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Rapid lactose recovery from buffalo whey by use of 'anti-solvent, ethanol'

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Abstract

In the present study the heat-induced partially deproteinated (0.1–0.2% w/v of residual protein after deproteination step) paneer whey (that resembles cottage cheese whey), originated from buffalo milk (containing 3.85–4.95% w/v of lactose) was tried for possible rapid lactose recovery, using 'anti-solvent' (ethanol). Optimization of various process parameters such as effective ethanol concentration (65–85% v/v), pH, temperature, seeding, etc. was carried out. On optimization of the process, the lactose recovery of more than 90% and the purity of 97–99% was obtained from paneer whey in 1 h of stirring time, at an effective ethanol concentration of 85% v/v. The 'roundness' (shape factor) and 'elongation' (aspect ratio) values of the lactose crystals recovered in the present study were found to be comparable to that of commercial analytical grade lactose sample. The crystallization kinetics has been also studied of the lactose recovery using anti-solvent 'ethanol' based recovery process.

Keywords: Lactose recovery; Whey; Anti-solvent; Ethanol

1. Introduction

The growing dairy industry in India and worldwide has a major concern of the disposal of dairy waste, which is increasing leaps and bounds with an increase in the production of processed milk products like cheese, shrikhand (obtained from curd, which resembles 'quarg' from Germany) and Indian cheese (i.e. paneer, which resembles cottage cheese and is most commonly consumed milk-based delicacy in India). Approximately 85% of total milk used for the manufacturing of cheese/paneer was discarded/drained off as whey (Sooch & Singh, 2002; Balasubramanyam, Singh, & Bhanumurthi, 1989). In 2001, the whey generated only in India, (paneer whey) was around 2.58×10^6 tonnes (Aneja, Mathur, Chandan, & Banerjee, 2002). It is a serious pollutant as it imposes a high biochemical oxygen demand (BOD) of 30,000–50,000 mg L^{-1} (Marwaha, Arora, & Grover, 2000). Whey contains approximately 5–6% w/v total solids of which more than 70% is accounted by lactose and only 4–9% by way of whey proteins. The heat-induced deproteination showed a recovery of around 58–79% of whey proteins based on various parameters, with around 0.1% w/v of residual protein left in the whey (Bund, 2005). Thus, resultant partially deproteinated whey consisting mainly of lactose is the main cause of the BOD in the effluent. Recovery of lactose from whey solves both the problems of improving economics of whey utilization and of pollution reduction as lactose recovery itself can reduce BOD of whey by more than 80%.

Based upon the quality of the lactose, this milk sugar finds application in many industries and in the various products at various degree of purity as crude, edible or pharmaceutical grade (Elvers, Hawkins, & Schulz, 1990; Paige & Davis, 1985).

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The key to make lactose recovery an economical process lies in developing a speedy recovery process from low concentration whey i.e. less evaporation cost (lactose content of 19–20% w/v) and with high purity in the first step of crystallization itself. The conventional lactose recovery process from cheese whey or permeate involves concentration of whey to 55–65% of total solids (high evaporation costs) followed by cooling to yield yellow colored raw lactose. The process is tedious with variation in crystallization time from 12 to 72 h (Kapil, Dodeja, & Sarma, 1991; Nickerson, 1979). The raw lactose is re-dissolved, charcoal treated and re-crystallized to yield various grades of the lactose, with a maximum yield of around 50–60% (Elvers et al., 1990; Kapil et al., 1991). The purity of the lactose is greatly influenced by the properties of the initial whey and its protein and mineral content.

In the past, limited attempts have been made to develop processes for the lactose recovery, different from 'traditional cooling method'. The use of solubility behavior of lactose was explored with the help of anti-solvents like ethanol (Leviton & Leighton, 1938; Whittier & Gould, 1931), methanol (Leviton, 1949), DMSO (Dincer, Parkinson, Rohl, & Ogden, 1999) for its recovery. However, most of them were tried with pure lactose solutions (Whittier & Gould, 1931) (shown inability to replicate results in actual whey systems), or spray dried cheddar cheese whey powders (Leviton & Leighton, 1938) involving a lot of pretreatments, post treatments, longer re-crystallization time. The yields were not greatly improved in comparison to conventional process (traditional cooling process) in spite of these multiple pretreatments.

With ultrafiltered deproteinated cheese whey and ethanol water mixture (72.9% w/w) the lactose recovery of 57% and 65% has been reported with solvent to solute ratio of 15:1 and 10:1 respectively in 20 h (Singh, Nielsen, & Chambers, 1991). However, the ultrafiltration involves substantial capital and recurring costs due to limited ultrafiltration membrane life and higher operating pressures. Hence, the small and medium scale dairy processors find it uneconomical to use ultrafiltration facility for the treatment of the whey, which results in draining of this whey causing serious environmental problem. For such processors, the heat-induced aggregation of whey protein would provide an economical alternative to ultrafiltration processes, followed by the recovery of lactose from deproteinated whey resulting in a complete and efficient whey management strategy.

