THE EFFECT OF MILK SOLIDS-NOT-FAT AND SUCROSE ON THE BEHAVIOR OF CERTAIN AMINO ACID ANTIOXIDANTS IN MILK FAT EMULSIONS

By

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INTRODUCTION

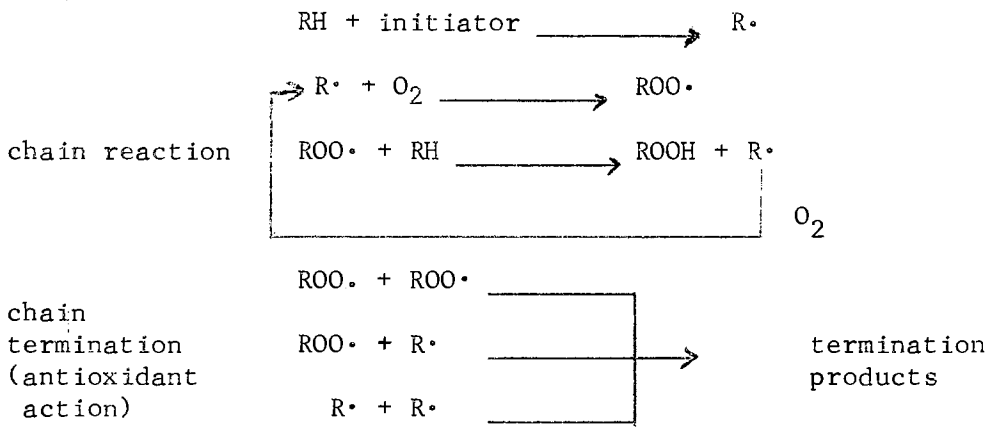
It is an established fact that flavor is one of the most important factors governing consumer acceptance of dairy products. Many of the desirable flavors of dairy products are due to the natural flavors of milk fat. However, some of the most serious flavor problems arise from this same milk fat. One serious flavor problem results from the reaction of milk fat with atmospheric oxygen causing an undesirable flavor termed "oxidized" (1, 4, 8). It is necessary to control this flavor in order to attain maximum storage stability of milk fat and maximum consumer acceptance of dairy products.

Certain phenolic antioxidants have been found which will prevent the formation of oxidized flavor in fats. El-Negoumy and Hammond (9), however, cast doubt on the effectiveness of these compounds in butter, hence additional efforts to find potent antioxidants are essential. Several authors have concluded that amino acids sometimes have antioxidant effects in various fat systems (18, 19, 20, 24). Surve (26) tested tryptophan, histidine and glycine in milk fat and found them to be effective antioxidants. This research was a continuation of the work started by Surve. The purpose of this study was to measure the antioxidant effects of histidine and cysteine in combination with sucrose and milk solids-not-fat (MSNF), substances which are present in many dairy products.

REVIEW OF LITERATURE

Oxidized flavor in lipids has been of great concern for many years and the literature on this subject is voluminous. This literature has been reviewed by several authors (1, 2, 6, 17, 27, 28). Oxidized flavor is actually a group of flavors, which originate from the unsaturated fatty acids in lipids. These react with atmospheric oxygen, to form a series of carbonyl compounds (4, 5, 8, 10, 13, 16), which cause the flavors. The mechanism of oxidation and the formation of carbonyl compounds have been summarized by Surve (26), Keeney (15) and others (8, 11, 12).

Antioxidants are used in many foods to prevent or delay the development of oxidation. These compounds function as inhibitors, to stop free radical formation, thus terminating the chain reaction mechanism. These mechanisms have been explained by the following equations (28).



where: R = any fatty acid or antioxidant radical

• = free bond on radical

H = hydrogen.

In general antioxidants may terminate oxidative chain reactions by donating an electron to a peroxy radical before or after being partially oxidized (28).

Several authors have studied the effect of added amino acids in connection with oxidation. Saunders et al. (24) found that the pro-oxidative action of histidine was retarded by the addition of phosphates and emulsifiers, or by pH values above or below the optimum range of 4.3 to 6.5. Histidine acts as an antioxidant at concentrations of less than 0.0001 M, and as a pro-oxidant at higher concentrations. Clausen et al. (3) reported that various amino acids act as synergists when used in combination with phenolic antioxidants. Marcuse (19, 20) found that tryptophan, histidine and glycine act as antioxidants in linoleic acid and its methyl ester. Surve (26) found that histidine, tryptophan and glycine were effective antioxidants in milk fat.

