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THE DETECTION AND DETERMINATION OF
NEUTRALIZING AGENTS AND/OR LACTIC ACID IN
LIQUID MILK AND DRIED MILK

III

A COLORIMETRIC ROUTINE METHOD FOR THE ESTIMATION OF
LACTIC ACID (LACTATE) IN MILK AND DRIED MILK¹

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I. PRINCIPLE OF THE METHOD

As among the methods for determination of lactic acid (lactate) in milk mentioned in the literature none is suitable as a routine method for practical laboratory use, and at the same time of sufficient accuracy³, we have tried to design such a method. We think we have succeeded in a very simple way, namely by adding diluted ferric chloride to a serum obtained by precipitating the milk proteins by means of an iron oxide sol as indicated by Tillmans and Luckenbach (1). When lactic acid is present a yellow color is formed, the intensity of which is estimated photometrically and serves as a measure of the quantity of lactic acid. This colour reaction originates from Landwehr (2); it was applied to the investigation of milk by Hillig (3), who however pre-treated the sample in a far more elaborate way.

The reaction is not specific, for, as Landwehr mentions, it is in fact a reaction with hydroxyl groups, so that besides oxyacids, water-soluble polyvalent alcohols and carbohydrates can also react positively. Moreover also carboxylic acids and phenols can give rise to colour reactions. Thus in our case especially lactose and citric acid might interfere. As appears, however, from the low colour values in fresh milk this interference is not of serious importance, as the blank value, at least in bulk milk, seems to vary within rather narrow limits.

II. PROCEDURE

Preparation of serum. To 25 ml of milk slowly and with continual shaking add 20 ml dialysed iron oxide sol (Oxydum Ferricum dialysatum liquidum, 5% Fe₂O₃). After 20 minutes the precipitate is filtered. When the serum is not clear it is poured back on to the filter.

The ferric chloride solution. Diluted solutions of ferric chloride containing no excess of hydrochloric acid are unstable. So a more concentrated stock solution (10%, spec. grav. 20/20 = 1,781), which is stable for some weeks, is prepared.

From this a 0,5% solution for immediate use is made by dilution.

The photometric determination. Of the serum 5 ml is pipetted, diluted with 25 ml water and to it 1 ml ferric chloride solution (0,5%) is added. The extinction is measured in the Pulfrich photometer in a 30 mm cuvette with filter S 43 (violet), taking five readings at each side. From these the quantity of lactic acid is found with the help of a calibration curve.

¹ For previous communications see Neth. Milk and Dairy J. 4 (1950) 22; 4 (1950)

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³ At time of composing this report the paper of J. Davidson, J. Dairy Res. 16 (1949) 209 had not been published.

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525.81

The calibration curve. This is obtained by adding known quantities of lactic acid to fresh milk and plotting the results of the measurements graphically.

Probably also a photo-electric colorimeter will be found useful.

An approximate determination can be made in a simple way by adding in a test glass to 5 ml of the serum 1 ml ferric chloride and comparing the intensity of standard colours in test glasses of the same diameter. These standard colours are obtained by adding known quantities of lactic acid to fresh milk and preparing sera from these in the above-mentioned way. These standard colours can be copied permanently by means of an artificial colour scale, obtained by diluting a 5% potassium bichromate solution with 0.1 *n* sulphuric acid.

III. EXPERIMENTAL DATA OBTAINED WITH DUTCH MILK

As our method was designed in the first place for the control of dried milk, we confined ourselves in the first instance to the investigation of whole milk (bulk) and whole milk powder (spray).

A. Pasteurized whole milk (bulk milk)

On 10 successive days in December 1948 photometer readings and pH measurements were made on pasteurized milk¹ two which known quantities of lactic acid had been added; from the mean values the calibration curve was deduced (see TABLE 1, FIG. 1 and FIG. 2). All photometer values are the means of 10 readings.

The series of milk samples with varying quantities of lactic acid were prepared by mixing 39 ml of milk with 1 ml of solutions of lactic acid of increasing concentrations, determined titrimetrically. (The slight dilution of the milk taking place in this way had no effect, as was shown by control determinations).

