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Extraction of lactose from paneer whey using ethanol and methanol

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Abstract
In the present study, by-product paneer whey which is usually drained off in small scale dairy industry was tried for economical and rapid extraction of lactose using solvents ethanol and methanol. Effect of solvent concentration (70%, 80% and 90%), effect of initial pH adjustment (4.5, 3.5 and 2.5) at solvent to solute ratio of 90:10 (v/v) and effect of crystallization time on lactose recovery were examined. The lactose recovery of more than 90% was obtained from paneer whey in 5 hr of crystallization time at an effective ethanol concentration of 90% (v/v) whereas recovery of more than 80% was observed in case of methanol used as solvent. Conditions which were more favorable in terms of crystallized lactose yield were at pH of 4.5 than the pH of 3.5 and 2.5 which showed low recovery of lactose. Crystal size distribution (CSD) analysis has been also studied of the lactose recovered which showed that the average diameter of crystals increases on increase in crystallization time. At last residual analysis of recovered lactose suggested that residues of solvents ethanol and methanol were present within permissible limit in the sample thus we can say the recovered lactose does not contain any harmful effect of solvent and is of good quality.

Keywords: paneer whey, lactose, solvents, ethanol, methanol, lactic acid, extraction

Introduction
Whey comprises of around 45-50 percent of total milk solids out of which 70 percent is milk sugar (mainly lactose). Recovering lactose from whey solves the problems of better economics by the utilization of whey and of reducing pollution as lactose recovery itself can reduce Biological Oxygen demand (BOD) of whey by more than80%.

In the food and confectionery industries, lactose is commonly used as a filler or flavor carrier. The principle application of lactose is in the preparation of infant formula where it is added to cow's milk (5% lactose) to boost the lactose content to a level similar to human milk (McSweeney and Fox, 2009) [7]. The purest form of α-lactose has been used by the pharmaceutical industry to manufacture pharmaceutical tablets and capsule. The typical process of recovering lactose from cheese whey includes concentrating whey to 55–65% of total solids (requires high evaporation costs) followed by cooling to yield yellow colored raw lactose. The process is a long and tiresome process with varying crystallization time from 12 to 72 hr (Kapil *et al.* 1991) [5]. The ultra-filtered 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 hr (Singh *et al.* 1991) [9]. However, the ultra- filtration involves substantial capital and recurring costs due to limited ultra-filtration membrane life and higher operating pressures. Therefore, for such processors, the heat induced aggregation of whey protein would provide an economical alternative to ultra- filtration processes, followed by the recovery of lactose from deproteinated whey resulting in a complete and efficient whey management strategy. Alcohols greatly reduce the solubility of the lactose in water thus, they are expected to accelerate crystallization by salting out and more rapid crystallization tend to produce □- stable lactose, while lower super saturation or dilute solution yield mostly □-hydrate (Majd and Nickerson, 1976) [6].

Objectives
1. To utilize paneer whey for economical and rapid extraction of lactose.
2. To standardize various process parameter such as effective solvent concentration, pH of whey concentrate and crystallization time.
3. To study the crystal size distribution (CSD) and residual solvent analysis of recovered lactose.

Materials and Methods
The experiment "Extraction of lactose from paneer whey using ethanol and methanol" was carried out in Department of Food Process Engineering, Vaugh Institute of Agricultural Engineering and Technology (VIAET), Sam Higginbottom University of Agriculture Technology and Sciences (SHUATS), Prayagraj during the session January-June 2019. The details of the experimental techniques that were employed during the course of investigation are as follows.

Procurement and purchasing of raw materials
Paneer whey was obtained from Student's Training Dairy SHUATS, Prayagraj and the chemicals and used were obtained from Department of Food Process Engineering, Vaugh Institute of Agriculture Engineering and Technology, (Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj). The materials used during the

study are as follows
1. Paneer Whey
2. Ethanol
3. Methanol
4. Citric Acid/Lactic Acid
5. Whatman Filter Paper
6. Sodium Tungstate
7. Sodium Carbonate
8. Benedict's reagent
9. Aluminum Foil
10. Bone Black
11. Activated Carbon

Equipment required

An electronic balance was used to weigh amount of lactose recovered, different chemicals for experimental purpose. The maximum limits of this balance were 200 grams. Water-bath was used for deproteination of whey where constant temperature of 90°C is required. Refrigerator was used to store whey sample and to maintain temperature during crystallization. Vacuum dryer was used to dry recovered lactose crystals. Hot plate was used during testing of lactose content in given whey sample. Microscope was used to analyze the crystal size distribution of recovered lactose crystals. Grinder was used to mill the crystal of lactose to make it a fine powder. Sealing machine was used to pack lactose powder in LDPE. Melting point apparatus was used to find out melting point of obtained lactose. A hot air oven was used to determine moisture content in the recovered lactose. pH meter was used to measure pH of obtained whey samples. Additional glassware such as Beaker, Volumetric flask, Funnel, measuring cylinder, Glass rod, Petri plates, Microscope slides, Pipettes, Burette were also used.

