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To the Graduate Council:

I am submitting herewith a thesis written by William S. Phillips entitled "The Influence of Heat and Vacuum Treatment on the Rancid Flavor of Milk." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairy Manufacturing.

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Major Professor

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THE INFLUENCE OF HEAT AND VACUUM TREATMENT ON THE RANCID FLAVOR OF MILK

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

William S. Phillips

August 1963

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CHAPTER I

INTRODUCTION

The dairy industry strives to serve the public with a safe wholesome milk supply which has an acceptable, standardized flavor. Off-flavors and odors in milk may be caused by feeds eaten by the cow, absorption of odors and flavors by the milk, and development of flavors and odors during processing and handling.

Rancid flavor may be caused by improper handling or processing which may activate the lipase system of milk. The short chain fatty acid content of the milk, which is responsible for the rancid flavor, is increased due to the hydrolysis of the milk fat by the lipase system. Some of the newer methods of handling milk have been found to activate the lipase system. The result is that much of the milk is on the threshold of being detectably rancid when it reaches the processing plant.

Vacuum treatments with or without steam injection have been effective in the removal of volatile compounds which cause many off-flavors in milk. These treatments are widely used in standardizing the flavor of milk during seasons of the year when feed flavors are generally a problem for the dairy industry. Likewise, in steam refining of edible oils, a vacuum steam distillation process, the low molecular weight fatty acids are largely removed. During this process the distillate, generally less than 0.5 per cent of the total product, is removed.

The purpose of this investigation was to determine if a vacuum treatment, in which a distillate is removed and replaced by water, could remove the flavor caused by hydrolytic rancidity from milk.

CHAPTER II

REVIEW OF LITERATURE

The Rancid Flavor

The rancid flavor in milk is caused by the presence of short chain free fatty acids primarily butyric acid (8, 12, 16, 30, 38). These acids are present in normal milk in small amounts. When the lipase system of the milk is activated, the milk fat is hydrolyzed and liberates more of these fatty acids (8, 16, 38). In a study of the free fatty acids in milk, Harper et al. (8) found that rancid flavored milk had comparatively high levels of butyric, caproic, caprylic, formic and acetic acids.

Rancid flavor has always been a problem in milk because milk contains lipase which may be easily activated by agitation (3, 12, 13, 14, 16, 27, 29, 37, 39, 40) or temperature changes during processing and handling (12, 14, 16, 19, 27, 40, 42). Throughout the history of the dairy industry there have been instances where new methods of handling and processing milk have activated the lipase system and produced rancid flavors. Homogenization is probably the most dramatic example of this. Dormer and Widner (5) discussed the speed with which raw milk became rancid after homogenization, and the prevention of this by heating the milk before homogenization.

More recently, the problem has occurred because of the activation of the lipase by improperly installed or improperly operated pipelines (3, 14, 20, 29, 37), farm bulk tanks (14, 19, 29, 42), and milk metering devices (17). Chen and Bates (3) in determining the causes and practical

measures of preventing the development of rancidity in herd milk produced by pipeline milker system, studied four types of pipeline installations. They found that foaming was the primary cause of rancidity development. Air leaks, obstructions in the system, position of valves, non-flush type gaskets, long pipeline, risers and centrifugal pumps were found to be the cause of foaming. Likewise, Jokay and Jensen (20), in a study of pipeline installations, found that excessive air intake was the number one cause of rancidity. Lines of 200 feet in length with moderate riser heights were used without causing significant increase in the acid degree value, provided that air intake and milk flow were properly regulated. Olson et al. (29) found that agitation with little foam formation caused only a small rise in the acid degree value after agitation and after 24 hours storage at 35 F. With agitation and extensive foam formation there was a larger increase in acid degree value after agitation, and after 24 hours storage at 35 F the milk had a very high acid degree value and was considered very rancid and unacceptable.

Irvin (14) found that by shortening a pipeline, altering its slope from one and one-half inches per ten feet to one inch per ten feet and removing the risers from the pipeline, rancid flavor in the milk was eliminated and the acid degree value was reduced. Likewise, Speer et al. (37) found that the greatest single factor contributing to rancidity in milk, when pipelines were used, is the presence of vertical risers in the vacuum line.

The results of a survey by Olson et al. (29) indicated that the bulk tank was not a factor tending to make the milk more susceptible to the development of rancid flavor. However, Johnson et al. (19) found that a delay of thirty minutes after the completion of milking, before turning on the refrigeration in the bulk tank, was effective in lowering fat acid values.

