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Consumer acceptance of cysteine-enhanced yogurt

Abstract
Within cells, cysteine can be synthesized from methionine by the enzyme \( \Gamma^3 \)-cystathionase. Cysteine is then utilized to synthesize glutathione, which has many functions in cells that contribute to good health. Certain subpopulations, however, especially the elderly, have decreased \( \Gamma^3 \)-cystathionase activity. Thus, dietary cysteine may be beneficial in maintaining health. In previous reports, a yogurt with enhanced cysteine content was made by incorporating whey protein isolate (WPI) into the mix’s dairy base combined with a process treatment to minimize cysteine denaturation. The gel quality of this yogurt matched or exceeded that of a yogurt that was formulated and processed to mimic an industrially manufactured product. More importantly, the cysteine content was 3 times greater, and the gel quality was stable throughout a 60-day shelf life. With this evidence, the question remained whether the flavor of an enhanced cysteine yogurt would be acceptable. Because addition of whey-based products in yogurt has been reported to affect flavor and texture properties, this study was undertaken to determine consumer liking of a high-cysteine yogurt. Formulas were adjusted to contain sugar and vanillin, and these mixes were processed to produce high-cysteine and low-cysteine yogurts. Yogurts were stored at 4°C for 1 week, then evaluated by a group of 119 consumers. Consumers rated yogurts based on their liking of appearance, thickness, flavor, aftertaste, and overall acceptability using a 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9). Overall, consumers rated the high- and low-cysteine yogurts similarly for flavor (6.1), aftertaste (6.1), and overall acceptability (6.3), with mean scores corresponding to “like slightly” to “like moderately.” Consumers liked the thickness of the high-cysteine yogurt more than the low-cysteine yogurt but liked the appearance of the low-cysteine yogurt more than the high-cysteine yogurt. The high-cysteine yogurt had approximately 3 times more cysteine than the low-cysteine yogurt. These results indicate that a high-cysteine yogurt may be a useful and acceptable food system to provide dietary cysteine.; Dairy Day, 2013, Kansas State University, Manhattan, KS, 2013; Dairy Research, 2013 is known as Dairy Day, 2013

Keywords
Dairy Day, 2013; Kansas Agricultural Experiment Station contribution; no. 14-179-S; Report of progress (Kansas Agricultural Experiment Station and Cooperative Extension Service);1093; Yogurt; Cysteine; Consumer preference

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Consumer Acceptance of Cysteine-Enhanced Yogurt

S. Bala and K.A. Schmidt

Summary
Within cells, cysteine can be synthesized from methionine by the enzyme γ-cystathionase. Cysteine is then utilized to synthesize glutathione, which has many functions in cells that contribute to good health. Certain subpopulations, however, especially the elderly, have decreased γ-cystathionase activity. Thus, dietary cysteine may be beneficial in maintaining health.

In previous reports, a yogurt with enhanced cysteine content was made by incorporating whey protein isolate (WPI) into the mix’s dairy base combined with a process treatment to minimize cysteine denaturation. The gel quality of this yogurt matched or exceeded that of a yogurt that was formulated and processed to mimic an industrially manufactured product. More importantly, the cysteine content was 3 times greater, and the gel quality was stable throughout a 60-day shelf life. With this evidence, the question remained whether the flavor of an enhanced cysteine yogurt would be acceptable. Because addition of whey-based products in yogurt has been reported to affect flavor and texture properties, this study was undertaken to determine consumer liking of a high-cysteine yogurt. Formulas were adjusted to contain sugar and vanillin, and these mixes were processed to produce high-cysteine and low-cysteine yogurts. Yogurts were stored at 4°C for 1 week, then evaluated by a group of 119 consumers. Consumers rated yogurts based on their liking of appearance, thickness, flavor, aftertaste, and overall acceptability using a 9-point hedonic scale ranging from dislike extremely (1) to like extremely (9). Overall, consumers rated the high- and low-cysteine yogurts similarly for flavor (6.1), aftertaste (6.1), and overall acceptability (6.3), with mean scores corresponding to “like slightly” to “like moderately.” Consumers liked the thickness of the high-cysteine yogurt more than the low-cysteine yogurt but liked the appearance of the low-cysteine yogurt more than the high-cysteine yogurt. The high-cysteine yogurt had approximately 3 times more cysteine than the low-cysteine yogurt. These results indicate that a high-cysteine yogurt may be a useful and acceptable food system to provide dietary cysteine.

Key words: yogurt, cysteine, consumer preference

Introduction
Yogurt popularity and sales continue to grow in the U.S. Yogurt consumption is influenced by many factors, but its nutritional profile and health benefits are most prominent. Whey proteins in milk are rich sources of the sulfur-containing amino acids, such as cysteine and methionine, and yogurt has the potential to be one of the best dietary sources of cysteine. Heat treatments in excess of 70°C denature whey proteins and decreases the bioavailability of some amino acids, cysteine in particular. High heat treatment conditions are desirable in yogurt, because denatured whey proteins contribute firmness and water-holding ability to the gel.

