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EFFECTS OF STORAGE ON VITAMIN-FORTIFIED CHEDDAR CHEESE SPREAD

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) A study on vitamin fortified, cheddar cheese spread was conducted at Natick Research and Development Laboratories (NLABS) to determine if the product would retain its acceptability and nutritional value in extended storage. One and one-half ounce packets of fortified and unfortified cheese spread were stored for up to three years at 4°C and 21°C and for up to two years at 38°C. At six-month intervals samples were analyzed for sensory and nutrient data. This testing resulted in the recommendation that thermoprocessed, flexibly packaged, cheddar cheese spread be fortified with vitamin A, ascorbic acid, thiamin, and pyridoxine.		

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PREFACE

This study was conducted to determine the effects of storage on the stability and acceptability of cheddar cheese spread and to evaluate the spread as a carrier of vitamin A, thiamin, ascorbic acid, and pyridoxine in the Meal, Ready-to-Eat, Individual.

This effort was undertaken 1973:O&MA work unit 33146558000, Storage Stability of Rations and Subsistence Items.

The work described in this report was performed in-house at NLABS, except that all nutrient analyses (proximate, mineral, and vitamin) were performed by Shankman Laboratories, Los Angeles, California, under contract numbers DAAG17-72-D-0002 and DAAK03-75-C-0015. The work was performed from November 1973 to March 1977.

The authors gratefully wish to acknowledge the contributions of Mr. Myer Glickstein for putting the cheese into storage and pulling samples at each withdrawal; Mr. Leon Klarman and Dr. Edward Ross for their data analysis support and Mrs. Joyce Barrett, Mrs. Judy Tamburro, and Ms. Nancy Irwin for their dedicated typing assistance.



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EFFECTS OF STORAGE ON VITAMIN-FORTIFIED CHEDDAR CHEESE SPREAD

INTRODUCTION

A basic requirement for all military rations is that they must meet the Daily Dietary Nutrient Allowances prescribed in AR 40-25 at time of consumption.¹ Each meal designed for combat ration use must provide one third of the prescribed daily nutrients. It is often necessary to store packaged operational rations for long periods of time and at extreme temperatures. This storage can result in considerable loss of vitamins that are sensitive to oxidation and to heat. It is therefore essential to fortify certain ration components to assure availability of the prescribed amounts of nutrients if the ration is consumed following long and/or adverse storage.

The purpose of this study was to test the stability of vitamins in a fortified cheddar cheese spread as well as the acceptability of the product when stored over a three-year period at 4°C and 21°C and two years at 38°C. Developed by the US Army Natick Research and Development Laboratories (NLABS) as a component of the Meal, Ready-to-Eat, Individual (MRE), thermoprocessed, flexibly packaged cheese spread is an item common to over one third of the menus (some of which contain less than the prescribed levels of vitamins). Because of its high overall acceptability, the cheese spread is very likely to be eaten by the soldier. It plays a role as a spread in the MRE similar to that of peanut butter, which has been shown to be a very acceptable and stable carrier of vitamin A, thiamin, and ascorbic acid.²

Little information is available in the literature on fortified, processed cheeses. Long-term storage studies conducted in the 1950s by Cecil and Woodroof³ on unfortified, processed American cheese in parchment-lined cans, however, showed that the quality of the cheese stored at 0°C (32°F) or lower was rated "good" in color, texture, and flavor after seven years. The quality rating for this processed American cheese was "good" after four years at 8°C (47°F), and "fair" after four years at 21°C (70°F). Cecil and Woodroof stated that panel members did not agree on the quality of cheese stored at 8°C and 21°C; some considered the changes that occurred to be desirable manifestations of aging, others thought the cheese increasingly undesirable.

¹ Medical Services Nutritional Standards. Army Regulation No. 40-25/BUMED Instruction No. 10110.3E/Air Force Regulation No. 160-95, 30 August 1976.

² M.H. Thomas and H. Spector. The effect of storage on fortified peanut butter. Quartermaster Food and Container Institute for the Armed Forces, December 1954.

³ S.R. Cecil and J.G. Woodroof. Long-term storage for military rations. Quartermaster Food and Container Institute for the Armed Forces, p. 93 (1962).

Studies by Salunkhe, Wu, Do, and Giffes⁴ on vitamin retention and acceptability of flexibly packaged, unfortified cheese spread showed that no significant changes in either thiamin or riboflavin levels occurred during storage for 54 months at 4°C. Discernible losses in both vitamins were found during storage at 21°C and 38°C. D.K. Salunkhe, M.T. Wu, J.Y. Do, and J.W. Giffes also reported that while the cheese spread changed in acceptability during storage at 4°C and 21°C, it was "still in a very acceptable condition" (i.e., acceptance ratings on a 9-point hedonic scale did not drop below "like slightly") even after 54 months storage at either temperature. Storage at 38°C, however, was terminated after 12 months when the mean acceptance ratings dropped to "dislike slightly."

MATERIAL AND METHOD

In this study, it was planned that 1½ oz of fortified, cheddar cheese spread would provide initially 50% of the Daily Dietary Nutrient Allowance (DDNA) for military personnel for vitamin A, thiamin, and pyridoxine and 63% of the DDNA for ascorbic acid (see Table 1). This fortified cheese spread should retain at least 70% of its initial levels of vitamin A, thiamin, pyridoxine and ascorbic acid during storage for 36 months at 21°C and for at least six months storage at 38°C. Therefore, this study undertook to determine the actual decrement in vitamin content and acceptance during storage and to assess the suitability of cheddar cheese spread as a vitamin fortified carrier.

Quantities of 1½ oz packages of vitamin fortified and unfortified cheddar cheese spread were purchased from Kraftco, R and D Division, Glenview, Illinois. The products were produced according to the military specification, Cheese Spread, Cheddar MIL-C-595-C, except that the spreads were packaged in polyolefin/aluminum foil/polyester pouches instead of cans. Also, the vitamins listed in Table 1 were required to be present at the specific levels for the fortified product.

Table 1. Vitamin fortification of cheddar cheese spread (per 1½ oz)

	Required Level
Vitamin A, IU	2500.0
Ascorbic Acid, mg	38.0
Pyridoxine, mg	1.0
Thiamin, mg	0.8

⁴D.K. Salunkhe, M.T. Wu, J.Y. Do, and J.W. Giffes. Effects of long-term storage on quality of processed foods I. Meal, Ready-to-Eat, Individual ration items packed in flexible retortable pouches. J. of Food Qual., 2:75-103 (1978).