¹ Standardized in accordance with the present regulations to a fat content of 2.5% and pasteurized in a Voltana pasteurizer at ca. 80 °C. — Pasteurization of milk, however, even at higher temperatures and for a longer time, was found to have no effect on the result.

TABLE 1. Pasteurized whole milk (H.T.S.T.) with and without the addition of lactic acid.

Lactic acid added <i>Melkzuur toegev.</i> 0.01%	Wednesday <i>Woensdag</i> 1/12		Thursday <i>Donderdag</i> 2/12		Friday <i>Vrijdag</i> 3/12		Monday <i>Maandag</i> 6/12		Tuesday <i>Dinsdag</i> 7/12	
	phot.	pH	phot.	pH	phot.	pH	phot.	pH	phot.	pH
0	0.202	—	0.209	—	—	—	0.196	6.68	0.174	6.60
1	0.284	—	0.267	—	—	—	0.245	6.60	0.245	6.52
2	0.312	—	0.370	—	0.310	—	0.300	6.52	0.289	6.44
3	0.385	—	0.375	—	0.352	—	0.393	6.45	0.358	6.38
4	0.440	—	0.429	—	0.422	—	0.428	6.38	0.430	6.32
5	0.484	—	0.493	—	0.492	—	0.496	6.32	0.485	6.26
8	0.597	—	0.614	—	0.621	—	0.666	6.10	0.644	6.10
10	0.740	—	0.691	—	0.681	—	0.725	6.08	0.696	6.06
20										
40										

TABEL 1. Gepasteuriseerde volle melk (H.T.S.T.) met en zonder toegevoegd melkzuur. Verband

FIG. 1. Whole milk (pasteurized). Relation between lactic acid content and photometer reading (Pulfrich).

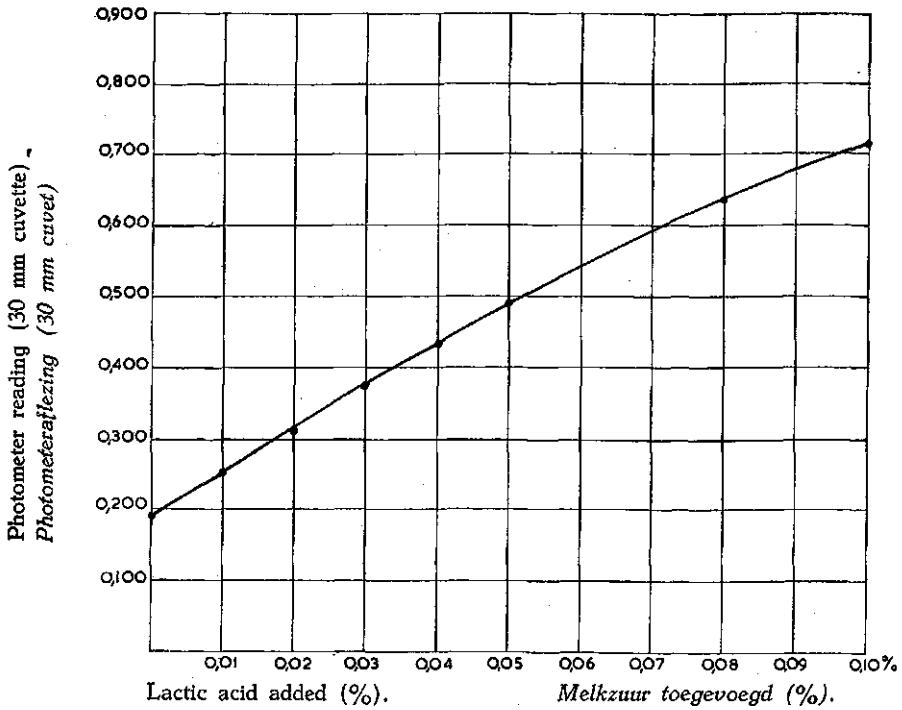


FIG. 1. Volle melk (gepast.). Verband tussen melkzuurgehalte en photometeraflezing (Pulfrich).

