AMINO ACIDS AS ANTIOXIDANTS IN MILK FAT

By

ALI H. SURVE

Bachelor of Veterinary Science

University of Bombay

Bombay, India

1962

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1965

AMINO ACIDS AS ANTIOXIDANTS IN MILK FAT

Thesis Approved:

J Brickley
Thesis Adviser

S. B. Musgrave
Faculty Representative

J. M. Logan
Dean of the Graduate School

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. James B. Mickle, his major adviser, for his encouragement, supervision and constructive criticism during the experimental work and the writing of this thesis. Appreciation also is expressed to Dr. S. D. Musgrave, Head of the Dairy Science Department, who was helpful in every way. The author wants to thank Mr. Paul E. Johnson, Dr. H. C. Olson, Mrs. Beverly Schrag, Mr. Robert L. Von Gunten and Mrs. Dorothy Woodruff for tasting the fat. The author also thanks Mrs. Evelyn Williams for her help with the experimental work, and Dr. R. D. Morrison, who helped design the experiment and analyze the data.

Deep appreciation goes to a wonderful friend Mr. G. N. Nadaf and to brothers Mr. M. S. Ahmed and Mr. A. S. H. Shaikh for their encouragement and support. Most of all, the author wishes to express his gratitude to his father Hasan and his mother Sairabi, whose sacrifice and patience are responsible for his being in the United States.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

One of the most serious defects of milk fat is oxidized flavor. As in any other fat, this defect is produced by the reaction of atmospheric oxygen with unsaturated fatty acids and their glycerides. This reaction leads to the formation of hydroperoxides, which on decomposition give rise to saturated and unsaturated carbonyl compounds and free radicals. The former are responsible for the flavor defect whereas the latter keep the process going and increase the intensity of the flavor.

One way to prevent this defect is by inhibiting the chain reaction. Any substance that will inhibit the initiation of this process and stop the free radicals from attacking new molecules will act as an antioxidant. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), isopropyl citrate (IPC), nordihydroguaiaretic acid (NDGA) and such other chemicals are used as antioxidants in many fats and fat containing foods (6). One of the more commonly used synergistic combinations to stabilize lard and other animal fats is a mixture of BHA, PG and citric acid in propylene glycol (11). El-Negoumy and Hammond (14) have recently shown that these conventional antioxidants are of doubtful value in butter. It is probable that these conflicting results were obtained because these antioxidants were not effective in milk fat or their potency was lost because butter is a complex emulsion. It, therefore, became essential to evaluate the effectiveness of these antioxidants in milk fat and to find, if necessary, other substances to be used as antioxidants in milk fat.

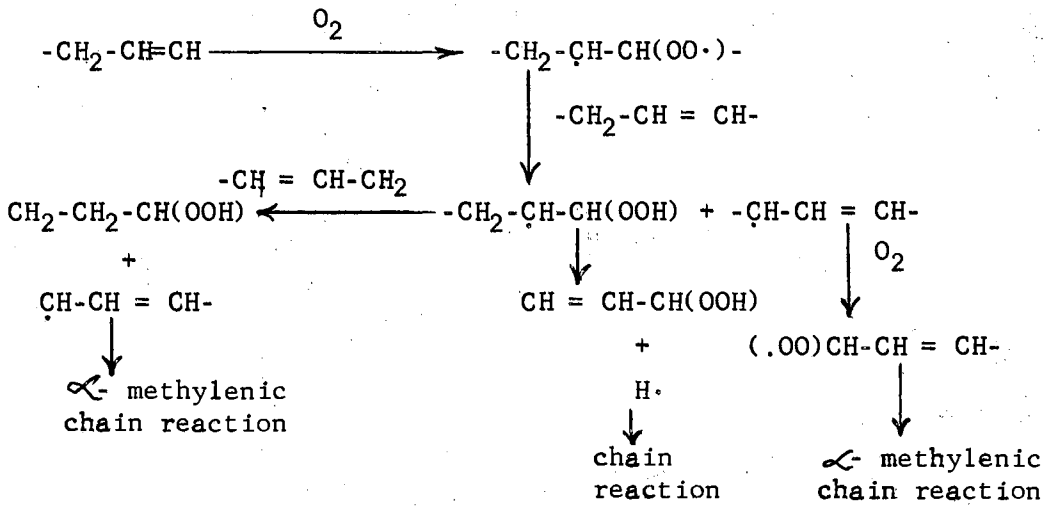
Marcuse (24) has shown that amino acids alone, in combination with various emulsifiers, or in emulsion with phosphate buffer act as antioxidants in linoleic acid and its methyl ester. He tried various amino acids and emulsifiers and found that tryptophan, histidine and glycine were good antioxidants and Span 20 was the most effective emulsifier. No work has been done to test amino acids as antioxidants in milk fat.

The objectives of this investigation were: (a) to test the potency of a mixture of BHA, PG, and citric acid as an antioxidant in milk fat (this mixture was used as an example of phenolic-type antioxidants); (b) to evaluate the antioxidative properties of tryptophan, histidine and glycine in milk fat; (c) to study the effect of added emulsifiers, Span 20 and lecithin, on the effectiveness of these antioxidants; and (d) to compare the potency of these antioxidants when added to dry fat and to an emulsion containing 80% milk fat and 20% phosphate buffer (pH. 7.0).

