THE EFFECT OF VARIOUS FACTORS ON THE INCIDENCE AND INTENSITY OF RANCID FLAVOR IN RAW MILK HANDLED IN BULK TANKS

By

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INTRODUCTION

In 1936 - 38 the farm bulk milk cooling tank began to be widely used across the United States. The first bulk tank in Oklahoma was installed in 1952 and since that time the use of these tanks has increased almost as rapidly as installation personnel can supply them. Milk processing plants are being reorganized to accommodate new bulk milk deliveries, and currently (1957) three Oklahoma processing plants are receiving 100% of their raw milk supply from producers using bulk cooling tanks.

Concurrent with the increased use of bulk tanks has been an increase in the incidence of rancidity in milk handled by this system. Since there is some disagreement as to the causes of rancidity in milk handled with bulk tank systems, there is a need for additional information on the influence of various factors on the development of this defect.

The work herein reported was undertaken to determine (1) the incidence of rancidity in a milkshed area using bulk milk tanks and every-other-day pickup, and (2) to determine if there is any correlation between rancidity and the equipment or methods used in handling the milk.

REVIEW OF LITERATURE

The short chain fatty acids developed in milk are responsible for the offensive odor of rancid milk (11). Davis (2) stated that these short chain volatile acids represent less than 5% of the titratable fatty acids liberated from butterfat by lipolytic hydrolysis. Since the rancid odor is much more apparent when the milk is hot (11), the odor may escape notice if the milk is examined when it is cold. The odor of rancid milk is not always the same. Sometimes the odor of butyric acid is easily recognized, but sometimes the odor is best described as dirty, or as goaty, which would mean that acids of 6, 8, and 10 carbon atoms are dominate.

In addition to the rancid odor, the milk may have a bitter taste. This aspect of rancid milk is not always observed. Some people miss this bitter flavor because their threshold for identification of this characteristic is too high.

Herrington (11) described a third characteristic of rancid flavored milk as a "feel". This develops slowly, leaving a puckering sensation in the mouth and an irritation in the throat. An extremely rancid milk sample could cause coughing, but not until after a short time had elapsed after tasting.

Rancidity in milk is caused by an entity believed to be an enzyme, and this substance is called lipase (2, 6, 11, 16, 17, 19, 23, 25, 30, 31, 34). Lipase will act as a catalyst under certain conditions to cause the hydrolysis of butterfat. Lipase has never been isolated from milk or prepared synthetically. In 1956 Frankel and Tarassuk (6) reported what they considered to be preliminary evidence of the presence of multiple lipolytic enzymes in milk. This confirmed a similar report by Herrington and Krukowsky (14) that in some milk a formaldehyde-tolerant lipase predominated, while in other milk, samples of the formaldehyde-sensitive lipase was more important. Most all samples contained both enzyme systems.

Pfeffer and co-workers (30) reported earlier that the lipolytic factor in milk is carried in the serum. They noted that as the fat content of cream was increased the lipase activity decreased. Also that separator bowl slime contained three times the lipolytic activity of the original milk.

One of the earliest methods of extracting fat to determine the amount of free fatty acids was to churn the milk and use the butter granules (18). With this method most of the water-soluble acids are lost. Another method is to extract the fat with solvents. This method recovers part of the water-soluble acids, but not all. Fat prepared in this way shows approximately 30% greater acidity when titrated than fat prepared by churning.

Thomas <u>et al</u>. (34) described a method of recovering fat by using a surface-active emulsion breaker. They reported that this method gave the most reproducible and practical results for large numbers of samples in the study of rancidity.

Breazeale and Bird (1) made a study of the various methods for the determination of acidity in butterfat and concluded that a 10 gram sample of fat in an alcohol-ether solution and titrated with alcoholic 0.05 N KOH, using phenolyphthalein solution as the indicator,

was the best method. The Thomas <u>et al</u>. (34) method used basically this same routine but used only a 1 ml sample of fat. Early investigators (18, 19) indicated the need for a re-evaluation of the data presented where the fat had been extracted by churning.

Harper <u>et al</u>. (9) have presented a method for a more complete recovery of free fatty acids from homogenized milk. They used the basic solvent extraction process with an increased amount of solvent being used for recovery.

The organoleptic method of judging milk samples is considered by most workers (1, 5, 6, 7, 9, 11, 25, 34, 35) to be a crude method for scoring the degree of rancidity. The threshold of rancidity perception is so high that it is of little value in research work where a small amount of free fatty acid must be measured. Krukovsky and Herrington (25) reported on organoleptic scoring and compared their results with the acid degree of the same samples. They found the perception threshold of two trained judges to be an acid degree of 0.80. The fat was extracted by churning and melting the resultant butter. Beazeale and Bird (1) have shown that different methods of extracting fat will give different "acid degrees", depending upon the efficiency of the method being used to retain the water soluble fatty acids.

Thomas <u>et al</u>. (34) report a threshold of rancidity to be an acid degree value of 1.3 for one judge who criticized the samples as "very slightly rancid". The second judge began rancidity detection at an acid degree value of 1.6 with a criticism of "slight rancid". The first "definitely rancid" criticism was reported at an acid degree value of 1.7.

Much research has been centered around the cow in the quest for information about rancidity. Several investigators (14, 30, 31, 33) have studied the lactation period as a factor in lipase activity. They concluded that there was no correlation between the rate of lipolysis and the stage of lactation. One report (26) stated that apparently all milk is capable of appreciable true lipolytic activity if subjected to suitable activating treatment. Some cows, particularly those in advanced lactation in winter months, may secrete milk which when cooled and held will show natural lipolytic activity.

