A Sulfur-based Method for Detecting the Adulteration of Nonfat Dry Milk

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ABSTRACT

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The emphasis of this study was to develop a procedure for detecting the adulteration of nonfat dry milk with whey protein products. It was necessary to determine the effects of mastitis and heat processing as these are two major factors which can affect the sulfur based method chosen for this study. Regression analysis showed no significant affect of mastitis on the sulfhydryl groups in milk protein. Statistical analyses indicated no significant difference between super heat and low heat skim milk powders. Whey protein concentrate and whey powders were analyzed to establish the sulfhydryl concentration for both sample groups. Blends of skim milk powder and whey protein products showed a direct linear relationship between the per cent of added whey product and sulfhydryl concentration. The consistency of the experimental values with previously reported values and the minimal effect of mastitis and heat processing on the sulfhydryl groups, suggest that the determination of sulfhydryls may be useful for detecting the adulteration on nonfat dry milk.

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TABLE OF CONTENTS

TABLE OF CONTENTS
(continued)

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Approximately one-half billion pounds of nonfat dry milk solids are used annually by the food industry in the preparation of food products. Nonfat dry milk is added to many food systems due to the stabilizing properties of milk protein. However, nonfat dry milk is an expensive source of protein compared to other sources of food protein. Whey protein concentrate, and modified whey protein products, which have functional properties similar to milk protein, can be prepared to simulate nonfat dry milk at approximately one-half the cost. For these reasons, some suppliers will adulterate nonfat dry milk to increase profit margins.

Several parameters have been investigated as being useful for detecting adulteration of nonfat dry milk. These include the quantitation of whey peptides, the measurement of the lactic acid or ash content, the presence of excessive amounts of sialic acid, and polarography to determine the cysteine/cystine ratio in milk products. These methods are limited because properties of whey protein products and nonfat dry milk can be altered by processing techniques and heat

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treatments. Furthermore, the natural composition of fluid milk can increase the frequency of false positive results (Olieman and van den Beden, 1983). The limitations of existing methods clearly demonstrate the need for a test, based on the chemical properties of milk, for detecting adulteration.

One property that may be utilized is the concentration and distribution of the sulfhydryl-containing amino acids. The amino acid composition of individual milk proteins, and particularly of the casein and whey protein fractions, is known (Gordon and Katan, 1974). Sulfhydryl-containing amino acids are not distributed uniformly between the casein and the whey protein fractions of milk. Since the amino acid composition of protein is determined genetically, the quantity of sulfur in a particular protein should be constant and subject only to variations in milk protein from individual cows. This suggests that determination of the concentration and distribution of the sulfhydryl-containing amino acids may be a useful chemical parameter for detecting the adulteration of nonfat dry milk with whey protein products.

An examination of the applicability of a sulfur-based method for detecting the adulteration of nonfat dry milk will be the focus of this study.

REVIEW OF LITERATURE

Nonfat dry milk is added to many food systems to take advantage of the stabilizing properties of milk protein. The requirement for high quality nonfat dry milk is necessary to meet the stabilizing needs of the food industry. The intentional adulteration of nonfat dry milk has presented a major problem to the food processor and a challenge to the food scientist. Research into methods of detecting adulteration based on the static chemical properties of milk is needed to ensure the food proceesor and the consumer that nonfat dry milk they receive is the quality they deserve. The major focus of this study will be the determination of the sulfhydryl concentration of nonfat dry milk as a method for detecting adulteration.

Milk Proteins

Milk protein is a complex mixture of individual proteins which can be divided into two major fractions, the caseins and the whey proteins. The casein fraction has been defined as the protein precipitated at pH 4.6 at 20 degrees Celsius and is composed of alpha-, beta-,

kappa-, and gamma- caseins. The whey protein fraction includes proteins that do not precipitate and is composed of beta-lactoglobulin, alpha-lactalbumin, bovine serum albumin, immunoglobulins, and enzymes (Brunner, 1976). The amino acid composition of the major milk proteins is known (Gordon and Katan, 1974). Sulfhydryl-containing amino acids are not distributed uniformly between the two fractions. The three amino acids which contribute to the total sulfur in milk protein are methionine, cystine, and cysteine. Casein contains methionine and traces of cystine while whey proteins contain significant levels of all three. However, reduced cystine and cysteine are the only amino acids which contribute to the total sulfhydryl pool as the chemical structure of methionine does not allow for a sulfhydryl contribution.