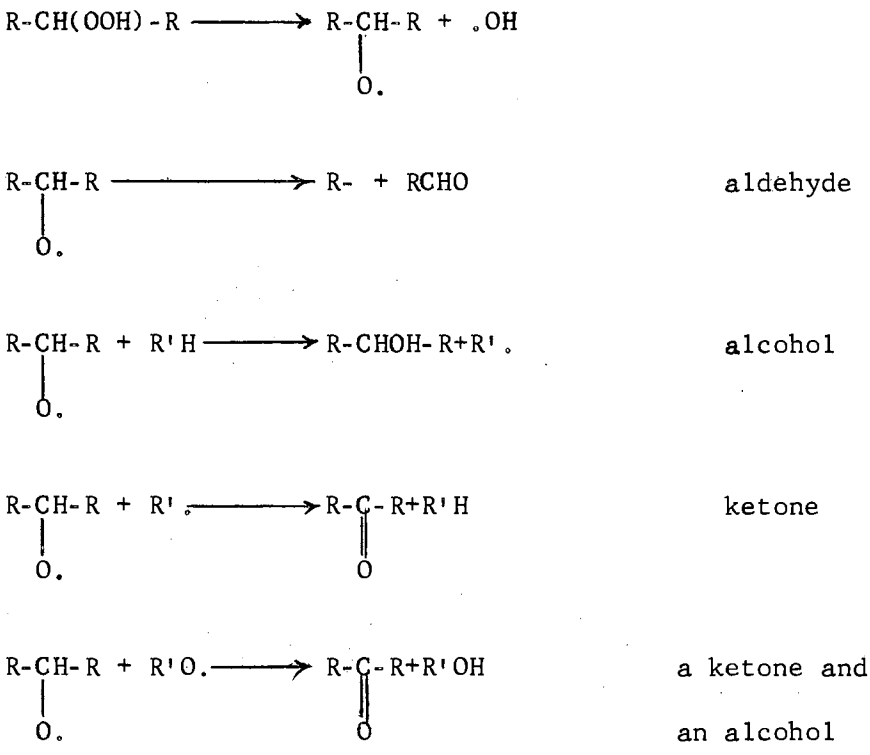
LITERATURE REVIEW

Oxidation of milk fat is a reaction of atmospheric oxygen with unsaturated fatty acids and their glycerides. This results in the development of various objectionable flavors thus greatly reducing the marketability of the product. The flavor defect produced in milk fat by oxidation is variously designated as fishy, tallowy, cappy, metallic, oxidized, oily, painty and grassy (9, 12, 15, 17). These flavors and the oxidation of fat in general have been extensively investigated as is evident from the voluminous literature reviewed by Brown and Thurston (5) and the papers presented at various symposia (1, 31).

Swern (38) reviewed the mechanism by which oxidation takes place and concluded that the initial point of oxidative attack was at the double bond because more energy is required to rupture the α-methylenic C-H bond. He further added that the "double bond attack" must occur to only a minor extent to "trigger" the α-methylenic chain reaction. These reactions he described with the following equations.



The hydroperoxides formed undergo degradation and give rise to carbonyl compounds, short chain fatty acids, alcohols and other compounds (5, 8). The process by which these carbonyl compounds are produced is described by Keeney (19) with the following equations.



The various oxidized flavor defects are produced by differing concentrations of various carbonyls. As Day (8) puts it "oxidized flavor is the additive effect of compounds, with each compound occurring at sub-threshold levels, but the combined concentrations equaling or exceeding the threshold of any one in the mixture." Stark and Forss (35) have shown that these compounds have flavor threshold values of one part in 10^9 parts of milk fat. The flavor threshold value has been defined (28) as the level at which the substance could be detected by 50% of the taste observers.

Various metals and enzymes act as catalysts in the oxidation of milk fat. Smith and Dunkley (34) proved that copper and ascorbic acid are prooxidants in milk. Ingold (18) reviewed the whole process of metal catalysis and showed how the heavy metals act as prooxidants. Xanthine oxidase has been shown to be a prooxidant in milk by Aurand et al. (3), but others (30, 33, 34) concluded that this enzyme plays no part in the development of oxidized flavor.

Dunkley (13) introduced the 2-thiobarbituric acid (TBA) test as a measure of oxidized flavor in dairy products. In this test a sample of fat is heated with the TBA reagent in the presence of an acid (41). During heating, one molecule of malonic dialdehyde reacts with two molecules of TBA to give a pink color which can be measured colorimetrically (32). Lea (21) has cautioned that the malonic dialdehyde present in an oxidized fat makes no contribution to its "off flavor" and the evaluation of TBA test results is somewhat empirical.

The active oxygen of the peroxides formed during oxidation can be reacted with potassium iodide to liberate iodine. The quantitative measurement of this iodine is the basis of the peroxide value (20). Peroxides themselves are odorless; therefore, any correlation with flavor intensity also is "empirical" according to Lea (21). El-Negoumy and Hammond (14) showed that there was no correlation between the oxidized flavor of milk fat and its TBA test or peroxide value. Lea (21) suggested that organoleptic evaluation must of necessity remain the ultimate criterion against which the performance of more objective tests will be judged. Pohle et al. (29), in their recent publication, said that flavor score could not be estimated for any given fat either from

the peroxide value or the TBA value. According to these authors, either objective test could be used to follow the development of oxidized flavor in a given product but the relative value of these tests would vary from one product to another.

Antioxidants are substances which inhibit the autoxidation of unsaturated lipids. Stuckey (37) found that there are "four possible mechanisms by which an inhibitor may function as a chain stopper for the free radical chain mechanism of lipid oxidation". "These are: (a) hydrogen donation by the antioxidant, (b) electron donation by the antioxidant, (c) addition of the lipid to the aromatic ring of the antioxidant, and (d) formation of a complex between the lipid and the aromatic ring of the antioxidant." Some of the more commonly used antioxidants in food and food products, such as fish (26) and meat (40), are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), nordihydroguaiaretic acid (NDGA) and mixtures which include one or more of above antioxidants (6). El-Negoumy and Hammond (14), however, have shown that these conventional antioxidants are of little use in butter.

