RELATIONSHIPS BETWEEN CERTAIN SHORT-CHAIN FATTY ACIDS, TOTAL FAT ACIDITY, AND RANCID FLAVORS IN MILK

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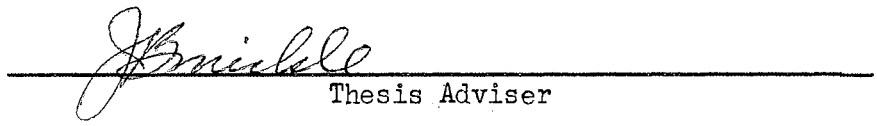

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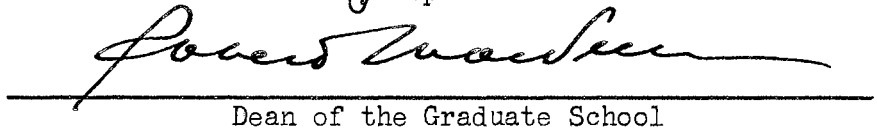

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INTRODUCTION

Milk is accepted primarily on the basis of its flavor, thus the maintenance of desirable flavors and the prevention of undesirable ones are of great importance to the dairy industry. Rancidity is one flavor which is quite objectionable in milk, and its occurence causes monetary losses to the dairy plant and the farmer.

The compounds which cause rancidity in milk are not definitely known. The flavor is defined basically in terms of organoleptic analysis, and this is subject to many human errors. Judges often do not agree as to whether a rancid flavor is present in a given milk sample. Even when they do agree that the flavor is present, there often is disagreement as to the intensity of the flavor. Various laboratory methods have been developed to measure rancidity; most of these involve the titration of milk fat for total acidity. These methods are more precise than organoleptic analysis, but there is no assurance that they measure the same substances which cause the flavor.

It is generally agreed that rancid flavors in milk are caused by the presence of certain short-chain fatty acids, especially butyric. However, the author could find only one published paper showing quantitative data concerning the fatty acids present in rancid milk.

Since there is not sufficient research data to adequately demonstrate what constitutes a rancid flavor, it would seem important to attempt to define this flavor in terms of the chemical compounds which

cause it. If such knowledge were available, progress toward controlling the flavor would be more rapid.

The first objective of this study, therefore, was to measure the amounts of formic, acetic, propionic, and butyric acids in milk and to determine if the amount of these acids present was related to the intensity of rancid flavors. The second objective was to determine if different conditions used to develop rancid flavors would cause differences in the fatty acids present in the milk and to determine if these differences in turn might be related to differences in the flavor.

REVIEW OF LITERATURE

It has been accepted generally that rancid flavors in milk were due to the hydrolytic splitting of milk fat by lipase enzymes. The liberated fatty acids, especially the short-chain ones, were thought to be responsible for the flavor and odor of rancid milk (11). The conditions affecting rancid flavors have been studied by many workers (2, 3, 4, 10, 12, 13, 15, 18, 19, 20, 21, 22, 31).

Herrington and Krukovsky (14) found wide variations in the acidity of fat in the milk of different cows. A great deal of variation was found in the rates of lipolysis of different milk samples and in the response of different samples to activation by warming and cooling (13). Herrington and Krukovsky (14) stated that the rate of lipase action was influenced by the rate at which the milk was cooled in certain critical temperature ranges. They found the upper limit of this range to be 20-25°C., while the lower limit was approximately zero in the case of naturally rancid milk and 10°C. in the case of temperature activated rancid milk (12). Tarassuk and Richardson (30) stated that from the upper limit of 15-20°C. the rate of lipase action seemed to increase with progressive cooling to lower temperatures.

Lipolysis in cold milk was accelerated by temperature activation. To obtain maximum lipolysis milk was pre-cooled to 5°C. or lower, warmed to 30°C., and then cooled to below 10°C. (20). The actual temperature was apparently more important than the length of

time the milk was held at 30°C. (20).

Rancidity developed readily in mixtures of milk composed of raw and homogenized-pasteurized milk. The maximum increase in acidity occurred when the ratio of raw to homogenized-pasteurized milk was approximately 1 to 1 (23).

The flavors and odors of rancid milk apparently were not always the same. It has been observed that the rancid flavor of homogenized milk was different from that of milk which became rancid spontaneously (11). Spontaneously rancid milk was defined by Herrington (11) as milk which became rancid as a result of cooling alone. Dunkley and Wood (2) stated that presence of low-molecular weight fatty acids (particularly butyric, caproic, and caprylic) generally caused rancid flavor. These acids caused odor, taste, and throat irritation but were not the only compounds which contributed to the flavor defect. When these authors churned butter from rancid cream, most of the samples had the characteristic rancid odor and taste, but some samples were bitter. Herrington (11) stated that sometimes the odor of butyric acid was easily recognized in rancid milk, but sometimes an odor described as "dirty" or "goaty" was present, which suggested that acids containing six, eight, or ten carbon atoms were present.

Gould (5) stated that homogenized raw milk usually was rancid when the acid degree of the fat was within the range of 1.5 to 2.0. However, he occasionally obtained milk fat with acid degree values as high as 11.5 which did not possess a rancid flavor. Gould (5) also stated that the free fatty acids in milk fat obtained by churning were not responsible for the typical rancid flavor of dairy products. These free fatty acids were found to be the water-insoluble

type by Gould and Johnson (6).

Kemp and Hetrick (17) used a simplified Ramsey-Patterson partition chromatographic method to separate the short-chain free fatty acids, butyric through capric, normally found in dairy products. They stated that the procedure had been applied to lipolyzed fluid milk and cream, but no data were presented. Propionic acid was present in some rancid dairy products but not necessarily in milk (17).

Hankinson, Harper, and Mikolajcik (7) used a modified Roese-Gottlieb procedure to extract known amounts of volatile fatty acids from the acid filtrates of milk. The extraction procedure and gas chromatographic analysis showed recoveries averaging 57, 87, 98, 81, 96, 97, and 99 per cent for formic (C_1), acetic (C_2), propionic (C_3), butyric (C_4), valeric (C_5), caproic (C_6), and caprylic (C_8) acids, respectively (7). According to these authors, the extraction procedure appeared specific for volatile fatty acids. They reported an average of 48.2, 21.3, 3.1, 3.4, 4.4, 7.6, and 9.7 micromoles of C_1, C_2, C_3, C_4, C_5, C_6, and C_8 acids, respectively, present in 100 ml. of the filtrate from a fresh, raw milk sample.

Morr, Harper, and Gould (25) reported that fresh, raw, mixed-herd skimmilk contained C_1, C_2, C_3, C_4, pyruvic, lactic, and four unknown acids. The C_1, C_2, C_3, and C_4 acids were found to range, respectively, 1.8-10.8, 0.0-4.6, 19.2-87.4, and 39.6-182.4 micromoles per 100 ml. of milk.

Harper, Gould, and Hankinson (9) reported that 13 samples of milk considered "free from off-flavors" contained C_1, C_2, C_3, C_4, C_5, C_6, and C_8 acids in the range of 9.8-43.1, 2.6-20.5, 0.0-1.5, 0.1-1.9, 0.0-1.9, 2.3-4.6, and 4.8-7.4 micromoles per 50 ml. of milk, respectively.

Seven samples of rancid-flavored milk contained C_1, C_2, C_3, C_4, C_5, C_6, and C_8 acids in the range of 25.5-54.9, 12.8-28.1, 0.0-1.5, 4.0-10.9, 0.9-1.7, 5.7-8.0, and 7.2-12.5 micromoles per 50 ml. of milk, respectively.

Rivers (29) reported that "spontaneously" and temperature activated rancid milk did not contain any detectable butyric acid, while milk activated by agitation contained increased amounts of propionic through stearic fatty acids.

EXPERIMENTAL METHODS

In general this work involved the analysis of rancid milk to determine the amounts of C_1-C_4 fatty acids present in it. Fresh milk was obtained and treated to develop a rancid flavor using several different methods of activation. The free fatty acids were extracted from this milk with an ether extraction procedure and measured by means of column chromatography. The chromatographic results were compared with organoleptic examinations of rancidity and acid degree values.