Calculations of the theoretical sulfhydryl concentrations have been made based on the range of individual proteins per gram of total milk protein as presented by Brunner (1976), and are shown in Table 1. The sulfhydryl concentration contributed by cysteine and half-cystine residues are reported per gram of total milk protein. The amount of sulfur per molecule of protein is much greater for the whey proteins than for the caseins. It is evident that only a limited percentage of

Table 1. The theoretical concentration range of sulfhydryl groups in milk protein.

Protein	Range[A]	-S-/Molecule	μMoles/g Milk Protein
αs_1-Casein	19.1-23.3	0	0
β-Casein	10.4-14.6	0	0
κ-Casein	4.2- 7.9	2	8.4-15.8
γ-Casein	1.5- 3.4	0	0
α-Lactalbumin	1.4- 3.5	8	11.2-28.0
β-Lactoglobulin	3.9- 6.6	5	19.5-33.0
Serum Albumin	0.1- 0.2	35	3.5- 7.0
Immunoglobins	(.015-.025)	(.03)	4.5- 7.5
Other			
			47.1-91.3

[A] Concentration Range in micromoles/g milk protein.

the sulfur in milk protein is due to casein. Beta-lactoglobulin and alpha-lactalbumin contribute a total of 65 per cent of the sulfhydryls found in milk protein. Overall, the whey proteins contribute 81 per cent of the total sulfhydryls.

In milk protein systems with reduced disulfide groups, calculations give a theoretical range for the sulfhydryl content of the total milk protein and whey fractions from 47 to 91 and 247 to 290, respectively. Since the amino acid composition of milk protein is determined genetically, the quantity of sulfhydryl groups in milk protein should be constant and subject only to variation in milk protein from individual cows.

Walstra and Jenness (1984) presented information showing the presence of sulfhydryl compounds in alpha-2-casein as shown in Table 2. This information has been incorporated with the data presented by Brunner and a value of 72.4 micromoles of sulfhydryls per gram of milk protein was calculated as the theoretical mean.

Factors Affecting the Quantity of Sulfhydryl Amino Acids

Factors that might affect the quantity of sulfhydryl-containing amino acids per gram of milk protein include disease, such as mastitis, and processing

Table 2. The theoretical mean for sulfhydryl groups in milk protein.

Protein	Moles/g milk protein[A]	μMoles-S-/g Milk Protein
Caseins		
αs_1	1.3	-
αs_2	0.3	6.0
β	1.2	-
κ	0.53	5.3
γ	0.12	-
Whey		
α-Lactalbumin	0.26	21.0
β-Lactoglobulin	0.54	27.0
Serum Albumin	0.017	6.1
Immunoglobulins	(0.021)	(7.0)
		72.4

[A] ($\times 10^{-5}$)

techniques. Mastitis is a bacterial infection of the mammary system of the cow. It may cause milk to be unsaleable or result in the temporary loss of one or more quarters of the udder. Acute mastitis results in a sudden swelling of the udder and the cow's going off feed. Chronic mastitis, which affects the udder over a period of time, is characterized by the production of flaky milk, a decrease in production, and development of lumps in the secretory tissue (Wiley, 1960).

Several changes in the composition of milk are associated with mastitis. These are a lowering of the fat, solids-not-fat, lactose and casein content of milk and an increase in the whey protein content of milk. The combined effect is an increase in total protein and a distortion of the normal protein distribution.

Many dairy products are required by law to receive specified heat treatments. Other dairy products, such as concentrated and dry milks, gain their identity only through heat processing. The purposes of heat processing may be summarized as follows: to meet public health requirements, either with pasteurization or sterilization, to destroy enzymes, to facilitate mixing and blending operations for ice cream and processed cheese, to achieve incubation temperatures in cheese and cultured dairy products, to impart desirable properties,

and to remove water (Jenness and Patton, 1959).

The purpose of drying milk and milk products is to remove most of the water in them with minimal physical and chemical changes. Dry milk powders are designated as low, medium, and high heat powders.