Clansen et al. (7), working with lard, showed that various amino acids act as synergists when used in combination with the phenolic antioxidants. "Synergism" as defined by Uri (39), "is the phenomenon in which a number of compounds, when present together in the same system, have a more pronounced effect than that which would be derived from a simple additivity concept." Marcuse (23) evaluated the use of amino acids as antioxidants and found them to have antioxygenic properties (except cysteine which was found to be prooxidant). Marcuse (24) also examined tryptophan, histidine and glycine as antioxidants in linoleic acid and its methyl ester. He found them to be potent antioxidants and concluded

that the antioxidative effect of amino acids was enhanced or the pro-oxidative effect lowered, by an addition of phosphate and emulsifiers.

EXPERIMENTAL PROCEDURE

The milk fat in this experiment was obtained from sweet cream butter which was stored at -15°C until used. As needed, aliquots of this butter were removed from storage and melted, the fat was decanted and filtered three times through double layers of fiber-bonded gauze-faced filter disks[1] to obtain dry milk fat.

Tryptophan,[2] histidine,[3] glycine[2] and a mixture of 20% BHA,[4] 6% PG[4] and 4% citric acid in propylene glycol (BHA mixture) were added to this fat and compared with a control which received treatment identical to the other samples except that no antioxidant was added. Oxidation was hastened by adding 5 ppm of copper sulfate to each fat sample (4, 10). The potency of these antioxidants was evaluated in dry fat and in emulsions containing 80% of the same fat. At the same time, the influence of 0.1% added emulsifiers (lecithin[5] and Span 20[6]) on the intensity of oxidation was studied. A split-plot experimental design, as shown in Tables II-IV, was used to evaluate these variables. Statistical analysis was conducted according to the procedure outlined by Steel and Torrie (36).

The level of emulsifiers to be used in the experiment was determined by preliminary trials in which different levels of emulsifier were incorporated into milk fat. A level of 0.1% did not give any foreign flavors to the fat and therefore was used.

To prepare the samples 500 g of fat (400 g in case of an emulsion sample) were weighed into an Erlenmyer flask. To this, 0.5 g of

---

1-6) See footnotes on page 12a.

emulsifier was added and the fat was then stirred for five to seven minutes. This mixture was divided into five equal parts. No antioxidant was added to one part (the control); to each of the other four aliquots, 0.05 g of BHA mixture (to give 0.01% BHA), 0.01 g tryptophan, 0.01 g histidine or 0.01 g glycine was added. After adding 0.1 ml of a 0.5% copper sulfate solution to each sample, these were again stirred for five to seven minutes to incorporate all the additives.

When preparing an emulsion, 20 ml of 0.02 M sodium phosphate buffer (pH 7.0) were added to 80 g of dry fat and poured into a 330 ml glass cylinder held in a water bath at 12-15°C. A stable emulsion (resembling butter) was formed in four to five minutes by cooling the mixture while agitating it with a dasher that so fitted the inside of the glass cylinder that it continuously scraped the solidified emulsion from the side of the beaker as it formed. This "laboratory churn" has been described in detail by Mickle et al. (25). The dry fat samples then were stored at 25 ± 2°C for 24 hours. The emulsions were held at the same temperature for 48 hours. At the end of this time, the samples were tested organoleptically for oxidized flavors. Peroxide and TBA values also were determined on aliquots of the same samples.

The organoleptic examination of these fats was done by five "trained judges." These judges were aware of the purposes of this research but at no time during the study were they aware of the individual sample descriptions. At each examination, a fresh sample of fat (or emulsion) was presented for tasting. This served as a standard to check the repeatability of the judges. The samples were coded and presented to the tasters in a random order.

These judges were asked to rank the samples from 1 to 5 according to the intensity of oxidized flavor. (A representative score sheet is shown in Table I.) Salted crackers and plain tap water were used between samples to reduce the taste carry-over.

The TBA test described by Patton and Kurtz (27) was used with some of the modifications suggested by other workers (41, 42). The procedure in brief was as follows:

Reagents: (a) TBA solution; 1 g TBA[7] dissolved in 75 ml of 0.1N NaOH and diluted to 400 ml with water; (b) Buffer solution; 59 g sodium citrate dissolved in 50 ml of concentrated HCl and diluted to 400 ml with water; (c) Trichloracetic acid (TCA) solution; 20 g TCA dissolved in 100 ml of water.

Procedure: 3.0 g of fat were weighed into a 250 ml flat bottomed flask. To this, 7.5 ml water, 3 ml TCA solution and 6 ml TBA reagent (4 ml TBA solution + 2 ml buffer solution) were added. The flask was connected to a condenser and refluxed for 30 minutes in a vigorously boiling water bath. The flask was cooled to room temperature by immersing it in a beaker of cold water and the liquid centrifuged at about 2,000 rpm[8] for five minutes. The fat layer then was siphoned off and the remaining liquid shaken for 30 seconds with 2 ml of petroleum ether. This mixture was centrifuged for five minutes. The petroleum ether layer then was siphoned out and the color of the clear solution was read on a Beckman Model B Spectrophotometer at 510 mu.

The peroxide test was run as outlined in A.O.C.S. (2) with certain modifications as stated by Swern (38). In brief the procedure was as follows: 50 ml of a 3:2 acetic acid-chloroform mixture were measured into an Iodine flask, in which 5 g fat had been weighed previously. Two ml of

7-8) See footnotes on page 12a.

freshly prepared 50% potassium iodide solution were added and allowed to react for fifteen minutes. At the end of this period 50 ml of water was added to the flask and the contents were titrated with a standard sodium thiosulfate solution using a 1% starch solution as an indicator. The peroxide value was calculated by the following formula:

$$\text{meq per kg} = \frac{\text{ml thiosulfate x normality of thiosulfate x 1000}}{\text{wt of the sample}}$$

TABLE I

A REPRESENTATIVE SCORE SHEET ON WHICH THE JUDGES RECORDED THEIR FLAVOR SCORES

SCORE SHEET

Name of the judge _____ Date _____

For your guidance

The desired flavor of butter oil is mild, sweet, clean and pleasant.

