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An X-ray diffraction analysis of crystallised whey and whey-permeate powders

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Abstract—Amorphous whey, whey-permeate and lactose powders have been crystallised at various air temperatures and humidities, and these crystallised powders have been examined using X-ray diffraction. The most stable lactose crystal under normal storage conditions, α-lactose monohydrate, forms preferentially in whey and whey-permeate powders at 50 °C, provided sufficient moisture is available, whereas anhydrous β-lactose and mixed anhydrous lactose crystals, which are unstable under normal storage conditions, form preferentially at 90 °C. Thus, faster crystallisation at higher temperatures is offset by the formation of lactose-crystal forms that are less stable under normal storage conditions. Very little α-lactose monohydrate crystallises in the pure lactose powders over the range of temperatures and humidities tested, because the crystallisation of α- and β-lactose is considerably more rapid than the mutarotation of β- to α-lactose in the amorphous phase and the hydration of α-lactose during crystallisation. Protein and salts hinder the crystallisation process, which provides more time for mutarotation and crystal hydration in the whey and whey-permeate powders.

Keywords: Whey; Lactose; Crystallisation; Amorphous; Caking

1. Introduction

Whey powder is a highly nutritious, low cost source of carbohydrates, proteins, minerals and vitamins, often used to fortify a wide variety of foods for human consumption, including bakery products, dry mixes, soups, drinking powders and confectionary. The caking of this powder during storage is a well-known phenomenon related to the presence of the carbohydrates or, more specifically, lactose, which assumes an amorphous state during the conversion of liquid whey to the powder in a spray dryer. Amorphous lactose is thermodynamically meta-stable and very hygroscopic, adsorbing moisture rapidly from the surroundings. If sufficient moisture is adsorbed, then the lactose plasticizes and experiences a decrease in its viscosity, so that the individual whey-powder particles become sticky and inter-particle bridges between adjacent particles form, leading to particle agglomeration and caking of the powder. The temperature at which this transition from an amorphous solid state to a viscous state occurs is known as the glass transition temperature,[1] which can fall below ambient temperature when the surrounding air humidity is sufficiently high, posing a problem during the storage of whey powder in humid areas. Caking of whey powder must be avoided in practice to ensure that the powder remains free-flowing and easy to handle.[2]

The plasticization of the lactose is accompanied by a higher molecular mobility of the individual molecules, which tend to spatially rearrange themselves to form a more thermodynamically-stable crystalline structure that is significantly less hygroscopic. Thus, the crystallisation and hence stabilisation of the lactose in whey powder occurs at the expense of particle stickiness and

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caking of the powder, when it is exposed to humid conditions. Saito[3] has suggested that the crystallisation of amorphous lactose in whey powder (by exposure to humid conditions) with a subsequent grinding procedure to break apart the agglomerated particles could provide a whey-powder product that is non-caking, even when it is exposed to humid conditions. The premise of the current work is that such a process has been developed, and that knowledge of the effect of various parameters (such as temperature and humidity) on the rate of crystallisation and the composition of the end product is required to optimise this process. The kinetics of whey powder crystallisation have been investigated by Ibach and Kind[4] at temperatures and humidities likely to be employed in such a crystallisation process. This work carries on from that study and investigates the composition of the crystallised powders using the X-ray diffraction (XRD) method.

Amorphous lactose may crystallise in different polymeric forms, including α-lactose monohydrate, anhydrous β-lactose, anhydrous α-lactose and mixed anhydrous forms of α- and β-lactose in molar ratios of 5:3, 3:2 and 4:1.[5] The types of crystals that form depend on the relative humidity and temperature of crystallisation, the powder composition and the time of exposure.[6,7] Moreover, lactose exists in two isomeric forms (α- and β-lactose) in solution, and different equilibrium ratios of these isomers can occur due to mutarotation of one isomeric form to the other, depending on the temperature and concentration of the solution and the time in solution.[7] Thus, any pre-processing of the liquid whey prior to the spray drying process, such as evaporation to concentrate the solution, can affect the ratio of α- and β-lactose in the spray-dried amorphous whey powder, and hence the types of crystals that form in a subsequent crystallisation process.

Drapier-Beche et al.[5] have found that amorphous lactose in skim milk powder stored at 20 °C and a relative humidity of 0.43 crystallises primarily as anhydrous β-lactose, whereas only α-lactose monohydrate forms at a higher relative humidity of 0.59. Both α-lactose monohydrate and anhydrous β-lactose crystallise at intermediate relative humidities. Jouppila et al.[6] have reported that freeze-dried amorphous lactose stored at room temperature and relative humidities between 0.54 and 0.76 crystallises mainly as a mixture of α-lactose monohydrate and anhydrous lactose crystals with the molar ratio $\alpha{:}\beta = 5{:}3$. They have shown that anhydrous β-lactose also crystallises at a low relative humidity of 0.44. Vuataz[8] has found that amorphous lactose in skim milk stored at 80 °C and a relative humidity of 0.55 crystallises within 3 min as anhydrous β-lactose, which progressively transforms to α-lactose monohydrate over a period of 13 min. However, amorphous lactose in skim milk stored at 98 °C crystallises eventually to anhydrous β-lactose. Finally, Drapier-Beche et al.[9] have shown that anhydrous lactose crystals with the molar ratio $\alpha{:}\beta = 5{:}3$ are unstable at ambient temperature and transform to α-lactose monohydrate with the uptake of moisture. These results indicate collectively that (1) α-lactose monohydrate and anhydrous β-lactose form preferentially at lower and higher temperatures, respectively, during the crystallisation of amorphous lactose-containing powders, provided sufficient moisture is available and (2) anhydrous β-lactose and anhydrous lactose crystals with the molar ratio $\alpha{:}\beta = 5{:}3$ are unstable at low temperatures and high humidities, recrystallising to the more stable α-lactose monohydrate form with the uptake of moisture. Clearly, α-lactose monohydrate is the most stable lactose crystal under normal storage conditions, which is an important consideration when choosing appropriate operating conditions for a whey-powder crystallisation process. Caking of crystallised whey powder due to recrystallisation of unstable lactose-crystal forms during storage could be avoided by the complete conversion of the amorphous-lactose fraction to α-lactose monohydrate in a prior whey-powder crystallisation process.