It has been difficult to determine the effects of heating on milk during the drying process. One property that seems to be affected by heat treatments is the reactivity of the sulfhydryl groups present in the serum proteins. These groups are buried or masked in the native protein and they are unreactive. However, when the protein is subjected to heat, the protein uncoils and the groups become more accessible and reactive (Jenness and Patton, 1959).

Josephson et. al. (1939) observed the activation of sulfhydryl groups in milk by heat treatment at about 167 degrees Farenheit, as measured by the nitroprusside test. In addition to showing the activation of the sulfhydryl groups, the susceptibility of these groups to oxidation was also demonstrated.

Patrick and Swaisgood (1976) examined the effect of direct ultra-high temperature heating and subsequent storage conditions on the sulfhydryl and disulfide groups in skim milk. UHT-treatment caused an increase in concentration of the reactive sulfhydryl groups with a

decrease in measurable half-cystine.

The concentration of reactive and "buried" sulfhydryl and disulfide bonds were measured for both refrigerator and room temperature storage. Results indicate that both reactive and total sulfhydryl concentrations decrease with time, but more rapidly with room temperature storage. With extended storage periods, a decrease was also noted for refrigerated storage. Storage at room temperature also resulted in greater oxidation to disulfide bonds. Therefore, the ratio of free sulfhydryls to disulfide bonds varies with heat treatment and storage conditions subsequent to heat treatment. However, when all disulfide groups have been reduced by chemical methods, the total concentration of sulfhydryls per gram of protein does not appear to be severely affected.

Methodology for Detecting Adulteration of Nonfat Dry Milk

Several parameters have been investigated as being useful for detecting adulteration of nonfat dry milk. Lyster (1964) developed a procedure to measure the free and masked sulfhydryl groups of heated milk and milk powder. The procedure is based on the use of p-chloromercuribenzoate (PCMB), which is specific for sulfhydryl groups, and utilizes Ellman's reagent as an

indicator. The indicator produces a yellow color in samples in which -SH is in excess oF PCMB, with the intensity of the color proportional to the excess. This method provides a measure of the free -SH groups, but total -SH content can be determined only after the addition of a denaturing agent. The analysis is limited by time since 8 minutes after the addition of Ellman's reagent, all samples developed a yellow color. In samples with low -SH content, error is increased because of the weakness of the color development.

Koning (1966) presented a method for detecting the presence of a neuraminic acid containing glycomacro-peptide (GMP) in rennet whey. This peptide is soluble in 12 per cent TCA and can be precipitated quantitatively and specifically by phosphotungstic acid. The quantity of neuraminic acid in the precipitate is determined by Warren's TBA-test. However, this method has limited value because the temperatures in the drying process are so high that some GMP is liberated.

A recently developed procedure to detect adulteration uses high pressure liquid chromatography to determine the presence of GMP in nonfat dry milk. The GMP is released into cheese whey from kappa-caseins by rennet during the manufacture of cheese (Olieman and van den Beden, 1983). The HPLC method is sensitive and can

accurately determine additions of more than 0.8 per cent rennet whey total solids to skim milk powder, with the quantity of whey added being determined from a standard curve. However, several factors indicate a need for additional test methods. The cost of the HPLC procedure would prohibit its use by many of the small laboratories in the dairy industry. The presence of GMP may result from enzymatic activity of protease produced by bacterial growth in milk, which would cause false positive results. The quantity of GMP in whey also is subject to the method used to prepare the whey and the time milk is exposed to rennet during the manufacture of cheese.

OBJECTIVES

1. To determine the effect of mastitis on the concentration of total sulfhydryl groups per gram of reduced milk protein.

2. To determine the effect of heat treatments during the manufacture of nonfat dry milk on the concentration of total sulfhydryl groups per gram of reduced milk protein.

3. To determine the total sulfhydryl concentration per gram of reduced whey protein for whey protein concentrate and whey powder.

4. To determine the minimum quantity of added whey protein that can be detected in nonfat dry milk.

EXPERIMENTAL PROCEDURES

Materials

Samples

Five sample groups were used in this study for the analysis of sulfhydryl concentrations. These were fresh fluid milks with somatic cell counts indicative of normal to severely mastitic milk, high heat skim milk powders, low heat skim milk powders, whey protein concentrate powders, and whey powders. The fresh milk samples were collected from individual cows at the Texas A & M University dairy and the dried samples were collected from USDA sources. The samples were representative of products found in the dairy industry and their composition and history were known.