Relation between lactic acid content, photometer reading and pH.

Wednesday Woensdag 8/12		Thursday Donderdag 9/12		Friday Vrijdag 10/12		Monday Maandag 13/12		Tuesday Dinsdag 14/15		Mean value Gemiddelde	
phot.	pH	phot.	pH	phot.	pH	phot.	pH	phot.	pH	phot.	pH
0.194	6.63	0.182	6.67	0.190	6.66	0.184	6.62	0.192	6.66	0.191	6.65
0.254	6.57	0.244	6.60	0.236	6.59	0.242	6.57	0.246	6.58	0.251	6.58
0.309	6.49	0.298	6.52	0.289	6.51	0.306	6.50	0.304	6.50	0.304	6.50
0.393	6.42	0.355	6.44	0.359	6.43	0.353	6.42	0.350	6.43	0.367	6.42
0.443	6.36	0.441	6.37	0.421	6.36	0.437	6.34	0.434	6.34	0.433	6.36
0.497	6.29	0.485	6.30	0.480	6.28	0.496	6.27	0.503	6.28	0.491	6.29
0.635	6.09	0.631	6.09	0.636	6.11	0.657	6.08	0.626	6.08	0.633	6.10
0.725	6.00	0.705	6.00	0.726	5.99	0.744	5.99	0.719	5.98	0.715	6.01
				0.902							
				0.951							

tussen melkzuurgehalte, photometeraflezing en pH.

B. Whole spray powder

To obtain mutually comparable results so much powder (a gram) was dissolved in 200 ml of water, that milk with 8,7 % solids-not-fat was obtained.

FIG. 2. Whole milk (pasteurized). Relation between lactic acid content and pH.

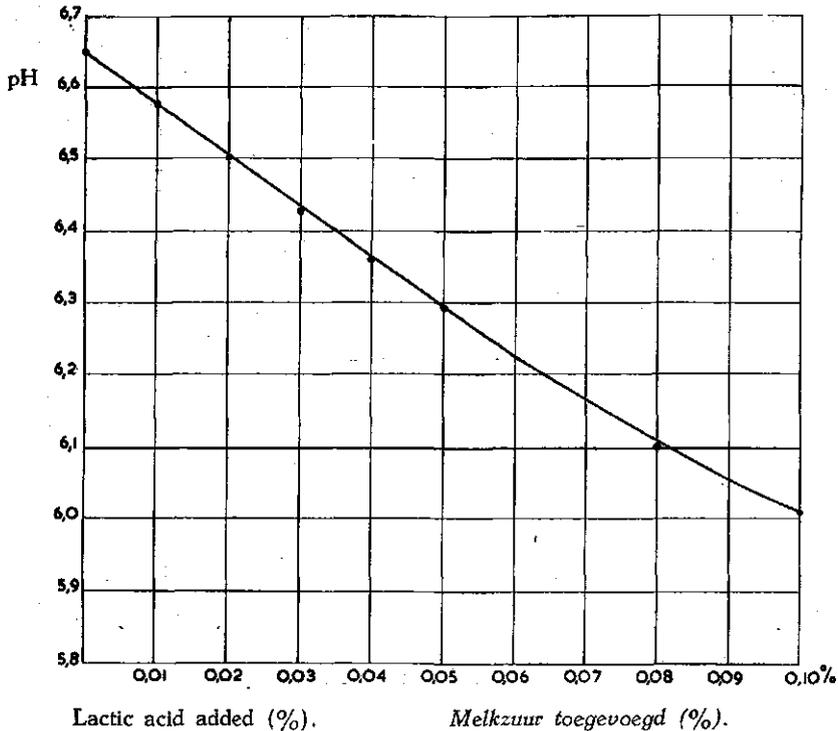


FIG. 2. Volle melk (gepast.). Verband tussen melkzuurgehalte en pH.

