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PARTICLE STRUCTURE IN SPRAY-DRIED WHOLE MILK AND IN INSTANT SKIM MILK POWDER AS RELATED TO LACTOSE CRYSTALLIZATION

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Abstract

The structure of instant skim milk and whole milk powders was studied by scanning electron microscopy with special respect to lactose crystallization during storage. X-ray diffraction analysis was used to confirm the crystallization. Some samples were ashed in a plasma asher and the residues were also examined by scanning electron microscopy. The structures of instant skim milk and whole milk powder particles were well-preserved after the ashing procedure.

The crystallization of $\alpha$-lactose hydrate occurred in instant skim milk and whole milk powders according to moisture uptake. In instant skim milk powder, $\alpha$-lactose hydrate crystals were observed on the particle surface. In whole milk powder, numerous droplets of free fat appeared on the surface of particles stored under conditions favoring the crystallization of $\alpha$-lactose hydrate except few lactose crystals. Only $\beta$-lactose was in the state of crystal in whole milk powder stored at 37°C for 5 months at a relative humidity of less than 20%.

In fresh powders of skim milk and whole milk, prismatic crystals of lactose formed in Heinz fluid, whereas in stored powders, they did not.

Key Words: Instant skim milk powder, whole milk powder, lactose, lactose hydrate, $\beta$-lactose, X-ray diffraction, crystallization, scanning electron microscopy, plasma asher, Heinz fluid.

Introduction

The structure of milk powder has been studied by many researchers since the start of its industrial production. Lactose and its crystallization in the milk powders were extensively studied, because lactose forms a continuous medium in which proteins, fat globules and air cells are dispersed.

It has been generally accepted that in milk powder (spray-, roller-, or freeze-dried) lactose occurs in the amorphous state (Nickerson, 1974). When milk powder was placed in a humid atmosphere, amorphous lactose took up moisture and crystallized in the form of $\alpha$-lactose hydrate. King (1965), in his review on the physical structure of milk powder, stated that the crystallization caused fine interstices and cracks along the sides and edges of crystals in the powder particles. Crystallization of lactose in milk powder was confirmed by X-ray diffraction technique (Tuckey and Ruehe, 1934; Knoop and Samhammer, 1962; Taneya, 1963) and/or infra-red spectroscopy (Goulden and White, 1958; Taneya, 1963; Bushill et al., 1965). The crystallization also accompanied changes in some physical properties, such as water absorption (Bushill et al., 1965), and porosity (Berlin et al., 1968a) and solubility (King, 1965) of the milk powder. Under the storage conditions which caused lactose crystallization, chemical deterioration such as browning (Saltmarch and Labuza, 1980a) and loss of lysine (Huss, 1970, 1974) occurred.

Whey powder as well as skim milk and whole milk powders were used (Roetman, 1979; Saltmarch and Labuza, 1980a,b; Warburton and Pixton, 1978a,b) to study the transition of lactose from amorphous to crystalline form.

Miyawaki and Maeno (1938) observed particles of whole milk powder, which were fixed with osmium tetroxide, by means of a light microscope. They concluded that irregular crystals of lactose due to the absorption of moisture covered the surface of the particles and that only a part of total lactose crystallized regardless of the level of moisture uptake. Buma (1966) used a polarized light microscope to detect lactose crystallization in skim milk and whole milk powders kept in a humid atmosphere. He, however, did not present micrographs showing lactose crystals, but claimed that crystallization cracks were observed. He also demonstrated that paraffin oil, i.e. mounting medium, could penetrate into vacuoles in particles of whole milk powder, but not of skim milk powder, after lactose crystallization due to moisture absorption. Electron micrographs of lactose crystals on the surface of skim milk powder particles were presented.

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by Taneya (1963) who had applied a replica technique for electron microscopic observation.

Scanning electron microscopy succeeded in showing the detailed structure of particle surface on which lactose crystals have been located (Warburton and Pixton, 1978a, b; Roetman, 1979) and confirmed the previous findings obtained by X-ray diffraction and other physical methods. Roetman (1979) supported King's remarks by showing the rather porous structure of post-crystallized skim milk powder by means of the scanning electron microscope. To make sure that the substance which appeared after the uptake of moisture on the particle surface was crystallized lactose, other experimental techniques were necessary. For this purpose, Roetman (1979) compared the surface structure of spray-dried lactose solution with that of milk powder.

