

INCREASE IN THE SENSITIVITY OF THE ORGANOLEPTIC
DETECTION OF LIPOLYSIS IN COWS' MILK
BY CULTURING OR DIRECT ACIDIFICATION

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Manuscript received: November 2nd, 1967

1. INTRODUCTION

Present practices employed on dairy farms and in dairy manufacturing plants in the United States have progressively raised the degree of lipolysis in raw milk prior to pasteurization. These same practices are being adopted in the Netherlands. At times, the extent of lipolysis may not be sufficient to produce a detectable rancid flavour in either the raw or pasteurized milk, but if the milk is used for a cultured product a rancid flavour is more likely to be noticed. A number of dairy plants in the U.S. have experienced a rancid flavour in their cultured products, but not in the uncultured products processed from the same raw milk. The type of cultured product is immaterial, be it yoghurt, sour cream, or butter churned from ripened cream. Even low-fat products such as cultured buttermilk and cottage cheese curd made from skim milk can have a rancid flavour.

Two factors are important for the production of a rancid flavour in the cultured product, namely:

- a. the level of lipolysis in the raw milk;
- b. the pH of the product.

The purposes of this report are twofold: first, to present experimental evidence showing that rancidity can be detected by taste at a lower level of fat hydrolysis in a cultured product or acidified milk than in the same milk at a normal pH; second, to suggest a simple method of increasing the sensitivity of organoleptic detection of rancidity in milk by acidification.

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2. LITERATURE

Numerous investigations have been made for the purpose of defining the causes or conditions which promote lipolysis in raw milk (1, 2, 3, 4, 5). Information gained from these investigations has made it possible to classify into two groups those factors which are important in contributing to increased lipolysis of milk fat:

- a. factors related to the cow (5);
- b. factors related to mechanical handling of raw milk (4).

When a manufacturing plant receives a significant volume of milk showing excessive lipolysis, action must be taken to determine the supply source of this milk and to correct the conditions which promote lipolysis of the milk prior to pasteurization.

The best method of securing a quantitative measure of the degree of lipolysis is the determination of the amount of free fatty acids in the milk fat. Another method is organoleptic evaluation of the milk by trained individuals who are sensitive to the rancid flavour. MACLEOD *et al.* (6), using the rapid screening test (BDI-test, 8), made a study of the relation of the fat acidity to the detectable rancid flavour in milk. The results of this study indicated that if a milk sample has a fat acidity of 1.5 or less, it will in all probability be free from rancid flavour. If the fat acidity lies within the range of 1.51 to 2.00, the milk may or may not be acceptable for use. Milk with a fat acidity greater than 2.00 should be rejected.

CHEN and BATES (5) also claimed that their flavour panel detected definite rancidity at a fat acidity of 2.0 or greater.

No references were found of work which correlated the fat acidity and pH of the sample with the sensitivity of organoleptic detection of rancidity.

3. MATERIALS AND METHODS

3.1. MATERIALS

Mixed milk obtained from the experimental dairy plant of the Institute was used in all trials.

When a comparison was made between normal milk and rancid milk, a single lot of raw whole milk was divided into two portions. One served as the control and the other was the experimental sample. Rancidity was induced by homogenizing the raw milk at 15 atm. and 30 °C, followed by a sufficient interval for development of a rancid flavour. Both lots were then heated to 85 °C for 10 min. The control lot was homogenized after heating at 85 °C and finally both lots were cooled to 5 °C.

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The milk samples received an additional heat treatment in flowing steam for 30 minutes if the milk was to be used for a cultured product. The milk was then cooled to 20 °C, inoculated with a mixed lactic starter (B-type) and incubated for 14 to 15 hrs. Judging the cultured milks was done immediately following incubation.

Acidified milk was prepared by adding a solution of 10% citric acid (w/v) to the different lots of cold (5 °C) pasteurized milk until the pH was 4.6–4.4.

Samples of milk with fat acidities within the range of 1 to 2.5 were prepared by mixing the control milk and rancid milk in definite proportions.