Recent reports from the Centers for Disease Control and Prevention indicate that 20.1 million Americans 40 years and older have cataracts and forecast that this number will increase to 30.1 million by 2020. Research has shown that as mammals age, the enzyme γ-cystathionase (which converts methionine to cysteine) tends to diminish, and the loss of this enzyme in the eye lenses has been implicated in cataract formation. All of these data indicate that people prone
to cataracts may benefit from consumption of dietary cysteine as a means to compensate for diminished γ-cystathionase activity.

We previously reported (Dairy Research 2012, pp 29-34) that yogurt with an elevated cysteine content could be made by formulating a yogurt mix with nonfat dry milk (NDM) and whey protein isolate (WPI) and using a heat treatment that assured pasteurization and minimized changes to cysteine. This enhanced-cysteine yogurt was found to have greater firmness, reduced syneresis, and most importantly, 3 times more cysteine content compared with yogurt made to mimic commercial manufacture. Moreover, these quality attributes were stable throughout a 60-day shelf life.

Different sensory techniques have been used to characterize flavor, color, appearance, and liking of yogurt. Whey proteins have been reported to influence the sensory properties of yogurt depending on the source and concentration of the whey protein. For instance, using descriptive analysis, the addition of whey protein products to a yogurt mix have been reported to increase the chalkiness, thickness, lumpiness, whey flavor, creaminess, and yellowness. On the other hand, consumer acceptance testing is used to evaluate the liking of products with panelists who are not trained. As early as the 1980s, reports in the literature have indicated that yogurts containing whey protein concentrate (WPC) had acceptable texture and appearance scores compared with yogurts containing other protein substitutes such as casein and sodium caseinate. More recently, researchers have reported that consumers liked the appearance, flavor, and texture of yogurts containing WPC (6%) and skim milk powder, and the overall impression of the yogurts was favorable.

As these results indicate, addition of whey products to yogurt may affect the flavor and texture profiles of the final product. Because we added a whey protein product and modified the process, the question of whether consumers would like a high-cysteine yogurt remained. Thus, this research project was undertaken to: (1) compare consumer liking of a high-cysteine and low-cysteine yogurt and (2) assess consumer willingness to buy such a product.

**Experimental Procedures**

Low-heat NDM, sugar, vanillin, WPI, and yogurt culture were obtained from commercial suppliers or local stores and maintained at -2 or -10°C (culture) until usage. Two yogurts were formulated: (1) a low-cysteine yogurt consisting of 12.5% (w/v) NDM and 5% (w/v) sugar; and (2) a high-cysteine yogurt consisting of 2.5% (w/v) WPI, 10% (w/v) NDM, and 5% (w/v) sugar. These ingredients were mixed in deionized, distilled water at 22 to 24°C for 30 minutes then heated, with the low-cysteine mix at 70°C for 20 minutes and the high-cysteine mix at 90°C for 7 minutes. Mixes were cooled to 43°C, and 1.6% (w/v) of vanillin flavor was added along with 0.6% (w/v) of the culture containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, stirred well, packaged, and incubated at 43 ± 1°C until pH 4.5 to 4.6. Samples were removed from the incubator and placed in cold storage (4 ± 1°C). On the following day, the mixes were stirred with a hand mixer for 5 minutes at speed 3 and returned to their containers and stored at 4 ± 1°C until the day of evaluation (sensory on day 7, physical and chemical analyses on day 1).

To evaluate low-cysteine and high-cysteine yogurts for consumer acceptance, a consumer panel was recruited from the general Kansas State University community. Panelists were screened on age (≥18 years), interest in consuming yogurt, and lack of food allergies or intolerances. Panel-
ists completed consent and demographic forms and were asked to describe their yogurt consumption frequency.

For the study, panelists were provided a ballot consisting of a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) for the attributes of appearance, thickness, flavor, aftertaste, and overall acceptability. At the end of each ballot, panelists were asked to indicate if they were willing to buy the product (yes or no) and if they had any specific comments to share. Panelists were provided water to clean their palates between samples, and each sample and ballot were presented individually.

About an hour before serving, approximately 15 g of each yogurt sample was placed in a 60-mL plastic cup, coded with a random three-digit number, covered with a lid, and returned to cold storage. Serving order (high- then low- and low- then high-cysteine) was randomized. After evaluation of the two samples, panelists were given coupons for free ice cream cones at Call Hall Dairy Store, Kansas State University, Manhattan, KS.