The vitamin A fortification was supplied by a vitamin A ester (palmitate) of edible grade, sufficiently refined so that it would not impart a fishy or other objectionable odor or flavor to the cheese spread. The ascorbic acid, thiamin, and pyridoxine conformed to the Food Chemicals Codex. The method of incorporating the vitamins into the product was not specified but was left to the discretion of the manufacturer who was experienced in fortification of food. The methods of analysis are described in references 5 and 6.

Storage

Vitamin fortified and unfortified lots of cheddar cheese spread were stored for a total of three years at 4°C and 21°C and for two years at 38°C. Samples were withdrawn for vitamin analysis and sensory evaluation at six month intervals. Table 2 indicates the test plan for storage withdrawal.

Table 2. Plan of withdrawal for fortified and unfortified cheese spread

Storage Temperature °C	Months of Storage					
	0	6	12	18	24	30
4		X	X	X	X	X
21	X	X	X	X	X	X
38		X	X	X	X	

Sensory Analyses

For each acceptance test, 36 consumers were selected at random from the 400 members of the NLABS Food Acceptance Taste Panel. Fortified and unfortified cheese spread samples labeled "Processed Cheese Spread" were independently presented in random order and rated on a nine-point hedonic scale at each session⁷ (see Appendix A). All test samples were allowed to reach room temperature before each test. A serving was approximately one quarter oz of cheese spread on an unsalted cracker. The cheese samples were presented to panelists in the NLABS acceptance testing area, which is designed to minimize noise and distraction. Means and standard deviations were obtained on all product ratings.

⁵The use of W. Horwitz (ed). Official methods of analysis of the association of official analytical chemists, AOAC, 11th Ed. (1970) was stipulated in contract No. DAAK 17-72-0002 and No. DAAK 03-75-0015.

⁶W. Horwitz (ed). Official methods of analysis of the association of official analytical chemists, ADAC, 11th Ed. (1970).

⁷D.R. Peryam and F.J. Pilgrim. Hedonic scale method of measuring food preference. Food Technol. 11:9 (1957).

Nutrient Analyses

Vitamin analyses were performed in duplicate by Shankman Laboratories.* Each sample withdrawn from storage was shipped to the laboratory via air freight. The contract for nutrient analyses allowed 21 days for analyses and reporting of results to NLABS. Each sample from storage consisted of eight pouches of product that the analytical laboratory composited just prior to analysis. For the initial prestorage samples, five replicates of each test variable were analyzed for proximate, mineral, and vitamin content. For each subsequent storage withdrawal, three replicates of each variable were analyzed for moisture and vitamin content only. The analytical methods were stipulated in the contract and are listed in Appendix B. Standard statistical methods (ANOVA and regression analysis) were used to analyze results.

RESULTS AND DISCUSSION

Sensory Analyses — Consumer Acceptance

Table 3 presents the consumer ratings of the fortified and unfortified cheddar cheese spreads stored at 4°C and 21°C over a 36-month period and at 38°C for 24 months. An analysis of variance was performed at each of three temperatures on data for the fortified and also the unfortified product. The means and standard deviations are given with the results of Duncan's Multiple Range Test (Table 3). Figure 1 illustrates the consumer ratings with broken lines representing fortified cheddar cheese spread and solid lines representing the unfortified cheddar cheese spread.

Initially, consumers rated, both the fortified and unfortified cheese spreads "like moderately." After 36 months storage at 4°C or 21°C, neither spread was judged to have changed significantly; both spreads stored at both temperatures were still rated between "like slightly" and "like moderately" after 36 months.

The fortified and unfortified cheese, spread stored at 38°C had declined in acceptability (significant $p < 0.05$) after, six months storage, at which time consumers rated both products "like slightly." After 18 months storage, the fortified and unfortified samples again dropped in acceptability: consumers rated both between "neither like nor dislike" and "dislike slightly," and both samples retained this rating through 24 months storage at 38°C.

Storage at 38°C significantly lowered the acceptance ratings for cheese spread compared to storage at 4°C and 21°C. Fortification was not a factor in the acceptance of cheese spread when stored for 36 months at 4°C or 21°C or for 24 months at 38°C.

*Shankman Laboratories, Los Angeles, California, Contract Nos. DAAG17-72-D-0002 and DAAK03-75-C-0015 (letter reports).

Table 3. Consumer hedonic scale ratings of fortified and unfortified cheese spread stored at 4°C, 21°C and 38°C up to 36 months**

Storage Time (Months)	Mean Standard Deviation	
	Fortified	Unfortified
4°C		
0	7.2 ± 1.28	7.2 ± 1.50*
6	6.7 ± 1.72	6.6 ± 1.48
12	6.5 ± 1.84	7.1 ± 1.00
18	6.7 ± 1.54	6.4 ± 1.52
24	6.4 ± 1.59	6.4 ± 1.31
30	6.2 ± 1.83	6.4 ± 1.79
36	6.7 ± 1.41	6.7 ± 1.31
21°C		
0	7.2 ± 1.28	7.2 ± 1.50
6	6.6 ± 1.56	6.6 ± 1.52
12	6.3 ± 1.64	6.7 ± 1.37
18	6.4 ± 1.81	6.7 ± 1.35
24	6.5 ± 1.83	6.5 ± 1.42
30	6.0 ± 1.89	6.3 ± 1.97
36	6.5 ± 1.44	6.6 ± 1.25
38°C		
0	7.2 ± 1.28*	7.2 ± 1.50
6	5.9 ± 1.78	6.0 ± 1.63
12	6.2 ± 2.07	6.3 ± 1.80
18	4.8 ± 2.26	4.5 ± 1.89
24	4.7 ± 2.13	4.8 ± 2.35

*Means followed by different letters are significantly different ($p < 0.05$) as determined by Duncan's Multiple Range Test.