3.2. METHODS

Quantitative measurement of the level of lipolysis was made by two methods:

- a. the rapid silica gel method of HARPER *et al.* (7);
 - b. the screening test for hydrolytic rancidity, using the BDI-reagent (8).
- Results are expressed as fat acidity or the number of mg equiv. KOH required to neutralize 100 gram of fat.

A judging panel of some ten graders was used to determine at which level of fat acidity rancidity could be detected in cultured and non-cultured milk with a temperature of 20 °C.

4. RESULTS AND DISCUSSION

4.1. LEVEL OF LIPOLYSIS IN MILK THAT PRODUCED RANCID FLAVOUR IN CULTURED MILK

It was tried to determine in cultured milk the fat acidity at which rancidity could be detected. Experimental lots of milk were therefore prepared as described in Section 3.1. Non-rancid control milk was blended in proper proportion with rancid milk to give samples with the following acid degree values as determined by the Rapid Screening test (BDI-test, 8): 1.0, 1.2, 1.5 and 2.0. The non-rancid milk had a fat acidity of 0.70. The results given in Table 1 show that rancidity could already be detected in the cultured sample which was made from milk with a fat acidity of 1.0, whereas the values were in the range of 1.5 to 2.0 in the uncultured milk. This represents a considerable increase in the level of lipolytic detection. If a fat acidity of 2.0 is required in normal milk before rancidity can be clearly detected (5), then in cultured milk it is possible to detect rancidity at approximately one half of the fat acidity of normal milk.

Immediately the following questions were asked:

1. Is there any increase in the fat acidity during culturing?

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Table 1. Fat acidities¹ at which rancidity could be detected in cultured and non-cultured milk.

sample	fat acidities ¹ of the non-cultured samples (BDI-test)	flavour of the non-cultured samples	flavour of the cultured samples
control	0.7	good/ <i>goed</i>	good/ <i>goed</i>
1	1.0	good/ <i>goed</i>	rancid/ <i>rans</i>
2	1.2	good/ <i>goed</i>	rancid/ <i>rans</i>
3	1.5	slightly rancid/ <i>iets rans</i>	rancid/ <i>rans</i>
4	2.0	rancid/ <i>rans</i>	rancid/ <i>rans</i>
<i>monster</i>	<i>zuurtegraden¹ van het vet van de niet-gezuurde monsters (BDI-proef)</i>	<i>smaak van de niet-gezuurde monsters</i>	<i>smaak van de gezuurde monsters</i>

¹ Screening test using BDI-reagent (8). Fat acidities determined in the non-cultured samples / BDI-proef (8). *Zuurtegraden bepaald in de niet-gezuurde monsters.*

Tabel 1. *Zuurtegraden¹ van het vet waarbij rans optreedt in niet en wel gezuurde melk.*

2. If so, is culturing uniquely responsible for the observed increase in fat acidity of the sample?
3. Is this increase sufficient to account for the observed difference in flavour between cultured and uncultured products?
4. If acids were added directly to the milk, would lowering of the detectable level of lipolysis still be achieved?

Answers to these questions are found by examining the data in Table 2 and Table 3. In Table 2 it is demonstrated that the fat acidity using the rapid silica gel method of HARPER *et al.* (7) has increased after culturing. Reduction in pH to the same level as that of cultured milk, by direct addition of citric acid, however, did not produce any increase in fat acidity, as appears from the following example. Before adding citric acid, the milk had a fat acidity according to Harper's method of 2.5; immediately and after storage for 96 hrs, values of 2.4 and 2.4 respectively were found. Since therefore the only difference between the samples listed in Table 2 and in the above mentioned example is the presence of starter organisms, it is logical to assume that the starter organisms are responsible for the increase in fat acidity. Although starter organisms are normally not considered to be very lipolytic, some investigators consider them to promote lipolysis to some extent (9). It is also possible that the starter organisms during their growth in milk produce organic acids which are determined together with the fatty acids. The answers to the questions 1 and 2 are in the affirmative.