Since, alcohols greatly reduce the solubility of the lactose in water, they are expected to accelerate crystallization by salting out and more rapid crystallization tend to produce α-stable lactose, while lower supersaturation or dilute solution yield mostly α-hydrate (Majd & Nickerson, 1976). Hence, in the present study the use of anti-solvent, ethanol was investigated for the speedy recovery of lactose from heat-induced deproteinated real whey of buffalo milk origin.

2. Material and methods

2.1. Sample acquisition and preparation

Paneer is obtained through heat/acid coagulation of casein component of standardized buffalo milk, entrapping through complex physico-chemical interactions almost all the fat, a part of denatured whey proteins and colloidal salts, as well as a part of soluble milk solids. The whey that separated during this process was named as 'paneer whey'/'whey' throughout the article here onwards. Paneer whey, used in the present study was procured intermittently from a local dairy. It was of buffalo milk origin. Variation in physico-chemical characteristics of paneer whey samples can be seen from Table 1. The whey had varied concentration of lactose from 3.6% to 5.04% w/v. The protein content of whey varied from 0.126% to 0.285% w/v. The pH of the whey varied from 3.6 to 5.0. The samples were stored at 8 ± 2 °C. Residual fat separation was observed after storage at this temperature which was skimmed off by filtration of wheys through a bolting cloth and centrifugation (centrifuge from Remi Instruments, India) at 8000 g for 35–40 min. These samples were again stored at a temperature of 8 ± 2 °C till further experiments. During storage study at a temperature of 8 ± 2 °C, for 1 month it was observed that the protein content of whey remained unaltered, however the lactose content decreased due to degradation. In the case of paneer whey, lactose level of 96.6% was retained at the end of 15 days of storage time, of the initial lactose content of 3.78% w/v (detailed data not given). Thus, the experiments were performed with in the 7 days of the procurement of whey.

2.1.1. Deproteination

Whey was deproteinated by pre-optimized process of heat-induced aggregation of whey proteins, at pH of 5.3 ± 0.1, temperature of 92 ± 2 °C, in treatment time of 60 min maintained in a constant temperature bath (procured from Hexatec Enterprises, Mumbai, India) followed by centrifugation at 8000*g* for 30 min for recovering, precipitated partially denatured whey proteins and supernatant with lactose (Bund, 2005).

2.1.2. Concentration

The supernatant from deproteination step was concentrated at temperature $\leqslant 95 \pm 3$ °C in a steam heated open porcelain pan. The resultant concentrated whey contained lactose 19.6–19.8% w/v. This concentrated whey was used for optimizing various conditions for the lactose recovery process using an anti-solvent, ethanol instead of a salt to precipitate the lactose.

2.1.3. General protocol for crystallization of lactose using anti-solvent, ethanol

Concentrated whey of known volume and known protein and lactose content, adjusted to a desired pH ($4.5–2.8 \pm 0.1$) was taken in 250 mL Erlenmeyer flasks, fitted

Table 1
Variation in physico-chemical characteristics of paneer whey samples procured intermittently from local dairy

Protein content by Folin lowry method (% w/v)	Lactose content by WillStäter's method (% w/v)	pH
0.238	3.78	3.6
0.267	3.69	3.6
0.26	5.04	4.37
0.144	3.8	4.46
0.284	3.6	4.62
0.126	4.14	4.62
0.285	4.1	5.0

with stoppers. It was kept in a constant temperature, cooling chamber on a magnetic stirrer (procured from Remi equipments, India). The ethanol (95% v/v), pre-chilled to the temperature of 7 ± 2 °C was added in an appropriate quantity at once to whey (with/without stirring), to achieve the effective concentration of ethanol of 65–85% v/v in the system. The process temperature during crystallization was maintained at 7 ± 2 °C. The samples were stirred at a speed (at rpm of 750–1000) sufficient enough to keep the contents inside the flask, in suspension for 5 h, with/without subsequent standing time of 12 h. The precipitate (recovered lactose) obtained (white in color), was filtered using the vacuum filtration. It was dried in vacuum oven at 60 °C for 4 h, and weighed. The recovered lactose was analysed for lactose, protein, ash content and melting point.

2.2. Reagents

Ethanol (95% v/v) was procured from M.S.S.I.D.C., State excise warehouse, Mumbai, India. Albumin, Bovine (BSA), prepared by Electrophoresis (RIA grade), used in the present study (as a model protein instead of whey proteins, for standard curve of protein analysis by Folin Lowry method) was procured from Sigma Chemical Co., St. Louis. Lactose monohydrate was procured from HiMedia Laboratories Ltd., Mumbai, India. The rest of chemicals used were all of analytical grade and procured from Merck, Mumbai, India.

2.3. Analytical methods

2.3.1. Estimation of protein

Estimation of the whey protein in the samples was done using (modified) Folin Lowry method as described below (Lowry, Roseborough, Farr, & Rondall, 1951).

Modified Folin lowry method −1 mL of the alkaline copper reagent was added to 0.1 mL of sample and vortex immediately. The solution was incubated at 30 °C for exactly 10 min. To this, 0.1 mL of Folin–Ciocalteau reagent (1:1 diluted with distilled water) was added and vortexed immediately. This was allowed to stand at 30 °C for exactly 30 min. The blue color thus produced was measured with the help of a UV–vis spectrophotometer (Chemito 2500, India) at 660 nm. The standard calibration curve was prepared in the protein-concentration range of 0–0.6 mg mL^{-1} of BSA.