The value of a is calculated in the following way. Let the fat content of the powder be V % and the moisture content 3 % (mean value), then the solids-not-fat = $(100 - V - 3)_1$ %, so in a gram of powder $a(1 - 0,01 V - 0,03)$ gram of solids-not-fat is present. This should be 8.7 % of the total weight of water + powder = $(200 + a)$ g.

So:

$$a(1 - 0,01 V - 0,03) = 0,087(200 + a)$$

or:

$$a = \frac{1740}{88,3 - V}$$

For example for powder with 28 % fat, $a = 28,85$ g, whereas for skim-milk powder (fat content 0,5 to 1,0 %) $a = 19,9$ g.

The calculated quantity of powder was shaken for 15 minutes with 200 ml water at 60 °C, then cooled. In the milk thus obtained the determination of lactic acid was performed as described above, after lactic acid had been added in the same way as in the samples mentioned under A.

The pH measurements were made at 20 °C, after the milk had been placed for 4 hours in the refrigerator at about 0 °C, as it was found that the equilibrium was not attained at once.

The results obtained with 18 samples of whole dried milk (spray) are collected in TABLE 2 and in FIG. 3 and FIG. 4.

TABLE 2. Whole dried milk (spray) with and without the addition of lactic acid. Relation between lactic acid content, photometer reading and pH.

Sample Monster Nr.	No lactic acid added <i>geen melkzuur toegevoegd</i>		0,01% lactic acid added <i>0,01% melkzuur toegevoegd</i>		0,05% lactic acid added <i>0,05% melkzuur toegevoegd</i>		0,10% lactic acid added <i>0,10% melkzuur toegevoegd</i>	
	photom.	pH	photom.	pH	photom.	pH	photom.	pH
1	0,187	6,53	0,225	6,49	0,465	6,18	0,715	5,88
2	0,175	6,68	0,271	6,60	0,473	6,28	0,699	5,97
3	0,165	6,67	—	—	—	—	0,688	5,98
4	0,154	6,70	—	—	—	—	0,711	6,02
5	0,176	6,53	—	—	—	—	0,715	5,98
6	0,150	6,69	—	—	0,427	6,30	0,661	5,97
7	0,181	6,60	0,244	6,54	0,479	6,25	0,735	5,98
8	0,170	6,60	0,232	6,54	0,498	6,25	—	—
9	0,182	6,60	0,257	6,52	0,503	6,23	0,727	5,91
10	0,168	6,65	0,227	6,57	0,479	6,26	0,717	5,92
11	0,176	6,69	0,226	6,60	0,487	6,30	0,722	5,95
12	0,178	6,60	0,227	6,52	0,475	6,20	0,721	5,85
13	0,180	6,60	—	—	—	—	0,759	5,88
14	0,172	6,70	—	—	—	—	0,748	5,96
15	0,183	6,68	—	—	—	—	0,741	5,93
16	0,177	6,68	—	—	0,499	6,29	—	—
17	0,184	6,68	0,247	6,59	0,493	6,27	0,733	5,95
18	0,174	6,67	0,245	6,60	0,451	6,28	0,717	5,92
Mean value <i>Gemiddelde</i>	0,174	6,64	0,240	6,56	0,477	6,26	0,719	5,94
Highest value <i>Hoogste waarde</i>	0,187	6,70	0,271	6,60	0,503	6,30	0,759	6,02
Lowest value <i>Laagste waarde</i>	0,150	6,53	0,225	6,49	0,427	6,18	0,661	5,88

TABLE 2. Volle melkpoeder (spray) met en zonder toegevoegd melkzuur. Verband tussen melkzuurgehalte, photometer-aflezing en pH.

These results should be considered as preliminary, for how far the samples in question were prepared from fresh milk in which no formation of lactic acid had taken place, was not known.

However, the nearly perfect agreement of the graphs FIG. 1 and FIG. 3 is an indication that this condition was satisfied to a fairly high degree.

FIG. 3. Whole dried milk (spray) reconstituted to milk of 8.7% solids-not-fat. Relation between lactic acid content and photometer reading (Pulfrich).

