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Evaluation of Yogurt with Enhanced Cysteine Content

S. Bala and K. A. Schmidt

Summary

Amino acids are the building blocks of protein and assist with metabolism in the body. In the human body, the amino acid cysteine can be synthesized from methionine by the enzyme γ -cystathionase. Because certain human subpopulations such as those prone to cataracts have decreased γ -cystathionase activity, dietary cysteine may be beneficial. Nutritionally, yogurt mix is one of the best dairy food sources of methionine and cysteine, but the heat treatment used in manufacturing yogurt decreases the dietary availability of cysteine. Last year, it was shown that supplementing yogurt mixes with whey protein isolate (**WPI**) (>90% protein) and processing yogurt mixes at a lower temperature produced yogurts with increased cysteine. Because the quality or cysteine content of the yogurt during the expected storage life is unknown, this study was conducted to determine if a combination of WPI addition and non-optimal process conditions could produce a yogurt with higher cysteine content and an acceptable shelf life. In this study, control yogurt mixes were made with nonfat dry milk (**NDM**) and processed at 90°C for 7 minutes, whereas the experimental yogurt mixes were made with NDM and WPI and processed at 70°C for 20 minutes. Both mixes were cooled, inoculated, fermented into yogurt, stored at 4°C, and evaluated periodically over a 60-day period. The experimental yogurts had ~2X more cysteine than the control yogurt; this trend was present throughout storage. After 60 days of storage, the water-holding capacity (**WHC**) and firmness was greater and the syneresis was less for the experimental yogurt than the control yogurt. These results show that yogurt supplemented with WPI and processed at less optimal conditions may be a good source of the conditional amino acid cysteine during storage.

Key words: yogurt, whey protein isolate, cysteine

Introduction

In 2011, the USDA reported an 85% increase in yogurt popularity and sales since 2002. This increased popularity may be attributed to the nutritional and health benefits of yogurt, such as improved digestibility and lactose utilization and antagonism toward enteric pathogens. When pasteurized at high temperatures, yogurt mixes have greater whey protein denaturation (**WPD**) but less dietary availability of cysteine. In milk, cysteine is a component of whey proteins. Heat causes whey proteins to unfold, which causes whey and casein proteins to aggregate. This aggregation contributes to high-quality yogurt, which is defined as exhibiting a firm gel, expressing minimal syneresis, and maximizing water-holding capacity (**WHC**). Yogurt firmness and syneresis are therefore related to protein content and WPD, because these properties are functions of the number and strength of the whey protein-casein interactions.

According to the Centers for Disease Control and Prevention (CDC, 2012), the current estimate is that nearly 20.5 million (17.2%) Americans who are 40 years and older have a cataract in one or both eyes; 30.1 million Americans are predicted to have cataracts by 2020. Studies of the occurrences and causes of cataracts have shown that elderly rats (24 to 26 months) had less or no γ -cystathionase (an enzyme that converts methionine to cysteine) in their eye lenses compared with young rats (5 to 6 months). Other researchers have reported that increased cataract formation was associated with decreased glutathione (**GSH**) contents in human eye lenses.

One of the substrates for GSH synthesis is cysteine (Figure 1); hence, the decreased activity of γ -cystathionase has been one of the focus in cataract research. These data suggest that people prone to cataracts may benefit from consuming dietary cysteine because it is a precursor for GSH synthesis.

Most commercial yogurts contain 9 to 14% milk solids, which are derived from the milk base and additional milk solids. Previous researchers have reported on the impact of various supplements (nonfat dry milk [NDM], whey protein concentrates [WPC], or whey protein isolate [WPI] in yogurt mixes. Typically, as WPC concentrations increased, yogurt had significantly greater firmness and reduced syneresis. Yogurts containing WPI had an even greater reduction in syneresis and increase in WHC. In our previous research, we formulated yogurts with various levels of WPI and observed greater firmness (1.4X) and cysteine contents ($\sim 4.5X$), but we used non-optimal process conditions. Although the yogurt was of good quality on day 1, we did not know whether the cysteine or gel quality would be sustained during the expected yogurt shelf life. Thus, this research project was undertaken to: (1) compare protein and cysteine contents in an experiment yogurt mix containing WPI and processed at less optimal conditions to a control yogurt, and (2) assess cysteine contents and gel quality of these yogurts during storage (day 1, 15, 30, 45, and 60).