2.3.2. Estimation of lactose

Lactose content was determined by WillStäter's iodometric titration (Pearson, 1976).

2.3.3. Ash content

The ash content of the recovered lactose was determined by heating and slowly igniting the accurately weighed lactose sample from 250 °C to $\leqslant$550 °C for 20 h, in a Muffle Furnace (AOAC, 1984).

2.3.4. Melting point

Melting points of the recovered lactose were measured using melting point apparatus of Campbell Electronics, Mumbai, India.

2.3.5. Crystal size analysis and distribution

The recovered lactose crystals were observed under microscope (40× magnification) of the Leica Gallen III mech. The images were photographed using camera (Pulnix) and analysed for crystal size and its distribution using Biovis Image Plus (software for image analysis and processing). The sample size of crystals was fixed to150 crystals. (It was observed that the standard deviation of average crystal diameter remained unchanged on sample size exceeding 90 crystals.)

2.4. Parameters investigated

2.4.1. Effect of the 'effective ethanol concentration'

The pre-cooled (7 ± 2 °C) ethanol was added at once to three pre-cooled (7 ± 2 °C), concentrated whey samples (pH 4.5–4.6), 15 mL each, to achieve the effective ethanol concentration of 65%, 75% and 85% v/v. All the samples were then stirred on magnetic stirrer in a cooling chamber maintained at 7 ± 2 °C, for 5 h and allowed to stand for further 12 h without agitation. The precipitate (recovered lactose) was separated using the vacuum filtration followed by drying in vacuum oven at 60 °C for 4 h. In the case of fourth sample (15 mL) of whey, to get effective concentration 85% v/v ethanol was added at once with stirring already on (pre-stirring). The rest of the procedure was the same as in the case of earlier three samples. The recovered and dried lactose was weighed to assess the % recovery of lactose.

2.4.2. Effect of 'initial-pH adjustment' (i.e. pH of whey before crystallization starts) and 'end-pH adjustment' on the quality of recovered lactose

The lactose showed high level of ash when recovered from the concentrated whey having pH 4.6. Thus, it was thought to increase the solubility of salts (mostly in chloride form) by acidification of whey prior to crystallization. To see the effect of 'initial-pH adjustment' 15 mL each of the concentrated whey was taken in two flasks. In the flask 1, the pH was kept as it is (pH 4.54). Where as, in flask 2

the pH of the whey was adjusted to 2.8 using HCl (1:1, conc. HCl and distilled water). The lactose was recovered as described in Section 2.4.1 at an effective ethanol concentration of 85% (v/v) and then analyzed for ash content and melting point.

To see the effect of 'end-pH adjustment' concentrated whey (pH adjusted to 2.8) 15 mL each was taken in three flasks. The crystallization of lactose was carried out in a similar way as stated above (initial-pH adjustment). Effect of the 'end-pH adjustment' was assessed by lowering the pH of the whey–ethanol system using 'alcoholic HCl' (1:1 proportion) to 4.1, 2.8 and 2.2, at the end of the crystallization process (5 min prior to the end of the stirring time of 5 h) without any further standing time. The lactose was recovered as described in Section 2.4.1 and analysed for lactose and ash content.

2.4.3. Effect of crystallization time

The lactose recovery was carried out from concentrated whey using ethanol at effective concentration of 85% v/v for different time intervals of stirring (1–5 h) (no standing time) keeping rest of the crystallization conditions same as described in Section 2.4.2. The initial and end-pH adjustment was carried out, by adjusting the pH of samples to 2.8 as described earlier in Section 2.4.2. The lactose was recovered as described in Section 2.4.1, weighed and analysed for lactose content.

2.4.4. Effect of seeding

The seeding of analytical grade lactose (1–3% w/w of the lactose content of concentrated whey) was carried immediately after the addition of pre-cooled (7 ± 2 °C) ethanol to concentrated whey samples. The initial and end-pH adjustment was carried out, by adjusting pH of samples to 2.8 as described earlier in Section 2.4.2. The crystallization was carried out with constant stirring for 1 h. The lactose was recovered as described in Section 2.4.1, weighed and analysed for lactose content.

2.5. Statistical analysis

The experiments were carried out in triplicates. The data were analysed by descriptive statistics tool provided by Excel, MS Office 2000. The graphs were plotted using mean values obtained from the data. The standard deviation is shown using Y-error bars.

2.6. Crystal size distribution (CSD) analysis

The lactose samples recovered at different time intervals (Section 2.4.3) from concentrated paneer whey were utilized for the CSD study.