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Fredeen <u>et al</u>. (8) showed that the susceptibility of milk to spontaneous rancidity varies with the individuality of the cow, her stage of lactation, and the season of the year. Advanced lactation, not advanced gestation, appeared to be the factor which contributed to the increase in rancidity toward the end of lactation. The results following injections of a mixture of pituitrin and stilbestrol were inconsistent.

Herrington and Krukovsky (13) showed that the acid degree values for freshly drawn milk from individual cows ranged from 0.25 to 0.35. After holding for 24 hours at 0° to 5° C. the values ranged from 0.4 to 2.4 on these same samples of milk. The major share of this acidity developed in the first 12 hours, but the peak value was not reached until the fourth day.

Thomas <u>et al</u>. (33) analyzed the various portions of a single milking and reported that the first portion (400 ml) drawn from the udder developed a higher fatty acid value than the second portion (85% of the total production), or the third portion (500 ml). The second and third portions were about equal in fatty acid value. They (33) also reported no correlation between fatty acid values and the breeds of cows.

Dodd and Foot (4) made the observation that cows that are hereditarily "fast milkers" are also producers of milk that is more susceptible to becoming rancid.

Pfeffer and co-workers (30) reported a direct relationship between the amount of milk produced and lipase activity. Less activity was observed when milkings were 10 pounds or less than when the milkings exceeded 15 pounds. They also reported lipase activity of milk from individual cows did not vary to any great extent from day to day. They also noted a decrease in lipase activity when cows were changed from pasture to dry lot feed. This decreased activity returned to normal activity within two weeks. The same reduction in activity was noted when cows were changed from dry lot feed to pasture. Here too, the lipase activity returned to normal within two weeks.

Homogenization of raw milk is an effective means of activating lipase (11). The violent shaking of warm raw milk will also activate lipase but to a lesser degree than homogenization (26). Milk churned for 15 minutes at 86° F. exhibited a fat dispersion roughly equal to 20% homogenized milk. It was concluded that the increase in the fat-aqueous interface due to fat dispersion may explain in part the mechanism of increased lipase action in agitated milk (10).

Herrington and Krukovsky (13) reported that milk cooled by cold air or cold water bath with moderate agitation had less lipase activity than milk cooled over a tubular cooler. Later investigators (11, 23, 31) reported that the amount of lipase activity induced by agitation is not determined by violence alone. The temperature of the milk and the presence of air in conjunction with agitation is important in the development of increased lipase activity.

However, Roahen and Sommer (31) reported that the developed fatty acid values of gravity separated cream was greater than that of centrifugally separated cream of the same fat content.

The different seasons of the year have a very definite effect upon the lipase activity in milk (17, 35). These investigators reported that lipase activity in the milk was at a maximum in December and January and at a minimum in May and June.

A phenomenon in the study of rancidity is the ability to alter the speed of enzymatic hydrolysis by fluctuation in temperatures (24). If the milk is cooled to 4° C. and then warmed to 30° C. and recooled to 4° C. the lipase activity is invariably accelerated. If the milk is taken through the same cycle with a warming temperature of 37° C. the lipase activity is reduced. Another cycle of warming to 30° C. will again speed up the hydrolysis. This increase in activity obtained by warming after cooling is practically instantaneous, but the amount of activation which can be produced by warming is related to the original activity of the milk (24). Milk originally low in lipolytic activity gets less of a boost.

The temperature to which milk is precooled is of greater importance than is the time held at that temperature before warming to activate the lipase (24). The rate of lipase action is influenced by the rate of cooling, being retarded by sufficiently rapid cooling. This rapid cooling is even more effective in

activated milk than in natural milk. The critical range through which milk must be cooled rapidly to retard lipolysis extends downward from 20° C. to approximately 0° C. for natural milk and 25° C. down to 10° C. for activated milk.

Comparisons of speed of cooling (15) have shown four times the amount of fatty acids developed in milk that took twenty five minutes to cool to 5° C. standing in an ice water bath compared to a duplicate milk sample cooled in one minute and fourty-two seconds through an iced aluminum coil. Another sample that took two hours and twenty minutes to cool in air without agitation developed three times the amount of free fatty acid as the very rapidly cooled sample.

The surface tension of milk has been used as an indication of rancidity. DeMott and Brandt (3) reported a correlation coefficient of 0.44 between surface tension values of 90 samples and the degree of rancidity as measured by taste. They found that the surface tension values became lower with increases in: length of storage period, milking vacuum level used, period of exposure to treatment in the milker, and volume of air bubbled through the milk. They also reported that the fat content of milk had little, if any, effect on the surface tension values.

Rancidity Wasderldow detected in milk samples with a surface tension above 46 dynes per centimeter, but was evident in most samples with values below 45 dynes (5).

Davis (2), in 1932, reported that the depression of lipase activity by copper, iron, nickel, cobalt, manganese, and chromium (in order of potency) varied directly with their catalytic powers

to induce fat oxidation later. It was suggested that destruction of lipase was catalysed by the traces of heavy metals according to their varying powers of activating oxygen. Lipase as a substance naturally associated with fats might thus be looked upon as a natural "antioxygen".

Krukovsky and Sharp (28) followed this theme and reported in 1940 that dissolved copper showed no inactivation of lipase in normal milk in the absence of dissolved oxygen. Normal milk lipase is susceptible to inactivation by dissolved oxygen, and this inactivation reaction is accelerated by heat and dissolved copper. Milk lipase was inactivated at:

135° F. in normal milk without copper.
125° F. in normal milk with added copper.
145° F. in deaerated milk without copper.
135° F. in deaerated milk with added copper.

The use of 0.2 - 0.4 ppm of copper in the presence of oxygen reduced lipase activity by about 20% (13). The addition of formalin to raw milk samples will cause a varying amount of reduction in lipolysis (31).