The extraction procedure used was patterned after that of Hankinson, Harper, and Mikolajcik (7). This procedure was as follows: (a) A 5 per cent solution of sulfuric acid was added to 100 ml. of milk to obtain a pH of 4.6. This mixture was warmed to 45-50°C. and filtered. (b) Fifty ml. of the filtrate were adjusted to a pH of 1.9 or less with 25 per cent sulfuric acid. (c) Fifty ml. of ethanol were added and the mixture was shaken about 10 times in a separatory funnel. (d) Then, 100 ml. each of ethyl ether and petroleum ether were added. This mixture was shaken for 45 seconds after the addition of each solvent; then the ether layer was decanted. (e) This extraction (Part d) was repeated twice. (f) The fatty acids in the combined ether extracts were converted to their sodium salts by neutralizing with alcoholic sodium hydroxide to a phenolphthalein end point. After evaporating the ether, the sodium salts were stored at room temperature until analyzed.

To prepare the fatty acids salts for chromatographic analysis, the sample was acidified to a pH of 1.9 or less with 1N sulfuric acid (not to exceed a total volume of two ml.). The acidified sample was transferred quantitatively onto a Celite column (16, 24, 26, 27). Then the first solvent was added. The flow rate of the column was adjusted to 70-90 drops per minute and the eluted solvent was collected in approximately 6.5 ml. fractions.[1] The solvents were added one at a time in the following order: 100 ml. of chloroform, 100 ml. of 5 per cent butanol in chloroform, 100 ml. of 10 per cent butanol in chloroform, and 100 ml. of 15 per cent butanol in chloroform (8, 28). These eluting solvents were equilibrated before use with 0.5N sulfuric acid.

The fractions were titrated with 0.02N alcoholic potassium hydroxide[2] using alcoholic phenolphathlein as an indicator. If the fractions were not titrated immediately, the fraction tubes were stoppered and stored at about 7°C. until analyzed, which was never more than 24 hours.

The column employed Celite 535[3] as a support for the sample and solvents. To prepare it for use, 300 grams of the Celite were shaken with 2000 ml. of 0.25N sulfuric acid for about one minute. The Celite was washed free from acid and was dried at 105°C. for 24 hours. Twenty grams of the dried Celite was then mixed with 12 ml. of 0.5N

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[1]The fraction collector was manufactured by Micro-Chemical Specialties Co., Berkeley, California.

[2]The alcohol was redistilled before use according to the procedure given in reference (1).

[3]Celite 535--a chemically inert silica obtained from Johns-Manville, Celite Division, New York 16, New York.

sulfuric acid in a mortar. This mixture was put in a 10 per cent acetone-in-hexane solution[4] (100 gm. of Celite to 1000 ml. of acetone-hexane solution) and stirred for about two hours. The hexane solution was equilibrated with 0.5N sulfuric acid solution before use.

This Celite was packed into a 1.3 cm. diameter column to a height of 34 cm. The column was washed with 50 ml. of chloroform to remove the acetone-hexane mixture before the sample was added.

Acid degree values were determined by the method of Thomas, Nielson, and Olson (32) as modified by Von Gunten (33). These values were expressed as the ml. of 1N base necessary to neutralize the acid in 100 gm. of fat. Before organoleptic examination each sample was pasteurized at a temperature of 167°F. for 17 seconds. After cooling to room temperature, the samples were coded and offered to three or four judges to be scored as either 1-not rancid, 2-slightly rancid, 3-distinctly rancid or 4-strongly rancid.

The raw herd-milk used in this study was obtained from the bulk tank of the Oklahoma State University Dairy Farm and the homogenized-pasteurized milk from the Oklahoma State University Dairy Plant. The free fatty acids were extracted from an aliquot of each sample after 0, 24, 48, and 72 hours then analyzed by chromatography. At the time of each extraction the sample was examined organoleptically and acid degree values were determined. All samples were stored in an ice bath at 0-4°C. until the last extraction.

Rancid flavors were produced in the milk by several different methods. In Experiment I, varying percentages (five, six, and ten)

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[4]The acetone was technical grade and the hexane was manufactured by the Phillips Petroleum Co., Bartlesville, Oklahoma

of raw milk were mixed with homogenized-pasteurized milk. In Experiment II, three samples of raw herd-milk were obtained from a bulk tank at about 5°C. These samples were temperature activated after 0, 24, and 48 hours of storage as described by Krukovsky and Herrington (20). Experiment III used the same procedure as Experiment II except that the raw milk was collected in the milking parlor from three Holstein cows (No. 947B, 176, and 132) and cooled immediately in an ice bath to about 1°C. in 10 to 15 minutes. In Experiment IV, the milk was collected as described in Experiment III from two Holstein cows (No. 176 and 132) and allowed to become rancid "naturally," that is, without any type of activation other than cooling to 1°C. in 10 to 15 minutes. Three samples from each cow were analyzed in this experiment.

RESULTS AND DISCUSSION

To evaluate the precision of the extraction procedure, known amounts of C_1-C_4 fatty acid were added to milk samples. These were then extracted and the recoveries were determined. These recoveries averaged 64.0, 73.7, 90.4, and 91.8 per cent for C_1, C_2, C_3, and C_4 acids, respectively (Table I). These recoveries compared favorably with those of Hankinson, Harper, and Mikolajcik (7) who reported 57, 86, and 81 per cent for C_1, C_2, and C_4 acids, respectively. These authors reported "quantitative" recovery of the C_3 acid.

Mixtures of known amounts of C_1-C_4 fatty acids were then analyzed to determine if the Celite column would give adequate separation and recovery. The results of a typical determination were graphed in Figure 1. This and other similar determinations showed the separation between acids to be adequate. The recoveries of known amounts of C_1-C_4 acids from six replicate determinations averaged 88.6, 99.4, 98.2, and 98.2 per cent for C_1, C_2, C_3, and C_4 acids, respectively (Table II). These recoveries were thought to be adequate to use as standards when analyzing the amounts of C_1-C_4 fatty acids present in milk.

In the work reported in this thesis the analyses of 18 fresh milk samples averaged 0.12, 0.03, 0.00, and 0.02 milliequivalents per 100 ml. of milk (meq./100 ml.) for the C_1, C_2, C_3, and C_4 fatty acids, respectively. These results were comparable to the analyses of 13 fresh milk samples which averaged 0.04, 0.02, 0.00, and

0.02 meq./100 ml. of milk for the C_1, C_2, C_3, and C_4 fatty acids, respectively, as reported by Harper, Gould, and Hankinson (9).

The acids present in milk were identified by the addition of a single known acid to a sample of milk. If the peak from this known acid coincided with the peak of an unknown acid and caused an increase in the size of that peak, the two acids (known and unknown) were assumed to be identical. The unknown acids also were identified by comparing the volume of liquid phase necessary to elute an unknown acid from the column with that required to elute a known acid.

The extraction procedure used in this work has been reported by others (7) to recover C_1, C_2, C_3, C_4, C_5, C_6, and C_8 fatty acids. In the present study, the C_4 acid was eluted from the Celite column with the first solvent that passed through the column. Since C_5, C_6, and C_8 acids were more soluble in chloroform than C_4 was soluble, it was thought that they also would appear with the C_4 acid in the first solvent through the column. Thus, the first peak off the column (hereafter labeled C_4) was probably a mixture of C_4, C_5, C_6, and C_8 acids.

In Experiment I (Table III) a rancid flavor was produced by mixing raw herd-milk with homogenized-pasteurized milk. The data in the three trials of this experiment revealed no consistent relationships when the amounts of fatty acids in the milk were compared with storage times, acid degree values, or flavor scores. There was a slight increase in acid degree values and flavor scores as the time of storage increased but the correlation coefficient for these two variables was only 0.66 (Figure 2).

It would appear from the data in this experiment that neither the

acid degree values nor the amounts of C_1-C_4 acids in the milk could be used to predict the rancid flavors (flavor scores) of the milk. However, most of the flavor scores in this experiment were less than 3.0, indicating that much of the milk did not develop a distinctly rancid flavor.

In Experiment II, a rancid flavor was produced in herd milk by temperature activation. This involved pre-cooling the milk to 0-5°C., then warming it to 30°C., and cooling it again to 10°C. or lower in a maximum of 15 minutes. After this temperature treatment the milk was stored at 0-4°C. The data from the three trials of this experiment are shown in Table IV.