Methodology

Pre-treatment of whey

The method followed for pre-treatment of whey was according to Bund *et al*. 2007 [4]. Paneer whey obtained was filtered to remove large particles, casein fines and fat globules from whey through clarification process. Paneer whey was then deproteinated by heating to 90 to 92°C for 15 min at pH 6.6. The presence of protein in whey increases the viscosity of concentrated whey and hinders the lactose crystal separation, and in extreme case even prevents the crystallization. Deproteinated paneer whey will be then concentrated so that resultant concentrated whey contains lactose 19-20%. this can be achieved by heating whey in open pan. This concentrated whey was used for optimizing various conditions for the lactose recovery process using ethanol and methanol.

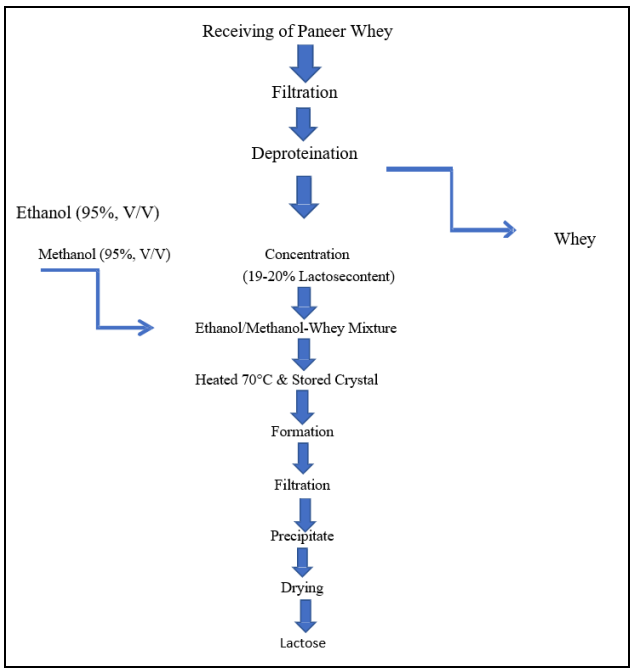

Fig 1: Process flow chart for extraction of lactose

Process of lactose extraction

Sample acquisition and preparation

Paneer whey used in the present study was procured intermittently from a local dairy. Its origin was of buffalo milk. The pH of the whey varied from 4.0 to 5.0. The samples were stored at temperature below 10°C. Residual fat separation was observed after storage at this temperature which was skimmed off by filtration of whey through a muslin cloth. These samples were again stored at a temperature below 10°C till further experiments.

Clarification of whey

The method followed for clarification of whey was according to Bund *et al*. 2005 [2].

Clarification was necessary to remove fat, suspended curd particles and other impurities (dust) from whey. Filtration was done at 55°C and muslin cloth was used to remove large particles, casein fines, and fat globules from whey.

Deproteination of whey

The method followed for deproteination of whey was according to Bund *et al*. 2005 [2]**.** Whey was deproteinated by pre-optimized process of heat-induced aggregation of whey proteins, at pH of 4.5, temperature of 92 ± 2°C, in treatment time of 60 min maintained in a constant temperature bath followed by filtration to remove protein.

The protein free lactose solution is ideal for recovery of lactose.

Concentration of whey

The method followed for concentration of whey was according to Bund *et al*. 2007 [4]. Paneer whey after separation of protein was concentrated to a lactose content of 19-20%. This was performed by a pre-concentration by evaporation process. The supernatant from deproteination step was concentrated at temperature of more than 95 ± 3°C in a heated open pan. This concentrated whey was used for optimizing various conditions for the lactose recovery process using a solvents ethanol and methanol to precipitate the lactose.

Procedure for using ethanol & methanol for lactose crystallization

The method followed for lactose crystallization using ethanol and methanol was according to Bund *et al*. 2007. [4] Concentrated whey of known volume and known lactose content, adjusted to a desired pH (4.5–2.5) was taken in the beaker; it was kept in a constant temperature. The ethanol (95% v/v)/ methanol (95% v/v), at ambient temperature was added in an appropriate quantity at once to whey (with/without stirring), to achieve the effective concentration of ethanol/methanol of 70–90% (v/v) in the system. The crystallization process has been carried out in normal temperature.

Crystallization of lactose (Crystal formation)

The method followed for crystallization of lactose was according to Majd and Nickerson (1976) [6]. The effective concentration of ethanol/methanol-whey mixture of 70–90% (v/v) was kept in constant temperature. The samples were stirred sufficient enough to keep the contents inside the beaker, in suspension for 5 h, with/without subsequent standing time of 12 h. The precipitate (recovered lactose) obtained (white in color), was filtered using filter paper. It was vacuum dried in an oven at 60°C for 4 h, and then weighed. The purpose of crystallization was to secure the formation of crystals that can be separated from the mother liquor.