Through a series of emperiments Krukovsky and Sharp (27) have gathered much information related to fat properties and their relation to rancidity. One result is that the total lipolytic action increases with the fat content up to 35 - 45% butter fat, but the acidity per unit of fat and the acidity per unit of plasma increases with the fat content only up to 8 - 10% butter fat, and then remains constant or decreases. They also discovered in using crystalline fractions of fat separated from milk, that the lower the temperature required for a fat fraction, the greater was the increase in acidity when the crystalline fractions were used as a substrate for milk lipase. This indicated that the rate of lipolysis is dependent upon the melting point of the fat or upon the degree of solidification of a fat at a given temperature.

Working with crystalline fat returned to globular form by homogenization they also found that the rate of lipolysis of resurfaced fat globules increased with increasing temperature of holding, whereas, the rate of lipolysis of fat globules with the original normal surface increased as the temperature of holding is lowered.

Previous work (10, 13, 26, 31) with milk has shown that agitation will accelerate lipolysis. The supposition that the normal stirring or agitation in a bulk milk tank could accelerate the action of lipase was attacked and disclaimed by several separate workers (20, 29). The mixing of warm milk with previously cooled milk during milking does not raise the temperature enough to be a factor in rancidity development (29).

The principal offense in the cause of rancidity in pipeline installations is excessive air intake (22, 23), especially from leaks in lines due to faulty fittings or improperly placed gaskets. Kelley and Dunkley (23) found that the chief causes of rancidity in bulk milk are: (1) low milk flow-rate in a line of excessive pitch, (2) elevation of warm milk under vacuum with air bubbling through it, (3) inclusion of a filter and numerous fittings in the vacuum section of the milk line and (4) continuous operation of a starved milk pump.

Jokay and Jensen (21, 22) found that the "fatty acid degree values" increased with the increase in the length of line and the increase in the number and height of risers. Their results support previous studies (23) that have shown rancidity to be initiated principally by conditions that cause foaming of milk when the fat is in the liquid state. They also warn that vibrations of milk

lines during in-place cleaning may loosen joints and cause air leaks. Irregular and especially high acid degree values in milk samples at various points of sampling are indications of air leaks.

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EXPERIMENTAL METHODS

A. COLLECTION OF MILK SAMPLES

Samples from Individual Cows. Two 4 ounce samples of milk 1. were taken from each milking of several cows in the Oklahoma State University herds. The cows in the Research herd (herd A) were milked with a De Laval pipeline milker in which the milk traveled approximately 19 feet from the cow to a vacuum-type bulk cooling tank. There was a 5 foot rise from the milk holder or weigh pail to a glass pipeline. One sample was taken from the weigh pail. The second sample was obtained from a 10 gallon stainless steel milk can with a vacuum fitted lid attached to the end of the line in place of the bulk tank. After each cow's milk had been drawn through the line, the vacuum was released on the can, the sample taken, and the remainder of the milk then emptied into the bulk tank for cooling without vacuum A composite sample was taken from the bulk tank at the completion of the milking.

The cows in the University Main herd (herd B) were milked with a De Laval pipeline milker in which the milk traveled approximately 14 feet from the nearest milking station to the sampling point near the bulk tank. This distance included a 5 foot 6 inch riser. The next three milking stations were at 8 foot intervals along a level stainless steel sanitary line. One sample was taken from the weigh pail or milk holder. The second sample was obtained

by a trap at a point near the bulk tank. The trap was devised by installing a tee in the sanitary line. A sanitary valve was fastened to the bottom of the tee, then a 10 inch line fastened below the valve with another sanitary valve on the end of this line. To operate the trap, the bottom valve was closed and the top valve opened to allow milk to flow into the trap. The top valve was then closed and the bottom valve opened to release the sample. Two samples were trapped from each cow's milk. The first, used to flush out any remains of the previous cow's milk, was discarded. A composite sample, representative of the milk from the cows selected, was prepared by taking aliquot amounts of each cow's sample according to the weight of milk produced.

One 4 ounce sample of milk was taken from the morning milking of all the cows in a private producer's herd in the Tulsa milkshed area (herd C). The samples were taken from the milk holder or weigh pail at the completion of the milking of each cow. The samples were promptly iced and delivered to the University laboratory for analysis.

2. <u>Samples from Producers' Bulk Tanks</u>. The milk samples from the individual producers' bulk tanks were collected by the milk tank drivers at the time of pick-up of the milk every other day. Portions of these samples were used for butterfat tests and the remainder of each sample was transferred to half pint bottles, labeled, and transported to the University laboratories for analysis. Because of the cold weather no refrigeration was required.

B. DETERMINATION OF THE ACID DEGREE VALUE OF FAT

The acid degree value of the fat was determined by the method described by Thomas (34) with some slight variations. This method

was used because it gave a numerical value which was reproducible and which revealed small variations in the acid degree value below the threshold of organoleptic detection. Briefly the method used was as follows: A 35 ml portion of a milk sample was placed in an 18 gram 30% Babcock test bottle by using a calibrated 50 ml glass syringe. Ten ml of BDI reagent (32) (30 grams of Triton X-100 and 70 grams of sodium tetraphosphate made up to 1 liter with distilled water) was added and the mixture agitated thoroughly. The bottle and contents were then placed in a boiling water bath. The contents of the bottle were mixed by shaking after 5 minutes and again after 10 minutes. After a total of 15 minutes in the boiling water bath the samples were centrifuged for one minute and sufficient 50% aqueous methanol added to bring the fat into the neck of the test bottle. The bottles were centrifuged for 1 minute, and then tempered in a water bath at 130° - 140° F. for 5 minutes. Using a 1 ml tuberculin syringe and a fine gauge hypodermic needle 1 ml of the clear fat was transferred to a 50 ml Erlenmeyer flask. The 1 ml of fat was dissolved with 10 ml of petroleum ether and 5 ml of absolute ethanol. Ten drops of a 1% solution of phenolphthalein in absolute ethanol was added as the indicator and the total titrated with 0.02 N KOH solution in absolute ethanol to the first definite shade of pink. The results are expressed in terms of "acid degree value" (ml of 1N base required to titrate 100 grams of fat).