The 2-thiobarbituric acid (TBA) test as reported by Dunkley (7) has been used as a measure of oxidized flavor in dairy products. This test is carried out by heating a sample of fat with TBA in the presence of acid (30, 31). During this reaction, one molecule of malonic dialdehyde reacts with two molecules of TBA to give a pink color which can be measured colorimetrically (25).

Pohle et al. (23) said that the flavor score of oxidized lipids could not always be predicted from TBA values; however, the test can sometimes be used to follow the development of oxidized flavor in a

given sample or in samples of similar composition. Some authors have reported (9) that there was little correlation between the oxidized flavor of milk fat and its TBA test, whereas others (26, 29) have found correlations between the TBA test and flavor scores ranging from 0.70 to 0.93.

EXPERIMENTAL METHODS

A 3 x 3 experimental design was used in this work. The nine milk fat samples were divided into three sets of three samples each. One set of samples contained 5% MSNF and a second set contained 5% sucrose. The third set of samples was used as a control and contained neither MSNF nor sucrose. A group of samples, one from each set, had 0.14% histidine added to it and a second group contained 0.14% cysteine. The third group of samples contained no added amino acid. Four of the nine samples were duplicated to obtain an estimate of the experimental error. This design can be visualized by referring to Table I. The concentrations of MSNF and sucrose were determined by preliminary trials in which selected levels of these constituents were added to different samples of milk fat. Levels of 5% were chosen since these concentrations did not add any detectable "unnatural" flavors to the fat. The 0.14% concentrations of amino acids were selected on the basis of information in the literature. At this level histidine was expected to act as an antioxidant but at higher concentrations it has been reported to act as a pro-oxidant (24).

The fat for this study was obtained by melting 92 score butter. The melted fat was decanted and filtered through a gauze-faced filter pad[1] to remove the last traces of water and coagulated proteins. This

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[1]Rapid-Flo Fibre-Bonded Milk Filter Disks. Johnson & Johnson, 4949 W. 65th St., Chicago 38, Ill.

TABLE I

EXPERIMENTAL DESIGN--ADDITIVES IN MILK FAT SAMPLES

Additives (sets)	Amino Acids Added (groups)		
	None	Histidine	Cysteine
None (control)	Milk fat	Milk fat & Histidine	Milk fat & Cysteine
MSNF	Milk fat & MSNF	Milk fat & MSNF & Histidine	Milk fat & MSNF & Cysteine
Sucrose	Milk fat & Sucrose	Milk fat & Sucrose & Histidine	Milk fat & Sucrose & Cysteine

anhydrous fat was stored at -15°C until used. During storage an "oxidized-like" flavor developed in the milk fat. This flavor was thought to be similar to that reported by Wyatt and Day (29). Therefore, the fat was "deodorized" before use at a temperature of 90°C and a pressure of 526 mm mercury for four hours. This treatment removed the "oxidized-like" flavor from the fat.

To prepare each sample, 70 grams of deodorized milk fat were heated to 90°C and 13 ml of water were added. If the sample contained additives, these were dissolved or suspended in the water prior to adding it to the fat. Five percent MSNF[2] and sucrose[3] were suspended or dissolved in 10 ml of distilled water, while 0.14% histidine and cysteine[4] were suspended in 3 ml of distilled water. These additives were calculated as a percentage of the fat weight.

To obtain a uniform mixture of water-in-fat, the samples were emulsified in a "laboratory churn" similar to that described by Mickle et al. (21). In this apparatus, the samples were cooled from 90 to 15°C with continuous agitation and a stable, semi-solid emulsion was formed in 4-5 minutes. These emulsions were placed in 250 ml beakers, covered with aluminum foil and stored at room temperature (20-24°C) until analyzed. Only three samples could be analyzed at one time. Thus, one sample was chosen from each set, at random, and the experiment involved the analysis of three lots of three samples each.

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[2]Spray process Extra Grade Non-fat dry milk. Distributed by Golden State Sales Corporation, San Francisco, Calif.

[3]Fine granulated sugar. The Great Western Sugar Company, Denver, Colorado.

[4]Histidine (free base) C.P. and cysteine hydrochloride, C.P. Mann Research Laboratories, New York.