Methods

Somatic Cell Count Determination

Somatic cell counts were performed on fresh milk samples to determine the degree of mastitis. The direct microscopic count procedure was utilized as outlined by the Milk Industry Foundation (1959). Freshly drawn milk (0.01 milliliters) was spread on a clean glass slide and allowed to dry. When completely dry, the slide was

dipped in xylol to dissolve the fat, then rinsed with 90 per cent ethanol to fix the milk smear. The slide was submerged in methylene blue staining solution for two minutes, rinsed to remove excess stain, then allowed to air dry. The individual leucocycte cells in ten microscopic fields were counted, averaged, and results were reported as somatic cells per milliliter of milk after multiplying the average cell count per field by a microscopic factor of 532,312.

Nitrogen Determination

The nitrogen content of the fresh milk samples, and dried powders, was determined using the micro-Kjeldahl procedure, which is accepted by the Association of Official Analytical Chemists as a standard method for nitrogen determinations (1975). Fresh milk samples were skimmed to remove the fat, and nitrogen was determined in the skim milk portion. The powders were reconstituted to a 10 per cent total solids solution for nitrogen determinations.

In the micro-Kjeldahl procedure, 3 ml of sample are digested in sulfuric acid. The milk and the acid were pipetted into a 100 ml volumetric digestion tube with a selenium catalyst and boiled at approximately 380 degrees

Celsius for one hour on a Tecator 1016 block digester. The cooled protein digest was diluted with distilled water and steam distilled using a Kirk distillation apparatus. In the distillation procedure, ammonia gas is liberated and condensed with 35 ml of distillate collected in 5 ml of boric acid trapping solution. The distillate was titrated with 0.0208 N HCl to a neutral grey endpoint. The titration volume was used to calculate the per cent total nitrogen in the sample by the following equation:

$$\% \text{ N} = \frac{\text{ml HCl sample} \times 14.007 \times \text{N HCl} \times 20 \times 100}{\text{sample weight (g)}}$$

Per cent nitrogen for the non-protein nitrogen fraction in the sample was also determined. The non-protein fraction was obtained using the Rowland (1938) fractionation procedure. Equal volumes of sample and 24 per cent trichloroacetic acid, were mixed in a 15 ml test tube and the proteins allowed to precipitate. After 20 minutes, the test tubes were centrifuged to partition the protein precipitate and the aqueous acid portion. The aqueous portion was filtered through Whatman No. 2 filter paper and the filtrate collected in a 5 ml test tube. The micro-Kjeldahl procedure was used to determine the per cent nitrogen in the non-protein filtrate. The non-protein nitrogen value was subtracted

from the total nitrogen value to obtain a protein nitrogen value. This value was multiplied by a conversion factor of 6.38 to estimate the per cent true protein in the sample.

Sulfhydryl Determination

The sulfhydryl concentration in the fresh milk samples and the reconstituted powders was determined by the method of Beveridge et. al. (1974). One hundred microliters of sample were pipetted into a 15 ml conical centrifuge tube. Five hundred microliters of 10 M Urea were added to the sample. Fifty microliters of 2-Mercaptoethanol was added to reduce disulfide bonds normally present, or those generated by heat treatments. After one hour, 10 ml of 12% TCA were added to the tube to precipitate the proteins. The sample was then centrifuged to collect the protein pellet and the process repeated twice. On the third rinse, 8 M Urea was used to dissolve the protein and Ellman's reagent was added to react with the sulfhydryl groups present to form a yellow color. After a 10 min. reaction time, the optical transmittance of the sample was measured on a Beckman spectrophotometer at a wavelenghth of 412 nm. The concentration of sulfhydryls

was directly related to the absorbance of each sample as sulfhydryl concentration increased with increasing absorbance. Absorbance readings were incorporated with the protein values and a sulfhydryl concentration per gram of protein was determined using the following equation:

SH= absorbance X 6 X 73.53 / grams protein

EXPERIMENTAL RESULTS

The primary purpose of this research was to develop a method for detecting adulteration of nonfat dry milk with whey protein products. To assess the applicability of a sulfur-based method for detecting adulteration, it was necessary to determine the effect of mastitis and heat processing on the sulfhydryl concentration of milk proteins, because these are two major factors which can affect the sulfhydryl concentration of milk proteins.

