CHAPTER IV

EXTENDED SHELF-LIFE STUDY OF A THERMALLY PROCESSED SWEET MILK AND EGG MIX

A. Abstract

A milk and egg mix processed at 92ºC with a 10 sec hold time was evaluated over a seven week refrigerated storage period for nutrient composition and functionality. The process was more than sufficient to destroy *Coxiella burnetti* and *Salmonella senftenberg*, the most heat resistant organisms of concern in processing a milk and egg mix. The spoilage organisms received a 425 D process which was more than adequate for providing a relatively safe product. There were also no significant differences in the chemical and physical changes over the storage period. The microbial counts also showed no significant differences over the storage period with colony forming units/ mL of less than 1 log ($p<0.05$)

The nutritional profile of the milk and egg mix was improved when dried eggs (solids and liquid proportion equivalent to whole egg) were replaced with dried egg white, cholesterol reduced egg yolk, and skim milk. The fat and cholesterol were reduced in the sweetened mixes by 22% and 37.5%, respectively, in the cholesterol reduced formulation (CRF) as compared to the control formulation (CF). The protein content of the milk and egg mix was not altered by the utilization of cholesterol- reduced egg yolk in the CRF as compared to the (CF). Addition of β-galactosidase decreased the lactose by 96%. The CF was more yellow than the CRF in the mixes and baked gels ($p<0.05$). There were also no differences in the gel strength among the baked gels made from the mixes.

B. Introduction

Consumer concerns with nutrition, food safety, and quality have caused manufacturers to recognize the importance of shelf-life evaluation (Man and Jones, 1994).

Shelf-life is defined as the number of days after production that a product can be consumed while keeping its quality standards, meeting consumer expectations, and most of all, remaining safe for consumption (Lewis and Dale, 1994). Factors which limit the shelf-life of food cause chemical, physical, and biological changes which result in sensory change.

Microbial growth is one of the major factors which limit the shelf-life of products (Walker, 1994). Spoilage organisms are neither pathogenic nor toxin producing (Stumbo, 1973). The most important genera of organisms causing food spoilage are *Pseudomonas, Achromobacter, Lactobacillus, Leuconostoc, Proteus, Micrococus, and Aerobacter*. Yeasts and molds also contribute to food spoilage. The maximum heat resistance of these organisms may be characterized by D_{150} values between 1 to 3 min and a z value in the range of 8 to 10 ^{o}F. Pasteurization time and temperature combinations based on these resistance values have been effective in extending the shelf-life of these products under refrigeration. Temperature fluctuation during storage of foods, particularly under refrigerated conditions, could have damaging results on the product quality and storage (Walker, 1994) . Thus, the control of temperature to ensure safety and quality of the product is needed.

For many years, the distribution of dairy products was only feasible to local and regional areas because of the short shelf life. Today, modern technological advances have created a mass market of new products which have an extended shelf-life. These products are able to be distributed to vast areas because of their increased shelf-life.

The objective of this study was to provide an adequate heat treatment to a milk and egg mix that would reduce the microbial load and increase chances for an extended shelf life product. The goal was to provide a process that would render a seven week extended shelf life under refrigerated conditions. The physical, chemical, and microbial

properties were monitored over the period for any changes.

C. Materials and Methods

1. Mix Formulations and Preparation

Formulations. Fluid sweetened milk and egg mix formulations for dessert application were evaluated. In the experiment, two formulations with two levels of lactose were tested in a straight factorial design. All other formulation parameters were the same as those described in Chapter III.

Preparation. The formulation was scaled up 24 times the standard formulation (Penfield and Campbell, 1990) in order to make batches of approximately 6.5 L for processing. All other preparation parameters were completed as previously described in Chapter III.

Thermal Processing. A laboratory scale pasteurizer (Microthermics UHT/ HTST Lab 25 - DH, Microthermics, Inc., Raleigh, NC) with a homogenizer (Niro Hudson Model NS2006A, Hudson, Wisconsin) in line was used in the thermal processing. The homogenizer was operated at a total pressure of 2500 psi (2000 - 1st stage; 500 - 2nd stage). This type of unit is a tubular heat exchanger. The product enters the system through a product tube and goes through two tubular product heaters (Microthermics, 1992). The temperature of the product in these heaters is controlled by adjusting the steam pressure used to generate the hot water, and the quantity of hot water bypassing the hot water generator. The product is cooled within the system by the use of chilled water. The system was operated at a flow rate of 1000 mL/ min with a 10 s hold time. Thermocouples were placed throughout the processing path (Table 18) so the different temperature changes within the heating line could be monitored and recorded.

Table 4.18. Unit placement of thermocouples between sections of the heating line.

Thermocouple	Unit Placement
1	Pump outlet - Preheater inlet
2	Preheater outlet - Homogenizer
3	Heater 2 outlet - Hold tube inlet
4	Hold tube outlet - Cooler inlet
5	Cooler outlet - Product outlet

The temperatures were recorded and retrieved using a computer program set up by Microthermics (Raleigh, NC) at their facility. The product entered the system at ~ 30 ºC before entering the preheater which was set at of 60 ºC. Next the product entered the homogenizer, and was homogenized at a total pressure of 2500 psi (2000 - 1st stage; 500- 2nd stage). The product continued to flow through heater no.2 which was set at 102 ºC (215ºF) before entering the hold tube and held at 92 ºC for 10 s. The product then reached the cooler set at a 23 ºC for product cool down. Before exiting the product outlet, the product was cooled down to ~ 30 ºC and collected into sterile containers. The sterile containers were put ice in a cooler until later transported back to the Virginia Tech facility (on that same day) and were placed in 4 ºC walk-in cooler.

Enzyme Addition. The enzyme was added to the sterile containers under a filtered positive air flow hood to rid the air of contaminants which would reduce the microbiological quality of the mix. The enzyme was added one day after processing and transportation back to the Virginia Tech facility. The β- galactosidase enzyme (1.8 mL; Lactozym ™ 3000L, Novo Nordisk, Franklinton, NC) was filtered through a sterile 0.45 um acrodisc (Gelman Sciences prod. no 4184, Fisher Scientific, Pittsburgh, PA) and added to 20 control formulation bottles and 20 cholesterol-reduced formulation bottles. The mix was then stored at 4 ºC before further analysis.

Baked Gel Preparation. The preparation procedures were completed as previously described in Chapter III.

2. Chemical Analyses

Lactose and Galactose Concentrations. Mix was stored for 5 days at 4ºC prior to the first analysis for lactose and galactose concentrations. Lactose and galactose concentrations were measured spectrophotometrically at weeks 1, 3, 5, and 7 of the storage period. The analysis procedures for lactose and galactose concentrations were completed as previously described in Chapter III.

Protein Concentration. Samples were stored at 4ºC for approximately 4 days prior to analysis. The analysis was completed on all 4 custard formulations the first week of the study to see if they were comparable in protein composition to the sweet milk and

egg mix reported in Chapter III. The analysis procedures for the protein concentration was completed as previously described in Chapter III.

Total Fat Concentration. All samples were stored at 4 ºC for approximately 4 days prior to analysis. The analysis was completed on all 4 milk and egg formulations only on the first week of the study to see if they were comparable in fat composition to the sweet milk and egg mix reported in Chapter III. The analysis procedures for the fat concentration was completed as previously described in Chapter III.

Cholesterol Concentration. Two mL of chloroform containing approximately 13-25 mg lipid obtained by the Bligh and Dyer (1959) method for lipid extraction was used to determine the cholesterol concentration. Sample vials that contained lipid amounts closer to the 30 mg amount were divided into 2 aliquots so that their absorbance would fall on the standard curve. The samples were stored for approximately 4 wks at 0ºC in sealed vials flushed with nitrogen. The chloroform was removed by a stream of dry nitrogen before continuing with the cholesterol procedure described by Kates (1986). Cholesterol concentrations were measured spectrophotometrically at 550 nm by a uv/vis spectrophotometer (Model lambda 3B, Perkin- Elmer, Norwalk, CT). Serum cholesterol (99%, cat. no. C7921, Sigma, St. Louis, MO) was used to make a standard stock solution containing l mg/mL of cholesterol. The stock was used to prepare working standards in the range of 0.05-0.60 mg. The amount of cholesterol in the samples was quantified by using the working standards and reading the samples against a linear regression curve.