An oxidized butter oil has a tallowy or cardboard-like flavor. It is prominent immediately after the sample has been spit-out. Therefore, do not swallow the sample if you can help it. Instead, keep it in your mouth for some time, spit it out and breath in deeply keeping your mouth open, and breath out through the nose keeping mouth closed.

Gargle with the water occasionally between samples.

Please use the following code to designate the flavor intensities.

"1"	no oxidized flavor
"2"	questionable
"3"	slightly oxidized
"4"	distinctly oxidized
"5"	very pronouncedly oxidized

Sample Code	Flavor Score	Remarks (if any)
a.		
b.		
c.		
d.		
e.		
f.		

Comments or suggestions that you would like to make regarding these samples.

FOOTNOTES FOR EXPERIMENTAL PROCEDURE

1. Filter disks: Johnson and Johnson, Chicago 38, Ill.

2. DL-tryptophan and glycine: Calbiochem, 3625 Medford Street, Los Angeles, Calif.

3. DL-histidine: Mann Research Laboratories Inc. 136, Liberty Street, New York 6, New York.

4. Butylated hydroxyanisole and propyl gallate: Eastman Chemical Products, Inc., Kingsport, Tennessee.

5. Soybean lecithin: Fisher Scientific Company, Fair Lawn, N. J.

6. Span 20 (sorbitan monolaurate): Atlas Powder Company, Wilmington, Delaware.

7. 2-thiobarbituric acid: Eastman Organic Chemicals, Rochester 3, New York.

8. International clinical-model centrifuge with a 6-inch diameter "streamlined" wheel. Fisher Scientific Co., Fort Worth, Texas.

RESULTS AND DISCUSSION

The relation between the organoleptic scores and the TBA values of the samples is shown in figure 1. In this diagram, the points were scattered and had no pattern indicating there was little relation between the two variables. The correlation coefficient for this comparison (calculated from the data in Tables II and III) was 0.11. Upon close examination, however, it was noticed that all the points representing the response due to the BHA mixture were at the upper left-hand corner of the graph and that these were separated from the rest of the data. When these 12 points were omitted from consideration, a fair linear relationship appeared between the organoleptic scores and TBA values. The calculated correlation coefficient in this case was 0.70. The TBA test is a measure of only one aldehyde (malonic dialdehyde) which is flavorless (21). On the other hand, the composition of the carbonyl mixture produced by oxidation in the same kind of fat varies from one experiment to another (8). This variation probably explains the poor correlation obtained between the organoleptic scores and the TBA values in this experiment.

In samples containing the BHA mixture the organoleptic scores were high and the corresponding TBA values were low when compared to the values obtained in the rest of the experiment. Thus, if one considers only the TBA readings it appears as if the BHA mixture almost stopped oxidation. On the other hand, the organoleptic scores indicate that this additive probably did little at all to check oxidation.

Most of the judges designated the flavor produced in the fat containing the BHA mixture as "metallic" or "tallowy." In milk fat, oct-1-en-3-one has been found to be responsible for metallic flavor (35) and n-octanal and n-nonanal to be responsible for tallowy flavors (16). Lillard and Day, in a recent publication (22) pointed out that some monocarbonyls, which are themselves initial degradation products of lipid hydroperoxides, undergo further oxidation to produce various additional carbonyls, including malonic dialdehyde. They also found that oct-1-en-3-one, a degradation product of lipid hydroperoxide, resists further oxidation, thus giving rise to metallic flavors and decreasing the amount of malonic dialdehyde produced. Thus, it may be that fat containing the BHA mixture produced large amounts of the above named carbonyls but very little malonic dialdehyde. Pohle et al. in their recent publication (29) showed that the TBA test can be used to follow the development of "off-flavor" in a given product but that the relative TBA values and falvor intensities may vary from product to product. It is possible that the fat containing the BHA mixture formed breakdown products which were different from those formed in the fat containing the other antioxidants, and this resulted in different TBA values.

The relation between the organoleptic scores and peroxide numbers is shown in figure 2. This graph indicates that there was little relation between the organoleptic values and the peroxides numbers of this milk fat. When several reagent blanks were run, the meq of sodium thiosulfate required to react with the liberated iodine in one kg of fat under identical conditions, ranged from about 7 to 14. Thus, the error of this technique was rather large compared to the peroxide numbers obtained (Table IV). Because of this, little correlation would be expected between these variables.

Since organoleptic examination was found to be the only reliable test to determine the oxidized flavors produced in this experiment, an analysis of variance was run only on those values. In this analysis the type of emulsifier and the various additives had statistically significant effects on the data ($P < 0.05$). To interpret these findings, the values were plotted (figure 3). This graph shows that all the amino acids acted as antioxidants, but that the BHA mixture behaved as a prooxidant. Histidine seemed to be the most potent antioxidant under the conditions of this experiment.

The behaviour of the BHA mixture conformed with what El-Negoumy and Hammond (14) found when working with other phenolic antioxidants in butter. Their data indicated that phenolic compounds do not act as antioxidants and that some of these compounds including a mixture of BHT and isopropyl citrate act as prooxidants. The antioxygenic properties of amino acids in this experiment also supports what Marcuse (24) found when working with linoleic acid and its methyl ester. He tested various amino acids and found tryptophan, histidine and glycine to be antioxygenic. Of these, histidine was the most effective. The mechanism by which amino acids act as antioxidants is not known, and the assumption that their metal-chelating property is responsible for this action (24) seems to be the only plausible explanation at the present time.

