QUANTITATIVE ASPECTS OF CRYSTALLINE LACTOSE IN MILK PRODUCTS

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         gronden

K. Roetman

QUANTITATIVE ASPECTS

OF CRYSTALLINE LACTOSE IN MILK PRODUCTS

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op vrijdag 19 maart 1982
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

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STELLINGEN   NN08201, 884

1. Diverse waarden voor de "beginoplosbaarheid" van α lactose
en β lactose, zoals deze vermeld zijn door Whittier, dienen
te worden herzien op basis van andere waarden voor het mu-
tarotatie-evenwicht.

E.O. Whittier, J.Dairy Sci. 27 (1944) 505.
K. Roetman & T.J. Buma, Neth. Milk and Dairy J. 28 (1974)
155.

2. De aanduiding "bereid met rauwe melk" voor fabriekskaas
moet als misleidend worden aangemerkt wanneer er ten dele van
rauwe melk is uitgegaan en het percentage daarvan niet is
gespecificeerd.

3. De gebruikelijke droogmethoden ter bepaling van het
vochtgehalte van droge melkprodukten zijn ongeschikt wanneer
die produkten kristallijne melksuiker bevatten.

4. De drie door Choi e.a. ontworpen methoden ter bepaling
van de kristallisatiegraad van melksuiker in droge melkpro-
dukten verschaffen weliswaar soms vergelijkbare resultaten,
maar dan is het gevonden niveau te hoog.

R.P. Choi, C.M. O'Malley & B.W. Fairbanks, J.Dairy Sci.
31 (1948) 619.
R.P. Choi, C.W. Tatter, C.M. O'Malley & B.W. Fairbanks,
J. Dairy Sci. 32 (1949) 391.
R.P. Choi, C.W. Tatter & C.M. O'Malley, J. Dairy Sci.
34 (1951) 845.

5. Alternatieve voedingsmiddelen kunnen een gevaar vormen
voor de volksgezondheid indien aan de niet-gemeten en niet-
meetbare kwaliteitsaspekten meer waarde wordt gehecht dan
aan de gemeten.

6. Het baseren van konklusies over de hygroscopiciteit van
melkpoeders op de gemeten taludhoek is per definitie
onjuist.

H. Hendrickx & H. de Moor, Meded. Rijksfac. Landbouw.
Gent 35 (1970) 279.
Dit proefschrift.

7. Er is heftige weerstand vanuit Nederland tegen de
voorstellen van de Europese Commissie (mandaat van 30 mei
1980) om algemene vrijstelling te verlenen van de bestaande
medeverantwoordelijkheidsheffing voor de eerste 30.000 kg
melk per bedrijf en om aan bedrijven die meer dan 15.000 kg
melk per hectare voedergewassen leveren een bijzondere
heffing op te leggen. Als voornaamste bezwaar wordt aange-
voerd dat deze voorstellen de meest doelmatige bedrijven
zullen straffen. Daarentegen is er stilzwijgen over de
voorgestelde extra heffing per bedrijf voor alle produk-
tietoename boven 0,5% per jaar. Deze reakties geven
aanleiding te veronderstellen dat zowel de regering als het
bedrijfsleven het tot dusver verguisde principe van produk-
tieregulatie per bedrijf aanvaarden.

8. Het feit dat rachitis (Engelse ziekte) bij borstkinderen
minder voorkomt dan op grond van het vitamine-D-gehalte van
moedermelk zou zijn te verwachten, is vermoedelijk niet te
danken aan een in de waterfase van moedermelk voorkomende
vitamine-D-metaboliet, maar aan het hoge lactosegehalte en
het lage fosfaatgehalte van moedermelk.

   D.R. Lakdawala & E.M. Widdowson, Lancet i (1977) 167.
   E. Leerbeek & H. Sondergaard, Br. J. Nutr. 44 (1980) 7.
   B.W. Hollis, B.A. Roos, H.H. Draper & P.W. Lambert,
   J. Nutr. 111 (1981) 384.

9. Het is principieel onjuist om zich bij het stellen van
normen aan stoffen waarmee het milieu wordt belast te richten
op het produkt melk.

10. Uit de tekeningen op bladzijde 8 van zijn brochure "Dit
is kaas" zou kunnen worden afgeleid dat Het Nederlands
Zuivelbureau het belang onderkent van de aanduiding van het
vetgehalte in de waar uit een oogpunt van konsumenten-
voorlichting. In dat geval geven de gepresenteerde illustra-
ties een onjuist beeld.

Proefschrift van K. Roetman
"Quantitative aspects of crystalline lactose in milk
products" Wageningen, 19 maart 1982.

Aan mijn ouders

WOORD VOORAF

Bij het tot stand komen van dit proefschrift betuig ik
allereerst mijn dank aan prof.ir. E.A. Vos, die het
vraagstuk van de rol van kristallijne melksuiker in
droge melkprodukten onderkende. Hij heeft door zijn
aanmoedigingen en adviezen mijn onderzoek voortdurend
gestimuleerd.
De medewerkers van het N.I.Z.O. en van het (toenmalige)
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verricht.
Van de velen die het onderzoek met hun adviezen hebben
begeleid wil ik met name dr.ir. J. Koops noemen. Zijn
hulpvaardigheid bij de vele analytische problemen heb
ik zeer gewaardeerd.
Dr. T.J. Buma en dr.ir. Th.E. Galesloot ben ik in het
bijzonder erkentelijk voor hun kritische kant-
tekeningen.
Aan prof.dr.ir. P. Walstra ben ik meer dank verschul-
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promotor wil optreden. Immers niet alleen in de periode
van onderzoek maar vooral in de fase van het bewerken
van de proefresultaten hebben zijn opmerkingen mijn
inzicht ten zeerste verdiept.
De Stichting J. Mesdagfonds van het Kaascontrolestation
"Friesland" te Leeuwarden, heeft met financiële steun
het onderzoek, tot en met de afronding ervan, mogelijk
gemaakt. Mijn werkgevers, DOMO Melkproduktenbedrijven
te Beilen en vervolgens het Zuivelcontrole-instituut te
Leusden ben ik erkentelijk voor de medewerking verleend
bij het voltooien van het proefschrift.

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CURRICULUM VITAE

De auteur is geboren te Zwollerkerspel op 19 mei 1943.
Na het behalen van het diploma Gymnasium-β te Zwolle,
werd in 1961 een aanvang gemaakt met de studie aan de
Landbouwhogeschool te Wageningen. Als studierichting
werd gekozen de zuivelbereiding en de melkkunde. In
1969 werd het ingenieursdiploma behaald, met als hoofd-
vak de zuivelbereiding en de melkkunde (verzwaard) en
als keuzevakken de organische scheikunde en de
voedingsleer. Hierbij werd het predikaat "met lof" toe-
gekend.
Van 1969 tot 1973 werd het onderzoek, beschreven in dit
proefschrift, verricht bij het Nederlands Instituut
voor Zuivelonderzoek (NIZO) te Ede. In deze periode was
er sprake van een detachering bij het instituut vanwege
de Landbouwhogeschool te Wageningen.
Van 1973 tot 1981 was de auteur in dienst van DOMO
Melkproduktenbedrijven te Beilen, laatstelijk als
bedrijfsdirekteur. In 1981 trad hij in dienst van het
Zuivelcontrole-instituut te Leusden.

CONTENTS

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QUANTITATIVE ASPECTS OF CRYSTALLINE LACTOSE IN MILK
PRODUCTS

Keywords: crystalline lactose, determination, milk
products, physical properties.

Summary

The occurrence of crystalline lactose in milk products
and its influence on their physical properties are
briefly reviewed. The importance of the quantitive
determination of crystalline lactose for scientific and
industrial purposes is indicated, and a summary is
given of our earlier work. This refers to the β/α ratio
of lactose in aqueous solution and in the amorphous
state, the structure of spray-dried milk products and
methods for the quantitative determination of
crystalline lactose in milk products. The relation
between these subjects and the quantitative aspects of
crystalline lactose in milk products is discussed.
   Some applications of methods for the quantitative
determination of crystalline lactose are given. These
refer to the improvement of the process for the
manufacture of pre-crystallized spray-dried whey and to
research into the hygroscopicity, free-flowingness and
caking of spray-dried whey.

1 INTRODUCTION

Cow's milk usually contains 4.6% lactose. In many milk
products lactose is a major constituent, and this

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determines to a large extent their physical properties. Because lactose has a low solubility (compared to other sugars) it frequently occurs in the crystalline state.

1.1 Properties of lactose

Some properties of lactose are briefly reviewed as a basis for understanding the main theme of this paper.

Lactose in aqueous solution

There are two stereoisomers, i.e. α and β lactose, which are in dynamic equilibrium. The change of the one form into the other is called mutarotation. The rate of mutarotation in aqueous solution is strongly affected by temperature (1). The equilibrium β/α ratio also depends on temperature. There is considerable dis- agreement in the literature on the effect of tem- perature on this ratio (Section 2).

Each stereoisomer has its own solubility; it is often called "initial" solubility. Due to mutarotation, solutions of both modifications reach the equilibrium β/α ratio and thus have the same "final" solubility. Lactose solutions can readily be supersaturated and the supersolubility indicates the concentration below which spontaneous crystallization does not normally occur. Fig. 1 gives the solubility curves according to Whittier (2). The final solubility shows a transition point at 93.5°C (3,4). Below this temperature the equilibrium solution is saturated with respect to α lactose and above it to β lactose; consequently α lactose crystallizes from aqueous solutions below 93.5°C and β lactose at higher temperatures.

Amorphous lactose

Amorphous lactose is obtained by rapid concentration of a lactose solution, thereby avoiding crystallization.

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solubility
(g anhydrous lac-
 tose / 100 g water)
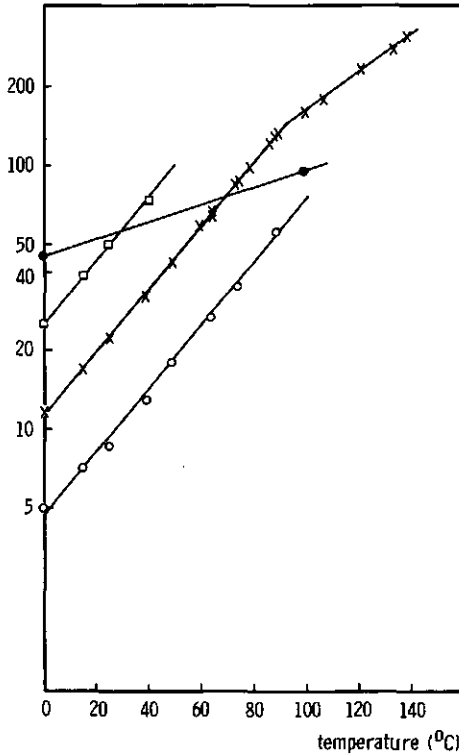
Fig. 1 The various solubilities of lactose
○ initial solubility of α lactose
● initial solubility of β lactose
X final solubility of lactose
▢ supersolubility

This can be achieved by removing water from the
solution by evaporation (as in spray-drying) or by
freezing. Because of its origin, amorphous lactose
consists of a mixture of α lactose, β lactose and
water. The lactose in dried milk and whey is usually in
the amorphous state (5). It has been assumed that the
β/α ratio in these products is fixed and related to the

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temperature of drying. In Section 2 we present evidence
for an equilibrium β/α ratio of approximately 1.25,
regardless of the temperature of drying.
Amorphous lactose can easily be "diluted" by contact
with water or by moisture uptake from the atmosphere.
During solubilization a certain critical concentration
is reached, allowing massive crystallization. This con-
dition favours the formation of minute crystals, which
can be used for seeding purposes (6,7).

Crystalline lactose in milk products

Crystalline lactose may exist in several modifications.
α lactose usually crystallizes as a monohydrate, β lac-
tose in an anhydrous form. Of the other modifications
we mention an anhydrous compound crystal, composition
$\alpha_5\beta_3$. In the following milk products lactose crystal-
lizes at moderate supersaturation at temperatures below
93.5°C, yielding α lactose hydrate:
- concentrated milk
  Lactose crystallizes after prolonged storage of the
  cooled concentrate (e.g. 24 h at 10°C). By spray-
  drying of such a concentrate pre-crystallized dried
  milk is obtained, sometimes unintentionally (8,9),
  sometimes on purpose (10,11,12).
- sweetened condensed milk
  Lactose always crystallizes in this type of product,
  because the supersaturation - already high at room
  temperature - is increased due to the high
  concentration of sucrose (13,14).
- concentrated whey
  In the normal industrial process concentrated whey
  leaves the evaporator at $\sim$ 60°C and is subsequently
  cooled to $\sim$ 10°C to enhance crystallization. By
  spray-drying of such a concentrate pre-crystallized
  dried whey is obtained with crystals of α lactose

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hydrate. The manufacture of dried whey containing
crystals of β lactose requires a special process
(15).

In some products the concentration of lactose is extre-
mely high. Because of supersaturation with respect to
both α and β lactose then, theoretically, both forms
may crystallize, resulting in crystals of α lactose
hydrate, β lactose and the $\alpha_5\beta_3$ compound. This can
apply to the following products:

- amorphous lactose
  Crystallization of α lactose hydrate due to moisture
  uptake is thought to be normal. Crystalline β lactose
  has also been observed (16-18).
- dried milk
  During the production of instant dried milk lactose
  will crystallize at high supersaturation if the pro-
  duct is not dried to below the moisture content
  critical for crystallization, or when the powder
  particles are re-wetted. Post-crystallization also
  occurs due to moisture uptake during storage. Usually
  α lactose hydrate is found (19), but crystalline β
  lactose (17) and the $\alpha_5\beta_3$ compound have also been
  observed (18).
- dried whey
  Crystallization may occur in the same way as men-
  tioned for dried milk. Therefore, predominantly α
  lactose hydrate can be expected. Sharp (17) could not
  find crystalline β lactose in samples of dried whey
  after absorption of water. Later Sharp and Doob (20)
  found crystalline β lactose as well.
- frozen products
  Very high concentrations of lactose may occur in the
  unfrozen liquid part of these products. In ice cream
  α lactose hydrate is usually found, although the pre-

sence of crystalline β lactose has also been observed
(21). In frozen milk concentrate α lactose hydrate
crystallizes (22-24).

Evaluating the literature, the presence of
crystalline lactose other than α lactose hydrate in any
kind of milk product is an exception. But it should be
realized that crystalline α hydrate will nearly always
contain a little β and, likewise, β lactose crystals
contain some impurity in the form of α lactose (25).
This may be of some importance for the accuracy of lac-
tose determinations.

1.2 The importance of quantitative aspects of
     crystalline lactose in milk products

The physical state in which the lactose exists is
thought to be important for the following properties of
milk products.
<u>Hygroscopicity</u>
The very hygroscopic amorphous lactose in dried milk
products can (partly) be replaced by crystalline α
lactose hydrate which is almost non-hygroscopic. This
conversion can be performed by pre-crystallization
(crystallization in the concentrate before spray-
drying), by post-crystallization (crystallization in
the amorphous state during or after spray-drying) or by
both. The wateractivity in a sample of dried whey or
dried milk containing crystalline lactose is increased
(and hence the hygroscopicity is decreased) when com-
pared with that of a similar sample without crystalline
lactose at the same water content excluding the water
of crystallization of the lactose (26-29). In most
patents for "non-hygroscopic" dried whey is claimed
that the product absorbs a negigible quantity of water

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from the atmosphere and that by consequence "caking" is prevented.

Caking

The amorphous lactose in dried whey and dried milk is diluted by water uptake from the atmosphere. When a certain moisture content (the "critical" moisture content) is reached the lactose crystallizes. This post-crystallization usually results in the formation of a hard cake (26). Numerous methods based on crystallization of the lactose have been reported for the manufacture of the so called non-caking or free-flowing product (30-34).

Agglomeration

Agglomeration of the powder particles is widely used to produce dried milk with "instant soluble" properties. Strong agglomerates, not readily broken by pneumatic transport and packaging are preferred. Agglomeration processes are based on collision of wet particles. In some patented processes post-crystallization is prescribed, because the presence of crystalline lactose is considered to be beneficial to the strength of the agglomerates (35,36). However the opposite effect has also been suggested (37).

Free fat content

Post-crystallization in dried whole milk, induced by moisture uptake or by alcohol treatment, strongly increases the apparent free fat content i.e. the portion of the free fat that can directly be extracted by organic solvents (38-40). According to Buma's model (41), increased particle porosity caused by post-crystallization (42,43) could be responsible. Little is known about the influence of pre-crystallization on apparent free fat content. For foam-dried whole milk an increase in apparent free fat content is reported when

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the lactose had been pre-crystallized (44).

Protein solubility

In the amorphous state, lactose (like other sugars)
protects the casein micelles against insolubilization
(45-48). This protection is thought to fail if post-
crystallization occurs (49,50). Crystallization of lac-
tose in frozen milk concentrate leads to a substantial
destabilization of the casein micelles (22,23,24,51).

Reconstitutability

In many patents a beneficial effect of crystalline lac-
tose on the reconstitutability is claimed for both
dried skim milk and dried whole milk. The crystalliza-
tion may be performed by pre-crystallization, by post-
crystallization or by both (33). Other workers deny
that crystalline lactose contributes to the instant
properties (52,53,54) or even claim a beneficial effect
of amorphous lactose (30,55).

Sandiness

Lactose crystals may cause the defect "sandiness" in
sweetened condensed milk and in ice cream. According to
number and size, the crystals are perceptible in the
mouth during consumption (56). At present sandiness in
ice cream is diminished by an improved (fast) freezing
process (57). In sweetened condensed milk sandiness is
prevented by the formation of impalpable, minute
crystals obtained by the use of appropriate seeding
material (6,58).

It can thus be concluded that the presence of
crystalline lactose is important for the quality of
several milk products. However, there is considerable
uncertainty about the magnitude of the effect on some
product properties. Sometimes it is even questionable
whether the presence of crystalline lactose is benefi-
cial or detrimental. Particularly in the patent litera-

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ture, no explanation is given for an alleged beneficial
effect. Usually quantitative determination of
crystalline lactose is not mentioned, or the method of
determination is obscure or questionable. Quantitative
assessment of crystalline lactose would be most helpful
for research purposes.

In the dairy industry interest in the crystallization
of lactose has changed during the past 25 years. Now
crystallization of lactose in sweetened condensed milk
and in ice cream is no longer a problem and attention
is focused on the crystallization of lactose in con-
centrated whey. This has become a large scale
industrial operation for the manufacture of lactose
from concentrated whey, of pre-crystallized dried whey
and of whey products with a high protein content.
Quantitative determination of crystalline lactose is
important for the improvement and the control of the
crystallization process. Industry also has a special
interest in the improvement of the quality of dried
whey and dried milk. Customers ask for products which
are easy to handle and ask for such properties as:
instant solubility, free-flowingness, absence of
caking, of hygroscopicity and of dustiness. As dis-
cussed above, crystalline lactose is likely to play a
part and its quantitative determination may be helpful
in the improvement of these products.

2 THE β/α RATIO IN NON-CRYSTALLINE LACTOSE

Some methods for the quantitative determination of
crystalline lactose are based on the determination of
isomers (Section 4). If the ratio of isomers of the
lactose in the product can be determined the fraction
crystallized can be calculated, provided the ratio of

isomers in the non-crystalline part is known. Therefore
it was important to know the β/α equilibrium ratio in
aqueous solutions and in amorphous lactose for the
examination of liquid and dried products respectively.
From our studies some controversies in the literature
could be explained, and new results on the mutarotation
equilibrium were established.

2.1 Temperature dependence of the equilibrium β/α ratio in aqueous solution (article I)

Results in the literature on the temperature dependence
of the equilibrium β/α ratio differ widely (Fig. 2). It
was therefore necessary to examine the cause of the
discrepancy and to find the correct values.
    Three different methods, all based on polarimetric
measurements, were used to determine the β/α ratio with
modern equipment. The first method was based on the
determination of specific optical rotations of α and β
lactose, and of the equilibrium solution at various
temperatures. A correction was made for impurities in
the crystals of α and β lactose, as determined by gas
chromatography (25). In the second method solutions,
equilibrated at various temperatures, were very quickly
brought to 25°C. By extrapolation to zero time of
polarimetric readings, performed at this temperature,
the original β/α ratio was calculated. The third method
was based on the mutarotation of mixtures of crystals
of α lactose hydrate and β lactose, dissolved in water
at a certain temperature. The ratio giving zero mutaro-
tation must have been equal to the β/α equilibrium
ratio at that temperature.
    The results of all three methods compared well. We
found that the β/α ratio decreased with increasing tem-

β/α ratio
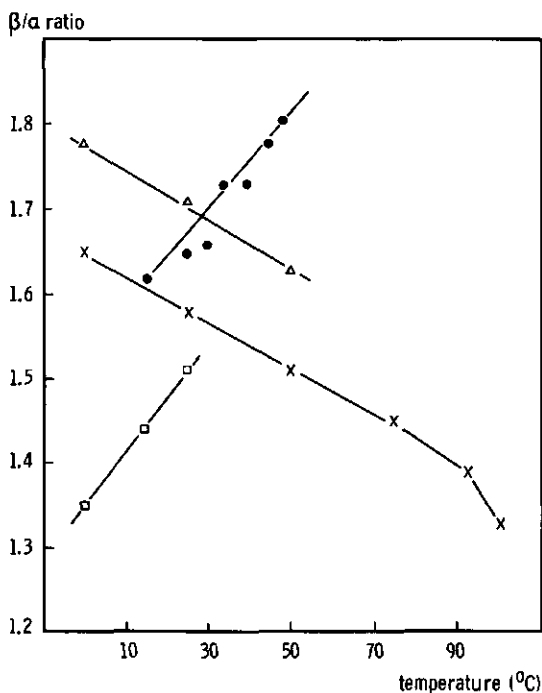
Fig. 2 Temperature dependence of β/α ratios in equilibrium lactose solutions reported by Hudson (□), Gillis (✗), Kendrew and Moelwyn–Hughes (●) and Nickerson (△), as reviewed from the literature in article I.

perature from about 1.64 at 0°C to about 1.36 at 100°C (Fig. 3). This was a confirmation of earlier results of Gillis (3), and we could also explain why the results of some other workers showed deviations.

2.2 The β/α ratio of lactose in the amorphous state (article II)

It is generally assumed that the α and β modifications in amorphous lactose are present in the ratio of an equilibrium solution at the temperature prevailing

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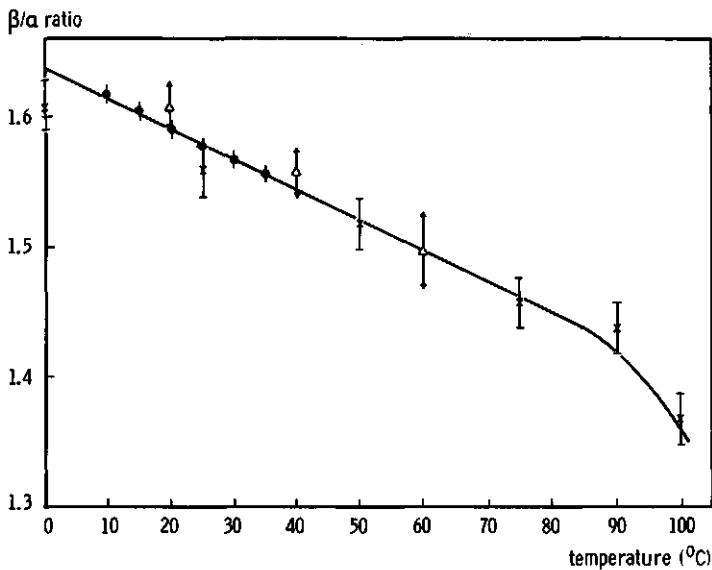
Fig. 3 β/α ratios in equilibrium lactose solutions at various temperatures obtained by three methods described in article I.

during the drying process. This β/α ratio is assumed to be fixed by drying. We found no relation between the outlet temperature of the drier (i.e. the prevailing drying temperature) and the β/α ratio of spray-dried milk products. In fact the β/α ratios found in amorphous lactose usually corresponded to temperatures which the solutions could never have reached during drying. In studies with lyophilized dried milk, mutarotation did occur in amorphous lactose at a rate depending on moisture content and temperature. Mutarotation was extremely slow at low temperatures and at low moisture contents (a shift of 0.1 in the β/α ratio at 25°C in lyophilized skim milk with 2% water may take months), and strongly increased at higher temperatures and higher water contents (a shift of 0.1 in the β/α ratio at 50°C was reached in about 3 hours in

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lyophilized skim milk containing 6% water).

These results lead to the conclusion that mutarotation in amorphous lactose can occur during drying as well as thereafter. Our results also show that there appears to be be a fixed equilibrium β/α ratio of about 1.25 in amorphous lactose, independent of temperature.

3 CRYSTALLINE LACTOSE AND THE STRUCTURE OF SPRAY-DRIED MILK PRODUCTS (article III)

Several methods for the quantitative determination of crystalline lactose in milk products can only be applied to dried products if a suspension in an aqueous medium can be made (Section 4). Both solution and growth of the suspended crystals during the performance of the test cause erroneous results. The rate of these processes depend on crystal surface area, hence on size and shape. Crystal size as such is also important in methods based on separation of crystals or on estimation of the refractive index.

Whether a product contains crystalline lactose can easily be detected using a polarizing microscope with crossed Nicols. In this way we observed that crystal size and powder structure of pre-crystallized dried whey could vary widely. In previous work (60) micrographs were shown of samples of dried whey made from concentrate with 54% and 60% total solids respectively. In the former sample the crystals were large (~ 100 µm) and they were mostly separated from the smaller powder particles (20-50 µm). In the latter sample small crystals were observed (~ 2 µm) enclosed in large powder particles (~ 100 µm).

More detailed information about the shape of lactose crystals and the resulting change in the structure of

spray-dried milk products was obtained by applying
scanning electron microscopy. Micrographs of dried lac-
tose solutions, dried whey and dried skim milk with and
without crystalline lactose were presented (article
III). The products containing crystalline lactose were
either pre-crystallized or post-crystallized. The shape
of the crystals in the two types of products was found
to be entirely different. Pre-crystallization resulted
in the well known tomahawk shape with a size of 50-100
μm and a specific surface area of about 0.05 m^2/g lac-
tose. In post-crystallized products needle-like
crystals were present with a size of 1-5 μm and a spe-
cific surface area of about 1 m^2/g.

The structure of the individual powder particles in
the post-crystallized products was observed to be more
or less porous as compared with that of the pre-
crystallized products and the products without crystal-
line lactose. Increased particle permeability and hence
increased free fat content as found for post-crystal-
lized products (Section 1.2) is therefore unlikely for
the pre-crystallized type. Crystals obtained by pre-
crystallization were often enclosed in the powder par-
ticles or were at least partly covered by whey or milk
solids.

For further research it would be interesting to
investigate the relation between the fraction of lac-
tose crystallized and some of the properties that may
be related to the physical structure of the powder par-
ticles, such as free fat content, gas permeability,
tendency to cake, free-flowingness, strength of agglo-
merates and reconstitutability in water. Particularly
for this last property it would be important to know
whether crystalline lactose could contribute to better
reconstitution of dried whey and dried milk. Con-

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ceivably the contact angle of powder to water, the
viscosity of the penetrating liquid and the capillary
contraction can be affected.

4 METHODS FOR THE QUANTITATIVE DETERMINATION OF CRYSTALLINE LACTOSE IN MILK PRODUCTS (article IV)

4.1 Principles of methods

Methods for the determination of the fraction of the
lactose which is crystalline, can be classified in four
types, based on different principles:

<u>Separation</u> (SEP methods)

If all the crystalline lactose can be separated from a
product, its proportion can be determined. After
separation from a suspension the fraction crystallized
can be calculated from, for instance, the lactose con-
tent of the original suspension and that of the
supernatant. We devised a method based on separation by
centrifugation, for concentrated whey, dried milk and
dried whey.