Fatty Acid Analysis. Two mL of chloroform containing approximately 13-25 mg lipid obtained by the Bligh and Dyer (1959) method for lipid extraction was used to determine the fatty acid profile. The samples were stored for approximately 4 wks at 0ºC in sealed vials flushed with nitrogen. The analysis was done on all four custard formulations to see if it was comparable in fat composition to the sweet milk and egg mix reported in Chapter III. The analysis procedures for determining the fatty acid profile was completed as previously described in Chapter III.

Moisture. The analysis was completed on all 4 mix formulations the first week of the study to see if it was comparable in moisture content to the sweet milk and egg mix

reported in Chapter III. The procedure for moisture analysis was completed as previously described in Chapter III.

Ash. The analysis was completed on all 4 custard formulations the first week of the study to see if it was comparable in ash content to the sweet milk and egg mix reported in Chapter III. The procedure for ash analysis was completed as previously described in Chapter III.

3. Physical and Microbiological Analyses

Color. Color measurements were obtained on the mix and baked gel from each formulation on weeks 1, 3, 5, and 7. Color measurements of the top and bottom surfaces of baked gel samples were taken at 3 different locations (Minolta Chromameter, Model CR-200, Minolta Camera Co., Ltd., Osaka, Japan). The gels were removed from the dish to measure the color of the bottom surface. The color measurements were made on all 4 formulations of baked custard, every other week for 7 wk, after the mix was prepared. The color measurements were reported as Hunter "L", "a", and "b" values. Calibration of the instrument was carried out using a white tiled Minolta Reference tile (L = 97.91, a = -0.68, b= +2.44). Means and standard deviations were calculated by the statistics mode on the chromameter. The color of the custard mix before baking was also evaluated by the same procedure. The mix was measured through a clear glass test tube held in the tube holding apparatus (Minolta Camera Co., Ltd., Osaka, Japan).

Gel Strength. Firmness was measured on all 4 formulations of baked custard, on weeks 1, 3, 5, and 7 after the mix was prepared. Firmness was measured as the force (g) needed to penetrate an 80 g baked custard sample. A Stevens L.F.R.A. Texture Analyzer (Model TA - 1000, Texture Technologies Corp., Scarsdale, NY) with a TA - 2 cone attachment operated at a speed of 2 mm/s and a distance of 5 mm was used in the analysis. Measurements were obtained from 2 different locations on the top surface of the custard and the values averaged. The custards were allowed to sit at room temperature for approximately 6 h before measurements were taken.

Syneresis. Syneresis was measured on all 4 formulations of baked custard every other week for seven weeks after the baked custards were prepared. Syneresis, the

drainage of a liquid from a baked custard gel, was measured as described by Penfield and Campbell (1990). The gelled custard sample was inverted on a cheesecloth supported on a funnel. The exudate was collected in a 10 mL graduated cylinder over a one h period at 22.2 - 23.3 ºC and the volume recorded.

Aerobic Plate Count. A standard plate count was performed on all 4 mix formulations on weeks 1, 3, 5, and 7. The procedure for aerobic plate count was completed as previously described in Chapter III.

4. Data Analyses

Data Collection. This experiment consisted of 2 replications of the actual thermal process used on 4 different batches of the sweet milk and egg mix. The analysis were completed on weeks 1, 3, 5, and 7. Some compositional analyses (protein, fat, moisture, ash, fatty acid methyl esters, and cholesterol) were performed on wk 1 to compare formulation compostions to the sweet milk and egg mix from previous analyses. All other tests were performed at weeks 1, 3, 5, and 7 were standard plate count (mix), lactose and galactose concentration (mix), color (mix & gel), texture (gel), and syneresis (gel).

Statistical Analyses. A t- test assuming equal variance was used for the following analysis: percent total fat, percent protein, percent cholesterol, percent moisture, percent ash, percent saturated fat, percent monounsaturated fat, and percent polyunsaturated fat. A straight factorial design was used for analysis on all the tests that were performed over the 7 wk period. The Statistical Analysis System (SAS Institute Inc., 1988) utilizing the PROC ANOVA procedure was used to analyze the data collected from the straight factorial design. Mean difference separations were determined using Tukey's HSD.

D. Results and Discussion

1. Thermal Processing

Thermal processing parameters were modified based on a patent for the production of an extended shelf-life sweet milk and egg based product (Dieu and CuQ, 1989). The thermal processing parameters were similar to those used in Chapter III with

the exception of a lower hold tube temperature (92ºC) with a 10 sec hold time. This heat treatment gave more than adequate D values for the destruction of *Mycobacterium tuberculosis*, *Coxiella burnetti*, *Salmonella*, and spoilage organisms of concern in this product. As stated before the time and temperature destruction of an organism when dealing with public safety is based on the hold tube (Swartzel, 1986).

Applying a hold tube temperature of 92ºC (197ºF) at 10 sec still gave a more than adequate process for safety. A 14,167 D process was given to *Coxiella burnetti*, which was the most heat resistant pathogen in the product; spoilage organisms received a 425 D process. In spite of the lower temperature applied in the hold tube, of this study, the product still received a more than adequate heat treatment.

Aerobic Plate Count. The 4 different formulations of the milk and egg mix had microbial counts (cfu/ mL) of less than l log for the 7 wk storage period. These low counts are attributed to several factors in addition to the thermal process. The use of pasteurized milk, commercially processed dried egg solids, and processed liquid egg yolk (pasteurized prior to cholesterol extraction) all played a role in providing lower counts due to their prior heat treatments. Additionally, the use of sterile containers in collecting the mix aided in lowering counts.

2. Chemical Composition of Sweetened Formulations

Proximate Analysis. The objective of this research was to develop a nutritionally enhanced milk and egg mixture with a shelf life of 6 wk under refrigerated conditions. Proximate analysis demonstrated a decrease in fat in the cholesterol reduced formulation (CRF) as compared to the control formulation(CF)(Table 19). There was a 22% reduction in the CRF. The higher fat content in the CF is attributed to the type of egg yolk used in the formulation. The calorie contents of the CF and CRF are very similar with CRF being slightly lower (Table 19). The CF had dried egg yolk while the CRF used a cholesterol reduced (CR) and fat reduced (FR) liquid egg yolk. Both treatments were formulated so that the yolk was proportional to egg white solids and total moisture level to be equivalent to a whole fresh egg. Thus, there was no difference in the moisture content between the CF and CRF as would be expected (Table 19). The milk and egg

sources both provided the proteins in the mix.

Table 4.19. Means[1] ± standard deviations for proximate analysis of sweetened milk and egg mixture.

Constituent (g/100g)	Formulation CF [3]	Formulation CRF [4]
Fat	$0.99^{a} \pm 0.12$	$0.77^{b} \pm 0.083$
Protein	$5.04^{a} \pm 0.13$	$5.07^{a} \pm 0.081$
Moisture	$78.35^{a} \pm 0.79$	$78.27^{a} \pm 0.74$
Ash	$0.93^{a} \pm 0.037$	$0.92^{a} \pm 0.030$
Carbohydrates [2]	$14.70^{a} \pm 0.91$	$14.97^{a} \pm 0.80$
Calories (kcal/ 100g)[5]	87.87	87.09

[1] Means and standard deviations for 4 replications
[2] Value not measured directly. Calculated as difference based on proximate analysis
[3]CF = Control formulation
[4] CRF = Cholesterol reduced formulation
[5]Value based on proximate analysis of three replications
[a, b] Means within a row with different letters are significantly different ($p \leq 0.05$)

Lactose and Galactose Concentration. As reported in Chapter III, the concentrations of lactose in the formulation without β-galactosidase treatments were not different. The formulations with the β-galactosidase enzyme added had significant decreases in the lactose concentrations and an increase in the galactose concentration (Table 20). There was an average of 96% reduction between the CF and CRF with β-galactosidase addition. Under refrigerated conditions, the lactose and galactose concentrations did not change over the 6 wk storage period. These results suggest that hydrolysis of the lactose was completed during the first week of storage.