Experimental Procedures

Low-heat NDM, WPI, and yogurt cultures were obtained from commercial suppliers and maintained at -2 or -10°C (culture) until usage. Two formulations were made: a control (C) mix consisting of 12.5% NDM and an experimental (E) mix consisting of 10% NDM and 2.5% WPI. Dried dairy powders were rehydrated in deionized distilled water at 22 to 24°C for 30 minutes. The C mix was processed at 90°C for 7 minutes to ensure $\sim 90\%$ WPD, whereas the E mix was processed at 70°C for 20 minutes to minimize WPD (and preserve cysteine). Both mixes were then cooled to 43°C , inoculated with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*, packaged into sterile containers, incubated until pH 4.6, and then placed in storage ($4 \pm 1^{\circ}\text{C}$) for up to 60 days.

Standardized, published methods were followed for all analyses, and three replications were performed. Mixes were made and analyzed for protein contents, WPD, and cysteine contents using a randomized complete block design. Stored yogurts were evaluated for cysteine contents, firmness, syneresis, and WHC on days 1, 15, 30, 45, and 60 using a split-plot design. All data were analyzed using SAS (SAS Institute, Cary, NC), and significant ($P < 0.05$) means and interactions were differentiated using Fisher's LSD tests.

Results and Discussion

The C yogurt was formulated and processed like a set-style commercial product. To ensure that the mixes were of different protein composition, mixes were analyzed for protein (true protein and whey) and WPD (Table 1). The E mixes had 32% and 189% more true protein and whey protein, respectively, than the C mixes. Mixes did not significantly differ in casein content ($\sim 3.34\%$). WPD was $\sim 19X$ less for E mixes than C mixes due to the processing difference. Table 1 also displays the effects of the manufacturing steps (formulation [unheated], processed [heat treatment], and fermentation) on cysteine. The heating step (process) dramatically affected cysteine, with the C mix exhibiting a 65% loss vs. the E mix exhibiting only a 21% loss compared with their respective unheated mixes. Interestingly, fermentation did not significant-

ly affect the cysteine content (the cysteine losses during fermentation were ~3% in both yogurt samples), but the E yogurt required 30 minutes more fermentation time than the C yogurt.

To make an effective delivery vehicle for a compound such as cysteine, the product needs to be acceptable to the consumer not only on day 1, but also throughout storage. On day 1, all yogurts had similar total solids content (12.35%), pH (4.42), syneresis (6.51%), and WHC (22.83%), but the E yogurt was ~2.1X more firm and had ~190% more cysteine than the control yogurt. Compared with a commercial, set-style yogurt (128 g) purchased at a local grocery store, the E yogurt had similar firmness (133 g).

With time, yogurt exhibits syneresis and shrinks away from the package. These two qualities, which are directly related to the whey protein–casein interactions, are considered defects by consumers; hence, the storage stability of this yogurt was evaluated. Results showed significant interactions for gel quality. Overall, firmness of the E yogurt was ~2.1X greater than the C yogurt (Figure 2), and both yogurts exhibited a significant increase in firmness (25%) from day 1 to day 15. The firmness of C yogurt was constant throughout the remaining storage period but the firmness of the E yogurt decreased from day 30 to day 45. The E yogurt exhibited 76% less syneresis than the C yogurt on day 1, and both yogurts decreased in syneresis from day 1 to day 15 (Figure 3), but yogurt syneresis remained constant thereafter. On day 1, the E yogurt had greater WHC than the C yogurt. The WHC of the E yogurt remained constant throughout storage, but the C yogurt increased in WHC (21%) from day 1 to day 15 and eventually decreased to its initial value at day 60 (Figure 4). More importantly, the E yogurt had greater cysteine content (398.4 mg/L) than the C yogurt (135.7 mg/L), and the cysteine content was not affected by storage time, which suggests that greater cysteine content would be stable and available throughout the storage life. These results indicate that WPI supplementation of yogurt mix combined with less optimal process conditions may produce a yogurt that effectively delivers cysteine.

Conclusions

In yogurt, cysteine content is a function of the type and amount of milk protein and the heat treatment of the mix. A yogurt made from mix with WPI and processed at less optimal conditions was shown to have greater cysteine content and acceptable gel quality. Further research involving sensory properties of this yogurt and investigation of how enhanced cysteine in yogurt affects GSH production in tissue cultures should be pursued.

Table 1. True protein and whey protein contents, whey protein denaturation (%) of yogurt mixes, and cysteine contents as a function of process treatments (means $\pm$ standard error)

	Composition	Control ¹	Experimental ²
Mix	True protein %	4.17 ^b $\pm$ 0.07	5.51 ^a $\pm$ 0.02
	Whey %	0.77 ^b $\pm$ 0.01	2.23 ^a $\pm$ 0.02
	WPD ^{3,4} %	70.28 ^a $\pm$ 0.73	3.45 ^b $\pm$ 0.88
Manufacturing step	Cysteine (mg/L)		
	Unprocessed mix	306.98 ^C $\pm$ 1.65	504.96 ^A $\pm$ 2.59
	Processed mix ⁴	138.52 ^D $\pm$ 4.21	400.15 ^B $\pm$ 12.08
	Fermented	135.74 ^D $\pm$ 4.64	398.32 ^B $\pm$ 19.79

^{a,b} Means (n=3) within rows with different lower case superscripts differ ($P < 0.05$).