Triton X-100 is a nonionic surface-active agent manufactured by the Rohm and Haas Company, Philadelphia, Pennsylvania.

C. ORGANOLEPTIC TESTS

All samples used for organoleptic examination were pasteurized before tasting by standing the bottles containing the samples in a rack suspended in a water bath and heating to approximately 143° F. for 30 minutes. Cold water was then run into the water bath to cool the samples to approximately room temperature. The samples were then coded and offered at random to be examined organoleptically by two experienced dairy products judges and classified as either not rancid, slightly rancid, or rancid. The characteristics of rancidity as described by Herrington (11) were used in this scoring.

D. COLLECTION OF DATA ON PRODUCERS' BULK TANKS

A milk Producers Cooperative Association which operates bulk tank pick-up routes over approximately one-half of the State of Oklahoma was used as the source of the bulk tank data presented in this report. Since time did not permit a visit to each bulk tank patron a questionnaire (see page 16) was given to each individual producer by the bulk tank pick-up driver. The drivers assisted the producers in completing their questionnaires and then returned the forms to the Association office.

The Producers Association field man checked all the reports to substantiate the data concerning each patron's milking system. Where a doubt existed as to the accuracy of the report a letter was mailed to the patron who would then answer the same question worded at greater length. The reports of all respondents used in this part of the survey were so verified.

BULK TANK STUDY

Producer
Brand of bulk milk tank being used
Approximate time bulk tank has been in operation
<u>Two</u> days milk production <u>in pounds</u>
Number of cows being milked
Uses pipeline system Yes No (Check one)
Please check the system you use <u>if you use a pipe line</u> :
Direct cow to tank through a vacuum release system above milk tank.
Direct cow to tank using pump in the pipe line.
Direct cow to vacuum tank.

Do you use a weigh can in the system checked above? Yes ____ No ____

Note: Lines 4 and 5 are asked so that we can compute the pounds produced per cow.

To supplement the information obtained by the questionnaires, it was decided to determine the types of milk pumps used by those respondents who indicated milk pumps in their sanitary lines. The Producers Association fieldman, assisted by a salesman familiar with all the bulk tank systems, was able to classify the milk pumps as either centrifugal or diaphragm type. Letters were written to the patrons when there were any doubts of agreement between the two men.

RESULTS AND DISCUSSION

A. CORRELATION BETWEEN CHEMICAL AND ORGANOLEPTIC TESTS FOR DETERMINING RANCIDITY

It is known that the ability to detect off flavors in foods varies between individuals. A measurement of the smallest amount of a particular flavor that a person can detect is called his "threshold" for that particular flavor. Since the "threshold" for rancidity will vary between individuals, this part of the study was undertaken to establish the relationship between the acid degree values of the fat and rancidity in milk as determined organoleptically. This relationship could then be used in practical application by the dairy technician to follow the acid degree value pattern in raw milk supplies to forecast the presence or absence of rancidity in the finished bottled product.

Based upon the organoleptic observations, the 286 samples may be divided into four general groups, in order of acid degree values. The results are shown in Table I. Group A was composed of 172 samples, none of which was rancid. The acid degree values were less than 1.50. In group B there were 66 samples, 8 of which were criticized as slightly rancid. The acid degree values ranged from 1.51 to 2.00. In group C there were 6 slightly rancid and 12 rancid samples among the 25. This was a total of 18 out of the 25 or 72% that were rancid in some degree. The acid degree values ranged from 2.01 to 2.50. In group D there were 23 samples, of which one was

COMPARISION OF ORGANOLEPTIC AND CHEMICAL METHODS FOR

Range of Acid Degree	Number of	Orga	noleptic Pl	lacing
Values	Samples	Not	Slightly	
	Na se	Rancid	Rancid	Rancid
	CDOIN			
	GĶOU.	r A 1		
0.41 = 0.50		1	•	6
0.51 = 0.60	5	5	-	
0.61 = 0.70	7	7	. 😅	-
0.71 = 0.80	5	5		5
0.81 - 0.90	14	14	æ	 .
0.91 - 1.00	2.9	29	12	G
1.01 - 1.10	20	20	•	8
1.11 - 1.20	2,5	25	.ca	-
1.21 - 1.30	20	20	æ	a
1.31 - 1.40	27	27	-	e
1.41 - 1.50	19	19	-	m
	1 70	170		
	1/2	1/2	æ	
<u> </u>	100.0	100.0	م ە ئىسى سەر بىر سەر سەر سەر مەر بىر س ەر مەر مەر مەر مەر مەر مەر مەر مەر مەر م	63
	GROU	РВ		
1.51 - 1.60	22	19	3	
1.61 - 1.70	14	12	2	·
171 = 180	12	10	2	
1 81 = 1 90	10	<u>0</u>	1	
1.01 - 2.00	Ω 10	9	1	
1.91 - 2.00	0	0	Ľ	
Total	66	58	8	0
<u>% of Total</u>	100.0	87.9	12.1	. 0
	GROU	PC		
2.01 - 2.10	5	3	ca	2
2.11 - 2.20	4	-	3	1
2.21 - 2.30	4	-	-	4
2.31 = 2.40	Q	3	2	4
2.41 = 2.50	2	1	1	- - 1
2.71 - 6.JV		*	Ŧ	Ŧ
Total	25	7	6	12
% of Total	100.0	28.0	24.0	48.0

DETERMINING THE INCIDENCE OF RANCIDITY IN MILK

Table I (Continued)

Range of Acid Degree	Number of	Organ	oleptic Pl	acing
Values	Samples	Not	Slightly	
·		Rancid	Rancid	Rancid
* *	GROU	PD		
2.51 - 2.60	2	a	1	1
2.91 - 3.00	1	-	-	1
3.11 - 3.20	1	~	B	1
> 3.80	19	6 0	80	19
Total	23		1	22
<u>% of Total</u>	100.0	a)	4.3	95.7
GRAND TOTAL	286.0	237	15	34
% OF GRAND TOTAL	100.0	82.9	5.2	<u> </u>

slightly rancid and the remainder rancid. The acid degree values for this group ranged from 2.51 to 19.56.