2.7. Crystallization kinetics

The crystal size distribution (CSD) data for lactose recovered at different time intervals (1–5 h) was utilized for determining the kinetic rate constants for lactose crystal growth for anti-solvent 'ethanol' based recovery process. The crystals were assumed to be spherical in shape as the average roundness factor for all the samples was in the range of 0.78–0.87. The perfectly spherical crystal will have the roundness factor 1. The total number of crystals in one mL (n) at end of specified crystallization time (1–5 h) were calculated. The growth rate expression (Eq. (1) below) for mixed suspension mixed product recovery (MSMPR) operating in a continuous mode was used in the present study for estimating crystal growth rate (G) of the lactose. Even though the present work was a batch study, the rationale to use the expression for the continuous mode was the similarity in the manner the lactose crystallizes. Due to nucleation, the lactose precipitating out is distributed simultaneously in the form of new crystals (nuclei) and growing crystals (growth rate), which can be accounted for quite simply by MSMPR expression. The growth rate expression is as follows

$$n = n_0 \exp\left(\frac{-L}{G\tau}\right) \tag{1}$$

where, 'n' is number of crystals mL^{-1}, 'n_0' number of embryo size crystals having diameter practically equal to zero present at the beginning of nucleation process, 'L' average crystal diameter (μm), 'G' crystal growth rate (μm s^{-1}) and 'τ' is crystallization time (s). On plotting $\log n$ vs L, the crystal growth rate (G) over time (τ) can be calculated taking into account, changing n due to nucleation. Then an empirical expression was used to correlate growth rate with supersaturation given as

$$G = k_g(C_b - C_s)^m \tag{2}$$

where, 'G' is crystal growth rate (μm s^{-1}), 'k_g' rate constant for growth [μm s^{-1} (g $mL^{-1})^{-m}$], 'C_b' residual lactose concentration (g mL^{-1}) in the supernatant after crystallization process is complete, 'C_s' is saturation solubility of lactose (g · mL^{-1}) at temperature of 7 ± 2 °C, in the solution having effective ethanol concentration of 85% v/v and 'm' exponential order of growth. Here, k_g is a function of the external processing conditions such as temperature, agitation rate and the presence of additives or impurities.

Similarly, n i.e. number of crystals formed mL^{-1} can also be correlated as a function of desupersaturation as given in expression 3.

$$n = k_n(C_b - C_s)^{m'} \tag{3}$$

where, 'n' is number of crystals mL^{-1}, 'k_n' rate constant for nucleation [number of crystals $mL^{-1} \cdot (g\ mL^{-1})^{-m'}$] and '$m'$' exponential order of nucleation. Here, k_n is a function of the external processing conditions such as temperature, agitation rate and the presence of additives or impurities.

3. Results and discussion

Crystallization typically includes the steps of generation of the supersaturated state, nucleation (with or without

seeding), and the crystal growth. In order to overcome the disadvantages observed during the conventional lactose recovery process, in the present study the approach of using an 'anti-solvent' ethanol instead of a salt to precipitate the lactose was studied.

The technique involves the addition of a second solvent (anti-solvent) in which the solute is insoluble, generating initially local (at the point of addition) and then eventually global supersaturation. The anti-solvent used in the present study is 'ethanol', due to its properties like inert nature and minimal solubility of lactose in it, at all the concentrations (in comparison to other alcohols viz. methanol, propanol, etc.) (Majd & Nickerson, 1976). The effective ethanol concentrations (65%, 75% and 85% v/v), selected in the present study were based on the reported solubility behavior of the lactose in alcohol (methanol), that showed a steep decrease in the solubility, from 90% to 10%, when the methanol (lactose solubility is larger in methanol than ethanol) concentration increased from 65% to 85%, respectively (Leviton, 1949).

The dried lactose, obtained at the end of the crystallization process was termed as 'recovered lactose'. The percentage lactose recovery was calculated on the basis of the lactose content (in g) of the concentrated whey sample, before crystallization.

3.1. Effect of the 'effective ethanol concentration'

Lactose recovery of 84.76%, 89.5% and 92.63% was obtained at effective ethanol concentration of 65%, 75% and 85% v/v, respectively as shown in Fig. 1. The lactose recovery was directly proportional to the final effective ethanol concentration in the system. The increase in the recovery due to an increase in the effective ethanol concentration could be attributed to the attainment of rapid supersaturation resulting in a precipitation of lactose. When the sample was pre-stirred, the lactose recovery was improved by 4%, at effective ethanol concentration of 85% v/v. The possible reason for the improvement in the lactose recovery because of the 'pre-stirring' could be due to the distributed nucleation and also an increase in the solid–liquid mass transfer rate resulting in enhanced crystal growth rate during ethanol addition.

3.2. Effect of the initial pH (i.e. pH of whey before crystallization starts) and end-pH on the quality of the recovered lactose

The high level of ash observed in the lactose recovered from the concentrated whey having pH 4.6 could be due to the simultaneous precipitation of inorganic salts along with lactose at these pHs on the addition of ethanol. Thus, the pH of whey was adjusted to 2.8, prior to crystallization, there by increasing the solubility of salts (mostly in their chloride form), in the final alcoholic aqueous solution. A marginal decrease in lactose recovery from 98.2% to 96.66% was observed, on lowering of the pH from 4.6 to 2.8. Where as some earlier researchers have reported no change on the lactose yield, recovered from ultrafiltration permeate of the cheese whey, on acidification of whey prior to crystallization (Singh et al., 1991).

