EFFECT OF MICELLAR CASEIN CONCENTRATE FORTIFICATION ON
PHYSICAL AND CHEMICAL PROPERTIES OF GREEK STYLE YOGURT

A Project Paper Presented to the Faculty of the Graduate School of Cornell University

In Partial Fulfillment of the Requirements for the Degree of Master of Professional
Studies in Food Science and Technology

By Wanyu Li

May 2013
ABSTRACT

Greek yogurt has gained wide popularity in recent years mainly due to its creamy texture, high protein content and low caloric content. Conventional Greek yogurt requires the removal of whey after fermentation. The objectives of this study are to fortify skim milk with micellar casein concentrate (MCC) obtained by membrane filtration in order to eliminate the need of whey removal, and to investigate the effect of milk protein fortification on the physical and chemical properties of the resulting yogurt. The proposed method would simplify the conventional making of Greek style yogurt and allow the production of Greek yogurt with an equivalent level of protein and low concentration of lactose. The quality of the yogurt including pH, titratable acidity, water holding capacity and texture was studied in order to optimize the processing method.

Keywords: Greek style yogurt, fortification, micellar casein concentrate (MCC)
BIOGRAPHICAL SKETCH

Wanyu Li was born and raised in Chengdu, China. She studied in Food Science and Technology at BNU-HKBU United International College (Zhuhai, China), and received her degree in Bachelor of Science (1st Class Honors) from Hong Kong Baptist University in 2011. In the same year, she went to Cornell University to continue her study in Food Science, expected to receive her degree in Master of Professional Studies in May 2013.
I would like to dedicate this work to the prettiest mom and smartest dad, Dan and Husheng.
ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my advisor Dr. Carmen Moraru, for her excellent guidance, caring, patience, and encouragement for this research.

I would like to show my deep appreciation to Dr. Guoping Feng for his invaluable instruction and support during my project. Also I would like to thank Dr. Anne Sauer for her great guidance and consideration. Special thanks to Deepthi Ananth (Cornell University ‘12) for her help with the experimental work.

I would like to thank all other people in my lab, who created a friendly working environment and who were always willing to help.

I would like to thank Chr. Hansen for providing yogurt cultures, and Cornell Dairy for providing milk samples for this research. I would like to thank Dr. Steven Mulvaney for letting me use the texture analyzer in his lab. I would like to thank Mr. Sean Samuel Schell for guiding and helping me in the Cornell Pilot Plant. I would like to thank people working on the 2\textsuperscript{nd} floor of Stocking Hall, who have ever given me a hand during my lab work.

Last, I would like to thank my parents and friends, who have always been encouraging and supportive.
JUSTIFICATION & OBJECTIVE

Greek yogurt has gained immense popularity among consumers in recent years. One of the challenges associated with the growth of Greek yogurt production is the large quantity of acid whey that results as a byproduct. In this project, an alternate making process for the Greek style yogurt based on fortification of milk with MCC was explored, which partially removed or did not require the removal of acid whey. The impact of this method on the physical and chemical properties of yogurt was evaluated.

Many studies have been done on protein-enriched yogurt and its physical and sensory properties, but none of them focused on the manufacture of Greek style yogurt using MCC fortification. The objective of this research is to assess the potential application of milk protein fortification as an alternative make process to the conventional make process, and also to investigate the effect of the fortification of skim milk with MCC on Greek yogurt manufacturing, and the chemical and physical properties of the resulting yogurt.
Table of Contents

JUSTIFICATION & OBJECTIVE ...........................................................................................................VI

INTRODUCTION .................................................................................................................................1

1.1. GREEK STYLE YOGURT ...............................................................................................................1
   1.1.1. The Market of Greek style yogurt ......................................................................................1
   1.1.2. Greek style yogurt manufacturing in current industry ......................................................3
1.2. THE PRODUCTION, UTILIZATION AND CHALLENGES ASSOCIATED WITH ACID WHEY ....7
1.3. MICELLAR CASEIN CONCENTRATE (MCC): MANUFACTURING AND UTILIZATION IN GREEK STYLE
    YOGURT MANUFACTURE ...........................................................................................................9

2. MATERIALS AND METHODS ......................................................................................................11

2.1. SAMPLE PREPARATIONS .........................................................................................................11
   2.1.1. Fortification of skim milk with MCC ...............................................................................11
   2.1.2. Inoculation and Fermentation .........................................................................................13
   2.1.3. Cooling, Mixing and Straining .......................................................................................14
   2.1.4. Post-straining ..................................................................................................................14
2.2. pH ...........................................................................................................................................16
2.3. TITRATABLE ACIDITY ............................................................................................................16
2.4. WATER-HOLDING CAPACITY ..............................................................................................16
2.5. TEXTURE PROFILE ANALYSIS (TPA) ..................................................................................17
2.6. VISCOSITY .............................................................................................................................17
2.7. STATISTICAL ANALYSIS ......................................................................................................17

