**Effect of Acidulant Addition on Yogurt Fermentation**

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**Summary**

Yogurt was manufactured by pre-acidifying the yogurt mix with citric acid, lactic acid, or concentrated lemon juice either before or after pasteurization to a target pH of 6.2, and then the traditional manufacturing process was continued. Adding citric acid or lemon juice to the yogurt mix after pasteurization resulted in a 13% reduction in fermentation time compared with the control. This reduction in fermentation time may result in greater efficiency for yogurt manufacturers, allowing for a more sustainable manufacturing process.

**Introduction**

In recent years, some consumers have expressed interest in purchasing products that have minimal impact on the environment, society, and the economy, which in short, is a definition of sustainable products. Consumers actively seek both food and nonfood sustainable products in the marketplace. In a recent survey, 50% of consumers responded that food products should contain labels that reflect their foods’ impact on the environment and that they would buy food products that had less impact on the environment, provided that food costs did not increase. In the same study, however, only a small percentage of consumers viewed themselves as “most responsible” for their carbon footprint, instead placing most of the responsibility to reduce carbon footprints on manufacturers, government, and retailers. This insight into the consumer mindset leads to a basic conclusion: consumers expect products that fit the sustainable profile in the marketplace; thus, processors and producers should meet this need.

Yogurt is a growing food category; sales increased 6.5% from 2004 to 2005. Interestingly, sales of natural and organic yogurt accounted for much of this growth. In a comparison of the environmental life cycle of dairy products, yogurt had the second greatest energy consumption per ton, only behind cheese, with 25% of the energy usage attributed to the manufacturing process. Fermentation generally is the rate-limiting step in yogurt manufacture because typical fermentation times can range from 2 to 6 hours for the bacteria to reduce the yogurt mix pH from 6.4 to 4.6 (legal definition of yogurt). If fermentation time could be reduced, companies might reduce the amount of non-renewable resources, such as energy and water, required to produce a unit of yogurt. Thus, yogurt manufacture, particularly yogurt fermentation, is an excellent model for studying sustainable practices in a processing facility.

Adding the enzyme β-galactosidase to yogurt mix decreased fermentation time decreased while increasing yogurt digestibility and sweetness. Other researchers combined an external acidulant, glucano-delta lactone, with the starter cultures to decrease fermentation time by about 30%, but the resulting yogurt was firmer and exhibited more syneresis (i.e., separation of liquid from the gel). Adding ingredients such as casein hydrolysates, whey protein concentrates, and fructooligosaccharides to yogurt mix stimulated the growth of probiotic bacteria in yogurt and decreased fermentation time by 50%.

Other researchers developed a protocol of adding a concentrated mother culture (15% starter cultures) to the yogurt mix and reported that the resulting yogurt was similar to one made traditionally but the fermentation time was 50% less. General Mills, Inc. was issued a patent that
described the process for directly acidifying yogurt when the pH reaches 4.8 to 5.2. Yogurt was successfully produced by adding a variety of acidulants such as citric acid, lactic acid, malic acid, gamma delta lactone, tartaric acid, and combinations thereof during the fermentation process. The yogurts had quality similar to that of yogurt made by the traditional process, but the fermentation time was reduced by 50%. This patent focused on minimizing the lag phase of the starter cultures by adding an acidulant (or acidulants) to quickly decrease pH to 4.6.

Reducing fermentation time has several advantages for yogurt producers including decreased labor cost, increased production efficiency, and more importantly, decreased non-renewable resource usage. The objective of this research was to determine a method of manufacturing yogurt in less time by pre-acidifying the mix with citric acid, lactic acid, or lemon juice, to jump-start acid production in the inoculated yogurt mix.

**Experimental Procedures**

To make yogurt mix, 13.5% nonfat dry milk was reconstituted in water, rehydrated overnight, pasteurized at 194°F for 10 minutes and quickly cooled to 10°F within 10 minutes. Yogurt mixes were acidified with citric acid, lactic acid, or lemon juice (80 ppm) either before or after pasteurization to a target pH of 6.2 ± 1. A direct set culture of 2:1 *Streptococcus thermophilus* (ST) and *Lactobacillus delbruekii* ssp. *bulgaricus* (LB) was added at 0.2%. Addition of ST and LB was considered to be the start of fermentation.