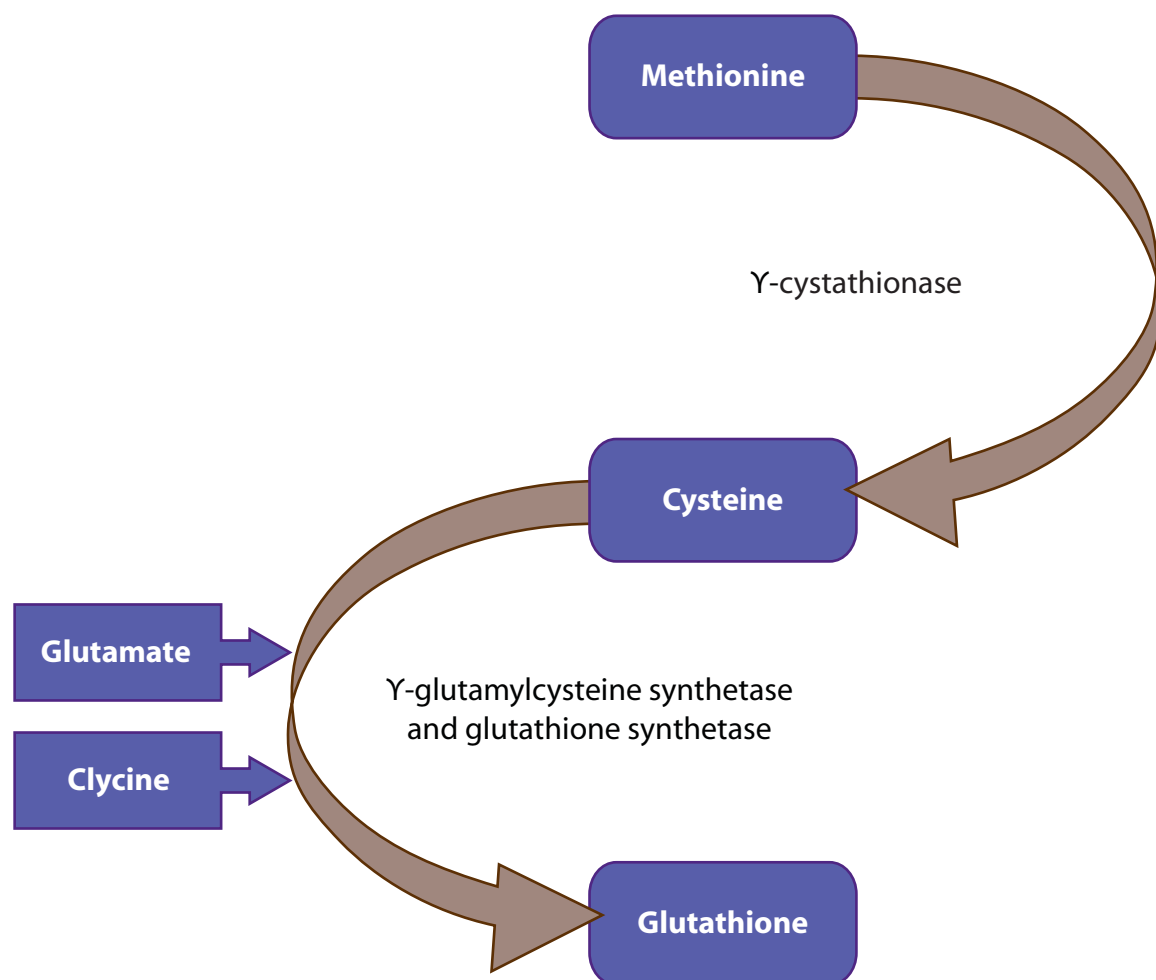
^{A-D} Means (n=3) with different upper case superscripts differ ($P < 0.05$).

¹ Nonfat dry milk (NDM) (12.5%).

² NDM (10%) + whey protein isolate (WPI) (2.5%).

³ Whey protein denaturation.

⁴ Control processed at 90°C for 7 minutes and experimental processed at 70°C for 20 minutes.