3. RESULTS AND DISCUSSIONS ................................................................................................18

3.1. FERMENTATION PROFILE ....................................................................................................18
3.2. WATER HOLDING CAPACITY (WHC) ................................................................................22
3.3. THE TEXTURE PROPERTIES OF MCC FORTIFIED GREEK STYLE YOGURT ...................23

4. CONCLUSIONS ..........................................................................................................................26

5. REFERENCES: .............................................................................................................................28

APPENDIX 1. SAMPLE PREPARATION FLOW CHART .................................................................33
Introduction

1.1. Greek style yogurt

1.1.1. The Market of Greek style yogurt

Greek yogurt, or strained yogurt, is gaining increasing popularity and market share in the last 5 years. It differentiates itself from other yogurt products for its higher protein content and consistency, which are achieved by straining the yogurt obtained via a regular yogurt making process. The removal of acid whey allows the protein content in Greek yogurt to be tripled as compared to regular yogurt (Andy Harris, 2002). According to Bernstein, the market share in yogurt industry has been growing from 4% in 2008 to 36% in 2012, with the potential to reach 50% in 2017 (Astley, 2012). The sales of Greek yogurt rose at a rate over 100% per year (York, E. B., 2012). In 2012, sales of Greek yogurt reached $1.6 billion, while non-Greek yogurt saw its sales decrease (Owen, 2013).

Also, according to UBS report, a growth of 40% in sales in 2013, and 120% in the upcoming 5 years was predicted (Needleman, 2012).
Chobani and FAGE are the two companies taking the lead of Greek yogurt industry in the US. It was mentioned “Chobani and FAGE took the market share more quickly than most other segments. The data strongly suggests that Greek yogurt has sustainable growth ahead from increasing shelf space at retail”(UBS, 2011). The vast growing demand for Greek yogurt, which requires almost 3 times more milk for the final yield compared with regular yogurt, stimulates the growth of dairy industry as well. A study conducted by the NPD’s National Eating Trends showed that the Greek yogurt in U.S. has been growing from 1% in 2009 to 5% in 2012 in terms of consumption, while many companies such as General Mills and Dannon have invested heavily to enter Greek yogurt industry (Astley, 2013).
A consumer survey (Table 1.) showed that the high protein content and thick texture helped create an image of Greek yogurt as a healthier choice among dairy products (Mutz-Darwell, Yurgec, & Fox, 2012).

Table 1. Summary of consumer focus group on Greek-style yogurt (Mutz-Darwell et al., 2012)

<table>
<thead>
<tr>
<th>Consumer feedback on Greek-style Yogurt</th>
<th>Health Benefits mentioned by consumers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Perceived health benefits relative to regular yogurt</td>
<td>• Higher protein</td>
</tr>
<tr>
<td>• The more substantial, thick and creamy texture</td>
<td>• Less sugar than regular yogurt</td>
</tr>
<tr>
<td>Feedback on how eating Greek-style yogurt make consumers feel:</td>
<td>• Probiotics/aids digestion/immune health</td>
</tr>
<tr>
<td>• Full/satisfied, without the guilt</td>
<td>• Low sodium content</td>
</tr>
<tr>
<td>• Healthy/rejuvenated/better for you</td>
<td>• Low calories, low fat, low saturated fat</td>
</tr>
<tr>
<td>• Indulgent but with something that is good for me</td>
<td>• “With It,” trendy</td>
</tr>
</tbody>
</table>

1.1.2. Greek style yogurt manufacturing in current industry

The traditional method of making Greek yogurt is to remove the whey from plain yogurt by straining until it achieves a desired body and consistency. At a large manufacturing scale, mechanical separation and ultrafiltration (for concentration) are applied currently in industry. The typical process of Greek yogurt manufacturing is shown in Fig. 1.

The raw material for yogurt is bovine milk, whose average chemical composition is shown in Table 2.
Table 2. Typical composition of bovine milk (de Wit 1998; Fox et al. 2000)

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (g/L)</th>
<th>Component</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>873</td>
<td>Whey proteins</td>
<td>6</td>
</tr>
<tr>
<td>Fat</td>
<td>37</td>
<td>β-Lactoglobulin</td>
<td>3.2</td>
</tr>
<tr>
<td>Lactose</td>
<td>48</td>
<td>α-Lactalbumin</td>
<td>1.2</td>
</tr>
<tr>
<td>Ash</td>
<td>7</td>
<td>Bovine serum albumin</td>
<td>0.4</td>
</tr>
<tr>
<td>Casein</td>
<td>28</td>
<td>Immunoglobulins</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Raw milk is received at the plant and filtered so that major impurities are removed. After that, milk is skimmed, homogenized, and pasteurized in a similar fashion as for pasteurized milk processing. Milk is then heated up to 90°C for 5 min, in order to induce denaturation of whey proteins. This extended heat treatment plays a significant role in the microstructure and physical properties of yogurt. The denatured whey proteins (β-lactoglobulin) will interact via disulfide bonding with κ-casein molecules located at the surface of casein micelles, and form casein-whey protein cross-links (Lucey & Singh, 1997). Moreover, the heat treatment kills pathogenic and spoilage microorganisms present in milk.

