sqrt{(L_o^* - L_f^*)^2 + (a_o^* - a_f^*)^2 + (b_o^* - b_f^*)^2} \]

where \( L_o^* \), \( a_o^* \) and \( b_o^* \) are the values for the control sample, and \( L_f^* \), \( a_f^* \) and \( b_f^* \) are the values for the sample. \( ΔE \sim 2.3 \) corresponds to just noticeable difference.

Statistical analysis
Data were analysed by a general linear model procedure of the Fisher’s protected-least-significant-difference test using SAS (SAS Inst., Cary, NC, USA). This test combines analysis of variance (ANOVA) with comparison of differences between the means of the treatments at the significance level of \( P < 0.05 \).

RESULTS AND DISCUSSION

Effect of protein ingredients addition on syneresis rate of tzatziki
The protein ingredients used as agents for improving water-holding capacity and decreasing syneresis were whey protein concentrate (WPC), albumin and sodium caseinate. Their measured properties are presented in Table 2. The choice of protein ingredients was based on their ability to decrease syneresis in tzatziki manufactured using commercial strained yoghurt. Figure 2a,b show the effect of the

Table 1 Formulations used for tzatziki manufacture

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yoghurt</th>
<th>Milk</th>
<th>Water</th>
<th>Whey protein concentrate</th>
<th>Albumin</th>
<th>Sodium caseinate</th>
<th>Active starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80</td>
<td>–</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1% whey protein concentrate</td>
<td>–</td>
<td>77.6</td>
<td>21.4</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>0.02</td>
</tr>
<tr>
<td>1% albumin</td>
<td>–</td>
<td>77.6</td>
<td>21.4</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>1% sodium caseinate</td>
<td>–</td>
<td>77.6</td>
<td>21.4</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>0.02</td>
</tr>
<tr>
<td>5% whey protein concentrate</td>
<td>–</td>
<td>74.5</td>
<td>20.5</td>
<td>5.0</td>
<td>–</td>
<td>–</td>
<td>0.02</td>
</tr>
<tr>
<td>5% albumin</td>
<td>–</td>
<td>74.5</td>
<td>20.5</td>
<td>–</td>
<td>–</td>
<td>5.0</td>
<td>0.02</td>
</tr>
<tr>
<td>5% sodium caseinate</td>
<td>–</td>
<td>74.5</td>
<td>20.5</td>
<td>–</td>
<td>–</td>
<td>5.0</td>
<td>0.02</td>
</tr>
<tr>
<td>5% protein mixture</td>
<td>–</td>
<td>74.5</td>
<td>20.5</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
<td>0.02</td>
</tr>
</tbody>
</table>
level of protein addition on the syneresis of tzatziki during cold storage at 4 °C. Each data point in the figure represents average values of three replications. The repeatability for syneresis expressed as the average standard deviation of the three replications was 0.3%. All three protein ingredients at both levels (1 and 5%) significantly decreased syneresis compared to the control sample. However, comparing the effect of the different concentrations of the same protein ingredient, it was concluded that increasing the concentration of WPC and sodium caseinate did not further decrease syneresis, while a more profound effect on syneresis was exhibited by increasing albumin concentration from 1 to 5%.

The effects of protein and total solids (TS) are difficult to study separately, as the two variables cannot be modified independently. Increasing TS improved yoghurt texture, based on sensory and instrumental evaluations. Generally, higher TS caused an increase in density and reduced pore size in the protein matrix of the yoghurt gel. This led to a reduction in syneresis and improvement of the water-holding capacity of the gel. Keogh and O’Kennedy (1998) explained that casein and β-lactoglobulin interact chemically on heating. This effectively increases the concentration of gel-forming protein in the yoghurt matrix and reduces syneresis through increased entrapment of serum within the interstices of the whey protein molecules attached to the surface of the casein. The effects of different levels of TS were studied by Tamime and Robinson (1999), and they found that the consistency was greatly improved as solids increased from 12 to 20 g/100 g. The greatest change was observed from 12 to 14 g/100 g, whereas levels above 16 g/100 g resulted in less pronounced change. According to Prentice (1992), increase in protein levels is the principal factor influencing texture and enrichment of milk with milk powder results in the development of chains and aggregates of casein micelles. Wu et al. (2001) demonstrated that water-holding capacity was related to the ability of the proteins to retain water within the yoghurt structure. These researchers further suggested that the fat globules in the milk may also play an important role in retaining water.