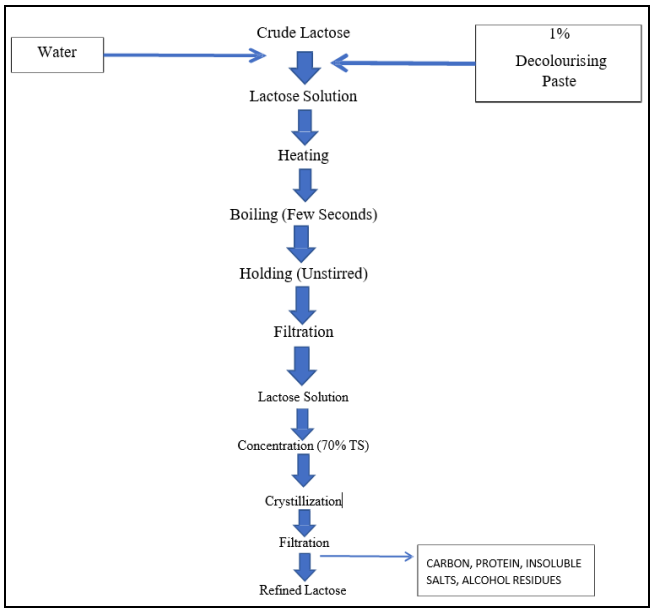

Fig 2: Process flow chart for refining of lactose

Refining process of crude lactose

The method followed for refining of lactose was according to Kumar, V. (2013). *By product technology, ICAR eBook.* Lactose was refined for high level of purity. Lactose was dissolved in hot water to a 50-60% concentration. About 1% of decolorizing paste consisting of 3 parts bone black and 1 part activated carbon was added. Quick dissolution is thought to require heating to a temperature of 105ºC. The crystals were cooled to approximately 20°C in about 6 h. After cooling, crystals were separated and dried. In the refining process, decolorizing carbon was used to adsorb color and probably removes other impurities to some degree.. Lime was used to adjust the reaction to that most favorable for the precipitation of protein and probably aids by combining with the protein to some extent. The carbon and the precipitated impurities were removed by filtration. The process of producing up to food grade lactose is by re-dissolving the crude lactose in clean water and to removing the impurities by a combination of adsorption and filtration processes, followed byre-crystallizing.

Parameters investigated

Recovery percentage (Yield)

The yield was calculated using % recovery formula as given in equation 1. Yield refers to the ratio of the mass of lactose crystal recovered to the initial mass of lactose in whey concentrate multiplied by hundred. Recovery percentage is given by

$$\% \text{ recovery} = \text{Mass of lactose crystals} \times \frac{100}{\text{Initial mass of lactose in whey concentrate}} \quad (1)$$

Effect of the 'Effective alcohol concentration'

The ethanol and methanol was added at once to cooled, concentrated whey samples (pH 4.5), 15 ml each, to achieve the effective ethanol and methanol concentration of 70%, 80% and 90% (v/v). All samples were stirred and kept undisturbed for 7-12 hrs without agitation for crystal formation. The precipitate (recovered lactose) was separated using the filter paper followed by drying in vacuum oven at 60°C for 4 h. The recovered and dried lactose was weighed to get the % recovery of lactose.

Effect of 'initial pH adjustment' (*i.e.* pH of whey before crystallization starts)

To see the effect of 'initial-pH adjustment' 15 ml each of the concentrated whey was taken in three beakers. In the beaker 1, the pH was kept as it is (pH 4.5) whereas, in beaker 2 and beaker 3 the pH of the whey was adjusted to 3.5 and 2.5 respectively using lactic acid. The lactose was recovered as described in Section 3.3.4 at an effective ethanol and methanol concentration of 90% (v/v). The precipitate (recovered lactose) was separated using the filter paper followed by drying in vacuum oven at 60°C for 4 h. The recovered and dried lactose was weighed to assess the % recovery of lactose.

Effect of crystallization time

The lactose recovery was accomplished from concentrated whey using ethanol at effective concentration of 90% (v/v) for different time intervals of stirring (1–7 hrs) keeping rest of the crystallization conditions same as described in Section 3.3.4. The lactose was recovered as described in Section 2.3.2 at an effective ethanol and methanol concentration of 90% (v/v). The precipitate (recovered lactose) was separated using the filter paper followed by

drying in vacuum oven at 60°C for 4 h. The recovered and dried lactose was weighed to estimate the % recovery of lactose.

Analytical Methods

Estimation of lactose content

Lactose content was determined by Benedict's method given by Benedict *et al*. 1909. Procedure: Whey sample of 5 ml was taken into a volumetric flask (50 ml). 2.5 ml of 10% sodium Tungstate was added drop by drop with continuous mixing to make the volume 50 ml with distilled water. Mixture was left in the flask for 10 minutes and then filtered. After that, filtrate was transferred into burette. In another beaker, 25 ml of Benedict's reagent with 30 ml of distilled water and 2 gm of anhydrous sodium carbonate was kept. Mixture was then mixed and heated till the solution became clear, it was boiled and titration was started, first rapidly by 2 ml till first shade of reduction was obtained. Then proceeded with titration drop by drop till complete reduction of blue color was obtained. Disappearance of blue color and appearance of reddish brown color (of cuprous thiocynate) was observed. Finally, the volume of filtrate exhausted in the titration ® was recorded.

Calculation

Every 25 ml of Benedict's solution is reduced by 0.0678 gm of lactose.

Melting point

Melting point of recovered lactose were determined using melting point apparatus.