Effect of Mastitis on Sulfhydryl Concentration

Twenty four fresh milk samples, with somatic cell counts indicitive of normal to severely mastitic milk, were collected from individual cows of the Texas A&M dairy herd. Somatic cell counts for these samples ranged from 17,000 to 10,000,000 per milliliter. Results of total sulfhydryl analysis are presented in Table 3. The data are the average values of duplicate determinations for twenty four samples. The mean value is consistent with the theoretical mean calculated based on the average sulfhydryl concentration per gram of total milk protein as reported by Walstra and Jenness (1984). The range of sulfhydryl concentrations is consistent with values reported by Patrick and Swaisgood (1976).

Table 3. Total sulfhydryl analysis for 24 fresh milk samples.

	TOTAL SULFHYDRYL CONCENTRATION[A]	
	EXPERIMENTAL	THEORETICAL
MEAN	73.10 ± 13.30	72.4
RANGE	46.5 - 96.5	47.1 - 91.3

A Micromoles of (-SH-) per gram of milk protein

Regression analysis was used to determine if a correlation existed between somatic cell counts and the sulfhydryl-containing amino acid residues per gram of protein. Figure 1 represents the calculated regression line. The line has a positive slope of 2.3×10^{-6} and a correlation coefficient of 0.36. Somatic cell counts within this range did not appear to have a significant affect on the sulfhydryl content per gram of milk protein.

Because most of these samples had somatic cell counts of less than 400,000 per milliliter, additional samples with higher somatic cell counts were analyzed. Eight additional samples with somatic cell counts greater than 500,000 per milliliter were analyzed.

Table 4 represents the results of the sulfhydryl analysis for 32 samples with somatic cell counts ranging from 17,000 to 10,310,000 per milliliter. The mean value shows a slight increase, however the range of sulfhydryl concentrations remained the same.

Regression analysis was used to determine if the samples with elevated somatic cell counts had an affect on the sulfhydryl-containing amino acid residues per gram of protien. Figure 2 includes the regression line for the 32 combined samples. The line has a positive slope of 2.3×10^{-6} and a correlation coefficient of 0.39.