Tzatziki samples containing albumin had lower syneresis rates than the others, whereas syneresis was reduced with increased proportions of sodium caseinate. This may be because the high whey protein-to-casein ratio induced shrinkage of the gel, which led to increased whey drainage or whey separation (Lucey 2004). Keogh and O’Kennedy (1998) explained that casein and β-lactoglobulin interact chemically on heating. This effectively increases the concentration of gel-forming protein in the yoghurt matrix and reduces syneresis through increased entrapment of serum within the interstices of the whey protein molecules attached to the surface of the casein. Saint-Eve et al. (2006) observed that sodium caseinate provides a gel with a heterogeneous structure with large pores. When yoghurts were enriched with whey protein, the protein network was more uniform and the pores of the gel were smaller than those of yoghurts to which sodium caseinate had been added.

**Effect of way of added moisture on syneresis rate of tzatziki**

Although the phenomena occurring during syneresis are not fully understood, it is agreed that increased syneresis with storage time is usually associated with severe casein network rearrangements (van Vliet et al. 1997) that promote

| Table 2 Protein solubility and pH of solutions at 25 °C (avg ± SD, n = 4) |
|------------------------|-----------------|--------|
| Protein (% w/w)        | Solubility (%)  | pH     |
| 5% whey protein concentrate | 33.0 ± 1.5b  | 6.70 ± 0.08b |
| 5% albumin              | 37.9 ± 1.7ab   | 7.24 ± 0.02b |
| 5% sodium caseinate     | 31.7 ± 0.7b    | 6.91 ± 0.01ab |

Means in the same column followed by the different superscript letters are significantly different (P < 0.05).
whey expulsion. As stirring the formed gel would add an extra destabilising effect, we tested the hypothesis that water would be better retained in stirred yoghurt products if it was added before fermentation.

Syneresis was significantly lower \((P < 0.05)\) for all tzatziki samples in which water was added before fermentation (Figure 3), except for the sample with 5% added albumin, in which there was no difference in syneresis. This was also true for the control sample, which exhibited an almost constant syneresis around 7\%, throughout the storage period. However, the control sample in which the water was added after fermentation had a much higher syneresis, at a level around 25\%. Samples with 5% albumin and 5% of the protein mixture exhibited the least syneresis.

Rheological properties of yoghurt have been well studied. Yoghurts have flow properties that are characteristic of a non-Newtonian and weakly viscoelastic fluid (Ramaswamy and Basak 1991, 1993; Benezech and Maingonnat 1993; Skriver et al. 1993). Keogh and O’Kennedy (1998) studied the role of milk fat, protein, gelatin and hydrocolloids (starch, locust bean gum/xanthan mixture) on the rheology of yoghurt. These authors showed that the consistency index \((K)\) and syneresis were more frequently influenced by the composition than the behaviour index \((n)\) and the critical strain \((\gamma_c)\). Lee and Lucey (2006) investigated the structural breakdown of the original (intact) yoghurt gels that were prepared \textit{in situ} in a rheometer, as well as the rheological properties of stirred yoghurts made from these gels. The same authors found that the rheological properties of yoghurts were greatly influenced by the physical properties of the original intact (set) yoghurt gels. Water in milk gels is physically trapped within the gel network, meaning that the tendency for whey separation is primarily linked to dynamics of the casein network rather than mobility of the water molecules (van Vliet and Walstra 1994).