Most studies concerning lactose crystals in milk powder showed that it is the $\alpha$-lactose hydrate form. As for crystals of $\beta$-lactose in milk powder, the following few papers have been published. Sharp (1938) detected the crystals of the $\beta$-form as well as $\alpha$-hydrate in dried milk after moisture uptake at room temperature, though detailed experimental conditions were not given. Knoop and Samhammer (1962) found $\beta$-lactose crystals in a few samples of spray-dried whole milk powder and a mixture of $\alpha$- and $\beta$-lactose crystals in some roller-dried milk powders. Recently, Würsch et al. (1984) reported crystallization of $\beta$-lactose in whole milk powder at high storage temperatures (55 and 60°C).

Asking procedure using low temperature plasma have been generally used for plant tissue because it leaves inorganic residues in forms which show initial structures of the tissue. Saito (1980) applied the ashing procedure to skim milk powder particles to learn about the distribution of inorganic components in the particles.

The objective of this study is: (1) to find the advantage of ashing samples with a plasma asher as a pretreatment for scanning electron microscopy, (2) to examine the effect of lactose crystallization on the structure of milk powders, and (3) to demonstrate the presence of $\beta$-lactose crystals in stored whole milk powder.

Materials and Methods

Instant skim milk powder and ordinary whole milk powder, both of which were produced by a spray-dry process, were obtained through a local store and directly from the manufacturer, respectively. The materials (moisture: $\leq 4\%$) packed in a commercial package (foil-laminated paper carton for 250 g or 300 g) or in a polyethylene bag were kept in a desiccator at room temperature until use.

In order to examine the inner structure, i.e. the cross sections, as well as the surface structure of the powder particles, the following pretreatment (Saito, 1980) for scanning electron microscopy was applied, whenever necessary: (a) chopping: about 0.2 g of the sample was chopped with a razor blade repeatedly for about 10 min, and (b) ashing: a small amount of the chopped sample was spread on a piece of quartz or on a stainless-steel plate ($7 \times 7$ mm) and subjected to the ashing procedure in a plasma asher (Yanagimoto LTA 2SN) at 40 watts with an oxygen supply (40 ml/min, 2 kg/cm$^2$) for 5 h. The ashing temperature, which was estimated from the above operating conditions according to the operation manual for the plasma asher, was 200°C or less.

The samples were examined without any fixation procedure. The samples excepting the ashed ones were sprinkled on a piece of a double adhesive tape attached to a specimen holder. In the case of the ashed sample, the plate carrying the ashed particles was pasted on the specimen holder with a small amount of silver paint. The samples were coated with gold by an ion-sputtering method. Observations with a scanning electron microscope (JEOL JSM U3 and JEOL 25 S II) were made using an accelerating voltage of 15–20 kV.

A computerized X-ray diffractometer (Rigaku Geigerflex RAD-IIA, Cu target) was used to obtain X-ray diffraction patterns.

Heinz fluid (Warburton and Pixton, 1978a, b; Saito and Taguchi, 1980) was prepared according to the private communication of Warburton (polyvinyl alcohol 10 g, distilled water 60 ml, glycerol 10 ml, 1.5% phenol solution 25 ml, chloral hydrate 100 g, and lactic acid 35 ml). A small amount of the sample (< 50 mg) was mixed with a drop of the Heinz fluid. A part of the mixture was diluted with another drop of the Heinz fluid and observed under a light microscope at 500X magnification.

Results and Discussion

Instant skim milk powder

Samples taken from several packages produced by 3 manufacturers in Japan were examined. Conditions of the particle surfaces differed somewhat probably due to differences in the manufacturing systems. Even particles in the same package did not have similar surface structure, i.e. small particles had smooth surfaces and no dents but large ones showed wrinkled surfaces and dents. However, only a small difference was observed in the cross sections of particles within the same sample. The cross sections showed vacuoles and compact walls. An example of a cross section is shown in Fig. 1A which is similar to those demonstrated by others (Verhey, 1972; Burna, 1978; Roetman, 1979).