**The nine-point scale is shown in Appendix A.

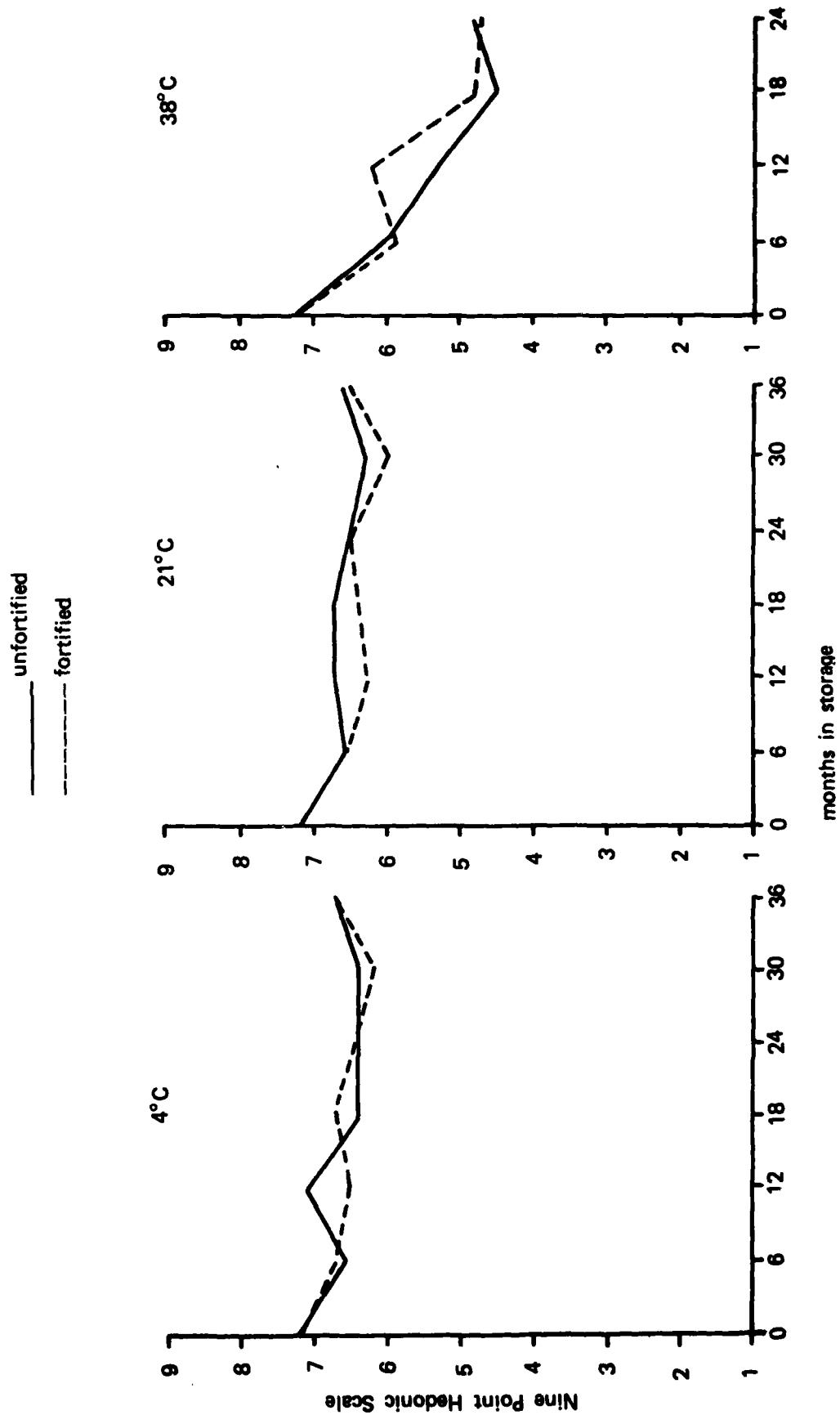


Figure 1. Consumer ratings of fortified and unfortified cheese spread stored at 4°C, 21°C and 38°C up to 36 months.

Proximate and Mineral Analyses

The mean values for the proximate and mineral analyses obtained on the fortified and unfortified cheese spread are given in Table 4 and show both products to be similar in protein, fat, and mineral content. The moisture content of the fortified cheese spread, however, was slightly higher than that of the unfortified spread.

Table 4. Proximate and mineral content of fortified and unfortified cheese spreads (per 100 g)

	Fortified	Unfortified
Moisture, g	42.6 ± 0.15	39.9 ± 0.91
Protein, g	14.1 ± 0.76	14.1 ± 0.44
Fat, g	41.7 ± 2.25	42.5 ± 1.54
Ash, g	4.9 ± 0.23	4.9 ± 0.22
Calcium, mg	371 ± 4.2**	412 ± 48.1**
Phosphorus, mg	692 ± 60.3	705 ± 55.2**
Iron, mg	0.87 ± 0.19**	0.95 ± 0.37
Sodium, mg	1554 ± 260	1712 ± 418
Potassium, mg	47 ± 16.0	52 ± 16.7
Magnesium, mg	15 ± 0.3	16 ± 0.5
Sodium Chloride, g	1.94 ± 0.12	1.87 ± 0.07

*N = 3

**N = 2

The carbohydrate, determined by difference, i.e., by subtracting the total percent moisture, protein, fat and ash from 100 percent, is zero for both the fortified and unfortified products. Attention is directed to the fact that the mean proximate value for each spread totals more than 100 g, 103.3 g for the fortified product and 101.4 g for the unfortified product. Analyses of previous procurements of cheese spread indicate that analytical errors were probably in the moisture and fat values, and that either or both values could be high. The specification for this product establishes limits for both fat and moisture at not less than 38% nor more than 42% each. Based upon data in Table 4, fortified cheese spread provides 427 calories per 100 g, and unfortified cheese spread 437 calories per 100 g.

Vitamin Content

Tables 5 and 6 summarize the vitamin content of fortified and unfortified cheese spread stored up to 36 months at 4°C, 21°C and 38°C. These data are provided on an "as is" basis, i.e., no correction for moisture content. An analysis of variance was performed on the data obtained for each nutrient at each temperature.

To determine the concentration of the vitamin attributable to the fortification, the value obtained for the unfortified product was subtracted from the appropriate fortified product. These data are presented on a moisture-free basis in Table 7. Regression analyses were performed on these data and those curves showing statistically significant changes are shown in Figures 2 through 4. Riboflavin has not been included because this vitamin was not added to the fortified product.

Desired and actual vitamin content of the initial samples

Table 8 compares, on a 100 g basis, the levels of nutrients which were desired for the initial samples of fortified cheese spread with the mean analytical values obtained (Table 5).