The samples were analyzed after 0, 4, and 8 days storage for oxidized flavor intensity. These time periods were chosen because of Hunziker's report that oxidized flavors usually developed in butter within a 4 to 8 day period (14). Flavor intensities were estimated with the TBA test which was conducted according to the procedure of Patton and Kurtz (22) with some of the modifications suggested by other workers (30, 31). Oxidized flavor intensity also was estimated by a taste panel of three "trained" judges. These were selected from an original group of eight people on the basis of their ability to replicate their flavor scores when evaluating samples with similar flavor intensities. The judges were asked to score four samples at each tasting period. These included the three experimental samples plus a sample of fresh milk fat which was used as a standard of reference. The judges were asked to evaluate the oxidized flavor intensity of the samples and to score this intensity using a scale of 1-6. An example of the score sheet used to record the judges opinions is shown in Figure 1.

Name_____ Date_____Judge No._____

The characteristic flavor of butterfat is nutty, mild, sweet, clean and pleasant, while oxidized butterfat has a tallowy or cardboard like flavor. Use the scale below to indicate your ranking of the experimental samples and write the number on the score sheet which best describes your feeling about the fat.

A fresh sample of butter is provided as a standard for comparison. Score each sample and then wait for 30 seconds, rinse your mouth with the standard sample between tastings of the experimental ones.

You are the judge! An honest opinion will be helpful in deciding your likes or dislikes concerning the flavor of this fat.

Rating Scale	Description
1.	very good - no oxidized flavor and no other undesirable flavor.
2.	questionable - neither good or bad, not sure if oxidized or not.
3.	slightly oxidized flavor - dislike slightly.
4.	distinctly oxidized flavor - dislike moderately.
5.	pronouncedly oxidized - dislike very much.
6.	extremely off flavor - not fit to eat.

Sample code and description	Flavor score	Remarks (if any)
a. Standard - fresh butter_____.	_____	_____
b. Experimental sample_____.	_____	_____
c. Experimental sample_____.	_____	_____
d. Experimental sample_____.	_____	_____

Comments or suggestions_____

_____

_____

Figure 1. Representative Score Sheet for Evaluating Oxidized Flavor Intensities by Taste Panel Members.

RESULTS AND DISCUSSION

Table II is a summary of the taste panel scores for oxidized flavor intensity. The TBA test results on the same milk fat samples are shown in Table III. The correlation coefficient for these two sets of data was 0.78 and this relationship is shown as a scatter diagram (Figure 2). This correlation coefficient agrees with the value of 0.70 obtained by Surve (26) from milk fat emulsions of similar composition and it is in general agreement with the recent data of Wyatt and Day (29) for deodorized milk fat samples. These correlation coefficients indicated that the two sets of data in this study probably had similar tendencies. Thus, the same decisions could be made by examining either set of data. The TBA test results appeared to be more repeatable than the results of the taste panel analysis. Therefore, these data were analyzed and the conclusions from this study were based only on the TBA data.

Statistical analysis (analysis of variance) of the TBA data (Table IV) indicated that the increase from one sampling time to the next was statistically significant ($P < 0.10$). This was the only trend in the data which was statistically significant ($P > 0.10$). The lack of significant differences was expected, though, since only four duplicate samples were analyzed and the difference between these duplicates was relatively large. Nevertheless, the data had certain trends which were worthy of comment.

When considering only the control set of samples, it appeared that histidine might have an antioxidant effect but that cysteine acted as a pro-oxidant (Figure 3). The conclusion drawn from the histidine data is in agreement with the findings of Surve (26). The addition of 5% MSNF had a pro-oxidant effect on most of these samples, but the added sucrose had little effect on the TBA values of those samples which contained no added amino acids (Figure 4).

The effect of combinations of additives were variable (Figures 5 and 6), and it appeared that some of these variations might be due to interactions among additives. These interactions would be worth further study, especially those between amino acid antioxidants and MSNF, additional knowledge about the effects of these additives on oxidized flavor in milk fat could be of value to the dairy industry.

TABLE II

FLAVOR SCORES[a] OF MILK FAT EMULSIONS CONTAINING MSNF OR SUCROSE AND/OR HISTIDINE OR CYSTEINE

Additives (sets)	Days of Analysis: 0 Day — Amino Acids Added (groups)			4 Day — Amino Acids Added (groups)			8 Day — Amino Acids Added (groups)		
	None	Histidine	Cysteine	None	Histidine	Cysteine	None	Histidine	Cysteine
None (control)	5 (4)[b]	4	6 (3)	5 (3)	3	14 (3)	7 (6)	7	17 (6)
MSNF	4	8	3	5	11	5	11	18	14
Sucrose	4 (4)	3	4 (4)	13[c] (4)	4	4 (3)	18[c] (3)	8	8 (4)

[a]Totals of three judges' scores.

[b]Number in parenthesis is analysis of duplicate samples.

[c]Thought to be an error.