The ash content of the recovered lactose decreased from 3.1% w/w to 2.2% w/w (i.e. almost 30%) due to the pH adjustment to 2.8 as could be seen from Fig. 2. Melting point (mp) is an important parameter, which can be used to distinguish between the α-lactose monohydrate (mp 201–202 °C) and β-lactose (253 °C) (Elvers et al., 1990). The melting point of the analytical grade lactose monohydrate procured from the market was observed to be 202 °C. The melting point of the lactose recovered from the whey (pH 2.8) was 205 °C much closer to analytical grade lactose than that of the recovered from whey at pH 4.6 (mp – 191 °C), possibly due to salting out of impurities such as inorganic salts or proteins at higher pH. The mp closer to 202 °C of the lactose recovered after the pH adjustment implied that the recovered lactose was mostly in the form of α-lactose monohydrate.

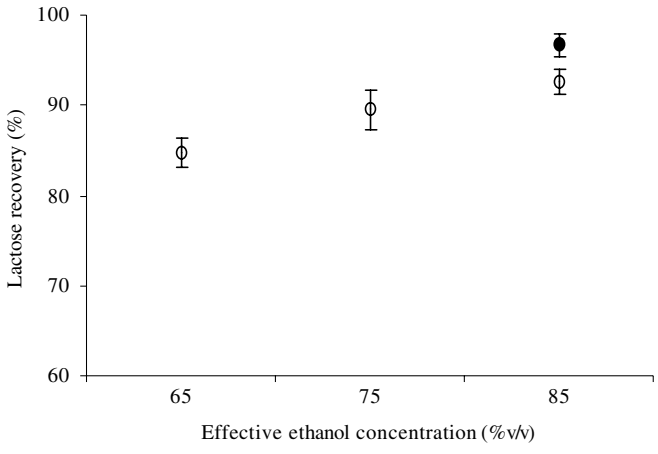

Fig. 1. Effect of 'anti-solvent' on lactose recovery during pre-stirring (●) and without pre-stirring (○).

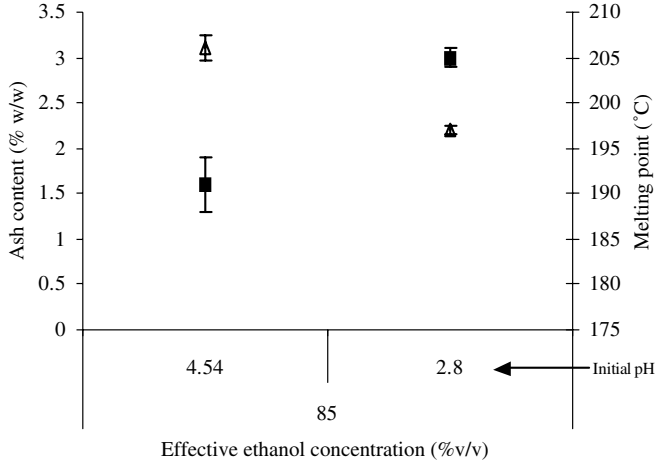

Fig. 2. Effect of initial pH of whey, on ash content (△) and melting point (■) of recovered lactose.

The visual inspection during lactose crystallization with anti-solvent showed an immediate development of turbidity on addition of ethanol to the concentrated whey solution, similar to the milky-ness reported by others (Nickerson & Lim, 1974). However, on stirring for 15–60 min the rapid precipitation in the form of hard or rigid crystals of lactose could be observed. Thus it was thought that the bulk of the lactose recovery took place in the initial 1–2 h of total stirring time. The elimination of 'standing time step' (12 h) from the crystallization process had no effect on the lactose recovery from the paneer whey.

The 'initial pH adjustment' of whey showed a decrease in the level of ash content in the recovered lactose. However, still it was substantially high (2.2% w/w). The possible reason for this could be the simultaneous change in pH due to the addition of ethanol (pH changed to 4.0–4.1 from initial adjusted pH, 2.8 on addition of ethanol). Hence a similar approach of acidification ('end-pH adjustment') was tried just before the end of crystallization period of 5 h.

Due to the end-pH adjustment the lactose recovery decreased from 97.43% (end pH 4.1) to 96.66% (end pH 2.8) and 95.91 % (end pH 2.2) as shown in Fig. 3. However, the ash content of recovered lactose decreased from 2.62% w/w (end pH 4.1) to 1.43% w/w (end pH 2.8), and 0.81% w/w (end pH 2.2). The lactose content of the recovered lactose also improved from 92% w/w (end pH 4.1) to 94.6% w/w (end pH 2.8) and 99% w/w (end pH 2.2).

It was observed that the overall variation in the lactose recovery was 3–5%, with improvement seen in the purity in terms of actual lactose content of the recovered lactose (99% w/w), at the cost of increase in one more step of end-pH adjustment. However, the use of this step would depend greatly upon the commercial/economic feasibility of the process and the required end use specification of lactose.

Using a conventional process, the lactose recovery of 64%, having purity of 95.95% was obtained, from heat-induced deproteinated paneer whey concentrated to 55% of total solids, which was cooled to 30 °C at a specific cooling rate, over a period of 12 h (Kapil et al., 1991). Where as lactose recovery of around 44% and 47% at the end of 5 h and 8 h of time was observed, when crystallization was carried out at 40 °C, with initial lactose content of 44%, in a batch crystallizer with 2.5 g of seed crystal added (Hartel, 2001).