Table 8. Initial vitamin content of fortified cheese spread (per 100 g)

Nutrient	Desired Levels	Actual Levels
Vitamin A, IU	5,880	3,080
Ascorbic Acid, mg	89	102
Thiamin, mg	1.88	3.0
Pyridoxine, mg	2.35	2.96

These data show that the mean initial level of vitamin A was lower than desired while the ascorbic acid, thiamin, and pyridoxine levels were more than adequate.

Vitamin A

The analytical values for vitamin A both initially and over time fluctuated widely. The standard deviations varied from 1.4% of the mean value to 37.7% of the mean value (see Table 7), with the initial samples having the largest standard deviations. Because of this fluctuation, the changes in vitamin A content that occurred during storage did not prove statistically significant although the regression curves showed a trend toward lower vitamin A content with increased time in storage at 38°C.

Table 5. Vitamin content of fortified cheese spread stored at 4°C, 21°C and 38°C up to 36 months mean $\pm$ standard deviation per 100 g (as-is basis)*
(N = 3 Duplicate Analyses)

Months in Storage	Vitamin A IU	Ascorbic Acid mg	Thiamin mg	Riboflavin mg	Pyridoxine mg
4°C					
0**	3081 $\pm$ 868ab	102 $\pm$ 6bc	3.00 $\pm$ 0.74	0.25 $\pm$ 0.02ab	2.96 $\pm$ 0.20b
6	1973 $\pm$ 259b	99 $\pm$ 6bc	3.32 $\pm$ 0.03	0.22 $\pm$ 0.01cd	2.60 $\pm$ 0.45b
12	3318 $\pm$ 155a	89 $\pm$ 15c	2.71 $\pm$ 0.15	0.27 $\pm$ 0.02a	2.75 $\pm$ 0.18b
18	2430 $\pm$ 215ab	114 $\pm$ 2ab	2.73 $\pm$ 0.08	0.26 $\pm$ 0.01ab	2.82 $\pm$ 0.10b
24	2178 $\pm$ 60ab	115 $\pm$ 3ab	3.11 $\pm$ 0.06	0.21 $\pm$ 0.02d	2.55 $\pm$ 0.14b
30	2635 $\pm$ 182ab	122 $\pm$ 3a	2.76 $\pm$ 0.04	0.22 $\pm$ 0.01cd	3.77 $\pm$ 0.10a
36	2303 $\pm$ 112ab	111 $\pm$ 15ab	3.22 $\pm$ 0.10	0.24 $\pm$ 0.01bc	2.88 $\pm$ 0.28b
21°C					
0**	3081 $\pm$ 868	102 $\pm$ 6a	3.00 $\pm$ 0.74a	0.25 $\pm$ 0.02a	2.96 $\pm$ 0.20b
6	2443 $\pm$ 32	95 $\pm$ 9a	2.79 $\pm$ 0.10ab	0.23 $\pm$ 0.01b	2.73 $\pm$ 0.06bc
12	2647 $\pm$ 206	76 $\pm$ 8b	2.05 $\pm$ 0.14bc	0.21 $\pm$ 0.01bc	2.44 $\pm$ 0.10c
18	2352 $\pm$ 69	107 $\pm$ 2a	2.00 $\pm$ 0.07bc	0.25 $\pm$ 0.02a	2.78 $\pm$ 0.19bc
24	2325 $\pm$ 97	103 $\pm$ 6a	2.14 $\pm$ 0.10bc	0.21 $\pm$ 0.01bc	2.57 $\pm$ 0.20c
30	2773 $\pm$ 176	102 $\pm$ 4a	1.68 $\pm$ 0.16c	0.18 $\pm$ 0.02c	3.60 $\pm$ 0.10a
36	2457 $\pm$ 50	99 $\pm$ 4a	1.80 $\pm$ 0.04c	0.22 $\pm$ 0.01b	2.78 $\pm$ 0.13 bc
38°C					
0**	3081 $\pm$ 868	102 $\pm$ 6a	3.00 $\pm$ 0.74a	0.25 $\pm$ 0.02a	2.96 $\pm$ 0.20a
6	2905 $\pm$ 345	69 $\pm$ 1b	1.15 $\pm$ 0.02b	0.22 $\pm$ 0.00b	2.45 $\pm$ 0.09b
12	2633 $\pm$ 79	69 $\pm$ 20b	0.36 $\pm$ 0.02b	0.21 $\pm$ 0.01b	2.27 $\pm$ 0.11b
18	2543 $\pm$ 58	42 $\pm$ 3c	0.20 $\pm$ 0.02b	0.20 $\pm$ 0.01b	2.38 $\pm$ 0.08b
24	2870 $\pm$ 35	35 $\pm$ 2c	†	0.20 $\pm$ 0.01b	2.12 $\pm$ 0.23b

*An analysis of variance was performed at each temperature. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by Newman Keuls' Multiple Range test. For example, 3081 $\pm$ 868ab indicates at 0 storage the value is not significantly different from other values with a or b.

**N = 5 (duplicate analyses)

†all values ≥ 0.001

Table 6. Vitamin content of unfortified cheese spread stored at 4°C, 21°C and 38°C up to 36 months mean $\pm$ standard deviation per 100 g (as-is basis)*
(N = 3 Duplicate Analyses)