No consistent relationships were observed when comparing any of the fatty acids with storage times, acid degree values, or flavor scores. The flavor scores and acid degree values tended to increase with time, but the correlation coefficient for the two variables was only 0.52 (Figure 3). This would seem to indicate that flavor scores could not be predicted accurately from acid degree values in this experiment. As in the previous experiment, no pronounced rancid flavor was produced by the temperature activation treatment used.

The results from the six trials of Experiment III are shown in Table V. In these trials, rancidity was produced in the milk by temperature activation as in Experiment II. The difference was in the type of milk samples used--one being milk from individual Holstein cows, and the other being mixed herd-milk. The latter was used in the two previous experiments.

In Experiment III, C_4 acids were present in all of the trials and increased with storage time. Propionic acid was not present in

the milk in a majority of the trials, and no consistent pattern was observed for C_1 and C_2 acids in relation to storage time, acid degree values, or flavor scores. The increase in C_4 acids with time was related to an increase in acid degree values (Figure 4) and flavor scores (Figure 5). The correlation coefficient for C_4 acids with acid degree values was 0.95 and for C_4 acids with flavor scores it was 0.74. The correlation coefficient for acid degree values with flavor scores (Figure 6) was 0.82.

The milk used in Experiment IV was obtained from two individual Holstein cows. No activating treatment was used except that which might be involved in cooling the milk from about 38° to 1°C. in 10-15 minutes. The results from the six trials of this experiment are shown in Table VI. The C_1, C_2, and C_3 acids showed no consistent pattern in relation to storage time. However, the C_4 acids, acid degree values, and flavor scores increased with the time of storage. The correlation coefficient for acid degree values with C_4 acids was 0.94 (Figure 7). Since only one trial had high acid degree values this might not have been a true indication of the relationship between acid degree values and C_4 acids. The correlation coefficient of 0.48 (Figure 8) for acid degree values with flavor scores indicated that flavor scores could not be predicted precisely from acid degree values.

To partially confirm the results of Experiments I through IV, known amounts of formic, acetic, propionic, and butyric acids were added to a milk sample. The flavor of this sample was evaluated organoleptically by four trained judges. The judges found rancid flavors when butyric acid was present, but the amounts of C_1, C_2, and

C_3 acids seemed to have no effect on the flavor. These results (Table VII) confirmed the findings of Experiments III and IV regarding the relation of C_4 acids with flavor scores. However, the judges indicated that many of the rancid milk samples did not have a "typical" rancid flavor. This might indicate that rancidity was actually more than one flavor or caused by more than one compound. The results would seem to indicate that the added butyric acid was not the entire cause of the rancid flavors reported in Experiments I through IV.

These results also confirm the opinions expressed earlier about the inconsistency of judges. All four of the judges had above average training in the organoleptic evaluation of milk. However, on the average they gave corresponding scores to duplicate samples only 50 per cent of the time. Out of a possible four points, one judge scored one sample a point or more higher than its duplicate in 85 per cent of the cases, while another judge gave one duplicate a score two points higher than its mate 31 per cent of the time.

Considering all the work in this study the results indicated no consistent pattern when comparing the amounts of C_1, C_2, or C_3 acids present in the milk with any of the other variables studied. These findings were new information and were contrary to what one might have inferred from the literature. In Experiments III and IV, the amounts of C_4 acids present in the milk increased with time, and this increase seemed to be related to similar increases in acid degree values and flavor scores. However, such a relationship was not apparent in the data of Experiments I and II. The correlation coefficients for C_4 acids with acid degree values were 0.95 and 0.94 in Experiments III and IV, respectively. These indicated that one variable might be predicted

from the other with reasonable accuracy, but the correlation coefficient of 0.74 for C_4 acids with flavor scores was not high enough to justify predicting one variable from the other. This latter finding might be due to the fact that flavor scores could not be determined precisely. On the other hand, it might be that additional data would have shown a more precise relationship.

The correlation of flavor scores with acid degree values usually was not high enough that one value could be used to predict the other with great accuracy.

The variations between the data of different trials and experiments were large. For example the amounts of C_4 acids present after 72 hours of storage in Experiment I were about the same as the amounts in many of the fresh milk samples in the other experiments. The milk in Experiment I developed a rancid flavor after 72 hours of storage, but rancidity could not be detected in the fresh milk samples.

In Experiments II and III, milk from different sources was activated with the same temperature treatment. In Experiment III, the treatment produced distinctly rancid flavors, but in Experiment II rancid flavors usually did not develop. These results were probably related to differences in the milk which apparently were not measured in this work.

The intensity of rancid flavor, as indicated by the flavor score, appeared to be related to the amount of C_4 acids present in Experiments III and IV. However, flavor score did not seem to be related to the amount of C_4 acids in Experiment I. If these data were valid, it might be that more than one rancid flavor was measured in this work; or, if only one flavor was measured, it had more than one cause.

SUMMARY AND CONCLUSIONS

This study was conducted to determine what relationships might exist between the amounts of C_1-C_4 fatty acids present in milk and the rancid flavors of that milk.

A rancid flavor was developed in fresh milk by mixing raw and homogenized-pasteurized milk, by using a temperature activation treatment on herd and individual cow's milk, and by cooling individual cow's milk to 1°C. ("naturally" rancid milk). The free fatty acids were extracted from the milk samples after 0, 24, 48, and 72 hours by an ether extraction procedure and were analyzed using Celite column chromatography. The chromatographic results were compared to storage times, organoleptic scores, and acid degree values.

No consistent patterns were observed when comparing the amounts of C_1, C_2, and C_3 acids to any of the other variables measured in this study. These findings were new information and were contrary to what one might have inferred from the literature. The results from individual cow's milk indicated that the C_4 acids increased with time and were related to similar increases in acid degree values and flavor scores. Such a relation did not appear in the data obtained from herd-milk. The data indicated that flavor scores could not always be predicted precisely from acid degree values. It also appeared that rancidity was more than one flavor or that more than one group of compounds caused it.

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A P P E N D I X

TABLE I

RECOVERY OF KNOWN AMOUNTS OF FATTY ACIDS
BY AN ETHER EXTRACTION PROCEDURE

	C_1	C_2	C_3	C_4
	%			
	72.1	75.6	95.9	94.2
	67.8	75.5	94.5	93.2
	66.4	74.9	92.4	92.6
	64.9	73.4	91.7	91.4
	64.9	73.4	89.6	90.8
	63.5	71.8	87.5	90.8
	63.5	71.1	86.8	89.8
	60.6	--	84.7	--
	59.9	--	--	--
	56.3	--	--	--
Average Recovery	64.0	73.7	90.4	91.8

TABLE II

RECOVERY OF KNOWN AMOUNTS OF FATTY ACIDS
BY CELITE COLUMN CHROMATOGRAPHY

	C_1	C_2	C_3	C_4
	%			
	79.9	104.5	98.3	94.7
	78.4	94.5	94.8	105.4
	92.1	92.6	98.6	92.3
	94.9	102.1	102.4	99.3
	101.4	103.1	96.4	97.1
	84.7	99.5	98.7	100.4
Average Recovery	88.6	99.4	98.2	98.2

TABLE III

FATTY ACIDS, FLAVOR SCORES, AND ACID DEGREE VALUES OF MILK ACTIVATED BY MIXING RAW AND HOMOGENIZED-PASTEURIZED MILK

Experiment I

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 1	C_4	0.03	0.03	0.02	0.02
	C_3	0.01	0.00	0.02	0.02
5% Raw	C_2	0.00	0.00	0.00	0.01
	C_1	0.02	0.00	0.00	0.13
Flavor Score		1.3	1.6	1.6	2.3
Acid Degree Value		1.9	1.9	2.2	2.7

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 2	C_4	0.02	0.03	0.02	0.02
	C_3	0.00	0.01	0.01	0.01
	C_2	0.00	0.02	0.02	0.00
6% Raw	C_1	0.00	0.09	0.00	0.00
Flavor Score		1.0	2.0	1.6	2.6
Acid Degree Value		1.0	2.3	2.6	3.5

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 3	C_4	0.00	0.02	0.02	0.02
	C_3	0.00	0.02	0.00	0.01
10% Raw	C_2	0.01	0.02	0.03	0.02
	C_1	0.00	0.04	0.00	0.00
Flavor Score		1.0	2.5	2.6	3.0
Acid Degree Value		0.8	2.8	3.6	4.3

[1]Milliequivalents of acid corrected to 100% recovery for 100 ml. of milk.