Lipid Profile. As reported in Chapter III, the type of egg played a major role in the difference in the type and proportion of fatty acids found in each formulation (Table 21; Table 22). There was a 8.7% reduction of saturated fatty acids in the CRF that used the cholesterol and fat reduced egg yolk. . There was also a 39% significant reduction in the monounsaturated fatty acids of the CRF. The amount of polyunsaturated fatty acids found in the CRF increased by 61%. Cholesterol was significantly lowered to 37.5% in the CRF formulation (Table 22).

A difference in the concentration of saturated fatty acids in the sweetened formulations, between the study reported in Chapter III and this study was observed. The saturated fatty acids reported in Chapter III for the sweetened mix is 57% higher than in this sweetened formulation. Both studies utilized the same lot of eggs. This may be attributed to the small amount of fat in the product, less than 1 g, causing some variation in the concentrations fatty acids.

3. Physical and Functional Characteristics of Sweetened Formulations

Color. There were no differences in color during the 7 wk storage for the mix formulations or the gels baked from those mixes (Figures 1-9). Hunter "L", "a", and "b" values were measured for the mix and top and bottom surfaces of the gel. In this color system, " L" illustrates dark to light (0 to100), "a" illustrates red (+) to green (-), and "b" represents yellow (+) to blue (-). The type of egg was the main effect contributing to the difference in color between the formulations. The "L" values for CRF mix indicated that it was lighter than the CF. There were significant differences in the "L", "a", and "b"

values

Table 4.20. Interaction means[1] ± standard deviations for lactose and galactose of sweetened milk and egg mixture.

	Constituent	
Formulations	Lactose (g/L)	Galactose (g/L)
CF [2] - NLR[3]	27.74[a] ± 5.17	14.60[a] ± 2.72
CF - LR[4]	1.38[b] ± 1.01	0.72[b] ± 0.53
CRF - NLR	29.63[a] ± 7.85	15.59[a] ± 4.13
CRF[5] - LR	1.16[b] ± 0.85	0.61[b] ± 0.45

[1] Means and standard deviations for 2 replications
[2] CF = Control formulation
[3] NLR = No lactose reduction
[4] LR = Lactose reduction
[5] CRF = Cholesterol reduced formulation
[a, b] Means within a column with different letters are significantly different ($p \leq 0.05$)

Table 4.21. Means[1] ± standard deviations for fatty acids in sweetened milk and egg mixture.

	Formulation	
Fatty acid (%)	CF[2]	CRF[3]
Butyric - C4	0.17[a] ± 0.33	0.77[b] ± 0.16
Caproic - C6	Trace	Trace
Caprylic - C8	0.17[a] ± 0.34	Trace
Capric - C10	0.51[a] ± 0.36	0.15[a] ± 0.30
Lauric - C12	0.57[a] ± 0.41	0.74[a] ± 0.12
Myristic - C14	1.94[a] ± 0.23	2.48[a] ± 0.39
Palmitic - C16	28.11[a] ± 0.82	23.48[b] ± 1.10
Palmitoleic - C16:1	3.40[a] ± 0.15	1.39[b] ± 0.01
Stearic - C18	10.06[a] ± 0.39	10.29[a] ± 0.20
Oleic - C18:1	41.50[a] ± 0.89	25.99[b] ± 0.61
Linoleic - C18:2	11.87[a] ± 0.30	29.32[b] ± 0.93
Linolenic - C18:3	0.14[a] ± 0.29	3.52[b] ± 0.17
Arachidonic - C20:4	1.57[a] ± 0.13	2.12[a] ± 0.60

[1] Means and standard deviations for 4 replications
[2] CF = Control formulation
[3] CRF = Cholesterol reduced formulation
[a, b] Means within a row with different letters are significantly different ($p \leq 0.05$)

Table 4.22. Means [1] ± standard deviations for lipid profile of sweetened milk and egg mixture.

Constituent	Formulation	
	CF [2]	CRF [3]
Saturated fat (%)	41.52[a] ± 1.15	37.91[b] ± 1.77
Monounsaturated fat (%)	44.90[a] ± 0.78	27.38[b] ± 0.61
Polyunsaturated fat (%)	13.58[a] ± 0.56	34.71[b] ± 1.18
Cholesterol (mg/100g)	40.87[a] ± 8.79	25.53[b] ± 2.59

[1] Means and standard deviations for 4 replications
[2] CF = Control formulation
[3] CRF = Cholesterol reduced formulation
[a, b] Means within a row with different letters are significantly different ($p \leq 0.05$)

of the mixes for the 4 different formulation ($p<0.05$) (Table 23). The "L" and "a" values of the mix were relatively close within each color attribute such that differences probably would have limited significance with respect to visual observations. The Hunter "b" values were low enough in the CRF, as compared to as compared to the CF, and may have contributed to a visual difference. The CF were significantly more yellow than the CRF.

No differences in the "L" values were observed for the top and bottom surfaces of the baked gels. There were significant differences in the "a" and "b" values of the top and bottom gels. The top surface "b" values were more yellow than the bottom surface and the CF were significantly more yellow than the CRF.

Gel Strength and Syneresis. There was no significant difference in the gel strength between the baked gels utilizing the milk and egg mix over the 7 wk storage period (Table 23). The strength of the gels made with the sweetened milk and egg mix reported in Chapter III were stronger than the baked gels in this study. This result could be attributed to the thermal process that was applied. The mix was homogenized in line in this thermal process. There were also higher preheating temperatures in this study than in chapter III. These factors may have caused denaturation of some of the egg proteins which are vital in the formation of a gel structure.

Syneresis, the drainage of a liquid from a gel, did not change over the 7 wk storage period in the 4 different formulations.