Procedure

Capillary tube was filled with crystals about 3 mm high. Capillary tube (open end down) was placed into the crystals and pressed it on the bottom of the crystallization dish to get the crystals into the tube. Crystals were made to slide to the bottom of the tube. Capillary tube was then placed in the MEL-TEMP melting point apparatus. MEL-TEMP was set at a high enough level to make a rapid determination of melting point. Melting process was observed though the magnifying lens. Finally, melting point of recovered lactose was observed and recorded.

Crystal size analysis

Recovered lactose crystals were observed under microscope (40 × magnifications) the images were captured using camera and analyzed for crystal size and its distribution using Image J (software for image analysis and processing). Average projected area and diameter of the recovered lacrosse crystal were examined.

Residual solvent analysis (Ethanol &methanol)

Residual solvent analysis for solvent methanol and ethanol were performed at Sigma test and research centre, New Delhi. Gas chromatography method was used for residual solvent analysis.

Procedure

For the determination of class II and class III residual solvents in drug substance a generic static headspace gas chromatography method was used. A sample was placed in a vial and heated. Then a single aliquot of gas was collected over sample and transferred to gas chromatography. A gas sample was collected after the equilibration between gas and liquid (or solid) phase was reached. It was a major tool for analysis of volatile organic compounds in environment, flavors and fragrance analysis for decades.

Table 1: Gas chromatography conditions for analysis of Class II (Methanol) and Class III (Ethanol) solvents

Instrument used	Agilent 6890A GC equipped with an FID and a 7694 HS sampler
Column	Agilent DB-624 (6%cyanopropylphenyl & 94% dimethylpolysiloxane) fused silica capillary column, (30m × 0.32mm×1.8µm)
Column oven Temperature	35ºC (Hold 0-3 min), raised to 110ºC at 4ºC/min, (Hold 3-21.75 min),
program	raised to 240ºC at 40ºC/min (Hold 21.75-25 min), at 240ºC (Hold at 25-30 min)
Inlet temperature	2000C
Carrier gas	Helium

Statistical analysis

The method followed for statistical analysis was according to Fisher and Yates (1969). The data recording during the course of investigation were subjected to statistical analysis by "Analysis of variance" technique for drawing conclusion. The significant and non-significant treatment affect was judge with help of F (variance ratio) table. The profound difference between the means was tested against the critical difference at a level of 5%. For examining the hypothesis proposed, the following ANOVA table was used. The skeleton of ANOVA is furnished in the given table 2.

Table 2: Skeleton of ANOVA

Source of variation	d.f.	S.S.	M.S.S.	F-Cal	F-Tab
Due to Treatment	r-1	RSS	RSS/(r-1)	MSSR/MS	SE
Due to Replication	t-1	TSS	TSS/(t-1)	MSST/MS	SE
Due to error	(r-1) (t-1)	ESS	ESS/(r-1)(t-1)		
Total	rt-1	TSS			

S.E. =Standard Errorr = Replication t =Treatment
d.f. = Degree of freedom F-Cal = Calculated F-value S.S. = Sum of Squares
F-Tab =Tabulated F-value M.S.S. = Mean sum of Squares Ve = Error mean Square
S.S.T. = Sum of squares due to treatment

Results and discussion

The present investigation entitled "Extraction of lactose from paneer whey using ethanol and methanol" was carried out in the Department of Food Process Engineering, viaet,

shuats, Prayagraj during the session 2018-2019.
The anti-solvent used in the present study is 'ethanol and methanol', due to their inert nature and minimal solubility of lactose, at all concentrations (in comparison to other alcohols like propanol). The effective ethanol and methanol concentrations (70%, 80% and 90% v/v), selected in the present study were based on the reported solubility behavior of the lactose in alcohol (methanol), that showed a sharp decrease in the solubility, from 90% to 10%, whereas the methanol (lactose solubility is larger in methanol than ethanol) concentration increased from 70% to 90%, respectively. The results obtained during the course of investigation are presented and discussed in this chapter under appropriate subheadings:

Extraction of lactose

Lactose was extracted by adopting solvent extraction method reported by Bund and Pandit (2007) [3]. Lactose was extracted using paneer whey with different levels of ethanol and methanol. Experiments were conducted to study the effect of different concentration of ethanol and methanol, pH of whey concentrate and crystallization time. The dried lactose, obtained after crystallization was called 'recovered lactose'. The percentage recovery of lactose was calculated on the basis of lactose content (in g) of the concentrated whey sample, before crystallization. The recovered lactose was checked for yield, melting point, crystal size analysis and residual solvent were conducted for recovered lactose sample.