TABLE IV

FATTY ACIDS, FLAVOR SCORES, AND ACID DEGREE VALUES OF TEMPERATURE ACTIVATED MIXED HERD-MILK

Experiment II

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 1	C_4	0.01	0.02	0.06	0.04
	C_3	0.01	0.00	0.00	0.02
	C_2	0.00	0.00	0.00	0.05
	C_1	0.00	0.00	0.00	0.01
Flavor Score		1.3	2.3	2.0	2.6
Acid Degree Value		0.8	1.1	1.4	1.8

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 2	C_4	0.00	0.00	0.00	0.00
	C_3	0.00	0.00	0.00	0.00
	C_2	0.00	0.00	0.00	0.00
	C_1	0.00	0.00	0.00	0.00
Flavor Score		1.0	1.3	1.3	1.0
Acid Degree Value		0.6	1.2	1.4	1.5

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 3	C_4	0.04	0.04	0.01	0.06
	C_3	0.01	0.02	0.00	0.01
	C_2	0.01	0.02	0.01	0.02
	C_1	0.05	0.08	0.12	0.03
Flavor Score		1.0	1.0	1.3	1.0
Acid Degree Value		0.9	1.1	1.2	1.2

[1]Milliequivalents of acids corrected to 100% recovery per 100 ml. of milk.

TABLE V

FATTY ACIDS, FLAVOR SCORES, AND ACID DEGREE VALUES OF TEMPERATURE ACTIVATED MILK FROM INDIVIDUAL COWS

Experiment III

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 1	C_4	0.03	0.23	0.37	0.32
	C_3	0.00	0.00	0.00	0.00
Cow 132	C_2	0.05	0.06	0.01	0.02
	C_1	0.47	0.15	0.01	0.03
Flavor Score		--	4.0	3.6	4.0
Acid Degree Value		1.6	10.1	13.2	16.3

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 2	C_4	0.02	0.15	0.26	0.32
	C_3	0.00	0.00	0.00	0.00
Cow 132	C_2	0.03	0.00	0.01	0.01
	C_1	0.07	0.00	0.03	0.03
Flavor Score		--	3.0	3.6	3.8
Acid Degree Value		1.0	8.4	12.8	14.0

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 3	C_4	0.05	0.10	0.16	0.13
	C_3	0.00	0.00	0.00	0.00
Cow 176	C_2	0.03	0.02	0.02	0.01
	C_1	0.53	0.20	0.05	0.07
Flavor Score		--	2.0	2.0	3.3
Acid Degree Value		0.8	4.8	7.0	8.4

[1]Milliequivalents of acid corrected to 100% recovery per 100 ml. of milk.

TABLE V (CONTINUED)

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 4	C_4	0.03	0.09	0.12	0.16
	C_3	0.00	0.00	0.00	0.00
Cow 176	C_2	0.01	0.01	0.01	0.00
	C_1	0.04	0.05	0.05	0.02
Flavor Score		--	2.8	3.3	3.3
Acid Degree Value		0.8	5.5	8.1	9.6

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 5	C_4	0.01	0.01	0.07	0.06
	C_3	0.01	0.00	0.01	0.00
Cow 947B	C_2	0.00	0.00	0.02	0.01
	C_1	0.00	0.02	0.08	0.04
Flavor Score		1.0	1.6	3.0	3.0
Acid Degree Value		0.8	2.3	4.2	5.8

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 6	C_4	0.02	0.04	0.12	0.21
	C_3	0.01	0.02	0.00	0.00
Cow 947B	C_2	0.04	0.00	0.01	0.01
	C_1	0.03	0.00	0.00	0.04
Flavor Score		1.0	2.0	4.0	4.0
Acid Degree Value		0.8	3.4	5.8	9.9

[1]Milliequivalents of acids corrected to 100% recovery per 100 ml. of milk

TABLE VI

FATTY ACIDS, FLAVOR SCORES, AND ACID DEGREE VALUES OF "NATURALLY" RANCID MILK FROM INDIVIDUAL COWS

Experiment IV

	Fatty Acids	Hours Storage			
		0	24	48	72
		meq.[1]			
Trial 1 Cow 176	C_4	0.03	0.01	0.02	0.03
	C_3	0.00	0.00	0.00	0.01
	C_2	0.06	0.01	0.02	0.04
	C_1	0.01	0.03	0.04	0.00
Flavor Score		--	1.3	2.0	2.0
Acid Degree Value		0.6	1.4	1.5	1.6

	Fatty Acids	Hours Storage			
		0	24	48	72
		meq.[1]			
Trial 2 Cow 176	C_4	0.02	0.02	0.05	0.06
	C_3	0.00	0.00	0.00	0.00
	C_2	0.14	0.01	0.05	0.04
	C_1	0.16	0.16	0.19	0.28
Flavor Score		--	2.0	2.5	2.0
Acid Degree Value		0.7	2.2	2.8	2.8

	Fatty Acids	Hours Storage			
		0	24	48	72
		meq.[1]			
Trial 3 Cow 176	C_4	0.03	0.04	0.04	0.04
	C_3	0.00	0.00	0.00	0.00
	C_2	0.04	0.02	0.02	0.03
	C_1	0.27	0.32	0.12	0.08
Flavor Score		1.0	1.6	2.0	3.3
Acid Degree Value		0.8	1.6	1.9	2.0

[1]Milliequivalents of acid corrected to 100% recovery per 100 ml. of milk.

TABLE VI (CONTINUED)

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 4 Cow 132	C_4	0.01	0.01	0.02	0.02
	C_3	0.00	0.00	0.00	0.00
	C_2	0.00	0.00	0.03	0.01
	C_1	0.00	0.03	0.04	0.05
Flavor Score		--	1.5	1.5	2.0
Acid Degree Value		0.4	0.9	0.9	1.0

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 5 Cow 132	C_4	0.03	0.10	0.17	0.32
	C_3	0.00	0.00	0.00	0.00
	C_2	0.02	0.01	0.05	0.05
	C_1	0.06	0.21	0.10	0.22
Flavor Score		--	2.0	2.3	3.7
Acid Degree Value		0.8	5.3	8.6	9.8

	Fatty Acids	Hours Storage 0	24	48	72
		meq.[1]			
Trial 6 Cow 132	C_4	0.02	0.05	0.04	0.04
	C_3	0.00	0.00	0.00	0.00
	C_2	0.00	0.03	0.02	0.03
	C_1	0.19	0.28	0.21	0.06
Flavor Score		1.7	2.0	2.0	4.0
Acid Degree Value		1.1	1.6	1.8	2.0

[1]Milliequivalents of acid corrected to 100% recovery per 100 ml. of milk.

TABLE VII

FLAVOR SCORES OF A MILK SAMPLE CONTAINING KNOWN AMOUNTS OF ADDED FATTY ACIDS

Sample No.		1	27	2	28	5	26	7	22	3	25
		meq./100 ml. milk									
Fatty Acids	C_4	-	-	0.03	0.03	0.06	0.06	0.12	0.12	-	-
	C_3	-	-	-	-	-	-	-	-	0.02	-
	C_2	-	-	-	-	-	-	-	-	-	0.03
	C_1	-	-	-	-	-	-	-	-	-	-
		Flavor Scores									
Judges	A	1.0	1.0	2.0	2.0	4.0	3.0	4.0	2.0	1.0	1.0
	B	1.0	1.0	1.0	1.0	3.0	3.0	3.0	4.0	1.0	1.0
	C	1.0	1.0	2.0	1.0	2.0	1.0	4.0	2.0	2.0	1.0
	D	1.0	2.0	2.0	3.0	1.0	3.0	3.0	4.0	1.0	1.0
Avg. Flavor Score		1.00	1.25	1.75	1.75	2.50	2.50	3.50	3.00	1.25	1.00

TABLE VII (CONTINUED)

Sample No.		6	23	4	21	8	20	9	19	10	18
		meq./100 ml. milk									
Fatty Acids	C_4	-	-	-	-	0.03	0.03	0.03	0.03	0.06	0.06
	C_3	-	-	-	-	-	-	-	-	-	-
	C_2	-	-	-	-	0.03	0.03	-	-	-	-
	C_1	0.08	0.08	0.10	0.10	-	-	0.08	0.08	0.10	0.10
		Flavor Scores									
Judges	A	1.0	1.0	1.0	3.0	1.0	1.0	2.0	1.0	4.0	2.0
	B	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
	C	1.0	1.0	2.0	1.0	1.0	2.0	2.0	2.0	2.0	2.0
	D	2.0	3.0	1.0	2.0	4.0	2.0	1.0	2.0	2.0	4.0
Avg. Flavor Score		1.25	1.50	1.25	1.75	1.75	1.50	1.50	1.50	2.25	2.50