Months in Storage	Vitamin A IU	Ascorbic Acid mg	Thiamin mg	Riboflavin mg	Pyridoxine mg
4°C					
0†	462 $\pm$ 158ab	1 $\pm$ 1	0.02 $\pm$ 0.01	0.26 $\pm$ 0.01a	0.04 $\pm$ 0.02
6	291 $\pm$ 119b	1 $\pm$ 1	0.01 $\pm$ 0.01	0.25 $\pm$ 0.01a	0.03 $\pm$ 0.00
12	650 $\pm$ 28a	2 $\pm$ 2	0.01 $\pm$ 0.01	0.24 $\pm$ 0.01a	0.05 $\pm$ 0.01
18	569 $\pm$ 97a	2 $\pm$ 0	0.01 $\pm$ 0.01	0.25 $\pm$ 0.01a	0.03 $\pm$ 0.00
24	418 $\pm$ 32ab	2 $\pm$ 1	0.01 $\pm$ 0.00**	0.21 $\pm$ 0.01bc	0.04 $\pm$ 0.00
30	632 $\pm$ 33a	3 $\pm$ 1	0.02 $\pm$ 0.01	0.20 $\pm$ 0.01c	0.04 $\pm$ 0.00
36	472 $\pm$ 23ab	4 $\pm$ 2	0.01 $\pm$ 0.01	0.22 $\pm$ 0.01b	0.03 $\pm$ 0.01
21°C					
0†	462 $\pm$ 158	1 $\pm$ 1bc	0.02 $\pm$ 0.01	0.26 $\pm$ 0.01a	0.04 $\pm$ 0.02
6	417 $\pm$ 127	0 $\pm$ 0c	0.01 $\pm$ 0.00	0.24 $\pm$ 0.01b	0.03 $\pm$ 0.00
12	527 $\pm$ 16	2 $\pm$ 1bc	0.02 $\pm$ 0.01	0.22 $\pm$ 0.01bc	0.03 $\pm$ 0.01
18	532 $\pm$ 19	3 $\pm$ 2ab	0.01 $\pm$ 0.00	0.27 $\pm$ 0.02a	0.02 $\pm$ 0.01
24	475 $\pm$ 157	2 $\pm$ 0bc	††	0.19 $\pm$ 0.02d	0.04 $\pm$ 0.01
30	705 $\pm$ 30	3 $\pm$ 1ab	0.02 $\pm$ 0.00	0.21 $\pm$ 0.01cd	0.04 $\pm$ 0.01
36	452 $\pm$ 6	5 $\pm$ 1a	0.01 $\pm$ 0.00	0.22 $\pm$ 0.02bc	0.04 $\pm$ 0.01
38°C					
0†	462 $\pm$ 158	1 $\pm$ 1	0.02 $\pm$ 0.01	0.26 $\pm$ 0.01a	0.04 $\pm$ 0.02
6	436 $\pm$ 172	0 $\pm$ 0	0.01 $\pm$ 0.00	0.23 $\pm$ 0.01b	0.02 $\pm$ 0.01
12	517 $\pm$ 54	3 $\pm$ 2	0.01 $\pm$ 0.00	0.23 $\pm$ 0.02b	0.03 $\pm$ 0.01
18	635 $\pm$ 42	3 $\pm$ 1	0.01 $\pm$ 0.00	0.25 $\pm$ 0.00ab	0.02 $\pm$ 0.01
24	550 $\pm$ 31	3 $\pm$ 0	††	0.15 $\pm$ 0.01c	0.02 $\pm$ 0.00

*An analysis of variance was performed at each temperature. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by Newman Keuls' Multiple Range test. See * footnote, Table 5.

**N = 1

†N = 5 (duplicate analyses)

††all values ≥ 0.001

Table 7. Differences in vitamin content of fortified and unfortified cheese spread stored at 4°C, 21°C and 38°C up to 36 months mean $\pm$ standard deviation per 100 g (moisture-free basis)*
(N = 3 Duplicate Analyses)

Months in Storage	Vitamin A IU	Ascorbic Acid mg	Thiamin mg	Pyridoxine mg
4°C				
0 †	4598 $\pm$ 1734	174 $\pm$ 11bc	5.19 $\pm$ 1.29	5.10 $\pm$ 0.38b
6	2954 $\pm$ 270	171 $\pm$ 9bc	5.73 $\pm$ 0.04	4.47 $\pm$ 0.79b
12	4737 $\pm$ 269	150 $\pm$ 25c	4.68 $\pm$ 0.26	4.70 $\pm$ 0.33b
18	3265 $\pm$ 424	194 $\pm$ 4ab	4.68 $\pm$ 0.16	4.81 $\pm$ 0.19b
24	3124 $\pm$ 71	196 $\pm$ 4ab	5.43 $\pm$ 0.09	4.40 $\pm$ 0.23b
30	3678 $\pm$ 376	210 $\pm$ 6a	4.79 $\pm$ 0.09	6.53 $\pm$ 0.18a
36	3260 $\pm$ 222	186 $\pm$ 27ab	5.57 $\pm$ 0.19	4.97 $\pm$ 0.49b
21°C				
0 †	4598 $\pm$ 1734b	174 $\pm$ 11a	5.19 $\pm$ 1.29a	5.10 $\pm$ 0.38b
6	3667 $\pm$ 288ab	164 $\pm$ 16a	4.82 $\pm$ 0.18ab	4.69 $\pm$ 0.13bc
12	3781 $\pm$ 374a	129 $\pm$ 15b	3.55 $\pm$ 0.25bc	4.21 $\pm$ 0.14c
18	3215 $\pm$ 179ab	182 $\pm$ 3a	3.46 $\pm$ 0.12bc	4.79 $\pm$ 0.29bc
24	3320 $\pm$ 279ab	177 $\pm$ 12a	3.78 $\pm$ 0.18bc	4.48 $\pm$ 0.37bc
30	3701 $\pm$ 286c	175 $\pm$ 8a	2.91 $\pm$ 0.28c	6.25 $\pm$ 0.19a
36	3581 $\pm$ 92ab	166 $\pm$ 6a	3.13 $\pm$ 0.07c	4.80 $\pm$ 0.23bc
38°C				
0 †	4598 $\pm$ 1734	174 $\pm$ 11a	5.19 $\pm$ 1.29a	5.10 $\pm$ 0.38a
6	4252 $\pm$ 839	117 $\pm$ 1b	1.95 $\pm$ 0.55b	4.15 $\pm$ 0.08b
12	3690 $\pm$ 106	114 $\pm$ 33b	0.60 $\pm$ 0.42c	3.86 $\pm$ 0.22b
18	3327 $\pm$ 45	68 $\pm$ 5c	0.33 $\pm$ 0.40c	4.06 $\pm$ 0.14b
24	4130 $\pm$ 114	57 $\pm$ 2c	0.00 $\pm$ 0.00c	3.67 $\pm$ 0.42b

*An analysis of variance was performed at each temperature. Means followed by different letters are significantly different ($p \leq 0.05$) as determined by Newman Keuls' Multiple Range Test. See * footnote, Table 5.