<u>Measurements of a property of the product to which
crystalline and dissolved lactose do not contribute to
the same extent, particularly of the refractive index</u>
(REF method)

The refractive index proved to be a suitable parameter
since crystals larger than about 0,1 μm do not
materially contribute to the refractive index of the
liquid in which they are suspended, when measured with
sodium light. A method was worked out for concentrated
whey, dried whey and dried milk.

<u>Determination of isomers</u> (IS methods)

Usually the crystalline lactose in dairy products is α
lactose hydrate (Section 1.1). Consequently, the frac-

tion crystallized can be calculated from the ratio of isomers of the lactose in the product and from the equilibrium ratio of isomers in the non-crystalline part therein. This follows from the assumption that for most products in most situations the β/α ratio in the non-crystalline part will be equal or nearly equal to the equilibrium β/α ratio. As this ratio is known (Section 2), only the ratio of isomers in the product has to be determined. Methods based on polarimetric or gas chromatographic determination of the latter were tried for dried products. The polarimetric method was also applied to concentrated whey.

Direct estimation of the water of crystallization of α lactose hydrate (CW methods)

In most products all the crystalline lactose is α lactose (Section 1.1). Its proportion can be derived from the content of water of crystallization, this being 5.00% of the crystals by weight. We tried to determine this content in two ways. The first method was based on the determination of all the water (water of crystallization included) of the product by e.g. the Karl Fisher titration, in conjunction with another water determination in which the water of crystallization was excluded (e.g. by drying under moderate conditions). The second method was based on the heat effect associated with the release of water of crystallization at high temperatures applying differential thermal analysis.

4.2 Evaluation of methods

The principles described before are all known in the literature or in dairy practice. However, most of the methods based on them have never been described in

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detail and have never been scrutinized and compared. Taking into account the principle of the method, the composition of the product tested and the relevant physical properties of the constituents of the product, equations were derived for the calculation of the fraction crystallized. In a similar way possible limitations and inaccuracies were estimated in order to optimize test conditions.

Attention was focused on concentrated whey, dried whey and dried milk. The SEP and REF methods mentioned can only be applied to dried products if a suspension in water can be made that contains the lactose crystals of the product. This is of course a complicating condition, which could cause erroneous results: during the test procedure no change in the quantity of crystalline lactose is permitted and undissolved material other than crystalline lactose should be absent or should not interfere with the result. We found a suitable procedure for pre-crystallized dried whey. For samples of post-crystallized dried whey and dried milk a change in the fraction crystallized during performance of the test could not always be prevented. Moreover undissolved non-lactose solids were found to be the cause of incorrect results for samples of dried milk. So the validity of the results obtained by either method depends on the type of dried product (pre-crystallized or post-crystallized).

From model experiments, recovery tests and comparison of results obtained by the various methods when applied to the same samples, the major conclusions were:
1. The polarimetric method (IS-POL) is paricularly useful for dried whey and dried milk. Inaccuracies can be caused by deviation of the β/α ratio in the non-crystalline lactose from the equilibrium ratio taken

for calculation of the result. Considerable deviations were found to occur in fast crystallizing solutions and were assumed to exist in some samples of post-crystallized dried products.

2. The refractive index method (REF) is particularly useful for process control for the crystallization of lactose in concentrated whey, because the method is quick and simple to carry out. The method cannot be advised for post-crystallized dried products.

3. The separation methods (SEP) cannot be advised for dried products. There are various modifications possible: based on determination of lactose and/or total solids content. In particular, the modifications based on lactose determination according to the Luff-Schoorl method were considered to be inaccurate.

4. The method based on the estimation of water of crystallization by different water determinations (CW-WAT) can only be applied to dried products. We found that a correction is necessary for a blank and that this blank value cannot be determined accurately for dried milk.

5. The gas chromatographic method (IS-GAS) and the method based on the determination of water of crystallization by differential thermal analysis (CW-DTA) were less accurate than comparable methods, i.e. IS-POL and CW-WAT respectively.

The results are summarized in Table 1.

5 POSSIBLE APPLICATIONS

The influence of crystalline lactose on various proper-ties of milk products is largely obscure (Section 1.2), so that there are many opportunities to apply the developed methods for the determination of crystalline

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Table 1. Evaluation of some methods for the quantitive deter-
mination of crystalline lactose in milk products.

Method	Main limitations	Accuracy	Ease of operation
Liquid products			
REF	Crystals should not be very small (> 1 μm)	good	very quick and easy
IS-POL	β/α ratio of non-crystalline lactose must be known, hence crystallizing solutions cannot be tested	good	laborious and expensive
SEP	Based on total solids	fair	very laborious
	Based on refractive index (for concentrated whey)	fair	fairly quick
	Other types unsuitable		
Dried products			
REF	Only for pre-crystallized products. No undissolved matter except crystalline lactose, hence not reliable for dried milks	fair	quick and easy
IS-POL	β/α ratio of non-crystalline lactose must be known, which may be a problem in post-crystallized products	good	laborious and expensive
SEP	Usually not suitable	poor	very laborious
CW-WAT	The blank value must be known precisely, hence not applicable to dried milk.	fair	fairly quick
IS-GAS	See IS-POL	fair	laborious and expensive
CW-DTA	Only suitable for pre-crystallized products	poor	laborious and expensive

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lactose in further research. We briefly studied
moisture absorption, free-flowingness and caking of
dried whey. We shall mention some preliminary results
in Sections 5.2 and 5.3.

Methods for the quantitative estimation of
crystalline lactose are also important for process
control and improvement of the process of lactose
crystallization in milk products. We shall refer to
some results of our previous work in Section 5.1. The
materials used are listed in an appendix.

5.1 Improvement of the process for the manufacture of
    pre-crystallized spray-dried whey

Some product properties of spray-dried whey are
improved by the presence of crystalline lactose
(Section 1.2). We feel that an increased yield (the
water of crystallization is considered as dry matter)
and improvement of the production process (e.g. a
reduction in the frequency of blocking and wet cleaning
of the drier) are equally important incentives for the
industry to apply pre-crystallization. A high fraction
of crystallized lactose is desirable with regard to all
three aspects.

The process conditions for the crystallization of the
lactose in concentrated whey were studied (59,60). The
refractive index method was found to be suitable to
determine the fraction crystallized in concentrated
whey. The rate of crystallization was investigated in
relation to total solids content, temperature and the
addition of seed lactose. As seen in Fig. 4 the rate of
crystallization strongly depended on the concentration
factor (expressed as total solids content). These tests
were performed in continuously stirred concentrates

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fraction crystallized (%)

```
100
 80                                    63,4
 60                                    59,5
                                       54,0
 40                                    42,6
 20
                                       38,6
  0   4   8   12  16  20  24           35,5
```

time (h)
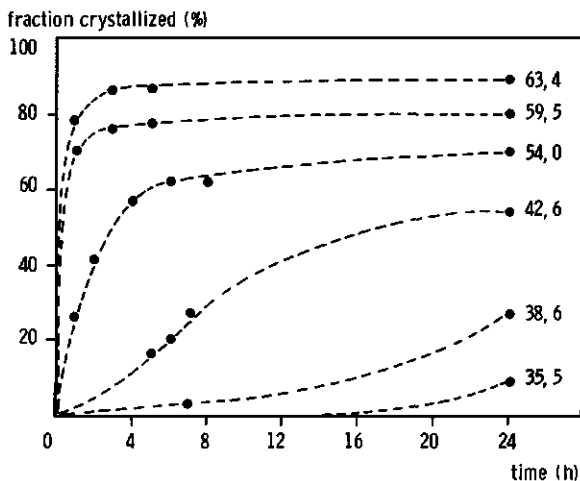
Fig. 4 The influence of the percentage of total solids
(parameter) on the crystallization of the lactose in unseeded
concentrated whey as a function of time after cooling to 20°C.

after cooling to 20°C, without addition of seed
lactose. We found that a change of temperature within
the range of 15-35°C had little effect on the rate of
crystallization for concentrates with total solids con-
tents of 50-60%, though the final fraction crystallized
materially increased with decreasing temperature (Table
2). The addition of seed lactose to concentrated whey
with a low total solids content was found to be
significant for the rate of crystallization for the
lower contents of total solids but it had no noticeable
effect when this was about 60%.

In addition to the traditional advantages of a high
total solids content of the concentrate (the capacity
of the drier is increased, the economy of the drying
process is improved) there are the following advantages
with respect to crystallization of the lactose:
- a high fraction of crystallized lactose can be
  obtained

Table 2. The effect of temperature on the crystallization of
lactose in three samples of concentrated whey.

Time after	Fraction crystallized (%)								
cooling (h)	total solids 52.8%			total solids 55.1%			total solids 61.2%		
	15°C	25°C	35°C	15°C	25°C	35°C	15°C	25°C	30°C
1	49	53	51	34	34	38	76	76	76
2	58	57	56	48	48	49	79	78	78
4	66	64	61	63	61	59	85	85	82
6	67	65	61	68	63	61	87	85	82
24	70	66	61	76	64	61	88	85	82

- the rate of crystallization is increased
- the rate of crystallization depends little on tem-
  perature, and addition of seed lactose is not needed
- the powder particles obtained after spray-drying are
  less vulnerable to damage by pneumatic transport,
  resulting in reduced losses in the exhaust air. A
  possible explanation is that the small crystals
  obtained at a high total solids content (Section 3)
  are enclosed in the powder particles and do no
  damage, whereas the larger crystals obtained at lower
  solids content are in between the powder particles,
  to some extent crushing them during agitation.

In modern evaporators, especially designed for whey,
total solids contents of 65% can be reached. Because of
the high rate of crystallization at these high con-
centrations it becomes interesting to study continuous
crystallization (61). This would dispense with the use
of large storage tanks for the crystallization process,

which are common nowadays. As the holding times,
applied at present, can be as long as 24 hours or more,
continuous crystallization may also reduce microbial
risks. We did a few tests (62), but had no opportunity
to investigate the subject further.

5.2 Water absorption by spray-dried whey as affected by
    crystalline lactose

We exposed various mixtures of amorphous lactose (L21)
and of α lactose hydrate (L3) with a total weight of
about 4 g each, in petri dishes to a relative humidity
(R.H.) of ∿ 100% at 25 ± 0,2°C. Water uptake was deter-
mined by weighing. As seen in Fig. 5 there is a maximum
water uptake, which is proportional to the content of
amorphous lactose in the mixture. Crystalline lactose
absorbed a negligible amount of water. This absorption
pattern is only to be expected. Amorphous lactose is
the hygroscopic component. Once sufficient water is
absorbed to crystallize it is converted into α lactose
hydrate which is practically non-hygroscopic. Hence
water absorption is followed by desorption proportional
to the amount of amorphous lactose. A similar pattern
is often suggested in the patent literature when a
reduction in hygroscopicity is claimed for spray-dried
milk products.
  We tested several samples of dried whey with frac-
tions of lactose crystallized (K) ranging from 0-70% at
similar conditions as applied for the mixtures of
amorphous and crystalline lactose. Now a different
water absorption pattern was found. All samples showed
continuing absorption during 48 hours of exposure and
there were rather small differences in absorption rate.
Only the results obtained in the early 10 hours of the

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moisture absorption
(mg water /g product)
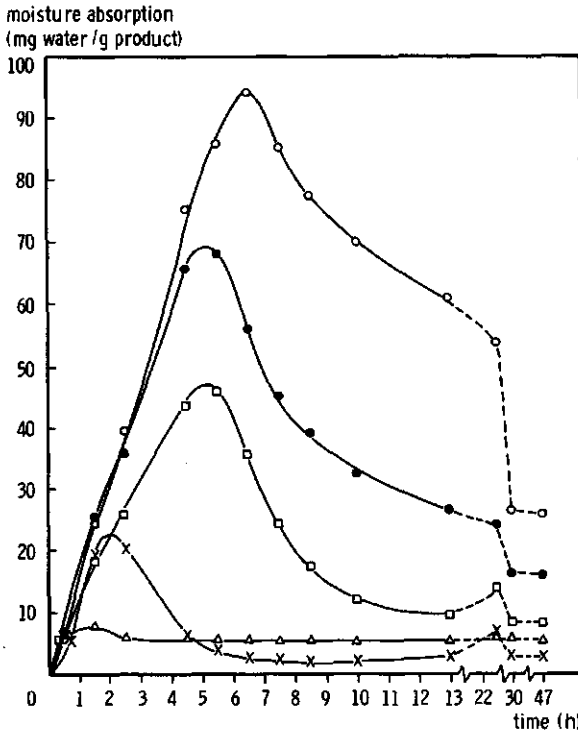
Fig. 5 Water absorption (mg/g product) by amorphous lactose, by crystalline α lactose hydrate and by mixtures of both at 100% relative humidity and 25°C.

O amorphous lactose (L21)

Δ α lactose hydrate (L3)

● 75% amorphous lactose and 25% α lactose hydrate

◻ 50%   "   "   " 50% α   "   "

✕ 25%   "   "   " 75% α   "   "

experiment seemed to be related to K. The test was repeated with a second set of samples of dried whey and with amorphous lactose. Now K was determined at several moments of absorption. Because a quick determination was needed after 2 and 4.5 h of absorption time, the refractive index method was used, although the method is not accurate if applied to post-crystallized pro-

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ducts (Section 4). Complete determination of all
samples with the more accurate polarimetric method
would have lasted several hours, permitting con-
siderable crystallization during the experiment.
As can be seen in Table 3, the lactose crystallized in
all samples, reaching finally approximately the same K-
value. (The decrease of K observed after 29 h for some
samples was probably caused by solution of crystalline
lactose as 25% water had been absorbed). The loss of
weight, observed for amorphous lactose, is clearly due
to considerable crystallization. During exposure the
differences in quantities of water absorbed by the
samples of dried whey disappeared, which is easily
understood since the K-values become similar during
crystallization. Release of water as caused by
crystallization of amorphous lactose was probably
masked by absorption by other constituents at the pre-
vailing high relative humidity.

Absorption tests were carried out with spray-dried
milk constituents (specified in the list of materials)
to produce further evidence for this hypothesis. Expo-
sure was not only performed at an extremely high rela-
tive humidity (98% R.H.), but also at a moderate 50%
R.H. For this test all products were dried at 25°C over
concentrated sulphuric acid (95-98%) during 130 h to
equilibrate the powders to the same relative humidity.
Quantities of 2.5 g of each sample, spread out in
weighing dishes ($\emptyset$ 4 cm) were placed in an air-
conditioned room at 25 $\pm$ 0,2°C. Water absorption was
determined by weighing. The results are shown in Table
4. At 98% R.H. all products, except for the salts and
the amorphous lactose, showed about the same absorption
rate. Release of water was not observed, even for
amorphous lactose, probably because crystallization was

Table 3. The fraction crystallized (K) of samples of dried whey
(WP 22, 1B, 5 and 7) and spray-dried lactose (L 21) during
water absorption at 25°C and 98% relative humidity.

Analyses	Spray-dried product				
	WP 22	WP 1 B	WP 5	WP 7	L 21
Initial K (IS-POL)	0	7	50	63	0
After 2 hours:					
K (REF)	37	85	97	95	32
water absorbed (mg/g)	120	81	76	67	50
After 4 1/2 hours:					
K (REF)	57	99	100	100	95
water absorbed (mg/g)	155	143	117	110	23
After 29 hours:					
K (IS-POL)	89	90	90	92	95
water absorbed (mg/g)	262	256	245	258	15

already complete within 3 hours. At 50% R.H. the
amorphous lactose and the mixture of lactose and salts
decreased in water content, after an initial increase.
For preparations simulating dried skim milk and dried
whey there was no such decrease. Although the isolates
used may behave differently from the components of milk
which they represented and interactions between them in
dried whey and dried milk may not be the same, these
results indicate that the presence of constituents
other than lactose mask the release of water due to
crystallization of the lactose.

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Table 4. Water absorption (mg/g product) by spray-dried milk components at 50 and 98% relative humidity (R.H.) and 25°C.

R.H.	Time of exposure	Preparation				
(%)	(h)	MC1	MC2	MC3	MC4	MC5
50	2	32	8	23	38	40
50	5.5	66	13	61	70	67
50	7.5	78	13	68	78	73
50	24	41	36	24	89	101
98	3	61	154	123	169	173
98	7	70	306	207	248	250
98	12	74	455	288	311	300
98	24	70	791	410	427	417

To demonstrate the influence of K on water absorption at moderate humidities some samples of dried whey (WP22, 2C, 2F, 2G, 10) were exposed to air of 50 and 75% R.H. in an analogous way. K was determined by the refractive index method during absorption (approximately one determination every two hours). At 50% R.H. no substantial crystallization occurred. There were differences in water absorption between the samples and these were correlated with differences in K (Fig. 6). At 75% R.H. substantial crystallization occurred after 7 hours of exposure in those samples already containing crystalline lactose from the beginning. Also here there was a difference in the amount of water absorbed as long as there were large differences in K. Neither at 50 nor at 75% R.H. could a decrease in water content be observed.
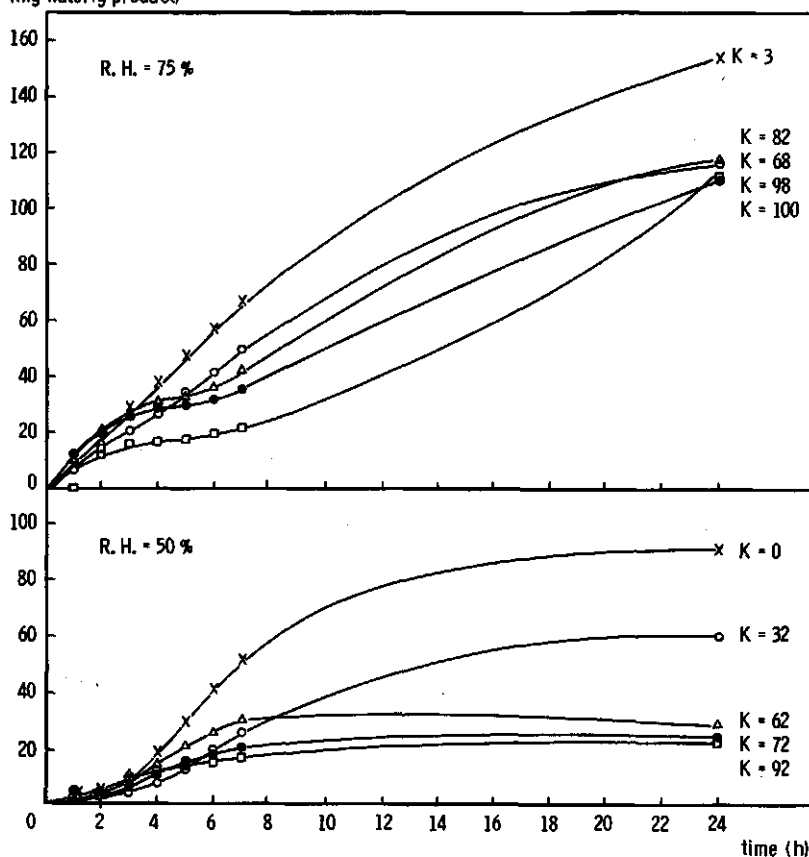
Fig. 6 Water absorption (mg/g product) by samples of spray-dried whey at 50 and 75% relative humidity (R.H.) (25 ± 0.2°C). Changes in the fraction crystallized (K) are indicated.

○ WP 2C initial K-value 22%
△ WP 2F   "   "   51%
● WP 2G   "   "   66%
◻ WP 10   "   "   90%
✗ WP 22   "   "   0%

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The following conclusions can be drawn from these experiments with dried whey.
1. The rate of water absorption depends on the relative humidity, as is only to be expected.
2. Release of water by lactose, due to its crystallization during absorption, is surpassed by water absorption by non-lactose constituents.
3. Differences in the initial values of K cause differences in water absorption. But if the relative humidity is high enough and the time for water absorption long enough to enable most of the lactose to crystallize, a final water content is reached which is independent of the initial value of K.

5.3 Free-flowingness and caking of dried whey as affected by crystalline lactose

Conversion of amorphous lactose into crystalline lactose allegedly improves free-flowingness and diminishes the caking tendency of dried whey (Section 1.2). To study the influence of the fraction crystallized (K) on these properties, experiments were carried out with samples of pre-crystallized dried whey (WP2A-G) with various values of K. Mixtures of the sample of K = 0 (WP2A) with large (L2) or small (L1) crystals of α lactose hydrate, and a mixture of the former with a commercial free-flowing agent, "tixolex" (T), also were tried.

Free-flowingness was characterized by measuring the angle of repose of a conic powder heap made by the flowing of a sample through a funnel at a fixed distance from a plane surface. The measurement was carried out as described by Sjollema (63). The intensity of caking was evaluated after water absorption

under standard conditions (72 h at 25°C, 75% RH and re-drying during 4 h at 50°C). In addition to visual observation (stirring of the caked powder with a glass rod in a petri dish), various mechanical tests were tried to find an objective criterion. The following procedure was thought to be useful. A Soxhlet extraction thimble filled with product was stored to absorb moisture under standard conditions. After re-drying as indicated, the thimble was carefully removed and the axial force needed for the powder plug (4 cm diameter, 10 cm height) to fall apart was recorded by a "tenderometer", developed by the "Sprenger Institute", Wageningen, the Netherlands. K values were determined before and after moisture absorption.

The results are given in Table 5. No clear effect could be observed of the initial vlue of K of spray-dried whey on the free-flowingness, as judged by the angle of repose. Addition of coarse crystals of lactose (L2) or of "tixolex" (T) strongly affected this property, whereas addition of seed lactose (small crystals, L1) dit not. The tendency to cake, both judged visually and by "tenderometer" was much reduced when K had been increased by pre-crystallization up to a value of about 50%. Addition of coarse lactose crystals before moisture uptake had a similar effect, whereas addition of "tixolex" had no effect at all. No caking could be observed if seed lactose had been added.

Obviously, free-flowingness as measured by the angle of repose and the tendency to cake are different properties, affected in a different way by the pre-crystallization. In our opinion, the structure of the powder is important in relation to both properties. The crystals in the samples WP2B-G were fairly large

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Table 5. The influence of crystalline lactose on the free-flowingness and tendency to cake of pre-crystallized whey powders and mixtures of crystalfree whey powder with crystalline lactose or a free-flowing agent (T).

Dried whey	Fraction crystallized (%) (IS-POL) initial	final	Free-flowingness (angle of repose, degrees)	Tendency to cake organoleptically	by tenderometer, in duplicate	
WP 2A	0	98	49	++++	32	28
WP 2B	14	97	53	++++	40	37
WP 2C	22	99	54	+++	26	22
WP 2D	35	97	58	++	23	17
WP 2E	55	98	58	++	9	8
WP 2F	51	100	52	+	11	10
WP 2G	66	98	52	+	12	14
Mixtures (w:w)						
WP 2A + L2 (1:1)	40	100	37	++	11	22
WP 2A + L1 (1:1)	40	92	51	−	0	0
WP 2A + T (99:1)	0	98	33	++++	40	45

(~ 100 μm). Very likely, they were partly covered with whey solids (Section 3) and, consequently, they may not have behaved like added lactose crystals but rather like powder particles, when measuring the angle of repose. To increase free-flowingness, the addition of many smooth particles will be most effective. This may explain that only small quantities of "tixolex" are needed, as compared to coarse crystals of lactose, to obtain the same small angle of repose. The lack of effect of added seed lactose may perhaps be explained by the irregular shape of these crystals as could be seen by electron microscope.

All pre-crystallized samples of dried whey showed some caking when the standard moisture absorption procedure was followed. Naturally, the test conditions very much affected the extent of caking. Because the crystals in this type of product were likely to be partly covered with whey solids, we suggest that caking is always possible in pre-crystallized dried whey, and that the "non-caking" properties claimed in some patents do not exist. Obviously the strength of the cake decreases with increasing fraction of the lactose crystallized. Added particles, be they lactose crystals or not, will have a similar effect. This effect will be larger when the number of particles is higer (compare coarse with seed lactose). The very small particles of "tixolex" may soak into the diluted amorphous lactose during water uptake, thereby losing their ability to prevent caking.

Further study of this subject would be useful, not only to verify the assumptions made, but also to find out how to improve product properties.

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LIST OF MATERIALS
(Particulars of the milk products and preparations used)

LACTOSE (L)
α lactose hydrate

L1	Commercial seed lactose, produced by grinding (Wessanen). Crystal size 0.1 – 2 μm.
L2	Commercial lactose (Lamers and Indemans, P-0200). Sieve fraction > 125 μm. Crystal size: length 250 μm, circumference ∿ 100 μm.
L3	Origin as of L2 but not fractionated.

spray-dried lactose

L21	Pilot plant NIZO. Drying of a 30% solution of lactose hydrate. Air-temperatures: inlet 215°C, outlet 80°C. Moisture content 2.6% (Karl Fischer). Lactose crystals absent.

SPRAY-DRIED WHEY (WP)

WP1B	Pilot plant NIZO. Fresh cheese whey concentrated to 34% TS and spray-dried after crystallization. K=7% (method IS-POL)
WP2 series (A-E)	Pilot plant NIZO. Fresh cheese whey concentrated to 34% TS. Part of it was spray-dried directly (WP2A), other parts (WP2B-E) at various moments of crystallization. K-values 0; 14; 22; 35 and 55% respectively (method IS-POL).
WP2 series (F,G)	Whey from the previous series concentrated to 43% TS and spray-dried with two times of crystallization. K-values 51 and 66% respectively (method IS-POL).
WP5	Commercial pre-crystallized dried whey (DOMO). K=50% (method IS-POL).
WP7	Commercial pre-crystallized dried whey (Acmesa). K=62% (method IS-POL).
WP10	Pilot plant NIZO. Fresh cheese whey concentrated to 55% TS. After crystallization (24h, 15°C) the concentrate was spray-dried. Air-temperature: inlet

	120°C, outlet 51°C. Moisture content 25%. The post-crystallized wet product was dried on a fluid bed to 4.8% water content. (Karl Fischer). K=90%. (method IS-POL).
WP22	Pilot plant NIZO. Fresh cheese whey concentrated to 35% TS and dried immediately. Water content 2.8% (Karl Fischer). K=0%. (method IS-POL).

SPRAY-DRIED MILK

MILK CONSTITUENTS (MC)	All preparations made in NIZO laboratory and pilot plant.
MC1	A spray-dried solution of lactose (SL), prepared similarly to L21.
MC2	A simulated milk salt solution (SMS), prepared according to Jennes and Koops (64), was spray-dried.
MC3	A mixture of SMS and SL (ratio by weight of dry matter 1:7.1) was spray-dried.
MC4	Whey proteins were prepared by dialysing fresh whey against distilled water. The retentate (R), SMS and SL were mixed in a ratio by weight of dry matter 1.4:1:7.1. The solution was spray-dried simulating spray-dried whey.
MC5	Lyophilized sodium caseinate, isolated from skim milk by iso-electric precipitation at pH=4.6, was dissolved by addition of sodium hydroxide. A mixture of this solution, R, SMS and SL (ratio by weight of dry matter 4.5:1:1:7.1) was spray-dried simulating dried skim milk.

VARIOUS

T	Commercial free-flowing agent (Tix.o.lex). Particle size $\sim$ 20 nm.

SAMENVATTING

K. Roetman, Kwantitatieve aspekten van kristallijne
melksuiker in melkprodukten.

Dit proefschrift is hoofdzakelijk gebaseerd op een
viertal tijdschriftartikelen over melksuiker. Deze
publicaties handelen over de β/α-evenwichtsverhouding
van lactose in waterige oplossing (artikel I), de β/α-
verhouding in amorfe lactose (artikel II), kristallijne
melksuiker en de mikrostruktuur van gesproeidroogde
melkprodukten (artikel III) en methoden voor de
bepaling van de kristallisatiegraad van melksuiker in
melkprodukten (artikel IV).