From these data the threshold for the detection of rancidity in milk seems to be at an acid degree value of 2.00. It could be assumed that any milk having an acid degree value of less than 2.00 would not taste rancid and any milk of over 2.00 would probably taste rancid. If all the milk samples in group B had been combined the resultant composite would very likely not have been criticized for rancidity. If the 66 samples of group B had been combined with the 172 samples of group A the resultant composite would represent 238 samples of milk of which only 8 samples were scored slightly rancid. This would be a dilution of almost 40 to 1 and would result in an acid degree value well below the threshold.

It appears that use of raw milk supplies having acid degree values in the range given for group C would not be wise as a mixture of this milk would surely have the rancid defect. However, if the raw milk supplies to a dairy had acid degree values in the same approximate incidence as groups A, B, and C, the resultant mixture of 263 samples would include only 26 samples with rancid criticisms. This is a dilution ratio of 10:1 and most probably the milk would not be criticized for rancidity. If milk of group C quality was to be used, great care would have to be taken to see that proper blending and dilution was accomplished.

The use of milk in group D is not recommended. Since all samples of this group were criticized for rancidity a single accident of grouping milk from several suppliers of this quality could result in loss of business worth far more than the profit derived from the sale of that volume of rancid milk.

B. INCIDENCE OF RANCIDITY IN MILK

1. <u>Milk from Individual Cows</u>. To establish the incidence of rancidity in milk from individual cows, acid degree values were run on milk samples from 123 cows in three herds. The results are shown in Table II. Herd A was composed of 9 Holstein cows getting normal rations except for free access to trench silage of good quality. Samples were taken May 17, June 27, and June 29. All samples used from herd A were taken after the milk had passed through the glass pipeline to the bulk tank. The samples were held 48 hours at 45° F. before the acid degree values were determined. It was found that 9 samples (33.3%) had acid degree values of 1.50 or below (group A), 13 samples (48.2%) had acid degree values between 1.51 and 2.00 (group B), 5 samples (18.5%) had acid degree values between 2.01 and 2.40 (group C). These latter 5 samples are above the threshold for rancidity established earlier in this report. No sample had an acid degree value above 2.40.

Herd B was composed of approximately 125 cows. Six cows each of the Holstein, Jersey, Guernsey, and Ayrshire breeds were selected for this testing. Each group included 2 "high producers", 2 "medium producers", and 2 "low producers" on the basis of the daily milk weights current at the time of sampling. The samples were taken on June 6, at which time the herd was on normal grain rations, slightly limited amounts of good silage, good quality hay, but very short pasture. All samples were taken after the milk had passed through the stainless steel line, which included a 5 foot 6 inch riser. After the samples were held 48 hours at 45° F. the acid degree values were determined. All 24 samples had acid degree values

Range of Acid Degree Values	Herd A 9 Cows Sampled 3 Times	Herd B 24 Cows	Herd C 72 Cows
	GROU	PA	
0.71 - 0.80	-	2	
0.81 - 0.90	-	7	-
0.91 - 1.00		3	2
1.01 - 1.10		6	-
1.11 - 1.20		4	6
1.21 - 1.30	-	2	1
1.31 - 1.40	5		6
1.41 - 1.50	4	-	10
Sub-Total	9	24	25
% of Total	33.3	100.0	34.7
	GROU	PB	
1.51 - 1.60	3	-	4
1.61 - 1.70	4	-	8
1.71 - 1.80	4	- 11 - 11 - 11 - 11 - 11 - 11 - 11 - 1	7
1.81 - 1.90	1	-	4
1.91 - 2.00	1	-	4
Sub-Total	13		27
% of Total	48.2		37.5
	GROU	PC	
2.01 - 2.10		-	
2.11 - 2.20	2	· · · · ·	2
2.21 - 2.30	-	-	2
2.31 - 2.40	3	1	4
2.41 - 2.50	12.2	-	1
Sub-Total	.5		9
% of Total	18.5		12.5

TABLE II

ACID DEGREE VALUES, OF MILK FROM INDIVIDUAL COWS

Table II (Continued)

Range of Acid Degree Values	Herd A 9 Cows Sampled 3 Times	Herd B 24 Cows	Herd C 72 Cows
ander werde wie eine eine eine Andrea von eine Andrea under geweichte Geweichen an der eine Andrea an der eine	GROUE	, D	
2.51 - 2.60	æ	-	3
2.71 - 2.80	=	· •	2
3.21 - 3.30	¢		1
3.31 - 3.40	_	a	1
> 3.40	æ		4
Sub-Total	co	مربع المربع ا هو	11
% of Total	ی میں ایس ایک	00	15.3
Total	27	24	72

* The acid degree value is the ml of 1N base necessary to neutralize the acid in 100 grams of fat.

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between 0.71 and 1.30, well under the limit of 1.50, below which no rancidity could be expected on organoleptic examination.