†N = 5 (duplicate analyses)

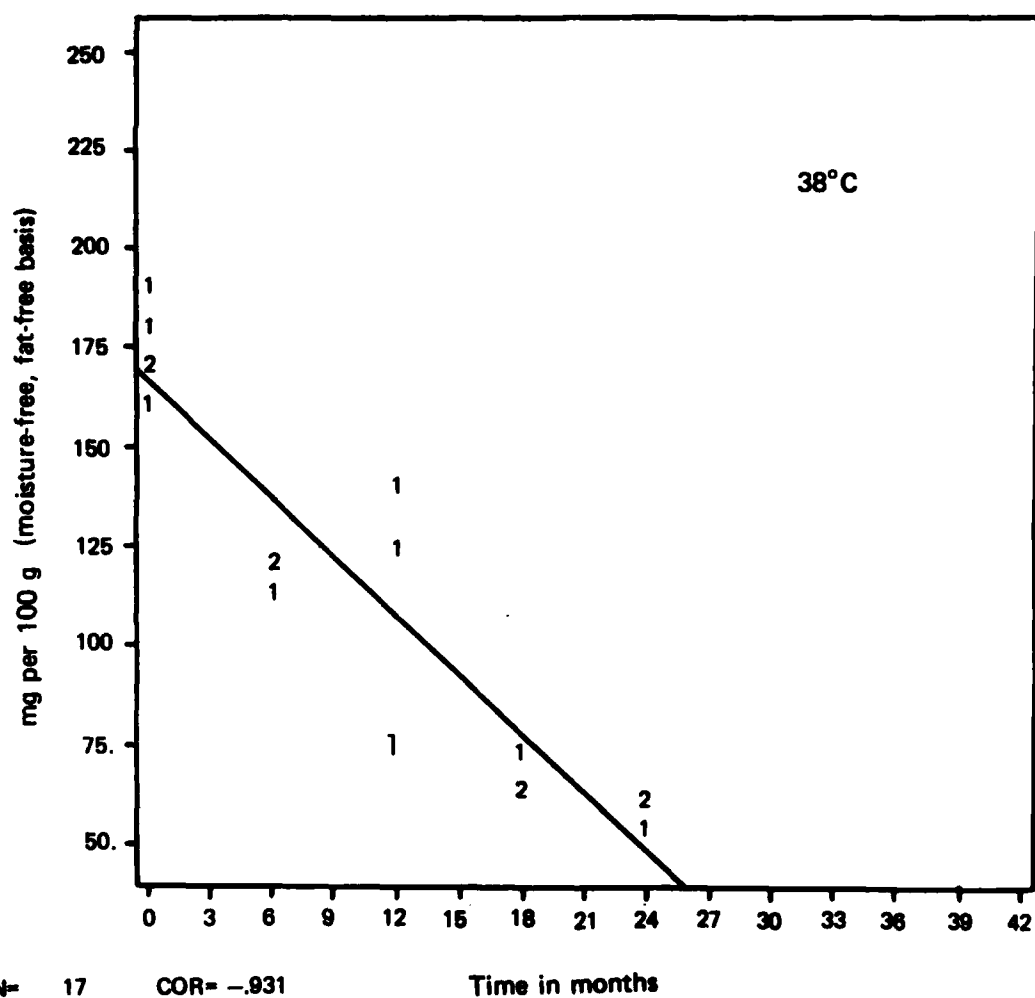


Figure 2. Regression curve for ascorbic acid based on differences for vitamin content between fortified and unfortified cheese spread stored at 38°C.

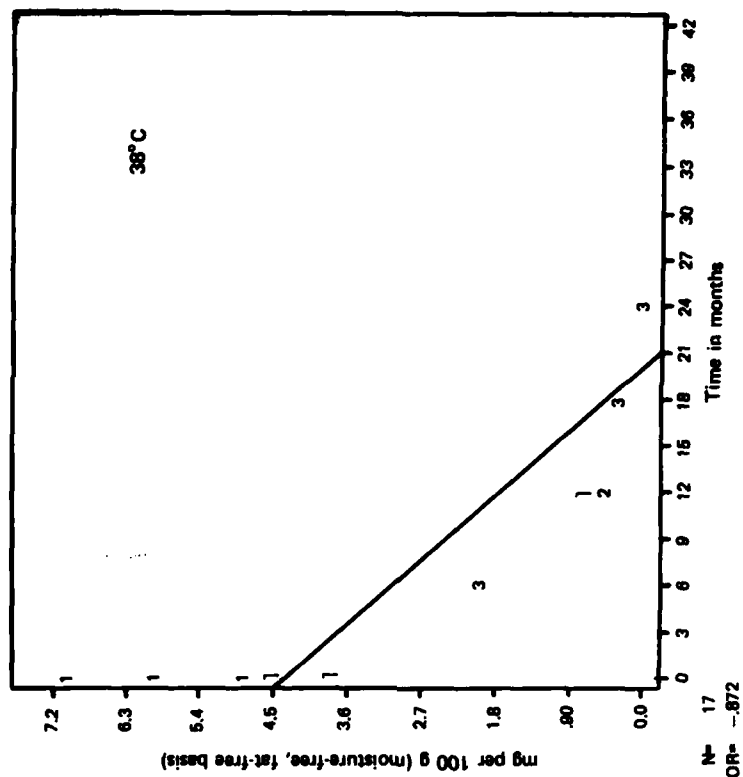
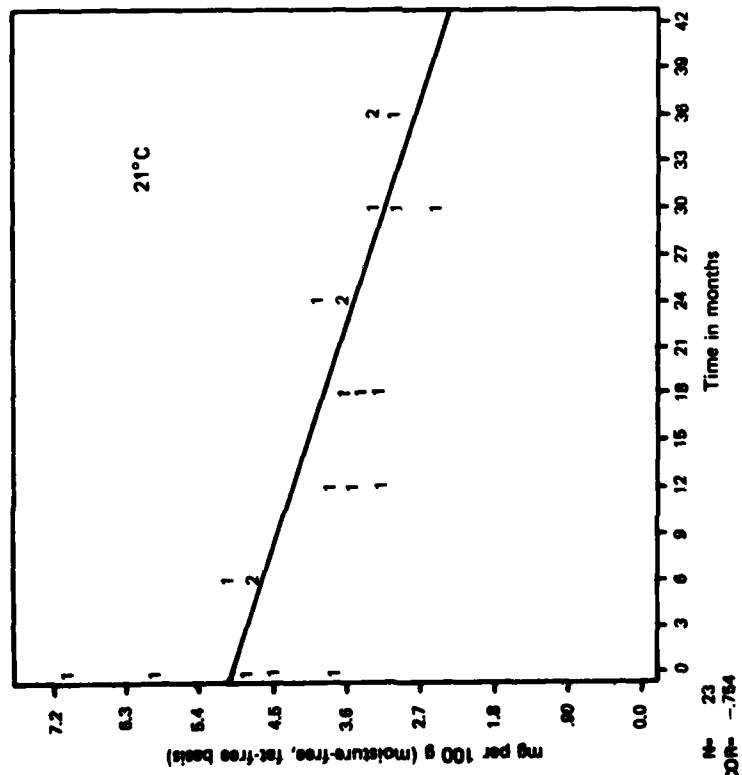


Figure 3. Regression curves for thiamin based on differences for vitamin content between fortified and unfortified cheese spread stored at 21°C and 38°C.

