Effect of Ultra-High Temperature Steam Injection Processing and Aseptic Storage on Labile Water-Soluble Vitamins in Milk

E. E. OAMEN,[1] A. P. HANSEN, and K. R. SWARTZEL
Department of Food Science
North Carolina State University
Raleigh 27695-7624

ABSTRACT

Whole milk standardized to 3.25% fat and 12.00% total solids was processed by UHT steam injection and aseptically packaged by a Tetra Pak AB3-250 into paper-foil laminate flexible containers with a gas barrier sealing strip to prevent gas exchange. Treatment involved heating normal and deaerated raw milk to 149°C for 3.4 s and 138°C for 20.3 s. Samples were stored at 24°C. Raw milk and two samples from each treatment were analyzed for vitamins B_2, B_6, B_{12}, C, and folic acid at intervals of 0, 2, 4, 8, 12, and 20 wk. The effects of thermal treatments and deaeration were negligible. The UHT processing for both thermal treatments and normal and deaerated milk averaged the following losses: 32.5% vitamin C, 17.9% vitamin B_{12}, 12.5% folic acid, 3.4% vitamin B_6, and 2.6% vitamin B_2. Storage at room temperature for 20 wk resulted in a 100% loss of vitamin B_{12}, 96% vitamin B_6, 85% vitamin C, 32% folic acid, and negligible change for vitamin B_2.

INTRODUCTION

The labile water-soluble vitamins C, B_6, B_{12}, and folic acid have shown significant loss during UHT processing (5, 6). Ford et al. (5) and Hall (6) reported the amount of destruction was dependent on the raw milk and the method of UHT processing.

Heating and cooling methods utilized in UHT processing involve direct heating with steam followed by expansion cooling or indirect heating and cooling through a heat conducting wall. The direct method has been shown to be less destructive to the nutritive value of the milk (5, 11). However, equivalent thermal treatments for both systems may not have been used. Folic acid loss during heat processing of milk has been shown to be dependent on the concentration of initial dissolved oxygen (5). Ascorbic acid is not destroyed by heat, but dehydroascorbic acid is rapidly destroyed when milk is heated (6). Dissolved oxygen is a major factor in the destruction of vitamin B_{12} during milk processing.

Significant losses of the labile water-soluble vitamins occur during storage of UHT milk, especially if subjected to light or if the package is partially permeable to gases (13). Storage of aseptically packaged milk in light-protected cartons at room temperature (15 to 19°C) for 180 d resulted in a loss of 50 to 60% of the original value of vitamin B_6 content. Twenty percent of this loss took place within the first 14 d of storage (5, 11). Losses during storage have been reported to be inevitable and unrelated to heat treatment, since similar losses were obtained for raw milk stored at −30°C (5, 6).

Loss of vitamin B_{12} during storage is influenced by levels of dissolved oxygen and ascorbic acid in milk. The presence of oxygen and the absence of ascorbic acid results in the rapid destruction of B_{12} (5, 6, 11).

Stability of ascorbic acid in milk during storage diminishes with increased levels of dissolved oxygen (6, 11, 13) while folic acid is stabilized by the presence of ascorbic acid. In the presence of oxygen, ascorbic acid is oxidized before folic acid. Loss of folic acid is not observed until complete elimination of ascorbic acid (5, 11).

Various aseptic packages are available for UHT-processed milk. Of these, the flexible multilayer polyethylene/aluminum foil/polyethylene/paper/polyethylene package offers many advantages. The use of a gas barrier sealing strip prevents gas exchange, and therefore,

Received March 23, 1988.
Accepted September 1, 1988.
[1]Present address: Wells Dairy Inc., Le Mars, IA 51031.

should decrease the loss of oxygen sensitive vitamins.

The objective of this study is to investigate the effect of direct UHT processing on normal and deaerated raw milk and subsequent aseptic storage in gas-tight flexible containers on the labile water-soluble vitamins found in milk.