In een inleiding wordt een kort overzicht gegeven van
de belangrijkste eigenschappen van melksuiker. Zo wordt
ingegaan op de verschillende vormen, de mutarotatie en
de kristallisatie zowel in de oplossing als in de
glasachtige (amorfe) toestand alsmede in melkprodukten.
Ook wordt ingegaan op de invloed van kristallijne
melksuiker op de fysische eigenschappen van melkproduk-
ten, zoals vochtopname, samenkoeking, oplosbaarheid
etc. Uit dit overzicht blijkt dat er onduidelijkheid is
over de mate en zelfs over de aard van de invloed van
kristallijne melksuiker op deze eigenschappen.
Geconcludeerd wordt dan ook dat voor verder onderzoek
kwantitatieve bepalingen van kristallijne melksuiker in
melkprodukten zeer gewenst zijn. Ook voor de verbete-
ring van het bereidingsproces van melkprodukten waarbij
de kristallisatie van melksuiker essentieel is, worden
kwantitatieve bepalingsmethoden van belang geacht.

In artikel I werd onderzoek beschreven, verricht naar
het mutarotatie-evenwicht tussen α- en β-lactose. Het
verband tussen deze β/α-evenwichtsverhouding en de tem-

peratuur wordt uit de literatuur niet duidelijk. Niet
alleen worden bij oplossingen met dezelfde temperatuur
zeer uiteenlopende waarden opgegeven, maar ook treft
men een tegengesteld verloop met de temperatuur aan. Om
klaarheid te scheppen in deze situatie werden drie
verschillende methoden toegepast ter bepaling van de
evenwichtsverhouding. Bij alle methoden werd gebruik
gemaakt van polarimetrische bepalingen met behulp van
moderne apparatuur. De eerste methode berust op de
bepaling van de specifieke rotaties van α-lactose,
β-lactose en van lactose in mutarotatie-evenwicht bij
verschillende temperaturen. Bij de tweede methode wer-
den de evenwichtsoplossingen bij verschillende tem-
peraturen zo snel mogelijk op 25°C gebracht om
vervolgens te worden gemeten. Bij de derde methode werd
gebruik gemaakt van mengsels van kristallen van
α-lactose-hydraat en van β-lactose. Alle drie methoden,
waarvan de eerste de meest nauwkeurige was, gaven goed
overeenkomende resultaten. De β/α-evenwichtsverhouding
bleek te dalen bij toenemende temperatuur en wel van
ca. 1,64 bij 0°C tot ca. 1,36 bij 100°C. Met de
uitkomsten die voorheen door Gillis waren verkregen
werd een treffende overeenkomst gevonden.

In artikel II werd ingegaan op de mutarotatie van
melksuiker in glasachtige toestand. Tot nu toe werd
aangenomen dat de melksuiker in gesproeidroogde en
gevriesdroogde zuivelprodukten een β/α-verhouding heeft
die vast ligt. Deze verhouding zou overeenkomen met de
evenwichtsverhouding in de vloeistof bij de temperatuur
van drogen. Bij ons onderzoek werd nagegaan welke
invloed de oorspronkelijk β/α-verhouding van de
vloeistof en de uitlaattemperatuur van de droger hebben
op de β/α-verhouding in het produkt. Aan de hand van
proeven met gevriesdroogde ondermelk werd bestudeerd of

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mutarotatie in amorfe lactose kan optreden. De resulta-
ten leiden tot de veronderstelling dat mutarotatie kan
optreden zowel tijdens het drogen in de oplossing als
daarna in de amorfe toestand. De mutarotatiesnelheid in
de amorfe lactose is daarbij afhankelijk van vochtge-
halte en temperatuur. In droge produkten werd geen ver-
band gevonden tussen de β/α-evenwichtsverhouding en de
temperatuur zoals dat is vastgesteld bij oplossingen
van melksuiker. De β/α-verhouding van gesproeidroogde
produkten bleek lager te liggen dan de evenwichts-
verhouding van oplossingen bij de geschatte droogtempe-
ratuur. Een β/α-evenwichtsverhouding van ongeveer 1.25
in amorfe lactose, onafhankelijk van de temperatuur,
lijkt waarschijnlijk.

In artikel III wordt aangegeven dat de structuur van
gesproeidroogde produkten verandert door kristallisa-
tie van de melksuiker. Met behulp van een microscoop
kan bij gebruik van gepolariseerd licht de aanwezigheid
van kristallijne lactose worden aangetoond. Omdat de-
tails van structuurwijzigingen van het poederdeeltje en
van de vorm van minuscule kristallen op deze wijze niet
kunnen worden verkregen, is bij dit onderzoek gebruik
gemaakt van een elektronenmicroscoop. Onderzocht werden
gesproeidroogde melksuikeroplossingen, wei en onder-
melk. Deze produkten waren gemaakt zonder kristallijne
lactose, met kristallen verkregen door voorkristallisa-
tie (d.w.z. vóór het sproeidrogen) en met kristallen
ten gevolge van nakristallisatie (d.w.z. tijdens of na
het sproeidrogen). De resultaten bevestigen enkele waar-
nemingen die andere onderzoekers op een andere wijze
hadden verkregen. Zo wordt door middel van de foto's
aangetoond dat door nakristallisatie de poederdeeltjes
een min of meer open structuur krijgen waardoor ze
toegankelijk kunnen worden voor gassen en vloeistoffen.

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Nieuw is dat de vorm van de melksuikerkristallen in voorgekristalliseerde en nagekristalliseerde produkten duidelijk verschilt. De eerste groep vertoont de bekende bijlvorm, terwijl in de tweede soort de kristallen naaldvormig zijn.

In artikel IV worden voorschriften voor diverse reeds bestaande methoden ter bepaling van de kristallisatiegraad van lactose ontwikkeld. Een nieuwe, snelle en eenvoudige methode, gebaseerd op de bepaling van de brekingsindex, werd daaraan toegevoegd. De "brekingsindexmethode", de "centrifugeringsmethode" en de "polarimetrische methode" werden onderzocht voor toepassing op geconcentreerde wei. Deze drie methoden te zamen met de "gaschromatografische methode", de "kristalwaterbepalingsmethode" en een methode gebaseerd op differentiële thermische analyse werden getoetst op geschiktheid voor toepassing op weipoeder en mager melkpoeder. De methoden werden beoordeeld op nauwkeurigheid, herhaalbaarheid en gemak van uitvoering.

De voornaamste conclusies zijn:
- de polarimetrische methode wordt in het zuivelonderzoek veelvuldig toegepast maar vaak met gebruikmaking van foutieve waarden voor de β/α-verhouding in het niet-kristallijne gedeelte van de melksuiker in het produkt. De methode is vooral geschikt voor weipoeder en melkpoeder;
- de brekingsindexmethode is bijzonder geschikt voor procescontrole bij de kristallisatie van melksuiker in geconcentreerde wei, omdat ze snel en eenvoudig is uit te voeren. Voor droge produkten is de toepasbaarheid beperkt tot voorgekristalliseerd weipoeder;
- de centrifugeringsmethode is minder geschikt voor weiconcentraat en ongeschikt voor droge produkten. Van

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deze methoden bestaan diverse varianten, gebaseerd op
lactose- en/of drogestofbepalingen. Met name de lac-
tosebepalingen volgens Luff-Schoorl zijn o.i. te
onnauwkeurig om een goed resultaat te behalen;
- de kristalwaterbepalingsmethode is minder bruikbaar
dan vaak wordt gedacht, omdat de blanco waarde nogal
varieert. De methode is uitsluitend bruikbaar voor
droge produkten. Er is gerede aanleiding te twijfelen
aan het eindresultaat bij nagekristalliseerde produkten
met een hoog vochtgehalte, omdat tijdens de bepaling
van het vochtgehalte met een "droogstoofmethode" ver-
dere kristallisatie mogelijk is.

Tenslotte worden enkele toepassingen beschreven van
kwantitatieve bepalingsmethoden van kristallijne
melksuiker. Allereerst wordt aangetoond dat deze metho-
den met succes kunnen worden gebruikt voor de ver-
betering van de bereiding van voorgekristalliseerd
weipoeder. Diverse adviezen aan de zuivelindustrie
waren het gevolg (zie "references" 59, 60 en 65).
Een tweede toepassing wordt gedemonstreerd bij de
bestudering van de wateropname door weipoeder. De
belangrijkste konklusie is dat de vochtopname door
weipoeder nauwelijks beïnvloed wordt door de kristalli-
satiegraad van de melksuiker zoals die oorspronkelijk
in het produkt kan worden bepaald. Wanneer nl. de rela-
tieve vochtigheid hoog genoeg is en de tijd voor
wateropname lang genoeg dan kristalliseert de
melksuiker verder waardoor een eindvochtgehalte wordt
bereikt dat onafhankelijk is van de oorspronkelijke
kristallisatiegraad. Een derde toepassing betreft
onderzoek naar de invloed van de kristallisatiegraad
van melksuiker op de "free-flowingness" en het
samenkoeken van weipoeder. Het werd aannemelijk gemaakt

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dat een dergelijke invloed sterk afhankelijk is van de vorm en de grootte van de kristallen en van de struktuur van het poeder.

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Temperature dependence of the equilibrium β/α ratio of lactose in aqueous solution

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Summary

Data concerning the β/α ratio of equilibrium aqueous lactose solutions found in the literature differ widely. A variety of values has been found for solutions of the same temperature, and both an increase and a decrease of the β/α ratio with temperature have been reported.

In the present investigations three different methods, all based on polarimetric determinations, were used to determine the temperature dependence of the equilibrium β/α ratio in aqueous lactose solutions.

The results of all three methods agree reasonably well. It was found that the β/α ratio decreases from about 1.64 at 0°C to about 1.36 at 100°C, in accordance with the earlier results of Gillis.

1 Introduction

Aqueous lactose solutions in equilibrium contain both α-lactose and β-lactose. When either form of crystalline lactose is dissolved in water, a change from one form into the other takes place until an equilibrium ratio between α-lactose and β-lactose is reached. This can be observed by the change in optical rotation, which is usually called mutarotation.

Widely disagreeing equilibrium β/α ratios at different temperatures have been reported in the literature (see Section 2). Some authors found the β/α ratio to decrease with temperature, but others reported an increase. The purpose of the study reported here was to solve the problem of the temperature dependence of the β/α ratio in equilibrium aqueous lactose solutions.

Table 1. The temperature-dependent specific optical rotations of α-lactose and β-lactose and of the equilibrium aqueous lactose solutions and the β/α ratios deduced from those data. $\lambda = 546$ nm; concentration 5%.

Temperature (°C)	$[\alpha_a]$	$[\alpha_\beta]$	$[\alpha_\infty]$	β/α ratio
10	108.06	39.50	65.66	1.62[1]
15	107.62	39.09	65.39	1.60[6]
20	107.23	38.68	65.12	1.59[2]
25	106.86	38.27	64.86	1.58[0]
30	106.54	37.86	64.59	1.56[7]
35	106.25	37.44	64.33	1.55[9]

x being the fraction of α-lactose and $(1 - x)$ the fraction of β-lactose at t°C. By inserting the specific rotation at equilibrium $[\alpha_\infty]_\lambda^t$ for $[\alpha_8]_\lambda^t$ in Eq. 2, the equilibrium β/α ratio is found. Recently Buma & van der Veen (9) determined $[\alpha_a]$, $[\alpha_\beta]$ and $[\alpha_\infty]$ at various temperatures in the range 10 – 35°C at a wavelength of 546 nm. From their results the β/α ratios in equilibrium solutions can be calculated in the temperature range 10 – 35°C. The accuracy of the β/α ratio is better than $\pm$ 0.005 as computed from the confidence limits (9). The results are shown in Table 1 and in Fig. 4.

3.2 Determination from optical rotations at the same temperature

When the temperature t_1 of an equilibrated lactose solution is instantly brought to another (measuring) temperature t_2, the content of α-lactose and β-lactose molecules and thus the β/α ratio is the same at that instant of time (zero time). Thereafter the ratio will start to change to the new equilibrium ratio appropriate to temperature t_2. When the optical rotation is determined at temperature t_2 at known intervals of time, the rotation at zero time (r_0) can be obtained by extrapolation. The new equilibrium rotation at temperature t_2 (r'_∞) can also be determined. This yields the specific rotation at zero time according to Eq. 3:

$$[\alpha_0]_\lambda^{t_2} = \frac{r_0}{r'_\infty} \cdot [\alpha_\infty]_\lambda^{t_2} \tag{3}$$

The β/α ratio at zero time and temperature t_2, being the β/α equilibrium ratio at temperature t_1, can now be calculated by inserting $[\alpha_0]_\lambda^{t_2}$, $[\alpha_a]_\lambda^{t_2}$ and $[\alpha_\beta]_\lambda^{t_2}$ in Eq. 2.

For β/α equilibrium ratios at different temperatures the same temperature of measurement and thus constant values for $[\alpha_a]_\lambda^{t_2}$, $[\alpha_\beta]_\lambda^{t_2}$ and $[\alpha_\infty]_\lambda^{t_2}$ can be used.
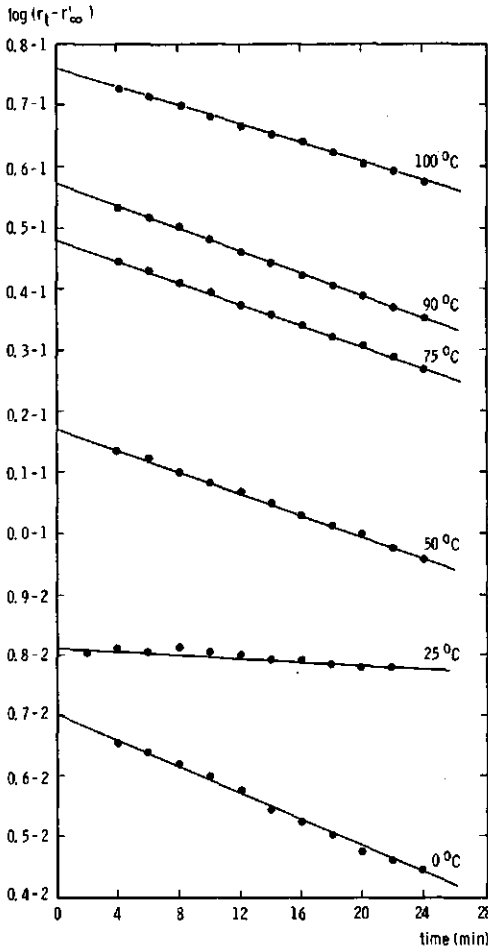
Fig. 2. Examples of extrapolation of $\log |r_t - r'_\infty|$ against time in order to determine r_0. Solutions equilibrated at various temperatures. Final concentrations approximately 10 g lactose in 100 ml.

We equilibrated lactose solutions carefully at various temperatures. Each solution was instantly brought to a measuring temperature of 25°C by mixing with a calculated quantity of distilled water at the appropriate temperature. Variations of temperature of the mixed solutions from 25°C were kept smaller than 0.5°C. After the solution had been transferred as quickly as possible into the 10.00-cm tube of a Perkin-Elmer 141 polarimeter, the rotatory power was determined at two-minute intervals for about half an hour. A thermostatically controlled (25.0 ± 0.1°C) tube was used and a wavelength of 365 nm applied. The optical rotation at the time of mixing (r_0) was found by extrapolation, for which examples are shown in Fig. 2. The rotation at equilibrium (r'_∞) was

Table 2. Initial (r_0) and final (r'_∞) optical rotations of lactose solutions equilibrated at various temperatures when brought instantly to 25°C, together with the calculated β/α ratios.

Equilibration		Approximate concentrations (g/100 ml)		r_0	r'_∞	β/α ratio	Average
tempera-ture (°C)	time (h)	initial	final				
0	72	20	10	14.48	14.53^1	1.60	
	72	20	10	14.61	14.68^8	1.61	1.61
	340	20	10	14.56	14.64^4	1.61	
	340	20	10	14.57	14.65^0	1.61	
25	24	10	5	7.33	7.31^0	1.56	
	24	10	5	7.37	7.26^0	1.57	
	24	20	10	14.74	14.68^1	1.56	
	24	20	10	14.70	14.66^1	1.57	
	72	30	15	22.18	22.10^5	1.56	1.56
	72	30	15	22.39	22.27^7	1.55	
	72	30	10	14.70	14.62^8	1.55	
	72	30	10	14.94	14.87^1	1.55	
	72	30	7.5	10.98	10.91^6	1.55	
	72	30 .	7.5	10.93	10.87^0	1.55	
50	1	20	10	14.91	14.75^9	1.52	1.52
	1	20	10	14.70	14.55^3	1.52	
	(min)						
75	20	30	10	14.90	14.59^2	1.46	1.46
	20	30	10	14.74	14.44^3	1.46	
90	15	36	10	15.05	14.67^3	1.44	
	15	18	5	7.53	7.34^6	1.44	1.44
	15	9	2.5	3.78	3.68^7	1.44	
	15	4.5	1.25	1.89	1.84^9	1.45	
100	5	40	10	16.33	15.72^7	1.37	1.37
	5	40	10	16.11	15.52^7	1.37	

determined after 24 h. The following values for the specific rotations at 25°C and 365 nm, were applied:

$$[\alpha_\alpha]^{25}_{365} = 258.9; \quad [\alpha_\beta]^{25}_{365} = 89.0; \quad [\alpha_\infty]^{25}_{365} = 154.8.$$

The accuracy of the method will depend predominantly on the instant change from the temperature of equilibration to the measuring temperature: muta-rotation during the change-over has to be avoided. Mutarotation gives too

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low values for the β/α ratios of solutions when equilibrated below and too high ones when equilibrated above the measuring temperature. The inaccuracy of the method is estimated to be $\pm$ 0.02 in the resulting β/α ratio. The results are given in Table 2 and also shown in Fig. 4.

3.3 The crystal mixture method

When a mixture of α-lactose and β-lactose crystals is dissolved in water, mutarotation occurs, unless the β/α ratio in the crystal mixture is exactly equal to the equilibrium β/α ratio in aqueous solution.

In the latter case the optical rotation at the time of dissolution (t = 0) of the mixture (r_0) is equal to the equilibrium rotation of the aqueous solution (r_∞), thus $r_0/r_\infty = 1$.

The equilibrium β/α ratio in aqueous solutions can be determined as follows. Three mixtures of α-lactose and β-lactose crystals with different β/α ratios are dissolved in water at a predetermined temperature, and r_0 as well as r_∞ are determined by polarimeter. The r_0/r_∞ ratios thus found are plotted against the α or β content of the crystal mixture, yielding a straight line at each temperature.

The intersections of these lines with the line $r_0/r_\infty = 1.00$ yields the equilibrium β/α ratios in aqueous solutions at the various temperatures. We applied this procedure with β/α ratios in the crystal mixtures of 1.10, 1.70 and 2.00 on an anhydrous weight basis. Corrections were made for the associated impurities in the lactose crystals as reported previously (9). The lactose concentrations in aqueous solution were 5.0% and the temperatures used were 20.0, 40.0 and 60.0°C.

Unfortunately the accuracy of the method appeared to be less than was desired. At 60°C the mutarotation rate is high and small deviations in the measurements result in a rather inaccurate estimation of r_0 by extrapolation to t = 0.

On the other hand, the mutarotation of lactose solutions prepared from mixtures with a β/α ratio of 1.70 is very small, also resulting in rather inaccurate estimations of r_0.

Notwithstanding the above difficulties, straight lines were obtained at all three temperatures, when r_0/r_∞ was plotted against the α-lactose content of the crystal mixtures as shown in Fig. 3. The intersections of these lines with the line $r_0/r_\infty = 1.00$ yielded the following values for the equilibrium β/α ratios of lactose in aqueous solution:

20.0°C: $\beta/\alpha = 1.61$ ($\pm$ 0.02);
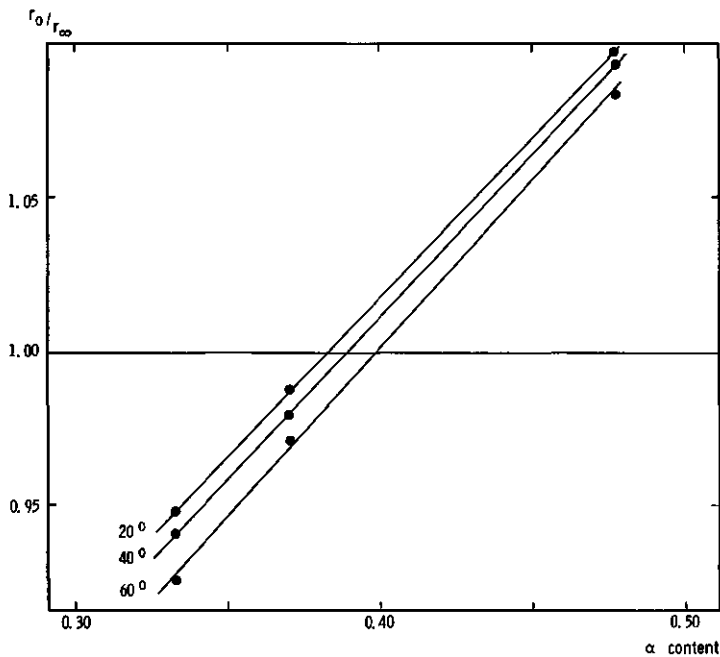40.0°C: $\beta/\alpha = 1.56$ ($\pm$ 0.02);
60.0°C: $\beta/\alpha = 1.50$ ($\pm$ 0.03).

Fig. 3. r_0/r_∞ as a function of the α-lactose content in the mixture of α-lactose and β-lactose crystals.

The figures in parentheses are rough estimates of the inaccuracies of the β/α ratios, mainly based on possible errors in the estimation of r_0 by extrapolation. The results are shown in Fig. 4.

4 Discussion

In Fig. 4 all the results obtained by the three above methods are collected. The estimated inaccuracies are also indicated by vertical bars.

A remarkable feature of the results obtained by the first method (Section 3.1) is that the temperature coefficient of the specific optical rotation of β-lactose and of equilibrium lactose solutions is almost constant in the temperature range of $10 - 35°C$, whereas that of α-lactose decreases considerably with temperature (Table 1). Thus the β/α ratio of lactose in equilibrium solutions changes with temperature in the same way as does the optical rotation of the α-lactose. The calculated β/α ratios are in very good agreement with those given by Gillis (5). Since he could not correct for the impurities present in the crystals

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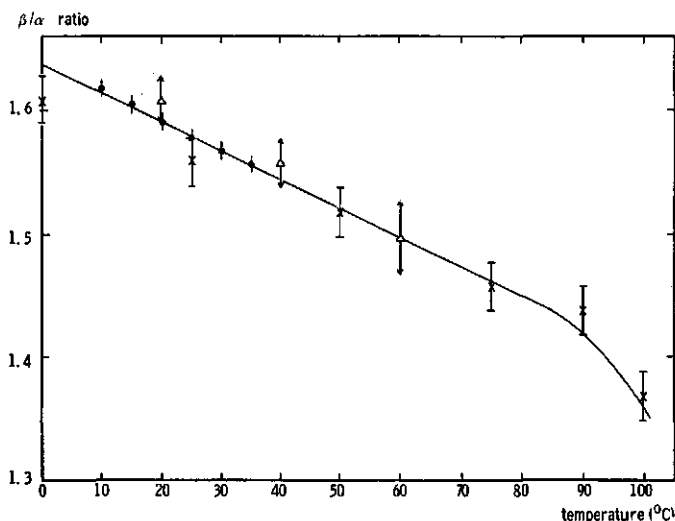Fig. 4. β/α ratios in equilibrium lactose solutions at various temperatures obtained by three methods.
● First method; × second method; △ third method.

of α-lactose monohydrate or β-lactose (9), it is a reasonable assumption that very pure materials were used.

The results obtained by the second method (Section 3.2) compare reasonably well with those obtained by the first method. There is a decline in the curve at high temperatures. This was ascribed by Gillis to experimental errors, especially to mutarotation during the rapid cooling of hot equilibrated solutions. However this would result in a change towards higher β/α ratios instead of to lower values. Therefore in our opinion the deviation from the straight line at high temperatures is significant. This was also suggested by Niquet (10).

The third method (Section 3.3) yields values for the β/α ratio which differ slightly from those obtained by the other two. However, the results of the three methods used show very clearly a decrease of the β/α ratio with increase in temperature. Undoubtedly the best values are those obtained by the first method.

The controversial data found in the literature may be explained by our results. Kendrew & Moelwyn-Hughes (2) used for the calculations the same specific rotations of α-lactose and β-lactose at all temperatures. As shown by Buma & van der Veen (9) this is not correct. The small temperature dependence of the specific rotations of α-lactose and β-lactose does convert an increase of the β/α ratio with temperature into a decrease. This can be demonstrated by

applying fixed values for $[a_\alpha]_{546}^t$ (e.g. 106.86) and $[a_\beta]_{546}^t$ (e.g. 38.27) in method 3.1 and comparing the calculated β/a ratios with those given in Table 1. Our results confirm those of Gillis (5) but still differ from those of Hudson (1). We think that the elaborate and difficult procedure used by the latter worker yielded results with relatively poor accuracy. The correction Nickerson (6, 7) made to the results of Gillis is in our opinion not justified, because it is apparently based on incorrect specific rotations.

In view of the latest results found for the specific rotations of lactose (9), the values given by Sharp & Doob (8) and also those determined earlier by Buma (11) must be revised. This will affect their calculations of the β/a ratio of lactose in dry products, which will be discussed at a later time (12).

Acknowledgements

Samenvatting

K. Roetman en T. J. Buma, *De β/α-evenwichtsverhouding van oplossingen van melksuiker in water in afhankelijkheid van de temperatuur*

Oplossingen van melksuiker in water bereiken bij elke temperatuur een mutarotatie-evenwicht tussen β- en a-lactose. Het verband tussen deze β/a-evenwichtsverhouding en de temperatuur wordt uit de literatuur niet duidelijk. Niet alleen worden bij oplossingen met dezelfde temperatuur zeer uiteenlopende waarden opgegeven, maar ook treft men een tegengesteld verloop met de temperatuur aan.

Om klaarheid te scheppen in deze situatie werden drie verschillende methoden toegepast ter bepaling van de evenwichtsverhouding. Bij alle methoden werd gebruik gemaakt van polarimetrische bepalingen met behulp van moderne apparatuur. De eerste methode berust op de bepaling van de specifieke rotaties van a-lactose, β-lactose en van lactose in mutarotatie-evenwicht bij verschillende temperaturen. Bij de tweede methode werden de evenwichtsoplossingen bij verschillende temperaturen zo snel mogelijk op 25°C gebracht om vervolgens te worden gemeten. Bij de derde methode werd gebruik gemaakt van mengsels van kristallen van a-lactose-hydraat en van β-lactose.

Alle drie methoden, waarvan de eerste de meest nauwkeurige was, gaven goed overeenkomende resultaten. De β/a-evenwichtsverhouding bleek te dalen bij toenemende temperatuur en wel van ca. 1,64 bij 0°C tot ca. 1,36 bij 100°C.

Met de uitkomsten die voorheen door Gillis waren verkregen werd een treffende overeenkomst gevonden.

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The β/α ratio of lactose in the amorphous state

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Summary

Little is known about the mutarotation of lactose in the amorphous state. The β/α ratio in amorphous lactose of spray dried and lyophilized products (e.g. dried milk and whey) is thought to be fixed. It is reported to correspond with the equilibrium ratio in solution at the temperature of drying.

In the present study investigations were made into the influence of the β/α ratio of the solution and the outlet temperature of the drier on the resulting ratio of spray dried products. Mutarotation in the amorphous lactose of lyophilized skim milk was also investigated.

The results lead to the hypothesis that mutarotation occurs during drying in the solution as well as thereafter in the amorphous state. Evidence is presented for a 'universal' equilibrium β/α ratio of about 1.25 in amorphous lactose. When this value is not reached mutarotation comes about at a rate which depends on moisture content and temperature. No equilibrium β/α ratio-temperature relationship could be found as was established for lactose solutions. The β/α ratio of spray-dried products proved to correspond with equilibrium ratios of solutions at higher temperatures than the drying solution could possibly reach.

1 Introduction

Amorphous lactose is a solid without a crystal structure. It is obtained by concentrating an aqueous lactose solution so fast and to such an extent that no crystallization occurs. Because of its origin, amorphous lactose consists of a mixture of α-lactose, β-lactose and water. The water content may vary between about 0 and 10 %.