TABLE III

TBA VALUES FOR MILK FAT EMULSIONS CONTAINING MSNF OR SUCROSE AND/OR HISTIDINE OR CYSTEINE

Additives (sets)	Days of Analysis								
	0 Day			4 Day			8 Day		
	Amino Acids Added (groups)								
	None	Histidine	Cysteine	None	Histidine	Cysteine	None	Histidine	Cysteine
None (control)	0.18 (0.19)[a]	0.19	0.40 (0.17)	0.24 (0.22)	0.12	0.62 (0.23)	0.40 (0.31)	0.37	1.90[b] (0.40)
MSNF	0.34	0.33	0.37	0.37	0.27	0.36	0.45	0.61	0.49
Sucrose	0.49[b] (0.29)	0.25	0.28 (0.27)	0.80[b] (0.23)	0.35	0.37 (0.32)	2.00[b] (0.32)	0.34	0.38 (0.41)

[a]Numbers in parenthesis are analysis of duplicate samples.

[b]Thought to be an error.

TABLE IV

ANALYSIS OF VARIANCE FOR TBA DATA

Source	df	SS	MS	F
Total	39	12.6474		
Time (Linear)	1	0.8227	0.8227	3.50[a]
Time (Quadratic)	1	0.1252	0.1252	0.53
Additive (MSNF, Sucrose)	2	0.1469	0.0735	0.31
Amino acids (Histidine, Cysteine)	2	0.0469	0.0235	0.10
Add. x a.a.	4	0.8313	0.2078	0.09
Time x Add.	4	0.0579	0.0145	0.06
Time x a.a.	4	0.0726	0.0181	0.08
Time x Add. x a.a.	8	0.6328	0.0791	0.0336
Error	12	2.8242	0.2353	

[a]Statistically significant ($P \leq 0.10$).