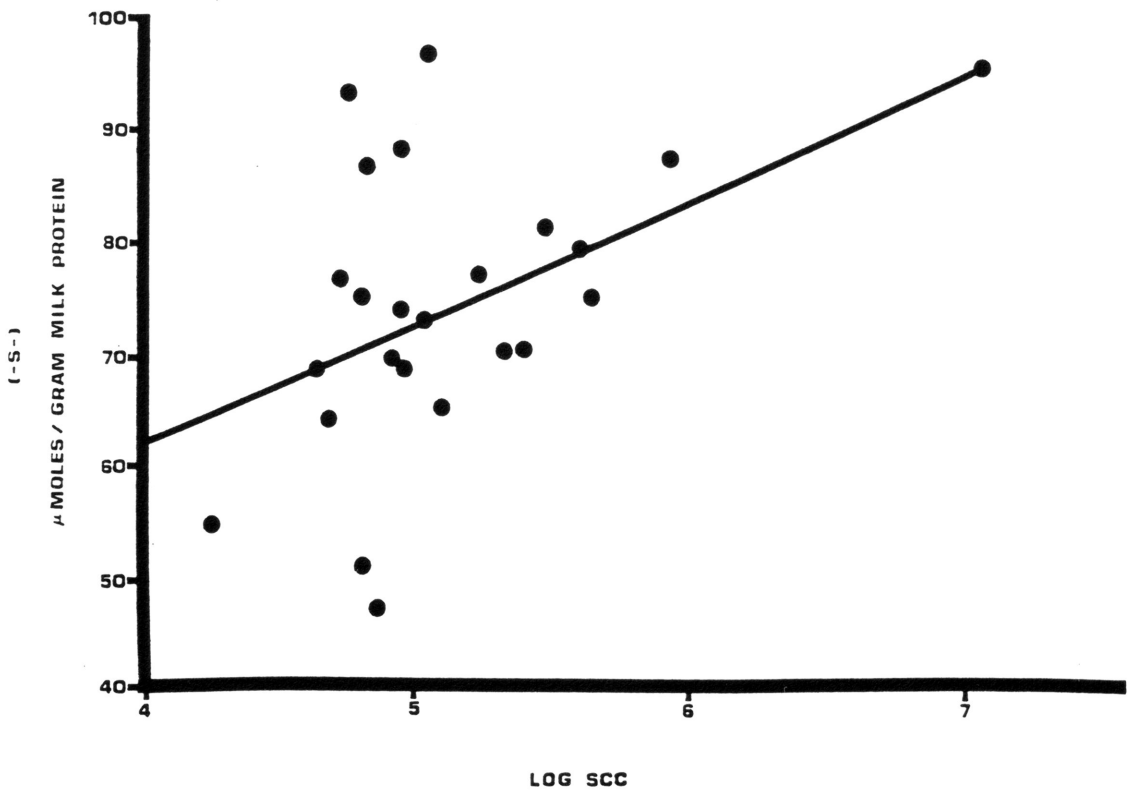

Figure 1. Regression analysis for 24 fresh milk samples.

Table 4. Total sulfhydryl analysis for 32 fresh milk samples.

	TOTAL SULFHYDRYL CONCENTRATION [A]	
	N = 24	N = 32
MEAN	73.1 ± 13.3	74.98 ± 13.0
RANGE	46.5 - 96.5	46.5 - 96.5

A Micromoles of (-SH-) per gram of milk protein

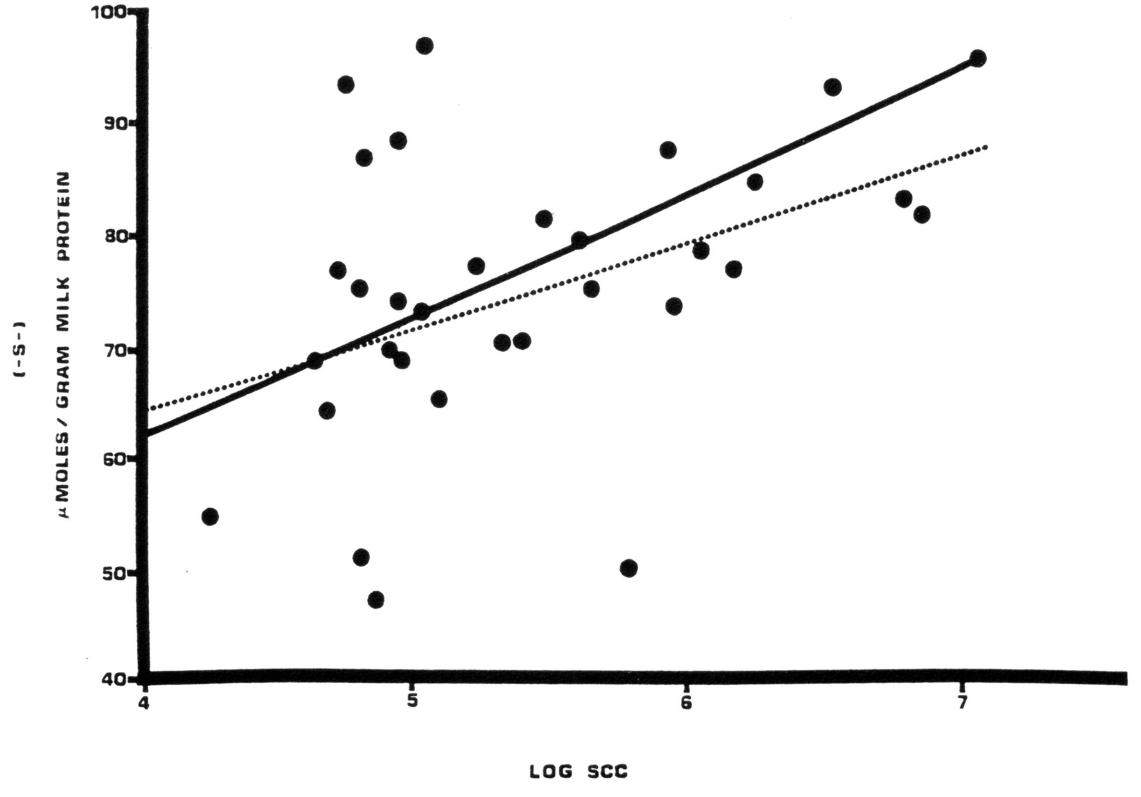

Figure 2. Regression analysis for 32 fresh milk samples.