Fig 3: Ethanol treated paneer whey for extraction of lactose. (a) Before crystallization and (b) Final Product

Fig 4: pH adjusted paneer whey treated with solvents for extraction of lactose. (a) Before crystallization and (b) Final Product

Fig 5: Paneer whey treated with methanol for extraction of lactose. (a) Before crystallization and (b) Final product

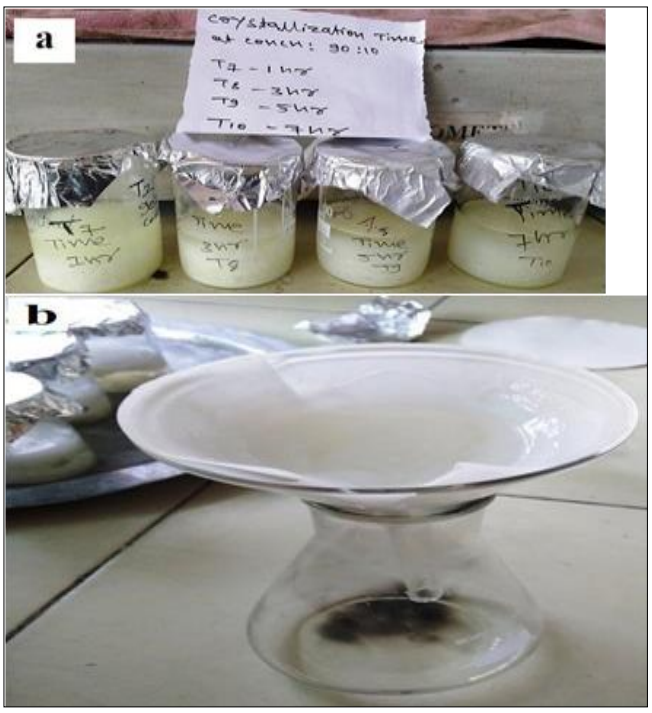

Fig 6: (a) Paneer whey treated with solvents to study the effect of crystallization time on lactose recovery and (b) Filtration of lactose using Whatman filter paper.

Effect of 'effective alcohol concentration' on lactose recovery

Lactose recovery of 71.36%, 80.58% and 91.17% was obtained from ethanol-whey mixture having effective ethanol concentration of 70%, 80% and 90% (v/v), respectively whereas lactose recovery of 62.74%, 70.58% and 82% was obtained from ethanol-whey mixture having effective methanol concentration of 70%, 80% and 90% (v/v), respectively as shown in Table 3 and Fig. 7. The lactose recovery was directly proportional to the final effective alcohol concentration in the system.

The increase in the recovery due to an increase in the effective alcohol concentration might be due to the rapid super saturation resulting in lactose precipitation. The result was found to be in agreement with results of Bund and Pandit (2007) [3].

They observed lactose recovery of 84.76%, 89.5% and 92.63% at effective concentration of 65%, 75% and 85%.

Table 3: Effect of solvents (ethanol and methanol) on lactose recovery

Treatment	Solvent Concentration (%)	Whey (%)	% recovery (Ethanol)	% Recovery (Methanol)
T1	70	30	91.17	82
T2	80	20	80.58	70.58
T3	90	10	71.36	62.74

Table 4: Effect of pH of whey concentrate on yield of lactose

Treatment	Solvent Concentration (%)	pH of Whey Concentrate (%)	% Recovery (Ethanol)	% Recovery (Methanol)
T4	90	4.5	90.29	80
T5	90	3.5	67.64	64.70
T6	90	2.5	47.05	41.17

Effect of the initial pH adjustment on lactose recovery

Lactose recovery of 90.29%, 67.64% and 47.05% was obtained at effective ethanol concentration of 90% (v/v) in ethanol-whey mixture, at pH range of 4.5, 3.5, and 2.5 respectively whereas lactose recovery of 80%, 64.70% and 41.17% was obtained at effective methanol concentration of 90% (v/v) in ethanol-whey mixture, at pH range of 4.5, 3.5, and respectively as shown in Table 4. and Fig. 8. At pH of 4.5 in 15–60 min the rapid precipitation in the form of hard or rigid crystals of lactose could be observed but at pH of 3.5 and 2.5 the crystallization process tends to be slow and tedious which results small size crystal formation. This was in agreement with results of Twieg and Nickerson (1968) [10] they found that low pH decrease crystallization rate due to decreased mutarotation.

Thus it was thought that the bulk of the lactose recovery took place in the initial 1–2 h of total standing time. Eliminating 'standing time step' (12 h) from the crystallization process did not affect the lactose recovery.

Effect of crystallization time on recovery of lactose

The visual inspection of crystallization process recommended that significant lactose recovery took place in early hours of crystallization. Thus for further optimization, the crystallization time for lactose recovery, was further reduced from 5 hr to 1 hr. In case of solvent ethanol the lactose recovery of 52.94%, 73.52% and 91.76% was observed for the crystallization time of 1, 3 and 5 hr, respectively as shown in Table 5 and Fig. 9. This indicated that around 50% of overall lactose recovery took place in first 1 hr itself, which was also confirmed with the visual observation of turbidity whereas in case of anti-solvent methanol the lactose recovery of 44%, 61.76%, 73.52% and 81.76% was observed for the crystallization time of 1, 3, 5 and 7 hr, respectively as shown in Table 5. and Fig. 9. The results are found to be in agreement with Bund and Pandit (2007) [3] they observed lactose recovery of 76%, 90.93% and 95.92% for crystallization time of 1, 3 and 5 hr respectively.