TABLE VII (CONTINUED)

Sample No.		11	24	12	16	13	15	14	17	29
		meq./100 ml. milk								
Fatty Acids	C_4	0.06	0.06	0.06	0.06	0.03	0.03	0.03	0.03	-
	C_3	-	-	-	-	-	-	-	-	0.11
	C_2	0.03	0.03	0.03	0.03	0.03	0.03	-	-	-
	C_1	-	-	0.10	0.10	0.08	0.08	0.10	0.10	-
		Flavor Scores								
Judges	A	3.0	3.0	2.0	2.0	1.0	1.0	1.0	3.0	-
	B	3.0	3.0	3.0	2.0	1.0	1.0	1.0	1.0	1.0
	C	2.0	2.0	2.0	2.0	1.0	1.0	2.0	3.0	-
	D	2.0	2.0	2.0	3.0	2.0	2.0	1.0	2.0	1.0
Avg. Flavor Score		2.50	2.50	2.25	2.25	1.25	1.25	1.25	2.25	1.00

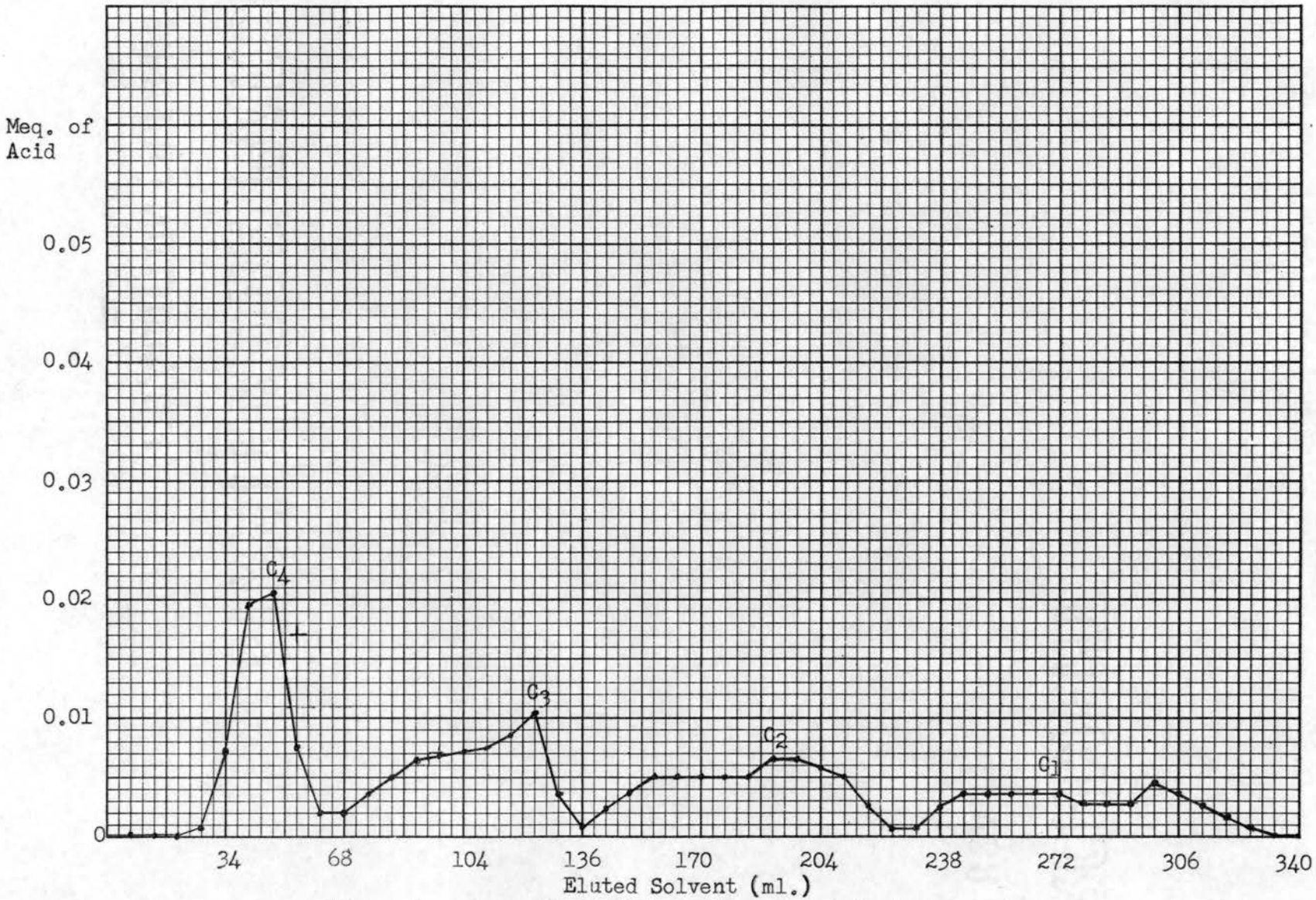

Figure 1. Separation of Known Amounts of C_1-C_4 Fatty Acids by Celite Column Chromatography.

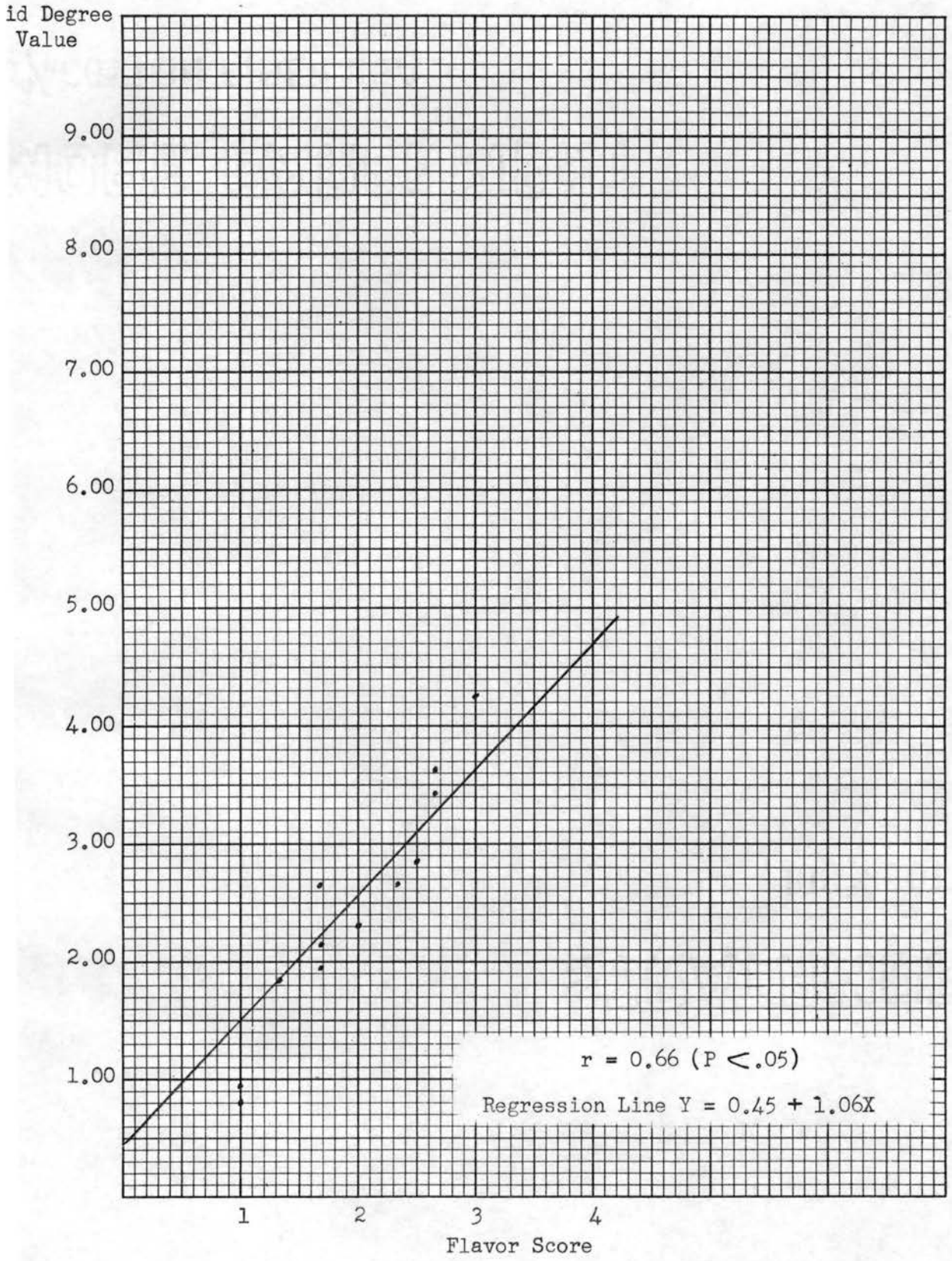

Figure 2. Comparison of Flavor Scores and Acid Degree Values of Milk Activated by Mixing Raw and Homogenized-Pasteurized Milk. Experiment I.