Van DeMark and March (42) studied the effect on lipase activity in milk of various blend temperatures of milk in bulk tanks after adding warm morning milk to cold evening milk. They found no development of rancidity detectable with blend temperatures of 40 F, 50 F, and 60 F, but was apparent after a maximum rise of 70 F.

Jenzen (17) found that a milk metering device, when used, had an effect on the flavor of milk. There was an increase in acid degree values when the devices were used because of additional air entering the milk in the pipelines.

Skean and Overcast (35), after a study of the effect of diluting rancid milk, estimated that as little as one-tenth of a total tank load of milk may, if strongly rancid, cause the entire truck load to be rancid.

Although the findings of the authors cited above do not always concur, it is the general concensus that much of the milk at the present is on the threshold of being rancid by the time it reaches the consumer. In a study of market milk samples, Skean and Overcast (36) found a general presence of high free fatty acid contents which were just below the threshold of detectable rancidity.

Tests for Rancidity

The three most widely used methods of determining the extent of rancidity in milk are: surface tension measurements, measurements of free fatty acids (acid degree), and organoleptic examination.

Herrington (12) and Nielson (28) discussed the shortcomings of the surface tension methods. The most important of which is that the surface tension of milk is influenced more by the high molecular weight fatty acids than by butyric acid, which is the most important in contributing to the rancid flavor. Dunkley (6) reported that variations in procedures for sampling and treatment of samples may cause differences in surface tension.

There have been several methods developed for measuring the free fatty acid content of milk and cream. Measurements of the free fatty acids have the advantage that they yield numerical values, which reveal very small changes in the fat. Johnson and Gould (18) found that the solvent method was superior to the churning method for recovery of pure fatty acids from cream, particularly the lower molecular weight acids, butyric and caproic.

Thomas et al. (41) developed a method which used the Bureau of Dairy Industry (BDI) reagent (a surface active emulsion breaker) for recovering the fat. This test is very useful for laboratories which use the BDI test for fat determination. Frankel and Tarassuk (7) developed a method which uses a mixture of 40 per cent ethyl ether and 60 per cent petroleum ether. They estimated that by using this method 90 per cent of the total fatty acids released from milk fat at any

given degree of hydrolysis can be determined. Harper et al. (9) described a chromatographic method for the rapid quantitative recovery of small amounts of fatty acids from milk and cream by directly separating the acids from the product without prior fractionation.

The organoleptic method is of little value in determining the degree of rancidity in the early stages of its development. Before any change can be detected organoleptically the free fat acidity may increase considerably. However, this method can be of great value when judges express their sensory reactions to flavors or odors in terms of scores, ranks, or the number of samples that are alike or different. Generally, the data obtained by these tests must be analyzed statistically to be of value. Schwartz and Foster (33) considered the organoleptical method of testing foods by a taste panel valuable, to the extent that it succeeds in defining consumer reaction to the food under study.

Boggs and Hanson (1) discussed the factors relating to the reactions to sensory difference tests. They state that to obtain high accuracy, experiments should be designed to: (a) have a minimum of within-sample variation, (b) limit the number of samples and characteristics of each that are judged in one period, (c) submit all the samples for which comparative data are desired at one time, (d) relate experimental samples to control samples, (e) mask all characteristics except the one under consideration, (f) eliminate samples having strong odor or flavor when possible, (g) judge sufficient replicates to show that trends can be repeated or replicate sufficiently that data can be analyzed

statistically. Mitchell (24, 25) found that distracting factors such as noise caused a drop in the sensitivity of tasters and that the tasters give a higher percentage of correct answers between the hours of eleven and twelve-thirty and one-thirty and two-thirty.

Boggs and Hanson (1) discussed four types of sensory difference tests: (a) paired and triangular difference test, (b) dilution test, (c) scoring test, (d) ranking test.

In the triangular taste test described by Roessler et al. (32), the judges are presented three samples identified only by numbers or letters. They are advised that two of the samples are identical and the other is different. The only decision they must make is which sample is different. The authors also present a table listing the number of correct answers necessary, for various numbers of tests, to establish significant difference of the five, one and one-tenth per cent levels of probability.