Table 5: Effect of crystallization time on yield of lactose

Treatment	Solvent Concentration (%)	Crystallization Time (hr)	% Recovery (Ethanol)	% Recovery (Methanol
T7	90	1	52.94	38.23
T8	90	3	73.52	61.76
T9	90	5	91.76	73.52
T10	90	7	92.8	81.76

Melting point

Melting point (mp) is an important parameter, which can be used to distinguish between the □-lactose monohydrate (mp 201–202°C) and □-lactose (253°C) The melting point of the analytical grade lactose monohydrate procured from the market was observed to be 202°C (Elvers *et al*. 1990). The melting point of lactose samples recovered using ethanol were 207°C, 205°C & 199°C whereas the melting point of lactose recovered using methanol were 205°C, 204°C and 201°C at pH of 2.5, 3.5 and 4.5, respectively as shown in figure 4.8. The melting point of the lactose recovered (pH 2.5) was 207°C much closer to analytical grade lactose than that of the recovered from whey at pH 4.5 (mp– 199°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 □-lactose monohydrate.

Table 6: Effect of pH on melting point of lactose

Solvent Concentration (%)	pH of whey Concentrate (%)	Melting Point (Ethanol)	Melting Point (Methanol)
90	4.5	199	201
90	3.5	205	204
90	2.5	207	205

Crystal size analysis

Photographs of lactose crystals observed under microscope (40 × magnifications) recovered at the end of 1 h and 5 h of crystallization time are shown in Fig. 4.9. The analysis of the crystal size of recovered lactose samples was performed using image J software. The crystal size analysis of various samples of recovered lactose showed that the average projected area (μm², as estimated by the image analyzer) of crystals enlarged from 14.37 μm² to 23.48 μm² as the crystallization time increased from 1 h to 5 h (Table 7.). The diameter of individual crystal is the average of the diameters measured at equal intervals around the centroid of the object. The average diameter of each lactose sample was determined from the individual diameter of the lactose crystals examined. The 'average diameter' of 4.27, 4.91 and 5.47 μm was observed for the lactose samples recovered in 1 h, 3 h, and 5 h of crystallization respectively. The 'average diameter' decrement on dip in crystallization time indicated less crystal growth. Similarly, the 'average diameter' of the recovered lactose was much smaller than that observed in analytical grade commercial lactose sample (15.4 μm). Bund and Pandit (2007) [3] also found that the 'average diameter' of crystals decrease on decrease in crystallization time which indicates lower crystal growth. The smaller

crystal size could be due to the rapid process of crystallization employed in the present case with an agent.

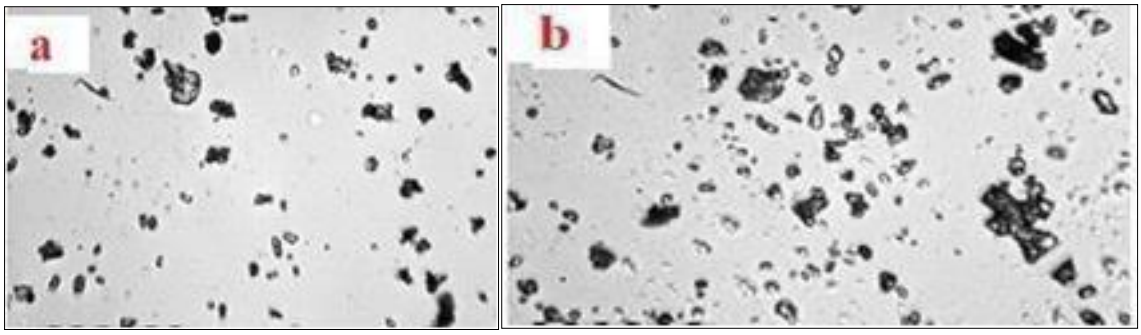

Fig 7: Lactose crystals observed under microscope (40× magnification) recovered at the end of 1 h (a) and 5 h (b) of crystallization time.

Table 7: Characteristics of crystals recovered from concentrated paneer whey

Samples	Average area (µm2)	Average Diameter (µm)
1 hr	14.37	4.27
3hr	18.87	4.91
5hr	23.48	5.47

Residual solvent analysis for ethanol and methanol

Residual solvent analysis for ethanol and methanol were done to check if recovered lactose samples contain any residues of ethanol and methanol. Since lactose is extracted from alcohol extraction process using ethanol and methanol residual solvent analysis of finished products is necessary for a number of reasons. High levels of residual organic solvents correspond a risk to human health due to their toxicity. Residual organic solvents also play a role in the physicochemical properties of the bulk substance. Residual organic solvents can create odour problems and color changes in the finished product and, thus, can lead to consumer complaints. Residual solvent analysis to detect residues of solvents methanol and ethanol in recovered lactose were performed at Sigma test and research centre, New Delhi. Residual solvent analysis test was performed by Headspace gas chromatography (HSGC) method and the results showed that residues of ethanol and methanol was present within permissible limit in the lactose samples extracted from paneer whey using ethanol and methanol as shown in table 4, Fig 8 and Fig 9.