In this work, we provide a comprehensive study of the different crystalline structures that form during crystallisation of amorphous lactose in whey powder at temperatures and humidities that are likely to be used in an industrial crystallisation process with reasonable conversion times.[4] Whey-permeate powder, which has had a portion of the high-value protein content removed by membrane filtration before the spray drying process, is likewise investigated, because it is also an important product in the dairy industry. Finally, the crystallised whey and whey-permeate powders are compared with crystallised lactose powder to demonstrate the effect of protein and salts on the crystalline composition of these powders. X-ray diffraction (XRD) is used to identify the different crystalline forms present, and a calibration curve is developed to quantify the α-lactose monohydrate and anhydrous β-lactose contents in the crystallised powders. Note that the paper of Ibach and Kind[4] presents rate data for the crystallisation of amorphous lactose and whey powders exposed to different temperatures and humidities, while the current paper focuses on a compositional analysis of the crystallised products produced in the previous work. The combined data set presented in this paper and in the paper of Ibach and Kind[4] builds on the crystallisation data already published in the literature[5,6,8,9] by (1) exploring a wider range of temperatures and humidities than previously investigated, which is likely to be useful for the development of an industrial crystallisation process, (2) quantifying the major species compositions in the crystallised powders, and (3) providing new hitherto-unreported crystallisation rate and composition data for whey and whey-permeate powders.

2. Experimental

2.1. Whey and lactose powder samples

Amorphous whey, whey-permeate and lactose powders were provided by Lactoprot, Meggle and Uelzena, respectively, which are local suppliers in Germany. The compositions of the whey and whey-permeate powders, which were provided by the suppliers, are given in Table 1. Each sample was sieved onto a specially-designed dish to form a monolayered particle bed with particles of 150 μm or smaller. The samples were then crystallised at different temperatures and humidities in a custom-made wind tunnel, where the crystallisation kinetics were tracked by observing the mass change of the samples with time as moisture was adsorbed (due to the hygroscopic nature of the powder) and subsequently released during crystallisation. Details of the equipment used and experiments conducted are found in Ibach and Kind.[4] The results of an X-ray diffraction (XRD) analysis of the crystallised whey, whey-permeate and lactose samples produced by these workers are presented in this paper.

Different ratios of α-lactose monohydrate and anhydrous β-lactose powders were used to develop an XRD calibration curve for the quantification of these lactose crystals in the crystallised whey, whey-permeate and lactose powders. Pure α-lactose monohydrate powder was supplied by Roth (Germany). Pure anhydrous β-lactose is not commercially available because of its inherit instability, and was therefore prepared using the methodology of Olano and Rios,[10] as follows: a 125 mL anhydrous MeoH solution (Roth, Germany) containing 50 mg of sodium hydroxide pellets (equivalent to a concentration of 0.04 g NaOH/mL methanol) was boiled under reflux for 10 min. Pure α-lactose monohydrate powder (10 g) was then sprinkled onto the surface of the methanol solution, which was then boiled for 2 h under reflux while being agitated with a magnetic stirrer. At the end of the reaction time, the product was filtered and thoroughly washed with methanol, dried under vacuum at room temperature, and stored at these conditions until required for the XRD analysis.

Anhydrous lactose crystal powder with molar ratio α:β = 5:3 was prepared according to the methodology of Simpson et al.[11] as follows: a solution of 1 wt % hydrochloric acid in 98 wt % aqueous methanol was made by adding 2.1 mL hydrochloric acid (35 wt % in water produced by Roth, Germany) to 100 mL anhydrous methanol solution. Pure α-lactose monohydrate powder (8 g) was then sprinkled onto the surface of this solution, which was then boiled for 1 h under reflux while being agitated with a magnetic stirrer. At the end of the reaction time, the product was filtered and thoroughly washed with methanol, dried under vacuum at room temperature, and stored at these conditions until required for the XRD analysis.

Table 1. Composition of amorphous whey and whey-permeate powders

	Whey powder (wt %)	Whey-permeate powder (wt %)
Lactose	73	78–80
Protein	12	3–4
Salt	3	10

2.2. Optical rotation measurement

The optical rotations of α-lactose monohydrate, anhydrous β-lactose and anhydrous lactose in the molar ratio α:β = 5:3 were measured using a polarimeter (Perkin–Elmer, type 241; sodium D line monochromatic radiation with $\lambda = 589$ nm; 1 dm long cuvette) to assess the purity of these compounds. In addition, optical rotation measurements of the pure lactose samples crystallised at various temperatures and humidities were made to verify the XRD analysis of these powders. Each lactose powder sample was dissolved in distilled water at 20 °C at a concentration of 1 g/100 mL solution. Timing started as soon as the first drop of distilled water touched the powder. The dissolved solution was injected into the measurement cuvette of the polarimeter, which was kept at a constant temperature of 20 °C by circulating water through the cooling jacket of the cuvette from a thermostatically controlled bath. Optical rotation measurements started approximately 2 min after timing began, and were continued over a period of 20 min to track the mutarotation of the lactose in solution. The measured optical rotation $[\alpha]_{\mathrm{m}}^{20}$ of the lactose in solution at the start of timing was extrapolated backwards using these data. This value was converted to the specific optical rotation $[\alpha]_{\mathrm{D}}^{20}$ using the equation

$$[\alpha]_{\mathrm{D}}^{20} = \frac{[\alpha_{\mathrm{m}}]^{20}}{lC} \tag{1}$$

where l is the length of the cuvette in dm (equal to unity in this case), C (g/100 mL solution) is the concentration of anhydrous lactose in the solution, and the specific optical rotation $[\alpha]_{\mathrm{D}}^{20}$ has units of $^\circ(\mathrm{dm})^{-1}(\mathrm{g}/100\ \mathrm{mL})^{-1}$, which we shorten to degrees (°) for the sake of brevity. In the case of α-lactose monohydrate, the concentration was corrected to account for the presence of the hydrate, which immediately dissociates from lactose when distilled water is added, by multiplying the weight of α-lactose monohydrate by the ratio of the molecular weights of anhydrous lactose to α-lactose monohydrate.