Starter cultures that contain the lactic bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophiles* are inoculated in milk after milk is cooled down to 40°C. Sometimes, lactic bacteria strains able to produce Extracellular polymeric substances (EPS) are used to yield an improved mouth thickness, creaminess and viscosity of yogurt (Folkenberg, et al., 2006). The milk is fermented at 43°C for 4-6 hours, until the pH reaches 4.55-4.6. As the pH drops to 4.6, the isoelectric point of casein, casein micelles
agglomerate by hydrophobic interactions and a gel network is formed (Lee & Lucey, 2004). The fermentation is stopped by rapid cooling with mild stirring applied to homogenize the product. Subsequently, whey is removed by using centrifugal separators. The speed and time of centrifugation process is standardized to reach the expected level of solids in the final product. Cream is then blended in for desired fat content. At last, the Greek yogurt is filled, packed, and stored under refrigeration until distribution.
Figure 2. Flow diagram for regular Greek yogurt manufacture
1.2. The production, utilization and challenges associated with acid whey

The liquid whey is a significant by-product of fermented dairy products. In Greek yogurt production, acid whey is strained away to reach a desirable concentration and texture. The whey removed in the production Greek yogurt accounts for 80-90% of the total milk volume and 50% of its nutrients (Bylund, 2003). Acid whey is composed of water (93%), lactose (5%), soluble proteins (0.85%), minerals, vitamins and trace amount of fat. β-lactoglobulin (BLG), which is absent in human milk, is the most abundant protein in whey (Pescuma, et al., 2006).

In the past acid whey used to be disposed as a waste, thus being a considerable environmental pollutant. Currently, it is utilized as livestock feed and land fertilizer or dried into acid whey powder.

Whey proteins can be concentrated and the resulting whey protein concentrate (WPC) used as a functional ingredient in food products such as pasta and beverages. The emulsifying, foaming, color formation (Maillard browning) and water binding properties of WPC make it a good option to replace some of the traditional food additives such as egg aluminum, milk powder in a wide range of foods including ice-cream and bakery goods (Gonzalez, 1996). As whey protein is denatured by heat, the WPC is processed by membrane filtration most effectively. Ultrafiltration is applied in WPC production with protein content varies from 20-89%. For whey protein isolates (WPI), ion-exchanger is used for molecule separation, through which lactose is completely excluded (Onwulata, & Huth, 2008). WPC is typically made from sweet whey, which has a pH above 5.6 and is the main by-product from rennet coagulated cheese production. The acid whey that results from Greek yogurt and acid-coagulated cheese has a pH less than 5.1 (Onwulata,
Compared with sweet whey, acid whey, especially that from Greek yogurt, tends to have fewer solids and more acidity, which make it less valuable or suitable for further processing. The acidic flavor and high mineral / salt content gives rise to undesirable attributes to this product, which limits its applications. It was reported that the waste whey management started to cost more money for the Greek yogurt manufacturers as their business scaled up. Most of the whey is taken by farmers and used as fertilizer or stock feed. However, as each farmer could only take limited amount of whey, disposing of the whey became more expensive it needed to be transported to farther places (Charles, 2012). At this point, the utilization of acid whey from the Greek yogurt industry is becoming a significant challenge for the Dairy Industry.

On the other hand, despite the fact that Greek yogurt has a higher nutrient than regular yogurt, losses of nutrients occur in Greek yogurt production during the whey straining process. The highest loss occurs for lactose and minerals such as sodium and potassium. Also, over 50% of several water-soluble vitamins are lost in the whey. Besides, losses in amino acid such as Histidine (11.38%), Arginine (8.67%) and Glycine (5.64%) were also reported, as shown in Table 3 (Nergiz, & Kemal, 1998).
Table 3. Chemical composition of unstrained yogurt and whey, and nutrient losses during straining (Nergiz, & Kemal, 1998)

<table>
<thead>
<tr>
<th></th>
<th>Yogurt</th>
<th>Whey</th>
<th>% Losses after straining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>3.7±0.20</td>
<td>0.3±0.002</td>
<td>7.3±0.70</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>3.4±0.57</td>
<td>0.03±0.006</td>
<td>0.8±0.10</td>
</tr>
<tr>
<td>Lactose (g)</td>
<td>4.7±0.40</td>
<td>3.32±0.136</td>
<td>72.1±5.08</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>56.1±8.91</td>
<td>38.9±4.71</td>
<td>70.2±8.61</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>143±13.32</td>
<td>96.4±4.47</td>
<td>68.2±7.02</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>163±30.32</td>
<td>107±23.09</td>
<td>65.6±7.82</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>155±10.95</td>
<td>77.8±9.08</td>
<td>50.2±2.73</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.04±0.008</td>
<td>0.02±0.002</td>
<td>51.8±2.73</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.13±0.013</td>
<td>0.077±0.012</td>
<td>60.5±12.90</td>
</tr>
<tr>
<td>Total solid (g)</td>
<td>12.6±0.76</td>
<td>4.2±0.171</td>
<td>33±1.38</td>
</tr>
<tr>
<td>Ash (g)</td>
<td>0.8±0.82</td>
<td>0.5±0.025</td>
<td>65.7±3.81</td>
</tr>
</tbody>
</table>