Herd C was a grade herd of 72 cows showing principally Holstein coloring and markings. In the opinion of the author the cows in the herd exhibited symptoms of malnutrition, being of small statures, narrow frames, poor legs, shallow barrels, and very poor finish. The herdsman stated that the animals had been on an overgrazed pasture "for some time". Samples were taken February 23 from the milk container or weigh pail at the milking stall. After the milk samples had been held 48 hours at 45° F. the acid degree values were determined. It was found that 25 (34.7%) of the 72 samples had acid degree values below 1.50, 27 samples (37.5%) had acid degree values between 1.51 and 2.00, 9 samples (12.5%) had acid degree values between 2.01 and 2.50, and 11 samples (15.3%) had acid degree values above 2.50. It is apparent from these results that the milk from 20 of these 72 cows (27.8%) would be expected to be rancid on organoleptic examination.

Of the three herds used in this investigation only herd B had no previous history of rancidity. Herds A and C were known to be supplying bulk tank milk criticized as rancid to some degree. The milk processor who had been buying the milk from herd C had requested the investigation to determine the amount of rancidity in the milk from among the individual animals.

The acid degree values of the samples from herd B confirmed what was expected, that is, that none of the cows produced milk which was above the threshold for rancidity. The rancid defect in the milk from herd A could probably have been eliminated by excluding the milk

from two of the animals. It was recommended that herd C eliminate the milk from the cows where the samples had shown high acid degree values.

2. <u>Mixed Herd Milk</u>. To establish the incidence of rancidity in mixed herd milk, acid degree values were run on samples taken from 249 herds. Two samples were taken from each herd and the average acid degree values determined. All samples were aged approximately 48 hours at 45° F. before the acid degree values were determined. The results are shown in Table III. Based upon the organoleptic groupings previously determined it was found that 204 (82%) of the herds sampled were in group A with acid degree values of less than 1.50. Group B had 19 samples (7.6%) with acid degree values between 1.51 and 2.00. Groups C had 13 samples (5.2%) with acid degree values between 2.01 and 2.50. Group D had 13 samples (5.2%) with acid degree values greater than 2.51.

It should be remembered that the milk in the bulk tank represented two days production. Since the maximum amount of fat hydrolysis is not reached until approximately the fourth day, the milk samples used to determine the acid degree values were held for this period, (2 days in the bulk tank plus 2 days storage at 45° F.) in order to obtain maximum hydrolysis. The bulk tank pick-up drivers were not required to taste the milk from the tanks; therefore, it is very possible that this accounts for the fact that rancidity of milk on the farm was rarely detected by smelling the cold milk.

The acid degree values presented in these data would probably not be obtained under commercial conditions unless the raw milk was stored for two days beyond the normal every-other-day pick-up during the same season of the year. Since most investigators agree that

Range of Acid Degree Values	e Number of Samples	Range of Acid Degree Values	Number of Samples
G	ROUP A	GROUT	c
0.61 - 0.70	1	2.01 - 2.10	3
0.71 - 0.80	6	2.11 - 2.20	2
0.81 - 0.90	38	2.21 - 2.30	1
0.91 - 1.00	40	2.31 - 2.40	5
1.01 = 1.10	19	2.41 = 2.50	2
1.11 - 1.20	43		
1.21 = 1.30	22	Sub-Total	13
1.31 - 1.40	23	% of Total Herds	5.2
1.41 - 1.50	12		
		GROUT	D
Sub-Total	204	2.51 - 2.60	1
% of Total Herds	82.0	2.61 - 2.70	ī
		2.71 = 2.80	4
G	ROUP B	2.81 - 2.90	2
1.51 - 1.60	7	2.91 - 3.00	
1.61 - 1.70	3	3.01 - 3.10	1
1.71 - 1.80	7	3.11 = 3.20	-
1.81 - 1.90	1. 1. 1. 1. 1. 1.	3.21 = 3.30	4
1.91 - 2.00	î	> - 3.30	4
Sub-Total	19	Sub-Total	13
% of Total Herds	7.6	% of Total Herds	5.2
TOTAL BELOW TUBECUO	1.0. 222	TOTAL ABOUE TURESHOLD	26
W BELOW THRESHO	LD 223	ABOVE THRESHOLD	10 4
6 BELOW INKESHOLD	09.0	& ABUVE THRESHULD	10.4

TABLE III

ACID DEGREE VALUES OF MIXED HERD MILK HANDLED IN BULK TANKS

raw milk exhibits a higher lipase activity in the winter months than at other seasons of the year, it would be unwise to allow a situation to develop in the winter where milk would be stored in the raw state for several days. Fortunately the Oklahoma surplus season is in the late spring and early summer when the raw milk is less prone to develop rancidity.

If a tank truck load of bulk tank milk were rejected by the processor for having a rancid odor it should be remembered that the individual milk sample taken for the butterfat test is large enough to use for determining the acid degree values of the milk from each producer. The test can be completed in approximately 30 minutes if the equipment and reagents are readily available. The individual herds producing the rancid flavored milk could then be identified and action taken to remove the causes of rancidity. It would be better for all concerned if an educational campaign, directed at the causes and prevention of rancidity, were conducted periodically to eliminate the possibility of rancidity ever being a problem in a tank truck load of milk.

C. INFLUENCE OF VACUUM ON THE INCIDENCE AND INTENSITY OF RANCIDITY

The survey of the Oklahoma City milkshed revealed a high incidence of rancidity in the milk from vacuum type bulk cooling tanks. The investigation reported herein was undertaken to determine if exposure to vacuum was a factor in the high incidence of rancidity in milk handled by vacuum type bulk tanks.