Some researchers have reported that using ethanol–water system' of 72.9% ethanol w/w, the lactose recovery was successful only if the ultrafiltration permeate of cheese whey were concentrated to 30% of solids (Singh et al., 1991). They also observed the lactose crystallization of 65% and 57% in 20 h at solvent to solute ratio of 10:1 and 15:1, respectively (Singh et al., 1991). Others have reported that approximately 16 h would be required for the crystallization of 80% of lactose from whey powder using alcohol (Leviton & Leighton, 1938).

However, in the present case (lactose concentration in the whey 19–20% w/v), the lactose recovery of more than 95% and the purity of 99% w/w could be obtained in 5 h of stirring time, at effective ethanol concentration of 85% v/v (Fig. 3) at a temperature of 7 ± 2 °C.

3.3. Effect of crystallization time

The visual inspection of crystallization process suggested that, substantial lactose recovery, took place in early hours of crystallization. Thus for further optimization, the crystallization time for lactose recovery, was further reduced from 5 h to 1 h. The lactose recovery of 76%, 90.93% and 95.92% was observed for the crystallization time of 1, 3 and 5 h, respectively as shown in Fig. 4. This indicated that around 80% of overall lactose recovery took place in first 1 h itself, which was also confirmed with the visual observation of turbidity. The lactose content (purity) of the recovered lactose remained almost unchanged (97–98% w/w) on reduction of crystallization time from 5 h to 1 h.

3.4. Effect of seeding

The seeding resulted in an increase in the lactose recovery from 76% (without seeding, as observed earlier) to

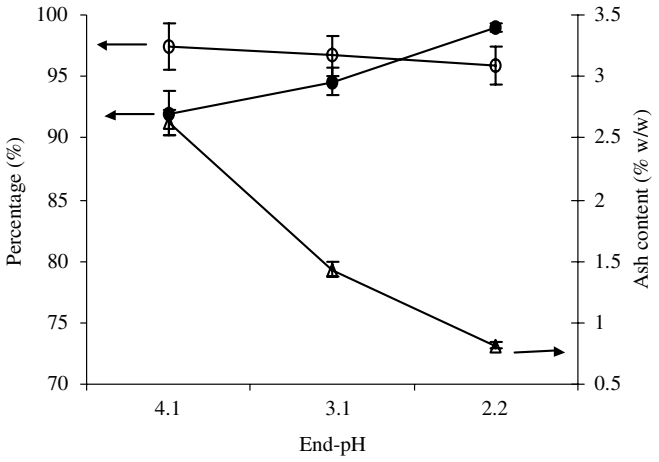

Fig. 3. Effect of 'end-pH adjustment' on lactose recovery (○), ash content (△) and lactose content (●).

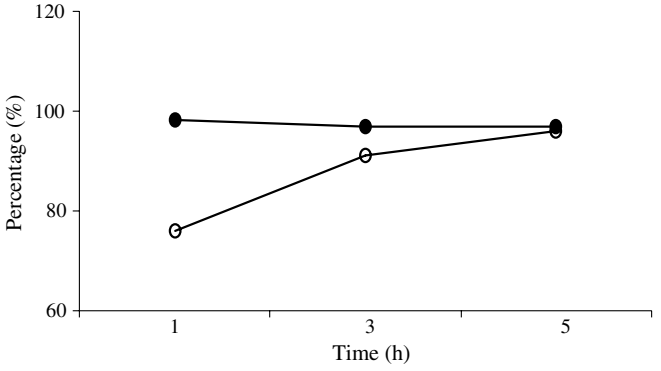

Fig. 4. Effect of crystallization time on lactose recovery (○) and lactose content of recovered lactose (●).

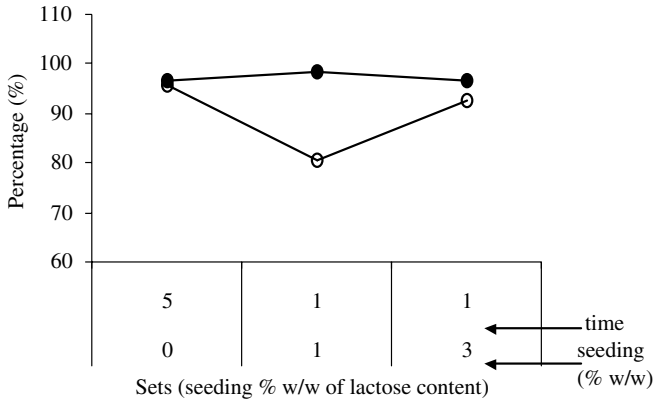

Fig. 5. Effect of seeding on lactose recovery (○), and lactose content of recovered lactose (●).

