

COMPARISON OF CERTAIN METHODS FOR THE DETERMINATION OF FAT HYDROLYSIS IN MILK

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1. INTRODUCTION

The trend in Europe has been to increase the storage time for raw milk, both on the farm and in the dairy plant before processing. Unless proper practices are followed in the handling of the milk, one result of this trend may be some increased lipolysis of the milk fat and the production of a rancid flavour in the milk. The degree of lipolysis is usually measured by determining the acidity of the milk fat. However, depending on the analytical method employed, different values for the fat acidity will be obtained. It is the purpose of the present report to compare four methods of determining fat acidity and to comment on their usefulness for control purposes in the identification of fat hydrolysis in milk.

2. MATERIALS AND METHODS

2.1 MILK SAMPLES

Mixed fresh raw milk samples for the determination of fat acidity were obtained at the experimental dairy of the Institute during the months of May and June 1967.

In some experiments the raw milk sample was divided into two portions. One portion served as the control with no induced lipolysis, and the other was homogenized in such a manner as to produce rancid milk. The control sample

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was heated in a water bath at 80 °C for 10 minutes, homogenized at 15 atm. and cooled to 5 °C. The experimental sample was warmed to 35 °C and homogenized at 15 atm. After standing 15 to 30 minutes, depending on the amount of lipolysis desired, the sample was heated at 80 °C for 10 minutes and cooled to 5 °C.

2.2 METHODS

The analytical methods compared were:

- I Rapid Silica Gel method for measuring total free fatty acids in milk (1);
- II Screening Test for hydrolytic rancidity, using the BDI reagent (2);
- III Churning method as described below;
- IV Extraction method according to FRANKEL and TARASSUK (3), as modified by RAADSVELD (4).

In this paper the methods listed above will be indicated by the numbers I, II, III and IV respectively. The methods will be briefly described below; for a detail account the reader is referred to the literature.

Method I. Silica gel is used for the separation of free fatty acids from the milk. The silica gel column is prepared in two sections: the bottom section contains 5 g dry silicic acid (Mallinckrodt No. 2847) mixed with 3 ml of 2 M KH_2PO_4 - K_2HPO_4 buffer (pH 6.5) and with 20 ml chloroform (alcohol free). A 10 g sample of the milk is acidified to pH 2.0 with 20 % H_2SO_4 and mixed with 15 g dry silicic acid until a free flowing powder is obtained. The mixture is slurried with 5 % n-butanol in chloroform and transferred quantitatively to the top of the bottom section. The solvent is allowed to flow through the column until 150 ml of 5 % n-butanol in chloroform have been collected. To the eluate 15 ml neutral absolute alcohol are added, after which the acids are titrated with 0.01 N alcoholic KOH, using phenol red as an indicator.

Method II. In the BDI test the milk is mixed with the BDI reagent in a proportion of 3.5 : 1. The reagent is prepared from 30 g of Triton X-100 and 70 g of sodium tetra phosphate, made up to 1 litre with distilled water. The mixture of milk and reagent is heated in boiling water for at least twenty minutes. The milk fat is separated by centrifugation. A known quantity of the fat is transferred to an Erlenmeyer flask, solved in a mixture of petroleum ether and N-propanol (4 : 1), and titrated with 0.02 N alcoholic KOH, using phenolphthalein as an indicator.

Method III. For method III the plastic cream, produced by centrifuging milk in tubes at $10,000 \times g$ for 30 min, was churned. The butter so obtained was melted at 45 °C, the fat was separated by centrifugation and then filtered. Approximately 3 g of melted fat were dispersed in 25 ml of a 1 : 1 mixture of ethyl ether and ethyl alcohol, and titrated with 0.1 N NaOH with phenolphthalein as the indicator.

Method IV. In method IV the fat acidity in milk was estimated as follows: 30 g of a sample of milk were weighed in a small centrifuge tube. After mixing with 500 mg disodium salt of EDTA, 20 ml neutralised ethanol were added. Subsequently the sample was extracted twice with an ethyl ether-petroleum ether mixture (v/v 2 : 1), using 25 ml and 20 ml of the mixture respectively. The mixture was shaken for 1 minute and centrifuged for 10 minutes at 1500 r.p.m. After each extraction the upper layers were removed by means of a pipette. The collected ether layers were titrated with 0.025 N alcoholic KOH, using 10 ml neutralised ethanol which contained 0.1 ml of a 0.2 % ethanolic solution of naphtholphthalein as an indicator. Since the average recovery of added fatty acids with this method has been proved to be about 90 %, the titration values were corrected by multiplying them by a factor of 1.11.

