Effect of Draining Volume on the Yield and Enrichment Ratio During Foam Fractionation of Greek Yogurt Whey

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Abstract
Foam fractionation was evaluated as a cost-effective method to add value to Greek yogurt whey (GYW), a co-product of Greek yogurt manufacturing. Two separate batches of GYW were obtained from a Greek yogurt manufacturer. Whey proteins present in GYW can be concentrated and manufactured as food ingredients using a low-cost foam fractionation. The objective of this study was to apply foam fractionation with different draining volumes to GYW and evaluate its enrichment and yield of whey protein. A benchtop foam fractionation setup was built in-house, and three different foam draining volumes were used to identify the optimal processing parameters for foam fractionation of GYW. All three levels of draining volume provided enrichment of whey protein in the foamate fraction, and the 33% draining volume resulted in the highest enrichment ratio of 1.59. The yield of the foam fractionation ranged from 41 to 52%, and several improvements can be implemented to increase the yield of the process, including the use of surfactants and enzymatic hydrolysis.

Keywords
whey protein, enrichment, novel methods, valorization

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Summary
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Introduction
The production of Greek-style yogurt in the U.S. market has increased to 770,000 metric tons in the year 2016. Greek yogurt is generally manufactured by removing a portion of the water and water-soluble components from yogurt resulting in GYW as a co-product. Greek yogurt whey is a dilute aqueous solution containing lactose, proteins, minerals, non-protein nitrogen compounds, and organic acids. The characteristic low pH and high mineral content of GYW make it difficult to process using normal dairy unit operations. In addition, Greek yogurt whey is difficult to process using traditional processing methods since it contains a relatively high amount of lactic acid and galactose. GYW. Greek yogurt whey, unlike cottage cheese whey, is lower in value due to its lower protein and lactose contents. Current disposal methods for GYW include land application, bioreactors, and animal feed, but each of these methods has its own limitations. A low-cost value addition process for fractionating the valuable components from GYW is needed.

Recently, researchers have developed a method to enzymatically convert lactose in GYW into glucose and galactose to produce a syrup-like ingredient. The new ingredient can be used as a replacement for high-fructose corn syrup, an ingredient that is essential
for the beverage industry. Also, various fermentation techniques have been examined to produce energy or health-promoting compounds and antimicrobials, such as organic acids.

While most of the current research in on the area of utilization of GYW has focused on lactose hydrolysis, the protein content in GYW can also be a component of interest due to the growing market for whey proteins. Because of the low protein concentration and high acidity, concentrating protein from acid whey using membrane processing would lead to long processing time and high energy input, making the process unsustainable for industrial production. One alternative protein concentration technique is foam fractionation. Foam fractionation is a process in which surface-active materials are removed by air flotation to form foam. Using the density difference between the bulk liquid and the foam, the protein-enriched foam can be separated gravimetrically to produce foamate (collapsed foam) with increased protein content. The objectives of the study were to design and develop a novel foam fractionation system and evaluate the efficiency of protein enrichment in GYW at different draining volumes.

**Experimental Procedures**

Two batches of GYW were procured from a commercial Greek yogurt manufacturer within the United States. The GYW was shipped frozen to Kansas State University and thawed completely before use. The foam fractionation setup was designed and fabricated in the Dairy Foods Laboratory of Kansas State University and a schematic view is shown in Figure 1. The foam fractionation setup consisted of a reservoir (1 liter), a diaphragm pump, a venturi-type air injector, a draining column, and a foamate outlet. In this study, three different draining column volumes were used to evaluate the efficiency of the foam fractionation. Three levels (low, medium, and high) of draining volume included 25% (0.25 liters), 33% (0.33 liters), and 50% (0.50 liters) of the reservoir volume. The GYW was recirculated within the reservoir using the pump via the air injector to incorporate air in the GYW as it was pumped, and consequently creating foam. As the draining column was completely filled with foam and overflowed, the draining foam was collected in a beaker. The foam fraction collected in the beaker is referred to as foamate and contains more surface-active compounds (such as protein) than the initial GYW. The foam fractionation was terminated when there was no foam coming out of the draining column for 5 min. The foamate and retentate were collected separately, weighted, and analyzed for protein content.