The answer to question 3 is not as definite. Experience with the Rapid Silica Gel method has demonstrated rather large increases in fat acidities in rancid *Neth. Milk & Dairy J.* 21 (1967)

Table 2. Effect of acidification by culturing on the fat acidity.

sample	fat acidity ¹ of milk	
	non-cultured	cultured
control	1.5	1.8
1	2.0	3.2
2	2.5	3.5
3	3.0	3.9
4	4.2	5.0

monster	<i>niet-verzuurd</i>	<i>verzuurd</i>
	<i>zuurtegraad¹ van het vet</i>	

¹ rapid silica gel method of HARPER *et al.* (7) /
snelle silica-gelmethode van HARPER *et al.* (7).

Tabel 2. *De invloed van het verzuren door zuurselbacteriën op de zuurtegraad van het vet.*

milk in comparison with normal milk (10). Hence, the increase in fat acidity that was secured by culturing is probably not sufficient to account for the difference in the level of detectable rancidity.

Further evidence that the above statement is true comes from examination of the data in Table 3. If the increase in fat acidity as a result of culturing is actually the only reason for detection of rancidity in cultured milk, but not in non-cultured milk, then direct addition of acid to milk should not have the same effect because, as was shown above, acid itself produces no increase in fat acidity.

On the other hand, if the pH of non-cultured milk is reduced by acidification to the same level as that of cultured milk, and rancidity can be detected in the acidified but not in the non-acidified sample, then it would appear that the pH of the medium is the main factor that is responsible, rather than micro-organisms.

In Table 3 are recorded the data from duplicate experiments designed to test the above hypothesis. These data show that panel judges were able with great regularity to detect rancidity at a much lower fat acidity in acidified milk than in the same milk that had not been acidified. Question 4 can be answered in the affirmative.

It is logical that this is true after calculations have been made to determine the ratio of acid to salt at the pH of a cultured or acidified milk, and the ratio

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Table 3. The intensity of rancidity in non-acidified and acidified milk.

experiment	composition of the milk mixture		fat acidity ¹	average score (scale 3-8) ²		number of graders scoring rancidity (scale 0-3) ³							
	% non-rancid	% rancid		non-acidified	acidified	non-acidified				acidified			
						0	1	2	3	0	1	2	3
1	100	0	0.4	6.6	6.6	9	0	0	0	9	0	0	0
	80	20	1.1	6.0	5.2	7	1	1	0	2	4	2	1
	50	50	2.2	5.6	4.3	2	4	2	1	0	2	3	4
	0	100	4.0	4.2	3.7	0	0	6	3	0	0	3	6
2	100	0	0.6	6.6	6.0	12	1	0	0	10	2	1	0
	94	6	1.0	6.6	5.4	12	1	0	0	5	3	4	1
	92	8	1.2	6.0	5.0	7	5	1	0	3	3	3	4
	86	14	1.5	5.5	4.4	3	4	6	0	0	4	3	6
	78	13	2.0	5.3	4.2	0	5	6	2	0	2	1	10
	0	100	6.9			not graded / niet beoordeeld							
<i>proef</i>	% niet rans	% rans	zuurtegraad van het vet ¹	niet aangezuurd	aangezuurd	0	1	2	3	0	1	2	3
	samenstelling van het mengsel			gemiddelde waardering (schaal 3-8) ²		aantal keurmeesters dat ransheid constateerde (schaal 0-3) ³							

¹ Screening test using BDI-reagent (8) / BDI-proef (8).
² 3 = not saleable / *zeer slecht*
 4 = undesirable / *slecht*
 5 = doubtful / *onvoldoende*
 6 = fair quality / *redelijk*
 7 = good / *goed*
 8 = excellent / *zeer goed*
³ 0 = not rancid / *niet rans*
 1 = slightly rancid / *iets rans*
 2 = rancid / *rans*
 3 = very rancid / *zeer rans*

Tabel 3. De mate van ransheid in wel en niet-aangezuurde melk.