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All values are expressed as fat acidities. The fat acidity represents the number of mg equiv. NaOH (KOH) required to neutralize 100 g fat. With methods II and III a solution of the isolated fat is titrated. With methods I and III, however, the titration values obtained are related to the fat content of the milk and the fat acidities are then calculated.

The fat content of the milk was estimated by the Gerber method.

All the analyses of any one sample of milk were performed during the same day.

3. RESULTS

3.1 ESTIMATION OF FAT HYDROLYSIS IN FRESH MILK

In order to determine the uniformity or otherwise of the results achieved by each of the four methods, a series of fresh milk samples was analysed for fat acidity. The results are given in Table 1, from which it appears that the fat acidity depends to a large extent on the method used for its determination. When method IV is used the highest values are obtained, whereas somewhat lower values are achieved by method I and still lower fat acidities are obtained by methods II and III. Two reasons may be given for these differences between the results.

- a. During the isolation of the fat by methods II and III, part of the fatty acids is lost in the plasma;
- b. By means of the methods I and IV acids other than those derived from the milk fat are also determined; they could be fatty acids present in the plasma and/or other organic acids.

3.2 RECOVERY OF FREE FATTY ACIDS IN RECONSTITUTED MILK

Recovery of free fatty acids was made in a natural medium which had been prepared by dispersing milk fat of known acidity in skim milk. Milk fat of known acidity was prepared by homogenizing raw cream at 15 atm/cm² and 35 °C and allowing a sufficient interval for fat hydrolysis to elapse before lipase was destroyed by heating to 80 °C. After 12 hrs at 5 °C the cream was churned. The recovered butter was melted, the fat filtered and the acidity of the purified fat determined. A given quantity of fat was then homogenized in HTST-pasteurized skim milk so as to produce milk with 3.5% fat. The milk was then analysed for fat acidity by each of the four methods. Methods II and III were used in the normal way; for methods I and IV the fat acidities were calculated after subtracting the titration value of the skim milk. One of

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Table 1. Comparison of four methods for the determination of fat acidity (mg equiv./100 g fat). Fresh milk samples.

| milk sample | method* | | | |
|-------------|---------|-----|-----|-----|
| | I | II | III | IV |
| 1 | 0.8 | 0.4 | 0.4 | 1.7 |
| 2 | 0.8 | 0.5 | 0.6 | 1.6 |
| 3 | 1.3 | 0.4 | 0.6 | 1.5 |
| 4 | 1.3 | 0.5 | 0.6 | 2.0 |
| 5 | 0.9 | 0.5 | 0.4 | 1.7 |
| 6 | 1.7 | 0.5 | 0.4 | 1.7 |
| 7 | 1.9 | 0.5 | 0.5 | 2.9 |
| 8 | 1.5 | 0.5 | 0.5 | 2.2 |

| melkmonster | methode* | | | |
|-------------|----------|----|-----|----|
| | I | II | III | IV |
| | | | | |

*I = Rapid Silica Gel method according to HARPER *et al.* (1)/snelle silica-gelmethode volgens Harper *et al.* (1).

II = BDI test/BDI-proef (2).

III = Churning method/karnmethode.

IV = Extraction method according to FRANKEL and TARASSUK (3), modified by RAADSVELD (4)/Extractiemethode volgens Frankel en Tarassuk(3), gewijzigd door Raadsveld (4).

Tabel 1. Vergelijking van vier methoden voor het bepalen van de zuurtegraad van het vet (mg eq./100 g vet). Bepalingen uitgevoerd in verse melk.

the analyses is given in Table 2; it shows that, if methods I and IV are used, the recovery is essentially 100%, but that the recovery is considerably lower when methods II and III are used. In this case about 30% of the fatty acids are lost in the plasma. As is shown below, the recovery of the fatty acids by methods II and III varies to some extent.