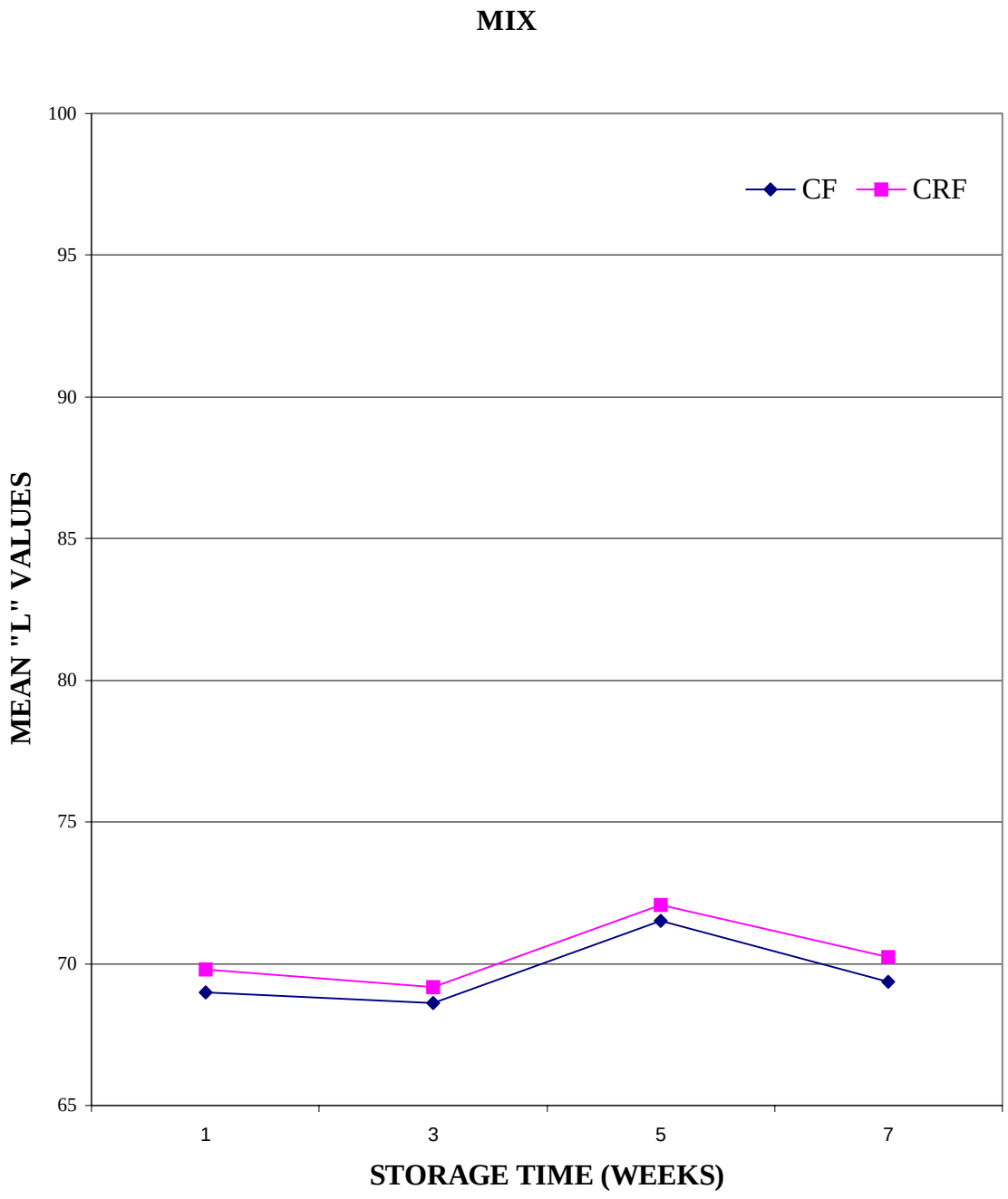

Fig. 1 - Mean "L" values for CF and CRF for the mix (n= 4) over a seven week storage period.

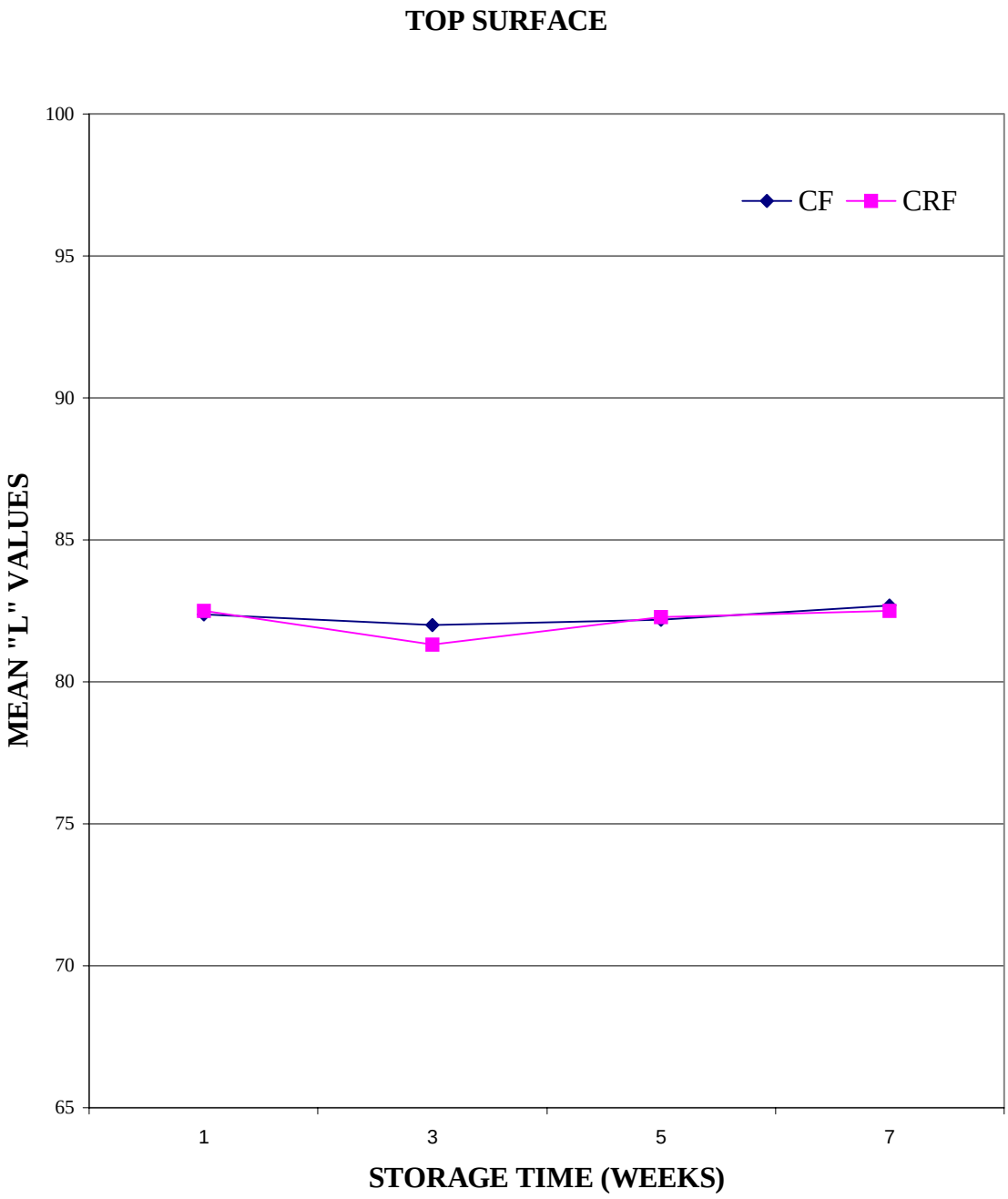

Fig. 2 - Mean "L" values for CF and CRF for the top surface of the baked gels (n= 4) over a seven week storage period.