2.3. X-ray diffraction measurements

The X-ray diffraction patterns for amorphous and crystallised whey, whey-permeate and lactose powders

were measured using a Guinier diffractometer (Huber 642), which uses CuKα_1 radiation (focussed quartz-monochromator: $2\theta_M = 26.642$; $\cos^2 2\theta = 0.799$, $\lambda = 0.1540598$ nm). Silicon crystals (originally less than 100 mesh in size and ground down further to produce a very fine powder) were used as the internal standard to enable the quantification of the lactose crystal concentrations in the crystallised whey, whey-permeate and lactose powders. This quantification was achieved using a calibration curve, which was developed by mixing known quantities of pure α-lactose monohydrate and anhydrous β-lactose powders in different ratios with a constant known quantity of silicon crystals, and analysing these powders using X-ray diffractometry. Mixtures with known quantities of pure α-lactose monohydrate, anhydrous β-lactose and amorphous lactose were also tested to demonstrate the effect of any non-crystalline components present on the accuracy of the calibration curve.

Each powder sample was ground with silicon powder in the ratio 75:25 using a mortar and pestle. These samples were then pressed by hand between two thin X-ray transparent plastic films, and immediately analysed by X-ray diffraction at ambient temperature and humidity. Intensities at diffraction angles (2θ) from 9.5 to 19° (step size 0.025°; time per step, 5 s) were measured, since the peaks most characteristic for the different lactose-crystal forms are found in this range. Intensities were also measured at diffraction angles in the range from 46.6 to 48.0°, around the location ($2\theta = 47.3°$) of the 220 reflection of silicon. Another strong peak is the 1 1 1 reflection of Si, which appears at 28.5° in the diffraction diagram. However, this peak was ignored in the composition analysis, because anhydrous β-lactose has a minor peak close to this diffraction angle, which interferes with the silicon peak. Nevertheless, intensities were also measured at diffraction angles in the range from 27.8 to 29.2° corresponding to this second silicon peak, to estimate the error on the crystalline composition measured using X-ray diffraction, as reported further in this paper. Amorphous whey, whey-permeate and lactose powders were also analysed at diffraction angles in the range from 9 to 36°. The X-ray diffractometer was operated with an anode current of 30 mA and an accelerated voltage of 40 kV. The divergence slit for the primary beam was 4 mm, corresponding to a beam divergence at the sample of 1.9°; the convergence angle of the (focussed) diffracted rays was 1.0°. The typical angular resolution has a full width at half maximum (FWHM) of 0.12° for the Si 1 1 1 peak.

3. Results

The specific optical rotation $[\alpha]_D^{20}$ of α-lactose monohydrate in solution was 89.0° with a standard deviation of 0.2° (calculated from seven different measurements), which is in good agreement with the value of 89.4° reported by Fox,[12] demonstrating the accuracy of the optical rotation measurements conducted in this work. Figure 1a shows the XRD diagram for pure α-lactose monohydrate. The peaks that best characterise this lactose crystal within the measured range of diffraction angles lie at approximately 12.4 and 16.4°. These values have also been reported earlier by Drapier-Beche et al.[5] for pure α-lactose monohydrate powder. Note that the XRD diagrams presented in this work were normalised by dividing through by the maximum intensity of the silicon peak at 47.3°.

The specific optical rotation of β-lactose in solution was 36.6° with a standard deviation of 0.6° (calculated from seven measurements), which is higher than the value of 35.0° quoted by Fox[12] for pure β-lactose. This indicates the presence of a small amount of α-lactose in the solution, and hence the presence of lactose forms

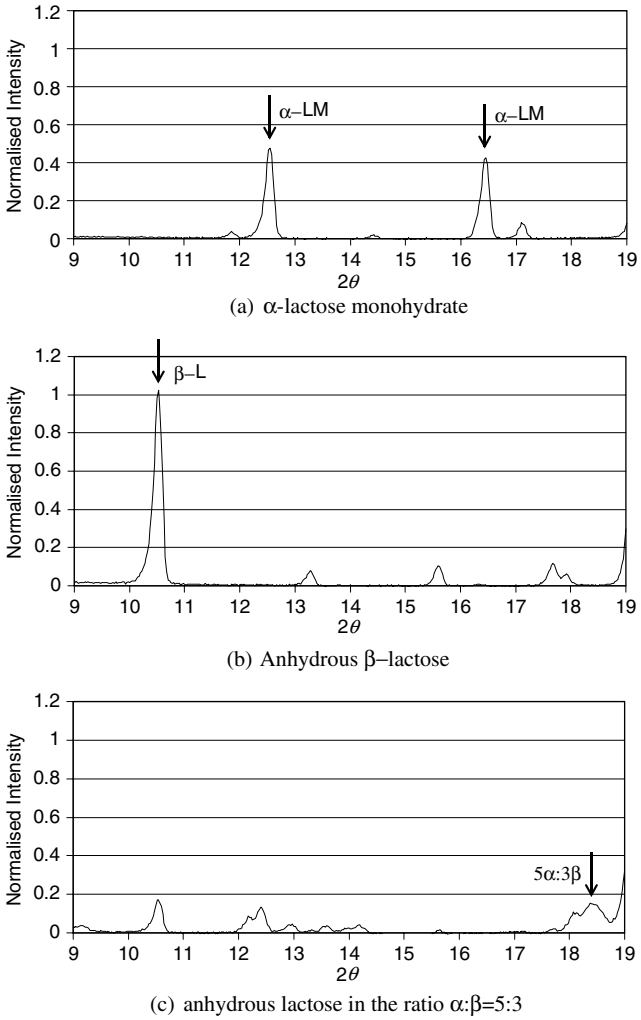

Figure 1. X-ray diffraction patterns for α-lactose monohydrate (α-LM), anhydrous β-lactose (β-L) and anhydrous lactose in the ratio $\alpha:\beta = 5:3$.

other than anhydrous β-lactose in the powder. The α-lactose monohydrate to anhydrous β-lactose conversion methodology used in this work has been optimised previously by Olano and Rios,[10] who achieved a maximum conversion of 93%, which demonstrates the difficulty of producing pure anhydrous β-lactose. Nevertheless, we have used the anhydrous β-lactose prepared in the way described above for the development of the calibration curve for the XRD analysis. The anhydrous β-lactose concentrations reported in this work are thus overestimated slightly by an amount that is difficult to determine without knowing exactly which other lactose-crystal forms were present in the sample. However, the quantities of these other lactose-crystal forms must have been very small, given that no peaks appear in the XRD powder diagram for anhydrous β-lactose in the range from 12.0 to 12.5° (Fig. 1b), where peaks representing anhydrous α-lactose and mixed anhydrous forms of α-and β-lactose in molar ratios of 5:3, 3:2 and 4:1 are normally found.[11,13] Figure 1b shows that the peak that best characterises anhydrous β-lactose lies at 10.4°, which has also been reported by Drapier-Beche et al.[5]