Carlin et al. (2) used the triangle taste test with a large number of tasters. Hening (11) used a triangle taste test on strawberries in which each sample was given a score. Mahoney et al. (22) used a triangular method where judges were instructed to check the preferred sample or samples to quantify the difference in flavor and indicate whether or not it was objectionable or not objectionable.

Kirkpatric et al. (21) used a dilution technique and a ranked paired comparison method for the selection of a taste panel. The dilution test was designed to determine at what level of concentration the tasters could detect proteolytic off-flavor in non-fat dry milk

and tallowy, storage, and rancid off-flavor in dry whole milk. The ranked paired comparison test was designed to determine if the tasters could rank samples of milk with varying degrees of cooked flavor in the proper order.

Taste panels have proven valuable in determining consumer preference and in evaluating the development or removal of flavors and odors that can be detected organoleptically.

Removal of Off-Flavors

Much work has been done on the removal of "off-flavors" from milk (4, 10, 23, 31, 34, 43). Cotner et al. (4) reported that vacuum treatment alone would improve the flavor of silage, alfalfa and rye off-flavored milks, and that steam injection definitely proved beneficial in removing alfalfa and rye off-flavors from milk.

Shipe (34) reported that the "feedy" flavor could be reduced by steam injection and vacuum treatment. Likewise, Wynn and Brunner (43) found that steam injection vacuum pasteurization was successful in removing the "feedy" or "cowy" flavor components from summer milk. McDowall (23) in discussing the use of the vacreator to remove weedy taints in cream, states that taints which can be detected by the sense of smell can be completely removed with adequate steam distillation treatment in the vacreator, but taints which can only be tasted are generally non-volatile in steam and cannot be removed by vacreator treatment. Hedrick and Trout (10) found that a vacuum treatment with or without steam injection gave a slight improvement in the flavor of normal, good-flavored, high-temperature short-time pasteurized milk.

Roberts (31) studied commercial operations using three types of vacuum machines for the removal of off-flavor in milk. In the first type machine no direct injection of steam was used and pasteurization did not occur. In the second type there was limited direct injection of steam, but pasteurization did not occur. In the third type a considerable excess of steam was injected, and it could be used as a pasteurizer. During the spring season, weekly scorings were made on fluid milk before and after processing. All the machines reduced the intensity of the feed flavors present in the raw milk. As the amount of steam treatment increased the amount of off-flavor removed increased. The third type of machine appeared to be able to handle satisfactorily the average amounts of feed flavor found in raw milk, but maximum steam treatment in this machine did not remove bitterweed, hydrolytic rancidity, or spoiled silage flavors.

In the literature reviewed, no one reported the specific removal of rancid flavor from milk by the use of vacuum treatments with or without steam injection. However, in steam refining of edible oils the free fatty acids with molecular weights below that of myristic acid, which are relatively volatile, are largely removed (15, 26). The principle upon which steam refining operates is that of vacuum steam distillation. This consist of blowing steam through hot oil (350-550 F) under vacuum (one-fourth inch pressure). The total product loss in the distillate generally is less than 0.5 per cent. Milk could not be subjected to such severe heat treatments. However, it appears logical that possibly with a lower operating temperature and larger amount of

distillate removed, the volatile short chain fatty acids which are responsible for the rancid flavor in milk could be removed by distillation.

CHAPTER III

EXPERIMENTAL METHODS

For this study, fresh raw milk at a temperature of 7 to 8 C was obtained from the University of Tennessee Creamery. At the time the sample was obtained the milk fat content was determined by the Babcock method.

Preparation of Sample

In the trials in which milk was treated for comparison by the taste panel, the sample was divided into two portions. The portion that was to be used as the control was heated to 80 C in a water bath to inactivate the lipase system. It was then cooled to 5 C in an ice water bath and stored at this temperature until used.

The second portion was placed in a Waring blender and stirred until the milk reached a temperature of 40 C (five to seven minutes depending upon volume of milk in the blender). The milk was then cooled in an ice water bath to 5 C for two to three hours. The lipase system was then inactivated by heating the milk to 80 C in a water bath. After inactivation the milk was cooled to 5 C in an ice water bath and stored at this temperature until used.

In the trials in which butyric acid was added to milk, the fresh raw milk was heated to 80 C in a water bath and cooled to 5 C in an ice water bath before the addition of the acid. Three milliliters of acid was added to 1,500 milliliters of milk and thoroughly mixed. The sample was stored at 5 C until used.