MATERIALS AND METHODS

Experimental Design

Summer milk was standardized to 3.25% fat and 12.00% total solids. One half of the raw milk (8 to 9 ppm dissolved oxygen) was deaerated to 1 ppm oxygen using AVA-SH Aro-Vac (Cherry-Burrell Corp., Cedar Rapids, IA). Normal and deaerated raw milk was preheated to 78°C and sterilized by UHT steam injection in a No-Bac Aro-Vac (Cherry-Burrell). Two processing conditions (149°C for 3.4 s and 138°C for 20.3 s) were selected from a recommended working range of UHT treatment of dairy products (8). The processed milk was cooled by expansion cooling and further cooled in a tubular heat exchanger to a filling temperature of 22°C. Aseptic packaging was accomplished by using a Tetra Pak Model AB3-250 filler (Tetra Pak, Shelton, CT). Milk was filled into 250-ml cartons composed of five-ply coextruded polyethylene/aluminum foil/polyethylene/paper/polyethylene with a plastic film vertical strip. Duplicate processing treatments on a different batch of raw milk were performed 1 wk later. All samples were stored at 24 ± 2°C.

Two cartons from each treatment were analyzed for vitamins B_2, B_6, B_{12}, C, and folic acid at intervals of 0, 2, 4, 12, and 20 wk of storage. Raw milk was analyzed at time zero and used as the basis for determining processing effects.

Determination

Vitamin B_2 content of the milk was determined by the autoanalytical method of Kirk (9) with some minor modifications, which involved the use of a 24-in dialyzer (M117-0043-01-E) that operates at room temperature and a different mixing coil (157-0226-01-C), all from Technicon Industrial Systems, Tarrytown, NY.

Vitamin C analysis was performed using a colorimetric measurement based on the reduction of 2,6-dichlorophenol-indophenol (10).

Vitamins B_6, B_{12}, and folic acid were determined by AOAC (1) and Difco (3) microbiological methods, using *Saccharomyces carlsbergensis* ATCC 9080, *Lactobacillus leichmannii* ATCC 4797, and *Lactobacillus casei* ATCC 7469, respectively. Samples of milk for B_{12} analysis had to be frozen (−20°C) due to a 3 mo delay in obtaining fresh media for B_{12} analysis.

Dissolved oxygen concentration was determined by means of a YSI Model 51B oxygen meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). The meter was calibrated with milk through which air had been bubbled for 20 min. Regression analysis with logarithmic transformation of the data (2) was used for the statistical analysis.

RESULTS

The data from the normal and deaerated milks were combined because deaeration of the raw milk before heat treatment did not have a significant effect on the destruction of the vitamins during UHT thermal treatment and subsequent storage for 20 wk. Table 1 gives concentrations of vitamins B_2, B_6, B_{12}, C, and folic acid in raw and UHT milk and represents means from eight samples, due to combining the normal and deaerated milk data. Figure 1 contains curves of average vitamin concentrations versus storage time in weeks. The UHT thermal processing had no significant effect on the original content of vitamin B_2. Vitamin B_6 exhibited a loss of 3.4%, B_{12} of 17.9%, C of 32.5%, and folic acid of 12.5%.

Storage time was a major factor in the loss of all the vitamins (Figure 1) with the exception of vitamin B_2, which was not significantly affected. Vitamins B_6, B_{12}, and C were the most severely affected. About 80% of vitamin B_{12} was lost at 12 wk, and 100% was lost before 20 wk. No detectable loss of B_{12} was measured during the 3-mo storage at −20°C. Vitamins B_6 and C were involved in gradual but extensive losses which amounted to 96 and 85%, respectively. Thirty-two percent of the folic acid activity was lost during the 20 wk of storage.

Dissolved oxygen in milk did not change significantly during storage. On the day of processing, oxygen concentration was about 1 ppm while after 20 wk storage the level of oxygen was .8 to .9 ppm.

TABLE 1. Concentration (μg/ml) and percent loss[1,2] of vitamins in raw and stored UHT milk.