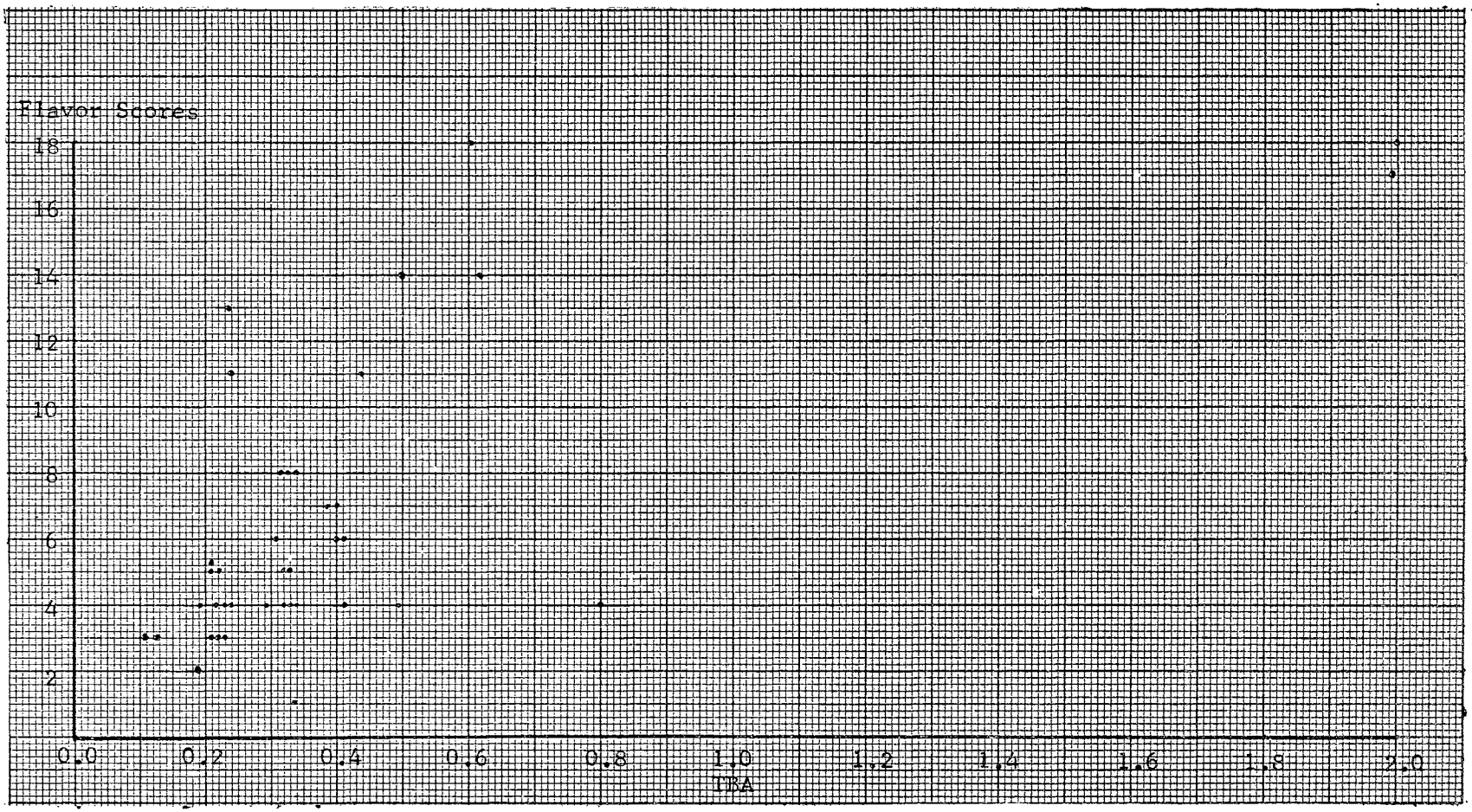

Figure 2. TBA Values vs Flavor Scores for Milk Fat Emulsions Containing MSNF or Sucrose, and/or Histidine or Cysteine

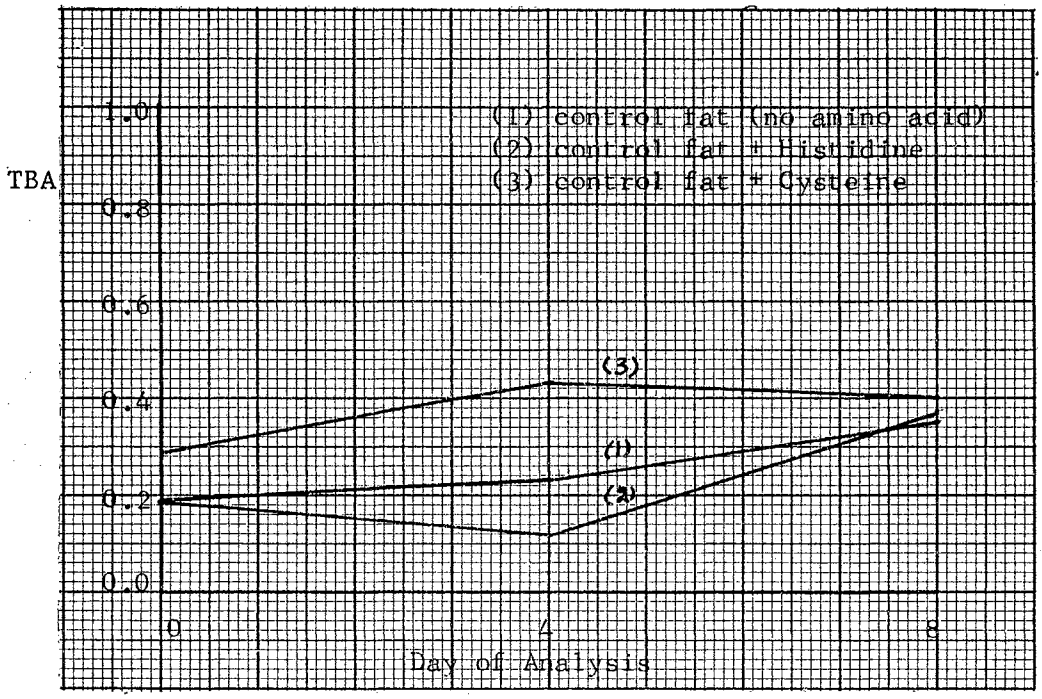

Figure 3. Effect of Histidine and Cysteine on Oxidized Flavor Intensity in Control Fat Sample