The theoretical value for the specific optical rotation of anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ is 69°, which is calculated from the specific optical rotations of 89.4 and 35° reported by Fox[12] for pure α- and β-lactose in solution, respectively. We have measured a specific optical rotation for this compound of 65.7° (average of two measurements), which is lower than the theoretical value of 69°, suggesting that β-lactose was present in the solution in more abundance than expected. Indeed, the XRD powder diagram for this powder (Fig. 1c) shows that there is a peak at 10.4°, which indicates an anhydrous β-lactose contamination in the powder. A peak also appears at 12.4°, but no second peak appears at 16.4°, which indicates that there was no α-lactose monohydrate in this sample. The peak at 12.4°, which is actually a double peak with diffraction angles at 12.1 and 12.4°, could represent anhydrous lactose crystals in the molar ratio $\alpha{:}\beta = 5{:}3$ and/or one of the other lactose crystals forms, such as anhydrous α-lactose and/or one of the mixed anhydrous lactose crystals of α- and β-lactose in molar ratios of 3:2 and 4:1. The analyses of Simpson et al.[11] and Buma and Wiegers[13] have shown that these other lactose crystal forms also have peaks close to 12.4°. Thus, only the peak at 16.4° should be considered when determining the presence of α-lactose monohydrate in a powder sample, because the peak at 12.4° could be contaminated by one of the other lactose-crystal forms.

The best peak to characterise the anhydrous lactose crystals in the molar ratio $\alpha{:}\beta = 5{:}3$ lies at approximately 18.2°, as reported by Drapier-Beche et al.[14] Using the XRD calibration curve, which will be developed later in this paper, we have calculated that the anhydrous β-lactose content in the anhydrous lactose crystals (molar ratio $\alpha{:}\beta = 5{:}3$) produced in this work was approximately 15%. The measured specific optical rotation of 65.7° is not far from the theoretical specific optical rotation of 63.9° calculated for anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ with a 15% β-lactose contamination. The difference in these values could be explained by experimental error and/or the presence of a small quantity of other lactose-crystal forms, such as anhydrous α-lactose and mixed anhydrous forms of α- and β-lactose in the molar ratios of 3:2 and 4:1.

The X-ray diffraction diagrams (Fig. 1) for α-lactose monohydrate, anhydrous β-lactose and anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ are used to identify these components in the whey, whey-permeate and pure lactose powders crystallised at different temperatures and humidities. Figure 2 shows the XRD calibration curves for α-lactose monohydrate and anhydrous β-lactose, which were developed by mixing different ratios of these compounds with a standard quantity of silicon-crystal powder, obtaining X-ray diffraction diagrams for these powders, calculating the areas under the peaks at 10.4° and 16.4°, which correspond to anhydrous β-lactose and α-lactose monohydrate, respectively, and finally normalising these quantities by dividing through by the area under the peak at 47.3°, which characterises the silicon standard. The three filled points on each curve represent powder mixtures of α-lactose monohydrate, anhydrous β-lactose and amorphous lactose in the ratios 20/20/60, 33.3/33.3/33.3 and 42.5/42.5/15, respectively. These points show that the compositions of α-lactose monohydrate and anhydrous β-lactose can be determined reasonably accurately, even when there is a non-crystalline component in the powder. This is important for the analyses of the crystallised whey and whey-permeate powders, in which protein makes up at least 3% of the total mass, compared with at least 73% for lactose (Table 1). The error on the crystalline com-

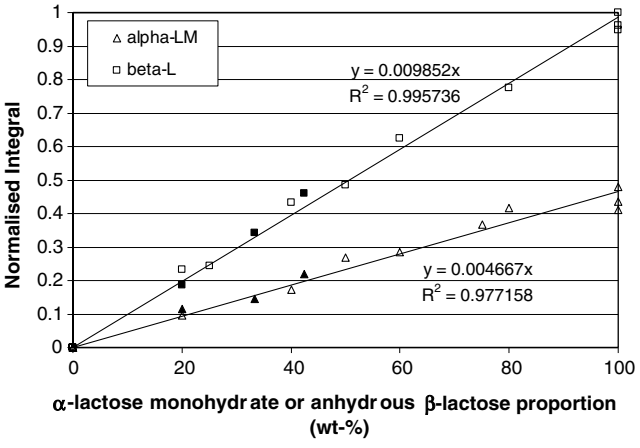

Figure 2. XRD calibration curves for α-lactose monohydrate (α-LM) and anhydrous β-lactose (β-L).

position calculated using these calibration curves is approximately 7%, which was estimated from the standard deviation of the ratios of the areas under the first and second silicon peaks for 26 different whey– or lactose–silicon powder mixtures, in which no anhydrous β-lactose was detected that would interfere with the silicon peak.

Figure 3 shows the X-ray diffraction patterns of amorphous whey, whey-permeate and lactose powders, which clearly have no major peaks that would otherwise indicate the presence of lactose crystals. Thus, we confirm here that the powders crystallised by Ibach and Kind[4] were indeed originally amorphous. Figures 4,5,6,7,8,9,10 and 12 show the X-ray diffraction patterns and the α-lactose monohydrate and anhydrous β-lactose

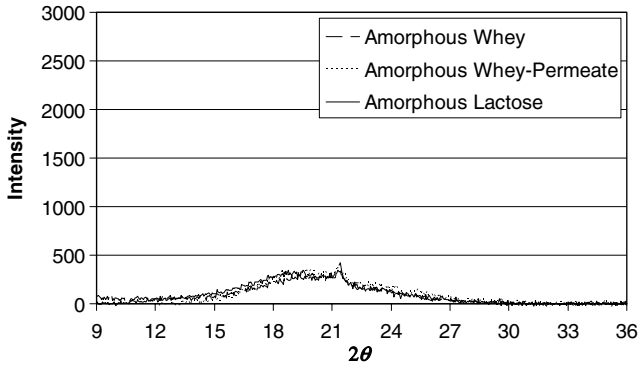

Figure 3. X-ray diffraction diagrams for amorphous whey, whey permeate and lactose powders.