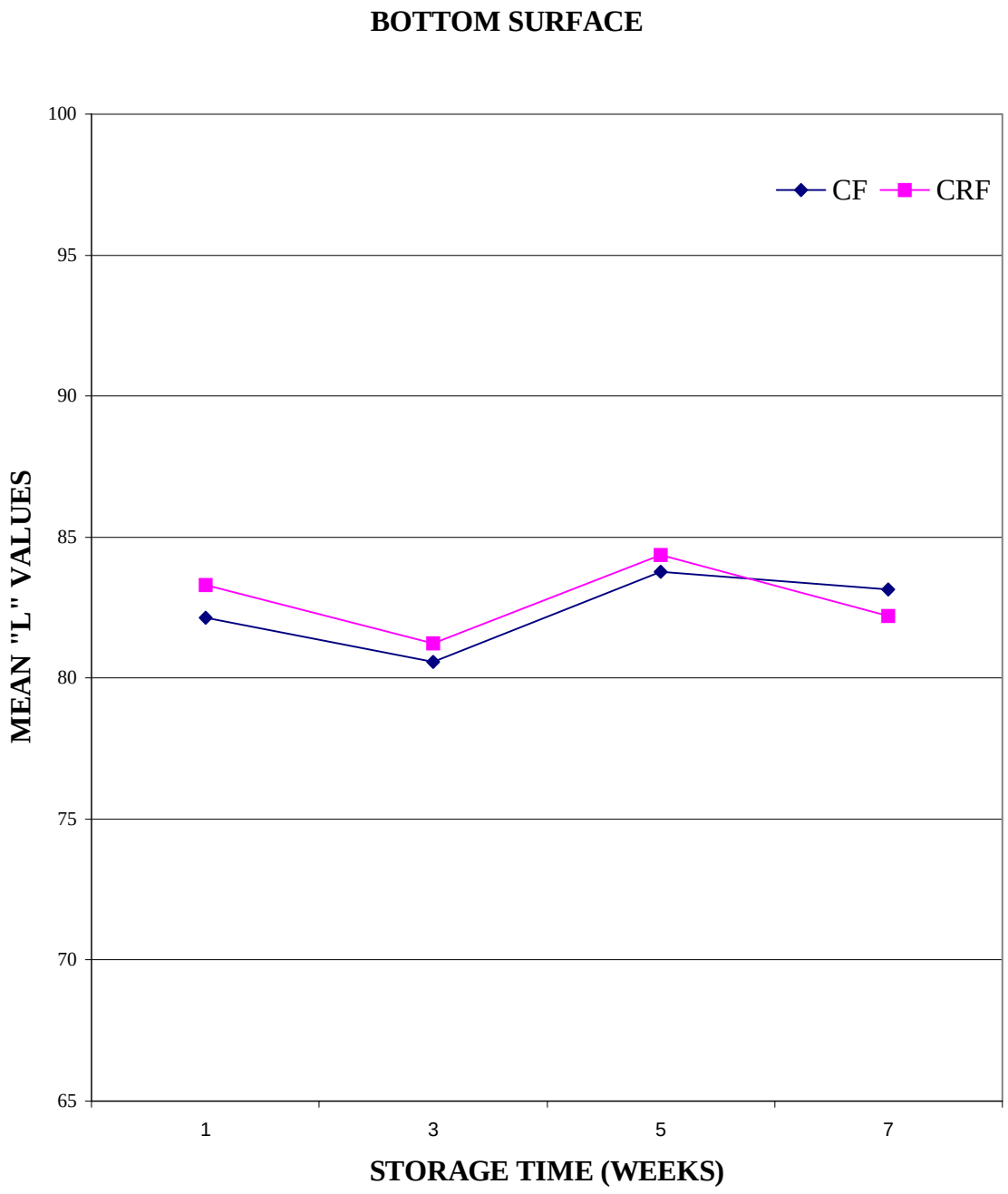

Fig. 3 - Mean "L" values for CF and CRF for the bottom surface of the baked gels (n= 4) over a seven week storage period.

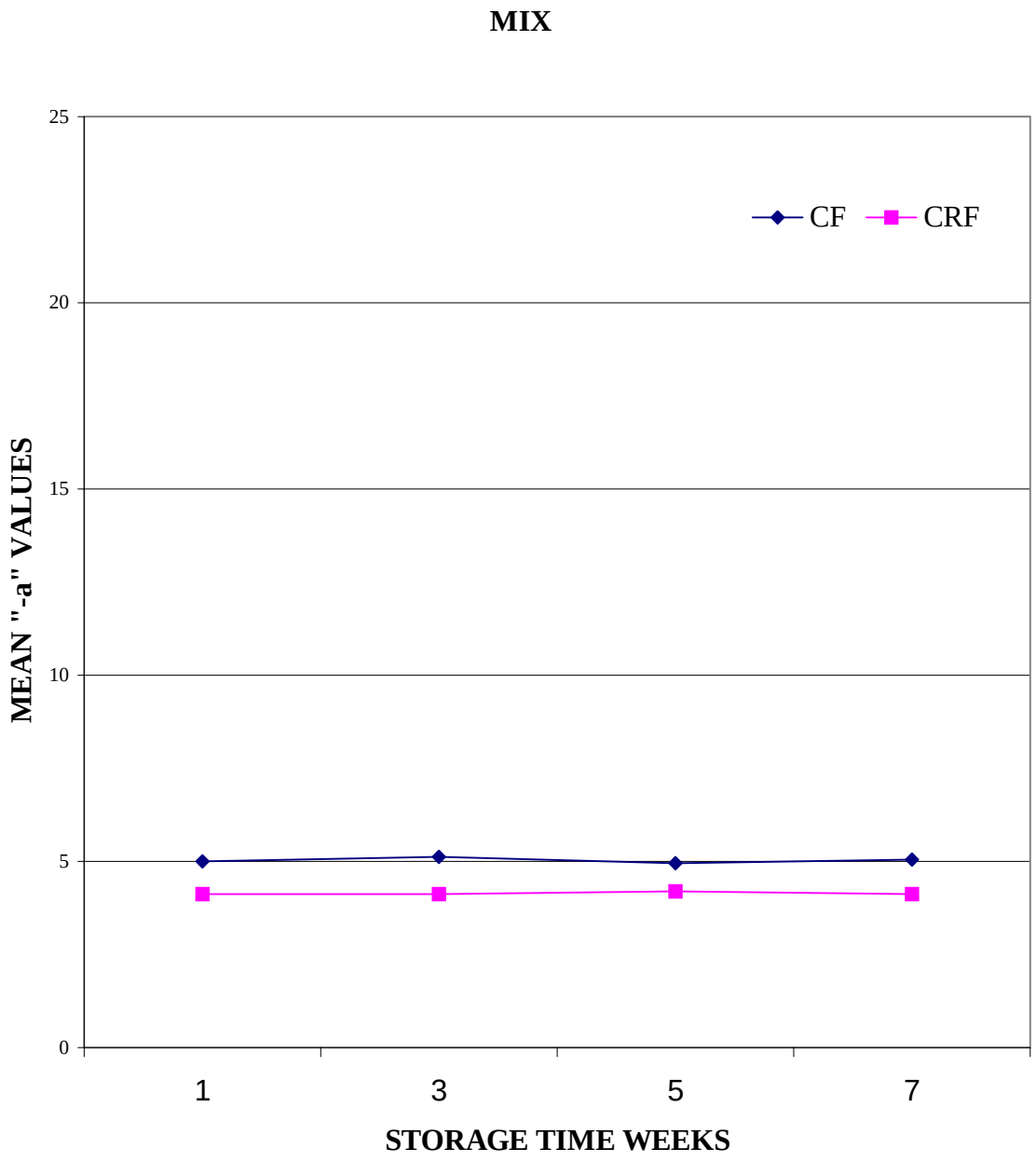

Fig. 4 - Mean "a" values for CF and CRF for themix of baked gels (n= 4) over a seven week storage period.