Since water was evaporated from the surface of the particle in the processing steps, a hard layer was produced at the surface of each particle. Therefore, water in the particle might not distribute evenly during the drying process. To learn about the difference in the compactness between outer and inner portions, ashing treatment by a plasma asher was applied. Since the treatment destroyed organic matter and left the inorganic residue, it was expected to illustrate differences in compactness.

The inner surface of a vacuole observed in an ashed sample was rough (Fig. 1D), although the particle surface was relatively smooth and dense (Fig. 1C). A kind of trench or dent was observed in the middle portion of the wall between the vacuoles and the particle surface (Fig. 1B, D). Thus unevenness in cross section, which was not observed in untreated samples, was detected after the ashing treatment.

According to the results of X-ray diffraction analysis, none of the instant skim milk powder samples included lactose crystals when they were examined at the beginning of the present experiment. It has been recognized that two-stage instantization, i.e. a rewet-redrying system which was the first invention to produce instant skim milk powder, caused the crystallization of lactose during the process (Bullock, 1962). No information about the manufacturing system for the instant skim milk powder used in the present study was available. However, recent developments in manufacturing, such as a single-stage instantizing
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process, have made it possible to produce instant skim milk powder without lactose crystallization.

The instant skim milk powder was sealed again after samples had been taken and was stored at room temperature. After storage for 21 months, an X-ray diffraction analysis confirmed the formation of a-lactose hydrate. At the same time, a crystal-like substance was detected on the particle surface. The powder stored for 51 months after the opening of the package showed relatively large deposits of the crystal-like substance covering the surface of the particles (Fig. 2A). The structure of the cross section of stored powder particles was smooth, showing no change after the formation of the crystal-like substance (Fig. 2B, C). The cross section was not porous and showed no cracks, at least under the conditions used for observation. Needle-type crystals were detected on the inner surface of vacuoles (Fig. 2C). The inner surface of some small vacuoles, however, lacked the crystals. The cross section of an ashed sample (Fig. 2D) revealed a fine porous structure similar to that one presented in Fig. 1D. The stored sample, which contained 34% of insoluble materials according to solubility tests (Saito and Taguchi, 1980), was treated with a 2% glutaraldehyde solution or 20% ethanol, both of which dissolved water-soluble substances but did not dissolve dried proteins. After the treatment, globular shapes of the particles were well preserved (Fig. 2E, F), but the crystal-like substance was washed out by these solutions, which indicated high solubility of the substance in an aqueous solution. When the crystal-like substance was removed, X-ray diffraction patterns showed that no crystals were present in the particles. The crystal-like substance disappeared after ashing, which indicated that it was not of inorganic nature. Hence it was concluded that the crystal-like substance consisted of a-lactose hydrate. Lactose crystallization did not seem to affect the structure of the cross section of particles. The needle-type crystals on the inner surface of vacuoles differed in form from the crystal-like substance, and were probably another form of a-lactose hydrate crystal, i.e. long prismatic crystals, because crystals other than a-lactose hydrate were not detected by X-ray diffraction patterns.

The development of lactose crystals was accelerated when a portion of instant skim milk powder was kept in a closed container together with a saturated sodium chloride solution at various temperatures between 0 and 37°C. Relative humidity in the container was 75% (Rockland, 1960). Since the difference due to storage temperature was not great, the changes due to time of storage at 20°C are shown in Figure 3. A slight change in the surface, namely the formation of a plate-like structure, occurred after 1 day of storage. Tiny prismatic crystals appeared after 2 days of storage (Fig. 3B), but no subsequent increase in size and number of the tiny crystals was observed. With time the plate-like structures continued to develop and in 4 days crystals, which resembled those demonstrated by Roetman (1979), were found (Fig. 3D).