In the trials using water and butyric acid, distilled de-ionized water was used. Two concentrations were used, one milliliter of acid

per 1,500 milliliters of water, and three milliliters of acid per 1,500 milliliters of water.

Treatment of Samples

The apparatus used for the treatment of samples is shown in Figure 1 with its various parts labelled (A through K). In each trial 500 milliliters of the sample were divided into two equal portions and placed in the apparatus. One portion (250 milliliters), hereafter referred to as the reflux sample, was placed in a 1,000 milliliter distilling flask (A). This was heated with a Glas-col heating mantle (C). The vapors were condensed in a condenser (E) and returned to the flask.

The second portion (250 milliliters), hereafter referred to as the exchange portion, was placed in another 1,000 milliliter distilling flask (B) which was also heated by a Glas-col heating mantle (D). The vapors from this sample were condensed in a condenser (F) and the distillate was collected in a graduated cylinder (G) in a bell jar (K). During treatment, distilled water was continuously added through a 50 milliliter burette (I) to the exchange sample to replace the distillate removed.

The temperature of the samples during treatment ranged from 56 to 64 C and was controlled by the Powerstat (J).

A Duo Seal vacuum pump was used to apply a partial vacuum (23.25 to 25.00 inches) through the bell jar to the entire system. The vacuum was controlled by a valve located at the vacuum gauge (H).

After treatment the samples were brought back to their original volume with distilled water.