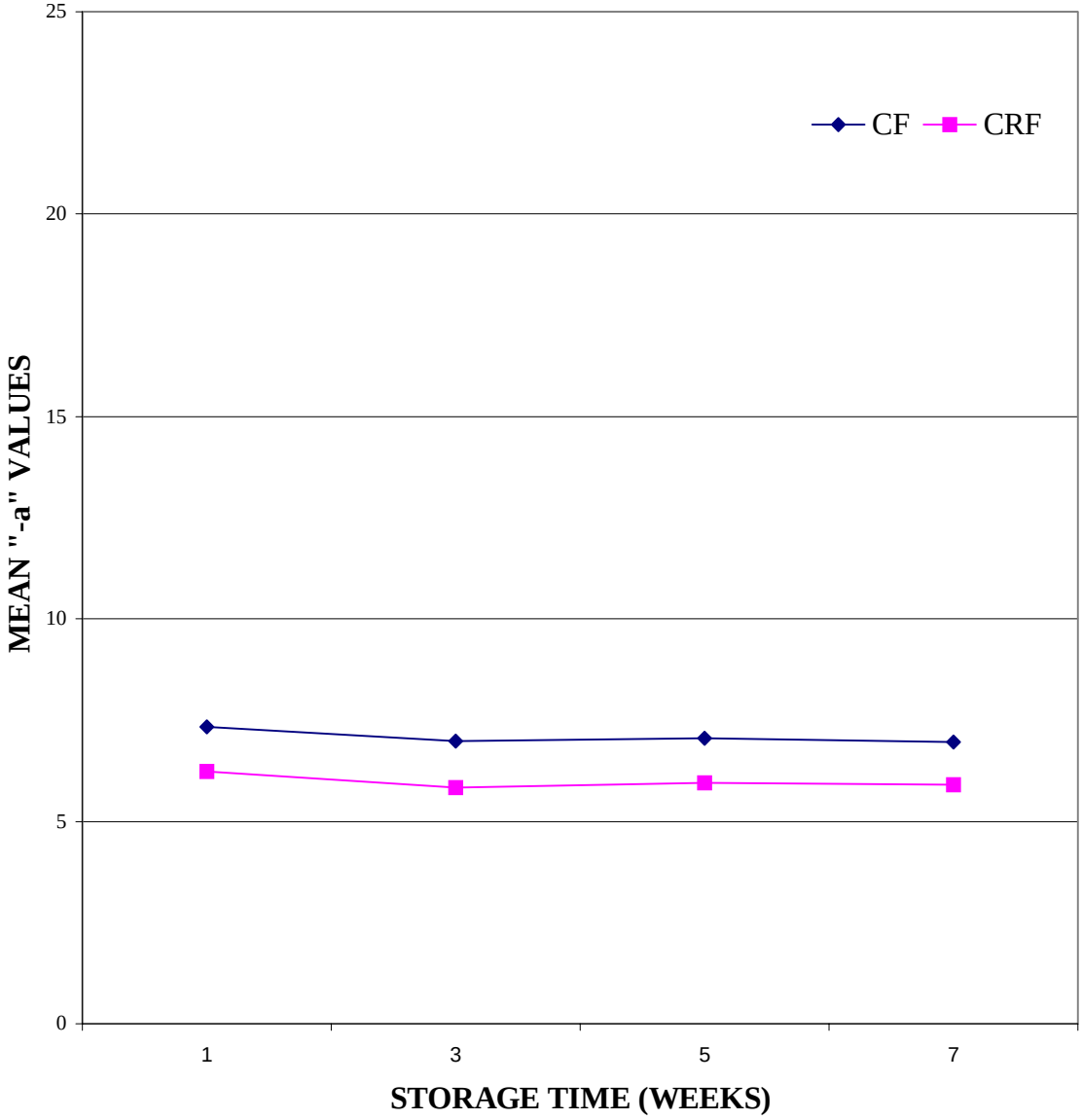

Fig. 5 - Mean "a" values for CF and CRF for the top surface of baked gels (n= 4) over a seven week storage period.

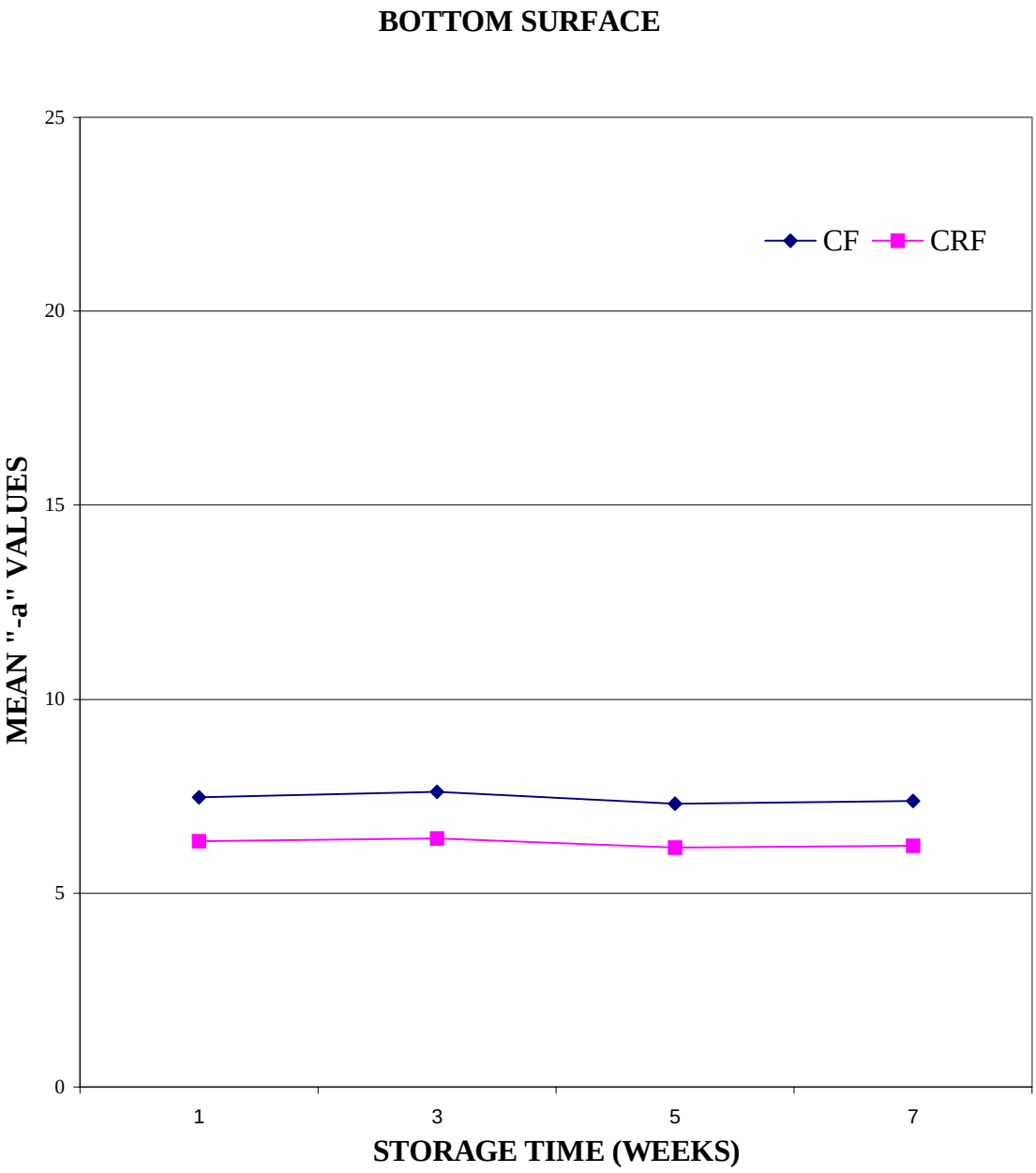

Fig. 6 - Mean "a" values for CF and CRF for the bottom surface of baked gels (n= 4) over a seven week storage period.