Quart samples of the evening milk from a cow known to produce milk susceptible of going rancid was taken from the weigh pail at the

milking stall. One sample was taken March 26 and a second sample 3 days later. Each sample was rushed to the laboratory where it was divided into two portions and the pint samples placed in an ice water bath over magnetic stirrers. A 20 inch vacuum was maintained in one pint sample with a water aspirator attachment. The other pint sample was cooled at atmospheric pressure. The magnetic stirring rods were adjusted to give each sample equal gentle agitation. Thirtyfive ml samples were taken from the two pint samples every thirty minutes for two hours. These 35 ml samples were placed in a 30% Babcock test bottle and rotated gently in the ice water bath to cool the contents very rapidly to approximately 33° F. After cooling,the samples were placed in a 45° F. storage room for approximately 48 hours before their acid degree values were determined. The results are shown in Table IV.

From these investigations it appears that the vacuum does not cause an increase in rancidity. In fact, it appears to cause a lowering of the amount of rancidity developed. In the first sample taken the acid degree values dropped from 3.33 in both the atmospheric pressure and the vacuum samples to 2.66 in both samples after 2 hours exposure to ice water and stirring. Approximately the same drop occurred in the samples taken on the second sampling day. It should be noted that it took 50 - 60 minutes to get the pint sample cooled down to $33 - 34^{\circ}$ F. The 35 ml samples that were taken at intervals were cooled much faster in the thin-walled Babcock test bottles. The sooner the samples were cooled to $33 - 34^{\circ}$ F., the higher the acid degree value after two days holding. This would indicate that the rate of cooling is a factor in the development

TABLE IV

Time of Exposure	Vac (20 i	uum nches)	Atmospheric Pressure	
Minutes	Acid Degree Value	Temperature ^O F.	Acid Degree Value	Temperature ^o F.
0	3 33	88	3 33	88
30	3.33	44	3.44	40
Trial 60	3.33	34	2.77	34
I 90	2.77	34	2.66	34
120	2.66	34	2.66	34
0	80 60 60 60	60 GB	1 1 1 1	ت ت
30	1.56	36	1.78	34
Trial 60	1.11	33	1.45	33
II 90	1.45	33	1.22	33
120	1.00	33	1.11	33

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THE EFFECT OF EXPOSURE TO VACUUM ON THE DEVELOPMENT OF RANCIDITY

of rancidity. However, it should be noted that all 4 of the samples in Table IV which reached $33 - 34^{\circ}$ F. within a 60 minute period apparently showed a decrease in acid degree values during the additional 60 minutes of holding with continued agitation.

The variation in the acid degree values between the two days warrants some discussion. During the course of gathering preliminary data for the work herein reported, it was noted that successive samples of milk from the same cows gave approximately the same acid degree values. Jokay and Jensen (22) reported that fluctuations of acid degree values from a single source to be attributed to air leaks. Since no examination was made of the milking system at the time of collecting the samples, no information is available to prove or disprove the air leaks theory. More investigation is intended along this line.

D. INFLUENCE OF VARIATIONS IN TYPES OF INSTALLATIONS ON INCIDENCE AND INTENSITY OF RANCIDITY

1. <u>Types of Bulk Tanks</u>. The survey of the Oklahoma City milkshed revealed that 10 different groups or brands of bulk milk cooling tanks were in use. To determine if the individual brand of tanks was a factor in the incidence and intensity, the acid degree value data on the milk from the 249 herds were grouped according to brand of tank. The results are shown in Table V.

There were 72 users of Brand A and 63 users of Brand B, the two most popular bulk tanks. There were only 8 samples of milk (5.9%) from these 135 bulk tanks that had acid degree values above the threshold of 2.00 to indicate rancidity. There were 33 users of Brand C tank, and milk samples from 3 tanks (9.1%) had acid degree

TABLE V

INFLUENCE OF BRANDS AND TYPES OF BULK TANKS

	Brand of Tank	Number of Tanks	Average Acid Degree Value	id Samples With Acid ue Degree Values Above		
				Numbers	%	
	Α.	70	1 16		56	
	A. D	72	1.10	. 4	5.0	
	a .	03	1.24	4	0.3	
	C	33	1.25	3	9.1	
	D	28	1.76	9	32.1	
	E	17	1.31	1	5.9	
	F	12	1.34	2	16.7	
	G	11	1.20	œ	87 99 99 44	
	н	5	1.35	1	20.0	
	I	4	1.52	. 1	25.0	
	J	4	1.50	1	25.0	
(
open Type	A, B, C, E, F, G, H, I, J,	221	1.24	17	7.7	
VACUUM TYPE	D	28	1.76	9	32.1	

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ON ACID DEGREE VALUES OF MILK

values above 2.00. There were 28 users of Brand D tank, and milk samples from 9 of these (32.1%) had acid degree values above 2.00. There were 17 or less producers using each of the remaining six brands of tanks. The number of samples of milk having acid degree values above 2.0 varied from 0 - 25.0%, but because of the small numbers involved, the percentages are not considered a true picture.

There were two distinctly different types of construction represented among the 249 tanks. There were 221 tanks of the open top type and 28 of the vacuum type. Seventeen (7.7%) of the 221 samples of milk from the open top tanks had acid degree values above 2.00, while 9 samples (32.1%) from the vacuum type tanks had acid degree values above the threshold for rancidity. These data indicate that there was a much higher incidence of rancidity in milk handled in the vacuum tanks than in the open tanks.