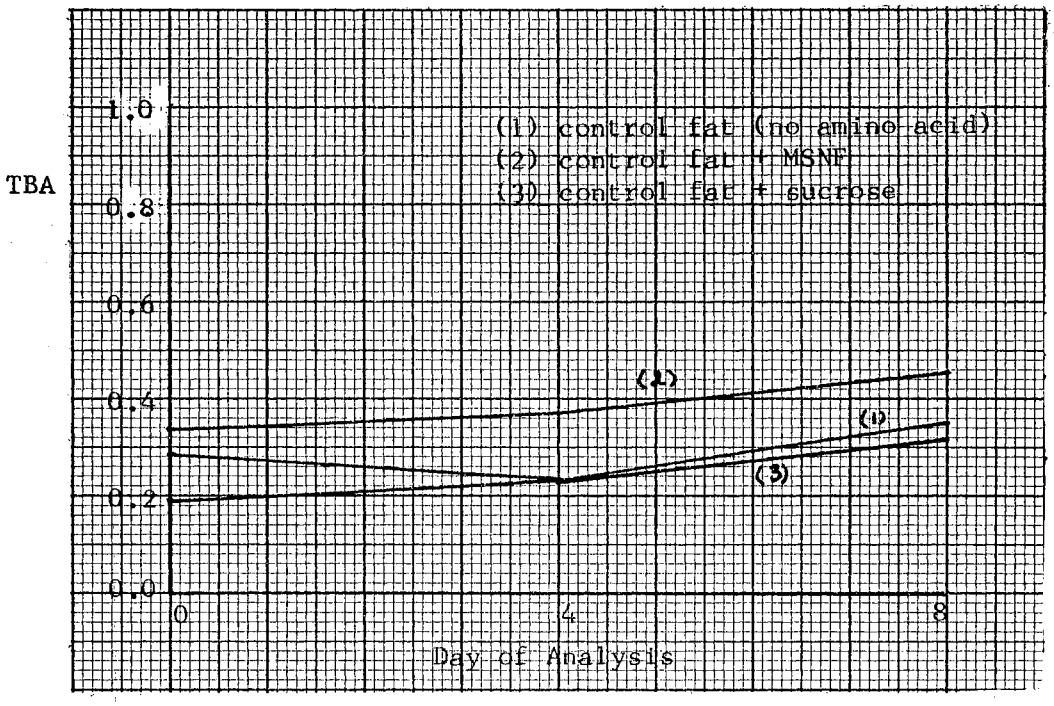

Figure 4. Effect of Added MSNF and Sucrose on Oxidized Flavor Intensity in Control Fat Samples