Table 8: Effect of solvents on the quality of recovered lactose

Sample	Test Parameter	Result	Test method
Lactose Powder	1. Methanol (ppm)	ND (DL=1ppm)	GC-HS
	2. Ethanol (ppm)	Permissible limit (DL=1ppm)	GC-HS

Note: ND= Not Detected, DL= Detection Limit

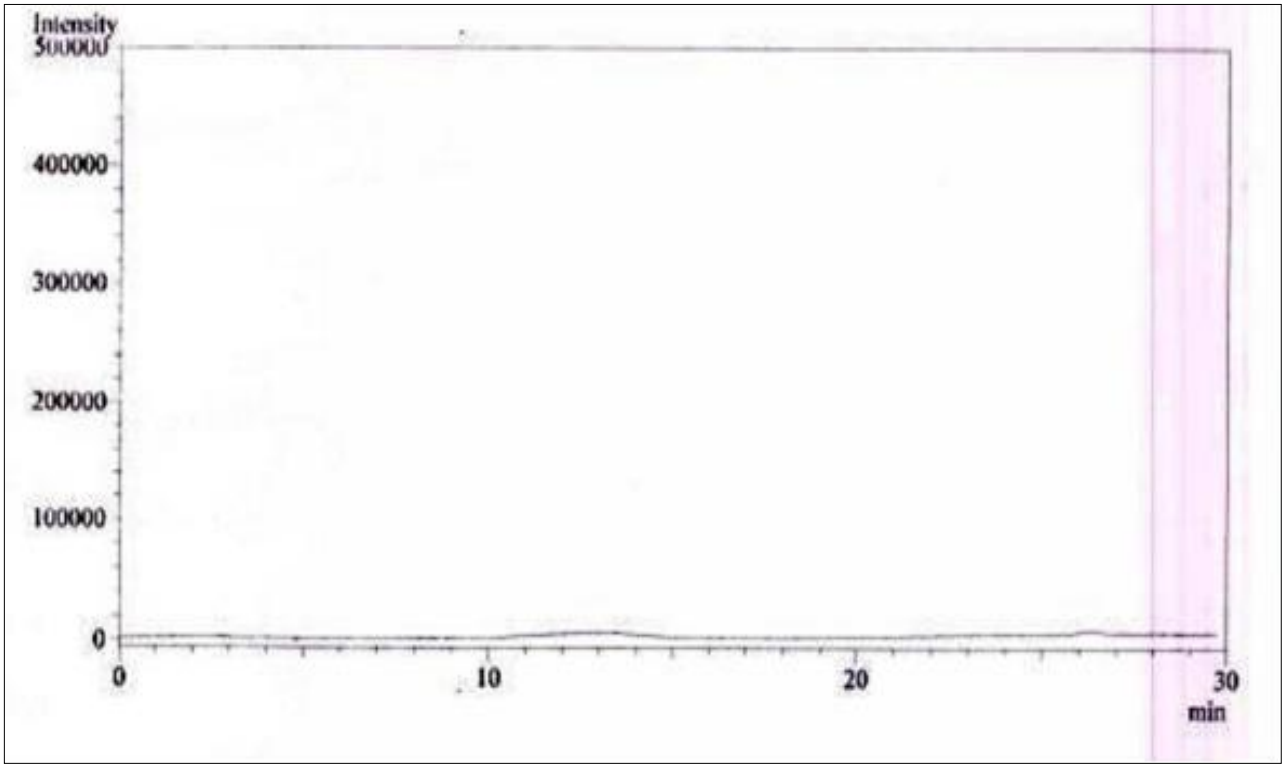

Fig 8: Chromatograph for detection of methanol in recovered lactose sample

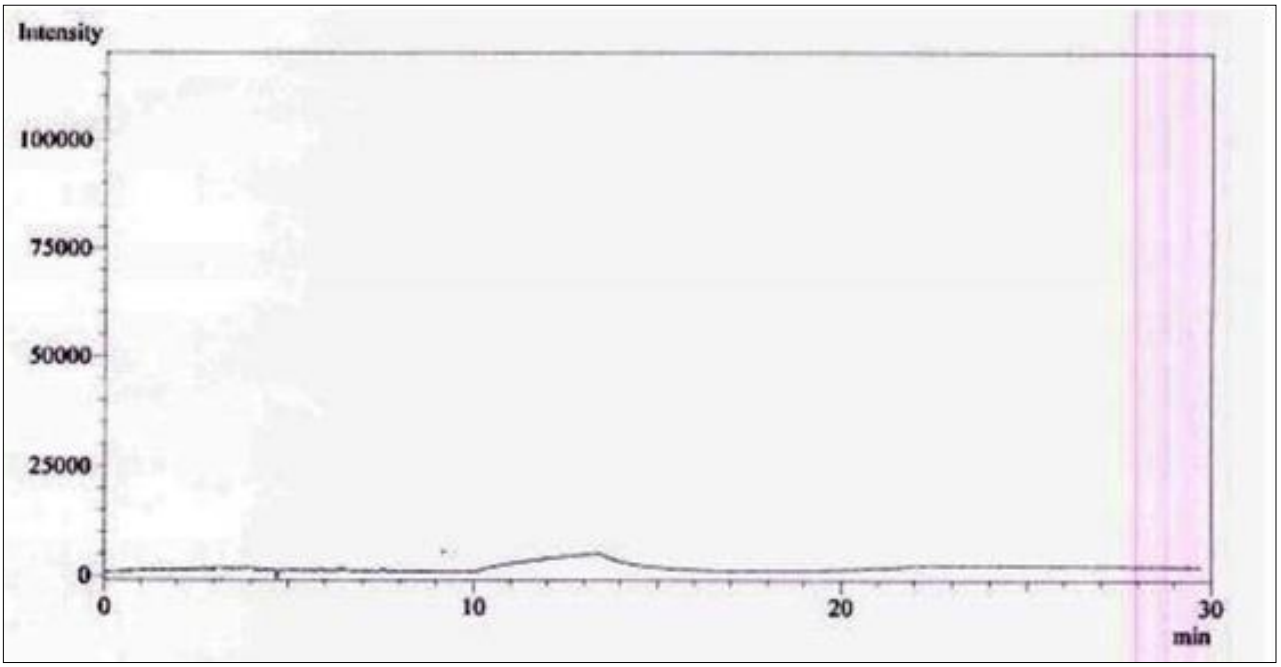

Fig 9: Chromatograph for detection of ethanol in recovered lactose sample

Summary
Extraction of lactose was introduced from homogeneous liquid solution (paneer whey), using a liquid phase (ethanol and methanol) which is insoluble with the lactose solution. Insoluble solvents (ethanol and methanol) are used in different ratios to optimize establishment of most feasible and economical process for lactose extraction. The present research that had been done in Department of Food Process Engineering, Vaugh Institute of Agricultural Engineering and Technology, Sam Higginbottom University of Agriculture Technology and Sciences, therefore, was directed towards investigating recovery of lactose under varied range of concentration, pH, and crystallization time. Further objective was to study and conclude that the recovered lactose is of food grade and does not contain any residues of ethanol and Methanol.

The result has been summarized below

1. Lactose recovery of 71.36%, 80.58% and 91.17% was obtained from ethanol-whey mixture having ethanol concentration of 70%, 80% and 90% (v/v), respectively.
2. Lactose recovery of 62.74%, 70.58% and 82% was obtained from ethanol-whey mixture having methanol concentration of 70%, 80% and 90% (v/v), respectively.
3. The lactose recovery was found to be directly proportional to the effective alcohol concentration.
4. The increase in the recovery due to an increase in the effective alcohol concentration could be attributed to the attainment of rapid super saturation resulting in a precipitation of lactose.
5. Less recovery of lactose was observed when methanol was used for extraction process this is because lactose solubility is larger in methanol when compared with ethanol.
6. Lactose recovery of 90.29%, 67.64% and 47.05% was obtained at pH range of 4.5, 3.5, and respectively at effective ethanol concentration of 90% (v/v).
7. Lactose recovery of 80%, 64.70% and 41.17% was obtained at pH range of 4.5,3.5, and 2.5 respectively at effective methanol concentration of 90% (v/v).