Effect of protein addition and way of moisture addition on viscosity, pH and colour of tzatziki

Protein addition increased yoghurt viscosity. Similar results were found by Abu-Jdayil (2003), who found greater viscosity in yoghurts of the labneh type, with higher protein content. Yoghurts containing WPC had lower viscosity values than the others. These results are in agreement with those reported by Modler et al. (1983) and Guzman-Gonzalez et al. (1999), who found that casein-based products tended to produce firmer gels with less syneresis than yoghurts fortified with whey protein. Modler and Kalab (1983), using electron microscopy studies, showed that yoghurts prepared with casein, SMP and MPC exhibited a high degree of fused micelles when compared to yoghurts stabilised with WPCs.

Adding the extra moisture to the system before fermentation and setting probably allows casein and whey proteins to arrange themselves in a network and trapping the extra moisture, rendering yoghurts less susceptible to syneresis compared to samples in which the extra moisture is added after fermentation. This is further strengthened by the observation that the viscosity of tzatziki samples with water added before fermentation was also found to be higher than that of all the respective samples in which the water was added after fermentation (Figure 4). For yoghurt products, steps such as mixing result in a viscosity reduction that is only partially restored after shearing is stopped. Recovery of structure is called ‘rebodying’ and is a time-dependent phenomenon. Structural recovery also affects the apparent viscosity of yoghurts (Lee and Lucey 2010). Arshad et al. (1993) reported that glucono-δ-lactone (GDL)-induced gels had only 30\% recovery of the original value of the dynamic moduli even after allowing 20 h for recovery after shearing.

As far as the effect of protein ingredients on pH changes, there was a small decrease in the pH from the time the fermentation was stopped till 48 h after storing the product at 4 °C (postacidification) and slowly continued till the 10th
day of storage. A decrease in pH during storage is expected as a result of the activity of LB (Tamime and Robinson 1999); this has been observed in lactic beverages (Oliveira et al. 2001). Postacidification occurred independently of the ingredient or the concentration used and was 0.20 pH units on average. A similar trend was observed by Damin et al. (2009). There was no significant pH difference between the control and the samples throughout the storage period for the 1% level of addition. On the other hand, at the 5% level of addition, a significant \( P < 0.05 \) difference was observed (Figure 5). This can be attributed to the higher buffering capacity brought by the added protein ingredients. There was a high correlation coefficient between the pH throughout the storage period and the respective syneresis, that is 0.90–0.95.

Furthermore, there was no significant effect of the way of extra water addition on the pH. There was, however, a tendency for a slightly higher pH during the first 2 weeks of storage for the samples with added water after fermentation (0.1–0.15 pH units, results not shown).

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Protein addition significantly affected the colour of yoghurt \((P < 0.05)\). An increase in the lightness and a decrease in yellow colour of yoghurts were observed. The results were in agreement with the observation of González-Martínez \textit{et al.} (2002), who studied the change in colour as affected by addition of casein. The higher milk protein content gave better protein coagulation. The coagulation of protein affected the structure and surface properties of yoghurt. Mor-Mur and Yuste (2003) reported that the increased protein coagulation enhanced the light absorption that resulted in the lighter tons.

Addition of water before fermentation also led to better colour retention of the tzatziki samples (Figure 6) for all treatments. In tzatziki samples in which water was added before fermentation, \(\Delta E^*\) is below 2.3, that is the value that corresponds to just noticeable difference. Albumin, even though significantly improving syneresis, led to a greater colour change and, therefore, it might not be acceptable for product formation.

CONCLUSIONS

The hypothesis that extra moisture can be better retained in stirred yoghurt-based products (e.g. tzatziki) by introducing the extra moisture before the formation of yoghurt was successfully tested. Results clearly show that, even without the addition of protein ingredients, in tzatziki samples with water added before fermentation, syneresis was lower and consistency was higher than in those where the water was added during the stirring of yoghurt. The use of albumin, WPC or a mixture of albumin, WPC and sodium caseinate further decreased the phenomenon of syneresis.

REFERENCES