Whole milk powder

The structure of whole milk powder particles resembled the spray-dried buttermilk particles described by Kalab (1980, 1981), i.e. the main particles had a crater-like scar and a minute globule surrounded with a low rim at the junction (Fig. 4A). However, the interior of the crater in whole milk powder was not as smooth as that of buttermilk particles, but was porous in the same manner as the rest of the particles (Fig. 4B). It is likely that the minute globule left the crater after breaking away from the main particle. The particles of whole milk powder were also similar to those shown by Buma and Henstra (1971) and Buma (1978) with respect to the minute globule. However, cracks, but no crateres, were observed in their micrographs. The cross section of whole milk powder (Fig. 4C) seemed to be porous owing to the presence of tiny vacuoles and fat globules, unlike the skim milk powder.

Plasma-ashing demonstrated that the interior of the whole milk powder particles (Fig. 4E) was of uniform porosity with no channels and thus differed from the instant skim milk powder. Ashing suggested that the solids-not-fat were evenly distributed in the dry particles. The rim of the crater was rather compact even after ashing (Fig. 4D, F).

Storage of the whole milk powder in a humid atmosphere caused the development of an adhered substance (Fig. 5A), which differed from the deposit on the surface of skim milk powder. The adhered substance was rather spherical in shape and had no sharp edges (Fig. 5B). The adhered substance was removed by washing with an organic solvent such as ethyl ether, and by ashing. Thus it was considered to be fat which emerged from the interior of the particles.

Portions of the whole milk powder were stored at 0 to 37°C for up to 1 month at 75% relative humidity in the same manner as the instant skim milk powder. Effects of the moisture uptake on the structure were evident after only 1 day of storage regardless of temperature. Powder structures after 1 month of storage are shown in Figure 6. The round shapes of an adhering substance were maintained at low temperature but not at 37°C. The development of a-lactose hydrate crystals was demonstrated by X-ray diffraction analysis in all samples that absorbed moisture. However, very few crystals of a-lactose hydrate were observed on the surface, although X-ray diffraction analysis revealed that a considerable amount of crystals existed in the particles. The particle surface was mostly covered with free fat (Buma, 1971; Buchheim, 1982) so that lactose at the surface portion probably could not crystallize or lactose crystals could not accumulate on the surface. Therefore, lactose crystals developed only inside of the particles and were rarely detected on the particle surfaces.

Another portion of the sample kept in an unsealed container, i.e. in a Petri dish with a slit between body and cover, in an incubator at 37°C (relative humidity < 20%) for 1 month showed no crystals, but the sample kept for 5 months under the same conditions demonstrated another type of X-ray diffraction pattern, which confirmed that all the lactose in the crystalline state was β-form. The pattern was typical for the crystals of β-lactose, i.e. there were peaks at 2θ = 10.5, 21.0 and 24.7° (Knoop and Samhammer, 1962; Buma 1967) whereas peaks at 2θ=12.4, 16.4, 19.5, 21.2 and 22.7°, which were typical for crystalline α-lactose hydrate, were missing. There were fewer physical alterations in the whole milk powder exposed to a relative humidity as low as 20% (Berlin et al., 1968a). Lactose crystallization, i.e. the formation of α-lactose hydrate, did not occur at 20% relative humidity, because the moisture content of the milk powder was too low. According to Heldman et al. (1965), equilibrium moisture content of low-heat whole milk powder with 26% fat at 38°C at 20.4% relative humidity was 4.25%. The moisture content was lower than the critical level for lactose crystallization (Choi et al., 1951; Buma, 1966; Huss, 1970; Berlin et al., 1968b). Thus the lack of humidity prevented the formation of crystals as α-lactose hydrate, but not as β-lactose. Würsch et al. (1984) demonstrated that lactose crystallized completely in the β-form in a whole milk powder stored in a sealed metal can for 60 days at 60°C but did not crystallize at 37°C and 45°C. In
this study, the lactose crystals of β-form developed during the storage at 37°C for 5 months. Mutarotation in amorphous lactose, namely the conversion of α-lactose to β-lactose, rapidly proceeded in milk powders at much higher temperatures than 37°C (Roetman and van Schaik, 1975; Olano and Martinez-Castro, 1983). However, mutarotation seems to occur at 37°C, if sufficient time, such as several months, is allowed. Roetman and van Schaik (1975) also stated that mutarotation in amorphous lactose was possible even at 25°C, and postulated that mutarotation continues depending on the moisture content and temperature until an equilibrium β/α ratio of about 1.25 is attained.

