Enhancing Cysteine Content in Yogurt

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Summary

Cysteine is considered a conditional amino acid for certain subpopulations. For example, in elderly people, cysteine has been associated with diverse functional properties as a general antioxidant as well as a specific role linked to cataract reduction or prevention. Yogurt is an excellent source of protein, the sulfur-containing amino acids methionine and cysteine in particular. Heat, however, can denature these amino acids and affect their bioavailability. A yogurt mix supplemented with whey proteins (an abundant source of cystiene) coupled with minimal pasteurization of yogurt mixes may increase the availability of cysteine in the final product.

In this study, yogurt mixes were supplemented with nonfat dry milk (**NDM**) or whey protein isolate (**WPI**; 90% protein), processed at 90°C for 7 minutes or 70°C for 20 minutes, then fermented into yogurt following a conventional procedure. Supplementing yogurt mix with WPI vs. NDM increased cysteine content by 140%. In contrast, overall cysteine contents decreased by 17% in mixes treated at 70°C for 20 minutes and 35% in mixes treated at 90°C for 7 minutes. Mixes supplemented with WPI and treated at 70°C for 20 minutes produced yogurts that had greater cysteine contents and slightly greater firmness and water-holding capacity, but the yogurts exhibited less syneresis compared with those made from mixes supplemented with NDM and treated at 90°C for 7 minutes. These results indicate that yogurt may be an excellent delivery vehicle for the conditional amino acid, cysteine.

Key words: yogurt, cysteine, heat treatment

Introduction

In 2009, USDA reported that yogurt popularity and sales had increased by 79% since 2000. Yogurt is an excellent source of whey proteins, but typical heat treatment of commercial yogurt mixes denatures about 90% of the whey proteins, resulting in decreased dietary availability of sulfur-containing amino acids, cysteine and methionine. Specifically, heat causes whey proteins to unfold, exposing free sulphydryl groups that initiate disulfide bonds with other sulfur groups. A high-quality yogurt can be described as firm, exhibiting low syneresis (release of a liquid from the gel that typically collects on the surface), and having a pleasant acid taste. The firmness and wheying off are directly related to protein content as well as whey protein denaturation. In addition, acid can denature milk proteins, especially the pH change that occurs during yogurt fermentation (6.4 to 4.6) and yogurt storage (4.6 to 4.2), but an amount of whey protein denaturation has not been reported.

The importance of dietary cysteine has been studied in rats and humans. Elderly rats (24 to 26 months) had less or no gamma-cystathinase, an enzyme in their eye lenses, compared with young rats (5 to 6 months). The decreased gamma-cystathinase activity was associated with a 50% decrease in cysteine and glutathione contents. In cells, cysteine is one of the components used to synthesize glutathione. Decreased glutathione content in human eye lenses has been associated with cataract formation; hence, if the substrate cysteine is decreased or depleted, the production of glutathione is decreased, too. According to the Centers for Disease Control and Prevention, an estimated 20.5 million (17.2%) U.S. citizens who are 40 years old or older have a cataract in 1 or both eyes, and the total number of people who will have cataracts by 2020 are estimated at 30.1 million. For humans, these data indicate that certain subpopulations may benefit from increased cysteine in their diets.

When manufacturing yogurt, a common practice is to boost nonfat milk solids (from 9 to 14%) by adding nonfat milk solids, typically nonfat dry milk (**NDM**), which in turn increases protein content from 3.24 to 5.22 g/100 mL. To produce a high-quality product (minimal syneresis, firm gel, and maximum water-holding capacity), most yogurt processors manufacture mixes to induce approximately 90% whey protein denaturation by heating the mix to approximately 90°C for 2 minutes or more. Researchers previously showed that yogurt supplemented with whey protein concentrate (**WPC**) rather than NDM had increased whey protein content (0.75 to 2.07 g), firmness (15.10 to 32.44 g), protein network hydration (2.44 vs. 2.47 g water/g solids), and water retention (72.7 vs. 88.4%), but cysteine contents were not reported. When contrasting whey protein isolate (**WPI**) with WPC, WPI has about 3 times more protein, allowing WPI to be an excellent source of the sulfur-containing amino acids. Thus, a research strategy combining WPI supplementation with low process treatments may result in a yogurt with enhanced cysteine content and acceptable quality. The specific objectives were to: (1) determine cysteine contents in yogurt made with different supplements (WPI vs. NDM) and (2) assess changes in cysteine content as functions of heat treatment and fermentation and evaluate resultant yogurt quality.