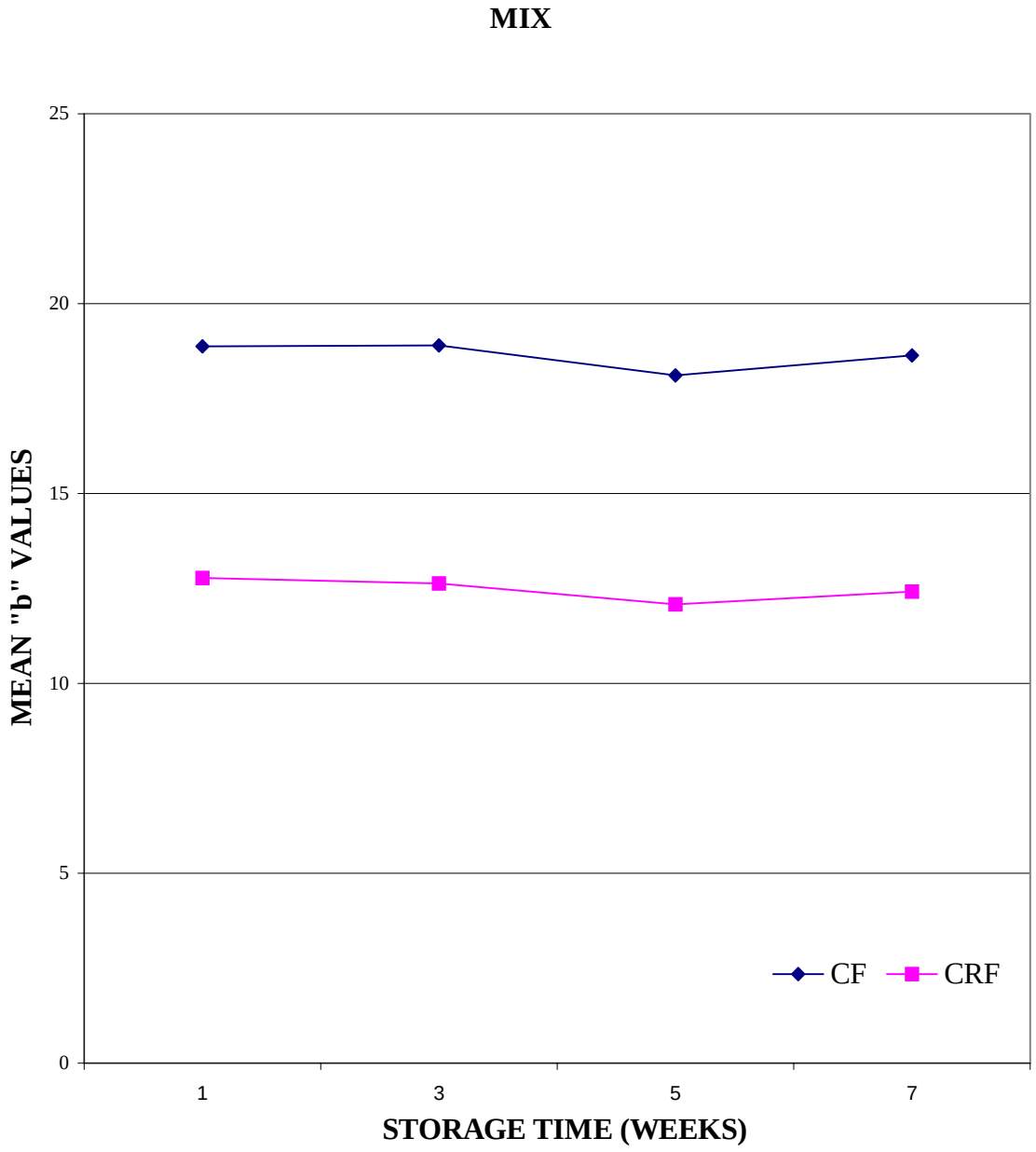

Fig. 7 - Mean " b" values for CF and CRF for the mix (n= 4) over a seven week storage period.

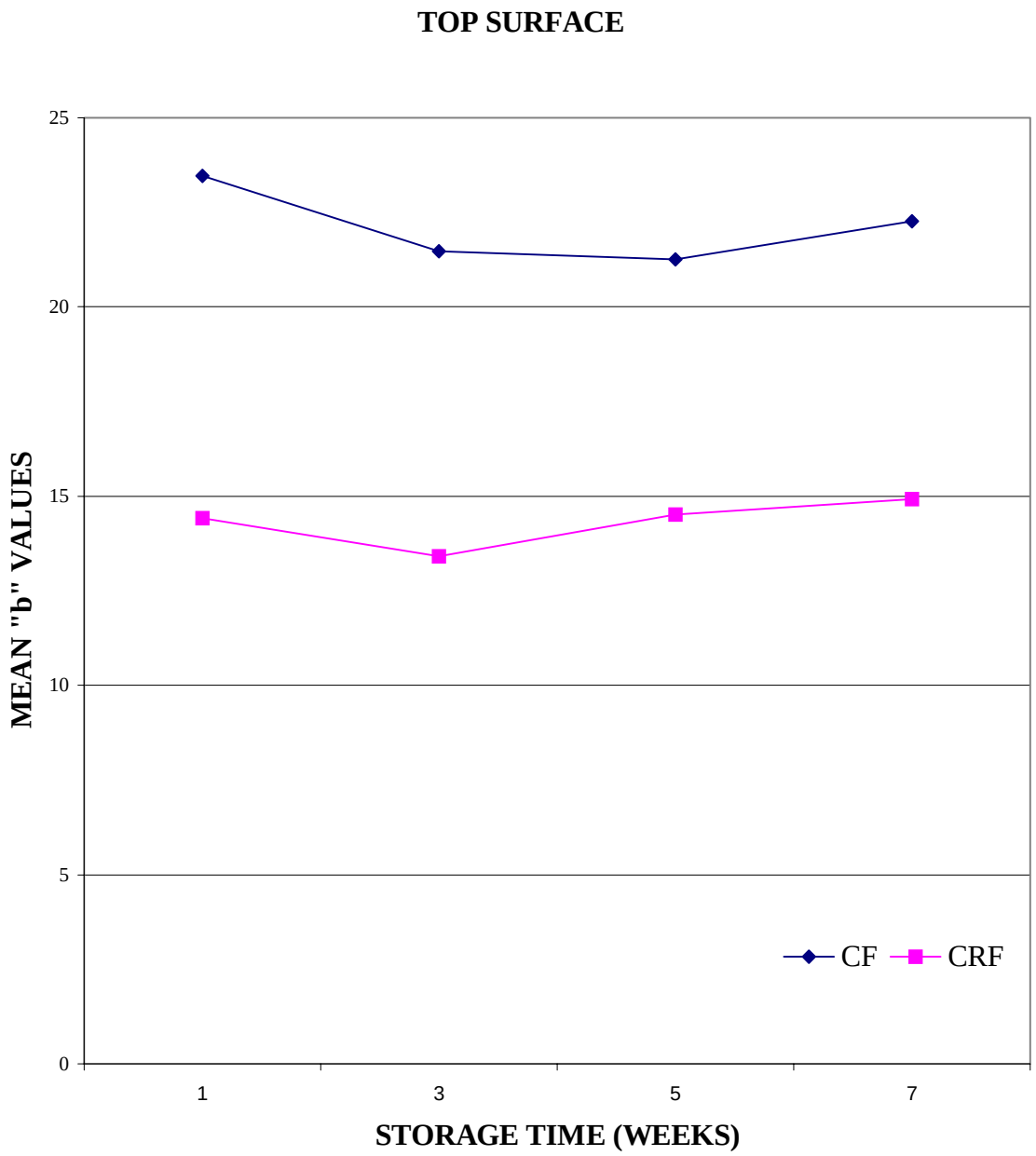

Fig. 8 - Mean " b" values for CF and CRF for the top surface of baked gels (n= 4) over a seven week storage period.

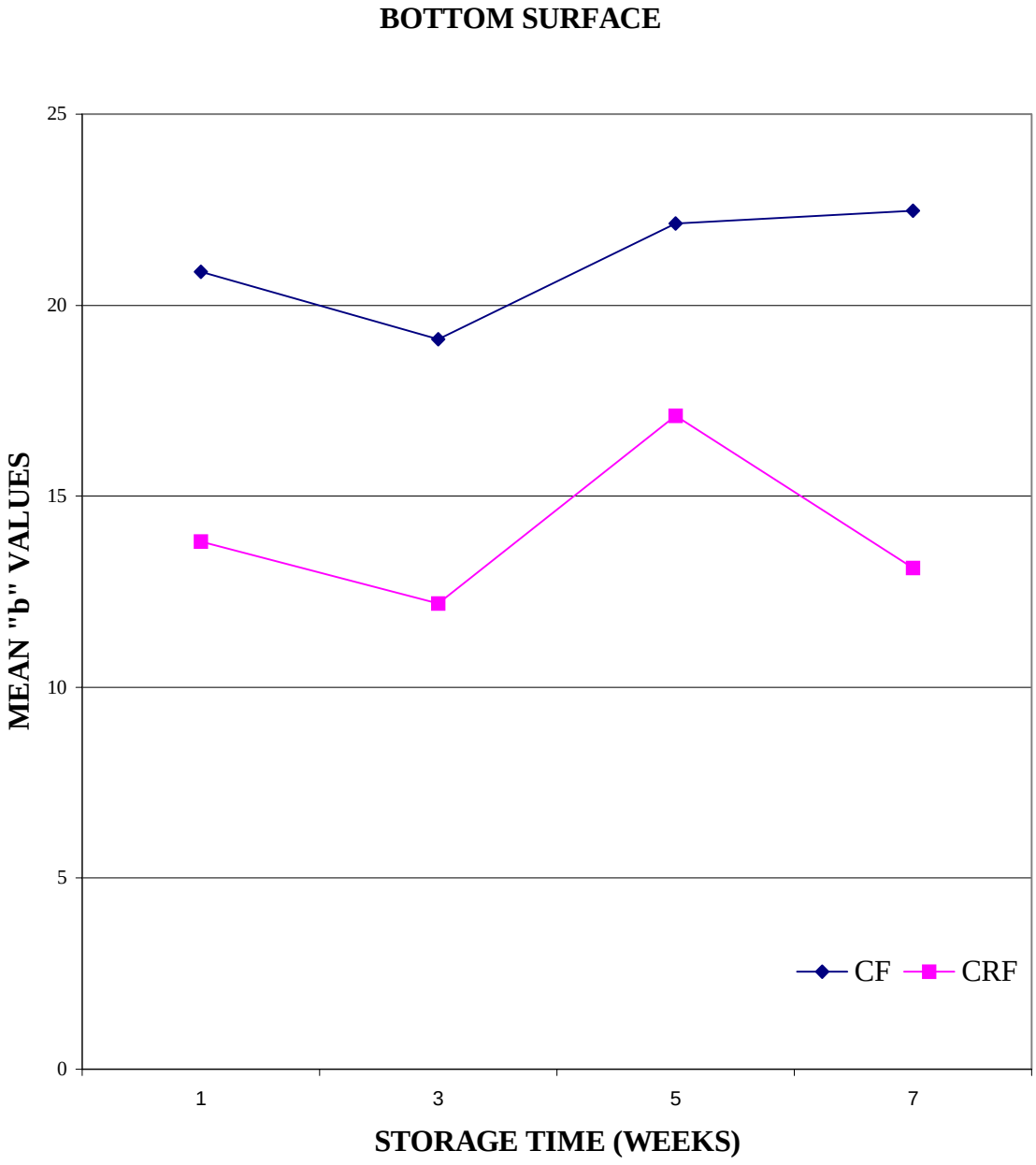

Fig. 9 - Mean " b" values for CF and CRF for the bottom surface of baked gels (n= 4) over a seven week storage period.