Many of the antioxidants tested in this experiment behaved differently in the presence of the two emulsifiers. The BHA mixture had no antioxygenic effects whether the emulsifiers were present or not. Tryptophan acted quite efficiently when no emulsifiers were added, but glycine gave better results with lecithin. Histidine was more effective when Span 20

was added to the fat, but the addition of lecithin had no effect on the antioxidant. Changing the physical state of the fat, from dry fat to an emulsion, had no statistically significant effect ($P > .05$) on the development of oxidized flavor.

TABLE II

ORGANOLEPTIC SCORES OF DRY MILK FAT AND MILK FAT EMULSIONS CONTAINING VARIOUS ANTIOXIDANTS AND EMULSIFIERS

Type of fat	Antioxidants	Added Emulsifier: None		Lecithin (0.1%)		Span 20 (0.1%)	
		(Total of 5 judge's rankings)					
Dry Fat	Control[a]	12	15	14	21	20	17
	BHA Mixture[b]	20	18	22	17	19	21
	Tryptophan	14	12	14	14	15	15
	Histidine	09	09	08	10	07	08
	Glycine	11	13	07	09	11	12
Emulsion[c]	Control	10	16	15	15	15	17
	BHA Mixture	19	21	18	20	16	21
	Tryptophan	11	11	14	15	15	14
	Histidine	10	07	08	11	07	07
	Glycine	09	09	11	13	13	13

[a]No antioxidant added

[b]Mixture of 20% butylated hydroxyanisole, 6% propyl gallate and 4% citric acid in propylene glycol

[c]Emulsion of 80% fat and 20% phosphate buffer

TABLE III

TBA TEST VALUES FOR DRY MILK FAT AND MILK FAT EMULSIONS CONTAINING VARIOUS ANTIOXIDANTS AND EMULSIFIERS

Type of fat	Antioxidants	Emulsifier: None		Lecithin (0.1%)		Span 20 (0.1%)	
		(absorbance at 510 mu)					
Dry Fat	Control[a]	0.560	0.495	0.620	0.850	0.950	0.920
	BHA Mixture[b]	0.135	0.110	0.145	0.160	0.145	0.112
	Tryptophan	0.730	0.610	0.610	0.760	0.660	0.750
	Histidine	0.375	0.310	0.400	0.620	0.095	0.335
	Glycine	0.320	0.520	0.510	0.465	0.080	0.580
Emulsion[c]	Control	0.220	0.315	0.550	0.420	0.460	0.620
	BHA Mixture	0.035	0.065	0.070	0.060	0.055	0.050
	Tryptophan	0.115	0.250	0.400	0.540	0.460	0.370
	Histidine	0.040	0.050	0.135	0.160	0.050	0.070
	Glycine	0.055	0.150	0.390	0.310	0.220	0.285

[a]No antioxidant added

[b]Mixture of 20% butylated hydroxyanisole, 6% propyl gallate and 4% citric acid in propylene glycol

[c]Emulsion of 80% fat and 20% phosphate buffer

TABLE IV

PEROXIDE VALUES FOR DRY MILK FAT AND MILK FAT EMULSIONS CONTAINING VARIOUS ANTIOXIDANTS AND EMULSIFIERS

Type of fat	Antioxidants	Emulsifier: None		Lecithin (0.1%)		Span 20 (0.1%)	
		(Meq./kg fat)					
Dry Fat	Control[a]	7.62	8.12	18.12	22.62	14.87	31.00
	BHA Mixture[b]	7.37	10.00	13.12	13.62	14.00	27.25
	Tryptophan	6.62	8.00	17.62	22.00	14.25	16.62
	Histidine	6.25	16.00	12.12	11.12	17.87	27.12
	Glycine	10.12	10.87	12.12	15.00	19.37	34.12
Emulsion[c]	Control	13.37	22.00	9.25	20.00	18.75	19.87
	BHA Mixture	12.87	29.37	13.00	21.50	21.25	26.25
	Tryptophan	11.87	25.87	9.00	17.75	17.37	21.62
	Histidine	10.12	10.87	7.87	20.87	17.12	27.62
	Glycine	12.87	27.87	8.50	26.12	13.25	25.62

[a]No antioxidant added

[b]Mixture of 20% butylated hydroxyanisole, 6% propyl gallate and 4% citric acid in propylene glycol

[c]Emulsion of 80% fat and 20% phosphate buffer

TABLE V

ANALYSIS OF VARIANCE OF
ORGANOLEPTIC SCORES

Source of variation	d.f.	Mean Squares
Replicates	1	12.150
Fat	1	2.817
Emulsifier	2	9.817*
Fat X Emulsifier	2	2.717
Error of Main Plot (Replicates X Treatment)	5	0.803
Antioxidants	4	212.625**
Antioxidants X Fat	4	2.775
Antioxidants X Emulsifier	8	52.200
Antioxidants X Fat X Emulsifier	8	29.400
Error of Sub-plot (total ss minus all calculated ss)	24	91.200

*Significant at the 5% level of probability.
**Significant at the 1% level of probability.