**Figure 1. Glutathione synthesis.**

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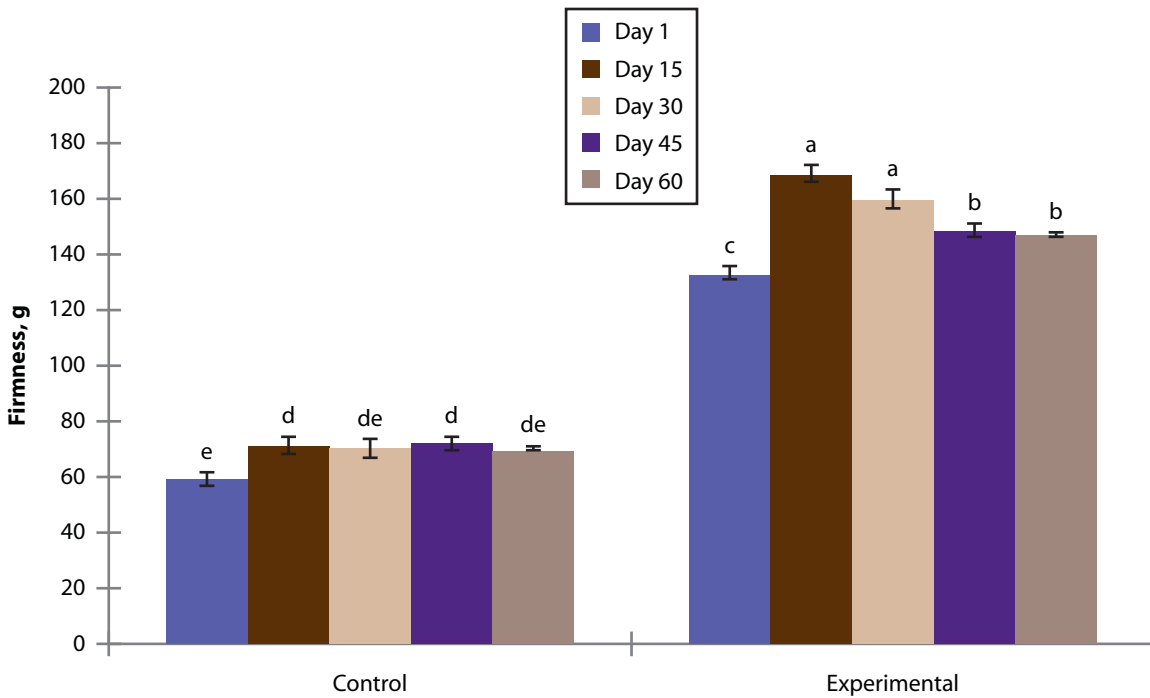


Figure 2. Yogurt firmness during 60 days of storage. Control: nonfat dry milk (NDM) (12.5%) processed at 90°C for 7 minutes. Experimental: NDM (10%) + whey protein isolate (2.5%) processed at 70°C for 20 minutes.

^{a-c} Bars with different superscripts differ ($P < 0.05$).