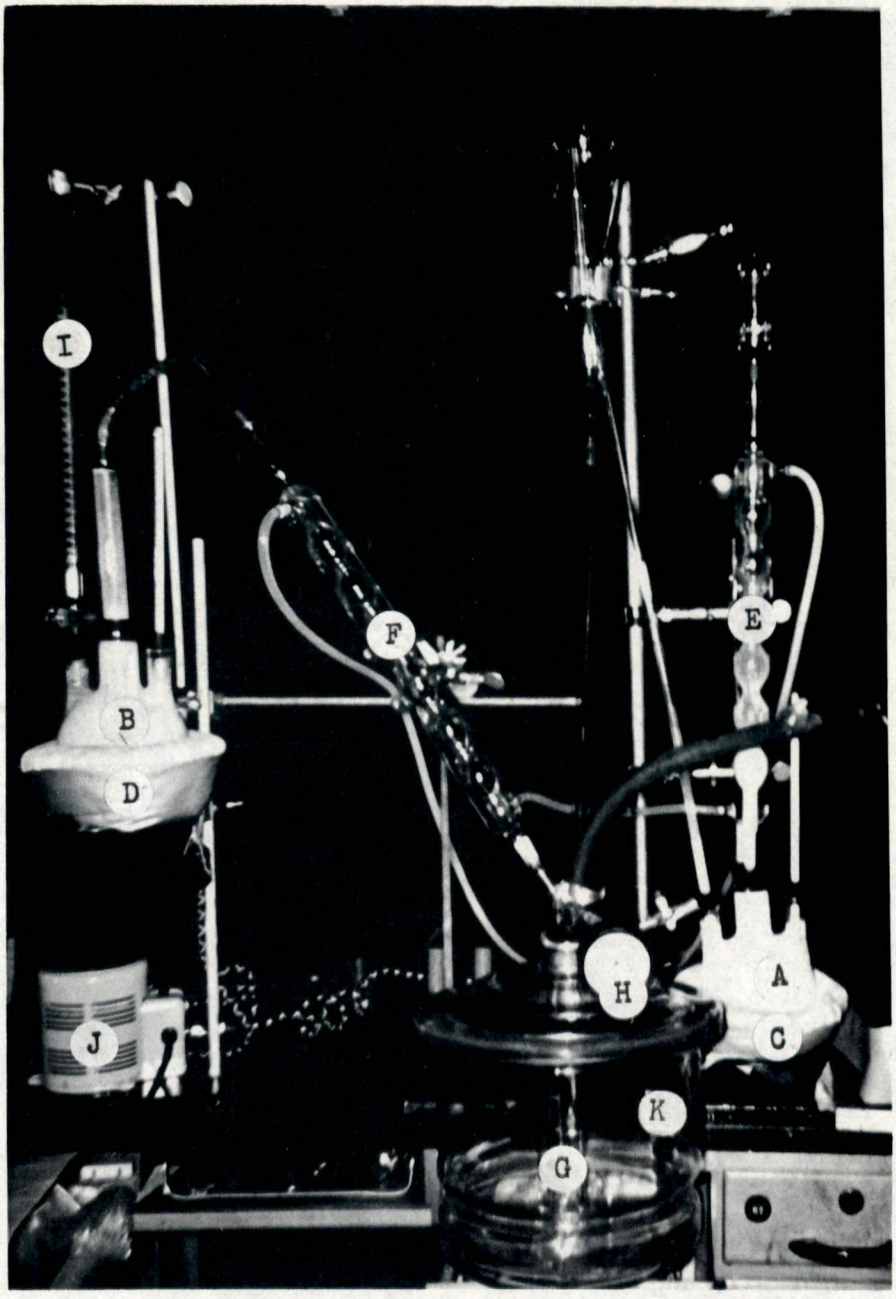


Figure 1. The Apparatus Used for the Reflux and Exchange Treatment of Milk

Foaming was a problem in the normal milk exchange sample. This was controlled by the addition of 5 milliliters of BDI reagent.

Extraction and Titration of Free Fatty Acids

Free fat acidities were determined on all samples before and after treatments. The procedure used on all milk samples was that described by Frankel and Tarassuk (7) with the exception that 0.020 N alcoholic KOH was used. The results are expressed as "free fat acidity" (FFA). FFA is the milliliters of 1 N alkali required to neutralize an ether extract of milk or cream containing 100 g of fat. Fifty milliliters of the distillates were titrated with 0.020 N alcoholic KOH, with the exception of the trials in which butyric acid was added to milk. The distillates of these trials were extracted and titrated using the method of Frankel and Tarassuk (7). The results are expressed as milliliter KOH.

In the trials using water and butyric acid, 1 milliliter of the sample or distillate was added to 10 milliliters of 95 per cent neutralized ethanol and 5 milliliters of the 40 per cent ethyl ether and 60 per cent petroleum ether mixture. Five drops of alcoholic phenolphthalein was added and this was titrated with 0.020 N alcoholic KOH. A 0.2 milliliter burette graduated to 0.001 milliliter was used for all titrations.

Taste Panel

The triangle taste test described by Roessler et al. (32) was used. In each trial nine samples were presented to the panel in three series of three samples. Each series consisted of two samples that were identical and one that was different. One series compared the

normal reflux sample with rancid reflux sample, one compared the rancid reflux sample with the rancid exchange sample, and one compared the rancid exchange sample with the normal reflux sample. The normal exchange sample was not used for the taste panel. The arrangements of the samples were prepared in advance and arrangements randomly picked. No two panels had the same arrangement. The taste panel was asked to select the sample that was different from the other two. The taste panel was made up of students and staff members in the University of Tennessee Dairy Department. Approximately one-half of the panel had no previous experience in judging milk.

CHAPTER IV

RESULTS AND DISCUSSION

The review of literature points out butyric acid as being primarily responsible for the hydrolytic rancidity of milk. Since rancid milk is detectable by smell, it appeared that vacuum treatment could improve the flavor especially if the treatment was designed to remove a portion of the water. Realizing that the boiling point of butyric acid is considerably higher than the temperature employed in milk processing, preliminary trials were conducted to determine if any of the butyric acid could be removed from milk or water. Table I shows the results of six trials using de-ionized distilled water and varying amounts of butyric acid. From these data it was apparent that considerable quantities of butyric acid could be removed by distillation at temperatures employed by milk processors. The amount removed ranged from 76.7 to 95.5 per cent.

A second series was conducted to determine the amount of added butyric acid that could be removed from normal milk. These data are shown in Table II. These results are similar to those obtained using water except that the quantities of butyric acid in the distillate from milk was considerably less than from the water. One explanation of this could be that the butyric acid was bound in some manner in the milk that prevented its distillation. Table II also shows that amounts of butyric acid removed by exchanging 100, 75 and 50 per cent of the water in the milk. These data indicate that sufficient butyric acid was removed that it might be possible to detect a change in the flavor of rancid milk.