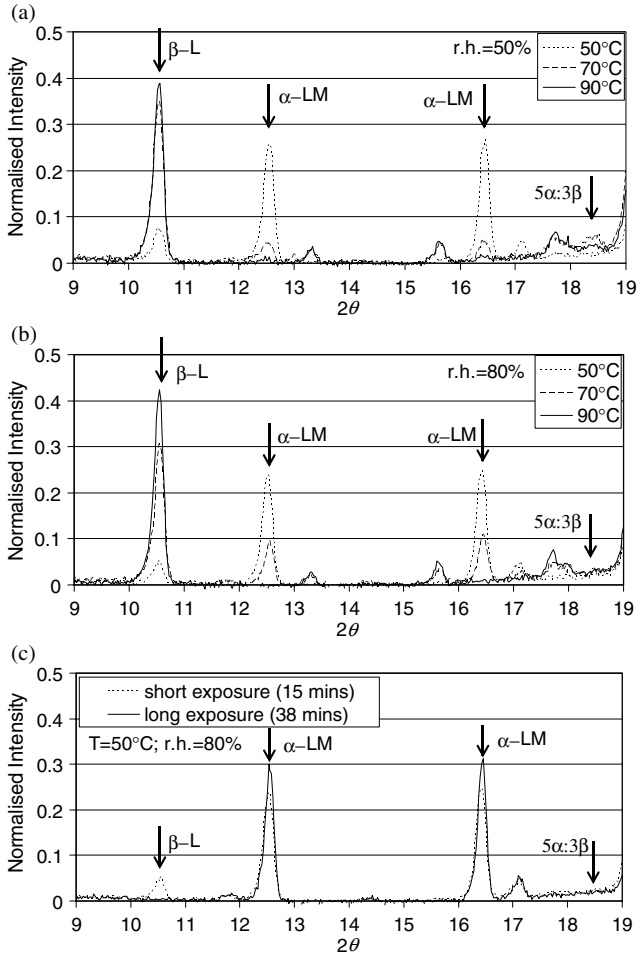

Figure 4. X-ray diffraction patterns for whey powder crystallised at various temperatures, humidities and exposure times.

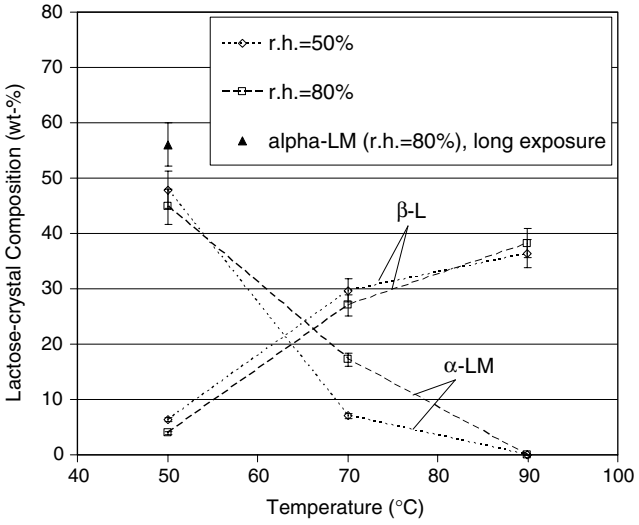

Figure 5. Compositions of α-lactose monohydrate (α-LM) and anhydrous β-lactose (β-L) in whey powders crystallized at various relative humidities and temperatures.

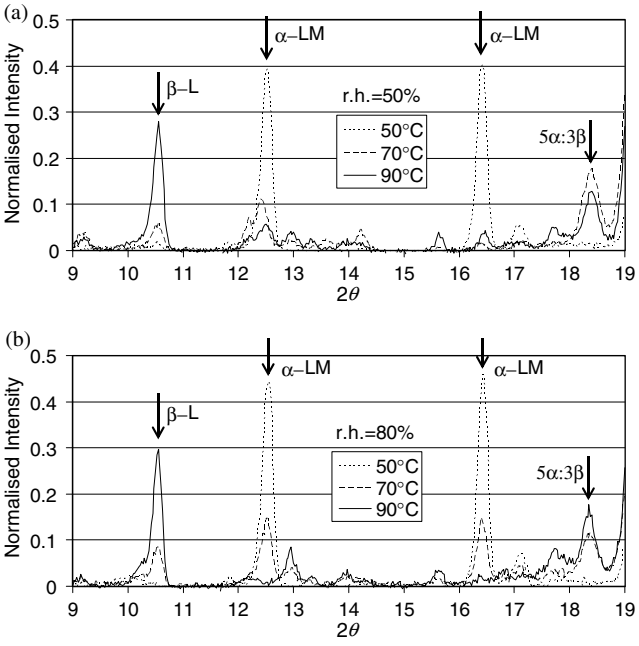

Figure 6. X-ray diffraction patterns for whey-permeate powder crystallised at various temperatures and humidities.

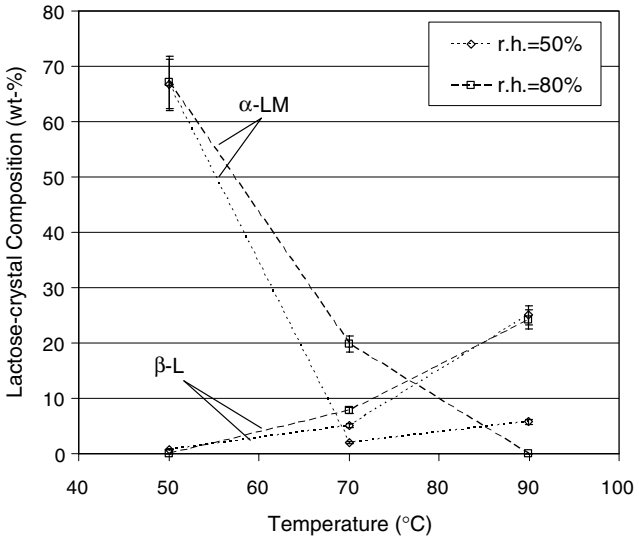

Figure 7. Compositions of α-lactose monohydrate (α-LM) and anhydrous β-lactose (β-L) in whey-permeate powders crystallized at various relative humidities and temperatures.