1.3. Micellar casein concentrate (MCC): manufacturing and utilization in Greek style yogurt manufacture

In the dairy industry, caseinate ingredients are utilized in yogurt production to improve the texture and stability of the product. A significant improvement in the firmness, whey holding capacity, and sensory aspects was found in yogurt fortified with caseinate salts in several studies. (Akalın, et al, 2012, Modler, & Kalab, 1983, Damin, 2009). The increase in firmness was believed to be a result of increasing interaction
between calcium and sodium caseinate (SC) during storage. Compared with other common dry ingredients used in milk fortification, including whey protein concentrate (WPC) and skim milk powder (SMP), yogurts fortified with Sodium caseinate (NaCn) displayed better physical and sensory properties; a coarse, smoother and more compact protein network is formed. (Akalın, 2012) A correlation was found between the degree of casein micelle fusion with yogurt firmness and susceptibility to syneresis. In Modler and Kalab’s study (1983), the yogurt fortified with caseinates presented the highest gel strength of 117.9g and lowest susceptibility to syneresis of 11.9, as compared with 77.1 and 23.0 in milk protein concentrate (MPC) fortified yogurt, respectively.

Micellar casein (MCC) is a new dairy ingredient that has been gaining increasing popularity in recently years due to its functional properties such as good water-binding capacity, emulsifying and thickening properties. As utilizations, fat substitute in whitener formulations, fortification of foods or protein-rich beverages can be mentioned.

MCC is obtained by microfiltration (MF) of skim milk, which removes serum protein based on their difference in size from casein micelles (Walstra et al., 1999). Hurt et al. (2010) developed a process for the manufacture of MCC that using a uniform transmembrane microfiltration system. In the first stage, skim pasteurized skim milk was microfiltered to 3X retentate at 50°C, followed by cooling down to 4°C and overnight storage. The second day the retentate from the 1st stage was diluted with pasteurized reverse osmosis water in ratio of 1:2 and diafiltrated to obtain a 3X retentate. In the 3rd stage, the retentate from the 2nd stage was diluted again with pasteurized reverse osmosis water (1:2) and the diafiltration repeated to obtain a 3X retentate. The MCC can be used directly as a liquid or dried into a powder. In this study, MCC powder obtained by spray-
drying (at an atomizer speed of 23,000rpm, (inlet air temperature 200˚C, outlet air
temperature 95˚C).

It has been reported that MCC is able to retain its functionality during dehydration,
storage and reconstitution. Even though poor reconstitution ability was found in MCC
possibly due to a slow release of casein micelles from the powder particles, it was
believed that improvement could be achieved by increasing reconstitution temperature
under high shear rate mixing (Schokker, E. P, et al., 2011).

2. Materials and Methods

2.1. Sample preparations

2.1.1. Fortification of skim milk with MCC

Skim, HTST pasteurized milk was obtained from Crowley Food (Binghamton, NY).
The milk was heated up to 40°C and then added with MCC using a High-performance
dispersing mixer (Ultra-Turrax T25, IKA, NC, USA) at 3000 rpm for 30 min (see
Appendix 1.). MCC powder obtained as described by Hurt et al. (2010) was used to
fortify the skim milk. The amount of MCC powder was determined based on the target
final protein content. The MCC fortified milk was stored overnight (18-20 hours) at 4°C
for full hydration of the MCC powder.

A control sample (regular plain yogurt made without MCC fortification) was be also
prepared for comparison. The composition of the different yogurt milks is shown in Table
4.
Table 4. Sample compositions before straining

<table>
<thead>
<tr>
<th>Samples†</th>
<th>%Skim milk</th>
<th>%MCC</th>
<th>Culture</th>
<th>%Protein</th>
<th>%Total Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>0</td>
<td>0.025</td>
<td>0.25</td>
<td>3.28</td>
</tr>
<tr>
<td>A</td>
<td>96.2</td>
<td>3.8</td>
<td>0.028</td>
<td>0.17</td>
<td>6.56</td>
</tr>
<tr>
<td>(skim milk + 3.8% MCC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>94.2</td>
<td>5.8</td>
<td>0.032</td>
<td>0.19</td>
<td>8.2</td>
</tr>
<tr>
<td>(skim milk + 5.8% MCC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>92.3</td>
<td>7.7</td>
<td>0.036</td>
<td>0.21</td>
<td>9.84</td>
</tr>
<tr>
<td>(skim milk + 7.7% MCC)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The amount of MCC (m\text{MCC}) used for fortification was determined using a partial mass balance in protein, as shown below:

\[
\text{Control: } T_{\text{S control}} = P_{\text{control}} + \text{Fat}_{\text{control}} + \text{Carb}_{\text{control}}
\]