3.3 ESTIMATION OF FAT HYDROLYSIS IN RANCID MILK AND IN THE CORRESPONDING FRESH MILK

An increase of free fatty acids in the milk, produced by lipase from the fat, was achieved by homogenization of raw milk. In order to evaluate each method for its capacity to measure the free fatty acids, the differences of the fat acidities before and after homogenization were compared, thus eliminating any possible effect of the non-fatty acids. The fat acidities were therefore estimated in fresh milk and in milk in which milk lipase had been activated by homogenization before heating as described under 2.2. Table 2 shows some of these results. From the values we formed the impression that for recovery of the free fatty

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Table 2. Recovery of the free fatty acids of milk fat dispersed in skim milk. Fat acidity of the dispersed milk fat 1.7.

| method | fat acidity | recovery % |
|--------|-------------|---------------|
| I | 1.7 | 100 |
| II | 1.2 | 70 |
| III | 1.3 | 76 |
| IV | 1.6 | 94 |

| <i>methode</i> | <i>zuurtegraad van het vet</i> | <i>terugggevonden %</i> |
|----------------|------------------------------------|-----------------------------|
| I | 1.7 | 100 |
| II | 1.2 | 70 |
| III | 1.3 | 76 |
| IV | 1.6 | 94 |

Tabel 2. *Het terugvinden van vrije vetzuren aanwezig in een hoeveelheid in ondermelk gemulgeerd melkvet. Zuurtegraad van het melkvet 1,7.*

acids method I is the best, closely followed by method IV. The greatest losses of fatty acids are found when use is made of the churning method and particularly of the BDI test. The results agree with those of JOHNSON and GOULD (5), who found the values obtained by the churning method to be about 70% of those given by the extraction method.

4. DISCUSSION

In accordance with Table 2, it may be assumed that approximately 70% of the fatty acids which are present in or produced from the milk fat, are recovered by methods II and III, as against approximately 100% by methods I and IV. It might therefore be expected that the values obtained by methods II and III would be approximately 30% lower than those obtained by methods I and IV. From the Tables 1 and 3 it follows, however, that the values of methods I and IV are proportionally higher. Consequently, other acids than those produced by fat hydrolysis are also determined by methods I and IV, possibly fatty acids present in the plasma and/or other organic acids. MORR *et al.* (6) have already shown that many organic acids are present in the milk plasma. More information is needed concerning which acids are included when methods I and IV are used.

We could prove that the results of method I were not influenced by the presence of lactic acid, acetic acid and citric acid. However, these acids appeared to be partly extractable by means of method IV. For this reason this method gives excessive fat acidities and should not be used for the estimation of fat acidity in cultured milk and in milk with a low level of developed acidity. In these cases method I is to be preferred, and this gives much lower values. These

Table 3. The estimation of the fat hydrolysis by four different methods in non-rancid milk and in rancid milk prepared from it.

| experiment | method* | fat acidities in | | difference in fat acidities | % of the difference found with method I |
|------------|---------|------------------|-----------------|-----------------------------|---|
| | | rancid milk | non-rancid milk | | |
| 1 | I | 2.9 | 1.3 | 1.6 | 100 |
| | II | 1.2 | 0.4 | 0.8 | 50 |
| | III | 2.0 | 0.6 | 1.4 | 87 |
| | IV | 3.0 | 1.5 | 1.5 | 94 |
| 2 | I | 3.8 | 0.8 | 3.0 | 100 |
| | II | 1.9 | 0.5 | 1.4 | 47 |
| | III | 2.7 | 0.6 | 2.1 | 70 |
| | IV | 4.2 | 1.6 | 2.6 | 87 |
| 3 | I | 4.8 | 0.9 | 3.9 | 100 |
| | II | 2.1 | 0.5 | 1.6 | 41 |
| | III | 2.6 | 0.4 | 2.2 | 56 |
| | IV | 4.9 | 1.6 | 3.3 | 85 |

| proef | methode* | ranse melk | niet-ranse melk | verschil in zuurtegraad | % van het verschil gevonden bij methode I |
|-------|----------|----------------|-----------------|-------------------------|---|
| | | zuurtegraad in | | | |

* See Table 1/zie tabel 1.

Tabel 3. Het bepalen van de vetafbraak volgens vier verschillende methoden in niet-ranse en in de daaruit bereide ranse melk.

observations are not in agreement with those of JAMOTTE (7), who found, by using method I, fat acidities in acidified cream of about 1.5 times as high as those obtained by an extraction method.

It might be assumed that, since methods II and III give a lower recovery of the fatty acids, they should be rejected. For determining the rate of fat hydrolysis in practice, however, those methods are to be preferred which, in the determination of fat hydrolysis in fresh milk, show the smallest variation. In such a case fat hydrolysis will be detected when the determined value exceeds the normal range of fat acidities of fresh milk to only a small extent. If we look at the variation of the fat acidities obtained by each of the methods given in Table 1, the following statement may be made. In the fresh raw milk samples investigated the variation is largest when methods I and IV are used, but it is smaller when methods II and III are used. Therefore the two latter are suitable for the detection of a low level of fat hydrolysis.