Yogurts were also assessed for consistency, cysteine content, pH, firmness, syneresis, and whiteness index following published methods for stirred-style yogurt. The design was a randomized complete block design with high-cysteine and low-cysteine yogurt as treatments (2) and panelists (112) as blocks. Statistical analysis was done using SAS (SAS Institute Inc., v 9.3, Cary, NC). Analysis of variance results for significant ($P < 0.05$) effects were further analyzed.

**Results and Discussion**

Table 1 shows the results of the chemical and functional tests of the high-cysteine and low-cysteine yogurts. Because only one batch was made, these numbers reflect the average of the repeated measures of one day’s production. The low-cysteine and high-cysteine yogurts varied (Table 1). The high-cysteine yogurt had 3 times more cysteine and 6.4 times less syneresis, was 1.5 times more firm and consistent, but had a lower whiteness index (indicating it was less white) than the low-cysteine yogurt. The pH of the yogurts did not differ. Despite the fact that these yogurts contained additional ingredients (flavor and sweetener) and were made as a stirred-style yogurt vs. set-style yogurt, these results show the same trends as our earlier studies.

One hundred and nineteen panelists (43 males and 76 females) participated in the evaluation. Demographically, 24 panelists ranged from 18 to 20 years, 55 ranged from 21 to 25 years, 20 ranged from 26 to 40 years, and 21 were >40 years. Twenty-eight percent were married, and 72% reported themselves as single. In ethnicity, 67% were Caucasian, 21% were Asian, 5% were African-American, 4% were Hispanic, and 3% indicated other. Frequency of yogurt consumption (self-reported) is shown in Figure 1. Overall, 17 panelists consumed yogurt daily, 45 consumed it 2 to 3 times weekly, 26 consumed it once weekly, 24 consumed it once monthly, and 7 never consumed yogurt. Based on recommendations from sensory scientists, these 7 consumers were removed from the dataset; thus, further analysis and results are based on the remaining 112 judgments.

The consumer liking results for appearance, thickness, flavor, aftertaste, and overall acceptance are shown in Figure 2. Analyses indicated no significant differences between low-cysteine and high-cysteine yogurt for the liking of aftertaste, flavor, and overall acceptability. In contrast, differences ($P < 0.05$) were detected in sensory scores for liking of appearance and thickness.
Appearance of the low-cysteine yogurt had greater liking (6.7 vs. 6.2) than the high-cysteine yogurt. These numbers correspond to “like slightly” (6) to “like moderately” (7). The difference in liking may be due to the color properties of yogurt, because the high-cysteine yogurt had a lower whiteness index than the low-cysteine yogurt (65.11 vs. 68.22; Table 1). In contrast, texture of the high-cysteine yogurt was liked more than the low-cysteine yogurt (6.4 and 5.8, respectively). These scores fall between “neither likes nor dislikes” (5) and “like moderately” (7). Considering the results in Table 1, instrumental analysis indicated that the high-cysteine yogurt was 1.5 times more consistent (thick) and firm than the low-cysteine yogurt, which may be responsible for the texture differences.

Overall, the aftertaste, flavor, and overall acceptability means for low-cysteine and high-cysteine yogurts ranged from 6.1 to 6.3, corresponding to “like slightly” to “like moderately.” The liking of flavor (6.1) and aftertaste (6.1) of the low-cysteine and high-cysteine yogurts was close to the “like slightly” rating, which agrees with previous work showing that yogurt liking did not differ in appearance and flavor with addition of WPC, or in this case, WPI.

The question concerning willingness to buy was used to subset the panelists for further analysis. Results indicated that 59 and 61%, respectively, of the panelists were willing to buy the high-cysteine and low-cysteine yogurts (Table 2). To further understand the willingness-to-buy-yogurt responses, Venn diagrams were made based on gender capturing the willingness to buy either or both yogurts (Figures 3 and 4). Overall, 56 females and 34 males indicated that they would buy at least one of these yogurts. From Figure 3, 15 females indicated they were willing to buy only the high-cysteine yogurt, 13 females indicated they were willing to buy only the low-cysteine yogurt, and 28 females indicated they were willing to buy both yogurts. On the other hand, 7 males indicated they were willing to buy only the high-cysteine yogurt, 10 males indicated they were willing to buy only the low-cysteine yogurt, and 17 males were willing to buy both yogurts. Overall, about 60% of the panelists showed willingness to buy the high-cysteine yogurt.