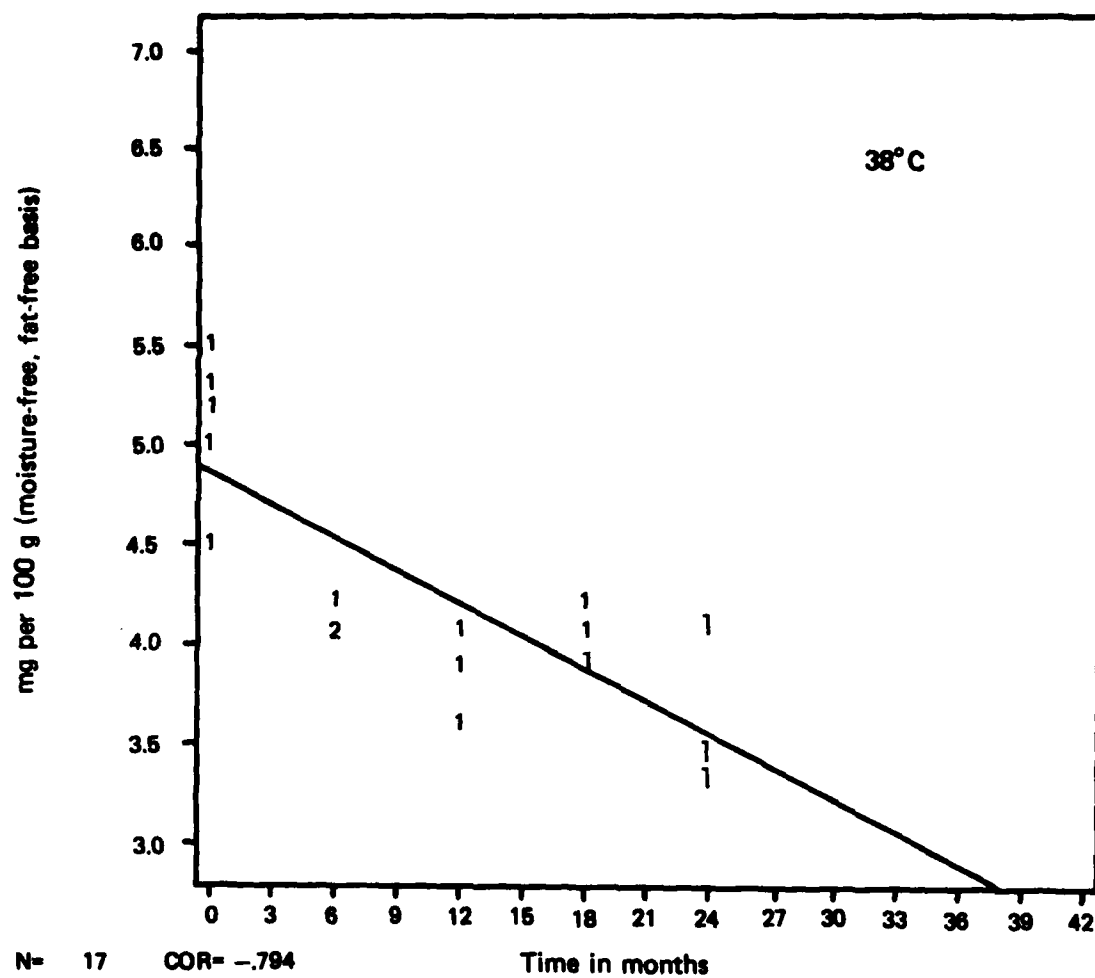


Figure 4. Regression curve for pyridoxine based on difference for vitamin content between fortified and unfortified cheese spread stored at 38°C.

Carotene

Analyses for carotene were conducted to determine the initial levels present in both the fortified and unfortified cheese spread and to assess the storage stability of carotene, since it is a precursor of vitamin A and makes a contribution to the total vitamin A content. The concentration of carotene in the initial samples of fortified and unfortified cheese spread was not significantly different, averaging respectively 0.15 ± 0.06 mg and 0.14 ± 0.00 mg per 100 g of product. These levels would contribute, respectively, 250 IU and 230 IU to the vitamin A content of 100 g of cheese spread, or approximately 100 IU per 1½ oz serving. Statistical analyses of the data indicated no significant changes in carotene occurred in either spread over time at any of the three temperatures studied.

Ascorbic acid

The mean values for ascorbic acid in the fortified cheese spread samples stored at 4°C and 21°C fluctuated. Statistical analyses of the data on both an "as is" and moisture-free basis (Tables 5 and 7) show ascorbic acid to be stable under these two storage conditions. Ascorbic acid was not stable in cheese spread stored at 38°C. Significant losses occurred even after six months storage ($p \leq 0.05$). Analysis of the ascorbic acid content on a moisture-free basis (Table 7) shows that retention during storage at 38°C of the vitamin as fortifier was 67%, 66%, 39% and 33% at 6, 12, 18 and 24 month intervals. When these data were subjected to regression analyses, the predicted retention of ascorbic acid was found to be 82% after six months storage, 65% after 12 months, and 29% after 24 months (Figure 2).

Thiamin

Thiamin was stable in the cheese spread stored for 36 months at 4°C. Changes in thiamin content during storage at 21°C were found statistically significant ($p \leq 0.05$) beginning with data for the 12 month withdrawal. Data in Tables 5 and 6 show a retention of at least 56% of the initial levels throughout 36 months' storage. The regression curves (Figure 3) show that at 38°C the predicted retention is 69% after six months' storage and 39% after 12 months at 38°C.

Riboflavin

Both fortified and unfortified cheese spread samples were analyzed for riboflavin content even though this vitamin was not added to the fortified spread. The data on Tables 5 and 6 show that the retention of riboflavin was good at all temperatures and that probably most of the statistical difference was due more to product variability rather than storage conditions. Levels of riboflavin showed retention for this vitamin after 36 months is 96% and 88% at 4°C and 21°C, respectively, and after 24 months is 80% at 38°C.