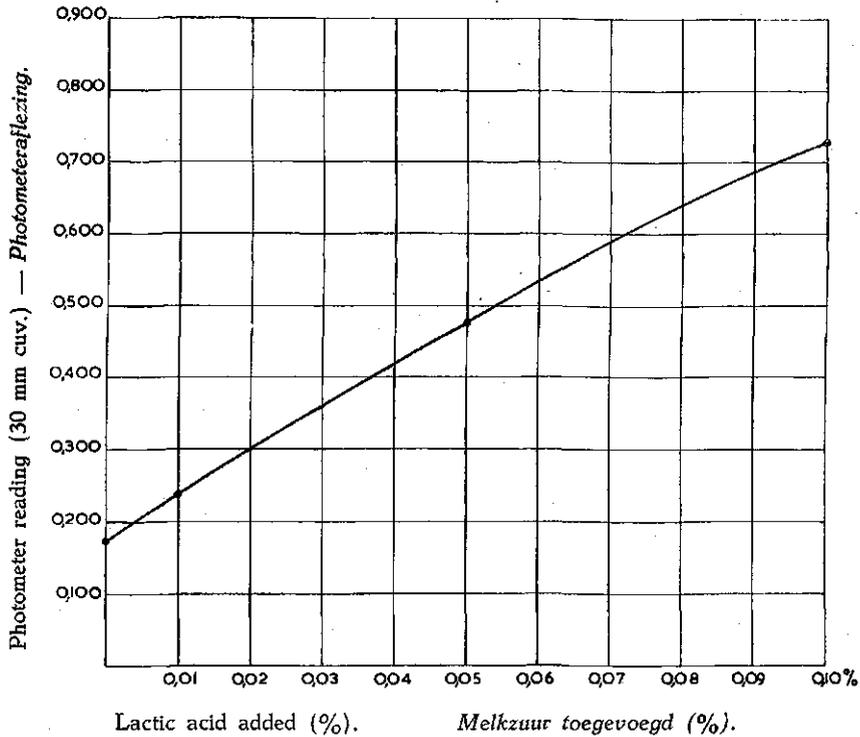


FIG. 3. Volle verstuivingspoeder als oplossing met 8,7% vetvrije droge stof. Verband tussen melkzuurgehalte en photometeraflezing (Pulfrich).

IV. DETECTION OF NEUTRALIZING AGENTS

The amount of neutralizing agent added can be computed in the following way.

From the pH value we can calculate graphically the amount of lactic acid which in the normal case (when no neutralizing agent had been added) would correspond to this pH. The difference between the latter value and the lactic acid content actually present (derived from the photometer reading) gives the quantity of acid neutralized by the alkali added.

FIG. 4. Whole dried milk (spray) reconstituted to milk of 8,7% solids-not-fat. Relation between lactic acid content and pH.