The hedonic scores for the 5 attributes were obtained based on the willingness to buy each yogurt, and the hedonic mean scores for the attributes are shown in Table 3. Sixty-seven panelists were willing to buy the high-cysteine yogurt, whereas 68 were willing to buy the low-cysteine yogurt; likewise, 45 and 44 panelists were not willing to buy the high-cysteine and low-cysteine yogurt, respectively. Obviously, the subset of not willing to buy had lower corresponding hedonic scores (by at least 1 point) for all 5 attributes than the hedonic mean scores from those willing to buy (Table 3). From those willing to buy at least one of the yogurts, significant differences were again observed only for liking of thickness and appearance. The liking of flavor, aftertaste, and overall quality were similar between these two groups. Considering the subset of the panelists willing to buy at least one of the yogurts, the 10 hedonic scores ranged from “like slightly” (6) to “like moderately” (7). Further research is warranted to optimize texture and appearance of the yogurt as well as to conduct animal studies to determine if a high-cysteine yogurt could increase glutathione contents in tissues or muscles.
Table 1. Chemical and functional properties of yogurts containing different levels of cysteine

<table>
<thead>
<tr>
<th></th>
<th>Consistency, cm</th>
<th>Cysteine, mg/L</th>
<th>Firmness, g</th>
<th>pH</th>
<th>Syneresis, %</th>
<th>Whiteness index</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-cysteine</td>
<td>6.4</td>
<td>409.6</td>
<td>39.20</td>
<td>4.50</td>
<td>0.790</td>
<td>65.11</td>
</tr>
<tr>
<td>Low-cysteine</td>
<td>10.1</td>
<td>128.5</td>
<td>25.63</td>
<td>4.49</td>
<td>5.065</td>
<td>68.22</td>
</tr>
</tbody>
</table>

1 High-cysteine yogurt: nonfat dry milk + whey protein isolate (NDM + WPI) base processed at 70°C for 20 minutes; low-cysteine yogurt: NDM base processed at 90°C for 7 minutes.

Table 2. Total and gender numbers of 112 people willing to buy high-cysteine and low-cysteine yogurts

<table>
<thead>
<tr>
<th>Willing to buy</th>
<th>High-cysteine yogurt</th>
<th>Low-cysteine yogurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>67 (F:43, M:24)</td>
<td>68 (F:41, M:27)</td>
</tr>
<tr>
<td>No</td>
<td>45 (F:31, M:14)</td>
<td>44 (F:33, M:11)</td>
</tr>
</tbody>
</table>

1 High-cysteine yogurt: nonfat dry milk + whey protein isolate (NDM + WPI) base processed at 70°C for 20 minutes; low-cysteine yogurt: NDM base processed at 90°C for 7 minutes.
F: Female, M: Male.

Table 3. Mean hedonic scores for high-cysteine and low-cysteine yogurts, based on willingness to buy

<table>
<thead>
<tr>
<th>Willingness to buy</th>
<th>Attribute²</th>
<th>High-cysteine yogurt³</th>
<th>Low-cysteine yogurt³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Appearance</td>
<td>6.6</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Thickness</td>
<td>6.9</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>Flavor</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Aftertaste</td>
<td>6.6</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>Overall acceptability</td>
<td>6.9</td>
<td>7.3</td>
</tr>
</tbody>
</table>

| No                 | Appearance | 5.4                   | 6.0                  |
|                    | Thickness  | 5.6                   | 4.8                  |
|                    | Flavor     | 4.9                   | 4.8                  |
|                    | Aftertaste | 5.0                   | 4.9                  |
|                    | Overall acceptability | 5.0 | 5.0                  |

1 Yes (n = 67) and no (n = 45) for high-cysteine yogurt; yes (n = 68) and no (n= 44) for low-cysteine yogurt. 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.
2 Ballot order.
3 High-cysteine yogurt: nonfat dry milk + whey protein isolate (NDM + WPI) base processed at 70°C for 20 minutes; Low-cysteine yogurt: NDM base processed at 90°C for 7 minutes.
Figure 1. Frequency of yogurt consumption by panelists, based on gender (female, n = 76 and male, n = 43).

Figure 2. Mean hedonic scores for liking of appearance, thickness, flavor, aftertaste, and overall liking of high-cysteine and low-cysteine yogurts. Bars with different letters within attribute differ ($P \leq 0.05$) (n = 112). Hedonic scale: 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely. High-cysteine yogurt: nonfat dry milk + whey protein isolate (NDM + WPI) base processed at 70°C for 20 minutes; low-cysteine yogurt: NDM base processed at 90°C for 7 minutes.
Figure 3. Venn diagram of female panelists (n = 56) who indicated willingness to buy high-cysteine and low-cysteine yogurts. High-cysteine yogurt: nonfat dry milk + whey protein isolate (NDM + WPI) base processed at 70°C for 20 minutes; Low-cysteine yogurt: NDM base processed at 90°C for 7 minutes.

Figure 4. Venn diagram showing the male panelists (n = 34) who indicated willingness to buy high-cysteine and low-cysteine yogurts. High-cysteine yogurt: nonfat dry milk + whey protein isolate (NDM + WPI) base processed at 70°C for 20 minutes; Low-cysteine yogurt: NDM base processed at 90°C for 7 minutes.