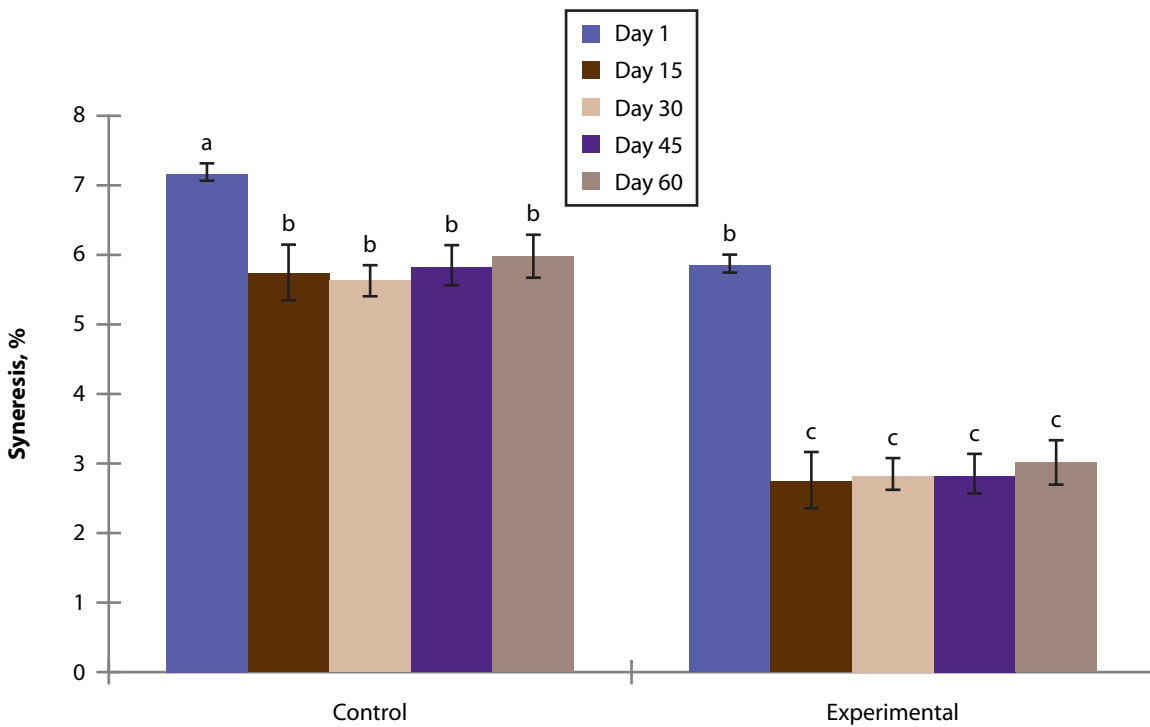


Figure 3. Yogurt syneresis during 60 days of storage. Control: nonfat dry milk (NDM) (12.5%) processed at 90°C for 7 minutes. Experimental: NDM (10%) + whey protein isolate (2.5%) processed at 70°C for 20 minutes.

^{a-c} Bars with different superscripts differ ($P < 0.05$).

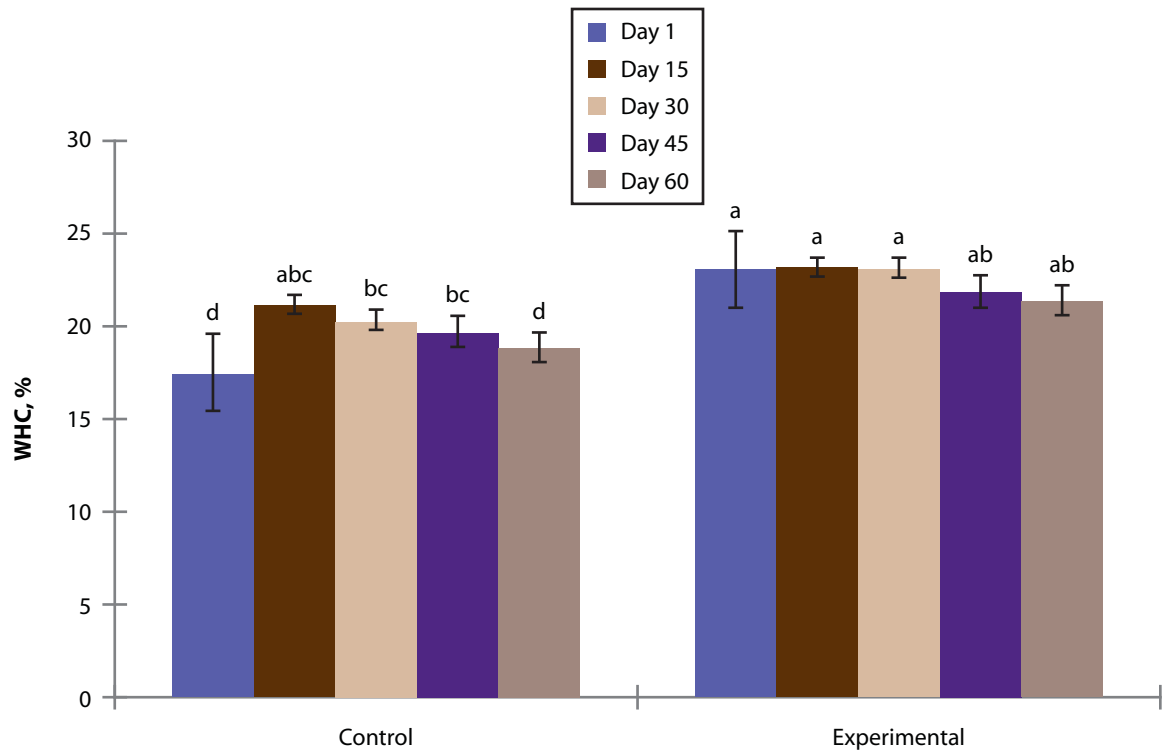


Figure 4. Yogurt water-holding capacity (WHC) during 60 days of storage. Control: nonfat dry milk (NDM) (12.5%) processed at 90°C for 7 minutes. Experimental: NDM (10%) + whey protein isolate (2.5%) processed at 70°C for 20 minutes.

^{a-c} Bars with different superscripts differ ($P < 0.05$).