Fortified samples (A, B, C): \[ T_{\text{S sample}} = (\text{Fat}_{\text{control}} + \text{Carb}_{\text{control}})/ [1 + (P_{\text{sample}} - P_{\text{control}})] + P_{\text{sample}} \]

\[
m_{\text{milk}}*P_{\text{milk}} + m_{\text{MCC}}*P_{\text{MCC}} = (m_{\text{milk}} + m_{\text{MCC}})*P_{\text{sample}}
\]

- \( T_{\text{S control}} \): Total solids of unfortified sample (control), %
- \( T_{\text{S sample}} \): Total solids of MCC fortified sample (A, B and C), %
- \( P_{\text{control}} \): Protein content of unfortified sample (control), %
- \( P_{\text{sample}} \): Protein content of MCC fortified sample (A, B and C), %
- \( P_{\text{MCC}} \): Protein content of MCC, %
The chemical composition (solids content and protein) of the samples was determined by Dairy One (Ithaca, NY). The following values were determined:

\[
P_{\text{MCC}} = 87.93\%, \text{ Casein}= 74.74\%, \text{ Fat}_{\text{MCC}} = 2.11\%, \text{ Ash}= 9.47\%, \text{ Lactose}= 0.53\%
\]

\[
P_{\text{control}} = 3.28\%, \text{ Fat}_{\text{control}} = 0.2\%, \text{ Carb}_{\text{control}} = 5\%
\]

2.1.2. Inoculation and Fermentation

Prior to inoculation, all skim milk samples were heated up to 90°C for 5 minutes, on a hot plate (PC520 Hot Plate Magnetic Stirrer, Corning, NY) under mild agitation. Samples were immediately cooled down to 45°C in an ice-bath.

To prepare the starter, 18 g of unfortified skim milk was combined with 2g of Greek yogurt culture (Y-051054 Chr. Hansen), at 45°C. The cultured milk was set in incubator for 10min at 43°C, for culture activation. This starter was then used to inoculate the milk and fortified milk samples, at a concentration of 0.025g/g for the control and adjusted amount (0.02g/g * (Total solids\textsubscript{A/B/C}/Totla solids\textsubscript{control})) for the MCC fortified milk. The inoculated milk was gently stirred for 5 minutes at 43°C so that the culture was dissolved and evenly distributed. After that, all samples were fermented in an incubator (Fisher Scientific, PA, USA) at 43°C until pH reached 4.6.
2.1.3. Cooling, Mixing and Straining

Once the fermentation was completed, all samples were taken out from the incubator and cooled fast in an ice bath. A 30-second stirring by hand mixer (Sunbeam, Boca Raton, FL) with a single dough hook at speed 1 was applied to all samples. Straining was then conducted using cheesecloth placed in a glass funnel set on a flask. All samples (including control) were stored in the refrigerator at 4°C during the straining process.

2.1.4. Post-straining

The straining process was stopped at a different time for each sample based on the amount of whey that was supposed to be removed in order to reach the same final protein content, of 9.84%. This protein content was chosen based on the protein content of commercial Greek yogurt (Chobani Greek yogurt, 0% fat, Plain). The percent whey removal for the samples was 69%, 35%, 18% and 0% for control, sample A, B and C respectively. All Greek style yogurt samples were mildly stirred for 5 sec and stored in the refrigerator at 4°C until testing.
Table 5. Chemical composition of yogurt samples

<table>
<thead>
<tr>
<th></th>
<th>%Protein</th>
<th>%Total</th>
<th>%Protein</th>
<th>%Total</th>
<th>%Protein</th>
<th>%Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%P_whey</td>
<td>%TS_whey</td>
<td>%P_strained</td>
<td>%TS_strained</td>
<td>%P_whey</td>
<td>%TS_whey</td>
</tr>
<tr>
<td>Control¹</td>
<td>0.33</td>
<td>6.09</td>
<td>3.28</td>
<td>9.07</td>
<td>9.84</td>
<td>15.70</td>
</tr>
<tr>
<td>A</td>
<td>0.55</td>
<td>6.69</td>
<td>6.56</td>
<td>12.59</td>
<td>9.84</td>
<td>15.82</td>
</tr>
<tr>
<td>B</td>
<td>0.75</td>
<td>6.99</td>
<td>8.20</td>
<td>14.36</td>
<td>9.84</td>
<td>15.97</td>
</tr>
</tbody>
</table>

¹ Control: Yogurt fermented from skim milk; A, B, C: Yogurt fermented from skim milk fortified with 3.8%, 5.8%, 7.7% MCC, respectively