TABLE I

TITRATION VALUES FOR DISTILLED DE-IONIZED WATER CONTAINING BUTYRIC ACID BEFORE TREATMENT, AFTER REFLUX TREATMENT, AND AFTER A TREATMENT TO EXCHANGE VARIOUS AMOUNTS OF WATER

Trial	Water Exchanged	Untreated	Reflux	Exchange	Distillate	Mixture Acid:Water
	----%	----------ml KOH[a]----------				----ml----
1	96.3	3.096	3.032	0.891	2.346	3:1500
2	97.2	0.383	0.370	0.142	0.284	1:1500
3	77.9	1.190	1.168	0.420	1.054	3:1500
4	75.6	1.253	1.166	0.470	1.167	3:1500
5	52.7	3.298	3.065	1.489	2.809	3:1500
6	52.7	0.383	0.381	0.181	0.364	1:1500

[a]ml 0.02 N KOH to titrate 1 ml in 10 ml neutralized ethanol and 5 ml of ether mixture.

TABLE II

FREE FAT ACIDITY OF MILK BEFORE AND AFTER THE ADDITION OF BUTYRIC ACID AFTER REFLUX TREATMENT AND AFTER A TREATMENT TO EXCHANGE VARIOUS AMOUNTS OF WATER

Trial	Water Exchanged	Normal Milk	Milk Plus Acid	Reflux	Exchanged	Distillate
	----%----	----------FFA----------				--ml KOH[a]-
7	95.2	2.400	14.096	12.688	10.560	0.861
8	95.2	2.448	15.696	14.608	11.344	0.828
Mean	95.2	2.424	14.896	13.648	10.952	0.845
Difference			+12.472	-1.248	-2.696	
9	76.6	2.208	15.120	14.880	12.480	0.894
10	76.6	2.624	16.448	16.112	13.392	0.837
Mean	76.6	2.416	15.784	15.496	12.936	.866
Difference			+13.368	-.288	-2.560	
11	55.0	2.640	14.776	13.644	11.554	0.756
12	57.3	2.640	14.554	13.160	11.108	0.714
Mean	56.2	2.640	14.665	13.402	11.331	.735
Difference			+12.025	-1.263	-2.071	

[a] ml 0.02 N KOH necessary to titrate the free fatty acids extracted from 10 ml of the distillate.

The results of the first trials with rancid milk (in which 100 per cent of the water was exchanged) are presented in Table III. The time of treatment ranged from 34 to 39 minutes, the temperature from 58.0 to 62.5 C, the vacuum from 23.50 to 25.00 inches, while the per cent water exchanged ranged from 87.1 to 103.2. The distillate from the rancid exchange sample required an additional 0.052 milliliters of 0.02 N KOH (above that required for the normal distillate) in order to neutralize the additional acid that was distilled over. This difference was not sufficiently great to enable the taste panel to distinguish between rancid reflux and rancid exchange samples but was great enough that the panel could not distinguish between the normal reflux and the rancid exchange sample. These data are presented in Table IV and the statistical analyses are based on the probability in triangular taste tests of Roessler et al. (32).

The results of the trials in which 75 per cent of the water was exchanged in rancid milk are presented in Table V. The time of treatment ranged from 29 to 36 minutes, the temperature from 57.0 to 61.0 C, and the vacuum from 23.50 to 25.00 inches, while the per cent water exchanged ranged from 75.6 to 81.2. At this level the distillate from the rancid exchange sample required an additional 0.036 milliliters of 0.02 N KOH (above that required for normal distillate) to neutralize the additional acid that was distilled over. This difference was not great enough to enable the taste panel to distinguish between the rancid reflux and the rancid exchange sample nor was it great enough to prevent them from distinguishing between the normal reflux and rancid exchange sample. These data are presented in Table VI.

TABLE III

FREE FAT ACIDITY OF NORMAL AND RANCID MILK BEFORE TREATMENT, AFTER REFLUX TREATMENT AND AFTER TREATMENT TO EXCHANGE 100 PER CENT OF THE WATER

Trial	Normal				Rancid			
	Untreated	Reflux	Exchange	Distillate	Untreated	Reflux	Exchange	Distillate
	FFA			--ml KOH[a]--	FFA			--ml KOH[a]--
13	3.240	2.126	1.903	0.132	12.892	13.119	12.827	0.210
14	3.240	2.126	1.903	0.132	12.720	13.006	12.800	0.189
15	3.008	2.745	2.104	0.120	11.392	11.803	11.227	0.170
16	2.079	2.638	1.825	0.133	12.033	11.301	9.863	0.175
17	2.335	2.258	1.962	0.116	11.222	10.800	9.973	0.147
Mean	2.780	2.379	1.939	0.126	12.052	12.006	11.338	0.178
Difference		-.401	-.640			-.046	-.668	