In dairy practice, amorphous lactose is prepared on a large scale as a component of dried milk and dried whey. The spray drying and lyophilizing processes normally convert dissolved lactose into the amorphous state (1).

Little is known about mutarotation during the drying process and afterwards. No equilibrium β/α ratios of lactose in the amorphous state have been

published as they have been for dissolved lactose (see Section 2). The purpose of this study is to investigate which factors actually determine the β/α ratio of amorphous lactose. This work is a continuation of researches made into the β/α ratio of lactose in aqueous solution (2).

2 Review of the literature

Generally the β/α ratio of amorphous lactose is estimated by the polarimetric method. This method has been described by Sharp & Doob (3) and later by Buma (4). A different method was applied by Choi et al. (6). The results published in the literature are related to roller-dried, spray-dried or freeze-dried milk and whey. Mostly the conditions of drying are not given and not always the absence of crystalline lactose is reported. In Table 1 data of β/α ratios are gathered from the literature concerning powders which have been selected for absence of crystals (e.g. fresh powders).

A relationship between the temperature of drying and the resulting β/α ratio was first mentioned by Troy & Sharp (5) and confirmed by Nickerson et al. (7) and Buma (4). The latter authors mentioned their agreement with the equilibrium ratios of lactose solutions found by Gillis (9). Nickerson et al. (7) found their results for spray-dried milk and freeze-dried milk to approach the equilibrium ratios of 1.33 at 100 °C and 1.65 at 0 °C as obtained by Gillis. Buma (4, 10) found a linear relationship for the β/α ratio of spray-dried lactose and the outlet temperature of the drier ranging from 1.48 at 53 °C to 1.36 at 90 °C. Comparing his results with those of Gillis he concluded that the outlet temperature minus 10 °C is the temperature at which the drying droplets become solid.

Table 1. β/α ratios of amorphous lactose in dried milk and whey reported in the literature.

β/α ratio range	Number of powders tested			Authors
	rolled-dried	spray-dried	freeze-dried	
1.58-1.58	2	–	–	Troy & Sharp (5)
1.58	–	1	–	Troy & Sharp (5)
1.31-1.49	6	–	–	Sharp & Doob (3)
1.33-1.67	–	5	–	Choi et al. (6)
1.36-1.67	3	–	–	Choi et al. (6)
1.19-1.39	? 3	?	–	Choi et al. (6)
1.30-1.37	–	3	–	Nickerson et al. (7)
1.61-1.69	–	–	3	Nickerson et al. (7)
1.50	–	1	–	Bockian et al. (8)

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It is generally accepted that the α- and β-modifications in amorphous lactose are present in the ratio of an equilibrium solution at the temperature prevailing during the drying process (1, 4, 5, 7, 8, 10, 11, 12). For some reason a β/α ratio of 1.5 is often assumed for spray-dried products (1, 12). Mutarotation in amorphous lactose may change its β/α ratio. In the literature no data are found concerning equilibrium β/α ratios of lactose in the amorphous state in relation to temperature, as they are known for lactose in aqueous solution (2). The β/α ratio is thought to be fixed by drying. Mutarotation however is noted when amorphous lactose crystallizes (4, 5, 13), which is contradictory to a fixed ratio.

3 Materials and methods

3.1 Materials
Amorphous lactose, both pure and incorporated in dried milk and whey, were used as test materials. The spray drying and freeze drying processes were used for their preparation.

Pure amorphous lactose was obtained by spray drying an equilibrated solution of α-lactose-hydrate (approximately 10-30 % solids) in the small laboratory drier or in the pilot plant drier of the Institute. Dried whole milk, skim milk and whey were made by evaporating (approximately to 45 % solids) and subsequently spray drying of the liquids in the NIZO pilot plant. Several commercial milk and whey powders and milk powders prepared in the pilot plant of the Agricultural University were processed in the same way. The outlet temperature of the drier was known for all of the powders.

Lyophilized skim milk was prepared in a special manner to avoid crystallization. Concentrated skim milk (approximately 45 % solids, and equilibrated at 25 °C for at least 5 h) was instantly frozen by immersion in liquid nitrogen and dried in a laboratory freeze-drier. We did not succeed in preparing pure amorphous lactose without crystals in this way.

3.2 Methods
All products were carefully checked for the absence of crystallized lactose by means of a polarizing microscope. A small sample of the powder was dispersed in paraffin oil and scanned for particles which showed up with the polarizer and analyser of the microscope crossed. As mentioned by King (1), the very beginning of crystallization cannot be observed in this way, but in our opinion the quantity involved would then be negligible.

The β/α ratio of powders was determined by the polarimetric method. The procedure described by Buma (4) was followed. The β/α ratio of solutions

was determined from polarimetric measurements at 25 °C as described previously (2).

In these methods, both for dried products and solutions, a Perkin Elmer 141 polarimeter was used at 25 °C, at a wavelength of 365 nm. The latest values $[\alpha_\alpha]_{365}^{25} = 258.9$, $[\alpha_\beta]_{365}^{25} = 89.0$ and $[\alpha_\infty]_{365}^{25} = 154.8$ for the specific rotations, were used for the calculation of the β/α ratio by means of Equation 1 which was derived earlier (2).

$$\beta/\alpha = (1.6725 - r_0/r_\infty) / (r_0/r_\infty - 0.5749) \tag{1}$$

The β/α ratio was occasionally also determined by a gas-chromatographic method, based on silylation. The procedure of Sweely et al. (14) was followed, with dimethyl sulphoxide as a solvent instead of pyridine. Because of this modification, mutarotation was strongly inhibited in the interval between dissolution and silylation (15).

4 Experiments and results

Several experiments were carried out to investigate which factors influence the β/α ratio in amorphous lactose.

4.1 The β/α ratio of the solution

To what extent the β/α ratio in spray-dried amorphous lactose is determined by the initial ratio in the solution was examined as follows. One portion (I) of a solution of α-lactose-hydrate of analytical quality in distilled water (concentration 10 %) was equilibrated at 5 °C for 90 h. The solution was then poured out of a cooled funnel, mounted on the laboratory centrifugal drier, directly onto the disc. Another part (II) was equilibrated by boiling for several minutes in a funnel with a heating jacket also mounted on the drier and was added to the disc immediately. The β/α ratio in both liquids and in the resulting powders were determined in duplicate by the polarimetric

Table 2. The β-α ratio of lactose solutions equilibrated at 5 °C and 100 °C, and of the corresponding amorphous lactose powders obtained by spray drying.

Solution:	10 % α-lactose-hydrate	I	II
	temperature °C	5	100
	β/α ratio	1.63	1.47
		1.62	1.43
Drier:	inlet temperature °C	200	200
	outlet temperature °C	100	110
Powder:	β/α ratio	1.31	1.27
		1.29	1.25

method. No crystalline lactose could be detected in the resulting powders. The results are given in Table 2.

4.2 The outlet temperature of the spray drier

The β/α ratios of 21 spray-dried powders of which the outlet temperature during preparation was known were determined in an attempt to find any correlation between the β/α ratio and this temperature. Some of the dried lactose solutions, whey and milk samples were obtained from dairy industries and others were prepared in the pilot plants of the NIZO and the Agricultural University of Agriculture. In none of the powders could crystalline lactose be detected. The outlet temperature during drying ranged from 70 to 115 °C. When this temperature was not exactly known, this is indicated. The β/α ratio was determined by the polarimetric and occasionally by the gas-chromatographic method. The results are given in Table 3.

Table 3. The β/α ratio of spray-dried products in relation to the outlet temperature of the drier

Spray-dried solution	Outlet temperature drier (°C)	β/α ratio by polarimeter		by gas chromatograph		
Lactose						
1	70	1.32	1.32			
2	80	1.25	1.24			
3	90	1.24	1.25			
4	115	1.14	1.14	1.12	1.14	
Whey						
1	~ 80	1.19	1.21	1.16	1.18	
2	~ 80	1.22	1.20	1.13	1.14	1.15
3	80	1.26	1.23			
4	~ 80	1.26	1.26			
Skimmed milk						
1	70	1.23	1.24			
2	~ 80	1.24	1.24			
3	~ 80	1.28	1.27	1.32		
4	80	1.33	1.26			
5	80	1.23	1.24			
6	84	1.24	1.24			
7	85	1.26	1.26	1.35		
8	86	1.25	1.25	1.33		
9	88	1.30	1.31			
10	90	1.27	1.29			
11	94	1.29	1.30			
12	98	1.25	1.26			
13	113	1.21	1.21			

4.3 Mutarotation in the amorphous state

To investigate whether mutarotation occurs in the amorphous state, spray-dried lactose, whey, skim milk and whole milk were stored for 16 months. The powders were kept in duplicate in completely filled air-tight tins both at about — 20 °C and at + 25 °C. Before and after the storage period the β/α ratio was determined by the polarimetric method, and the moisture contents were determined by the Karl Fischer titration. No crystalline lactose could be found, either before or after storage. The results are shown in Table 4.

To investigate whether mutarotation is detectable at elevated temperatures and increased moisture contents, freeze-dried skim milk was used. Because a lower β/α ratio is expected at higher temperatures (2), such a shift will be most easily detected by using such a product with a high original β/α ratio. The lyophilized skim milk was prepared as indicated above (Section 3) and part of the powder was exposed to the air at room temperature to increase the moisture content. No crystalline lactose was found in these powders. All powders were kept at —40 °C immediately after preparation.

To hold the powders at elevated temperatures, a small amount was packed in a plastic bag, which was evacuated before sealing. In doing so, a thin layer of wrapped powder was obtained. This bag was placed similarly in a second

Table 4. β/α ratio and moisture content of several spray-dried products before and after prolonged storage at different temperatures.

Spray-dried product	β/α ratio (by polarimeter)	Moisture content (%)
Lactose solution (30 % lactose hydrate)		
Freshly prepared (after 3 days)	1.25-1.24	2.34-2.79
Kept for 16 months at —20 °C	1.24-1.24-1.25-1.22	2.55-2.47
Kept for 16 months at 25 °C	1.24-1.23-1.25-1.24	2.53–2.52
Whey		
Freshly prepared (after 7 days)	1.26-1.23	2.83 -2.89
Kept for 16 months at —20 °C	1.26-1.25-1.27-1.26	2.96-2.92
Kept for 16 months at 25 °C	1.23-1.21-1.23-1.23	2.87-2.85
Skim milk		
Freshly prepared (after 6 days)	1.23-1.24	2.41-2.40
Kept for 16 months at —20 °C	1.23-1.23-1.27-1.24	2.49–2.51
Kept for 16 months at 25 °C	1.22-1.22-1.25-1.23	2.48-2.48
Whole milk		
Freshly prepared (after 2 days)	1.23-1.23	2.08-2.00
Kept for 16 months at —20 °C	1.23-1.24-1.24-1.26	2.04-2.00
Kept for 16 months at 25 °C	1.22-1.22-1.23-1.24	1.94-1.98

bag in which milk powder was inserted as a barrier against penetrating moisture. The whole was then mounted vertically in a water-bath at the desired temperature. After the holding time the packet was instantly cooled by immersion in ice water for 30 min to fix the β/α ratio. The barrier milk powder was found to prevent sufficiently the transport of moisture to the test powder. An increase of 0.1 % in the moisture content of the test powder was recorded after holding for 1 h at 100 °C and subsequent cooling. No increase was found after keeping for 10 min at 100 °C and subsequent cooling. Long holding periods had to be avoided because of undesirable changes in the product, recognizable by discoloration, smell and reduced lactose content. Lyophilized milk powder kept for 24 h at 80 °C, for example, was completely spoiled.

After the heat treatment the powder was checked again for absence of crystalline lactose. A few powders containing crystals were rejected. The β/α ratio of the crystal-free powders was determined by polarimeter and occasionally by gas chromatograph. Moisture contents were determined according to the method of Karl Fischer.

The results of three series of experiments are given in Table 5. A slightly different procedure was followed for series I: no barrier powder was used in these first experiments. Increases in moisture contents must be allowed for here (e.g. 1.2 % for 5 h at 50 °C; 2.3 % for 3 h at 65 °C; 4.0 % for 1 h at 80 °C and 1.2 % for 1 min at 100 °C).

5 Discussion

5.1 Influence of the β/α ratio of the solution before drying
In the experiment in Section 4.1 the liquids I and II differed by approximately 0.18 in initial β/α ratio (Table 2). The real difference is thought to be approximately 0.26 because the β/α ratio of the boiling liquid II will correspond with the equilibrium ratio of 1.36 at 100 °C (Fig. 1). The deviation is ascribed to mutarotation in the hot sample for analysis, because some cooling was not avoided. Both liquids I and II reach about the same β/α ratio when spray-dried. Thus we found that the β/α ratio in spray-dried amorphous lactose was not influenced by the original ratio in the solution. However, this ratio changes considerably during the process of spray drying, even from 1.6 to 1.3 (liquid I).

5.2 Influence of the outlet temperature during spray drying
The β/α ratio of several solutions, spray-dried at various outlet temperatures of the drier, are given in Table 3 (see also Section 4.2). The order of magni-

Table 5. Influence of temperature, holding time and moisture content on the β/α ratio of freeze-dried skim milk.

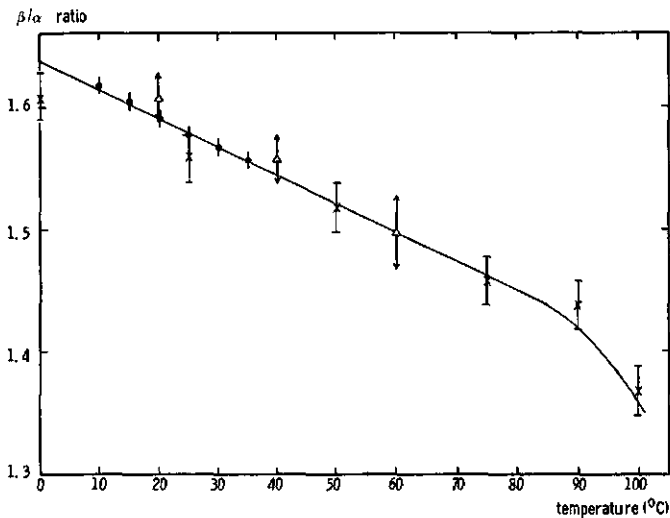| Temp. (°C)	Series I (β/α ratio concentrated milk 1.54)				Series II (β/α ratio concentrated milk 1.54)				Series III (β/α ratio concentrated milk 1.54)					
	powder I-1 (2.26% moisture)		powder I-2 (4.08% moisture)		powder II-1 (2.32% moisture)		powder II-2 (5.83% moisture)		powder III-1 (2.01% moisture)			powder III-2 (7.45% moisture)		
	holding time	β/α ratio (polari-meter)	holding time	β/α ratio (polari-meter)	holding time	β/α ratio (polari-meter)	holding time	β/α ratio (polari-meter)	holding time	β/α ratio polari-meter	gas chromatograph	holding time	β/α ratio polari-meter	gas chromato-graph
25	0	1.48	0	1.42	0	1.52	0	1.48	0	1.48	1.51	12 days	1.24	1.28
	10 days	1.46			16 days	1.47	10 days	1.44	12 says	1.46	1.54			
	24 days	1.43												
37.5									29 h	1.44	1.48	29 h	1.25	
50	1 h	1.46			3 h	1.45	1 h	1.30	1 days	1.35	1.41	24 h	1.22	
	3 h	1.43			5 h	1.44	3 h	1.26	3 days	1.31	1.35			
	5 h	1.39			6 h	1.45	6 h	1.26	9 days	1.23				
	7 h	1.38			24 h	1.36								
	24 h	1.24												
65	1 h	1.36			1 h	1.38	1/2 h	1.26						
	2 h	1.30			3 h	1.34	1 h	1.24						
	3 h	1.25			4 h	1.33	3 h	1.26						
	4 h	1.25												
75									4 h	1.23	1.29			
80	1 min	1.46	5 min	1.25	15 min	1.33	5 min	1.26						
	5 min	1.39	15 min	1.24	30 min	1.24	10 min	1.27						
	15 min	1.32	30 min	1.26	45 min	1.25	15 min	1.26						
	25 min	1.30	45 min	1.25	60 min	1.24	30 min	1.27						
	60 min	1.24	60 min	1.24	75 min	1.23	45 min	1.28						
	90 min	1.26			90 min	1.24	60 min	1.27						
100	1/2 min	1.28			1/2 min	1.45	1/2 min	1.38	10 min	1.21	1.26			
	1 min	1.26			1½ min	1.27	1 min	1.30						
	2 min	1.23			3 min	1.26	2 min	1.25						
	4 min	1.23												
	6 min	1.24												
	8 min	1.24												
	20 min	1.26												

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Fig. 1 β/α ratios in equilibrium lactose solutions at various temperatures (2).

tude of the polarimetric β/α ratios is confirmed by the gas-chromatographic method.

When the lactose powders (all prepared in the same drier) are considered, a correlation between the β/α ratio and the outlet temperature is likely. No such correlation could be established for skim milk powder, not even when the data were restricted to two series of powders (Nos 1, 4, 10 and 9, 11, 13) each prepared in the same drier. When all data given in Table 3 are taken into account it is not clear that such a correlation exists.

5.3 Mutarotation in the amorphous state

The experiments mentioned in Section 4.3 deal with mutarotation in the amorphous phase. The results shown in Table 4 indicate that no mutarotation could be detected in several spray-dried products with a (normal) low moisture content, not even during a long period of time either at —20 °C or at + 25 °C. According to the results given in Table 5, however, mutarotation is possible at all the temperatures mentioned, 25 °C included. In addition, there seems to be a tendency for the β/α ratio to stabilize on a value of about 1.25 regardless of the temperature. If there is indeed such a 'universal' equilibrium ratio in amorphous lactose, the lack of mutarotation recorded in Table 4 for spray-dried products would be explained: these powders possessed the equilibrium ratio already.

As can be seen in Table 5 the equilibrium ratio of the concentrated milk is

which the particle approaches the temperature of the outlet air. This period varies again according to the construction of the drier, size of droplet, etc. The duration of this second stage of drying in the NIZO pilot plant drier was determined to be a maximum of $2\frac{1}{2}$ min with about one minute for 90 % of the particles (19). During this time mutarotation proceeds relatively fast because of high temperature and relatively high moisture content (Section 5.3). After drying, mutarotation continues depending on moisture content and temperature until an equilibrium β/α ratio of about 1.25 is attained.

According to this hypothesis the β/α ratio found in a spray-dried product depends only on the extent to which the equilibrium is reached. Mutarotation in amorphous lactose due to crystallization, even at room temperature, which was noted by others (5, 13) and also frequently by us, can now be explained.

This theory does not conflict with the results found before by Buma (4, 10). Buma took samples directly from the same place in the drier chamber and cooled them immediately. Also according to our concept a relationship between outlet temperature and β/α ratio may then be found. It is interesting to know that a decrease of about 0.07 in β/α ratio was later found in some of the same powders Buma used in his experiments (13), indicating mutarotation towards a lower 'equilibrium' ratio.

Acknowledgment

Samenvatting

K. Roetman en M. van Schaik, *De β/α-verhouding van melksuiker in glasachtige toestand*

Er is weinig bekend over mutarotatie van melksuiker in glasachtige toestand. Tot nu toe werd aangenomen dat de melksuiker in gesproeidroogde en gevriesdroogde zuivelprodukten een β/α-verhouding heeft die vast ligt. Deze verhouding zou overeenkomen met de evenwichtsverhouding in de vloeistof bij de temperatuur van drogen.

Bij ons onderzoek werd nagegaan welke invloed de oorspronkelijke β/α-verhouding van de vloeistof en de uitlaattemperatuur van de droger hebben op de β/α-verhouding in het produkt. Aan de hand van proeven met gevriesdroogde ondermelk werd bestudeerd of mutarotatie in amorfe lactose kan optreden.

De resultaten leiden tot de veronderstelling dat mutarotatie kan optreden zowel tijdens het drogen in de oplossing als daarna in de amorfe toestand. De mutarotatiesnelheid in de amorfe lactose is daarbij afhankelijk van vochtgehalte en temperatuur. In droge produkten werd geen verband gevonden tussen de β/α-evenwichtsverhouding en de temperatuur zoals dat is vastgesteld bij oplossingen van melksuiker. De β/α-verhouding van

gesproeidroogde produkten bleek lager te liggen dan overeenkomstig de evenwichtsverhouding van oplossingen bij 'de temperatuur van drogen' mogelijk is. Een β/α-evenwichtsverhouding van ongeveer 1.25 in amorfe lactose, onafhankelijk van de temperatuur, lijkt waarschijnlijk.

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lization') usually due to a high moisture content of the powder caused by moderate drying or by moisture uptake. In the dairy industry spray-dried products with crystalline lactose are produced either unintentionally (2) or purposely to improve product properties (3).

Crystallization of the lactose changes the powder structure. According to King (1) amorphous lactose is the continuous phase in spray-dried milk powder particles in which vacuoles, casein micelles and fat globules are dispersed. In his concept postcrystallization 'provokes development of a network of fine interstices and cracks along the sides and edges of tiny crystals'. This may explain the increased permeability for gases and fat solvents (4).

By precrystallization and postcrystallization the powder structure is also changed by the mere presence of crystals between or enclosed in these particles (5).

Lactose crystals in dried milk products can be observed microscopically with polarized light (4, 5), but the detailed structure of the particles remains obscure. Even the pictures of lactose crystals in dried milk given by Taneya (6) who applied electron microscopy (replica technique) do not give any detailed information. Buma & Henstra (7, 8) and Verhey (9) studied the structure of dried milk products (containing no crystalline lactose) by scanning electron microscopy. The present paper deals with a study in which use was made of this technique, of the presence of crystalline lactose in spray-dried milk products and of the effect of crystallization on particle structure.

2 Materials and methods

2.1 Materials
The products used were spray-dried in the NIZO experimental drier. Crystallization was performed both in the liquid to be dried or in the product after drying. The following materials were prepared.

2.1.1 Amorphous lactose. A solution of 30 % lactose hydrate was dried at an air inlet temperature of 215 °C and an air outlet temperature of 80 °C (215 °C – 80 °C). The percentage of crystalline lactose (K) determined polarimetrically (Section 2.2) was 0.

2.1.2 Amorphous lactose, partly crystallized by moisture uptake. Product 2.1.1 was spread in a thin layer on paper. After exposure to the air for 24 h it was gently dried at 50 °C.

2.1.3 Spray-dried seeded lactose solution. A solution of 50 % lactose hydrate to which 15 % seed lactose was added, was dried at 180 °C – 80 °C. K = 65 %.

2.1.4 Dried whey without crystalline lactose. Whey with a total solids content

of 49 % was dried at 195 °C - 85 °C. K = 0 %.

2.1.5 Dried whey with lactose partly crystallized by moisture uptake. Product 2.1.4 was crystallized in the same way as product 2.1.2. K = 90 %.

2.1.6 Dried whey with lactose partly crystallized in the concentrated liquid. The same concentrated whey as used for the preparation of product 2.1.4 was held for 3 h at 25 °C and dried under the same conditions. K = 33 %.

2.1.7 Dried skim milk without crystalline lactose. Concentrated skim milk (46 % total solids) was dried at 185 °C - 85 °C. K = 0 %.

2.1.8 Dried skim milk with lactose partly crystallized by seeding the concentrated liquid. The same concentrated skim milk as used for the preparation of product 2.1.7 was seeded with 0.01 % seed lactose and kept for 24 h at 20 °C. It was dried under the same conditions as for product 2.1.7. K = 27 %.

2.1.9 Dried skim milk with lactose partly crystallized due to a high moisture content. Concentrated skim milk (45 % total solids) was spray-dried to a moisture content of 15 - 16 % at 185 °C - 60 °C. Subsequently it was dried to 6 % moisture on a fluidized bed. K = 56 %.

2.1.10 Dried skim milk with crystalline lactose (commercial instant product, rewetting procedure). K = 48 %.

2.2 Methods

The percentage of the total amount of lactose present in the α-modification (P_a) was determined polarimetrically as described previously (10). Crystalline lactose (K in %) was calculated according to:

$$K = P_a — (100 - P_a)/1.25 \tag{1}$$

assuming a β/α equilibrium ratio in the amorphous part of the lactose of 1.25 (10) and all crystalline lactose being α-lactose.

Samples for scanning electron microscopy were prepared and examined according to the method described by Buma & Henstra (7).

3 Results

Fig. 1 gives the structure of the wall and interior of a chopped particle of amorphous lactose (2.1.1). The solid wall has a smooth surface without cracks and folds. The same holds for the entrapped small particles which are interconnected by lactose bridges, presumably due to moisture uptake during preparation for electron microscopy.

In Fig. 2 a chopped particle of the same lactose powder after partial crystallization due to moisture uptake (2.1.2) is shown. A porous mass of coherent
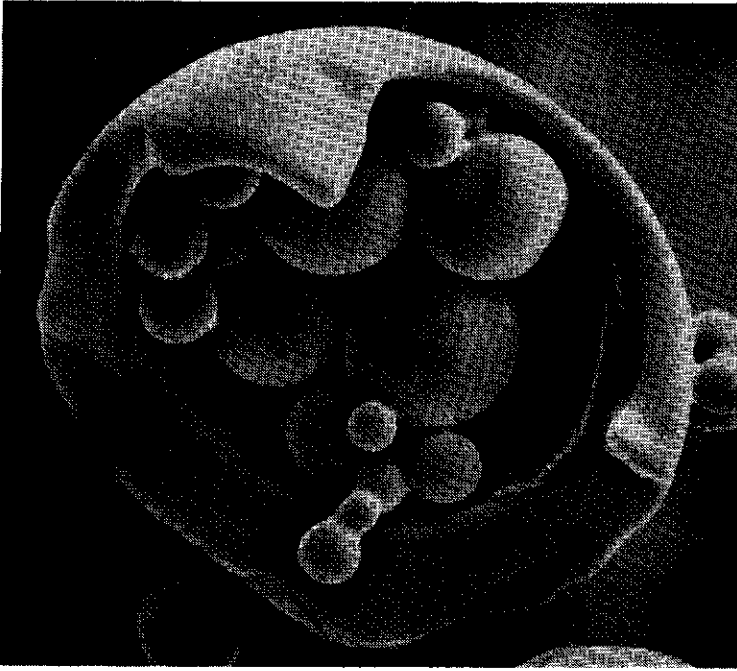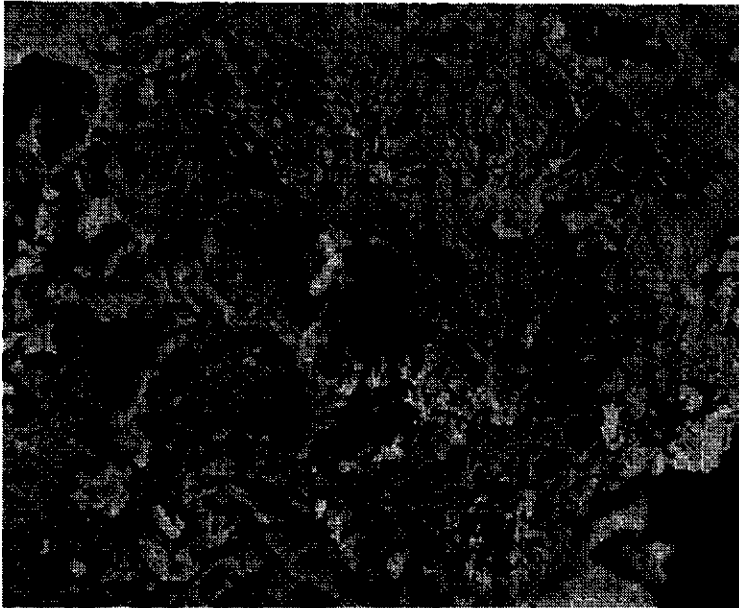
Fig. 1. Amorphous lactose; × 1000.