Yogurt mixes were fermented in a BioFlo 3000 (New Brunswick Scientific Co., Edison, NJ) fermentation unit until a pH of 4.6 and 0.9% titratable acidity were reached as per federal regulations. To monitor the fermentation process, pH and titratable acidity were measured every hour starting at inoculation following standardized methods. Microbiological analyses for ST and LB were assayed 10 minutes after inoculation and at the end of fermentation following standard methods. Seven treatments were prepared and 3 replications were done. As the experiment was designed to include a control, data were analyzed using procedure GLM and Dunnett’s method in SAS 9.1 (SAS Institute Inc., Cary, NC) to determine differences from the control.

**Results and Discussion**

Table 1 presents the results for fermentation time and LB and ST counts. Fermentation time was significantly affected by the acidulant treatment as well as by when the acidulant was added (before or after pasteurization). Neither citric acid nor lemon juice added before pasteurization affected fermentation time, but adding citric acid or lemon juice after pasteurization decreased fermentation time by 13.4% and 13.6%, respectively.

There were no differences in ST or LB counts expressed as colony forming units (cfu) per milliliter at the beginning or end of fermentation for any treatments, indicating that the experimental treatments did not affect the microbial populations compared with the control. This could be important to a yogurt manufacturer who uses the Live & Active Cultures seal, a program assuring that yogurt contains 10⁶ bacteria per milliliter at the time of manufacture. The experimental treatments did not affect the viability of starter cultures and would meet the Live & Active Cultures requirements.

Adding citric acid or lemon juice to yogurt mixes after pasteurization resulted in shorter fermentation times and a more efficient yogurt manufacturing process. Changes in titratable acid
and pH during fermentation were very similar to the control yogurt, with the exception of citric acid and lemon juice added after pasteurization (Figure 1). Yogurt mixes that were pre-acidified with citric acid or lemon juice after pasteurization developed acidity at an accelerated rate compared with control yogurt.

Lactic acid addition either before or after pasteurization resulted in a longer fermentation time than the control. Lactic acid is the result of lactose fermentation by ST and LB, and its external addition slowed fermentation in this study. This could be due to the low acid tolerance of ST, which may have prevented ST growth during the early phases of fermentation.

Overall, the order of acidulant addition affected fermentation time. Researchers have reported that if yogurt mix is pasteurized at pH <6.55, the whey proteins tend to self-aggregate rather than aggregate with the casein proteins during heat treatment. Perhaps the self-aggregation process of the whey proteins inhibited some of the proteolytic reactions necessary to induce the desired symbiotic relationship between ST and LB that allows for accelerated fermentation.

In summary, fermentation time could be reduced by adding certain acidulants to yogurt mix after pasteurization. It is important to note that although the fermentation time decreased about 45 minutes and the pre-acidification process required an additional 2 minutes, an overall reduction in the time required to manufacture yogurt was demonstrated. Decreasing the fermentation time of yogurt while maintaining desirable sensory and physiochemical properties, should appeal to both yogurt manufacturers and consumers seeking sustainable products. A decreased process time could potentially save energy, thus reducing the carbon footprint of the yogurt manufacturer. Further testing is warranted to ensure that the functional and sensory properties of these yogurts are not adversely affected.
Table 1. Average fermentation times and counts of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*

<table>
<thead>
<tr>
<th>Acidulant treatment</th>
<th>Addition order</th>
<th>Fermentation, hours</th>
<th>Start of fermentation</th>
<th>End of fermentation</th>
<th>Start of fermentation</th>
<th>End of fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>5.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.83</td>
<td>7.62</td>
<td>7.78</td>
<td>7.77</td>
</tr>
<tr>
<td>Citric</td>
<td>Before</td>
<td>5.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.23</td>
<td>7.14</td>
<td>7.55</td>
<td>7.48</td>
</tr>
<tr>
<td>Lemon</td>
<td>Before</td>
<td>5.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.93</td>
<td>7.00</td>
<td>7.77</td>
<td>7.96</td>
</tr>
<tr>
<td>Lactic</td>
<td>Before</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.15</td>
<td>7.22</td>
<td>7.65</td>
<td>7.72</td>
</tr>
<tr>
<td>Citric</td>
<td>After</td>
<td>4.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.81</td>
<td>7.54</td>
<td>7.68</td>
<td>7.54</td>
</tr>
<tr>
<td>Lemon</td>
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<td>4.66&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>6.98</td>
<td>7.78</td>
<td>7.65</td>
</tr>
<tr>
<td>Lactic</td>
<td>After</td>
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<td>6.46</td>
<td>6.52</td>
<td>7.90</td>
<td>7.68</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Means having different superscript letters differ (\(P < 0.05\)).

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**Figure 1.** pH development during fermentation of yogurt samples with acidulant added after heat treatment.