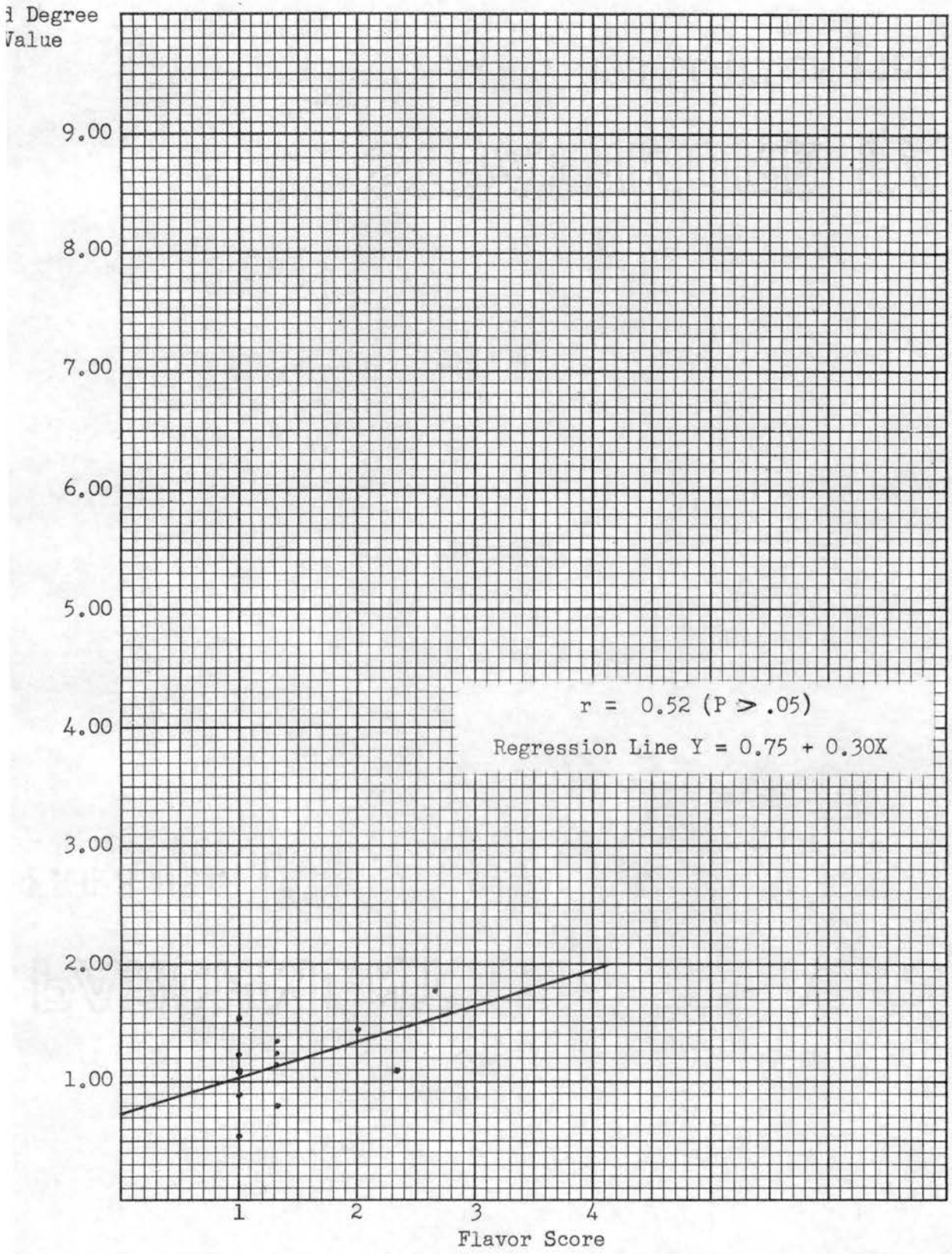

Figure 3. Comparison of Flavor Scores and Acid Degree Values of Temperature Activated Mixed Herd-Milk. Experiment II.

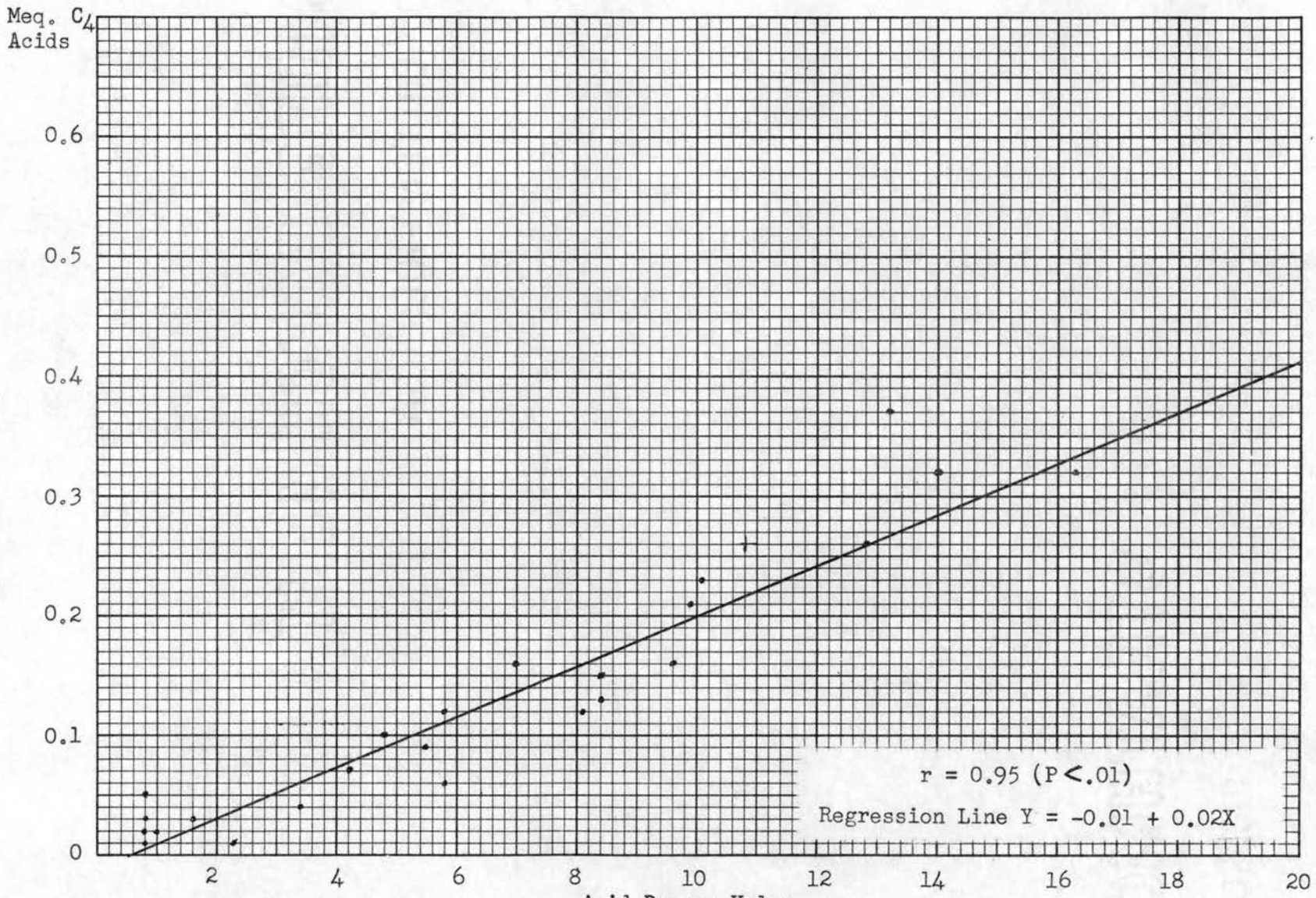

Figure 4. Comparison of Acid Degree Values and Milliequivalents of C_4 Acids from Temperature Activated Milk of Individual Cows. Experiment III.