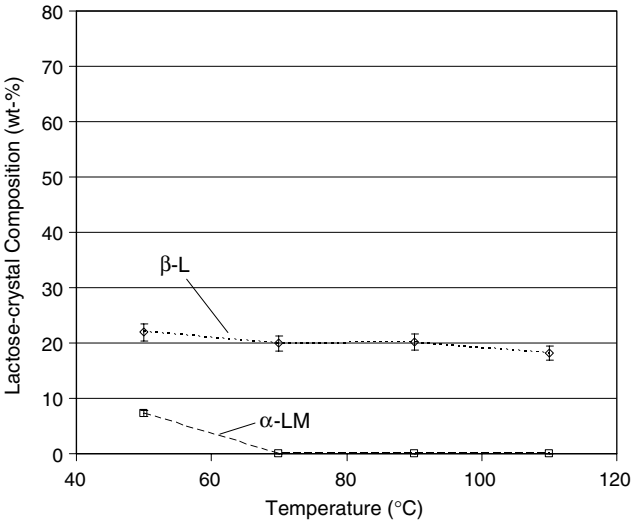

Figure 9. Compositions of α-lactose monohydrate (α-LM) and anhydrous β-lactose (β-L) in pure lactose powders crystallized at a relative humidity of 50% and various temperatures.

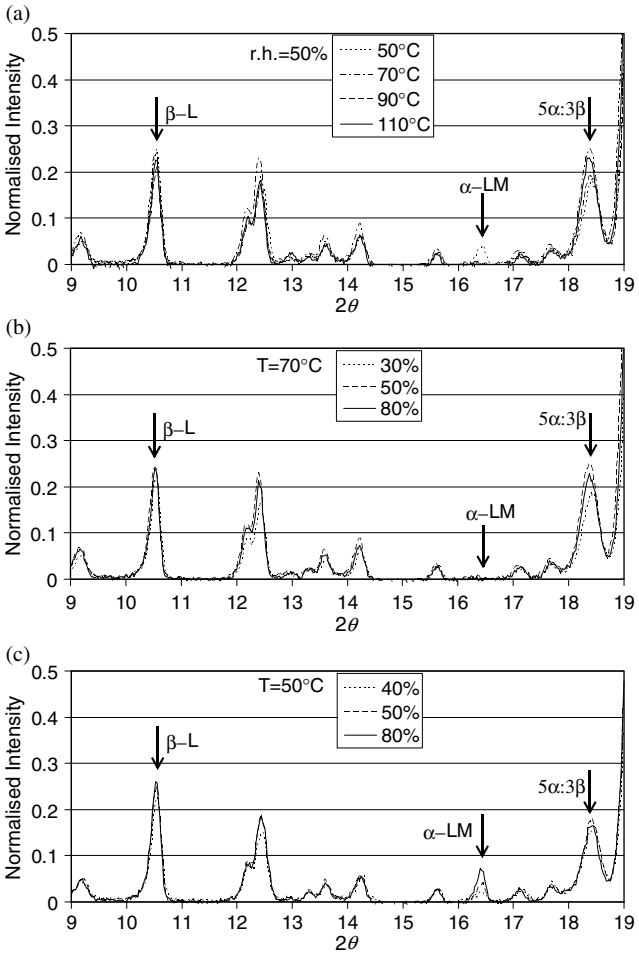

Figure 8. X-ray diffraction patterns for pure lactose powder crystallised at various temperatures and humidities.

compositions for whey, whey-permeate and pure lactose powders crystallised by Ibach and Kind[4] at various temperatures and humidities. The arrows in the XRD patterns (Figs. 4,6,8 and 12) highlight the locations of the characteristic peaks for α-lactose monohydrate, anhydrous β-lactose and anhydrous lactose in the molar ratio α:β = 5:3. The α-lactose monohydrate and anhydrous β-lactose compositions shown in Figures 5,7,9 and 10 were extracted from the X-ray diffraction patterns using the XRD calibration curves given in Figure 2, where the normalised integral is defined as the ratio of the area under the peak at 10.4° (for anhydrous β-lactose) or 16.4° (for α-lactose monohydrate) to the area under

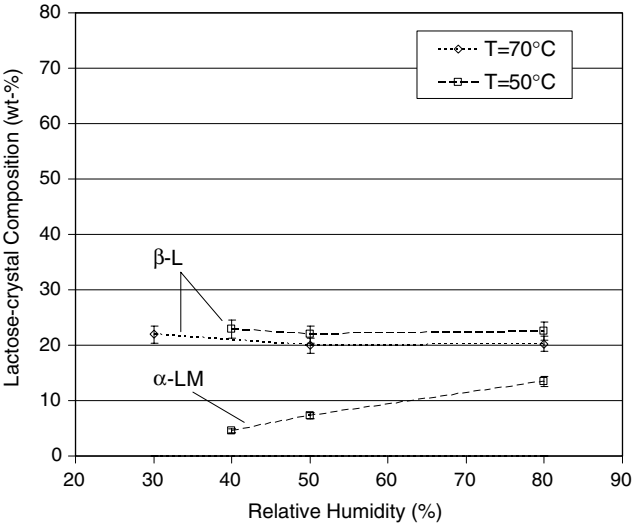

Figure 10. Compositions of α-lactose monohydrate (α-LM) and anhydrous β-lactose (β-L) in pure lactose powders crystallized at various relative humidities and temperatures.

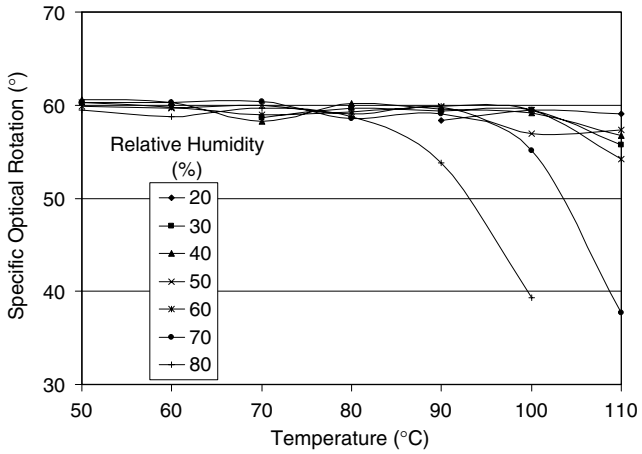

Figure 11. The specific optical rotations of pure lactose samples crystallised at various relative humidities and temperatures.

the silicon peak at 47.3°. Finally, Figure 11 shows the optical rotation measurements of the pure lactose samples crystallised at various temperatures and humidities. Similar measurements were not conducted for the whey and whey-permeate samples, which were found to be too opaque in solution for accurate polarimeter readings. Note that the X-ray diffraction diagrams for the amorphous powders (Fig. 3) could not be normalised, because no silicon standard was added for the XRD analysis of these powders. These diagrams were scaled roughly to provide a better impression of the height of these curves relative to the height of the curves in the XRD diagrams for the crystallised powders shown in Figures 4,6 and 8.