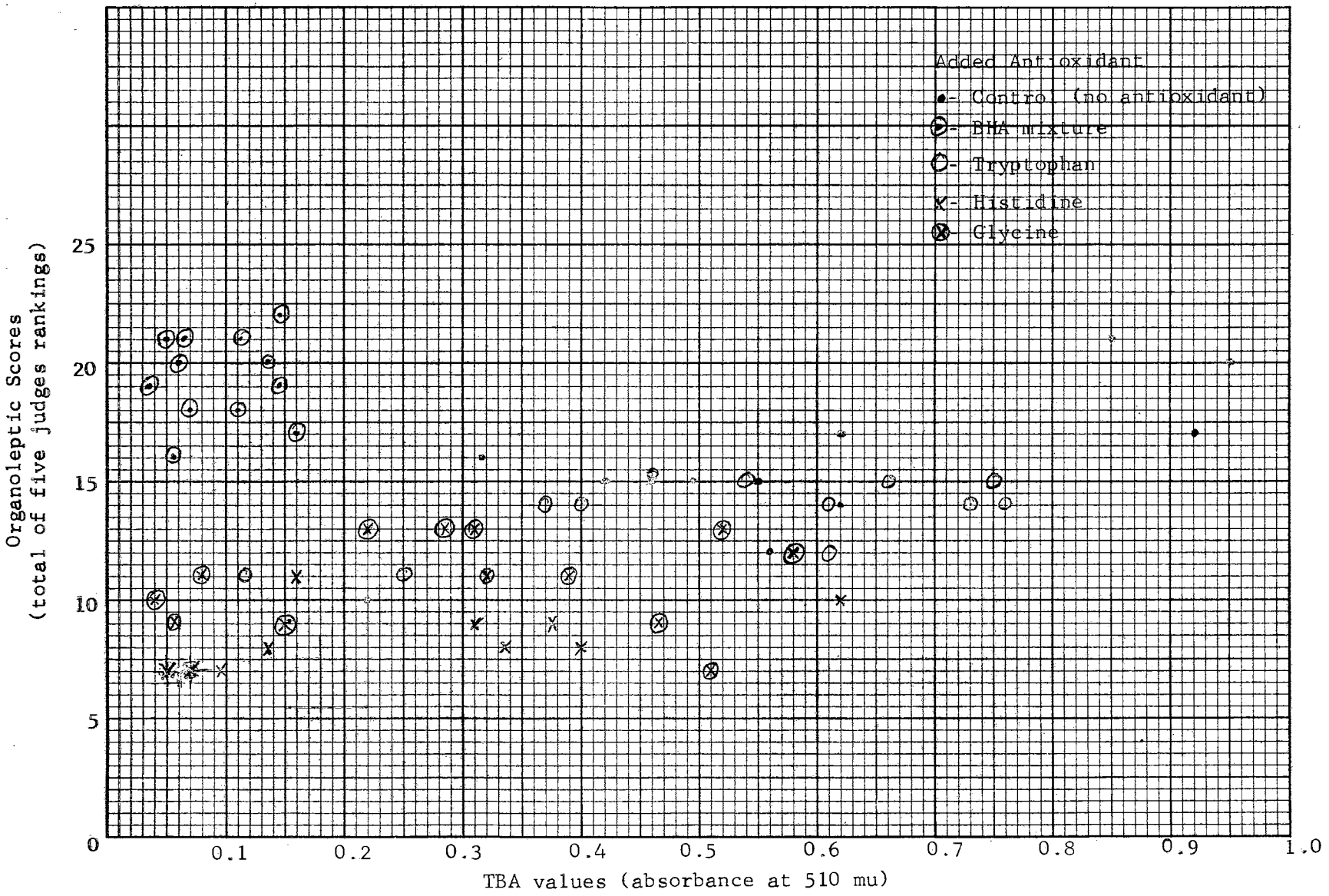


Figure 1. Organoleptic scores vs TBA values for milk fat samples containing antioxidants.