The total protein content of GYW, foamate, and retentate was analyzed using the Kjeldahl method. The protein concentration was calculated using the nitrogen conversion factor of 6.38. Enrichment ratio and protein yield (%) were used to evaluate the foam fractionation efficiency and calculated using equations 1 and 2, respectively.

\[
\text{Yield, \%} = \frac{\text{Mass of protein in the foamate}}{\text{Mass of protein in the GYW}} \times 100 \quad [1]
\]

\[
\text{Enrichment ratio} = \frac{\text{Concentration of protein in the foamate}}{\text{Concentration of protein in the GYW}} \quad [2]
\]
Results and Discussion

Two lots of GYW were analyzed by using the Kjeldahl method for average protein content, and results are provided in Figure 2. The GYW protein concentration served as the reference to examine foam enrichment of protein in the foamate. As shown in Figure 2, after foam fractionation, the protein concentration in foamate was greater than the retentate, showing an enrichment of protein in the foamate fraction. The results supported the hypothesis of this study that foam fractionation can enrich protein from GYW. Other studies have shown that a foam fractionation process is more efficient at low protein concentrations, ranging from 0.01 to 0.1%. Therefore, GYW may have the advantage to better utilize foam fractionation compared to sweet whey, which contains more protein and is consequently less efficient for foam fractionation. Also, the low pH of GYW can also promote foam fractionation, as some research has shown that extracting proteins close to their isoelectric point enhances the foamability of the proteins. Greek yogurt whey contains alpha-lactalbumin and beta-lactoglobulin, and the two proteins have isoelectric points of 4.4 and 5.2, respectively, which is close to the pH of the GYW generated from Greek-yogurt production.

The protein concentration in the foamate was found to be different based on the draining volume, and both medium and high draining volume showed significant differences in protein concentration compared to the original GYW ($P < 0.05$). The greatest difference appeared in the medium draining volume, indicating it was the optimal for protein enrichment. Table 1 shows that the final foamate volume is similar among three different draining volumes, meaning that the amount of foamate generated from GYW may be related to the protein type and concentration, rather than the draining volume. However, in future studies, the amount of foamate generated from acid whey should be studied as it can impact the final yield of the protein enrichment in the foamate. Possible factors that may impact foamate generation include surfactants, protein concentration, and the pH of the acid whey.

We also observed that the medium draining volume had the greatest enrichment ratio of 1.59, followed by 1.33 from high draining volume and 1.11 from low draining volume (Table 1). The enrichment ratio is another representation of the protein concentration shown in Figure 2. It indicated that foam fractionation can increase the protein concentration in foamate by 1.59 times. Compared to other studies with acid whey, the enrichment ratio was within the reported ranges. The direct fractionation of GYW without pretreatment made the technique robust and easy to adapt in existing processing plants. For future work, lactose content in the foamate can be measured to determine the purity of the enriched whey protein. With relatively high purity whey protein, the protein enriched fraction can be further processed into usable food ingredients, such as protein powders.

The percent yield and enrichment ratio from the foam fractionation of GYW is provided in Table 1. The medium draining volume (33%) gave a yield of 52.1%. The yield from this study was not as high as some other foam fractionations of whey protein. However, use of some surfactants in the feed could potentially increase the yield. The surfactants can interact with both protein and air, and consequently can improve the fractionation yield. Several studies have used food-grade surfactants to increase the yield of foam fractionation. Another method to increase yield could be enzymatic hydrolysis.
Similar to the isoelectric point approach, partial hydrolysis of the whey protein can unfold the protein and increase the structure flexibility. It has been shown that whey protein hydrolysate can have improved foamability compared to the unhydrolyzed whey protein. The modification of whey protein in GYW can potentially improve the fractionation yield.

**Conclusions**
In this study, GYW was foam-fractionated to enrich whey protein concentration. The foam fractionation of GYW enriched the protein content in the foamate. With different draining volumes, the medium (33%) draining volume showed the best enrichment ratio (1.59) and yield (52.1%). The technique provides a novel processing method to add value to GYW into potential food ingredients. Improvement of fractionation yield and purity of protein in the foamate should be further studied.

**Table 1. Final foamate volume, enrichment ratio, and yield of protein from foam fractionation of acid whey using different draining volumes**

<table>
<thead>
<tr>
<th>Draining volume</th>
<th>Foamate volume (mL)</th>
<th>Enrichment ratio</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>326</td>
<td>1.11</td>
<td>42.8</td>
</tr>
<tr>
<td>Medium</td>
<td>309</td>
<td>1.59</td>
<td>52.1</td>
</tr>
<tr>
<td>High</td>
<td>298</td>
<td>1.33</td>
<td>40.9</td>
</tr>
</tbody>
</table>
Figure 1. Schematic representation of the foam fractionation setup. A: acid whey reservoir (1 L); B: external pump; C: venturi-type injector; D: draining column; and E: foamate outlet.
Figure 2. Protein concentration changes after foam fractionation with different draining volumes. The asterisk indicates the significant protein concentration increase in foamate within each draining volume (n = 2, P < 0.05).