8. Rapid precipitation in form of hard or rigid crystal of lactose could be observed at pH 4.5 but at pH 3.5 and 2.5 the crystallization process tends to be slow and tedious which results small size crystal formation.
9. In case of anti-solvent ethanol the lactose recovery of 52.94%, 73.52% and 91.76% was observed for the crystallization time of 1, 3 and 5 hr, respectively in case of anti-solvent methanol the lactose recovery of 44%, 61.76%, 73.52% and 81.76% was observed for the crystallization time of 1, 3, 5 and 7 hr, respectively.
10. The visual inspection of crystallization process suggested that, substantial lactose recovery, took place in early hours of crystallization. This indicated that around 50% of overall lactose recovery took place in first 1 hr itself.
11. The crystal size analysis of various recovered lactose samples showed that the average projected area (μm^2) of crystals increased from 14.37 μm^2 to 23.48 μm^2 as the crystallization time increased from 1 h to 5h.
12. The 'average diameter' of 4.27, 4.91 and 5.47 μm was observed for the lactose samples recovered in 1 h, 3 h, and 5 h of crystallization respectively. The 'average diameter' increases on increase in crystallization time.
13. Residual solvent analysis of recovered lactose samples showed that no residues of ethanol and methanol were present within permissible limit in the samples.

Conclusion
In the present study, optimization of various parameters for anti-solvent (ethanol and methanol) based lactose recovery process for the paneer whey has been carried out. It can be concluded that ethanol extraction process has given higher amount of lactose than methanol extraction process. The lactose recovery was found to be directly proportional to the effective alcohol concentration. At pH 4.5 rapid precipitation of lactose was observed in compared to pH of 3.5 and 2.5 which shows slow crystallization of lactose as a result small size crystal are formed. Longer crystallization time was observed in case of anti-solvent methanol which shows recovery of 81.76% in 7 hr in compared to ethanol

which showed recovery of 91.76% in 5 hr. The crystal size analysis of the recovered lactose samples demonstrated that the average projected area and diameter of the crystal increased on increase in crystallization time. The lactose extracted from this process was of high quality and contained residues of ethanol and methanol within permissible limit.

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