²: The amount of whey that was strained out

\[ \frac{m_{\text{unstrained}} \times \%P_{\text{unstrained}} - m_{\text{whey}} \times \%P_{\text{whey}}}{m_{\text{unstrained}} - m_{\text{whey}}} = \%P_{\text{strained}} \]

³: \( \%P_{\text{whey}} \)

⁴: \( \%TS_{\text{whey}} \)

⁵: Results from Dairy One, on average of 3 batches of measurement

\[ \frac{m_{\text{unstrained}} \times \%P_{\text{unstrained}} - m_{\text{whey}} \times \%P_{\text{whey}}}{m_{\text{unstrained}} - m_{\text{whey}}} = \%P_{\text{strained}} \]

\[ \frac{m_{\text{unstrained}} \times \%TS_{\text{unstrained}} - m_{\text{whey}} \times \%TS_{\text{whey}}}{m_{\text{unstrained}} - m_{\text{whey}}} = \%TS_{\text{strained}} \]

\( P_{\text{unstrained}} \) - Protein content of yogurt sample before straining, %

\( TS_{\text{unstrained}} \) - Total solids of yogurt sample before straining, g

\( m_{\text{unstrained}} \) - Weight of yogurt sample before straining, %

\( m_{\text{whey}} \) - Weight of whey strained out, g

\( P_{\text{strained}} \) - Protein content of yogurt sample after straining, %

\( TS_{\text{strained}} \) - Total solids of yogurt sample after straining, %
2.3. **pH**

The pH was measured using pH meter (XL20, Fisher Scientific, PA, USA). The pH analysis was repeated twice for each sample. Values were based on the averages of 5 samples.

2.4. **Titratable Acidity**

Titratable acidity was measured by titration and expressed as percentage lactic acid. Five grams of curd was diluted with 10 ml distilled water and titrated with 0.1 N NaOH using phenolphthalein (0.1\%) as an indicator, at room temperature, to an end-point of faint pink color.

\[
\text{Titratable Acidity (wt/wt) } = \frac{V_{(\text{NaOH})} \times N_{(\text{NaOH})} \times MW_{(\text{lactic acid})}}{(V_{\text{sample}} \times 1000)}
\]

\[
MW_{(\text{lactic acid})} = 90 \text{g/mol}
\]

\[
V_{(\text{NaOH})} \quad \text{Volume of NaOH, mL}
\]

\[
N_{(\text{NaOH})} \quad \text{Normality of NaOH}
\]

\[
MW_{(\text{lactic acid})} \quad \text{Molecular weight of lactic acid}
\]

\[
V_{\text{yogurt}} \quad \text{Volume of yogurt Sample (Control, A, B, C), mL}
\]

2.5. **Water-Holding Capacity**

The water-holding capacity (WHC) was determined by separating the free whey from the sample after centrifugation, using a method adapted from Remeuf et al. (2003). Briefly, 10g of yogurt from each sample were centrifuged (RC-5B, Sorvall, OH, USA) for 15 min at 5500rpm and 4°C. The liquid whey was separated and weighed. The water-holding capacity (WHC, g/ kg) was calculated as:

\[
\%\text{WHC} = \frac{(m_{\text{yogurt}} - m_{\text{whey}})}{m_{\text{yogurt}}} \times 100
\]
m_{yogurt} – Weight of yogurt sample, g
m_{whey} – Weight of whey separated from yogurt sample after centrifugation, g

The experiment was repeated three times and the data analyzed statistically.

2.6. Texture Profile Analysis (TPA)
   The hardness of the yogurt samples was conducted using TA.XT2 Texture Analyzer (Texture Technologies Group, Scarsdale, NY) equipped with a 5 kg load cell. The firmness was measured as the maximum force during first compression cycle by using the PO.5S ½” spherical stainless probe. Measurements were conducted at 10± 0.5°C. Each test was replicated three times.

2.7. Viscosity
   Viscosity was measured by a Brookfield viscometer (Brookfield Engineering Laboratories, Inc., MA, USA), with spindle LV4, at 5 rpm. All viscosity measurements were replicated three times.

2.7. Statistical analysis
   All results were based on 5 processing replicates, with 2 analytical replicates for the measurements of pH and TA analysis, and 3 analytical replicates for measurements of WHC and TPA.

   Statistical analysis was used to determine the significance of protein fortification on Greek yogurt quality. The data obtained were processed by one-way ANOVA using the JMP software (version Pro 9.0.2, SAS Institute Inc., Cary, NC). The means were compared with Turkey’s HSD test at the $P < 0.05$ level.
3. Results and Discussions

3.1. Fermentation profile

During the first 2 hours of fermentation, the pH dropped faster for the higher MCC content samples, which was possibly caused by a higher amount of culture (in terms of weight) (Figure 3). The rate of decrease in pH was accelerated during the 3rd and 4th hour fermentation in the unfortified sample, while it remained constant or dropped slightly in all fortified samples. The higher proportion of MCC was added, the bigger influence on the acidification rate was noticed. At the end of the 4th hour of fermentation, no significant difference in pH was shown among the fortified samples.