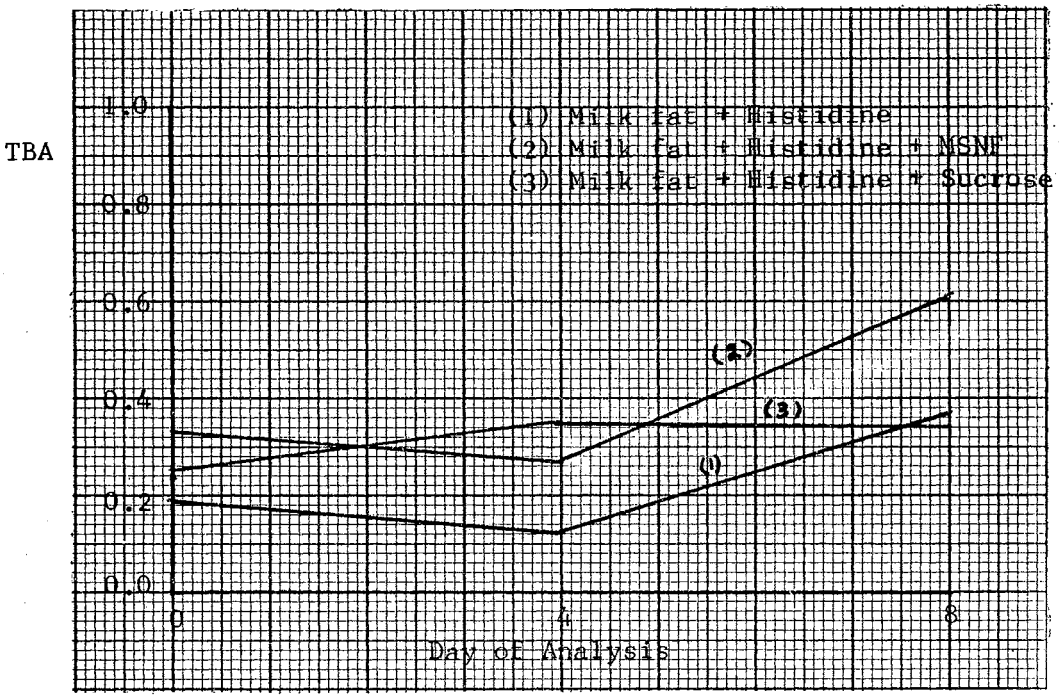

Figure 5. Effect of MSNF and Sucrose on Milk Fat Samples Containing Histidine

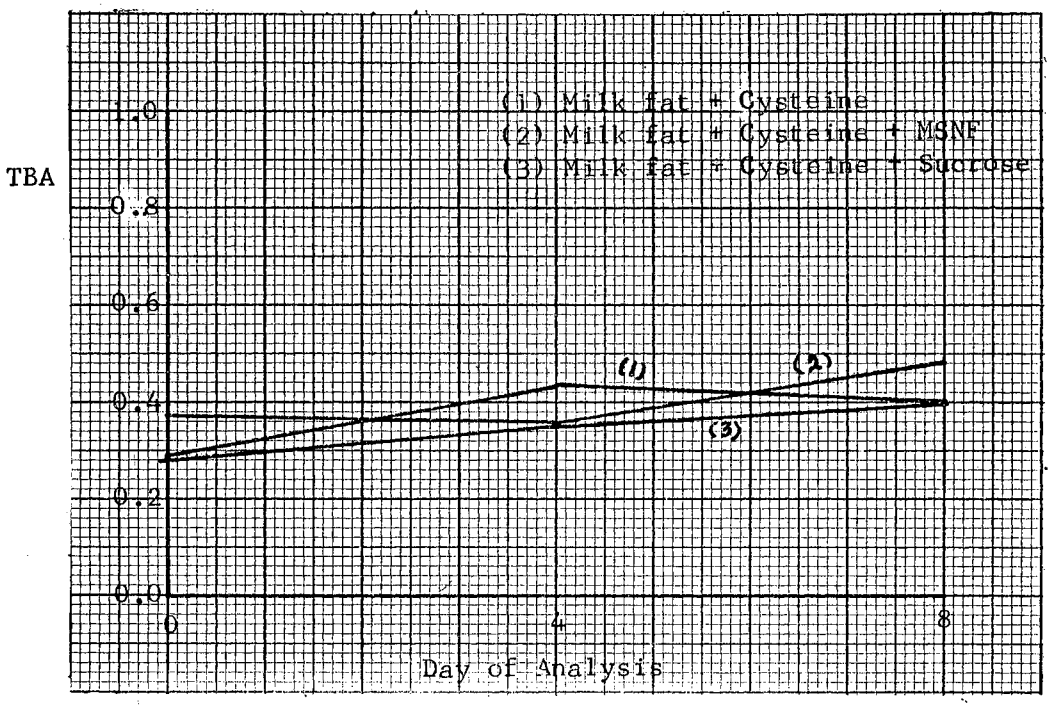

Figure 6. Effect of MSNF and Sucrose on Milk Fat Samples Containing Cysteine

SUMMARY AND CONCLUSIONS

This research was a continuation of the work started by Surve (26) in which he demonstrated that certain amino acids were effective antioxidants in milk fat emulsions. The object of this research was to measure the effect of MSNF and sucrose on the antioxidative properties of the amino acids histidine and cysteine. Milk fat emulsions containing 5% MSNF or sucrose and/or 0.14% histidine or cysteine were prepared. The oxidized flavor intensities of these mixtures were measured after 0, 4 and 8 days storage at room temperature (20-24°C) using a taste panel and the TBA test.

The correlation coefficient for taste panel scores vs. TBA tests was 0.78, indicating that these two sets of data had similar tendencies. Oxidized flavor intensities and TBA tests increased with storage time ($P \leq 0.10$). Histidine acted as an antioxidant in this study, but cysteine was a pro-oxidant. The addition of 5% MSNF increased the oxidized flavor intensities of these samples while sucrose had little or no effect on the flavor.

LITERATURE CITED

(1) Brown, W. C. and Thurston, L.M. A Review of oxidation in milk and milk products as related to flavor. J. Dairy Sci. 23: 629. 1940.

(2) Chipault, J. R. Antioxidants for use in foods. In Autoxidation and Antioxidants. Vol. II, edited by Lundberg, W. O., Interscience Publishers, New York. 1962.

(3) Clausen, D. F., Lundberg, W. O., and Burr, G. O. Some effects of amino acids and certain other substances on lard containing phenolic antioxidants. J. Am. Oil Chem. Soc. 24: 403. 1947.

(4) Day, E. A. and Lillard, D. A. Autoxidation of milk lipids. I. Identification of volatile monocarbonyl compounds from autoxidized milk fat. J. Dairy Sci. 43: 585. 1960.

(5) Day, E. A. Autoxidation of milk lipids. J. Dairy Sci. 43: 1360. 1960.

(6) Dugan, L. R., Jr. Stability and Rancidity. J. Am. Oil Chem. Soc. 32: 605. 1955.

(7) Dunkley, W. L. Evaluation of the thiobarbituric acid test as a measure of oxidized flavor in milk. Food. Technol. 5: 342. 1951.