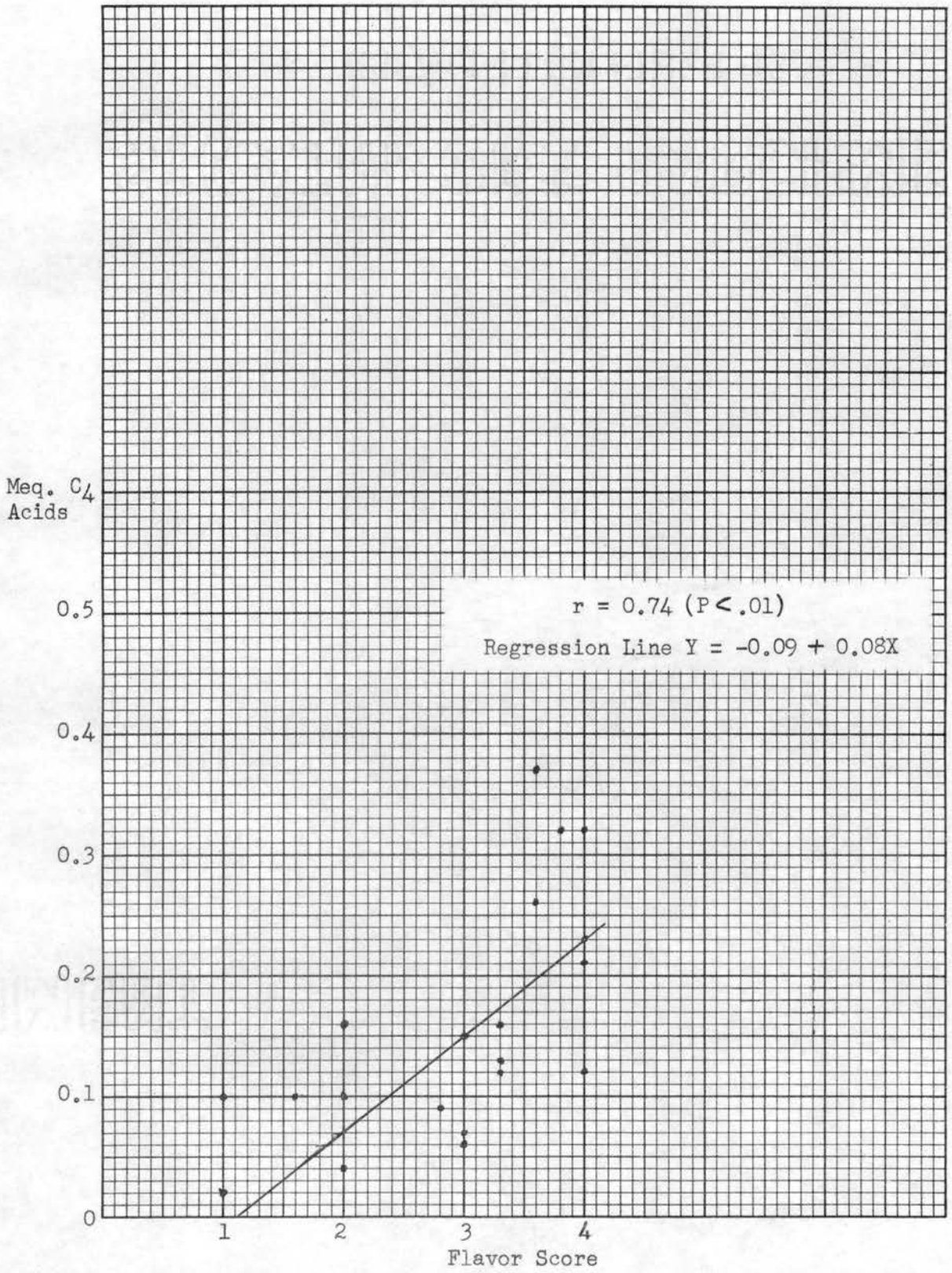

Figure 5. Comparison of Flavor Scores and Milliequivalents of C_4 Acids from Temperature Activated Milk of Individual Cows. Experiment III.

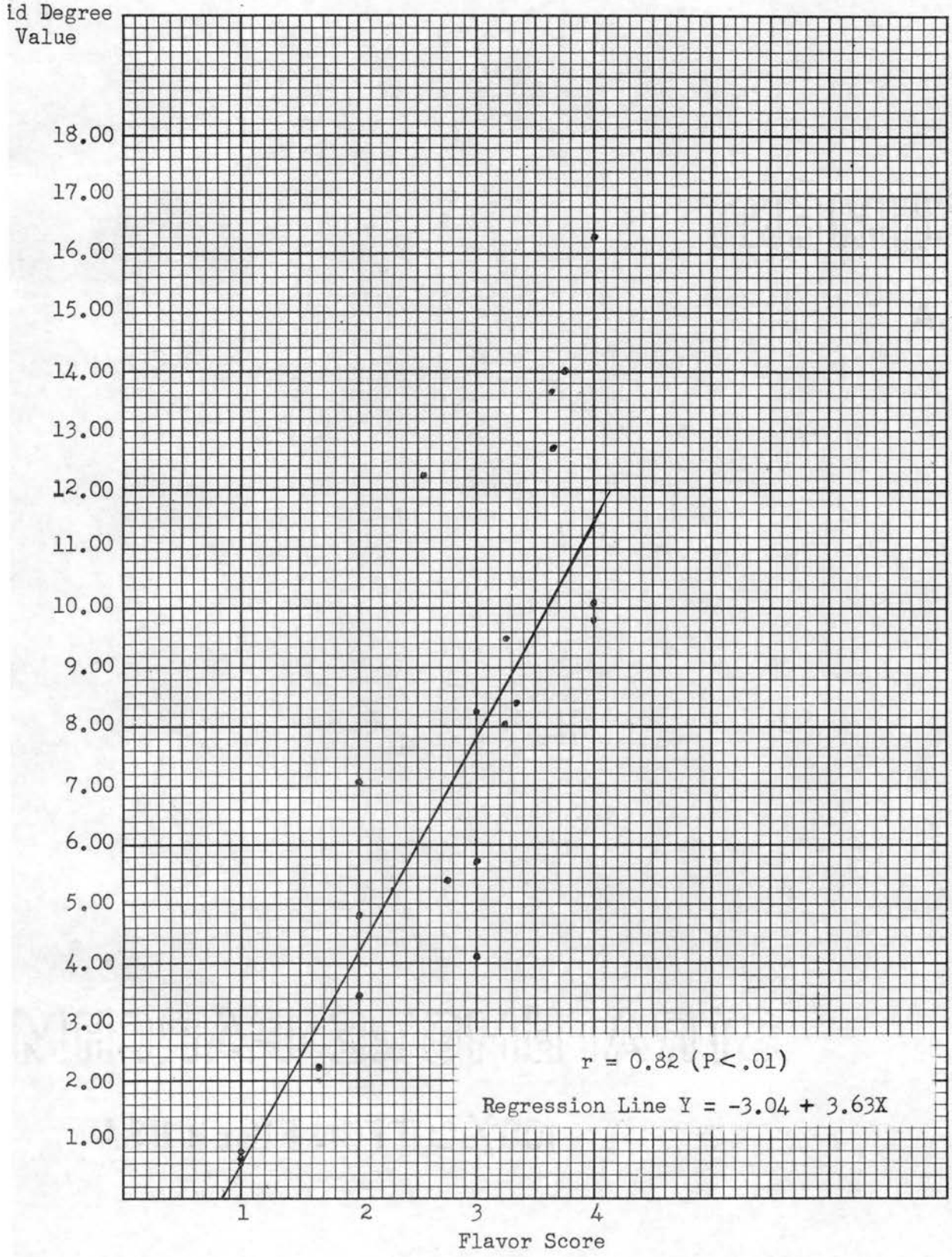

Figure 6. Comparison of Flavor Scores and Acid Degree Values from Temperature Activated Milk of Individual Cows. Experiment III.