Towards the end of the fermentation, after about 4 hours, the slower decrease in pH change in control sample suggested the occurrence of pH buffering. It was found before that the production of acid by lactic acid bacteria (LAB) increases the buffering in the vicinity of pH 5 in regular yogurt, which slows down the pH drop and provides a proper environment for growth of LAB (Lucey, 2004). Also, it was found that skim milk with high solids had less pH reduction but with higher titratable acidity (TA) once the pH reached 5.0, also due to buffering (Collins, 1951).
The observed changes in pH might suggest that stronger buffering took place in MCC fortified samples, as they all slowed down during the 3rd and 4th hour fermentation. This could be a result of a higher buffering capacity of casein, in comparison with the whey protein (Akalin, et al, 2012).

Wilkowske (1953) pointed out that to reach the same TA value, the pH would be higher from milk with high solids than that from milk with lower solids (Figure 4). This explains the pH value of each sample at the end of fermentation. For example sample C, which had highest solids during fermentation, had a significantly higher pH as compared with the control sample.
Figure 4. The relationship of TA and pH in reconstituted nonfat milk of various concentrations (Wilkowske, 1953).

Titratable acidity (TA) measures the level of lactic acid in the sample (Figure 5.). A higher TA was shown in sample with higher level of MCC fortification. This agreed with Wilkowske’s (1953) finding that the apparent acidity of reconstituted skim milk increases as the percentage of solids increases.

A positive correlation between MCC content and TA was found for all samples. The higher level of MCC fortification, the faster and more acid was formed in the sample. A significant difference in levels of TA was found between control and the MCC fortified samples, and also between sample A and C before straining. However, no significant difference in TA existed after the samples were stained when they had reached the same level of protein content. This suggested a possible correlation between the protein content and TA.
Figure 5. The effect of MCC fortification method on the titratable acidity of Greek style yogurt

Control: Yogurt fermented from skim milk; A, B, C: Yogurt fermented from skim milk fortified with 3.8%, 5.8%, 7.7% MCC, respectively

A high acid-buffering capacity (BC) of Labneh (strained yogurt) was found by Al-Dabbas et al. (2011). In that study, the Labneh sample was titrated with HCl to reach pH 1.5, and then titrated back to its original pH by NaOH. The BC was determined by dividing the TA over the change in pH from its original value to 1.5. Casein proteins are composed of amino acids including Glutamic acid (20.2%) and Aspartic acid (6.4%). It was believed that the presence of high level of glutamic and aspartic acid led to a higher BC by maintaining the acid-base equilibrium. The high level protein content was associated with the formation of the casein network, which trapped constituents such as organic acids, salts and CCP, thus increased the BC (Al-Dabbas et al., 2011).

In this study, before straining, the TA was largely dependent on the protein content which resulted in different BC. Samples with a higher protein content showed higher TA,
possibly due to higher BC. After straining, all samples’ protein contents were expected to be at the same level. The TA reached the same level as the samples were concentrated by straining.

3.2. Water Holding Capacity (WHC)

Syneresis is a significant defect of yogurt (Lucey, 2002). WHC, which is determined by measuring the quantity of acid whey expelled from the gel after centrifugation, was used to evaluate the syneresis potential of yogurt.

As it showed in the Table 6, WHC was the highest in the control sample, and decreased as the MCC level increased. In other studies, WHC varied on the basis of two parameters: (1) the moisture content of the product; (2) the water binding capacity. In the current work, there was a significant difference in moisture content among control, A, B and C, because the acid whey, which contained 93-94% water, was removed from the sample in a different degree (from 69% in the control to 0% in the sample with the highest level of MCC fortification).

In this case, the ratio of mass of the sample after centrifugation and before straining was calculated. According to the results, only 20.46% (w/w) of the control sample remained after centrifugation, while it doubled in sample with 5.8% MCC fortification and almost 50% of sample C remained in the end. This ratio indicated a significant increase in water binding capacity of the product by fortification of MCC. It was well documented in other studies that the addition of milk protein concentrate significantly decreases the syneresis indices of yogurt (Akalin, 2012, Isleten & Karagul-Yuceer, 2006).
Besides contributing to improved texture and product stability, the increased water binding capacity also suggests a reduction in cost per unit product.

3.3. The texture properties of MCC fortified Greek style yogurt

The gel strength is one of the attributes of yogurt texture. As shown in table 6., the firmness and adhesiveness decreased as the MCC fortification level increased, and less acid whey was strained away. There was significant difference in terms of firmness and adhesiveness between control and the MCC fortified yogurt, while there was no significant difference among fortified sample with different MCC content.

Several studies showed that a significant increase in firmness was found in yogurt that was fortified with milk protein concentrate. In this study, since fortification was conducted with casein only, the lower ration of whey protein to casein likely resulted in a lower level of cross-linking between κ-casein and β-lactoglobulin, which is known to increase gel strength and water holding capacity of yogurt.