80.2% (1% w/w seeding) and 92.6% (3% w/w seeding) at the end of 1 h of crystallization time as shown in Fig. 5. The lactose recoveries obtained due to seeding in 1 h of crystallization time were comparable to that obtained with no seeding at the end of 5 h of crystallization time. The change in the lactose content (quality and purity of the lactose recovered) obtained was negligible. The lactose recovered on seeding showed the melting point in the range of 202–203 °C, very close to the melting point of α-form of lactose.

3.5. Crystal size distribution (CSD) analysis

Photographs of lactose crystals observed under microscope (40× magnification) recovered at the end of 1 h and 5 h of crystallization time are shown in Fig. 6. The crystal size analysis of various recovered lactose samples showed that the average projected area (μm^2, as estimated by the image analyzer) of crystals decreased from 25.40 μm^2 to 14.92 μm^2 as the crystallization time decrease from 5 h to 1 h (Table 2). The diameter of individual crystal is an average of the diameters measured at equal intervals around the centroid of the object. The average diameter for every lactose sample was determined from the individual diameter of the lactose crystals examined. The 'average diameter' of 4.19, 4.84 and 5.33 μm were observed for the lactose samples recovered in 1 h, 3 h, and 5 h of crystallization respectively. The 'average diameter' decrease on decrease in crystallization time indicated lower crystal growth. Similarly, the 'average diameter' of the lactose recovered was much smaller than that observed in analytical grade commercial lactose sample (15.4 μm). The smaller crystal size could be due to the rapid process of crystallization employed in the present case with ethanol as an anti-solvent.

The crystal habit in this case is expressed in terms of 'roundness (shape factor)' and 'elongation (aspect ratio)'. The roundness was calculated as the following formula – Roundness = (perimeter * perimeter)/(4 * π * area). The 'elongation' is the ratio of the longest length to the width at the right angles to that length. In the present case, the average roundness (0.78–0.86) and average elongation (1.4–1.5) observed for the recovered lactose crystals was similar to that observed in the commercial analytical grade lactose (average roundness, 0.72 and average elongation 1.41). The crystal size distribution of the recovered lactose samples can be seen from Fig. 7. It can be seen that the peak of the distribution (maximum number) shifted from average diameter of 6–4 μm with a decrease in crystalliza-

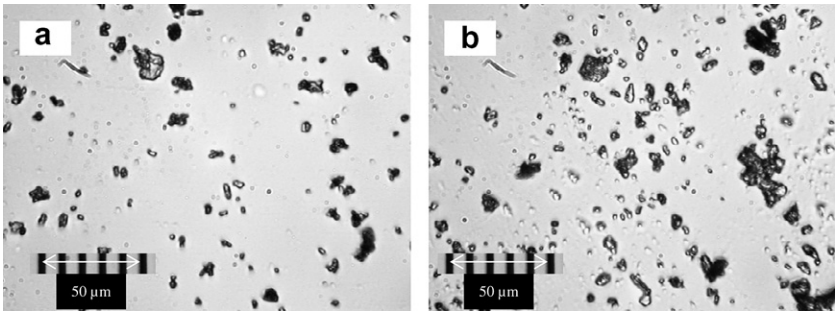

Fig. 6. Lactose crystals observed under microscope (40× magnification) recovered at the end of 1 h (a) and 5 h (b) of crystallization time.

Table 2
Characteristics of crystals recovered from concentrated paneer whey–ethanol system

Samples	Average area (μm^2)	Average diameter (μm)	Standard deviation average diameter (±)	Average elongation	Average roundness
CT[a] 1 h, 0% seed	14.92	4.19	1.26	1.4	0.86
CT[a] 3 h, 0% seed	19.95	4.84	1.67	1.4	0.85
CT[a] 5 h, 0% seed	25.40	5.33	2.33	1.4	0.78
CT[a] 1 h, 1% seed	19.25	4.68	0.61	1.5	0.86
CT[a] 1 h, 3% seed	25.16	5.46	2.4	1.5	0.79

[a] CT – crystallization time.

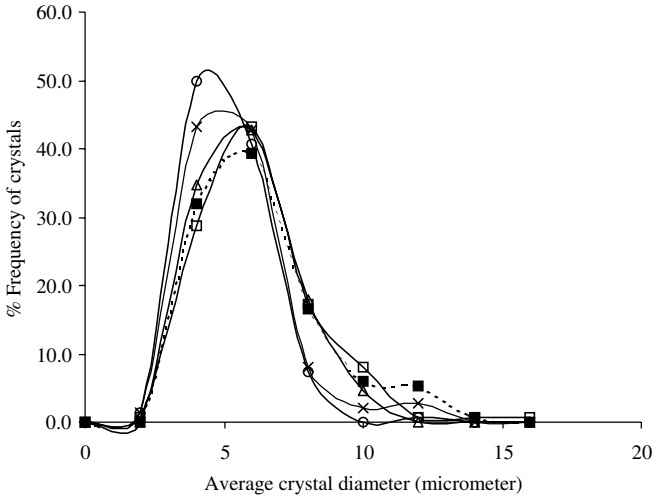

Fig. 7. Crystal size distribution of recovered lactose samples. Crystallization time (CT) – 1 h, seeding (% w/w) (S) – 0 % (○), CT 3 h, S – 0 % (△), CT – 5 h, S – 0 % (□), CT – 1 h, S – 1 % (×), CT - 1 h, S – 3% (■).