4. Discussion

Figure 4a and b show that the amount of anhydrous β-lactose increased while the amount of α-lactose monohydrate decreased as the crystallisation temperature of the whey powder increased, which agrees with the observations of Vuataz[8] for the crystallisation of skim milk. The amounts of α-lactose monohydrate and anhydrous β-lactose formed at 50 °C were approximately 46 and 5 wt %, respectively (Fig. 5). Given that the total lactose content in the whey powder was 73 wt % (Table 1), then 22 wt % of the total weight of the sample must have been accounted for by lactose-crystal forms other than α-lactose monohydrate and anhydrous β-lactose. Indeed, anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ was also detected, as indicated by the small rise at a diffraction angle of 18.2° (Fig. 4a). At the intermediate temperature of 70 °C, more α-lactose monohydrate formed at 80% relative humidity than at 50% relative humidity (17 wt % and 7 wt %, respectively), which agrees with the observation of Drapier-Beche et. al.[5] that higher humidities encourage the development of α-lactose monohydrate. However, no α-lactose monohydrate formed at 90°C, while approximately 37 wt % anhydrous β-lactose crystallised at both relative humidities tested. Once again, a high proportion of the mass of these samples (36%) must have been accounted for by lactose-crystal forms other than α-lactose monohydrate and anhydrous β-lactose. These other crystalline forms, which include anhydrous lactose in the molar ratios $\alpha{:}\beta = 5{:}3$, 3:2 and 4:1, are known to be unstable at ambient storage conditions.[9] This suggests that lower temperatures and higher humidities are most appropriate for crystallising whey powders, so that α-lactose monohydrate, which is the most stable lactose-crystal form at ambient storage conditions, is produced in the greatest quantities. The time for crystallisation of whey powder at a temperature of 50 °C and a relative humidity of 80% is approximately 10 min, compared with only 4 min for whey powder crystallised at a temperature of 90 °C and the same relative humidity. Thus, it appears that the advantage of quicker crystallisation times at higher temperatures is offset by the production of lactose crystal forms that are less stable under normal storage conditions.

Ibach and Kind[4] have measured the crystallisation times for the whey, whey-permeate and pure lactose powder samples investigated here, which is defined as the time at which each sample reached an equilibrium moisture content after having progressively gained moisture (due to its hygroscopic nature) and subsequently lost moisture during crystallisation. These workers have assumed that, once the equilibrium moisture content had been reached, all the amorphous lactose had crystallised, and additional moisture in the structure was due to the hygroscopic nature of any protein and salts present. The time required for complete crystallisation of each lactose sample was easily determined, because the different crystallisation stages could be clearly distinguished. However, these stages were difficult to differentiate in the case of the whey and whey-permeate powders crystallised at high humidities (>70%), when crystallisation was relatively quick, because the protein and salt contents continued to adsorb moisture well after the crystallisation process was complete. In these cases, an intermediate equilibrium moisture content was temporarily established before the moisture content rose once again towards the final equilibrium moisture content. This intermediate equilibrium corresponded to the point at which the lactose content had adsorbed moisture (due to its hygroscopic nature) and then begun to lose this moisture as it crystallised, during which time the protein and salts continued to adsorb moisture. Figure 4c shows the diffraction patterns for two whey powder samples crystallised at a temperature and relative humidity (80% and 50 °C, respectively) at which such an intermediate equilibrium moisture content was observed. One

whey powder sample was exposed to these conditions until shortly after the moisture content had begun to rise from the intermediate equilibrium value (15 min), while the other sample was exposed to these conditions until the final equilibrium moisture content was attained (38 min). Assuming that the whey powder had completely crystallised within 15 min, then it appears that anhydrous β-lactose, together with any other unstable lactose-crystal forms present, transformed progressively to α-lactose monohydrate, whose composition increased from 45 to 56 wt % (Fig. 5). This is a phenomenon previously observed by Vuataz[8] for skim milk crystallised at 80 °C and 55% relative humidity, which confirms the unstable nature of anhydrous β-lactose when it is exposed to high relative humidities and moderate temperatures.

Note that two samples were crystallised at each air temperature and relative humidity tested during the whey, whey-permeate and lactose crystallisation experiments to check for repeatability. In the case of the first sample, exposure to the given set of conditions was stopped once it became clear that the final equilibrium moisture content was attained and thus crystallisation had ended. For the second sample, the same exposure times were used, except at conditions where the sample experienced a clear intermediate equilibrium moisture content, in which case exposure to the given set of conditions was stopped shortly after the intermediate equilibrium was reached. This only occurred in the whey and whey-permeate powders exposed to high humidities, and was not observed in the lactose samples. It was assumed that complete crystallisation of the lactose in the whey and whey-permeate powders had occurred shortly after this intermediate equilibrium, and that recrystallisation of any unstable lactose-crystal forms present to α-lactose monohydrate could continue beyond this point. XRD analyses were generally conducted on the second crystallised-powder samples.

Figures 6 and 7 show that whey-permeate powder experiences similar trends to whey powder when it is crystallised. Thus, lower temperatures and higher relative humidities encourage α-lactose monohydrate formation, whereas higher temperatures encourage anhydrous β-lactose, although the relative humidity appears to have very little effect on its formation over the range of values tested. However, significantly more anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ formed in the whey-permeate powder than in the whey-powder crystallised at temperatures of 70 and 90 °C, as shown by a comparison of Figures 4 and 6. Indeed, a comparison of Figures 1c and 6 shows that the height of the characteristic peak in the powder diffraction diagram for 'pure' anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ (at 18.2°) is not significantly different than the height of the corresponding peak in the powder diffraction diagram for the whey-permeate powders crystallised at 70 and 90 °C, which suggests that a large proportion of the crystallised whey-permeate powder must have been composed of this particular lactose-crystal form. The combined α-lactose monohydrate and anhydrous β-lactose contents in the whey-permeate powder crystallised at 90 °C and relative humidities of 50% and 80% were 31 and 24 wt %, respectively (Fig. 6). Given that the total lactose content in the whey-permeate powder was 79 wt % (Table 1), then the remaining lactose-crystal forms must have accounted for 48% and 55%, respectively, of the total mass of these powders. These crystalline forms are unstable under normal storage conditions, converting to the more stable α-lactose monohydrate with the uptake of moisture.[9]

More α-lactose monohydrate formed in the whey-permeate powder than in whey powder crystallised at 50 °C (67 wt % compared with 46 wt %) and virtually no anhydrous β-lactose formed at this temperature (Fig. 7). The remaining lactose content of 12 wt % must have been composed of either a small amount of anhydrous lactose in the molar ratio $\alpha{:}\beta = 5{:}3$ or one of the other anhydrous lactose-crystal forms. These results show once again that the most stable crystalline form at ambient storage conditions (α-lactose monohydrate) is produced in the greatest quantities at lower temperatures, although the disadvantage of using a lower temperature is a longer crystallisation time. Note that Ibach and Kind[4] have shown that the crystallisation of whey-permeate is generally faster than the crystallisation of whey powder. They have explained that the diffusional pathways of the lactose molecules in the solid matrix of whey powder are shorter, due to the presence of more protein and salts, so that the rate of crystallisation is slower.