[a]ml of 0.02 N KOH necessary to titrate 50 ml of the distillate

TABLE IV

SELECTION OF THE DIFFERENT SAMPLE BY THE TASTE PANEL
AFTER EXCHANGE OF 100 PER CENT OF THE WATER

	Normal Reflux vs Rancid Reflux		Rancid Reflux vs Rancid Exchange		Normal Reflux vs Rancid Exchange	
Trial	Correct	Incorrect	Correct	Incorrect	Correct	Incorrect
13	4	1	3	2	2	3
14	3	2	4	1	2	3
15	6	1	2	5	4	3
16	4	3	2	5	4	3
17	5	3	4	4	1	7
Total	22[a]	10	15[b]	17	13[b]	19

[a] $P<0.05$

[b] Not significant $P=0.05$

TABLE V

FREE FAT ACIDITY OF NORMAL AND RANCID MILK BEFORE TREATMENT, AFTER REFLUX TREATMENT AND AFTER A TREATMENT TO EXCHANGE 75 PER CENT OF THE WATER

Trial	Normal				Rancid			
	Untreated	Reflux	Exchange	Distillate	Untreated	Reflux	Exchange	Distillate
	----------FFA----------			-ml KOH[a]-	----------FFA----------			-ml KOH[a]-
18	4.480	3.064	2.448	0.124	11.216	11.256	9.808	0.176
19	3.257	2.674	1.971	0.089	12.720	12.377	11.845	0.134
20	4.509	3.188	3.000	0.123	12.480	12.274	12.069	0.077
21	2.353	1.705	1.453	0.087	11.874	11.684	11.321	0.176
22	1.911	1.721	1.711	0.104	12.316	12.016	11.463	0.140
Mean	3.302	2.470	2.117	0.105	12.121	11.921	11.301	0.141
Difference		-.832	-.353			-.200	-.620	

[a] ml of 0.02 N KOH necessary to titrate 50 ml of the distillate

TABLE VI

SELECTION OF THE DIFFERENT SAMPLE BY THE TASTE PANEL AFTER EXCHANGE OF 75 PER CENT OF THE WATER

	Normal Reflux vs Rancid Reflux		Rancid Reflux vs Rancid Exchange		Normal Reflux vs Rancid Exchange	
Trial	Correct	Incorrect	Correct	Incorrect	Correct	Incorrect
18	5	2	1	6	3	4
19	6	2	6	2	6	2
20	3	5	2	6	4	4
21	4	4	4	4	6	2
22	7	1	1	7	6	2
Total	25[a]	14	14[b]	25	25[a]	14

[a] $P < 0.05$

[b] Not significant $P = 0.05$

The results of the trials in which 50 per cent of the water was exchanged in rancid milk are presented in Table VII. At this level the time of treatment ranged from 20 to 22 minutes, the temperature from 58.0 to 62.0 C, and the vacuum from 23.25 to 24.00 inches, while the amount of water exchanged ranged from 50.4 to 59.6 per cent. The distillate from the rancid exchange sample required and additional 0.023 milliliters of 0.02 N KOH (above that required by the normal distillate) in order to neutralize the additional acid that was distilled over.

The data presented in Table VIII indicate that this difference was not great enough to prevent the panel from distinguishing between the normal reflux and rancid exchange sample. However, they were able to distinguish between the rancid reflux and rancid exchange sample.

In summarizing the data presented, it was definitely established that, when added to distilled de-ionized water or normal milk, butyric acid could be removed in the distillate at temperatures employed by milk processors. The results also indicate that there is a decrease in the FFA of normal and rancid milk caused by refluxing and a greater decrease caused by the exchange treatment. The reason for the decrease caused by refluxing is not apparent. One possible explanation may be that the volatile free fatty acids were not condensed and were removed by the vacuum pump. Another possible explanation may be that they were condensed but adhered to the side of the condenser and were not returned to the flask.