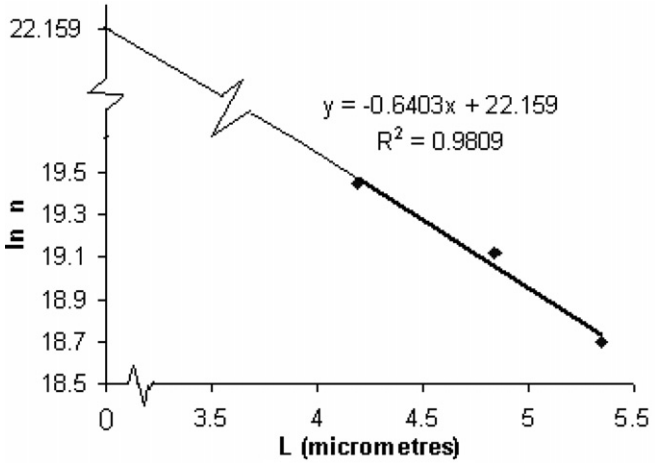

Fig. 8. Calculation of (n_0) and G from lactose crystallization data.

tion time from 5 h to 1 h. However, on seeding, the maxima shifted back to 6 μm, even though the crystallization time was only 1 h. This could be due to the higher crystal size of the seed (analytical grade commercial lactose), making larger initial surface area available for further deposition of the crystallizing lactose.

3.6. Crystallization kinetics

The kinetic rate constants for crystal growth obtained using the data from the lactose crystallization from paneer whey using ethanol (effective concentration 85% v/v) in 1–5 h of crystallization time are shown in Table 3 and Fig. 8. In the case of paneer whey/ethanol system the growth rate (G) obtained was 4.33E−4 μm s^{-1} for 1 h crystallization time and the number of embryo size crystals (n_0) observed were 4.2E+9 crystals mL^{-1}. Whereas in the case of paneer whey/methanol system the G observed was little higher 5.26E−4 μm s^{-1} for 1 h crystallization process and n_0 was much lesser i.e. 2.04 E+9 crystals mL^{-1} (unpublished data). This indicated a possibility of higher rate of nucleation in paneer whey/ethanol system in comparison to paneer whey/methanol system, resulting in faster desupersaturation. This could have resulted in smaller size crystals in paneer whey/ethanol system in comparison to paneer whey/methanol system. In the case of paneer whey/ethanol system the rate constant of growth (k_g) and the exponential order of growth (m) obtained were 0.0123 [μm s^{-1} (g $mL^{-1})^{-m}$] and 0.68 respectively. The value of 'm' obtained for the paneer/ethanol system (0.68) is close to 1, which indicates that lactose crystallization from paneer whey (in the present process conditions) using ethanol as anti-solvent behaves nearly as the first order process. This suggested that the mass transfer and diffusion of lactose molecules from solution to the crystal surface is mainly responsible for the crystal growth in paneer whey/ethanol system whereas the effect of impurities appears to be negligible. The rate constants obtained for the nucleation with respect to desupersaturation are given in Table 4. The rate constant for nucleation 'k_n' and exponential order of nucleation 'm'' were 1.52E+09 [(Number of crystals mL^{-1}) (g $mL^{-1})^{-m'}$] and 0.329, respectively.

Table 4
Kinetic nucleation rate constants

Crystallization time (s)	n (number of crystals recovered mL^{-1})	k_n [Number of crystals mL^{-1}] (g $mL^{-1})^{-m'}$	m'
3600	2.85E+08	1.52E+09	0.329
10800	2.01E+08		
18000	1.32E+08		

4. Conclusions

In the present study, optimization of various parameters for anti-solvent (ethanol) based lactose recovery process for the paneer whey has been carried out. The lactose recovery was found to be directly proportional to the effective ethanol concentration. The 'initial' and 'end-pH'

Table 3
Kinetic growth rate constants

Crystallization time (s)	Average diameter (μm)	$1/G\tau$ (μm)	G (μm s^{-1})	n_0 (number of embryo crystals mL^{-1})	k_g [μm s^{-1} (g $mL^{-1})^{-m}$]	m
3600	4.19	0.6403	4.33E−4	4.2E+9	0.0123	0.68
10800	4.84		1.44E−4			
18000	5.33		8.66E−5			

adjustment reduced the ash content, there by improving the quality of the recovered lactose. The crystal size analysis has thrown some light on the effect of change in the crystallization time and seeding on the size distribution. Similarly, it was observed that the lactose samples recovered under different processing conditions in the present study compared well with the commercial sample of analytical grade lactose in terms of 'crystal habit' expressed as average roundness and average elongation of the crystals. This information can be used to obtain lactose of desired crystal size distribution and crystal habit. The lactose crystal growth rate constants were obtained for ethanol-based lactose recovery process from paneer whey.

From the lactose recoveries obtained (always more than 90%, and purity around 97–99%) in the case of the paneer whey, in just 1 h of crystallization time, it could be concluded that the, anti-solvent (ethanol) based lactose recovery process has an edge over the conventional lactose recovery processes.

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