Parrisch and Brown (1982) suggested that the conversion of α-lactose to β-lactose proceeds in the solid state and that the presence of a base favors the crystallization of β-lactose at 27°C. Würsch et al. (1984) mentioned that Amadori products, which are associated with browning by the Maillard reaction and are basic amines, might promote the crystallization of β-lactose. According to the results by Coulter et al. (1948) and Tarassuk and Jack (1948), the browning may not proceed to a detectable level under the conditions of the present study. However, it could be possible that at least the initial stage, which produces colorless condensation products and water and involves Amadori rearrangements (Hodge, 1953), of the Maillard reaction might proceed to some extent. Since milk powders were in a highly supersaturated state with respect to β-lactose as well as α-lactose,
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Fig. 4. Scanning electron micrographs of whole milk powder. A = typical particle. B = crater-like scar. C = cross section. D, E, F = ashed particles. E and F show cross section and surface, respectively. Bars = 10 μm.

Fig. 5. Scanning electron micrographs of whole milk powder stored for 4 weeks at 75% relative humidity at room temperature. Entire view (A) and enlarged surface pattern (B) are shown. Bars in A and B = 100 and 10 μm, respectively.

β-lactose gradually crystallized at 37°C during the prolonged storage. The factors which initiated the crystallization of β-lactose are unknown at present.

The surface structure of powder particles containing β-lactose crystals is presented in Figure 7. The structure was quite different from that of the other stored powders (Figs. 5, 6) and from that demonstrated by Würsch et al. (1984). The substance which adhered to the powder particles and which was described by the latter authors as a molten material, presumably free fat, was

Fig. 6. Scanning electron micrographs of whole milk powder stored for 1 month at 75% relative humidity at various temperatures. A, B, C, D = stored at 0, 10, 20, 37°C, respectively. Bars = 100 μm.

Fig. 7. Scanning electron micrographs of whole milk powder stored at 37°C (<20% relative humidity) for 5 months. Bar = 10 μm.

Fig. 8. Optical micrographs of fresh (A) and stored (B) instant skim milk powder and fresh whole milk powder (C) mounted in Heinz fluid. Lactose crystals were formed during the mounting procedure (A, C). Bars = 10 μm.
not observed in the powder examined in the present study. Since the whole milk powder was kept in unsealed container, accumulation of water (Würsch et al., 1984) produced via the Maillard reaction, if any, was avoided. Moisture seems to be required for the formation of the adhering substance or the molten material. Hydration of other constituents, particularly proteins (Berlin et al., 1968b) may expel free fat onto the surface of the particles.

**Crystal formation by Heinz fluid**

Heinz fluid, one of the mounting media for light microscopic observation, dissolved the dry milk particles but not the lactose crystals (Saito and Taguchi, 1980). The addition of the fluid to a saturated solution of lactose at twice the volume or more caused crystallization of lactose. The microscopic observation of instant skim milk powder mounted in the Heinz fluid detected many prismatic or diamond-shaped crystals (Fig. 8A). Since no crystals were detected by scanning electron microscopy and X-ray diffraction analysis in the fresh powder sample before mounting, a rapid crystallization of amorphous lactose must have occurred during the mounting procedure with the Heinz fluid, i.e. the Heinz fluid had caused lactose crystallization. The stored instant skim milk powder, however, was shown to contain only minute crystals and some insoluble material but no large prismatic crystals were present (Fig. 8B). Lactose in the stored instant skim milk powder did not develop into large crystals in the Heinz fluid, possibly because it was already in a fine crystalline form or because the Heinz fluid did not solubilize the stored instant skim milk powder completely. Similar results were obtained with the whole milk powder (Fig. 8C).

It seems possible that the Heinz fluid can be used to find the state of lactose in instant skim milk and whole milk powders.