The pure lactose powder experienced different trends to the whey and whey-permeate powders, as shown in Figures 8–11. Figure 8a shows that the temperature did not significantly affect the composition of the lactose powder crystallised at a relative humidity of 50%. Approximately 20% anhydrous β-lactose and hardly any α-lactose monohydrate formed at this relative humidity, irrespective of the temperature used, which ranged from 50 to 110 °C, as shown in Figure 9. The relative humidity also had very little effect on the composition of the powder crystallised at a constant temperature of 70 °C, as shown in Figure 8b. In this case, the anhydrous β-lactose composition was constant at 22% for relative humidities ranging from 30% to 80% (Fig. 10). The crystal form α-lactose monohydrate only appeared at 50 °C (Fig. 8c), although its composition was rather low, increasing from 5 to only 13 wt % as the relative humidity was increased from 40% to 80% (Fig. 10). This suggests that the crystallised lactose powders were composed mostly of anhydrous α-lactose and mixed anhydrous forms of α- and β-lactose in the molar ratios of 5:3, 3:2 and 4:1. Indeed, a comparison of Figures 1c

and 8 shows that the characteristic peak in the powder diffraction diagram for 'pure' anhydrous lactose in the molar ratio α:β = 5:3 (at 18.2°) is smaller than the corresponding peak in the powder diffraction diagram for the crystallised lactose samples, which suggests that anhydrous lactose in the molar ratio α:β = 5:3 is slightly purer in the crystallised lactose samples.

The crystallisation times were significantly faster for the lactose powders than for the whey and whey-permeate powders.[4] At a relative humidity of 50%, lactose crystallisation times ranged from 2 to 20 min for temperatures ranging from 50 to 90 °C, whereas the whey and whey-permeate crystallisation times ranged from 8 to 80 min and 8 to 50 min, respectively, over the same range of temperatures. We propose that, in the case of pure lactose, the onset of crystal nucleation and growth was so rapid that no mutarotation of the lactose from one isomeric form to the other was possible in the amorphous phase, and α-lactose had very little time to subsequently take up water and crystallise as α-lactose monohydrate. According to this theory, the lactose isomers (α- and β-lactose) should have crystallised in the same ratio as they existed in the amorphous phase. This is confirmed in Figure 11, which shows that the specific optical rotation of the crystallised lactose (a measure of the ratio of α- to β-lactose in the powder) is relatively constant over a wide range of temperatures and humidities and close to the specific optical rotation of 59.2° measured for the original amorphous lactose. In the case of the whey and whey-permeate powders, the crystallisation of lactose was slowed down by the presence of protein and salts, so that the lactose isomers had more time (1) to mutarotate to find an equilibrium appropriate for the given temperature and relative humidity, before they crystallised in these forms and (2) to become hydrated during crystallisation in the case of α-lactose.

Nevertheless, Figure 12 shows that mutarotation is possible in pure amorphous lactose at a high temperature and relative humidity of 110 °C and 70%, respectively. In this case, the composition of anhydrous β-lactose in the crystallised powder was 88% and there was no evidence of α-lactose monohydrate, although a small amount of anhydrous lactose in the molar ratio α:β = 5:3 formed. This can also be seen in Figure 11, which shows a decrease in the specific optical rotation of lactose crystallised at high temperatures and relative humidities towards the value for pure β-lactose of 35°. The high temperature and relative humidity clearly increased the mutarotation rate of the lactose in the amorphous phase, so that the ratio of the two isomers (α- and β-lactose) approached an equilibrium, which, in this case, favoured the appearance of β-lactose. Indeed, Fox[12] has reported that anhydrous β-lactose crystallises preferentially in a super-saturated solution at temperatures above 93.5 °C.

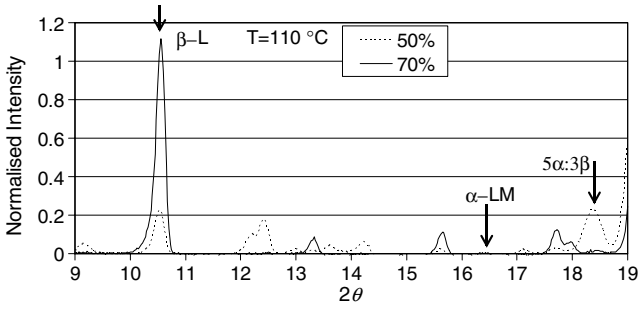

Figure 12. X-ray diffraction patterns for pure lactose powder crystallised at 110 °C and two different relative humidities (50% and 70%).

5. Conclusions

The amounts of α-lactose monohydrate, anhydrous β-lactose and mixed-anhydrous lactose that crystallise in whey and whey-permeate powders depend on the air temperature and relative humidity at which crystallisation occurs. Lower temperatures and higher humidities encourage α-lactose monohydrate formation, which is the most stable lactose crystal under normal storage conditions. Unstable anhydrous β-lactose and mixed anhydrous lactose crystallise at higher temperatures, with the advantage that crystallisation is faster. However, the lactose-crystal forms that appear during crystallisation of pure lactose are relatively insensitive to the temperature and humidity. In this case, crystallisation is so rapid that no mutarotation of the α- and β-lactose isomers can occur in the amorphous phase, and very little water can be taken up by α-lactose to form the hydrate during crystallisation. The presence of protein and salts in the whey and whey-permeate powders reduces the crystallisation rate, so that mutarotation and crystal hydration can occur in these powders, provided sufficient moisture is available.

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