(8) El-Negoumy, A. M., Miles, D. M. and Hammond, E. G. Partial characterization of the flavors of oxidized butter-oil. J. Dairy Sci. 44: 1047. 1961.

(9) El-Negoumy, A. M. and Hammond, E. G. Effect of antioxidants on the flavor of cold storage butter. J. Dairy Sci. 45: 848. 1962.

(10) Foress, D. A., Dunstone, E. A. and Stark. W. Fishy flavor in dairy products. II. The volatile compounds associated with fishy flavor in butter-fat. J. Dairy Res. 27: 211. 1960.

(11) Gaddis, A. M., Ellis, R. and Currie, G. T. Carbonyls in oxidizing fat. III. The distribution of volatile and non-volatile carbonyls. Food Res. 25: 495. 1960.

(12) Gaddis, A. M., Ellis, R. and Currie, G. T. Carbonyls in oxidizing fat. V. The composition of neutral volatile monocarbonyl compounds from autoxidized oleate, linoleate, linolenate esters and fats. J. Am. Oil Chem. Soc. 38: 371. 1961.

(13) Hammond, E. G. and Hill, F. D. The oxidized metallic and grassy flavor components of autoxidized milk fat. J. Am. Oil Chem. Soc. 41: 180. 1964.

(14) Hunziker, O. F. The butter industry. The author. La Grange, Illinois. 1940.

(15) Keeney, M. Secondary degradation products. In Symposium on foods: Lipids and their oxidation. Edited by Schultz, H. W., Day, E. A. and Sinnhuber, R. O. The Avi Publishing Company, Inc. Westport, Connecticut. 1962.

(16) Lillard, D. A., and Day, E. A. Degradation of monocarbonyls from auto-oxidizing lipids. J. Am. Oil Chem. Soc. 41: 549. 1964.

(17) Lundberg, W. O. Oxidative rancidity in food fats and its prevention. In Autoxidation and Antioxidants. Vol. II, Edited by Lundberg, W. O. Interscience Publishers, New York. 1962.

(18) Lundberg, W. O. Antioxidant. U. S. patent 2, 523, 127. Sept. 19, 1950.

(19) Marcuse, R. Antioxidative effect of amino acids. Nature. 186: 886. 1960.

(20) Marcuse, R. The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. J. Am. Oil Chem. Soc. 39: 97. 1962.

(21) Mickle, J. B., Patel, C. V., Malkus, M. V., Leidigh, M. E., Baker, H. J., and Morrison, R. D. Flavor studies of solidified water-in-milk fat emulsions. Food Technol. 18: 237. 1964.

(22) Patton, S., and Kurtz, G. W. 2-thiobarbituric acid as a reagent for detecting milk fat oxidation. J. Dairy Sci. 34: 669. 1951.

(23) Pohle, W. D., Gregory, R. L. and Van Giessen, B. Relationship of peroxide value and thiobarbituric acid value to development of undesirable flavor characteristics in fats. J. Am. Oil Chem. Soc. 41: 649. 1964.

(24) Saunders, D. H., Coleman, J. E., Hampson, J. W., Wells, P. A., and Riemenschneider, R. W. Autoxidation of fatty materials in emulsions. I. Pro-oxidant effect of histidine and trace metals on the oxidation of linoleate esters. J. Am. Oil. Chem. Soc. 39: 434. 1962.

(25) Sinnhuber, R. O., and Yu, T. C. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Res. 23: 626. 1958.

(26) Surve, Ali H. Amino acids as antioxidants in milk fat. M.S. Thesis, Oklahoma State University, 1965.

(27) Swern, D. Primary products of olefinic autoxidations. In Autoxidation and Antioxidants. Vol. I. Edited by Lundberg, W. O. Interscience Publishers. New York. 1961.

(28) Uri, N. Mechanism of Antioxidant. In Autoxidation and Antioxidants. Vol. I. Edited by Lundberg, W. O. Interscience Publishers. New York. 1961.

(29) Wyatt, C. J. and Day, E. A. Evaluation of antioxidants in deodorized and non-deodorized butter-oil stored at 30°C. J. Dairy Sci. 48: 682. 1965.

(30) Yu, T. C. and Sinnhuber, R. O. 2-thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technol. 11: 104. 1957.

(31) Yu, T. C., and Sinnhuber, R. O. Further observations on the 2-thiobarbituric acid method for the measurement of oxidative rancidity. J. Am. Oil. Chem. Soc. 41: 540. 1964.

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