Another reason for the difference in firmness may be the significant difference in pH of yogurt after straining. It has been observed before that a decrease of pH from 4.50 to 3.85 resulted in an increase of 20% of gel hardness of yogurt. It was assumed that higher intra-micellar repulsion was created by the positive charge of caseins under low pH, below their isoelectric point. As a result, the size of casein particles increased and a more rigid gel structure was formed (Sodini, 2004).
There was no significant difference in viscosity among the fortified samples. The viscosity of the control could not be measured since the maximum measuring capacity of the viscometer was exceeded.

It has been reported that firmness of yogurt dependents on various factors including total solids content, culture composition and type of protein used for fortification (Oliveira et al, 2001). In this study, the casein content could be the main factor that determined the hardness and adhesiveness of the yogurt.
Table 6. Effects of MCC fortification method on chemical and physical properties of Greek style yogurt

<table>
<thead>
<tr>
<th>Samples</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Straining</td>
<td>4.49± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.64±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.70±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.73±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>After Straining</td>
<td>4.53±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.83±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.89±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.94±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Titratable Acidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Straining</td>
<td>0.95±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.16±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.28±0.10&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.36±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>After Straining</td>
<td>1.36±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.31±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.32±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>%Water Holding Capacity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66±4.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54±3.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51±3.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48±3.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 %m&lt;sub&gt;unstrained&lt;/sub&gt;/ m&lt;sub&gt;centrifuged&lt;/sub&gt;</td>
<td>20.46</td>
<td>35.10</td>
<td>41.80</td>
<td>48.00</td>
</tr>
<tr>
<td>Texture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>0.58±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.06±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adhesiveness (-g*s)</td>
<td>122.84±27.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.57±2.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.61±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.99±3.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Viscosity mPs (cP)</td>
<td>N.A.*</td>
<td>14027±5610&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7818±1515&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8499±2723&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Control: Yogurt fermented from skim milk; A, B, C: Yogurt fermented from skim milk fortified with 3.8%, 5.8%, 7.7% MCC, respectively

<sup>2</sup>%m<sub>unstrained</sub>/ m<sub>centrifuged</sub> The mass ratio of yogurt before straining over yogurt after centrifugation

<sup>abc</sup>: Means ± standard deviation in each row with the different superscripts are significantly different P<0.05

N.A.*: The viscosity of control sample exceeded the maximum measurement capacity
4. Conclusions

An alternate process for the manufacture of Greek style yogurt was developed by using micellar casein concentrate to fortify the milk at the desired protein concentration in the final product. Fortification of yogurt with MCC significantly changed the pH, acidity, WHC and textural properties of the final product. The increased total solids resulted in a higher buffering capacity of milk during fermentation, thus the final product presented a higher pH value at the same titratable acidity compared to the control. The MCC fortification significantly improved the WHC of the yogurt, but it resulted in poor gel strength. In this study, the MCC used was produced a year and half before the experiment. A significant loss reconstitution ability of spray dried MCC was shown during storage of dry MCC. Using fresh MCC could increase the reconstitution ability of MCC thus improve the physical and flavor profile of the yogurt.

Besides the chemical and physical properties of the yogurt, sensory tests would be necessary in order to evaluate the flavor profile and customer acceptability of the MCC fortified yogurt, immediately after production and during storage. A previous study found that the phosphoserine residues on casein are able to bind with calcium to prevent the depletion flocculation, thus increasing the firmness of yogurt (Dickinson et al., 2003). This suggests the potential texture development of the MCC fortified yogurt during storage.
In general, it was challenging to replace the straining process completely by MCC fortification and obtain a product with similar quality characteristics as traditional Greek yogurt. The overall properties of the yogurt were significantly different even with the same level of protein. Nonetheless, the developed alternate method was possible to reduce the amount acid whey strained during the process. This could represent a huge benefit for the Dairy Industry, by removing the stream of acid whey, which is becoming an increasing problem for the industry and potentially a burden for the environment.
5. References:


Hurt, E., Zulewska, J., Newbold, M., & Barbano, D. M. (December 01, 2010). Micellar casein concentrate production with a 3X, 3-stage, uniform transmembrane
pressure ceramic membrane process at 50°C. *Journal of Dairy Science*, 93, 12, 5588-5600.


Mutz-Darwell, S., Yurgec, M. & Fox, K. (2012), Achieve rich, creamy texture in Greek-style yogurt with a cost effective approach


30


Remeuf, F., Mohammed, S., Sodini, I., & Tissier, J. P. (January 01, 2003). Preliminary observations on the effects of milk fortification and heating on
microstructure and physical properties of stirred yogurt. *International Dairy Journal*, 13, 9, 773-782.


York, E. B. (March 16, 2012) Greek Yogurt Causes a Stir with Triple Digit Gains, *Chicago Tribune*