Storage	Treatment	Vitamin B_2	Vitamin B_6		Vitamin B_{12}		Vitamin C		Folic acid	
(wk)		(μg/ml)	(μg/ml)	(% Loss)	(μg/ml)	(% Loss)	(μg/ml)	(% Loss)	(μg/ml)	(% Loss)
	Raw	1.86	.333		2.63×10^{-3}		21.32		1000	
0	138°C, 20.3 s	1.82	.320	3.4	2.17×10^{-3}	17.9	14.49	32.5	.0900	12.5
	149°C, 3.4 s	1.80	.323		2.14×10^{-3}		14.29		.0850	
2	138°C, 20.3 s	1.85	.203	36.8	2.00×10^{-3}	8.6	12.17	16.3	.0805	8.0
	149°C, 3.4 s	1.86	.203		1.94×10^{-3}		11.93		.0805	
4	138°C, 20.3 s	1.79	.169	47.3	1.78×10^{-3}	17.4	8.90	38.0	.0805	8.0
	149°C, 3.4 s	1.81	.170		1.78×10^{-3}		8.94		.0805	
8	138°C, 20.3 s	1.86	.099	69.7	1.36×10^{-3}	37.8	7.17	49.8	.0788	10.1
	149°C, 3.4 s	1.86	.096		1.32×10^{-3}		7.28		.0786	
12	138°C, 20.3 s	1.81	.043	86.2	4.40×10^{-4}	79.8	4.85	65.8	.0784	10.6
	149°C, 3.4 s	1.82	.046		4.30×10^{-4}		4.99		.0780	
20	138°C, 20.3 s	1.81	.014	95.6	0	100	2.15	84.6	.0591	32.4
	149°C, 3.4 s	1.82	.014		0		2.30		.0592	
SEM		.006	.002		1.3×10^{-5}		.052		.0003	

[1] At 0 wk of storage, percent loss from processing. At 2 to 20 wk of storage, percent loss during storage from time 0.

[2] Percent loss is a mean value from the two thermal treatments for both normal and deaerated samples.

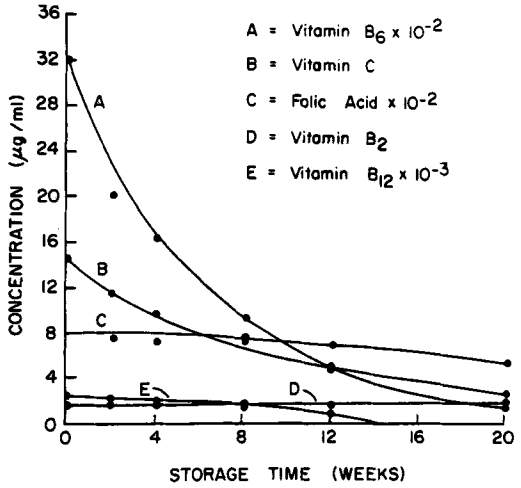

Figure 1. Degradation of vitamins B_2, B_6, B_{12}, C, and folic acid in UHT milk during storage.

Least square multiple regression analysis demonstrated that thermal treatment, normal and deaerated milk, storage time, and their interactions had no significant effects on vitamin B_2. Thermal treatments were significant at the .05 level ($P<.05$) to losses in vitamins B_6, B_{12}, C, and folic acid when included with the other variables in the regression model but became insignificant when analyzed in a model with a single variable. Storage was highly significant ($P<.01$) to vitamins B_6, B_{12}, C, and folic acid (Figure 1).

The change in B_6 concentration with storage time was significant, with a first order pattern of log B_6 versus storage time yielding a straight line (R^2 = .98) with a rate constant (k) of .067 μg/(ml wk) at 24°C. The reaction equation obtained for vitamin B_6 was log B_6 = .512 − .067t with k (slope) having a standard error of .00093. Attempts at fitting the data to other reaction orders yielded lower correlation coefficients. Vitamin C degradation yielded log C = 1.157 − .040 with k having a standard error of .00056 and R^2 = .98. Again, attempts at fitting the data to other reaction orders yielded lower correlation coefficients. A plot of the change of vitamin B_{12} concentration during storage yielded a (zero order) straight line through the origin (B_{12} = −.000059 + .00011t). The reaction order for folic acid was difficult to determine. The plot in Figure 1 indicates a zero order; however, a first order model yields the largest correlation coefficient (R^2 = .85).