That a technique such as method III is indeed more suitable for the de-
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Table 4. Comparison of methods III and IV for the estimation of the fat acidities of milk collected in cans and of bulk tank milk.

| experiment | type of collection ¹ | flavour ² | fat acidity using method | | free fatty acids using method |
|------------|---------------------------------|----------------------|--------------------------|-----|-------------------------------|
| | | | III | IV | IV mg eq./l |
| 1 | C | 0/14 | 0.6 | 2.3 | 1.0 |
| | B | 3/14 | 1.0 | 2.9 | 1.0 |
| 2 | C | 0/11 | 0.6 | 2.3 | 0.9 |
| | B | 3/11 | 1.0 | 2.9 | 1.1 |
| 3 | C | 0/11 | 0.6 | 3.0 | 1.0 |
| | B | 7/11 | 1.6 | 4.0 | 1.4 |
| 4 | C | 0/19 | 0.6 | 2.3 | 1.0 |
| | B | 3/19 | 1.0 | 2.9 | 1.1 |
| 5 | C | 0/19 | 0.6 | 3.0 | 1.0 |
| | B | 7/19 | 1.6 | 4.0 | 1.4 |
| 6 | C | 0/19 | 0.6 | 2.5 | 0.9 |
| | B | 9/19 | 1.4 | 3.6 | 1.2 |

| <i>proef</i> | <i>wijze van ophalen¹</i> | <i>smaak²</i> | <i>III</i> | <i>IV</i> | <i>vrije vetzuren volgens methode IV in mg eq./l</i> |
|--------------|--------------------------------------|--------------------------|------------------------------------|-----------|--|
| | | | <i>zuurtegraad volgens methode</i> | | |

¹C = fresh milk collected in cans/*verse melk opgehaald in bussen*

B = bulk milk, stored and collected in tanks/*melk bewaard en opgehaald in tanks*

²0/14 = none of the 14 graders considered the sample to be rancid, etc./*geen van de 14 keurmeesters beschouwden het monster als rans, etc.*

Tabel 4. *Vergelijking van de methode III met de methode IV. Zuurtegraden bepaald van het vet van melk opgehaald in bussen en in tanks.*

tection of a low level of fat hydrolysis than method IV is demonstrated in Table 4. During a series of experiments carried out at the Institute the suitability for conversion into manufactured products of fresh milk delivered in cans was compared with that of bulk collected milk stored in tanks on the farm. The fresh milk and the stored bulk milk were separate batches. RAADSVELD estimated the fat hydrolysis in the milk on its arrival at the experimental dairy of the Institute. The results show that fresh milk and slightly rancid milk may have almost equal fat acidities using method IV, but different fat acidities using method III.

Finally it must be emphasized that methods II and III are good control methods for following the changes of fat acidities during different stages of converting milk into manufactured products.

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SUMMARY

The fat acidity was determined in a number of fresh and rancid milk samples by four different methods:

- I Rapid Silica Gel method according to HARPER *et al.* (1);
- II BDI test (2);
- III Churning method;
- IV Extraction method according to FRANKEL and TARASSUK (3), as modified by RAADSVELD (4).

The best recovery of fatty acids was achieved by methods I and IV. Due to their small variation when used for fresh milk, methods II and III are suitable for the detection of a low level of fat hydrolysis. The usefulness of the four methods is discussed.

SAMENVATTING

STADHOUDERS, J., TUCKEY, S. L. en RAADSVELD, C. W., *Vergelijking van een viertal methoden voor het bepalen van de vethydrolyse in melk.*

Van een aantal monsters verse en ranse melk werd de zuurtegraad van het vet bepaald met behulp van de vier volgende methoden:

- I De snelle silica gelmethode volgens HARPER *et al.* (1);
- II De BDI-proef (2);
- III De karnmethode;
- IV De extractiemethode volgens FRANKEL en TARASSUK (3), zoals die werd gewijzigd door RAADSVELD (4).

Vetzuren worden het best teruggevonden door toepassing van de methoden I en IV. Bij de bepaling van de zuurtegraad van het vet in verse melk vertonen de methoden II en III de geringste variatie. Deze laatste zijn daarom geschikter om na te gaan of een geringe vethydrolyse heeft plaatsgehad. De bruikbaarheid van de methoden werd besproken.

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