Fig. 2. Amorphous lactose, partly crystallized by moisture uptake; × 1000.
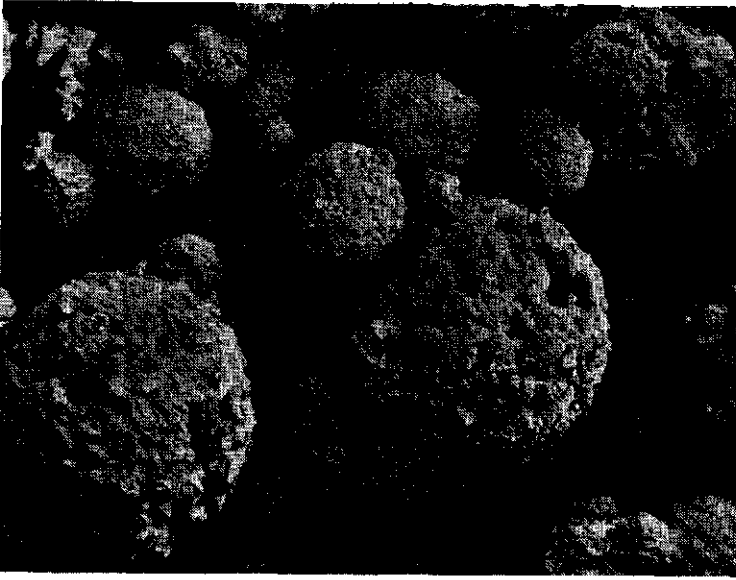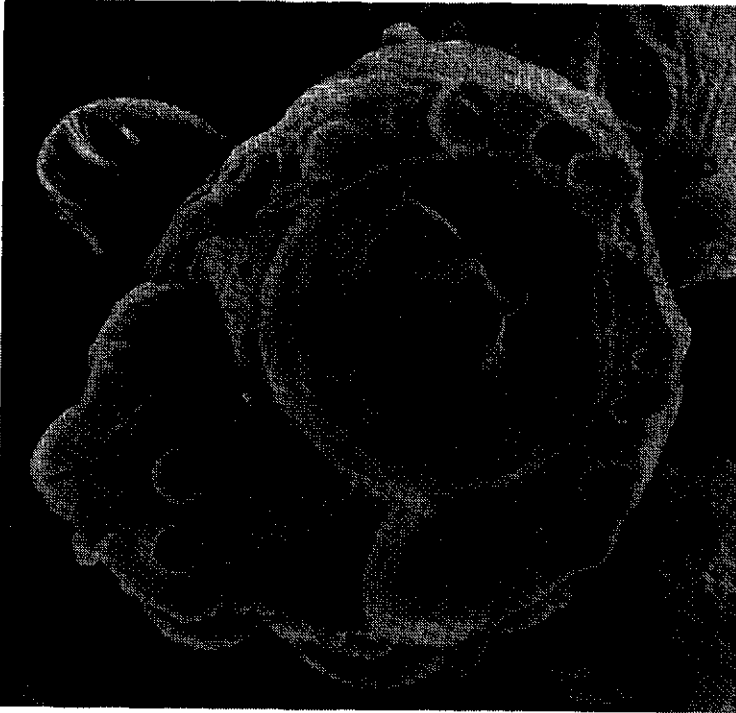
Fig. 3. Spray-dried seeded lactose solution; × 300.

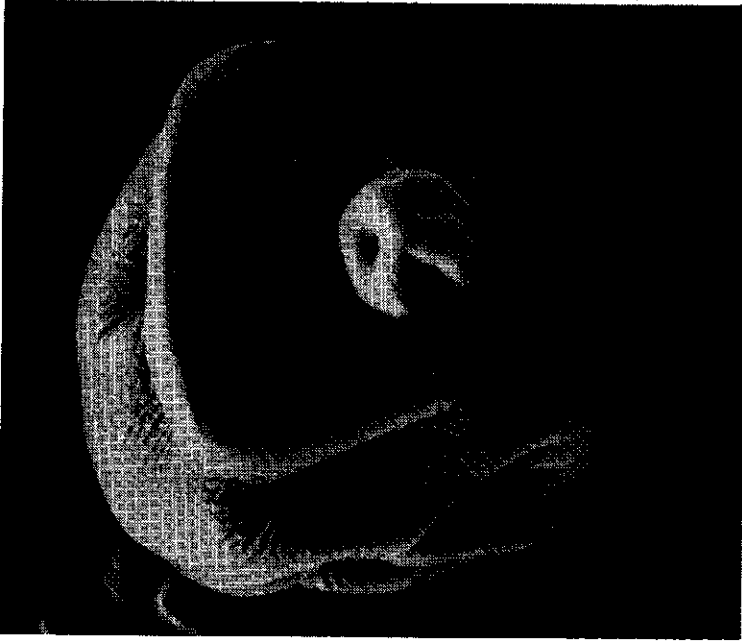

Fig. 4. Spray-dried whey without crystalline lactose; × 700.

Fig. 8. Spray-dried skim milk with partly crystallized lactose obtained by seeding the concentrate; × 1000.
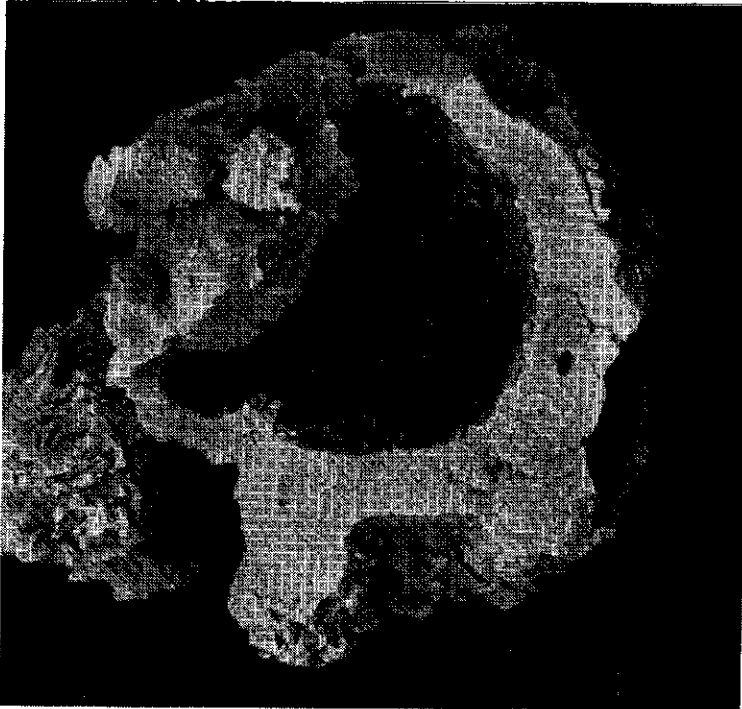

Fig. 9. Spray-dried skim milk with partly crystallized lactose obtained by moisture uptake; × 1600.

Fig. 10. 'Instant-soluble' spray-dried skim milk containing crystalline lactose (rewetting procedure); × 2000.

ing in polarized light. In this figure a lactose crystal is present on the particle surface, covered with milk solids. The rest of the surface shows dents and folds as in the product without crystals (Fig. 7).

In particles of dried skim milk containing lactose which was partly crystallized due to moisture uptake of the powder (2.1.9) both the inner wall of the vacuole and the surface are rather rough (Fig. 9). This roughness is caused by lactose crystals of the type depicted in Fig. 2 and 5. The particle wall has a more or less brittle structure, which may cause porosity.

Fig. 10 represents a commercial 'instantly soluble' dried skim milk (2.1.10) revealing also superficial roughness, although to a smaller extent than in Fig. 9. The powder particles in Fig. 10 are interconnected by bridges with a more porous structure than those observed in Fig. 1.

4 Discussion

The photographs of products without crystalline lactose are identical with those given by others (7, 8, 9). It is evident from the pictures of products with crystalline lactose that a distinction can be made between the 'precrystallized' and 'postcrystallized' types of powder.

4.1 'Precrystallized type'
When the lactose in the concentrated liquid crystallizes before spray-drying the usual tomahawk modification is formed. The crystals are often enclosed in the powder particles or are partly covered with whey or milk solids. This will reduce improved product properties like non-caking and free flowing (3). The structure of the particle wall and surface is similar to that of products without crystalline lactose. Increased permeability due to precrystallization does not seem likely when studying the pictures.

4.2 'Postcrystallized type'
When the lactose crystallizes after spray-drying the shape of the crystals is different: needle-like crystals are formed.

From the pictures it is seen that all 'postcrystallized' products have a more or less porous structure. The structure is open when the lactose content of the product is high (dried whey, dried lactose solutions). In dried skim milk there are just tiny cracks and fine interstices. Finally the mechanism of caking due to 'post-crystallization', as first described by Troy & Sharp (11), is illustrated here.

The representative selection of the pictures given here attributes further evidence that King's remarks (Section 1) about the particle structure of postcrystallized dried milk are basically right. Further it confirms the results of other workers, who used the air pycnometer and the polarizing microscope to demonstrate the particle porosity and structure of spray-dried milk products (4, 5, 12).

Acknowledgments

Samenvatting

K. Roetman, *Kristallijne lactose en de structuur van gesproeidroogde melk-produkten, waargenomen met behulp van de elektronenmicroscoop*[1]

In de literatuur wordt aangegeven dat de structuur van gesproeidroogde produkten verandert door kristallisatie van de melksuiker. Met behulp van een microscoop kan bij gebruik van gepolariseerd licht de aanwezigheid van kristallijne lactose worden aangetoond. Omdat details van structuurwijzigingen van het poederdeeltje en de vorm van

minuscule kristallen op deze wijze niet kunnen worden verkregen, is bij dit onderzoek gebruik gemaakt van een elektronenmicroscoop. Onderzocht werden gesproeidroogde melksuikeroplossingen, wei en ondermelk. Deze produkten waren gemaakt zonder kristallijne lactose, met kristallen verkregen door voorkristallisatie en met kristallen ten gevolge van nakristallisatie.

De resultaten bevestigen enkele waarnemingen die andere onderzoekers op een andere wijze hadden verkregen. Zo wordt door middel van de foto's aangetoond dat door nakristallisatie de deeltjes een min of meer open structuur krijgen waardoor ze toegankelijk kunnen worden voor gassen en vloeistoffen. Nieuw is dat de vorm van de melksuikerkristallen in voorgekristalliseerde en nagekristalliseerde produkten duidelijk verschilt. De eerste groep vertoont de bekende bijlvorm, terwijl in de tweede soort de kristallen naaldvormig zijn.

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Methods for the quantitative determination of crystalline lactose in milk products*

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Summary

Crystallization of lactose may occur during the processing of several milk products such as concentrated whey, dried whey and dried skim milk. Methods for the quantitative determination of crystalline lactose are important for process control and for studying the influence of crystallization on some product properties.

In the present work methods known in the literature or in dairy practice were described and, if necessary, improved. A quick and simple method, based on the determination of refractive indices, was developed. Three methods based on refractive index, separation and polarimetric determination of the isomer ratio respectively, were evaluated for their suitability to examine concentrated whey. The same methods, together with those based on gas chromatographic determination of the isomer ratio, water determinations (drying versus Karl Fischer titration) and differential thermal analysis were evaluated for their suitability to test dried whey and dried skim milk. The criteria handled were accuracy, ease of operation and possible limitations for application.

1 General aspects

1.1 Introduction

Crystalline lactose is present in several milk products. Sometimes it is there as a result of a carefully designed process, and sometimes unintentionally and even unwanted.

Crystallization of lactose in concentrated whey is a large-scale industrial operation. It is not only used to obtain crystalline lactose and whey products with low lactose contents as commodities, but also to improve process conditions, yield and product properties in the production of spray-dried whey. Crystallization of lactose in the manufacture of dried whey and dried milk can be brought about in the concentrate before drying or in the dry product itself. 'Pre-crystallization' and 'post-crystallization' or sometimes a combination of both are described in patents which are mostly related to dried whey and sometimes to dried milk (1). Crystalline lactose in dried milk products may also be present unintentionally, for example due to prolonged cold storage of concentrated milk (2), or unwanted, for example by moisture uptake. Some patents claim improved product properties, such as reduced hygroscopicity and caking, improved reconstitutability, stronger agglomerates in 'instant' powders and better free-flowingness, due to partial crystallization of lactose. Some types of deterioriation of dried products, such as caking, increased free fat content and reduced protein solubility, are also ascribed to lactose crystallization. The size of the lactose crystals can affect the quality of other important dairy products, such as ice cream and sweetened condensed milk, too large crystals causing 'sandiness' and inhomogeneity.

Crystalline lactose can simply be detected by means of a polarizing microscope, while scanning electron microscopy permits observation of greater detail (3). In many instances, however, knowledge about the quantity of crystalline lactose is needed. This applies to process control in dairy industry, where quick and simple methods are required, as well as to research into properties of milk products. There is for example still considerable uncertainty concerning the effect of crystallization of lactose on the physical properties of dried milk and whey.

The aim of the present work was to elaborate and evaluate methods for the quantitative determination of crystalline lactose in milk products. Attention was focused mainly on concentrated whey, dried whey and dried skim milk.

1.2 Selection of methods

Four groups of methods were distinguished based on different principles.

1.2.1 Measurement of a property of the product to which crystalline and dissolved lactose do not contribute to the same extent. Crystals, if above a certain size, do not contribute to properties such as refractive index and electric conductivity. A method based on the refractive index was worked out for concentrated whey, dried whey and dried milk. Later it was found that the same

principle was described to determine the percentage of 'grain' in molasses (4). Measurement of the electric conductivity has been applied by the sucrose industry (5); we did not succeed in obtaining satisfactory results with this procedure when applied to suspensions of crystalline lactose. Scattering of light would be almost exclusively due to the crystals, but differences in size and shape would considerably affect the results; hence, this principle was also considered unsuitable.

1.2.2 Separation. The fraction of the lactose crystallized in a product may be determined by removing the crystals. This can be achieved by centrifugation or by filtration of suspensions of lactose crystals, resulting in the separation of the solid and the liquid phase. The fraction crystallized can be calculated from the difference in lactose content or in total solids content of the original suspension and that of the supernatant or of the filtrate.

To the best of our knowledge such a method has not been described in the literature for liquid products, although it appears to be applied in some forms in dairy practice. For dry products a method is given by Choi et al. (6). We devised methods for concentrated whey, dried milk and dried whey.

1.2.3 Determination of the ratio of isomers. Usually the crystalline lactose in a product will be either α or β lactose. If the ratio of isomers of the lactose in the product can be determined and the ratio of isomers in the non-crystalline part is known, the fraction crystallized can be calculated.

The polarimetric determination of α and β lactose in solutions is well known. Gillis (7) applied an extrapolation technique of data obtained by polarimetric measurements to determine the amounts of α and β lactose in solutions. Sharp & Doob (8) used this kind of technique to determine the quantities of either modification in dried milk and dried whey. Sharp & Doob's method is frequently used to find the fraction crystallized from the shift in the relative amounts of α lactose. Buma (9) described more precisely how to determine and calculate the amount of crystalline lactose in dried products according to this polarimetric method. This method has been investigated for dry and fluid products.

The gas chromatographic analysis of carbohydrates and related polyhydroxy compounds after conversion into their trimethylsilyl derivatives has been introduced by Sweely et al. (10). Newstead & Gray (11) used this procedure to determine α and β lactose. This method was used with some improvements. As water interferes with the silylation reaction the method is suitable only for dry products.

Choi et al. (12) described a method of determining the amounts of α and β

lactose in dried milk by estimation of solubilities. We did not try the method because the procedure is laborious and is thought to be rather inaccurate.

1.2.4 Estimation of water of crystallization. When lactose in a product is crystallized as α lactose hydrate the fraction crystallized can be derived from the content of water of crystallization, which accounts for 5.00 % by weight of the crystalline lactose. Various methods may be applied to estimate the water of crystallization.

In some methods for determining the water content of a product the water of crystallization is included in the result, but in others it is not. The difference in results between such methods would thus give the amount of water of crystallization and hence the α lactose hydrate content. We devised such a method for dried whey and dried milk.

Choi et al. (13) described a method based on toluene distillation. Here the water of crystallization is released slowly during distillation. By extrapolation to zero time the total amount of water of crystallization is estimated.

When differential thermal analysis is applied to a sample of α lactose hydrate an endothermic dehydration peak was found due to the release and vaporization of water of crystallization (14). We have determined the fraction of the lactose crystallized in dried whey and dried milk with this principle.

The amount of α lactose hydrate can also be estimated directly by infrared spectroscopy as indicated by Susi & Ard (15). We had no opportunity to investigate this method.

1.3 Complications

Correct sampling of a product containing crystalline lactose may be crucial. If sedimentation of crystals is possible, for example in concentrated whey, it requires special attention. Dried milk and dried whey can also be unhomogeneous, due to sedimentation of crystals before drying.

Changes in the fraction crystallized during the determination should be prevented. In rapidly crystallizing fluid products the supersaturation of lactose in the sample can be reduced by dilution to retard the crystallization. In dry products such as dried milk and whey the lactose is usually in the amorphous state (16). Amorphous lactose can easily be 'diluted' by contact with water or by moisture uptake from the atmosphere. During this process a 'critical' concentration is reached allowing massive crystallization. This condition favours the formation of minute crystals and is commercially used to make seed lactose (17). Many of the methods discussed above, can only be applied to dry products if a suspension in water can be made which contains only those lactose

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crystals which were originally in the product. Here, crystallization of the amorphous lactose should not be allowed to take place. As moreover both dissolution and growth of the original crystals cause erroneous results, the conditions for preparation of such suspensions are critical.

Crystalline lactose may exist in several modifications (16). The most common one is α lactose hydrate, being the only form containing water of crystallization. The anhydrous modifications are: α lactose, β lactose and a compound of α and β lactose in a ratio of 5:3 (18). Methods based on the estimation of water of crystallization cannot be used unless the crystalline lactose in the product is α lactose hydrate. Methods based on determination of isomers will give erroneous results if crystals of both isomers or of the $\alpha_5\beta_3$ type are present. In milk products lactose usually crystallizes at moderate supersaturation. Then either α lactose hydrate is found (at temperatures $<$ 93.5 °C) or β lactose ($>$ 93.5 °C). Consequently in concentrated whey and milk, and thus in the resulting 'pre-crystallized' dried products α lactose hydrate is found. The processes usually applied to produce dried milk and dried whey cannot lead to the formation of crystals of β lactose (19). However, if crystallization were to take place at high concentrations of lactose (e.g. 'post-crystallization') the solution could be supersaturated with respect to both α and β lactose. Theoretically, then both forms may crystallize, irrespective of the temperature. Usually α lactose hydrate is found in post-crystallized dried whey and dried milk (20, 21) but incidentally the occurrence of β lactose (21, 22, 23) and of the $\alpha_5\beta_3$ compound (18) has been observed.

1.4 Framework of the present paper

First the selected methods have been studied individually. Subsequently they have been applied to a number of milk products and preparations. The latter experiment allowed a better comparison of the methods.

Particulars of milk products and preparations, used for the evaluation of the methods, have been collected in the list of materials.

2 Refractive index method (REF method)

2.1 Principle of the method and theoretical aspects

In the milk industry it is known that the refractive index of concentrated whey decreases during crystallization of the lactose. A method was developed based on this observation. The refractive index (n) of a solution is usually measured from the angle of total reflection at a glass/solution interface. Refraction

(bending of the light ray) and reflection take place in a layer of roughly a quarter wavelength (λ) thickness at each side of the interface (24). Therefore it is reasonable to assume that particles larger than this dimension, i.e. $\lambda_o/4n_s$ (where subscript o denotes vacuum and s solvent), do not materially contribute to the refractive index of the liquid, as measured in a prism refractometer. With water as the solvent ($n_s = 1.33$), and with sodium light for measuring ($\lambda_o = 589$ nm), the critical dimension of, for example, lactose crystals for contributing to refraction would be some 110 nm.

According to Goulden (25) and Glover & Goulden (26) the refractive index of a multicomponent system can be given by:

$$n = n_s + \Sigma\, d \cdot c \cdot r \tag{1}$$

where d = (mass) density, c = mass fraction and r = specific refraction increment of a component. This implies that the refractive index of a solution increases linearly with the concentration of a solute if expressed in unit mass per unit volume of solution, assuming no volume changes (contraction) during solution. The amount of crystalline lactose in the sample (q_k) can be calculated from the difference in refractive index (Δn) between the 'initial' refractive index of a suspension of crystalline lactose (n^I) and the 'final' refractive index with all the lactose dissolved (n^F). Using the superscript I for the initial and F for the final state of the sample and the subscript l for lactose and x for other solutes, we derive from Eq. 1:

$$\Delta n = d^F \cdot c_l^F \cdot r_l + d^F \cdot c_x^F \cdot r_x \;-\; d^I \cdot c_l^I \cdot r_l - d^I \cdot c_x^I \cdot r_x \tag{2}$$

With q for mass quantity of a solute, v for total volume and $\bar{v}_l$ for the apparent partial specific volume of lactose hydrate substitution of $d^F \cdot c_l^F = q_l^F/v^F$; $d^F \cdot c_x^F = q_x/v^F$; $d^I \cdot c_l^I = (q_l^F - q_k)/(v^F - q_k \cdot \bar{v}_l)$ and $d^I \cdot c_x^I = q_x/(v^F - q_k \cdot \bar{v}_l)$ in Eq. 2 leads to

$$\Delta n = \frac{(r_l \cdot v^F - q_l^F \cdot r_l \cdot \bar{v}_l - q_x \cdot r_x \cdot \bar{v}_l)q_k}{(v^F)^2 - v^F \cdot \bar{v}_l \cdot q_k} \tag{3}$$

Hence,

$$q_k = \frac{(v^F)^2 \cdot \Delta n}{r_l \cdot v^F - q_l^F \cdot r_l \cdot \bar{v}_l - q_x \cdot r_x \cdot \bar{v}_l + \bar{v}_l \cdot v^F \cdot \Delta n} \tag{4}$$

The fraction of lactose crystallized (K) can now be calculated. For a product p containing crystals of α lactose hydrate, K is given by:

$$K = 0.95\,(q_k/q_p \cdot L_p) \tag{5}$$

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in which the factor 0.95 is used to convert q_k from lactose hydrate to anhydrous lactose. L_p is the lactose content (mass fraction, anhydrous basis) and q_p is the quantity of the product taken for examination.

To check the method, recovery tests can be done by adding crystalline lactose to a solution. As the initial state is now a solution in which q_k is dissolved to obtain the final state, substitution of $d^F \cdot c_1^F = (q_1^l + q_k)/(v^l + q_k \cdot \bar{v}_l)$; $d^F \cdot c_x^F = q_x/(v^l + q_k \cdot \bar{v}_l)$; $d^l \cdot c_1^l = q_1^l/v^l$ and $d^l \cdot c_x^l = q_x^l/v^l$ in Eq. 2 leads to:

$$\Delta n = \frac{(r_1 \cdot v^l - q_1^l \cdot r_1 \cdot \bar{v}_1 - q_x \cdot r_x \cdot \bar{v}_l)q_k}{(v^l)^2 + v^l \cdot \bar{v}_1 \cdot q_k} \tag{6}$$

The method may be used both for suspensions (e.g. concentrated whey) and dry products. Application to dry products is only possible when the lactose crystals are suspended first.

2.2 Procedure

An Abbe refractometer (Carl Zeiss, model A), thermo-controlled at 20 ± 0.2 °C, was used. Liquid products with a lactose content below (50 g/100 g water were sampled as such. With higher contents the product was diluted by thoroughly mixing with an equal weight of lactose solution (22.0 % lactose hydrate). Exactly 3.50 g of the dry products were suspended in 25.0 ml of a lactose solution (22.0 % lactose hydrate) and completely dispersed within 3 min by stirring at 20 °C, without crushing the crystals or producing excessive foaming.

The refractive index (n^l) of all suspensions was determined in triplicate within 2 min after preparation. The whole procedure (including the preparation of the suspension) was repeated two times. Because of the presence of crystals the refractometer may be difficult to read. Slight lifting of the upper prism increased the accuracy of reading n^l. The 'final' refractive index (n^F) was determined in triplicate at 20 °C in each of the three different samples. The lactose in these samples was dissolved by keeping the latter in covered conical flasks in a water bath at 65 ± 5 °C for half an hour. Δn was thus calculated from the averages of nine determinations of both n^F and n^l.

2.3 Check of prerequisites

For an accurate determination three prerequisites must be met.

2.3.1 The crystals in the suspension should be near to equilibrium with the sur-

rounding lactose solution to prevent any change in refractive index within the time required for a measurement, i.e. within 5 min. The equilibrium concentration depends on the radius of curvature of the crystals, according to the Kelvin equation, assuming all crystals are uniform and equivalent to spheres:

$$RT \ln \frac{S_c}{S_\infty} = 2\gamma v_m / r_c$$

in which:

R   = gas constant $(8.314 \text{ N} \cdot \text{m} \cdot \text{mol}^{-1} \cdot \text{K} \cdot)$
T = temperature (K)
r_c = radius of curvature of a crystal (m)
S_c = equilibrium concentration of crystals with r_c (kg/kg)
S_∞ = equilibrium concentration of a crystal with r_c is infinite = solubility (kg/kg)
γ = interfacial tension between crystal and solution $(\text{N} \cdot \text{m}^{-1})$
v_m = molar volume of the crystalline material $(\text{m}^3 \cdot \text{mol}^{-1})$.

We calculated for crystals of α lactose hydrate with $V_m = 2338 \times 10^{-7}$ $\text{m}^3 \cdot \text{mol}^{-1}$ and $r_c = 1, 0.1$ and $0.01 \mu\text{m}$ equilibrium concentrations of 16.76, 17.35 and 24.5 % lactose hydrate respectively at T = 293 K, assuming S_∞ to be 16.7 % lactose hydrate (27) and γ to be 20 mN m^{-1}. The latter value is very uncertain. For sucrose crystals in a saturated solution $\gamma = 5$ mN m^{-1} has been reported (28), and because of its far smaller solubility a higher value is to be expected for lactose.

We tried to find experimentally the equilibrium concentration for small crystals ($\sim 0.1\text{-}2 \mu\text{m}$) (L1) and large ones ($\sim 100\text{-}250 \mu\text{m}$) (L2). Suspensions of 2.625 g crystals (corresponding to the amount of lactose in 3.50 g dried whey) in 25.00 ml lactose solution with various concentrations of lactose hydrate (16.7, 18.0, 20.0, 22.0, 25.0 %) were stirred continuously at 20 $\pm$ 1 °C. The refractive index was determined at various times. Within the precision of the method ($\sim 2 \times 10^{-4}$) no change was found within 5 min for either small or large crystals at a concentration of 22.0 %. An excess solubility ($S_c - S_\infty$) of about 5 % lactose would correspond with an effective radius of curvature r_c of about 0.015 μm, which seems reasonable for crystals of 0.1-2 μm with many sharp edges.

Apparently, growth of crystals is negligible at this supersaturation within the time scale of the experiment. But higher concentrations may occur in the test solutions. Following the procedure for dried products, the concentration is increased by dissolution of the non-crystalline lactose of the product. To check this, various amounts of crystalline lactose (L1, L2) were added to con-

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centrated lactose solutions imitating suspensions of dried whey in which the fraction crystallized (K) was 20, 40, 60 and 80 %. No substantial decrease of the refractive index was found within 5 min, using the large crystals (Δn was 5 × 10^{-4} at most). In some suspensions of small crystals, however, a decrease up to 9 × 10^{-4} was observed after 5 min. (As to discuss later the method was indeed less accurate if small crystals were present.) In liquid products, such as concentrated whey with a high content of total solids, the concentration of lactose can also be high. These products are diluted with a lactose solution of 22.0 % lactose hydrate, not only to retard crystallization, but also to enable complete dissolution of the lactose so that n^F can be determined.

During the determination of n^I crystalline lactose should not be present. This can easily be checked by means of a polarizing microscope; we never found crystalline lactose in the solutions after heating.