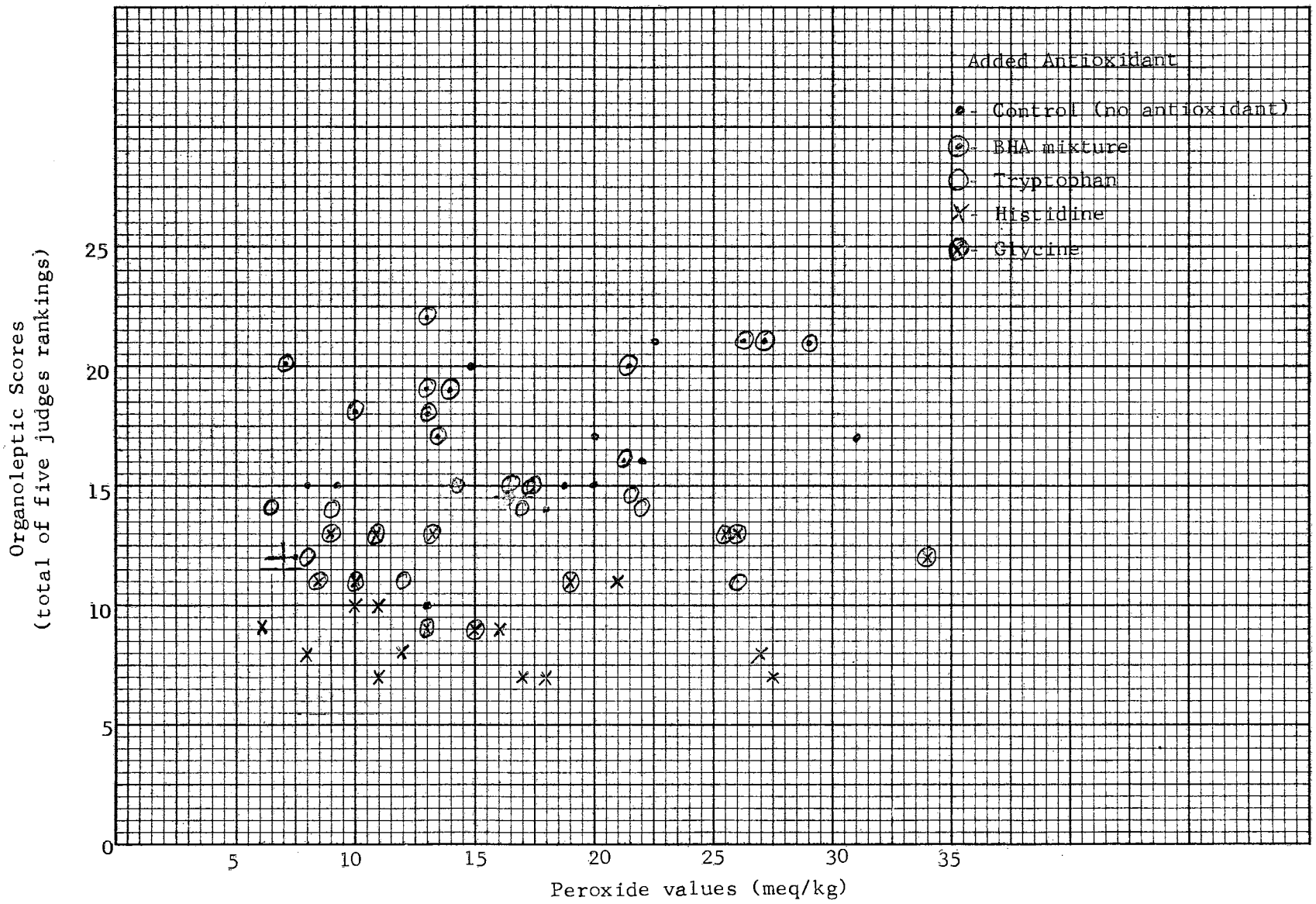


Figure 2. Organoleptic scores vs peroxide values for milk fat samples containing antioxidants.

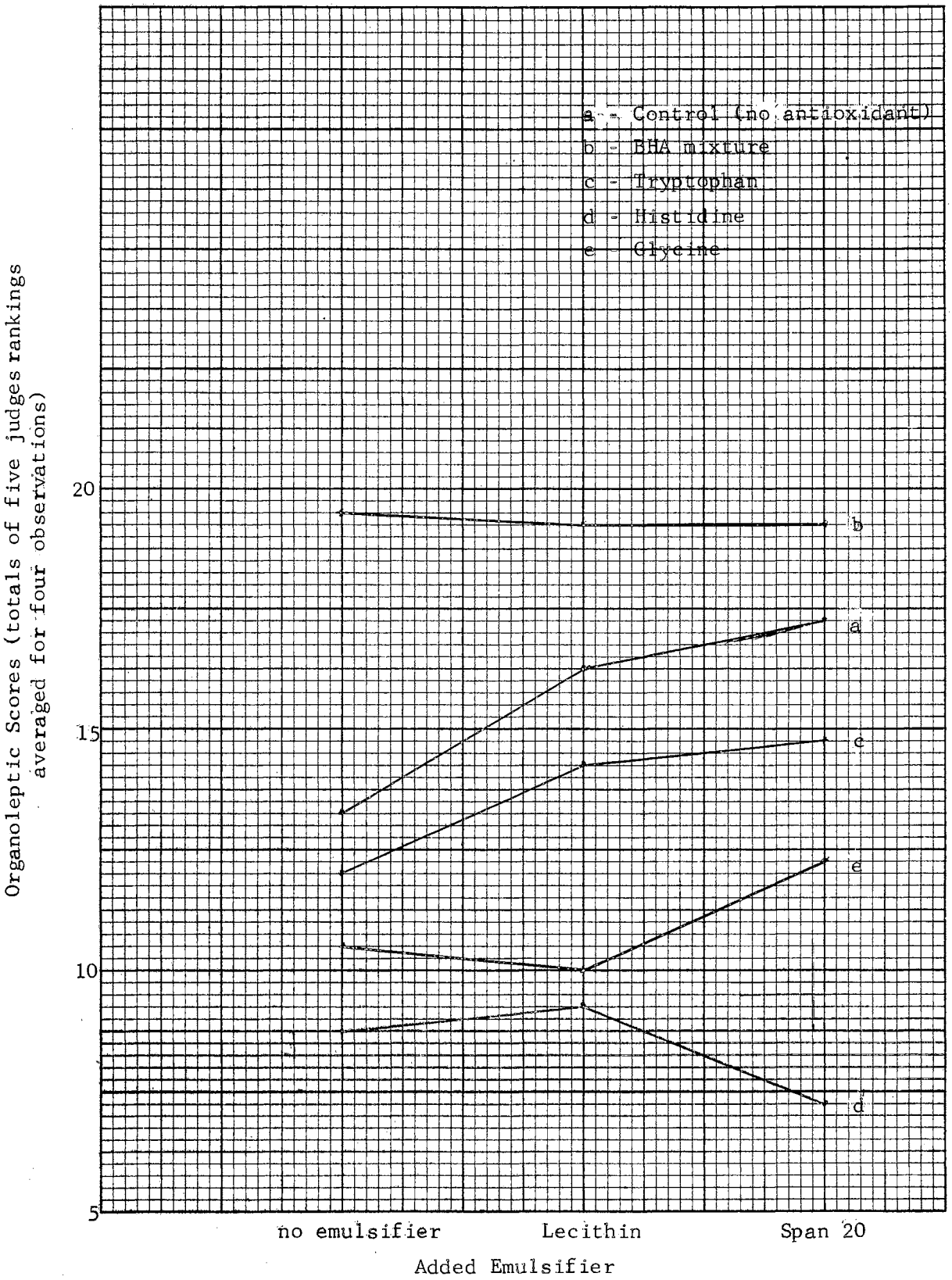


Figure 3. Oxidized flavor in milk fat containing different antioxidants used in combination with lecithin and Span 20. (The points are connected for ease of visual observation, but do not indicate any linear relationships.)

SUMMARY AND CONCLUSIONS

The object of this experiment was to test amino acids and a mixture containing BHA, as antioxidants in milk fat. It also was determined whether certain emulsifiers when added to dry fat or to its emulsion had any influence on the effectiveness of these antioxidants. Tryptophan, histidine, glycine and a BHA mixture were incorporated into dry milk fat and into an emulsion containing 80% milk fat and 20% phosphate buffer. Lecithin or Span 20 was added to some of the samples. Oxidation was initiated by adding 5 ppm of copper sulfate and hastened by storing the fat at 25°C for 24-48 hours. The flavor developed in the samples was evaluated by organoleptic examination and TBA tests and peroxide values were determined on aliquots of each sample.