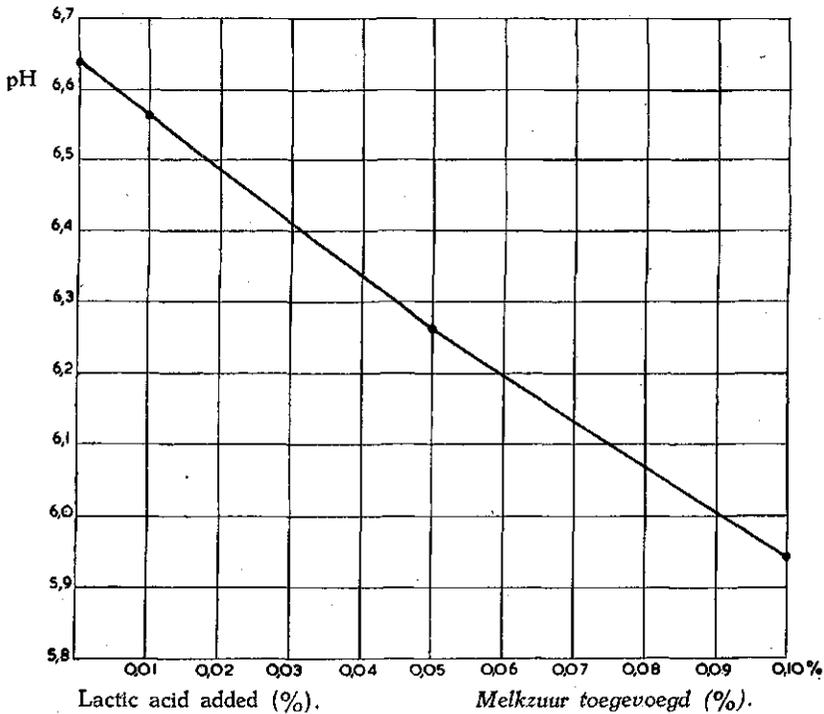


FIG. 4. Volle verstuijvingspoeder als oplossing met 8,7% vetvrije droge stof. Verband tussen melkzuurgehalte en pH.

Part of a sample of fresh milk (A) was acidified by addition of a known quantity of lactic acid (sample B); to a part of sample B a known quantity of sodium bicarbonate was added (sample C). The result of the analysis of the three samples is given in TABLE 3.

V. OUTLOOK

The investigation as to the usefulness of the above methods will be continued by comparing the results with those found by the veratrol and possibly other methods; it will also be applied to fresh milk and different kinds of dried milk (full cream and skimmilk roller and spray powder), etc.

TABLE 3. Examples of calculating the added quantity of neutralizing agent from photometer reading and pH.

Sample	Lactic acid added x 0,01%	NaHCO ₃ added		Photometer reading	pH	Lactic acid x 0,01%				
		x 0,01%	Equiv. to lactic acid x 0,01%			From photom.	From pH	Neutralized acidity cal- culated	Actually neutralized	Difference
A	—	—	—	0,20	6,60	0,2	0,6	-0,4	—	-0,4
B	5,0	—	—	0,51	6,30	5,4	4,9	0,5	—	0,5
C	5,0	3,7	4,0	0,49	6,48	5,1	2,3	2,8	4,0	-1,2

Monster	Toegevoegd melkzuur x 0,01%	x 0,01%	Aequiva- lent met melkzuur x 0,01%	Photometer- aflezing	pH	Meikzuur x 0,01%				
						Photo- metrisch	uit pH	Geneutr. zuurtegr. berekend	Werkelijk ge- neutraliseerd	Verschil
		Na HCO ₃ toev. ev.								

TABEL 3. Voorbeelden van de berekening van de toegevoegde hoeveelheid neutralisatie-middel uit de photometer-aflezing en de pH.

SUMMARY

A simple method is given for the determination of lactic acid (lactate) in milk and dried milk, suitable for practical routine work. The determination is made by addition of diluted ferric chloride to a serum, obtained by adding iron oxide sol to the milk (according to the method of Tillmans and Luckenbach). From the intensity of the yellow colour measured photometrically the lactic acid or lactate content is derived with the help of a calibration curve. In analysing dried milk the latter is dissolved in such a way that the solids-not-fat in the reconstituted milk is 8,7 %.

Two provisional calibration curves are given, showing the course of the photometer readings in the case of whole milk and whole spray powder respectively, to which varying amounts of lactic acid have been added.

By comparing the lactic acid content found in this way with the pH of the milk (or reconstituted milk) an estimate of the amount of neutralizing agents which may have been added is possible.

The investigation as to the suitability and exactness of this method will be continued.

ACKNOWLEDGEMENT

We thank Mr. N. J. Tofte Jespersen (cand. agron.) for his most valuable collaboration.

SAMENVATTING

Venekamp, J. T. N. & Kruisheer, C. I. *Over het aantonen van neutralisatiemiddelen en/of melkzuur in melk en melkpoeder. III. Een colorimetrische melkzuur- (lactaat)bepaling, geschikt voor serieonderzoek.*

Daar onder de in de literatuur aangegeven methoden voor de bepaling van vrij plus gebonden melkzuur (lactaat) in melk, er geen is, geschikt voor serieonderzoek in de praktijk en tevens van een voldoende nauwkeurigheid¹, werd gepoogd een dergelijke methode te ontwerpen.