2.3.2 Constituents other than α lactose hydrate should contribute equally to n^I and n^F. If crystalline β lactose is present it will dissolve at 20 °C because of its high solubility (27). We checked this by adding 10 ml of a solution of 22.0 % lactose hydrate to 1.0 g of crystalline β lactose (L25). After 5 min 94 % and 96 % were dissolved in duplicate tests. This result is acceptable, because it will not cause a substantial contribution to Δn. Amorphous lactose without crystals dissolved completely within one min of stirring a mixture of 1.0 g of spray-dried lactose (L21) and 10 ml of a solution of 22.0 % lactose hydrate at 20 °C. To check whether other milk and whey constituents contribute equally to n^I and n^F the method was applied to dried milk and dried whey containing no crystalline lactose (as checked by polarizing microscopy), usually Δn proved to be zero for dried whey, but for dried milk undissolved non-lactose constituents can contribute to Δn (Section 6.3).

2.3.3 As mentioned above, the method is applicable only if the crystals are larger than ~0.1 nm. Crystals obtained by post-crystallization may be smaller than 0.1 nm (3). This may cause erroneous results (Section 6.3).

2.4 Simplified equations for the calculation of K

To calculate the fraction crystallized from the results of the REF method, as applied to concentrated whey, dried whey and dried milk, several parameters must be known (Section 2.1). As their determinations and calculations are time-consuming, we used in this paper simplified equations by assuming constant values for them. These approximations are discussed below.

Goulden (25) gives $r_1 = 0.140$ ml/g lactose hydrate at 20 °C and $\lambda = 589$

nm. The value of $\bar{v}_l$ was calculated from published densities and refractive indices of lactose solutions (29). Assuming $\bar{v}_{H_2O} = 1$ we found $\bar{v}_l$ to range from 0.6356-0.6366 for 22-40 % lactose hydrate. For $\bar{v}_l$ the value 0.63 was adopted. As r_l and $\bar{v}_l$ are given for lactose hydrate attention should be paid to an appropriate conversion for other parameters (e.g. q_l^F) into lactose hydrate. For dried whey and dried skim milk the average compositions were taken as analysed in 1971 by the Netherlands Institute for Inspection of Milk and Milk Products. For 203 samples of dried whey the anhydrous lactose content, L (%) = 71.3 $\pm$ 1.9 % and the water content = 2.6 $\pm$ 0.6 %, leaving 26.1 % for the other constituents (R). The mass density at 20 °C (d) of a 'final' solution of 3.50 g dried whey in 25.0 ml 22.0 % lactose hydrate solution was determined to be 1.128 (1.275 − 1.279) g ml^{-1} for three samples (WPs 5, 6, 7) by a precision density meter (DMA O2C Anton Paar). At the end of the determination no crystals of lactose could be detected by polarizing microscopy. Since the mass of the final solution is 30.675 g (3.50 g dried whey + 27.175 g lactose solution) its volume (v^F) will be 27.19 ml. Based on the content of water and anhydrous lactose, the residual dry matter (R) was calculated for the three samples of dried skim milk was found L % = 50.3 $\pm$ 2.2 %. The water content was and the concentration of lactose and the residual dry matter content of the final solution, r_R was calculated to be 0.155 (0.152-0.158) ml g^{-1}. In 111 samples of dried skim milk was found L = 50.3 $\pm$ 2.2 %. The water content was 3.5 $\pm$ 0.5 %, leaving 46.2 % for the other constituents. In a similar way as mentioned for dried whey the density of the final solution for three samples of dried milk (MPs 8, 9, 10) was found to be 1.126 (1.1256-1.1264) g ml^{-1}. The volume of the final solution (v^F) was thus 27.24 ml, and r_R = 0.174 (0.169-0.178) ml g^{-1}. Concentrated whey varies widely in dry matter content. Hence, L, R and d have to be determined in every sample of this product.

Based on these data the following simplified equations can be given:

for concentrated whey
$$K = \frac{100\Delta n}{L \cdot d(15 - 10L \cdot d - 10R \cdot d + 67\Delta n)} \tag{7}$$

for diluted concentrated whey
$$K = \frac{200\Delta n}{L \cdot d(15 - 5L \cdot d - d - 5Rd + 67\Delta n)} \tag{8}$$

for dried whey
$$K = \frac{237.5\Delta n}{(3.5 + 21\Delta n)L} \tag{9}$$

for dried skim milk
$$K = \frac{240\Delta n}{(3.5 + 21\Delta n)L} \tag{10}$$

in which L and R are mass fractions (g/g) and d is given in g ml^{-1}.

Table 1. Comparison of the change of the refractive index (Δn) determined by experiment and by calculation in suspensions with added crystalline lactose hydrate.

Series	Lactose hydrate added to	Crystal size	Quantities added (mg)	Equation for calculation	$(\Delta n_{exp.} - \Delta n_{cal.}) \times 10^4$	Difference significant ($\alpha = 0.05$)
1	25.0 ml 22.0 % lactose hydrate	small (L1)	250(250)2500	Eq. 6	-1.6 ± 1.8	+
2	25.0 ml 22.0 % lactose hydrate	large (L2)	250(250)2500	Eq. 6	-0.8 ± 1.9	−
3	25.0 ml 22.0 % lactose hydrate + 2250(250)0 amorphous lactose (L21)	large (L2)	250(250)2500	Eq. 3	2.8 ± 2.0	+
4	30 g concentrated whey (34 % total solids)	large (L2)	500(500)2500	Eq. 6	-1.6 ± 1.5	−
5	15 g concentrated whey (34 % total solids) + 15 g 19 % lactose hydrate solution	large (L2)	500(500)2500	Eq. 6	-0.6 ± 1.1	−
6	15 g concentrated whey (43 % total solids) + 15 g 19 % lactose hydrate solution	large (L2)	500(500)2500	Eq. 6	0.8 ± 1.6	−
7	3.50 g dried whey (WP 5) +: 25.0 ml 22.0 % lactose hydrate	large (L2)	100(100)2500	Eq. 6	0.7 ± 1.3	+
8	3.50 g dried skim milk (MP16) + 25.0 ml 22.0 % lactose hydrate	large (L2)	250(250)2500	Eq. 6	-0.5 ± 2.7	−

2.5 Check of the method by addition of crystalline lactose hydrate

Several tests were carried out (Table 1). Increasing quantities of α lactose hydrate were dispersed in lactose solutions (series 1, 2), in concentrated whey with or without dilution (series 4, 5, 6) or added to dry products and then dispersed in a lactose solution (series 3, 7, 8). For each mixture of series 1, 2 and 3 both n^I en n^F were determined, whereas only n^F was estimated for mixtures of the other series. For n^I of each of the latter series the value of n^F was taken, determined in the corresponding sample without added crystalline lactose. Δn was not only determined by experiment but was also calculated according to the appropriate equation. Therefore the composition of the mixtures should be known. For series 4, 5 and 6 all relevant determinations were made. For series 7 and 8 the average composition of dried whey and skim milk (Section 2.4) was taken for calculation. The amounts of dissolved lactose in the initial and final solutions of series 1, 2 and 3 were determined from the moisture contents of the α lactose hydrate and amorphous lactose used. Hence Δn could also be found here from data gathered for lactose solutions by McDonald & Turcotte (29).

The differences between the values found for Δn from our calculations and those from the literature were $(5 \pm 7)10^{-5}$ for series 1 and 2 and $(0 \pm 7)10^{-5}$ for series 3 and thus were not significant ($\alpha = 0.05$). From the results of the tests, shown in Table 1, it can be concluded that there are only minor differences, although some of them are significant. The increases in refraction, due to the addition of large crystals of lactose hydrate to the various products, are so well recovered that at most small deviations in K (~ 0.05) may be expected when the method is applied to products with similar crystals. Our tests with seed lactose (L_1) indicate that larger deviations may be expected if the crystals are very small.

2.6 Repeatability

The impact of variations of individual parameters in Eq. 4 and 5 on the value of K for dried whey is illustrated in Table 2. The repeatability of n of suspensions of lactose depends strongly on the quantity and size of the crystals present. In the case of suspensions of 2.625 g (the equivalent of all the lactose in 3.50 g of dried whey) of small (L1) and of coarse lactose crystals (L2) in 25.0 ml of a 22.0 % lactose hydrate solution we found s = 1.2×10^{-4} and s = 0.6×10^{-4}, respectively (n = 10). For all other individual variables in Table 2 the estimated deviations were smaller than those causing 1 % increase in K. The repeatability of K is therefore predominantly governed by the reading of the

Table 2. Variations calculated for each parameter to give an increase of K by 1 % if 3.50 g of dried whey with an average composition is suspended in 25.0 ml of a 22.0 % lactose hydrate solution.

Parameter	$K = 50\ \%$	$K = 51\ \%$	Δ
Δn	54.1×10^{-4}	55.2×10^{-4}	1.1×10^{-4}
ν^F (ml)	27.19	27.94	0.75
η (ml/g)	0.140	0.137	0.003
q^F_1 (g)	8.60	9.27	0.67
$\bar{v}_1$ (ml/g)	0.636	0.686	0.050
q_R (g)	0.91	1.52	0.61
r_R (ml/g)	0.155	0.259	0.104
L_p (g/g)	0.713	0.699	0.014
q_p (g)	3.50	3.43	0.005

refractometer.

For K of pre-crystallized dried whey (WP6) we found s = 1.6 percentage units (average $K = 70\ \%$, n = 10). From repeated determination in several samples of pre-crystallized dried whey we found for the method s = 1.4 percentage units (df = 18). For K for concentrated whey the standard deviation is estimated as s ≈ 1.0 percentage units.

3 Separation methods (SEP methods)

3.1 Principle of the method

The method is similar to the refractive index method in as far as a property of the liquid phase is determined. Here crystals of α lactose hydrate are separated quantitatively from the solution. The fraction of the total lactose in the suspension that is crystalline (K_s) is calculated from the lactose contents of the suspension (L_i) and of the liquid phase after separation (L_f). L_i is made up from lactose in the liquid and in the solid phase and is, expressed on an anhydrous basis, given by:

$$L_i = 1 - \left(\frac{K_s \cdot L_i}{0.95} \right) L_f + K_s \cdot L_i \tag{11}$$

in which all contents are given as mass fractions.
Hence:

$$K_s = (L_i - L_f)/(L_i - 1.0526\,L_i \cdot L_f) \qquad \text{(method SEP-L)} \quad (12)$$

The fraction crystallized (K_s) can in a similar way be calculated from the total solids contents (30) of the suspension (D_i) and of the liquid after separation (D_f):

$$K_s = (D_i - D_f)/(L_i - 1.0526 L_i \cdot D_f) \qquad \text{(method SEP-TS)} \quad (13)$$

Selective removal of constituents other than lactose from the suspension would lead to an increased value of L_f and a decreased value of D_f. Eq. 12 and Eq. 13 would thus give too low a value and too high a value for K_s, respectively. A correct result can still be obtained by relating the lactose to the water in which it is dissolved:

$$L_i = \frac{L_f}{(1 - D_f)}\left[(1 - D_i) - \left(\frac{K_s \cdot L_i}{0.95} - K_s L_i\right)\right] + K_s \cdot L_i \qquad (14)$$

Hence,

$$K_s = \frac{(L_i - L_i D_f - L_f + L_f D_i)19}{19L_i - 19L_i \cdot D_f - L_i \cdot L_f} \qquad \text{(method SEP-LTS)} \quad (15)$$

Dry products have to be suspended in a lactose solution in such a way that the quantity of crystalline lactose does not change. Many fluid products can be handled directly, but sometimes dilution with a lactose solution is necessary. If a lactose solution is added, the fraction crystallized in the original product K is calculated from

$$K = K_s/F \qquad (16)$$

Table 3. Calculated values of ΔK_s (deviation from the true value K_s) caused by 1 % variation (relative) in lactose and total solids contents or by removal of non-lactose solids for a hypothetical sample of concentrated whey with $L_i = 39.00$ %, $D_i = 49.00$ %, $L_f = 25.00$ %, $D_f = 37.50$ % and $K_s = 48.72$ %.

Deviation of 1 % relative for:				ΔK_s (%) according to:		
L_i (%)	L_f (%)	D_i (%)	D_f (%)	Eq. 12	Eq. 13	Eq. 15
39.39	—	—	—	+ 0.86	− 0.48	+0.53
—	25.25	—	—	− 0.70		− 0.53
—	—	49.49	—		+ 2.07	+0.51
—	—	—	37.87		− 1.28	− 0.32
39.39	24.75	—	—	+ 1.54		
—	—	49.49	37.12		+ 3.32	
39.39	24.75	49.49	37.12			+ 1.85
Removal of non-lactose solids (g/100 g concentrate)						
2 g				− 1.80	+ 5.28	0
5 g				− 4.76	+13.17	0
10 g				− 10.48	+26.19	0

in which F is the fraction of the lactose in suspension originating from the dry product or the concentrate.

In Table 3 we show the importance of correct determinations of the lactose and the total solids content and the influence of removal of non-lactose solids.

3.2 Procedure

We devised the following procedure.
1. Dry products are suspended and whey concentrates are diluted with a 22.0 % lactose hydrate solution, as described in Section 2.2.
2. Crystalline lactose is removed from the suspension by centrifugation at 20 ± 1.5 °C (~ 1500 g, 3 min total residence time). The supernatant is decanted immediately.
3. For concentrated whey the lactose content of the suspension and that of the supernatant are determined in duplicate according to the method of Luff-Schoorl (Section 3.4). Total solids contents are determined in duplicate by the aluminium foil method (Section 3.5). For dried whey and dried milk the lactose content in the dry product is determined in duplicate by the method of Luff-Schoorl or polarimetrically (Section 3.4) and the water content in duplicate by the Karl Fischer titration (Section 5.2.1).

A simplified procedure was devised by Pisecki (30) for concentrated whey, based on the industrial practice to estimate total solids in concentrated whey from refractometer readings. The total solids are read directly from a sucrose scale, or from a table giving refractive indices for sucrose solutions. This does not give the true contents of total solids. However, satisfactory results are obtained for the fraction crystallized in concentrated whey if the total solids of the suspension (D_i^s) and of the supernatant (D_f^s) are read from the sucrose scale and substituted in Eq. 13. For dried whey of average composition (Section 2.4) we have $L_i = 0.73 \, D_i^s$ yielding

$$K_s = \frac{95 \, D_i^s - 95 \, D_f^s}{69 \, D_i^s - 73 \, D_i \cdot D_f^s} \qquad \text{(method SEP-STS)} \quad (17)$$

3.3 Check of prerequisites

For an accurate determination two prerequisites must be met.
− There should be no substantial change in the amount of crystalline lactose during the determination.

Since the procedure for the suspending of lactose crystals in dry products is

the same as discussed earlier, the same deviations can be expected for any change in the fraction crystallized (Section 2.3).

− An effective separation of the solid and the liquid phase is necessary.

In the beginning we also studied filtration. A filtration procedure must be quick, as no change in the amount crystallized is allowed and filters with small pores ($\sim 1 \mu$m) have to be used to retain small crystals. These conditions are difficult to meet for viscous suspensions such as concentrated whey. We used pressure filtration with Sartorius filters (type SM 16225), applying pressures up to 1 MPa, but did not succeed in meeting the conditions mentioned. Therefore, centrifugation was the only choice.

To check whether centrifugation is effective, the following experiments were carried out. A quantity of 2.50 g of small (L1) or large (L2) crystals of α lactose hydrate was suspended at 20 °C separately in each of four solutions. These solutions (checked for the absence crystalline lactose) were:

a. 25.0 ml of a 22.0 % lactose hydrate solution;
b. solution a plus 3.50 g of dried whey (WP27A), dispersed as described in Section 2.2;
c. solution a plus 3.50 g of dried skim milk, dispersed in b;
d. 25.0 ml of concentrated whey with 35 % total solids.

After adding the crystalline lactose the suspensions were centrifuged immediately in a thermo-controlled centrifuge (Sorvall Superspeed RC2-B, distance to the axis 5 cm). The speed was increased to 5000 rpm ($\sim 1500\ g$), followed directly by stopping. Total residence time was ~ 3 min at 20 ± 1.5 °C. Crystalline lactose was absent in the supernatants as checked by polarizing microscopy. It thus appears that the lactose crystals were effectively removed from each of the four solutions. The refractive index of the four solutions without added crystals had not changed after centrifugation, indicating that sedimentation of components other than lactose was absent. The refractive index of each suspension with large crystals was equal to that of the corresponding supernatant. However, for suspensions of small crystals in solutions b and c, a decrease was observed of $\Delta n = 23 \times 10^{-4}$ and 10×10^{-4} respectively, which was probably due to ensuing crystallization. Although no separation of non-lactose solids was observed in these tests, it may happen with some products (e.g. dried milk with a high insolubility: Section 6.3).

3.4 Determination of lactose

The polarimetric and reductometric methods were chosen, because they are frequently used in dairy chemistry and are thought to be sufficiently accurate. In polarimetric measurements the lactose content was determined from the

optical rotation (r_∞) and the specific rotation ($[\alpha_\infty]_\lambda^i$), both at β/α equilibrium. The concentration of anhydrous lactose (g/ml) was calculated according to

$$L = \frac{r_\infty}{[\alpha]_\lambda^i \cdot u} \qquad (18)$$

in which u is the length of the polarimeter tube in dm. Measurements were carried out after an equilibration time of 24 h at 25 ± 0.1 °C. Initially, our determinations were made with a 'Zeiss Kreispolarimeter 0.01 °C' (indicated by Pol-Z). The specific optical rotation was given by Buma (31): $[\alpha_\infty]_{589}^{25} = 55.28 ± 0.03$.

Subsequently a 'Perkin Elmer 141' polarimeter was used at a wavelength of 365 nm (Pol-PE). The lactose contents were calculated with $[\alpha_\infty]_{365}^{25} = 154.8 ± 0.2$ (31, 37).

The reductometric method according to Luff-Schoorl (LS) as described by Schoorl (32) was applied using the complex tartrate solution according to Weide & Krugers Dagneaux (33).

Correction for the volume of precipitate has to be applied in some cases due to clarification of solutions. With the LS method no clarification was applied. For the polarimetric determinations the solution was clarified as described later with Carrez reagents (Section 4.2.2). When a solution of 10 g dried whey or dried skim milk is clarified with 20 ml Carrez I and 15 ml Carrez II, the average volume of the precipitate is calculated to be 1.9 and 3.7 ml, respectively, from the volume of the individual components. Lactose solutions containing

Table 4. Comparison of polarimetric and reductometric methods for the analysis of pure lactose solutions.

Method	Anhydrous lactose (g/l)	Number of determinations	Recovery (%)	Standard deviation (g/l)
Pol-PE	11.87	8	100.3	0.086
	23.75	8	100.2	0.078
	35.62	8	100.3	0.088
Pol-Z	47.52	6	99.9	0.090
	95.00	6	99.9	0.058
	190.01	6	99.8	0.043
LS	0.950	6	98.2	0.69
	1.900	7	98.1	0.69
	2.850	6	99.0	0.28

various amounts of lactose (L4) were analysed. The results are given in Table 4. The standard deviation of Pol-PE and Pol-Z is about ten times smaller than that of LS. The values found by LS deviate more from the true value than those found by Pol-PE and Pol-Z.

Concentrated whey with ~65 % total solids was analysed after dilution with a sufficient amount of water to dissolve all crystalline lactose. The lactose content (g/l) in the final solution was determined according to Pol-Z and LS. The values found were 59.2 ± 0.33 (Pol-Z, n = 9) and 63.9 ± 0.34 (LS, n = 9) respectively, which differ significantly. Using an enzymatic method which is said to be specific for lactose (34) we found 64.2 ± 1.2 (n = 9). This result differs significantly from the average value obtained by Pol-Z but not from that found by LS. Other workers have also reported too low results when using the polarimetric method for concentrated whey. House (35) ascribed this to optically active protein compounds which were not removed by clarification. Although we think that further research is needed, we decided to use only LS for concentrated whey.

Samples of dried whey and dried milk were analysed after dissolving in water (~ 50 °C), according to Pol-PE and LS. The standard deviations for the percentage of lactose were: s = 0.22 (n = 25) for dried whey (WP6) by Pol-PE; s = 0.42 (n = 8) for dried whey (WP27C) by LS; s = 0.13 (n = 18) for dried skim milk (MP9) by Pol-PE; s = 0.66 (n = 8) for dried skim milk (MP26) by LS. Twelve samples of dried whey and dried skim milk were analysed by both Pol-PE and LS. The respective standard deviations for the percentage of lactose (calculated from twofold determinations) were s = 0.05 and 0.21 using Pol-PE, and 0.41 and 0.55 appplying LS. The average difference of the results obtained by these methods (Pol-PE − LS) was for dried whey + 1.2 % ± 0.7 and for dried skim milk + 0.7 % ± 0.8. Both methods were used for dry products.

3.5 Determination of total solids

Methods for the determination of total solids should exclude the water of crystallization of lactose hydrate. For liquid products the 'aluminium foil' method (AFM) of Schulz et al. (36) was chosen, because it was expected to be quick and accurate. If necessary, the sample was diluted with water to give lactose concentrations ≤ 25 g/100 g water to be sure that all lactose was dissolved.

A 15.83 % (anhydrous basis) solution of lactose gave a total solids content

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Table 5. Comparison of six methods to determine K in mixtures with known K values.[1]

	Series	Known K values	SEP-L	SEP-TS	SEP-LTS	SEP-STS	REF	Cd
Known K values	I		0.3	-1.3	-0.1	-0.7		1.7
	II		-11.2	-2.5		4.2		6.0
	III		-1.6	0.4	-1.2	1.0	1.0	1.9
SEP-L (Eq. 12)	I	*		-1.6	-0.4	-1.0		1.7
	II			8.7		15.3		6.0
	III			2.0	0.4	2.6	2.6	1.9
SEP-TS (Eq. 13)	I				1.2	0.6		1.7
	II		*			6.7		6.0
	III		*		-1.6	0.6	0.6	1.9
SEP-LTS (Eq. 15)	I					-0.6		1.7
	II							
	III						2.2	
SEP-STS (Eq. 13)	I		*					
	II		*					
	III			*	*		2.2	1.9
REF (Eq. 6)	I		*					
	II							
	III		*		*		0.0	1.9

[1] Results of an analysis of variance of the values of K_s found for the addition of 1.00 (1.00) 4.00 g of α lactose hydrate to 25.0 ml of a 22.0 % lactose hydrate solution (series I); of 0 (0.50) 2.00 g α lactose hydrate to 28.50 g of concentrated whey with 33.5 % total solids (AFM) and 24.8 % anhydrous lactose (LS) (series III); and of the values of K found for mixtures of 2.50 (0.50) 0 g of amorphous lactose (L21) and 0 (0.50) 2.50 g of α lactose hydrate (series II). The average differences between the results of K_s (%) or K (%) obtained by the various methods are shown (e.g. the top row gives the known percentage minus determined by method based on lactose contents, etc.). Futher it is indicated whether methods differ significantly (*), and at what critical difference (cd), according to Tukey's test ($\alpha = 0.05$).

of 15.85 % (s = 0.04, n = 9). For a diluted whey concentrate with a total solids content of 24.4 %, s = 0.10 (n = 10) was found.

The Karl Fischer titration was used to determine water in dried milk and dried whey. See Section 5.2.1 for details.

3.6 Recovery tests

Three series of recovery tests were carried out, of which the results are given in Table 5. In all tests the α lactose hydrate added was type L3. K_s was determined by the various SEP methods. In all cases lactose was estimated by LS and total solids by AFM. For series II, K was calculated for the dry mixture from K_s. K_s was also incidentally determined by the REF method. The results obtained by the various methods were evaluated by analysis of variance. Substantial differences between methods occur. Tukey's test showed that some differences were significant (α = 0.05). The results obtained by the SEP-L method seem to deviate most from those obtained by other methods.

In other experiments various amounts of crystalline lactose hydrate (L3) were added to dried whey (WP22) and dried skim milk (MP13). Neither product contained crystalline lactose. K_s was determined by various SEP methods according to the procedure described in Section 3.2 for dry products. The amount of added lactose hydrate was calculated from K_s by multiplication by the total amount of lactose present in the various suspensions. The results, given in Table 6, show poor recoveries for both dry products.

From these recovery tests we conclude that for all modifications rather large differences from the true values are possible. This is understandable, as a very high accuracy is necessary in the determination of the parameters used for the

Table 6. Recovery of crystalline lactose hydrate, added to 3.50 g of dried whey or dried skim milk and dispersed in 25.0 ml of a 22.0 % lactose hydrate solution, as determined by separation methods.

Added amount of α lactose hydrate (mg)	Recovered when added to dried whey			Recovered when added to dried skim milk		
	SEP-L (Eq. 12)	SEP-TS (Eq. 13)	SEP-LTS (Eq. 15)	SEP-L (Eq. 12)	SEP-TS (Eq. 13)	SEP-LTS (Eq. 15)
0	−139	311	−17	−258	656	−31
250	143	385	207	40	924	258
500	596	759	640	318	1133	520
1500	1553	1804	1619	1437	2164	1614
2500	2792	2867	2811	2525	3276	2707

calculation of K_s. Because deviations in K_s will be enlarged for the determination of K in dry products rather poor results may be expected for them.

4 Isomer ratio method (IS methods)

4.1 Introduction

Because crystalline lactose in dairy products is usually α lactose hydrate (Section 1.3), the fraction crystallized (K) can be obtained from the determination of the β/α ratio (Q) of all the lactose in the product provided that the β/α ratio (Q^*) of the non-crystalline part of the lactose in the product is known.

K can be calculated by subtraction of the non-crystalline part of the α lactose from the total fraction of α lactose (A) of the lactose in the product:

$$K = A - (1 - A)/Q^* \tag{19}$$

or, since $Q = (1 - A)/A$, directly from

$$K = \frac{Q^* - Q}{Q^*(1 + Q)} \tag{20}$$

When only β lactose is crystallized, the method can be used in an analogous way. However, when both α and β lactose are in the crystalline state the method fails.

The value to be taken for Q^* is essential, but it cannot be determined by experiment. The equilibrium ratio is thought to be the best approximation. Therefore for liquid products $Q^* = 1.63 - 0.0023\ T$ was taken at temperatures T (°C) from 0° to 80 °C, according to earlier results (37). For amorphous lactose in spray-dried products the value $Q^* = 1.25$ (38) was used.

The β/α isomer ratio of the product (Q) can be determined by polarimetry; since the specific rotations of β and α lactose are known, determination of the optical rotation and of the lactose content of (or a solution of) the product yields Q. The quantities of α and β lactose can also be determined by gas-chromatography of their trimethylsilyl derivatives.

4.2 The polarimetric method (IS-POL method)

4.2.1 Principle of the method

To determine the relative amounts of α and β lactose the total amount of lactose in the sample should be completely and instantly dissolved. Now mutarotation occurs; hence the rotation at time t after solution (r_t) changes, until a
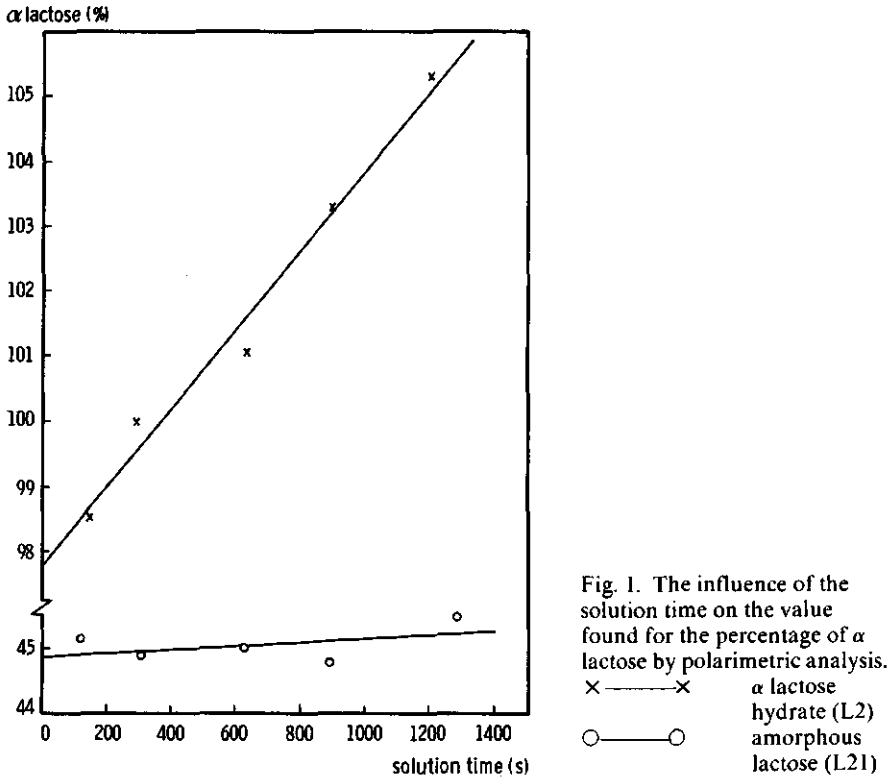
Fig. 1. The influence of the solution time on the value found for the percentage of α lactose by polarimetric analysis.
×———× α lactose hydrate (L2)
○———○   amorphous lactose (L21)

mal for amorphous lactose (38). The slope does not differ significantly from zero, and the solution time is thus not very critical. Mutarotation is slower here because the β/α ratio in the product ($Q = 1.23$) does not differ much from the final ratio in solution at 25 °C (1.58).