The difference in untreated and exchanged samples can be explained by the partial removal of the free fatty acids in the distillate. There

TABLE VII

FREE FAT ACIDITY OF NORMAL AND RANCID MILK BEFORE TREATMENT, AFTER REFLUX TREATMENT AND AFTER A TREATMENT TO EXCHANGE 50 PER CENT OF THE WATER

Trial	Normal				Rancid			
	Untreated	Reflux	Exchange	Distillate	Untreated	Reflux	Exchange	Distillate
	FFA			ml KOH[a]	FFA			ml KOH[a]
23	5.621	4.342	4.326	0.122	12.624	13.042	12.111	0.130
24	5.321	4.373	4.342	0.108	12.205	11.968	11.195	0.136
25	4.080	4.144	3.256	0.122	11.112	10.304	6.432	0.147
26	2.200	1.775	1.517	0.105	11.592	10.600	10.367	0.144
27	2.175	1.667	1.517	0.123	11.850	11.308	11.742	0.139
Mean	3.879	3.260	2.992	0.116	11.877	11.444	10.315	0.139
Difference		-.619	-.268			-.443	-1.139	

[a] ml 0.02 N KOH necessary to titrate 50 ml of the distillate

TABLE VIII

SELECTION OF THE DIFFERENT SAMPLE BY THE TASTE PANEL AFTER EXCHANGE OF 50 PER CENT OF THE WATER

Trial	Normal Reflux vs Rancid Reflux		Rancid Reflux vs Rancid Exchange		Normal Reflux vs Rancid Exchange	
	Correct	Incorrect	Correct	Incorrect	Correct	Incorrect
23	4	3	4	3	6	1
24	1	6	5	2	3	4
25	6	1	4	3	6	1
26	5	2	4	3	6	1
27	3	4	4	3	7	0
Total	19[a]	16	21[a]	14	25[a]	5

[a] $P < 0.05$

was a greater titration value for the distillate of the rancid milk than for the distillation of the normal milk at all three levels, indicating that more free fatty acids were removed from rancid than from normal milk. As the per cent water exchanged increased, the titration value of the distillates of the rancid samples increased, indicating a larger amount of free fatty acids were removed.

At the 100 per cent level (Table IV), the taste panel was able to determine the normal reflux from the rancid reflux milk ($P<0.05$). However, they were unable to determine the rancid reflux from the rancid exchange or the normal reflux from the rancid exchange ($P>0.05$). If there had been one more correct answer in the rancid reflux vs rancid exchange series, the difference would have been significant ($P = 0.05$) according to Roessler et al. (32).

At the 75 per cent level (Table VI), there was a significant difference in the normal reflux and rancid reflux sample, and a significant difference in the normal reflux and rancid exchange samples ($P<0.05$). There was no significant difference in the rancid reflux and rancid exchange sample ($P>0.05$).

At the 50 per cent level (Table VIII), there was significant difference in the normal reflux and rancid reflux samples, the rancid reflux and rancid exchange samples, and the normal reflux and rancid exchange samples ($P<0.05$).

Although this taste panel was unable to detect rancid reflux from rancid exchange samples at the 100 per cent level, there was an indication that a sufficient amount of free fatty acids was removed to

alter the flavor to the extent that they could not detect rancid exchange from the normal reflux samples. However, the inexperience and inability to detect the rancid flavor by some members of this panel may have altered the results. Since the reliability of the results increases with the number of samples tasted, an increase in the number of samples may have altered the results. A need for further study, possibly using a taste panel of trained milk judges which would score the samples, is indicated.

CHAPTER V

SUMMARY AND CONCLUSIONS

An attempt was made to remove the free fatty acids (primarily butyric acid), responsible for the flavor of rancid milk, by a treatment which would exchange various amounts of water in milk at temperatures normally used by milk processors.

To confirm that butyric acid could be removed by this treatment trials were made in which butyric acid was added to distilled de-ionized water. After treatment the major portion of the butyric acid was found in the distillate. Trials were also made in which butyric acid was added to milk. The amount of acid removed in the distillate from the milk was not as great as that in the trials using water.

The FFA of rancid milk was reduced by the treatment at all levels of exchange and the free fatty acids were detectable in the distillate. As the amount of water exchanged increased, the titration value of the distillates increased.

The taste panel was able to detect a difference ($P < 0.05$) in the normal reflux and rancid exchange samples at the 50 and 75 per cent exchange level but was unable to detect a difference ($P \geq 0.05$) between the normal reflux and rancid exchange sample at 100 per cent exchange level.

The results indicate that some of the free fatty acids responsible for rancid flavor can be removed by a process which will exchange a portion of the water in the milk, and that possibly at the 100 per cent level of exchange enough may be removed to alter the flavor of the milk.

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