DISCUSSION

Processing was less detrimental to vitamin content than storage. Regression analyses of the data on stored samples indicated that all the independent variables except storage time had no significant effects on any of the vitamins. Vitamin B_2 was the most stable vitamin during both processing and storage. Vitamins B_6, B_{12}, and C were the most severely affected, whereas folic acid was mildly affected.

Riboflavin is stable to both heat and subsequent storage in UHT milk providing that the milk is protected from the light. Our result agrees with other researchers (11) who have reported little or no change in riboflavin due to UHT processing and storage.

Losses of B_6 from UHT processing reported in the literature are less than 10% (12), which agrees with 3.4% loss observed in our laboratory. Losses in vitamins after UHT processing are largely dependent upon the content of dissolved oxygen in the milk as reported by Renner (12). Factors responsible for the loss of B_6 during storage are largely unexplained. The possible presence of some natural antagonists might function to inactivate vitamin B_6 in milk. Additional mechanistic work is needed in this area. The concentration of B_6 began declining on the 1st d of storage and continued declining throughout the storage period. After 2 wk of storage, 37% of the B_6 was lost; after 4 wk, approximately 50% was lost. Previous researchers (5, 12) encountered the same problem; however, they found lower losses. Some authors (4, 5) reported storage to have similar detrimental effects on UHT milk as it did to raw milk stored at −30°C.

Oxygen is the main component responsible for the loss of vitamin C in UHT milk. Oxygen tends to oxidize ascorbic acid to dehydroascorbic acid (11, 12). Although ascorbic acid is stable to heat treatment, its oxidative product is heat-labile (6, 11). The loss of vitamin C during UHT processing may be related to the level of dissolved oxygen and the amount of dehydroascorbic acid formed before processing; however, the concentration of dehydroascorbic acid was not measured.

On the day of processing, levels of oxygen were about 1 ppm and dropped to .8 to .9 ppm at 20 wk. This final drop in oxygen could be due to the oxidation reactions occurring in the package. Loss of vitamin C during storage was

probably due to the oxygen already in the package. Ford et al. (5) and Thompson (13) reported that all of the vitamin C disappeared in UHT milk after 2 wk storage in oxygen permeable containrs. Renner (12) reported tests for vitamin C depletion in UHT milk containing different oxygen levels. In UHT milk containing 8 to 9 ppm of oxygen, 100% of the vitamin C was gone in 28 d. When the oxygen was reduced to 3 to 6 ppm, over 50% of the vitamin C remained at 160 d. At an oxygen concentration of 1 ppm, 70% of the vitamin C remained at 160 d. We observed losses of 84.6% after 5 mo. This result could imply slow leakage of oxygen into the package and its consumption in chemical reactions as it entered the package thus keeping the oxygen level in the package below 1 ppm.

Losses of vitamin B_{12} due to UHT processing reported by Renner (12) are in the range of 10 to 20%. Losses found in this study due to UHT processing averaged 17.9%. During heating, various forms of vitamin B_{12} are oxidized and converted to cyanocobalamin (7). This form of vitamin B_{12} is not metabolically active until it is reduced. Porter and Thompson (11) reported 100% loss of vitamin B_{12} when milk was sterilized in the bottle. During storage of the UHT milk over a 20-wk period, all of the vitamin B_{12} activity was lost in this study. This loss may be due to exposure of vitamin B_{12} to dissolved oxygen in the container causing oxidation of the vitamin. Due to a shortage of vitamin B_{12} medium, the UHT milk samples for 2, 4, and 8 wk of storage were frozen until the 12th wk when the medium became available. The authors experienced a major difference between the concentration of the vitamin in the frozen and unfrozen samples. Frozen samples contained more vitamin B_{12} per milliliter of milk than unfrozen samples. One explanation for this result may be that denaturation of the milk proteins from freezing may release some of the bound vitamin yielding a higher value for B_{12}.