of acid to salt at the pH 6.5 of normal milk. The dissociation constants of three fatty acids and the calculated acid-to-salt ratios at two different pH-values are given in Table 4. From the calculations it follows that the ratio of acid to salt is much higher at pH 4.6 than at pH 6.5. This means that at pH 6.5 there is less undissociated fatty acid to taste. We are therefore of the opinion that at pH 6.5 a rancid flavour is not as easily detectable as when the pH is 4.6. It should not be assumed that the salt form of the fatty acid is without taste. If this were true, the difference in the level of detection of rancidity between cultured (acidified) products and normal milk would be much greater. It is possible that yet other
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Table 4. Dissociation constants for normal fatty acids in aqueous solutions at 25 °C (11).

acid	dissociation constant K	pK-value	ratio acid to salt ¹ at:	
			pH 4.6	pH 6.5
butyric acid / <i>boterzuur</i>	1.50×10^{-5}	4.82	1.675	0.021
caproic acid / <i>capronzuur</i>	1.41×10^{-5}	4.85	1.778	0.022
caprylic acid / <i>caprylzuur</i>	1.41×10^{-5}	4.85	1.778	0.022

zuur	<i>dissociatie-constante</i> K	pK-waarde	<i>pH 4,6 pH 6,5</i> verhouding zuur:zout ¹ by	

¹ Calculated from the equation $\log \text{acid/salt} = \log (\text{H}^+) - \log K_{\text{acid}}$ (12).

Tabel 4. *Dissociatie-constanten van enige normale vetzuren in water bij 25 °C (11).*

factors are of importance. Nevertheless, when water solutions of butyric acid and caprylic acid in 100 μM concentrations at pH 4.6 and pH 6.5 were judged organoleptically, there was indeed greater intensity of the fatty acid flavour in the samples at pH 4.6.

4.2. A RECOMMENDED PROCEDURE FOR INCREASING THE SENSITIVITY OF ORGANOLEPTIC DETECTION OF RANCIDITY

Milk samples which are to be judged for suspected rancidity should be treated as follows:

1. add sufficient citric acid to lower the pH to 4.4–4.6;
2. taste the sample in addition to smelling it. Classify it as being rancid or not rancid.

If a rancid odour or flavour cannot be detected after acidification, the milk is satisfactory to use for any cultured products.

The above procedure can be used to identify farmers who are delivering milk with a low level of lipolysis.

SUMMARY

It was demonstrated that rancidity could be detected at a lower level of lipolysis in cultured milk than in non-cultured milk. The same result was obtained when the milk was not cultured but acidified by e.g. citric acid. This phenomenon is caused mainly by the effect of the pH on the ratio fatty acid to fatty acid salt.

When cultured milk products are prepared, a low level of lipolysis in the milk

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will sooner give rise to flavour defects than when non-cultured products are made.

The observation is also of importance to the organoleptic evaluation of raw milk delivered to the dairy. After acidification of the milk by means of e.g. citric acid, low levels of lipolysis can easily be detected.

ACKNOWLEDGEMENT

We thank Ir. H. T. BADINGS for his assistance in the organization of the organoleptic evaluations, and Drs. H. A. VERINGA for his valuable suggestions in the presentation of the data.

SAMENVATTING

TUCKEY, S. L. EN STADHOUDERS, J., *Vergroting van de gevoeligheid in de organoleptische waarneming van vethydrolyse in melk, na verzuring door melkzuurbacteriën of direct aanzuren.*

Er werd aangetoond dat een geringe vethydrolyse duidelijker werd geproefd in door zuurselbacteriën verzuurde melk dan in het niet-gezuurde produkt. Direct aanzuren van de melk met een zuur tot pH 4,6 leidde tot hetzelfde resultaat. Het verschijnsel wordt voornamelijk veroorzaakt door de verandering in de verhouding vetzuur/vetzuurzout onder invloed van de pH.

Bij de bereiding van zure melkprodukten zal derhalve een geringe vethydrolyse eerder aanleiding geven tot smaakafwijkingen dan bij de bereiding van niet-zure produkten.

De waarneming is tevens van belang voor het organoleptisch aantonen van vetsplitsing in rauwe melk in de fabriek of op de boerderij. Na aanzuren van de melk kan een geringe vetsplitsing reeds worden geproefd. Een voorschrift daartoe werd opgesteld.

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