In most dried products with crystalline lactose a mixture of amorphous and α lactose hydrate is present. The lower the β/α ratio is at the time of solution (more crystalline α lactose present), the more important is quick solution, to allow for reliable extrapolation. It must however be emphasized that all lactose should be dissolved before the clarification and filtration step, so that no crystalline lactose is removed from the solution. The time taken was ample but always shorter than 5 min, leading to a deviation of at most 2 % α lactose (Fig. 1).

4.2.3.2 Determination of r_∞. The optical rotation was determined after 24 h at 25 ± 0.1 °C. The equilibrium β/α ratio, hence the equilibrium rotation,

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should definitely have been reached (38). We preferred not to use additives to accelerate mutarotation.

4.2.3.3 The β/α ratio in the non-crystalline lactose (Q*). Equilibrium β/α ratios, needed for the calculation of K, were taken from earlier determinations on lactose in aqueous solutions (37) and in the amorphous state (38).

However, the dissolved lactose in a liquid product may not have the equilibrium ratio. For instance, when concentrated whey leaves the evaporator at 60 °C and is rapidly cooled to 10 °C, the mutarotation rate is retarded about 100 times (38). The β/α ratio may still be 1.50 (corresponding to 60 °C), while a value of 1.62 is taken for the calculation of K.

In the case of extensive and rapid crystallization, large deviations are also possible. The rate of crystallization is now primarily determined by the rate of mutarotation (40), and the β/α equilibrium will be disturbed. Large deviations were found in the following experiments. To 50 g of a 50 % lactose solution, 5 g seed lactose was added at 25 °C. After 30 min of stirring the β/α ratio in the supernatant was determined to be 3.6, and in a second experiment with 3 g seed lactose we found 3.1. The equilibrium ratio at 25 °C is 1.58.

The β/α equilibrium ratio of the amorphous part of the lactose in spray-dried and lyophilized products is ~ 1.25. For spray-dried products without crystalline lactose, produced under normal conditions, the β/α ratio may in fact range from 1.15 to 1.35 (38). In pre-crystallized spray-dried products any difference from the ratio as found in products without crystals is unlikely. The true value of the β/α ratio in post-crystallized products may, theoretically, exceed considerably the assumed value of 1.25, because crystallization may proceed much faster than mutarotation in amorphous lactose (38).

4.2.4 Some applications and recovery tests
4.2.4.1 Lactose. The average values and standard deviations for r_o/r_∞ and A were calculated from the results of eight experiments with α lactose hydrate (L3). The crystals were dissolved to a concentration of 7.50 g/200 ml. Determinations were made according to the procedure for dry products (Section 4.2.2). The values found were r_o/r_∞ = 1.644 ± 0.0127 and A (%) = K (%) = 98.2 ± 1.2.

Mixtures of amorphous (L21) and crystalline α lactose hydrate (L3) were made in duplicate by adding 0 (1) 5 g amorphous lactose to crystalline lactose to a final weight of 5 g. The K values of these mixtures were calculated from the accurate weight after correction for water contents. The average difference between experimental and calculated results was small (ΔK (%) = 0.5 ± 0.5), but significant (*t* test α = 0.05).

4.2.4.2 Dried whey. The average values and standard deviations for r_o/r_∞, A and K were calculated from the results of 25 determinations of the lactose in the same dried whey (WP6). Values found were: r_o/r_∞ = 1.447 ± 0.0164; A (%) = 79.4 ± 1.5; K (%) = 63.0 ± 2.7.

Quantities of 0 (1) 4 g of α lactose hydrate (L3) were added to 6.5 (1.3) 1.3 g of dried whey (WP27A) in order to obtain six mixtures with the same quantity of lactose (~ 5.7 g) and K ranging from 0-80 %. The dried whey was checked for the absence of crystalline lactose by polarizing microscopy. The proportions of crystalline lactose in the mixtures were calculated from the lactose content of the dried whey and the water content of the α lactose hydrate. The results of K from duplicate determinations were increasingly higher than the calculated values as the added amount of crystalline lactose increased: ΔK (%) = 1 (at K = 0 %), 2 (at K = 20 %), 3 (at K = 40 %), 4 (at K = 60 %) and 5.5 (at K = 80%). This effect was significant (Spearman rank correlation r = 0.975). As the solution time was assumed to be responsible, we varied this time by varying the concentration from 2.5 to 10.6 g/200 ml; this concerned six samples of the mixture with K = 80 %. The solution time now ranged from 150 to 400 s. By extrapolation to zero time, K = 79 % was found. This shows that the recovery is good if a correction is made for the solution time. For pre-crystallized dried whey (WP27E) as such with K = 62 % there were no deviations caused by prolonged solution time. This is to be expected because of a relatively low β/α ratio at the time of solution (Section 4.2.3.1).

4.2.4.3 Dried skim milk. The average values and standard deviations for r_o/r_∞, A and K were calculated from the results of eight determinations, using sample MP13. The values found were r_o/r_∞ = 1.237 ± 0.0055, A (%) 60.3 ± 0.5 and K (%) = 28.5 ± 0.9. Sample MP9 (without crystals), K = 0 %, gave r_o/r_∞ = 1.065 ± 0.0013 and A (%) = 44.6 ± 0.1 (n = 18).

A recovery test was done in the same way as for dried whey. The same significant increase of K with higher additions of crystalline lactose was also found here. As in the case for dried whey these poor recovery results are not considered to be important: they are due to prolonged solution time caused by added crystals.

4.3 The gas chromatographic method (IS-GC method)

4.3.1 Procedure
The procedure as given by Newstead & Gray (11) to determine α and β lactose by gas chromatographic analysis was followed. The method was improved by Olling (41): as pyridine strongly accelerates mutarotation (42), dimethyl sul-

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phoxide, in which mutarotation is much slower, was used as a solvent. As water interferes with the silylation reaction it can only be applied to dry products.

An amount of product containing 5-10 mg lactose was dissolved in 0.5 ml dry dimethyl sulphoxide in a closed reaction vial. Silylation was carried out by subsequent addition of 0.2 ml hexamethyl disilazane and 0.1 ml trimethyl chlorosilane. By the addition of 1.0 ml pure pyridine, the original polar and apolar layers become one phase and a precipitate forms, which is removed by centrifugation. The TMS derivatives of α and β lactose are separated by gas chromatography, applying the following conditions: instrument Perkin Elmer F-11 (dual column, dual flame version). I.D. columns 1.40 m $\times$ 2 mm containing 4 % XE-60 on 100-120 mesh Varaport 30, carrier gas 15 ml N_2/min, temperature 220 °C; injection of 0.3-0.6 μl of the reaction mixture at 270-300 °C.

4.3.2 Results and discussion

The method was applied to determine the β/α ratio of spray-dried and crystalline lactose, dried whey and dried milk. Part of the results obtained have been published earlier (38). In α lactose hydrate small amounts of β lactose were always found. This confirms the results given by other authors (39). For spray-dried lactose (L21) the β/α ratio was found to be 1.19 $\pm$ 0.038 (n = 8).

K can be calculated according to Eq. 20. As is the case for the IS-POL method, the β/α ratio in the non-crystalline lactose (Q^*) has to be known. The method does not seem to have any particular advantages over the IS-POL method.

5 Methods based on determination of the water of crystallization of α lactose hydrate (CW methods)

5.1 Introduction

If all crystalline lactose in a product is α lactose hydrate, the fraction crystallized (K) can be derived from the fraction of water of crystallization (W), which accounts for 5.00 % by weight of the crystalline lactose. Hence,

$$K = 0.19 \, W/L \tag{25}$$

L being the fraction of lactose (expressed as anhydrous lactose) in the product.

The method is only valuable if W can be determined accurately. Water evaporating from milk products during drying tests may be classified into:

a) water formed during heating by 'pyrolysis';

b) water of crystallization in α lactose hydrate;

c) water tightly bound to other substances, particularly to proteins and some salts;

d) water less tightly bound.

The difference between c and d is only gradual. There are no unequivocal methods of assessing the different kinds of water. Most methods based on drying yield values including all of d. The amounts of water removed from the proteins (c) and from lactose hydrate (b), as well as the quantity of internal water formed (a), depend on the drying conditions. Moreover, weight gain from oxidation or weight loss from decomposition, as caused by severe heating may introduce errors.

W can be determined directly or indirectly. Indirect determination can be based on the difference in water content found when applying two different methods; or it can be based on the toluene distillation method (13). As a direct method differential thermal analysis (DTA) could be applied.

5.2 Method based on difference in water content (CW-WAT method)

5.2.1 Introduction and procedure

Since the Karl Fischer method (KF) determines the total water content b + c + d (Section 5.1) this method was chosen. Comparing the water contents determined by KF and by drying, K can be estimated if the latter does not remove b and if the difference in water content, when applying the KF method and the drying method to the non-crystalline lactose part of the product, is known.

KF determinations were performed with an automatic titrator (Metrohm, type E452). The procedure described by Thomasow et al. (43) was followed, inserting the sample by means of the Metrohm plug device. Because dehydration of α lactose hydrate in methanol is very slow (44), the usual procedure applied in the KF titration of the hydrate is sluggish and therefore inaccurate. Improvement was obtained by dissolution of the lactose hydrate in the product before titration in a mixture of methanol and formamide (1:1). Titration of dried milk was done in methanol since the powder particles coagulated in the methanol formamide mixture.

Drying was done according to the German standard method (GS) (45). In this procedure the sample is dried for 6 h at 87 °C, which results in negligible loss of water of crystallization (46).

The difference in water content (ΔM, expressed in percentage units) was calculated by subtracting the mean of two results by the drying method from the

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mean of three results by the KF method. After correction of ΔM for a blank (Section 5.2.2) the value for W in Eq. 25 was obtained. Because an accurate assessment of W is impossible for products with much water, the method is restricted to dry products.

5.2.2 Determination of the blank value

Nine samples of commercial spray-dried whey and skim milk were checked for the absence of crystalline lactose by polarizing microscopy. Following the procedure given in Section 5.2.1, ΔM was determined. Dried whey gave ΔM (%) = 0.47 ± 0.09 and dried skim milk ΔM (%) = 0.87 ± 0.09. For a second series of 21 samples of dried skim milk ΔM (%) = 0.76 ± 0.20 was found. These samples were taken from six lots of spray-dried produced from the same milk in the NIZO pilot plant. There was no significant difference in ΔM between these lots of powder.

It was checked whether an approximation of the blank values could be given on the basis of the water content of the individual constituents. The major constituents are: proteins, salts and lactose. Whey proteins were prepared by dialysing fresh whey against distilled water and freeze-drying the retentate. Casein was isolated from skim milk by iso-electric precipitation at pH 4.6. The precipitate was washed and freeze-dried. A solution of milk salts was simulated as described by Jenness & Koops (47), and spray-dried. Amorphous lactose was prepared by spray-drying a solution of α lactose hydrate (L3). L3 was also used as a source of crystalline lactose. The ΔM values found for the individual constituents are given in Table 7. All the main constituents of dried milk and whey seem to contribute to ΔM. The value found for amorphous lactose was confirmed by analysis of other spray-dried lactose solutions: 0.36 %, 0.42 % and 0.52 %. From the average composition of dried whey and dried

Table 7. Difference in water content between the Karl Fischer method (KF) and the German Standard Drying Method (GS) for the major individual constituents of dried whey and dried skim milk both as such (ΔM) and in g per 100 g dry product (ΔM^*) (calculated from the KF water content).

Constituents	Water (%)			
	KF	GS	ΔM	ΔM^*
Whey proteins	10.73	9.08	1.65	1.85
Casein	10.75	9.17	1.58	1.77
Milk salts	4.19	2.73	1.46	1.52
Amorphous lactose	1.74	1.34	0.45	0.46
Crystalline lactose	5.06	0.00	5.06	5.33

skim milk without crystalline lactose, differences of 0.70 and 1.01 % water, respectively, were calculated. These results are only approximations, because of variation in composition, uncertainty in ΔM of the constituents, and possible interactions between the latter. Moreover, lyophilized precipitated casein may behave differently from (spray-)dried casein micelles, and the water of crystallization of spray-dried milk salts may depend on drying conditions, etc. Therefore, the calculated blanks appear to agree reasonably well with the experimental values.

In conclusion, neither dried whey nor dried milk has a constant blank value of ΔM. In particular, the contribution of amorphous lactose to ΔM is smaller when part of the lactose is crystalline. A deduction of $0.45\,K\,L$ was made for the crystalline lactose present in which 0.45 was taken as the contribution of amorphous lactose to ΔM (%) and KL represents the fraction of anhydrous lactose (L) of the product in the crystalline state. The corrections for dried whey and dried skim milk without crystalline lactose were assumed to be 0.45 % and 0.9 %, respectively. Then the total correction C of ΔM (%) for the blank is for dried whey $C\,(\%) = 0.45 - 0.45\,K\,L$ and for dried skim milk $C\,(\%) = 0.9 - 0.45\,K\,L$. Substition of $W = (\Delta M - C)/100$ in Eq. 25 yields:

$$\text{for dried whey: } K = (0.208\,\Delta M - 0.093)/L \tag{26}$$
$$\text{and for dried skim milk: } K = (0.208\,\Delta M - 0.187)/L \tag{27}$$

in which ΔM is expressed in percentage units.

Correction for a blank is thus necessary. Anderson & Berlin (48) used a similar method to estimate crystalline lactose in dried whey. In our opinion, a correction for a blank should also have been applied in their experiments.

5.3 Method based on toluene distillation (CW-T method)

This method, devised by Choi et al. (13), is based on the slow release of water of crystallization by toluene distillation, which is supposed to be a first order reaction. The quantity of water distilled off (x) is estimated as a function of time. Total water content is estimated by Karl-Fischer titration (a). The linear part of log (a-x) as plotted against time, is extrapolated to zero time. The difference between this intercept and the total amount of water is reported to yield the content of water of crystallization. Some critical remarks must be made.
1. Sharp et al. (49) demonstrated that part of the moisture in dried whey and skim milk (both without lactose hydrate) and in casein is released as slowly as the water of crystallization. This is in agreement with our finding that the

water of amorphous lactose and of proteins is not removed so easily (Section 5.2.2).

2. Both Choi et al. (13) and Sharp et al. (49) found that the rate of removal of water of crystallization depends on crystal size. Crystals smaller than 50 μm could not be analysed by this method. Hence extrapolation for post-crystallized products that usually contain very small crystals (1 < μm), will be inaccurate. Moreover, for products containing crystals in a wide size distribution, the release of water of crystallization cannot follow first-order kinetics, thus making extrapolation impossible.

3. Substituting in log $(a-x)$ for a the percentage of water determined by Karl Fischer titration may give incorrect results, because there is a significant difference between the results of the Karl Fischer titration and of the toluene distillation method for some products (48, 50, 51, 52, 53).

The method was difficult to carry out because of frequent failure due to coagulation in the powder-toluene mixture. Moreover, in most of the trials with dried whey and skim milk no reasonable extrapolation could be made (54). We therefore did not investigate the method further.

5.4 Method based on differential thermal analysis (CW-DTA method)

5.4.1 Introduction and procedure
Berlin et al. (14) found an endothermic dehydration peak between 97 and 167 °C with a maximum at 144 °C when heating a sample of a lactose hydrate at 10 °C/min in a covered sample pan, due to the release and vaporization of water of crystallization. Later it was indicated that the fraction of lactose crystallized in dried whey could be estimated quantitatively by thermogravimetry (48). Therefore, we tried to determine W (defined in Section 5.1) and hence K, by differential thermal analysis, which should yield the enthalpy of vaporization.

A Du Pont type 900-1B DTA Scanner was used. Amounts of approximately 5 mg were weighed accurately (± 0.001 mg) in covered sample pans. The pans were not sealed and an empty one was used as a reference. Melting of pure indium was used for calibration, to obtain absolute values for the enthalpy expended. Samples of a lactose hydrate, dried whey and skim milk were scanned from 90 to 180 °C at 5 °C/min. Peak areas were measured planimetrically.

5.4.2 Results and discussion
In Fig. 2 an endothermic peak is shown for a lactose hydrate from 110 to 160 °C. Similar peaks were found for dried whey and skim milk containing these crystals, and when crystals were absent no peaks were observed. A re-
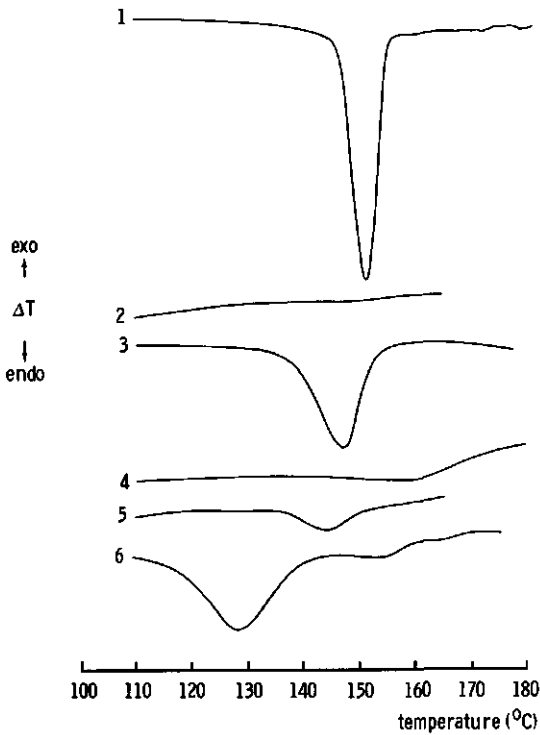
Fig. 2. DTA scans of ca. 5 mg of α lactose hydrate (1), dried whey without crystalline lactose (2), pre-crystallized dried whey (3), dried skim milk without crystalline lactose (4), pre-crystallized (5) and post-crystallized (6) dried skim milk. Heating rate 5 °C/min.

markable feature is the shift of the peak towards lower temperatures in post-crystallized dried skim milk. Such a shift was also observed in the various samples after grinding. This is a confirmation of the results of Lerk et al. (55).

To find the specific peak area several scans were run with crystalline α lactose hydrate from various sources. Commercial lactose (L2) was used directly and after crushing in a mortar. There is a significant difference in specific peak area between product L2 before and after grinding (Wilcoxon's test, $\alpha =$ 0.05). When varying the amount of sample taken (L4) from 2 to 11 mg, no significant difference was observed (Spearman rank correlation test, $\alpha =$ 0.05). For the specific peak area the value 0.105/mg α lactose hydrate was taken.

Several samples of dried whey and dried skim were examined. The fraction crystallized was calculated from:

$$K = 0.95 \, O/S \cdot L = 9.05 \, O/L \tag{28}$$

in which O = peak area found/mg product, S = specific peak area of α lactose hydrate, and L = fraction of anhydrous lactose in the product.

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Table 8. Peak areas per mg of product obtained from DTA scans, and the resulting values of K for dried whey and dried skim milk.

Product	Peak area/mg	$L(\%)^*$	$K(\%)$
α lactose hydrate			
L2	0.084 (s = 0.0023, n = 7)		
L2 (ground)	0.101 (s = 0.0078, n = 10)		
L4	0.106 (s = 0.0055, n = 10)		
L5	0.105 (s = 0.0136, n = 5)		
Dried whey			
WP 22 (without crystals)	0.0012 (s = 0.0002, n = 3)	73.0	1
WP 27B (pre-crystallized)	0.025 (s = 0.0018, n = 3)	75.5	30
WP 27C (pre-crystallized)	0.039 (s = 0.00025, n = 17)	76.4	46
WP 27D (pre-crystallized)	0.047 (s = 0.0029, n = 6)	77.4	55
WP 27E (pre-crystallized)	0.057 (s = 0.00023, n = 10)	78.4	66
WP 27E (ground)	0.065 (s = 0.0056, n = 7)	78.4	75
Dried skim milk			
MP 13 (post-crystallized)	0.029 (s = 0.0012, n = 3)	50.4	52
MP 19 (post-crystallized, ground)	0.043 (s = 0.0100, n = 10)	50.6	77
MP 23 (without crystals)	0.00 (n = 2)	50.1	0
MP 25A (without crystals)	0.00 (n = 2)	51.8	0
MP 25B (pre-crystallized)	0.015 (s = 0.0026, n = 4)	52.8	26
MP 25B (ground)	0.019 (s = 0.0015, n = 3)	52.8	33
MP 26 (without crystals)	0.00 (n = 3)	50.3	0

* Anhydrous lactose content as determined by the polarimetric method (Pol-PE).

As can be seen in Table 8 for products without crystalline lactose zero values were indeed found. For pre-crystallized dried whey and milk the results appeared to be fairly correct as compared to those of other methods, whereas too high values were found for post-crystallized dried milk (Section 6). Since in post-crystallized products the crystals are very small the size of the crystals may be important. Crushing crystals in a mortar seems to increase the value found for K (Table 8), which also indicates that the presence of small crystals leads to deviating results.

The main problem in the application of the CW-DTA method is to establish a correct base line, which is reflected in the large standard deviations.

6 Comparison of results obtained by the various methods

6.1 Introduction

The previous sections give results of individual experiments with the various methods of determining K. In the present section the results obtained by different methods will be compared, by applying them to the same milk prod-

ucts. Concentrated whey, dried whey and dried skim milk were selected, being products for which crystallization of the lactose is important in the dairy industry. The various SEP methods, the REF and the IS-POL methods were used to examine the fluid and the dry products. In addition, the CW-WAT, CW-DTA and IS-GAS methods were applied to the dry products.

6.2 Comparison of results for concentrated whey

6.2.1 Particulars of determinations
Several concentrates were prepared as described in the list of materials. During crystallization the concentrates were continuously and slowly stirred. Prior to sampling the crystals were intensively mixed with the liquid by vigorous stirring.

The refractive indices needed (REF, SEP) were determined after dilution with a lactose solution in the case of concentrate with total solids content > 40 % ($\sim$ 50 g lactose/100 g water).

The density of the final solution, required in Eqs. 7 and 8 was obtained from the corresponding refractive index, as indicated in the tables for sucrose solutions. No difference was found for K in doing so, as compared to the result obtained by weighing the content of a calibrated measuring flask of 500 ml at 20 °C.

The determination of the parameters of the liquid in which all the lactose had been dissolved, was not always carried out for all samples of the same concentrate. In the case of series W1 and W4, n^F, L_i, D_i and d only were determined in a sample taken immediately after concentration. In the analysis of series W2 this was done for L_i and D_i. In the samples taken directly, crystalline lactose was absent, as checked by polarizing microscopy.

The value of K determined by IS-POL was calculated with $Q^* = 1.60$ at 15 °C, at which temperature the concentrates were kept (Section 4.1).

6.2.2 Results and discussion
The results are collected in Table 9. The determination of K by IS-POL was carried out in duplicate. The duplicate results are indicated as a and b. For samples of series W4 (b) differed more from (a) than for W3. As (b) was determined $\sim$ 30 min after (a) (because only one polarimeter was available), and since the concentrates of W4 were crystallizing and those of W3 were not, the differences are explained by continuing crystallization. Moreover, in rapidly crystallizing concentrates the β/α ratio (Q^*) may differ considerably from the equilibrium ratio (Section 4.2.3.3), causing too low values for K. The method therefore has a distinct disadvantage when applied to concentrated whey un-

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Table 9. K values for concentrated whey, according to REF, SEP and IS-POL.

Method	Sample series	K (%) of sample					
		A	B	C	D	E	F
REF	W1	11	29	38	45		
SEP-L		7	27	35	43		
SEP-TS		11	23	32	39		
SEP-LTS		8	27	33	44		
SEP-STS		11	30	40	47		
REF	W2	9	20	30	46	48	66
SEP-L		6	16	28	45	43	62
SEP-TS		11	22	36	51	47	69
SEP-LTS		7	17	29	46	44	63
SEP-STS		11	20	31	48	48	66
REF	W3	1	0	22	66	85	
SEP-L		1	−4	13	66	79	
SEP-TS		2	7	23	70	90	
SEP-LTS		1	−1	16	67	81	
SEP-STS		2	0	23	69	87	
IS-POL a*		1	1	17	61	81	
IS-POL b*		1	1	19	62	83	
REF	W4	22	34	46	53		
SEP-L		17	30	42	51		
SEP-TS		24	34	48	55		
SEP-LTS		20	31	43	51		
SEP-STS		23	36	48	55		
IS-POL a*		15	29	39	47		
IS-POL b*		22	34	43	48		

* Duplicate results (b $\sim$ 30 min after a).

Table 10. Results of analysis of variance of K values given in Table 9 for concentrated whey.[1]

Method	REF	SEP-L	SEP-TS	SEP-LTS	SEP-STS
REF		3.4	−1.2	2.3	−1.3
SEP-L	*		−4.6	−1.1	−4.6
SEP-TS		*		3.5	−0.1
SEP-LTS	*		*		−3.6
SEP-STS		*		*	

[1] Average differences between calculated K values (%) (e.g. top row gives REF-SEP.L; REF-SEP.TS etc.). Significant differences between methods are indicated (*). Critical difference (Tukey, $\alpha = 0.05$) = 1.84 %.

Table 11. K values for dried whey (WP), dried skim milk (MP) and dried lactose solutions (L) obtained by various methods.

Sample	Type of crystalli-zation	REF	IS-POL	CW-WAT	IS-GAS	CW-DTA	SEP L	TS	LTS
WP									
1A	0	1	1	−2	1				
1B	pre	12	7	3	10				
1C	pre	32	28		30				
1D	pre	39	38	34	37				
1E	pre	49	46	43	45				
2A	0	−1	0		5				
2B	pre	17	14		16				
2C	pre	30	22	25	24				
2D	pre	37	35	34	36				
2E	pre	55	55	54	50				
2F	pre	53	51	51	47				
2G	pre	70	66	69	62				
5	pre	54	50	54					
6	pre	70	63	65					
7	pre	63	62						
22	0	1	0	2					
27A	0	0	0	−1		1	8	7	8
27B	pre	38	32	34		30	40	46	42
27C	pre	46	46	45		46	68	55	65
27D	pre	55	56	57		55	70	56	67
27E	pre	63	62	62		66	88	70	84
31	pre + post	81	72						
32	pre + post	90	75	76					
36	post	19	4						

Sample	Type of crystalli-zation	REF	IS-POL	CW-WAT	IS-GAS	CW-DTA	SEP L	TS	LTS
MP									
8	0	−4	0						
9	0	0	0						
10	0	3	0						
13	post	112	23	35	22	52	45	101	57
14	0	27	−1	−1	0			23	8
15	0	27	1	10	0				
16	0	0	−1	5	−2				
17	0		−1	−13					
19	post	139	56	66	54	77	3		
23	0	45	1			0	6		
25A	0	11	0	−10	−3	0	27		
25B	pre	41	26	15	23	26	37	44	39
26	0	1	0	−4	−3	0	8	9	8
L									
24A	pre + post	97	71						
24B	pre + post	83	62						
24C	post	13	2						

dergoing crystallization.