Increasing somatic cell counts did not appear to have a significant affect on the sulfhydryl-containing amino acid residues per gram of milk protein.

Effect of Heat Processing on Sulfhydryl Concentration

Twenty samples of super heat treated skim milk powder and twenty samples of low heat treated skim milk powder were analyzed to determine the effect of heat treatment on the total protein content and total sulfhydryl content of the powders. A comparison of the protein concentrations for the super heat and low heat powders is presented in Table 5. The data are the average of duplicate determinations for protein content. The protein values for both samples are consistent with published values for protein content. The low heat powder shows a slightly lower protein content but statistical ananlysis could find no significant difference between the two sample groups.

A comparison of the sulfhydryl concentrations for super heat and low heat powders is presented in Table 6. The data are the average of duplicate determinations for sulfhydryl concentrations. The sulfhydryl values are within the range of concentrations as reported by Patrick and Swaisgood (1976). The high heat powders show a

Table 5. Protein content of super heat and low heat skim milk powders (%).

	TOTAL PROTEIN CONCENTRATION [A]	
	SUPER HEAT	LOW HEAT
MEAN	35.03 1.00	34.45 0.77
RANGE	33.24 - 36.71	33.02 - 36.41

A TN - NPN X 6.38

Table 6. Total sulfhydryl analysis for super heat and low heat skim milk powders.

	TOTAL SULFHYDRYL CONCENTRATION [A]	
	SUPER HEAT	LOW HEAT
MEAN	74.62 ± 6.12	71.82 ± 4.77
RANGE	64.34 - 86.38	63.34 - 83.52

A Micromoles (-SH-) per gram of milk protein

slightly higher sulfhydryl concentration per gram of protein, but statistical analysis showed no significant difference between the two sample groups. Statisitical analyses demonstrate no significant affect of heat treatment on the protein content and sulfhydryl content in super heat and low heat skim milk powders.

Sulfhydryl Concentration of Whey Protein Products

Twenty samples of whey protein concentrate and twenty samples of whey powder were analyzed to determine the protein and sulfhydryl concentration of each sample group. The results of the protein analyses are presented in Table 7. The data represent the average of duplicate determinations for protein concentration. The protein values are consistent with published values for whey protein concentrate and whey powder.

The results of the sulfhydryl analyses are presented in Table 8. The data are the average of duplicate determinations for sulfhydryl concentration. The sulfhydryl values are within the range of concentrations calculated based on the range of sulfhydryl concentration per gram of total milk protein as reported by Brunner (1976).

Table 7. Protein content of whey protein concentrate and whey powder (%).

	TOTAL PROTEIN CONCENTRATION [A]	
	WHEY POWDER	WPC
MEAN	10.64 ± 0.33	55.05 ± 1.45
RANGE	10.97 - 11.12	30.39 - 35.39

A TN - NPN X 6.38

Table 8. Total sulfhydryl analysis for whey protein concentrate and whey powder.

	TOTAL SULFHYDRYL CONCENTRATION [A]	
	WHEY POWDER	WPC
MEAN	252.42 ± 12.90	231.20 ± 24.47
RANGE	217.78 - 269.41	168.17 - 264.96

A Micromoles of (-SH-) per gram of protein

Determination of the Sensitivity of a Sulfur-based Method

To determine the sensitivity of the sulfur-based test for detecting the adulteration of nonfat dry milk, super heat and low heat powders were combined with whey protein concentrate and whey powders in ratios of 9:1, 1:1, and 1:9 parts of skim milk to whey protein product. The results of the total sulfhydryl analysis for blends of nonfat dry milk and whey protein concentrate are presented in Table 9. There is a significant increase in the sulfhydryl concentration as the amount of whey protein concentrate increases.