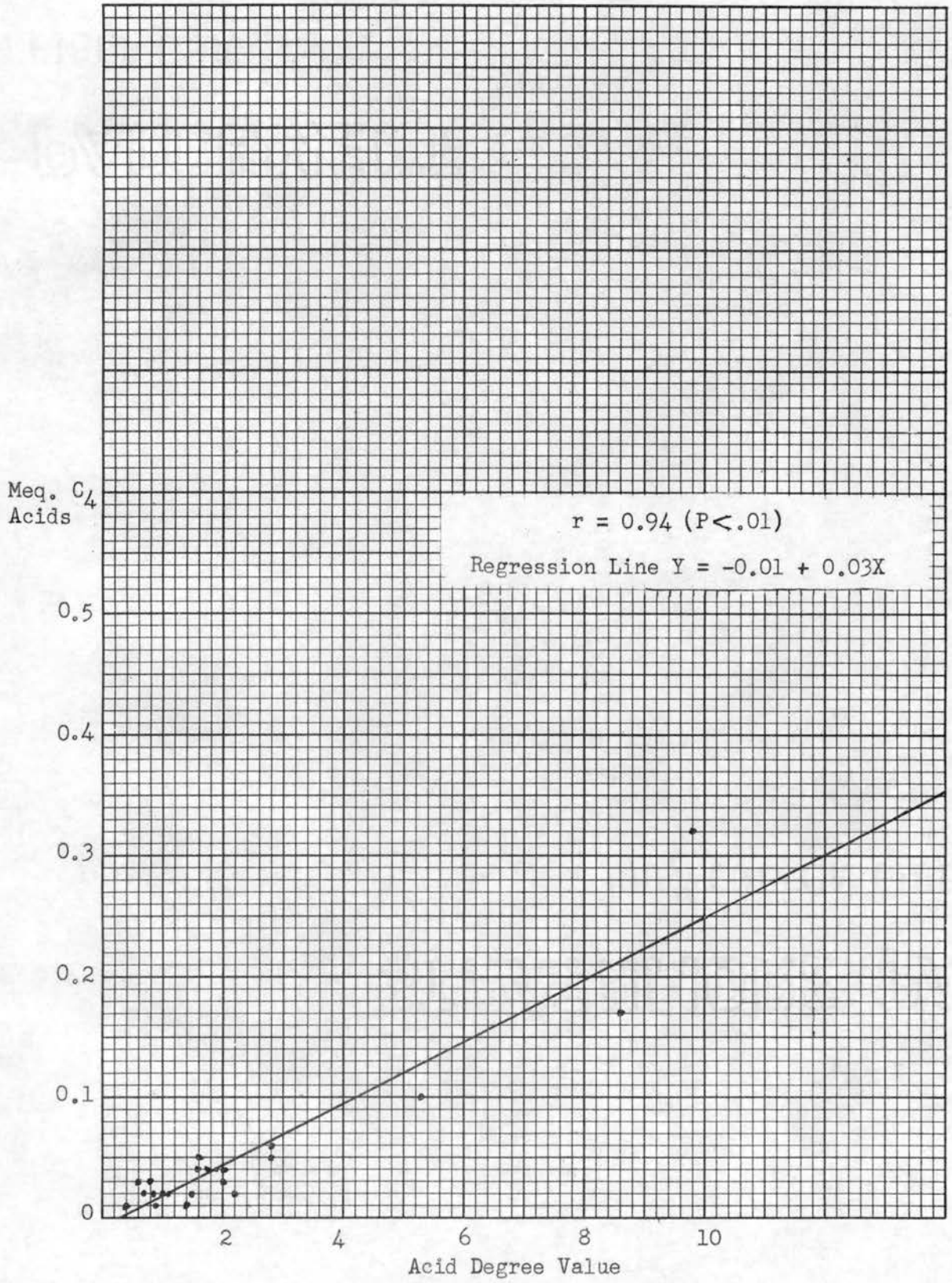

Figure 7. Comparison of Acid Degree Values and Milliequivalents of C_4 Acids from "Naturally" Rancid Milk of Individual Cows. Experiment IV.

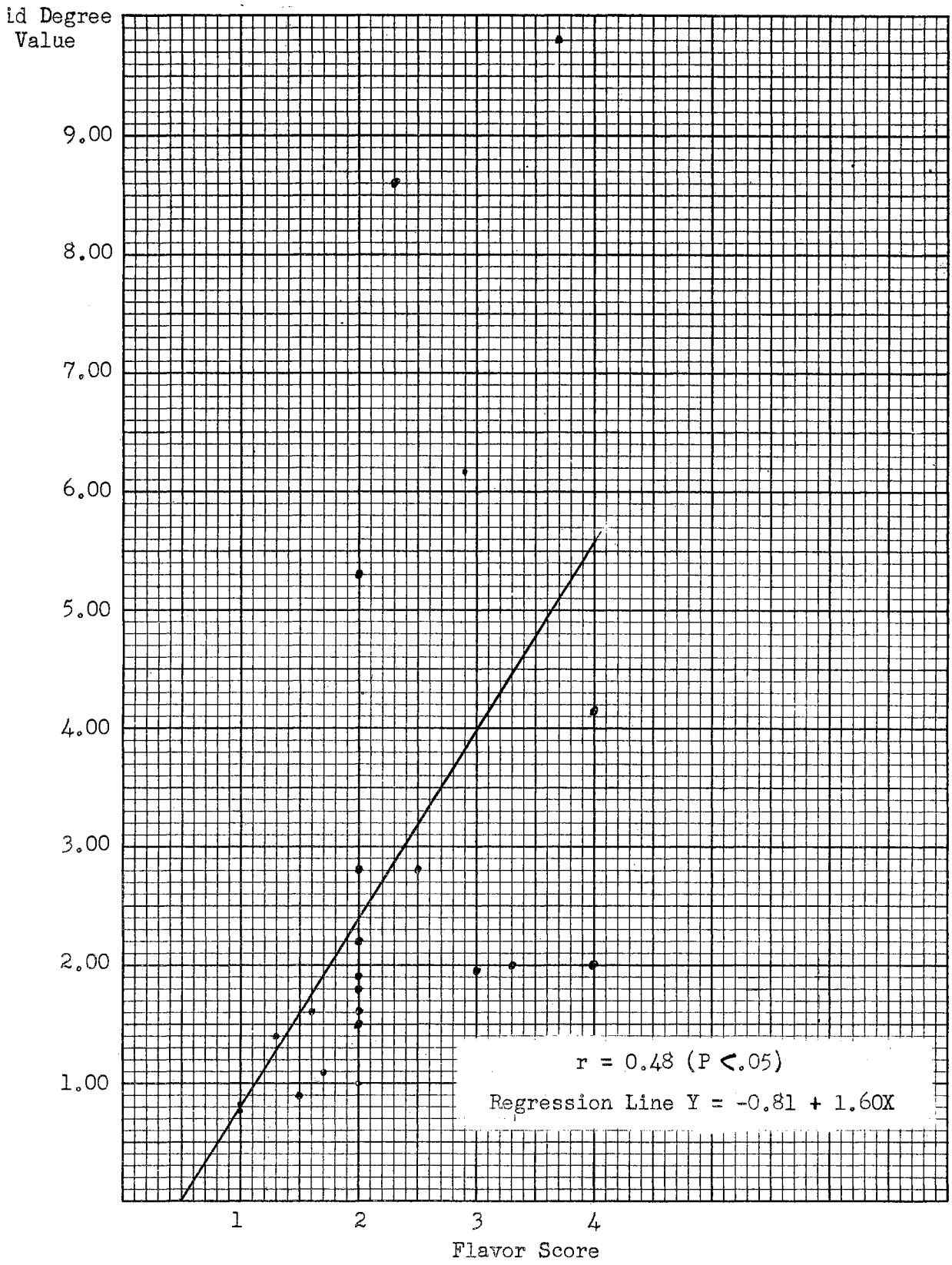

Figure 8. Comparison of Flavor Scores and Acid Degree Values from "Naturally" Rancid Milk of Individual Cows. Experiment IV.

VITA

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Master of Science

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