**Conclusions**

Structures of instant skim milk and whole milk powder particles were well-preserved after ashing in a plasma asher. The ashing treatment did not detect the influence of lactose crystallization on the particle structure. It did, however, succeed to give some information about uniformity in distribution of constituents in the powders.

α-Lactose hydrate crystals developed rapidly in instant skim milk and whole milk powders according to moisture uptake. However, the changes in the surface structure caused by lactose crystallization were quite different in both powders. The moisture uptake proceeded through the surface of the skim milk powder particles, so that the lactose crystallization started at the surface and covered it. On the other hand, in whole milk powder, few lactose crystals but numerous droplets of free fat appeared on the surface of particles stored under conditions favoring the crystallization of α-lactose. It is highly probable that crystallizing lactose facilitated the movement of free fat onto the surface of particles due to the moisture uptake by other constituents, because the crystallization may have weakened the structure of the amorphous lactose phase in the particles. However, the relationship between lactose crystallization and the emergence of free fat is not fully understood.

Crystallization of β-lactose proceeded very slowly in whole milk powder at 37°C, when humidity was low enough to inhibit the crystallization of α-lactose hydrate. The X-ray diffraction analysis confirmed that β-lactose alone was in the crystalline state in whole milk powder stored at 37°C for 5 months at a relative humidity of less than 20%.

**References**


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Discussion with Reviewers

E.A. Davis: Please provide examples of X-ray diffraction patterns of lactose crystals.

P. Allan-Wojtas: As X-ray diffraction technique was used to show differences between the \( \alpha \)- and \( \beta \)-forms of lactose crystals and also between lactose and fat, please show one or two representative diagrams.

Author: X-ray diffraction patterns of the stored whole milk powder are presented in Figure 9. The peaks of \( \alpha \)-lactose hydrate crystals (Fig. 9A) and \( \beta \)-lactose crystals (Fig. 9C) are demonstrated. The pattern showing no peaks (Fig. 9B) is the same as that of the fresh powder.

P. Allan-Wojtas: What converts the original skim milk powder into an instant skim milk powder while crystallization of lactose is prevented in the recent developments in the manufacturing process?

Author: I have not confirmed experimentally yet, but it must be amorphous lactose. There are two methods to produce instant skim milk powder, namely (1) to agglomerate fine particles, and (2) to produce large particles at the time of spray-drying. In the case of agglomeration process, lactose makes particles stick together. Recently, the agglomeration process, as well as the lactose–particle process, have been carried out by a single-staged process, which means that instant skim milk powder is produced from liquid milk in a single drying operation. Therefore, the rapidity of moisture evaporation during the process prevents crystallization of the lactose.

M. Kalab: You have found that there were differences between the surfaces of commercial skim milk and whole milk powder particles, whereby lactose crystals developed to a lesser extent on the whole milk particles because of the presence of fat. To eliminate the effects of differences in the manufacturing process and to confirm that the presence of fat in the whole milk powder was responsible for the surface features in that powder,
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did you observe similar differences in experiments with original skim milk and whole milk powders exposed to a humid atmosphere?

Author: I did not examine the original (non-instant) skim milk powder. However, 3 samples of the instant skim milk powder produced by different manufacturers were examined after 1 month storage at 75% relative humidity. Similar results were obtained from these samples in respect to lactose crystallization on the particle surface.

D.P. Dylewski: In your paper you state that the hydration of proteins may expel free fat onto the surface of whole milk particles. Could you explain further what you mean by “free” fat and how it might differ from “bound” lipid?

Author: Free fat means the fat which is not in the form of fat globules. Initially all of the fat in milk exists as globular fat. Mechanical or physical force damages fat globules and produces free fat. In the whole milk powder, fat globules embedded in lactose matrix could be equivalent to “bound” lipid.

M. Saltmarch: What practical significance would you say the discovery of \(\beta\)-lactose crystals could have in instant skim milk powder?

Author: The finding about \(\beta\)-lactose crystals is a matter of interest in lactose crystallization rather than in the properties of instant skim milk powder. However, the effect of the crystallization of \(\beta\)-lactose on physical properties of milk powder particles may provide a new area for the use of milk powder in the food industry.