2. <u>Types of Pipeline System</u>. The results of the survey made of the Oklahoma City milkshed indicated that the use of a pipeline milking system might be a factor in the incidence and intensity of rancidity in the milk. The data on the acid degree values of the milk were grouped according to the type of milking system used. The data are shown in Table VI. From the 243 milking systems for which data were obtained, 69 were equipped for pipeline milking and 174 were non-pipeline systems. Of the 174 producers who did not use a pipeline, seven, or 4%, had milk with acid degree values indicating that it could be criticized as rancid. Of these seven producers only one had a rancid sample on both days the samples were taken. The average acid degree value for the 174 milk samples from installations that did not include a pipeline system was 1.14. Nineteen, or

ACID DEGREE VALUES OF MILK HANDLED BY DIFFERENT TYPES OF MILKING SYSTEMS

Types of Milking Systems	Number of Installations Reported	Average Acid Degree Value	Samples Degree V	With Acid alues Above 2.00
•			Numbers	%
Non-Pipeline	174	1.14	7	4.0
Pipeline	69	1.69	19	27.5
PIPELINE SYSTEM USING:				
Vacuum Releaser	23	1.57	5	21.7
Diaphragm Type Pump	15	1.81	4	26.7
Centrifugal Type Pump	11	1.23	1	9.1
Vacuum Tank	20	1.99	9	45.0

TABLE VII

ACID DEGREE VALUES OF MILK FROM DIFFERENT TYPES

OF VACUUM TANK INSTALLATIONS

Type of Installation	Range of Acid Degree Values	Number of Installations	Samples With Acid Degree Values Above 2.00	
			Number	%
Pipeline to				
Tank.	1.09 - 3.66	20	9	45.0
Milk Poured into Tank at Atmospheric				
Pressure.	0.84 - 1.49	5		
Vacuum Hose Used to Empty Milk Container				
into Tank.	1.22 - 1.78	3		
TOTAL		28	9	32.1

27.5% of the 69 samples of milk from pipeline systems had acid degree values above 2.00 and could be considered rancid.

Twenty of the 28 vacuum type tanks were operated with pipeline systems. (See Table VII) Nine, or 45%, of the samples of milk from these 20 systems had high acid degree values which would indicate rancidity. Of the 8 vacuum tanks operated without pipelines, 5 were operated without vacuum and were filled by pouring through a conventional milk strainer. The other 3 tanks were operated under a vacuum and were "vacuum filled" from the milk pail by way of a hose. There were no milk samples from systems without pipelines that had acid degree values high enough to indicate rancidity. All samples of milk from the vacuum type group that indicated rancidity came from installations that included pipelines.

The results of the survey indicated 23 bulk tank installations using pipeline milking systems with a vacuum releaser device installed in the milk line. The milk samples from 5, or 21.7%, of these 23 systems had acid degree values above 2.00 which indicated that organoleptic examination would find them rancid. The survey also indicated 26 pipeline milking systems using milk pumps in the pipelines. Fifteen of the milk pumps were of the diaphragm type, and samples of milk handled by this type of pump had an average acid degree value of 1.81. Four of these 15 samples had acid degree values over 2.00. Eleven of the milk pumps were of the centrifugal type, and samples of milk handled by this type of pump had an average acid degree value of 1.23. Only one of the 11 samples had an acid degree value of over 2.00.

SUMMARY AND CONCLUSIONS

A study was conducted to determine the incidence and intensity of rancidity in raw milk handled by bulk tank systems in Oklahoma, and to obtain other information pertaining to the influence of various factors on the defect.

Since the human senses of taste and smell are unable to detect and measure small amounts of materials responsible for the rancid flavor in milk, it was necessary to establish a chemical equivalent to organoleptic ratings. This chemical rating system is a measurement of the fat hydrolysis, which is responsible for the rancid flavor in milk. It is expressed as acid degree value, which is the ml of lN base required to neutralize the free fatty acids in 100 gm of butterfat. The threshold at which the two trained judges could organoleptically detect rancidity was established at acid degree value of 2.00. This value was determined by comparing the organoleptic observations on 286 samples of milk with the acid degree values of the same samples.

The incidence of rancidity in milk from individual cows and mixed herds as measured by acid degree values was determined. The acid degree values of the milk from 123 individual cows in three herds ranged from 0.71 to 10.21 with 25 samples rating above the threshold for detection of rancidity. These data show that there is a wide variation in the susceptibility to rancidity in the milk from individual cows. The acid degree values of mixed herd milk handled in bulk tanks placed 223, or 89.6% of the 249 total samples, below the threshold for rancidity, while 26 samples, or 10.4% of the total, were above the threshold for rancidity.

The survey of an Oklahoma milkshed revealed a higher incidence of rancidity in milk handled in vacuum type bulk tanks than in that handled in open tanks.

A comparison of the acid degree values of the milk from different brands and types of tanks disclosed 221 open type tanks with samples from 17, or 7.7%, developing acid degree values above the threshold for rancidity. There were 28 vacuum type tanks with samples from 9, or 32.1%, above the threshold for rancidity.

Laboratory trials of exposing milk to a vacuum treatment indicated that the vacuum was not a factor in the acceleration of lipase activity. It should be stated that none of the samples of milk from the 8 vacuum tanks used without pipelines was rancid, while 45% of the samples from the remaining 20 vacuum tanks with pipelines were rancid.

A study was made of the influence of pipeline systems on the incidence of rancidity of milk handled in bulk tanks. Of 174 samples of milk from non-pipeline systems only 4% had acid degree values in the range indicative of rancidity, while of 69 samples from pipeline systems, 27.5% had values in the range where rancidity would probably be detected organoleptically.

These data show that the use of a pipeline system is a far more important factor as a cause of rancidity than is the use of a bulk tank. From the data it appeared that vacuum releasers and pumps in the pipeline systems are important factors to consider as causes of rancidity. In a limited number of comparisons it was observed that there was a much higher incidence of rancidity in milk from the systems having diaphragm pumps than from those having centrifugal pumps.

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Thesis: THE EFFECT OF VARIOUS FACTORS ON THE INCIDENCE AND INTENSITY OF RANCID FLAVOR IN RAW MILK HANDLED IN BULK TANKS

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