Daartoe wordt aan het serum, verkregen door praecipitatie van de melkeiwitten met ijzersol volgens Tillmans en Luckenbach (1), verdund ijzerchloride toegevoegd. Bij aanwezigheid van melkzuur ontstaat een gele kleur, waarvan de intensiteit colorimetrisch wordt gemeten. Met behulp van een ijkromme kan hieruit de aanwezige hoeveelheid melkzuur worden bepaald. De ijkromme wordt afgeleid door aan verse melk opklimmende hoeveelheden melkzuur toe te voegen en het resultaat der colorimeteraflezingen grafisch uit te zetten.

De reactie is niet specifiek, immers zoals Landwehr (2) vermeldt, reageren ook meervoudige alcoholen en koolhydraten positief. In ons geval zouden dus lactose en citroenzuur speciaal storend kunnen werken. Blijkens de lage waarden verkregen bij verse melk is deze storing echter niet van ernstige aard, daar de blanco waarde zich, althans bij mengmelk, tussen vrij nauwe grenzen schijnt te bewegen.

Experimentele gegevens: Daar de methode voornamelijk bestemd was voor toepassing op melkpoeder, bepaalden wij ons in de eerste plaats tot het onderzoek van gepasteuriseerde melk en volle melkpoeder.

Een voorlopige ijkromme voor gepasteuriseerde volle melk werd afgeleid uit metingen op 10 dagen in de maand December; aan deze monsters werden stijgende hoeveelheden melkzuur toegevoegd. Het verloop van de gemiddelde photometeraflezing en de pH is weergegeven in TABEL 1, FIG. 1 en FIG. 2.

Voor het onderzoek van melkpoeder werd volle verstuivingspoeder gebruikt. Om onderling vergelijkbare cijfers te verkrijgen werd in 200 ml water zoveel poeder (a gram) opgelost, dat de vetvrije droge stof van de verkregen melk 8,7% bedroeg. Deze hoeveelheid varieert met het vetgehalte (V) en wel bedraagt deze (aannemende dat het vochtgehalte 3% bedraagt):

$$a = \frac{1740}{88,3 - V}$$

De resultaten verkregen met 18 monsters volle verstuivingspoeder vindt men in TABEL 2, FIG. 3 en FIG. 4.

Men diene deze resultaten als voorlopig te beschouwen; immers in hoeverre de onderzochte monsters melkpoeder bereid waren uit volkomen verse melk, waarin nog geen melkzuurvorming van betekenis was opgetreden, was ons niet bekend. De vrijwel volkomen overeenstemming tussen de grafieken FIG. 1 en FIG. 3 wijst er echter op, dat aan deze voorwaarde vermoedelijk was voldaan.

Uit het op bovenstaande wijze gevonden melkzuurgehalte en de pH van de melk (of van de melk verkregen na oplossing van het poeder) kan worden afgeleid in hoeverre neutralisatiemiddelen zijn toegevoegd, namelijk als volgt: Grafisch kan worden bepaald welk melkzuurgehalte normaal (d.w.z. wanneer geen neutralisatiemiddelen waren toegevoegd) met de gevonden pH zou corresponderen. Het verschil van deze waarde en het

¹ De publicatie van J. Davidson (J. Dairy Res. 16 (1949) 209) was ten tijde van het opstellen van dit verslag nog niet verschenen.

werkelijke melkzuurgehalten (afgeleid uit de photometrische waarneming) geeft de hoeveelheid melkzuur, welke door het toegevoegde alkali is geneutraliseerd.

Het onderzoek naar de bruikbaarheid van de bovenstaande methoden zal worden voortgezet.

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3. Hillig, F. J., Ass. Off. Agr. Chem. **20** (1937) 130.