Experimental Procedures

Low-heat NDM, WPI, and yogurt cultures were obtained from commercial suppliers and maintained at −2 or −10°C (culture) until usage. To simulate a typical commercial yogurt (based on milk solids), mixes were formulated as shown in Table 1 and the calculated cysteine content was based on NDM and WPI protein content. Dairy powders were rehydrated in deionized, distilled water at 22 to 24°C for 30 minutes, then subdivided and treated at 70°C for 20 minutes or 90°C for 7 minutes, cooled to 43°C, inoculated with *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus,* packaged into sterile 120-ml cups, and incubated until pH 4.6. Cups were removed from the incubator and cooled by placing in storage (4 ± 1°C) until the following day.

Two replications were made, data were analyzed, and significant means and interactions were differentiated. Cysteine contents and pH were assessed before heat treatment, after heat treatment, and after 1 day of yogurt storage. Cysteine content was analyzed using Ellman's reagent. Yogurt mixes and/or samples were analyzed for total solids, pH, syneresis, water-holding capacity, and firmness using standard published methods.

Results and Discussion

Table 2 shows the cysteine content in the mixes and yogurt samples as functions of heat treatment and fermentation. Supplementation with WPI vs. NDM resulted in a 142% increase in cysteine content (comparing the non-heated mixes), and these values agreed with the predicted calculated values reported in Table 1. As expected, the statistical analyses showed that heat treatment significantly affected cysteine content. On average, mixes treated at 70°C for 20 minutes had a 17% loss in cysteine, whereas those treated at 90°C for 7 minutes had a 37% loss. Supplementation also affected the cysteine content, and the mixes containing WPI had greater cysteine contents compared with mixes without WPI. More surprising, however, was that cysteine contents were not affected by the fermentation process (in either mix or heat treatment) because the decrease in cysteine contents from heated mix to fermented yogurt was on average approximately 1.5 mg cysteine/L. These data indicate that if cysteine is available at the start of fermentation, it will be available 1 day after storage.

Overall, milk proteins are good buffering agents. The WPI is an abundant source of protein, but a poor source of lactose, which is necessary for the fermentation process. Because the mixes containing WPI have greater protein and less lactose (data not shown), mix pH, mix total solids, and fermentation times also were monitored. All mixes had similar total solids content, approximately 12.4%, which indicates consistency in formulation. When analyzing the pH data, an interaction was observed between the heat treatment and formulation. As shown in Table 3, the pH of mixes containing WPI was greater than mixes without WPI, but the pH of yogurts containing WPI was less than yogurts without WPI. On the other hand, fermentation time was a function of heat treatment because mixes treated at 70°C for 20 minutes required 30 minutes more to ferment than mixes treated at 90°C for 7 minutes, which needed approximately 5.5 hours. The greater heat treatment may not only initiate some component degradation, facilitating culture growth, but also should reduce microbial counts and destroy enzymes from the initial milk sources that might compete or interfere with the yogurt culture.

To make an effective delivery vehicle for a compound such as cysteine, the product needs to be acceptable to the consumer. To evaluate quality, 3 physical properties were measured — water-holding capacity, firmness, and syneresis — all of which affect consumer preference and are directly related to protein content and whey protein denaturation. In this study, mixes without WPI treated at 90°C for 7 minutes and resultant yogurts were most similar to a commercial plain, set-style yogurt; hence, it was considered a control sample. All these properties had significant interactions and the means are shown in Table 4. Mixes containing WPI treated at 90°C for 7 minutes produced yogurts that were approximately 8 times more firm, held approximately 4 times more water, and exhibited almost no syneresis compared with the control yogurt. But mixes containing WPI treated at 70°C for 20 minutes produced yogurts that were 1.4 times as firm and exhibited slightly greater water-holding capacity and less syneresis than the control yogurt. More critically, the cysteine content of the yogurt containing WPI was 4.5 times greater than that of the control. These results indicate that WPI supplementation of yogurt mix, combined with a more minimal heat treatment may result in a yogurt that is an effective delivery vehicle for cysteine.