Table 4.23. Interaction means [1] ± standard deviations for physical measurements of sweetened milk and egg mixture.

Properties	Formulation			
	CF[2]-NLR[3]	CF-LR[4]	CRF[5]-NLR	CRF-LR
Gel strength (g force)	12.25 ± 0.54	12.29± 0.48	12.49 ± 0.37	12.40 ± 0.39
Syneresis (mL)*	1.96 ± 1.25	1.61 ± 0.38	1.60 ± 0.40	2.03 ± 0.36
Color				
Top surface				
Hunter "L"	82.34 ± 1.32	82.29 ± 1.46	81.59 ± 1.81	82.71 ± 0.97
Hunter "a"*	-7.12 ± 0.26	-7.04 ± 0.16	- 5.96 ± 0.20	-6.01 ± 0.20
Hunter "b"*	22.06 ± 1.49	22.18 ± 1.30	14.14 ± 1.14	14.50 ± 0.71
Bottom surface				
Hunter "L"	82.01 ± 3.00	82.81 ± 1.40	81.92 ± 2.54	83.62 ± 1.76
Hunter "a"*	- 7.51 ± 0.23	- 7.36 ± 0.15	-6.35 ± 0.15	-6.22 ± 0.21
Hunter "b"*	20.68 ± 2.09	21.61 ± 1.84	13.33 ± 2.14	14.78 ± 2.36
Mix				
Hunter "L"*	69.54 ± 1.36	69.70 ± 1.17	70.29 ± 1.36	70.35 ± 1.09
Hunter "a"*	5.04 ± 0.06	-5.01 ± 0.20	.08 ± 0.22	-4.18 ± 0.12
Hunter "b"*	18.62 ± 0.69	18.65 ± 0.33	12.55 ± 0.62	12.40 ± 0.38

[1] Means and standard deviations for 2 replications
[2] CF = Control formulation
[3] NLR = No lactose reduction
[4] LR = Lactose reduction
[5] CRF = Cholesterol reduced formulation
* Significant differences at ($p \leq 0.05$)

E. Conclusion

A nutritionally enhanced milk and egg mix can be formulated and thermally processed for today's health conscious consumers. The product offers lower cholesterol and fat contents while providing beneficial nutrients such as calcium and protein. Fat is a major concern for individuals who are watching calorie intake, as well as individuals who are monitoring fat and cholesterol intakes because of health conditions. The product could also be consumed by the lactose intolerant individuals who shy away from dairy products because of gastrointestinal discomfort. Because of all the positive attributes of this product, the elderly population would be a good target market. The elderly population tends to have a poorer intake of nutrients, and may be more aware of fat and cholesterol in their diets due to health problems.

The heat treatment applied to this product ensures a relatively safe product with an extended shelf life. The extended shelf life of this product makes distribution of this product to food service industries ideal. Also, the added convenience makes it very marketable to the consumer with less time to spend in the kitchen preparing a meal.

F. References

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APPENDIX A

Bligh and Dyer Lipid Extraction

I. Solutions:

Methanol:Chloroform (2:1)

Methanol: Chloroform: Water (2:1:0.8)

II. Procedure:

1. Add 1 g or mL of sample to a 16 x 125 screw cap test tube
2. Add 4 mL of methanol:chloroform mixture. Tighten cap. Vortex
3. Shake or vortex every 15 min for 1-2 hrs.
4. Centrifuge tubes in a table top centrifuge at maximum rpm for 10 min.
5. Transfer supernatant to another 16 x 125 mm screw cap test tube.
6. Add 5 mL of methanol:chloroform:water to the pellet and re-extract. Vortex the pellet and solvent. Centrifuge again as above.
7. Add the second supernatant to the first.
8. Add 3 mL of chloroform and 3 mL of distilled water to the combined supernatants. Mix and centrifuge as above.
9. Weigh a labeled 10 mL crimp top vial. Record the weight.
10. Withdraw the lower chloroform phase from the centrifuged tube in step 8 and add to the weighed vial.
11. Evaporate the chloroform under a stream of nitrogen.
12. Add 2 mL of chloroform to the lipid, seal and freeze till methylation procedure.

% Lipid Calculation: (Wt. empty vial) - (Wt. vial after chloroform remove) = mg lipid

mg lipid/ Wt. sample * 100 = % lipid

Cholesterol Analysis

I. Solutions:

Stock Ferric Chloride Solution: 85% Orthophosphoric Acid

Working Ferric Chloride Solution: 4 mL of 85% orthophosphoric acid in 50 mL volumetric flask and diluted to a total volume of 50 mL with concentrate sulfuric acid.

Stock Standard Solution: Add 100 mg of 100% cholesterol to a total volume of l00 mL of glacial acetic acid.

Working standards: 50, 100, 200, 300, 400, and 600 uL of stock. (Take the standards and a blank through the procedure that follows.

II. Procedures:

1. Place a lipid aliquot containing approximately 0.3 mg of cholesterol in a 16 x 150 glass test tube with a teflon liner screw cap.
2. Place aliquots of the above working standard in test tubes.
3. If samples were stored in chloroform, evaporate solvent under nitrogen.
4. Add 6 mL of glacial acetic acid to the sample tubes. Mix
5. Add appropriate aliquots of glacial acetic acid to working standards and blank to reach a total volume of 6 mL mix.
6. Add 4 mL of working ferric chloride to samples, standard, and blank tubes.

Mix

7. Cool for 10 min. and read absorbance on a spectrophotometer set at 550 nm.
8. Calculate the concentration of the samples from the standard curve.

Boehringer Mannheim Assay (Lactose and Galactose Concentration)

I. Solutions:

12% Trichloracetic acid

1 Normal Sodium Hydroxide solution

1/10 Normal Sodium Hydroxide solution

Boehringer Mannheim Test Kit Reagents

II. Procedure:

1. Two mL of liquid milk and egg mix were added to 20 mL of 12% trichloracetic acid.
2. Samples were mixed and placed in large centrifuge tubes.
3. Tubes placed in a Sorvall Refrigerated Superspeed Centrifuge at 6000 rpm for 20 min.
4. Ten mL of clear supernatant were removed from the tubes and placed in separate 50 mL beakers

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5. The supernatant from the samples is adjusted to a pH of 7.0 + .2 using 1 N and 1/10 N sodium hydroxide solution.
6. After samples were pH, they were brought to a total volume of 25 mL using distilled water.
7. The samples were further analyzed according to instructions provided in the Boehringer Mannheim test kit.

VITAE

Tracy D. Sutton was born in Wilson, NC on November 5, 1971. She graduated from James B. Hunt High School in June, 1990, and entered North Carolina Agricultural and Technical State University (NCA&T) in the fall of 1990. She graduated from NCA&T State University in December, 1994 with a Bachelor of Science degree in Food Science. In the Summer of 1995, she started Virginia Polytechnic Institute and State University (VPI&SU) in the Food Science department under the supervision of Dr. Duncan. She finished her Master of Science degree in Food Science and Technology in October, 1997.