Pyridoxine

The overall retentions of pyridoxine following 36 months' storage at 4°C and 21°C are, respectively, 97% and 94% (significant at $p \leq 0.05$) (Table 5). The sharp increase in the

sample values at 30 months for both 4°C and 21°C appears to be due to analytical variability, rather than to an increase in pyridoxine content. Even though changes after 12 and 24 months' storage at 38°C were significant ($p < 0.05$), retention was still 87% and 73%, based on regression analysis (Figure 4). Thus, cheese spread is considered suitable as a carrier for pyridoxine.

Retention of vitamins

Table 9 summarizes the projected retention of vitamins in fortified cheese spread during storage at 21°C and 38°C based on the regression curves of the moisture-free data (Figures 2 through 4). Percents of initial vitamin levels retained at the end of 36 months' storage at 21°C and after 6, 12, and 24 months' storage at 38°C are given.

Table 9. Vitamin fortified cheese spread: projected nutrient retention during storage¹

	Percent			
	21°C 36 Mo	38°C		
		6 Mo	12 Mo	24 Mo
Vitamin A	79	92	84	68
Ascorbic Acid	100	82	65	29
Thiamin	56	69	39	0
Pyridoxine	100	93	87	73

¹ Based on regression analysis of differences between fortified and unfortified products.

CONCLUSIONS

Thermoprocessed, flexibly packaged cheddar cheese spread can be fortified with vitamin A, ascorbic acid, thiamin and pyridoxine to provide initially 50% of the Daily Dietary Nutrient Allowances for these vitamins. If 70% of these initial levels are retained throughout storage, fortified cheese spread will still supply one third of the DDNA. As Table 9 shows, except for thiamin, at least 70% of the initial levels of the other three vitamins (A, ascorbic acid and pyridoxine) will be retained throughout storage for three years at 21°C or six months at 38°C. Only 56% of the initial level of thiamin can be expected to be present after 36 months' storage at 21°C. Thus, 1½ oz of stored cheese spread fortified with 0.80 mg of thiamin may not provide one third of the DDNA. Therefore, the fortification requirement for thiamin in 1½ oz of cheese spread should be increased from 0.80 mg to 1.0 mg. This increase will assure adequate levels of thiamin after 36 months storage at 21°C, and after six months, at 38°C. The increase also will not affect the consumer acceptability of the initially fortified cheese spread because in this study, the actual level of thiamin in the initial fortified sample was 1.28 mg per 1½ oz.

No significant differences in acceptability were noted between the fortified and unfortified cheese spreads. Consumer panelists accorded fortified and unfortified cheese spreads similar acceptance ratings throughout the storage study. Over three years' storage at 4°C and 21°C, the consumer acceptance of both fortified and unfortified cheese spread changed from "like moderately" to "like slightly." After storage for both six and 12 months at 38°C the fortified cheese spread still rated "like slightly." After two years at 38°C, the fortified and unfortified cheese spreads both received "neither like nor dislike" ratings.

RECOMMENDATIONS

It is recommended that cheddar cheese spread be fortified with vitamin A, ascorbic acid, thiamin and pyridoxine to supply in each 1½ oz serving (packet) the following levels of fortification after processing.

Vitamin A, IU	2500
Ascorbic Acid, mg	38
Pyridoxine, mg	1.00
Thiamin, mg	1.00

For thiamin the recommendation increases the amount from 0.80 mg to 1.0 mg required for cheese at time of procurement. The higher level of thiamin is needed to compensate for losses found during 36 months at 21°C. This study also shows that an ample overage of vitamin A is needed to achieve the specified level.

ADDENDUM

As a result of this study, but before it was completed, vitamin fortification requirements for cheese spread (Class 1 product of MIL-C-595) were included in the Limited Production Purchase Description, LP/P DES 27-74, for Cheese Spread, Cheddar; Peanut Butter; and Jelly, Flexibly Packaged. Vitamin-fortified cheese spread has been procured as a component of the Meal, Ready-to-Eat, Individual since 1980.

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APPENDIXES

- A. Hedonic Scale**
- B. Methods of Analyses**

APPENDIX A **Hedonic Scale**

Preference

Like	9
Extremely	
Like	8
Very Much	
Like	7
Moderately	
Like	6
Slightly	
Neither Like	5
Nor Dislike	
Dislike	4
Slightly	
Dislike	3
Moderately	
Dislike	2
Very Much	
Dislike	1
Extremely	

Comments: _____

APPENDIX B

Methods of Analyses⁶

American Association of Official Analytical Chemists, 13th Edition (1980)⁶

Assay	Reference
Moisture	As appropriate
Total Fat	As appropriate
Protein	2.057
Crude Fiber	7.065
Ash	14.006
Phosphorus	2.021
Chloride as NaCl	18.034
Cholesterol	14.149
Fatty Acid Profile	28.051
Total Fatty Acids	
Atomic Absorption Spectrophotometer ⁸	ASAP
Calcium	ASAP
Iron	ASAP
Sodium	ASAP
Potassium	ASAP
Magnesium	ASAP
Other	
Iodine	Anal. Chimica Acta 10, 78 (1954) ⁹

⁸Perkin-Elmer, Analytical methods for atomic absorption spectrophotometry, a technical manual (1964).

⁹W.T. Binnerts. Determination of iodine in milk. Anal. Chimica Acta., 10:78 (1954).

APPENDIX B

Methods of Analyses (cont'd)

Methods of Vitamin Assay — Third Edition (1966)¹⁰

Assay	Pages
Vitamin A	70-79
Carotene	104-115
Thiamin	127-140
Riboflavin	158-164
Niacin	172-176
Pyridoxine	212-219
Vitamin E ^{11,12}	366-396
Ascorbic Acid	299-306
Folacin	227-234
Vitamin B ₁₂	262-270

¹⁰ Association of Vitamin Chemists, Inc., Methods of vitamin assay, Third Edition, 1966.

¹¹ Bro-Rasmussen F. and W. Hjarbe. Determination of α -tocopherol by chromatography on secondary magnesium phosphate. Acta. Chem. Scand., 11:34 (1957).

¹² Dicks-Bushnell, M.W. Column chromatography in the determination tocopherol: florisil, silicic acid and secondary magnesium phosphate. J. Chromato, 27:96 (1967).