Conclusions

In yogurt, cysteine content is a function of type and amount of milk protein and heat treatment. Mixes supplemented with WPI and treated at 70°C for 20 minutes produced yogurts with the greatest cysteine content, more water-holding capacity and firmness, and less syneresis compared with the control yogurt. Syneresis and firmness are related to the number of whey protein-casein interactions, which are induced by denaturation. In this experiment, the yogurt made from mixes supplemented with WPI and treated at 90°C for 7 minutes had almost no syneresis and great firmness compared with the other yogurts, perhaps to the point that consumers would question whether the product is a yogurt. To pursue this concept, further research monitoring whey protein denaturation and shelf life is needed.

Table 1. Yogurt mix formulations, protein content, and predicted cysteine content

Mix	Formula: Nonfat dry milk (%)	Formula: Whey protein isolate (%)	Composition: Protein (g/1000 mL)	Composition: Predicted cysteine content (mg/1000 mL)
Nonfat dry milk solids	12.5	0.0	45.0	350
Nonfat dry milk solids + whey protein isolate	9.0	3.5	70.6	750

Table 2. Mean cysteine contents of yogurt mixes, unheated or heated to 70ºC for 20 minutes or 90ºC for 7 minutes and yogurt (1 day old)

Item	Mix: NDM solids[1]	Mix: NDM + WPI[2]	Average
	Cysteine content (mg/1000 mL)		
Unheated	301.3 ± 5.6	729.8 ± 11.8	515.3[a] ± 8.1
70ºC for 20 min	240.1 ± 5.0	631.8 ± 14.6	435.8[b] ±9.8
90ºC for 7 min	141.4 ± 10.2	546.8 ± 16.8	343.8[c] ± 13.5
Yogurts 1 day old			
70ºC for 20 min	236.0 ± 4.4	630.7 ± 13.9	433.1[b] ± 9.1
90ºC for 7 min	140.4 ± 9.1	545.2 ± 17.5	342.5[c] ± 13.3
Average	211.6[b] ±6.76	616.6[a] ± 14.9	

[a-c] Means within item or between mixes with different superscript letters differ ($P < 0.05$).
[1] Nonfat dry milk (12.5%).
[2] Nonfat dry milk (9%) and whey protein isolate (3.5%).

Table 3. Mean pH of yogurt mixes, unheated or heated to 70ºC for 20 minutes or 90ºC for 7 minutes and yogurt (1 day old)

Item	Mix: NDM[1]	Mix: NDM solids + WPI[2]
	pH	
Unheated	6.57[bc] ± 0.02	6.61[a] ± 0.01
70ºC for 20 min	6.56[c] ± 0.02	6.61[a] ± 0.02
90ºC for 7 min	6.55[c] ± 0.01	6.56[c] ± 0.02
Yogurts 1 day old		
70ºC for 20 min	4.55[d] ± 0.01	4.49[e] ± 0.02
90ºC for 7 min	4.53[d] ± 0.01	4.46[e] ± 0.01

[a-e] Means with different superscript letters differ ($P < 0.05$).
[1] Nonfat dry milk (12.5%).
[2] Nonfat dry milk (9%) and whey protein isolate (3.5%).

Table 4. Mean physical properties of yogurt with different formulation and heat treatment

	Mix			
	NDM solids[1]		NDM solids + WPI[2]	
Properties	70°C for 20 minutes	90°C for 7 minutes	70°C for 20 minutes	90°C for 7 minutes
Syneresis (%wt/wt)	8.10^{a}±0.12	2.17^{b}±0.03	1.66^{c}±0.20	0.47^{d}±0.06
Firmness (g)	20.55^{d}±0.63	51.42^{c}±0.97	70.86^{b}±0.65	413.80^{a}±8.0
WHC[3] (%wt/wt)	16.95^{d}±0.25	20.52^{c}±0.66	23.84^{b}±0.23	62.71^{a}±0.56
pH	4.55^{a}±0.01	4.53^{a}±0.01	4.49^{b}±0.02	4.46^{b}±0.01

^{a,b,c,d} Means within rows with a different superscript letter differ ($P < 0.05$).
[1] Nonfat dry milk (12.5%).
[2] Nonfat dry milk (9%) and whey protein isolate (3.5%).
[3] Water-holding capacity.