An analysis of variance was made leaving IS-POL out of consideration. As can be seen in Table 10 the results for K, obtained by the various modifications of SEP, do not compare well. The significant difference between SEP-L and SEP-TS may have been caused by separation of non-lactose solids. If so, SEP-TS gives too high and SEP-LTS would yield correct results. As seen in Section 3.3 separation of non-lactose solids from concentrated whey is very unlikely. In addition, there is fair agreement between REF, SEP-TS and SEP-STS. Moreover we found that the refractive indices of the supernatants compared well with those of the corresponding suspensions before centrifugation. We therefore assume that the low results obtained by SEP-L and SEP-LTS were caused by inaccurate lactose determinations. As illustrated in Table 3, a low result by SEP-L, as caused by deviating lactose contents would correspond with low results by SEP-LTS, although to a lesser extent in the cases that the solids contents were correct. Deviating results by SEP-L and SEP-LTS were also found in the recovery tests (Section 3.6).

Although it cannot be concluded which method gives the true result, it is reasonable to assume that the methods REF, SEP-TS and SEP-STS come close. SEP-STS performs surprisingly well, considering the fact that it is based on rough approximations of total solids contents and of the total lactose content of concentrated whey.

6.3 Comparison of results for dried whey and dried skim milk

6.3.1 Particulars of determinations
Spray-dried whey and skim milk were prepared as described in the list of materials. Approximately 500 g of each of the well-mixed powders were stored in air-tight containers. In the samples assigned type 'o' in Table 11, crystalline lactose was absent as checked by polarizing microscopy. Pre- and post-crystallization of lactose in the other samples are also indicated in this table.

For all calculations of K of a particular sample the same (polarimetric) lactose content was used. For the calculation of K of a certain sample with the SEP methods the values for L_i and D_i were the same.

6.3.2 Results and discussion
6.3.2.1 Discussion of the results by REF. As can be seen from the results, collected in Table 11, those obtained by REF differ markedly from the others in the case of dried skim milk. The method yields for samples without crystalline lactose results around zero (MPs 8, 9, 10, 16, 26) or positive values (MPs 14, 15, 23, 25A). K values for some post-crystallized samples were even higher

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than 100 % (MPs 13 and 19). The main causes for these deviations were further investigated.

Too high results for K must have been caused by too high values for Δn. As n^F was found to be ~ 1.3836, which is normal for dried skim milk, n^I must have been too low, caused by crystallization of lactose during determination, by undissolved constituents in the initial solution, or both.

To check this, Δn was determined for samples of the product without crystals (MP26), post-crystallized (MP13) and pre-crystallized (MP25B), varying the concentration (x) of the lactose solution used for dispersion. As can be seen in Fig. 3 only sample MP26 gave Δn (x) = 0 over the whole range of x. With water (x = 0) for samples MP13 and MP25B, Δn (o) = 18 × 10^{-4} and 8 × 10^{-4}, respectively. Because no crystals of lactose could be found in the initial solutions by polarizing microscopy, these values for Δn (o) must be ascribed to undissolved material other than crystalline lactose. The amounts of sediment in these solutions were determined by centrifuging the suspensions at 1000 g for 30 min. Determinations of the protein content of the samples and the corresponding supernatants were carried out. From the results it was calculated that the suspensions contained 10, 220 and 50 mg of undissolved protein for the MP samples 26, 13 and 25B, respectively.

Δn (o) caused by this quantity of proteinaceous material can be calculated from the following equation, which is analogous to Eq. 3:

$$\Delta n = \frac{(r_e \cdot v^F - q_e^F \cdot r_e \cdot \bar{v}_e - q_R \cdot r_R \cdot \bar{v}_e)q_o}{(v^F)^2 - v^F \cdot \bar{v}_e \cdot q_o} \quad (26)$$

in which:

specific refraction increment of milk proteins	r_e	= 0.2 ml/g (25)
volume of final solution	v^F	= 27.1 ml (determined)
total protein in final solution	q^F_e	= 1.26 g (determined)
apparent specific volume of protein (neglecting bound water)	$\bar{v}_e$	= 0.75 ml/g (56)
non-protein constituents in final solution	q_R	= 2.15 g (determined)
specific refraction of the non-protein contuents	r_R	= 0.15 ml/g (estimated).

The quantity of undissolved proteinaceous material q_o can be calculated after substitution of these values in Eq. 26 according to:

$$q_o = 147 \, \Delta n/(1 + 4 \, \Delta n) \quad (27)$$

giving 0, 263 and 117 mg for MP samples 26, 13 and 25B, respectively. Considering the inaccuracies in the various approximations made, the calculated and found values agree reasonably well.

If crystallization of lactose occurs during determination, the initial solution must have been supersaturated. The equilibrium lactose concentration in 25 ml of a lactose solution containing 3.50 g of MP sample 13 or 25B can be calculated to occur at ~ 14 % lactose hydrate, using the K values determined by IS-POL (Table 11). Therefore the decrease of $\Delta n(x)$ in Fig. 3 for both samples at concentrations $x < 14$ % should probably be explained by dissolution of crystalline lactose. For $x > 14$ % $\Delta n(x)$ is almost constant for sample MP25B, but it increases strongly for sample MP13, indicating crystallization during determination. This difference in behaviour can probably be ascribed to the difference in size of the crystals in pre-crystallized and post-crystallized products. If 3.50 g dried skim milk ($K = 28$ %) is dissolved in 25 ml of a 22 % solution of lactose hydrate, some 500 mg crystalline lactose is present, yielding a calculated supersaturation of ~ 70 % during the procedure for the
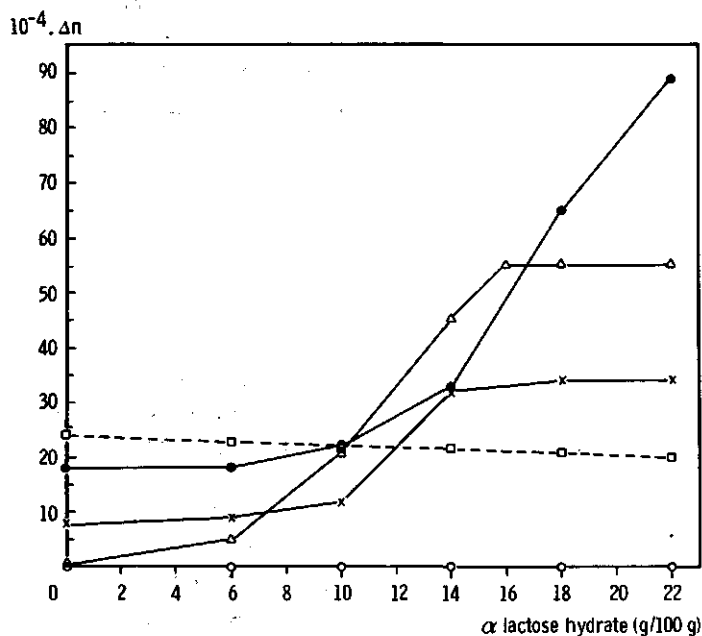

Fig. 3. Δn as determined according to the refractive index method, using lactose solutions with various concentrations.
Experimental results:
●——●   post-crystallized dried skim milk (MP 13)
×——×   pre-crystallized dried skim milk (MP 25B)
○——○   dried skim milk without crystalline lactose (MP 26)
△——△   pre-crystallized dried whey (WP 27C)
Calculated results:
□— — —□   dried skim milk with an average composition and $K = 28$ %.

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determination of n^1. However, as most crystals are enclosed in amorphous lactose (3) initially much higher supersaturations are possible. It is therefore difficult to estimate what growth of the crystal surface may be expected. From data of van Kreveld & Michaels (57, 58) it can be derived that the average linear growth of the surface of α lactose hydrate crystals kept for 5 min at 30 °C, would be roughly 0.2 μm and 2 μm at 55 % and 120 % supersaturation, respectively. Between MP samples 13 and 25B a difference of Δn (22) ~ 56 $\times$ 10^{-4} is found (Fig. 3). Such a big difference can be explained by crystallization during determination of the former, as is illustrated by the following calculation for which several assumptions have been made:
a) both samples contain 500 mg crystalline lactose in 3.50 g (K ~ 28 %);
b) crystals are equivalent to spheres with a radius (r) of 1 μm for the post-crystallized and of 50 μm for the pre-crystallized type;
c) there is a growth of 0.9 μm of the crystal surface in the initial solution before the reading of n^1. (Here, it is assumed that the effective average supersaturation is > 70 %).
The surface area (Z) of the crystals (number N) in the initial solution of the sample is given by

$$Z = 4\pi r^2 N \tag{28}$$

where N is derived from $\frac{4}{3}N\pi r^3 d = 5 \times 10^{-4}$ kg, in which the density d of α lactose hydrate is 1540 kg/m^3. Substitution in Eq. 28 yields:

$$Z = (1/r) \times 10^{-6}\,\text{m}^2 \tag{29}$$

For $r = 10^{-6}$ m and 50 $\times$ 10^{-6} m, Z becomes 1 and 0.02 m^2 respectively, yielding for a growth of the crystal surface of 0.9 μm additional quantities of 1.4 and 0.03 g α lactose hydrate before the reading of n^1. From Eq. 5 and Eq. 10 follows:

$$\Delta n = q_k/(253 - 6q_k) \tag{30}$$

Substitution of 1.4 and 0.03 g yields $\Delta n = 57 \times 10^{-4}$ and $\Delta n = 1 \times 10^{-4}$, respectively. This agrees well with the results of Fig. 3.

Unreliable results for K, due to crystallization during the determination procedure, can thus be expected for other post-crystallized products as well. For post-crystallized dried whey and spray-dried lactose solutions we indeed found too high values, as compared to IS-POL (Table 11). Because in these cases $\Delta n(o)$ was invariably zero, the increased values could not be ascribed to undissolved constituents.

We conclude that REF cannot be applied to post-crystallized products. The method is unreliable for pre-crystallized dried milk as well, because undissolved proteinaceous material may contribute to Δn.

6.3.2.2 Discussion of the results by SEP

The results obtained by the SEP methods often disagree with those found by most other methods. The dispersion of the dry product in a lactose solution was performed in the same way for SEP as for REF (Section 3.2). Therefore the same difficulties may be encountered here. Post-crystallized products will crystallize during the determination procedure, causing aberrant results for all modifications of SEP. Consequently, the method cannot be applied to products of this type. Also for products without crystalline lactose (WP27A, MP14, MP26) incorrect results were obtained. If separation of non-lactose solids were the cause, SEP-LTS should at least yield zero values, but apparently this is not the only deviation. As discussed in Section 6.2.2, SEP-L and SEP-LTS were considered to be insufficiently accurate methods for concentrated whey. In the case of dry products, the deviations of K as found for the suspensions, are multiplied by a factor ~ 3.3 for dried whey and ~ 4.2 for dried skim milk (cf. Eq. 16). As in addition recovery was poor in all three modifications of SEP for dried whey and dried skim milk (Section 3.6), we consider the method to be insufficiently accurate.

In the method of Choi et al. (6), which is a similar separation method, a weighed amount of 5 g dried milk or 3.5 g dried whey was added to 25.00 g of a 22.8 % lactose hydrate solution at 25 °C. After dispersion of the product by shaking the mixture by hand for 1 min the crystals were separated by centrifugation at 1500 rpm for 2 min. In their method K was calculated from lactose and total solids contents. Consequently, it must be assumed that their method has similar restrictions to our own, especially if used for post-crystallized products. We tested Choi's dispersion procedure but found it to be no improvement. On the contrary, it was very difficult to dissolve the dry product and this resulted in larger deviations of K.

6.3.2.3 Discussion of the results by IS-POL, CW-WAT, IS-GAS and CW-DTA

The results obtained with dried whey, given in Table 11, agree fairly well for the methods IS-POL, CW-WAT, IS-GAS and CW-DTA. The same is true in the case of dried skim milk, for both IS methods. CW-WAT seems to be unreliable here. From the values of ΔM determined in dried skim milk and dried whey without crystalline lactose (Section 5.2.2), standard deviations of K % due to variation of the blank, are calculated as being maximally $s = 7$ and $s = 2.3$, respectively. As the correction for the blank (C) is derived from these values, the poor results obtained for dried skim milk should primarily be ascribed to the large deviations which are possible for C. Too high values may be found by CW-WAT due to crystallization of the lactose during the drying procedure according to the German standard method. During 6 h at 87 °C there is ample

Table 12. Results of an analysis of variance of K values given in Table 11 for dried milk products.[1]

Method	REF	IS-POL	CW-WAT
REF		3.0	2.7
IS-POL	*		−0.3
CW-WAT	*		

[1] Average difference between calculated K values (%) (e.g. top row gives REF-IS.POL; REF-CW.WAT, etc.). Significant differences between methods are indicated (*). Critical difference (Tukey, $\alpha = 0.05$) = 1.88 %.

opportunity for mutarotation (38) and crystallization may well occur, especially in products with a high initial moisture content and in post-crystallized products.

The values of K found by WAT-DTA for post-crystallized dried skim milk (MPs 13 and 19) differ considerably from those found with the two IS methods. As indicated in Section 5.4.2 the method is not reliable for this type of product.

An analysis of variance was carried out for the results obtained by REF, IS-POL and CW-WAT taking into consideration the following samples of pre-crystallized WPs 27B, C, D, E; 1 B, D, E; 2 C, D, E, F, G; 5 and 6. The results are shown in Table 12. A similar analysis was performed for the results obtained by both IS-methods for the following samples of pre-crystallized WPs 1 B, C, D, E; 2 B, C, D, E, F and G. No significant difference was found. Because the same isomer ratio of non-crystalline lactose (Q^*) was applied, we may conclude that the different procedures to determine Q do not cause significant differences in K. The results obtained by both methods can however be equally influenced by uncertainty in Q^*.

Since IS-POL, IS-GAS and CW-WAT compare well in the case of pre-crystallized dried whey, and REF shows somewhat higher results, it cannot be decided which method gives the true value. For post-crystallized products, evidence was presented that all methods lead to unreliable results except the two IS methods. The only disadvantage of the latter could be that the value chosen for Q^* (1.25) may in some cases be too low for this type of product (Section 4.2.3.3), causing too low K values.

7 Evaluation of the suitability of the methods used

The main criteria for the suitability of the various methods are: limitations for different types of products, accuracy, and practicability (in particular speed

and ease of execution and costs). These aspects are briefly discussed for each method.

7.1 Limitations

7.1.1 REF method. The method fails if in dry products the crystalline lactose present is not in the α lactose hydrate modification. In the case of liquids the method could in principle be adapted to determine also other types such as crystalline β lactose. REF cannot be used for post-crystallized dry products, not only because the fraction of lactose in tiny crystals ($<$ 100 nm) may be considerable, but predominantly because substantial crystallization may occur during the determination. Its application is not possible when non-lactose solids contribute to Δn as can frequently be observed when analysing dried (skim) milk.

7.1.2 IS methods. Determination of either crystalline α lactose hydrate or β lactose is possible. The method cannot be used if both α and β crystals or $\alpha_5\beta_3$ crystals are present. The β/α ratio of the non-crystalline part of the lactose in the product (Q^*) should be known in order to obtain accurate results (Section 7.2).

7.1.3 SEP methods. Their application is not possible in the case of post-crystallized dried products because of crystallization during the determination. These methods should be neither used for other dried whey and dried milk products, because of high inaccuracy (Section 7.2). For liquid products the modifications SEP-L and SEP-LTS are too inaccurate to be advisable.

7.1.4 CW methods. Both CW methods are restricted to dried products containing crystals of α lactose hydrate. The application of CW-DTA to post-crystallized products is inaccurate (Section 7.2), whereas CW-WAT cannot be used in the case of dried products in which crystallization occurs while drying for 6 h at 87 °C (e.g. post-crystallized products and products with a high moisture content).

For some of the limitations mentioned it cannot be checked easily in advance whether they apply. For instance, the absence of crystals other than α lactose hydrate is difficult to ascertain. However it is so rare in most dairy products (Section 1.3) that it can be overlooked. Whether a product is pre- or post-crystallized can often be detected with the polarizing microscope. Knowledge about the processing will also be helpful to make the right decision (Section 1.1).

7.2 Accuracy

7.2.1 REF method. The method showed good results in recovery tests, both for liquid and dried products (Section 2.5). The repeatability is predominantly governed by the accuracy of reading the refractometer. For K of concentrated whey the corresponding standard deviation is estimated as s $\approx$ 1.0 and for pre-crystallized dried whey as s $\approx$ 1.5 percentage units (Section 2.6). The method is thought to give correct results in the case of concentrated whey. For pre-crystallized dried whey the results were slightly higher than those obtained by other methods.

7.2.2 SEP methods. Recovery tests carried out on fluid products were rather unsatisfactory, especially when based on lactose contents. Recovery tests performed on dried products were poor (Section 3.6). The repeatability was also poor in the case of dried products: pre-crystallized dried whey (WP27C) giving, for SEP-L, -TS and -LTS, s = 3.1; 1.8 and 2.7, respectively (n = 7); and dried skim milk (MP13) yielding s = 3.0; 3.0 and 2.8 percentage units, respectively (n = 7).

The results of SEP-TS and SEP-STS did compare well with those of other methods when analysing concentrated whey, but not in the case of dried products.

7.2.3 IS-POL method. Inaccurate K values may be found if the β/α ratio of the non-crystalline fraction of the lactose deviates considerably from the equilibrium value taken for calculation. In rapidly crystallizing liquids these deviations were substantial (Section 4.2.3.3). Consequently, the method should not be used for these products. In dried products the deviation from the assumed β/α ratio of 1.25 depends on the manner of preparation (spray-drying, freeze-drying) and on the storage conditions there after (time, temperature, moisture content). Substantial deviations ($>$ 0.10) are not expected in the case of crystal-free or pre-crystallized spray-dried products (38). Evidence that this is also true for post-crystallized products is insufficient. Recovery tests did not always agree, due to inaccurate determination of r_0 caused by slow dissolution of added crystalline lactose (Section 4.2.4). The repeatability of the method is good as was determined from 19 duplicate determinations carried out on dried whey (s = 1.1 percentage units) and from 18 duplicate determinations performed on dried skim milk (s = 0.6 percentage units). The method is thought to give correct results for dried whey and dried skim milk (Section 6.3).

7.2.4 IS-GAS method. The method is thought to give correct results for dried whey and dried skim milk, because the K values agreed with those found by IS-POL. The repeatability for amorphous lactose was rather poor (Section 4.3.2).

7.2.5 CW-WAT method. The results for pre-crystallized dried whey are thought to be correct. The repeatability was good: $s = 0.7$ percentage units for WP27C ($n = 7$). The method is too inaccurate to apply to dried skim milk (Section 6.3.2.3).

7.2.6 CW-DTA method. The method gave some support as to the correctness of other methods when applied to pre-crystallized dried whey and dried skim milk. In our opinion, the method itself cannot successfully be used, because of poor repeatability (Table 8), predominantly caused by the difficulty in finding a correct base line (Section 5.4.2).

7.3 Practicability

7.3.1 REF method. REF can be performed with simple and cheap apparatus. No skilled labour is needed. As the determination is rapid, the method is particularly useful for rapidly crystallizing liquids such as highly concentrated whey. Because of these properties REF is convenient for process control in the dairy industry.

7.3.2 IS methods. Both IS-POL and IS-GAS can only be applied successfully with sophisticated and expensive equipment. Skilled labour is needed to run the time-consuming tests and to calculate the results. These methods should be restricted to well-equipped laboratories.

7.3.3 SEP methods. These methods can be performed with less advanced equipment. A centrifuge complying with the conditions mentioned in Section 3.3 should be available. The time needed to run a test will depend strongly on the modification used. Generally spoken, the method is laborious, except when SEP-STS is used. The latter also needs relatively little analytical skill.

7.3.4 CW methods. CW-WAT is relatively simple, but rather time consuming. The use of an automatic Karl-Fischer titrator is advised. In our opinion CW-DTA should not be applied, because there are cheaper and easier alternatives, which have at least the same accuracy.

List of materials (particulars of the milk products and preparations used)

Lactose (L)

α lactose hydrate

L1	Commercial seed lactose, produced by grinding (Wessanen). Crystal size 0.1-2 μm
L2	Commercial lactose (Lamers and Indemans, P-0200). Sieve fraction > 125 μm. Crystal size: length ~ 250 μm, circumference ~ 100 μm
L3	Origin as of L2 but not fractionated
L4	Commercial lactose (Analar BDH 10139)
L5	Lactose crystallized twice from distilled water (Research Laboratory CCF)

spray-dried lactose

L21	Pilot plant NIZO. Drying of a 30 % solution of lactose hydrate. Air temperatures: inlet 215 °C, outlet 80 °C. $M = 0.026$ (Karl Fischer). Lactose crystals absent
L24 series (A, B, C)	Pilot plant NIZO. Drying of a 50 % solution of lactose hydrate. Air temperatures: inlet 160 °C (A) or 180 °C (B, C), outlet 80 °C. To a part (B) 15 % seed lactose was added. Samples contain pre- and post-crystallized lactose (A, B) or post-crystallized lactose (C)

β lactose

L25	Sample provided by Research Laboratory CCF

Concentrated whey (W)

W1 series (A-D)	Pilot plant NIZO. Fresh cheese whey concentrated to 34 % *TS*. Analysed at various moments of crystallization (15 °C, 28-56 h)
W2 series (A-D)	Pilot plant NIZO. Fresh cheese whey concentrated to 34 % *TS*. Analysed at various moments of crystallization
W2 series (E, F)	Whey from W2 series concentrated to 43 % *TS*. Analysed at two moments of crystallization

W3 series (A-E)	Pilot plant NIZO. Fresh cheese whey concentrated to 18 % TS (A), 31 % TS (B), 40 % TS (C), 52 % TS (D), 64 % TS (E). Analysed after approximately 24 h of crystallization
W4 series (A-D)	Pilot plant NIZO. Fresh cheese whey concentrated to 46 % TS. Analysed at various moments of crystallization

Spray-dried whey (WP)

WP1 series (A-E)	Made from concentrate W1. Part of it was spray-dried directly (WP1A). For other parts (WP1B-E) moments of drying correspond to moments of analysing concentrate (W1A-D)
WP2 series (A-E)	Made from the 34 % TS concentrate W2. Part of it was spray-dried directly (WP2A). For other parts (WP2B-E) moments of drying correspond to moments of analysing concentrate (W2A-D)
WP2 series (F, G)	Made from the 43 % TS concentrate W2. Moments of drying correspond to moments of analysing concentrate (W2E, F)
WP5	Commercial pre-crystallized dried whey (DOMO). $R = 0.243, L = 0.718$
WP6	Commercial pre-crystallized dried whey (Borculo). $R = 0.239, L = 0.731$
WP7	Commercial pre-crystallized dried whey (Acmesa). $R = 0.272, L = 0.703$
WP22	Pilot plant NIZO. Fresh cheese whey concentrated to 35 % TS and dried immediately. $L = 0.730, M = 0.0278$ (Karl Fischer)
WP27 series (A-E)	Pilot plant NIZO. Fresh cheese whey concentrated to 46 % TS, Part of it was spray-dried directly (WP1A). Other parts dried at various moments of crystallization
WP31, WP32	Pilot plant Stork. Product partly pre-crystallized, partly post-crystallized
WP36	Pilot plant NIZO. By spray-drying of crystal free concentrated whey at an extremely low outlet temperature (58 °C) a post-crystallized product was obtained. Inlet temperature 185 °C. Two-stage drying.

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Spray-dried skim milk (MP)

MP8	Commercial product (Acmesa). $R = 0.461$, $L = 0.503$
MP9	Commercial instant product (Coberco). $R = 0.465$, $L = 0.518$
MP10	Commercial product (Coberco). $R = 0.465$, $L = 0.507$
MP13	Commercial instant product (Berneralpen Milchgesellschaft)
MP14	Commercial product (Kallo)
MP15	Commercial product (Kallo)
MP16	Commercial instant product (Coberco)
MP17	Pilot plant NIZO. *TS* content of the concentrate 46 %. Air temperature inlet 130 °C, outlet 80 °C. $L = 0.527$, $M = 0.036$ (Karl Fischer)
MP19	Pilot plant NIZO. Powder with post-crystallized lactose made in a way similar to that of WP36
MP23	Pilot plant NIZO. *TS* content of the concentrate 46 %. Dried immediately. Air temperatures inlet 225 °C, outlet 80 °C. $L = 0.501$, $M = 0.0240$ (Karl Fischer)
MP25 series (A, B)	Pilot plant NIZO. *TS* content concentrate 46 %. A part was dried immediately (A), the other part was seeded and kept at 15 °C for 24 h and dried (B)
MP26	Pilot plant NIZO. *TS* content concentrate 45 %. Air temperatures inlet 177 °C, outlet 86 °C

Samenvatting

K. Roetman, *Methoden voor de bepaling van de kristallisatiegraad van melk-suiker in melkprodukten*[1]

De belangstelling voor de kristallisatie van melksuiker in melkprodukten is de laatste 25 jaar verlegd van de produktie van gesuikerde gecondenseerde melk en comsumptie-ijs naar de bereiding van weipoeder en weiderivaten (lactose, ontzout weipoeder, lactose-arme weipoeders). Ook voor de verbetering van de eigenschappen van melkpoeder is de kristallisatie van melksuiker meermalen van belang geacht, gezien de octrooien op dit gebied.

Goede methoden voor de bepaling van de kristallisatiegraad zijn onmisbaar voor de procescontrole bij de bereiding en voor het onderzoek naar de invloed van de kristallisatie op de eigenschappen van de eindprodukten. Tot nu toe zijn er diverse methoden bekend in de zuivelindustrie en in de literatuur. Ze zijn gebaseerd op afscheiding van de kristallen (bijv. door centrifugeren), op bepaling van de β/a verhouding of op bepaling van het kristalwater. Sommige methoden zijn nooit precies beschreven, laat staan op hun waarde onderzocht.

In deze publikatie worden voorschriften voor de diverse reeds bestaande methoden gegeven. Een nieuwe, snelle en eenvoudige methode, gebaseerd op de bepaling van de brekingsindex, werd daaraan toegevoegd. De 'brekingsindexmethode', de 'centrifugeringsmethode' en de 'polarimetrische methode' werden onderzocht voor toepassing op geconcentreerde wei. Deze drie methoden tezamen met de 'gaschromatografische methode', de 'kristalwaterbepalingsmethode' en een methode gebaseerd op differentiële thermische analyse werden getoetst op geschiktheid voor toepassing op weipoeder en magermelkpoeder. De methoden werden beoordeeld op nauwkeurigheid, herhaalbaarheid en gemak van uitvoering.

De voornaamste conclusies zijn:
– de polarimetrische methode is in het zuivelonderzoek veelvuldig toegepast maar vaak met gebruikmaking van foutieve waarden voor de β/a verhouding in het niet-kristallijne gedeelte van de melksuiker in het produkt. De methode is vooral geschikt voor weipoeder en melkpoeder;
– de brekingsindexmethode is bijzonder geschikt voor procescontrole bij de kristallisatie van melksuiker in geconcentreerde wei, omdat ze snel en eenvoudig is uit te voeren. Voor droge produkten is de toepasbaarheid beperkt tot voorgekristalliseerd weipoeder;
– de centrifugeringsmethode is minder geschikt voor weiconcentraat en ongeschikt voor droge produkten. Van deze methoden bestaan diverse varianten, gebaseerd op lactose- en/of drogestofbepalingen. Met name de lactosebepalingen volgens Luff-Schoorl zijn o.i. te onnauwkeurig om een goed resultaat te behalen;
– de kristalwaterbepalingsmethode is minder bruikbaar dan vaak wordt gedacht, omdat de blanco waarde nogal varieert. De methode is uitsluitend bruikbaar voor droge produkten. Er is gerede aanleiding te twijfelen aan het eindresultaat bij nagekristalliseerde produkten met een hoog vochtgehalte, omdat tijdens de bepaling van het vochtgehalte met een 'droogstoofmethode' voortschrijdende kristallisatie mogelijk is.

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