Organoleptic examination was the most dependable means of evaluating oxidized flavors in this work, but TBA and peroxide values could not be relied upon to evaluate this flavor. The BHA mixture was a prooxidant whereas the three amino acids tested had antioxygenic properties. The added emulsifiers, lecithin and Span 20, had different effects on these antioxidants; in some cases they made the antioxidant more efficient, but in most cases they did not. Histidine, in combination with Span 20, was the most efficient antioxidant tested. Changing the physical state of the fat from dry fat to an emulsion had little effect on the action of these antioxidants.

LITERATURE CITED

1. Anonymous. Proceedings Flavor Chemistry Symposium. Campbell Soup Company, Camden, New Jersey. 1961.

2. American Oil Chemists' Society. Official and Tentative Methods. Amer. Oil Chem. Soc., Chicago, Ill. 1961.

3. Aurand, L. W., Woods, A. E. and Roberts, W. M. Some factors involved in the development of oxidized flavor in milk. J. Dairy Sci. 42:961. 1959.

4. Briggs, L. H. The autoxidation of butterfat. J. Dairy Res. 3:61. 1931.

5. 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.

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

7. Clansen, 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. 41:468. 1964.

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

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

10. DeLuca, A. P., Teichman, R., Ronssean, J. E., Jr., Morgan, M. E., Eaton, H. D., MacLeod, P., Dicks, M. A. and Johnson, R. E. Relative efficiency of various antioxidants fed to lactating dairy cows, on incidence of copper-induced oxidized milk flavor and apparent carotene and tocopherol utilization. J. Dairy Sci. 40:877. 1957.

11. Dugan, L. R., Jr., Kraybill, H. R., Ireland, L. and Vibrans, F. C. Butylated hydroxyanisole as an antioxidant for fats and foods made with fat. Food Technol. 4:457. 1950.

12. Dugan, L. R., Jr. Development and inhibition of oxidative rancidity in foods. Food Technol. 15:10. 1961.

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

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

15. Forss, D. A., Dunstone, E. A. and Stark, W. Fishy flavour in dairy products. II. The volatile compounds associated with fishy flavour in butter fat. J. Dairy Res. 27:211. 1960.

16. Forss, D. A., Dunstone, E. A. and Stark, W. The volatile compounds associated with tallowy and painty flavours in butter fat. J. Dairy Res. 27:381. 1960.

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

18. Ingold, K. U. Metal Catalysis. 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.

19. 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.

20. Lea, C. H. Methods for determining peroxides in lipids. J. Sci. Food Agri. 3:586. 1952.

21. Lea, C. H. The oxidative deterioration of food lipids. 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.

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

23. Marcuse, R. Antioxidative effect of amino acids. Nature. 186:886. 1960.

24. 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.

25. 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.

26. Olcott, H. S. Marine 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.

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

28. Patton, S. and Josephson, D. V. A method for determining significance of volatile flavor compounds in foods. Food Res. 22:316. 1957.

29. Pohle, W. D., Gregory, R. L. and VanGiessen, 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.

30. Rajan, T. S., Richardson, G. A. and Stein, R. W. Xanthine oxidase activity of milks in relation to stage of lactation, feed and incidence of spontaneous oxidation. J. Dairy Sci. 45:933. 1962.

31. Schultz, H. W., Day, E. A. and Sinnhuber, R. O. Symposium on foods: Lipids and their oxidation. The Avi Publishing Company, Inc. Westport, Connecticut. 1962.

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

33. Smith, G. J. and Dunkley, W. L. Xanthine oxidase and incidence of spontaneous oxidized flavor in milk. J. Dairy Sci. 43:278. 1960.

34. Smith, G. J. and Dunkley, W. L. Prooxidants in spontaneous development of oxidized flavor in milk. J. Dairy Sci. 45:170. 1962.

35. Stark, W. and Forss, D. A. A compound responsible for metallic flavour in dairy products. I. Isolation and identification. J. Dairy Res. 29:173. 1962.

36. Steel, R. G. D. and Torrie, J. H. Principles and procedures of statistics. McGraw-Hill Book Company, Inc. New York. 1960.

37. Stuckey, B. N. Antioxidants. 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.

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

39. Uri, N. Mechanism of antioxidation. In Autoxidation and Antioxidants. Vol I. Edited by Lundberg, W. O. Interscience Publishers. New York. 1961.

40. Watts, B. M. Meat 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.

41. Yu, T. C. and Sinnhuber, R. O. 2-thiobartituric acid method for measurement of rancidity in fishery products. Food Technol. 11:104. 1957.

42. 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.

VITA

Ali Hasan Surve

Candidate for the Degree of

Master of Science

Thesis: AMINO ACIDS AS ANTIOXIDANTS IN MILK FAT

Major Field: Dairy Science

Biographical:

Personal Data: Born in the Village of Savnas in the Taluka Khed, of District Ratnagiri, (of the then) Bombay State, India, May 1, 1934, the son of Hasan and Sairabi Surve.

Education: Attended grade school in the city of Bombay and graduated from the M. H. Saboo Siddik Technical High School, in 1954. Completed the pre-veterinary requirements from Ismail Yusuf College, Bombay, in 1956. Received the Bachelor of Veterinary Science Degree from the University of Bombay, India, in 1962. Attended the Oklahoma State University from January 1964 until January 1965; a candidate for the Master of Science degree in May 1965.

Experience: Worked as Veterinary-and-Animal Husbandry Officer with the Maharashtra State Government for three and a half years.

Organizations: American Dairy Science Association, India Student Club, International Moslem Students' Association and International Relations Council.