The results of the total sulfhydryl analysis for blends of nonfat dry milk and whey powder are presented in Table 10. There is a significant increase in the sulfhydryl concentration as the amount of whey powder increases.

The samples chosen for this part of the study had sulfhydryl values that were less than the mean. Even when using the least detectable combinations to make the blends, an increase in sulfhydryl concentration was evident.

Table 9. Total sulfhydryl analysis for blends of nonfat dry milk and whey protein concentrate.

TOTAL SULFHYDRYL ANALYSIS[A]	
% WPC	-SH- / g protein[B]
0	61.70
10	75.07
50	152.08
90	226.75
100	234.48

A Based on TN protein

B Micromoles (-SH-) per gram protein

Table 10. Total sulfhydryl analysis for blends of nonfat dry milk and whey powder.

	TOTAL SULFHYDRYL ANALYSIS[A]
% WP	(-SH-) / g protein[B]
0	61.70
10	81.30
50	138.82
90	196.59
100	217.65

A Based on TN protein

B Micromoles (-SH-) per gram protein

DISCUSSION

The adulteration of nonfat dry milk with whey protein products has become a major concern to the food industry. In order to alleviate the problem, methods for detecting adulteration based on the chemical properties of milk must be studied. Knowledge of the effects of disease and heat processing on the properties of milk protein is essential to the development of a procedure, as these are two major factors which can affect analytical tests for detecting adulteration. The study of the effects of disease and heat processing on the sulfhydryl amino acids in milk protein was the major emphasis of this research.

Previous studies that have measured the sulfhydryl amino acids in milk protein have suggested possible processing and storage affects, and "burying" of the reactive sulfhdryl amino acids. In this study, super heat and low heat skim milk powders were analyzed to determine the effects of heat processing on the sulfhydryl amino acids. Statistical analysis demonstrated no significant difference between the sulfhydryl concentrations of the two sample groups. This indicates that the method chosen for this study was not affected by heat processing.

Josephson (1939) and Patrick and Swaisgood (1976) observed a reduction in the free sulfhydryl groups and oxidation of sulfhdryl bonds with long term storage. Long term storage did not appear to have a significant affect on the sulfhydryl concentrations of the sample groups studied. The sulfhydryl values obtained at the beginning of the study did not differ from the sulfhydryl values obtained after eight months of storage. If burying or oxidation of sulfhydryl bonds had occurred, the reduction of disulfide bonds by chemical methods allowed for complete measurement of all sulfhydryl groups. This indicates that the method chosen for this study was not affected by storage conditions after processing.

Mastitis is another factor that may affect the sulfhdyryl amino acids in milk protein. Sulfhydryl analysis of milk samples with somatic cell counts indicative of normal to severely mastitic milk were analyzed. Regression analysis found no significant difference in samples with low somatic cell counts and high somatic cell counts. The method utilized in this study was not affected by mastitis and the high somatic cell counts associated with the disease.

Samples of nonfat dry milk and whey protein concentrate, and samples of nonfat dry milk and whey

powder were mixed together in ratios of 9:1, 1:1, and 1:9 parts of skim milk to whey protein product. A significant increase in sulfhydryl concentration was noted with increasing levels of whey protein product. A direct linear relationship between sulfhydryl concentration and per cent added whey protein product was observed using a Young plot. Even when the least detectable combination was used to make the blends, adulteration was evident at a 10 per cent level.

CONCLUSION

The values obtained in this study are consistent with previously reported values for sulfhydryl concentration in nonfat dry milk. The regression analyses suggest little correlation between increased somatic cell counts and the total sulfhydryls per gram of reduced milk protein. The statistical analysis for super heat and low heat skim milk powder show no significant effect of heat processing on the protein content and sulfhydryl content per gram on milk protein. At a confidence interval of 99.5 per cent, sulfhydryl values of less than 55.37 or greater than 92.86 micromoles of sulfhydryls per gram of protein are suspect to illegal practices.

The consistencey of the mean values and the minimal effect of mastitis and heat processing on the sulfhydryl groups in milk protein suggest that the determination of total sulfhydryls may be useful for detecting the adulteration of nonfat dry milk with whey protein products.

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