Loss of folic acid due to UHT processing is in the range of 10 to 20% as reported by Renner (12). Folic acid losses in this study amounted to 12.5% due to UHT processing. Losses in folic acid are associated with the oxygen content in the milk and the level of ascorbic acid which acts as a protective influence (5). Folic acid in the raw milk is present in the reduced metabolically active coenzyme form often conjugated to one or more glutamates in peptide linkage (7). During heat treatment, these labile active forms are either oxidatively destroyed or oxidized and converted to pteroylgutamic acid, which is stable and unless reduced is not metabolically active. Ascorbic acid is known to be protective to these labile active forms and prevents the formation of pteroylglutamic acid (6).

Folic acid losses in the milk during the first 12 wk of storage amounted to 10.6%. After 20 wk of storage, 32.4% of the folic acid activity was lost. The losses in storage could be attributed to the oxygen in the milk and to the loss of a large amount of ascorbic acid in the latter part of the storage study. Oxidative loss of a large amount of ascorbic acid leaves the labile forms of folic acid unprotected, thereby forming stable compounds with a consequent loss of folic acid activities.

It is evident from the study that UHT processing had a minimum effect on losses of B_2 (3%) and B_6 (3%). Greater losses occurred in folic acid (12%), B_{12} (18%), and vitamin C (32%). Significant losses occurred during 20 wk storage in the range of 32 to 100% with the exception of B_2, which showed no loss. The level of oxygen in the package or other oxidizing agents in the milk may be responsible for the loss of these vitamins during storage.

REFERENCES

1 Association of Official Agricultural Chemists. 1975. Official methods of analysis. 12th ed. Washington, DC.
2 Barr, A. J., J. H. Goodnight, J. P. Sall, and J. T. Helwig. 1976. SYSREG procedure *in* A user's guide to SAS. SAS Inst. Inc., Raleigh, NC.
3 Difco Laboratories, Inc. 1972. Manual for microbiological and clinical laboratory procedures. Difco Laboratories, Inc., Detroit, MI.
4 Ford, J. E. 1967. The influence of the dissolved oxygen in milk on the stability of some vitamins towards heating and subsequent exposure to sunlight. J. Dairy Res. 34:239.
5 Ford, J. E., J.W.G. Porter, S. Y. Thompson, J. Toothill, and J. Edwards-Webb. 1969. Effect of UHT processing and of subsequent storage on the vitamin content of milk. J. Dairy Res. 36:447.
6 Hall, H. S. 1970. Sterilization of milk. Natl. Inst. Res. Dairying-Bienn. Rev., Paper No. 3560.
7 Herbert, V., N. Colman, and E. Jacob. 1978. Folic acid and vitamin B_{12}. Modern nutrition in health and disease. 6th ed. Lea & Febiger, Philadelphia,

PA.
8 Kessler, H. G. 1981. Food engineering and dairy technology. Verlag A. Kessler Publ. House, Freicing, Germany.
9 Kirk, J. R. 1974. Automated method for the analysis of riboflavin in milk, with application to other selected foods. J. Assoc. Offic. Agric. Chem. 57:1085.
10 Omaye, S. T., J. D. Turnbull, and H. E. Sauberlich. 1979. Selected methods for the determination of ascorbic acid (vit. C) in animal cells, tissues and fluids. Methods Enzymol. 62:3.
11 Porter, J.W.G., and S. Y. Thompson. 1972. The nutritive value of UHT milk. Page 56 *in* Int. Dairy Congr., IDF Monogr. UHT milk, Annul Bull., Part V.
12 Renner, E. 1979. Nutritional and biochemical characteristics of UHT milk. Pages 21–52 *in* Conf. on UHT processing and aseptic packaging of milk and milk products. North Carolina State Univ., Raleigh.
13 Thompson, S. Y. 1969. Nutritional aspects of UHT processed products. Proc. Seminar, Agric. Inst., Rep. Ireland.