Behaviour of lactose with the presence of lactic acid and Ca as affected by pH

Jayani Chandrapala[1,2]*, Claire Gauthier[1] and Todor Vasiljevic[1]

[1] Advanced Food Systems Research Unit, College of Health and Biomedicine, Victoria University, Werribee campus, VIC 3030, Australia
[2] School of Science, RMIT University, Bundoora, Victoria 3083, Australia

Contradictory statements about the effects of pH change on crystallisation behaviour of lactose exist in the literature. Considering the importance of addressing the processability issue of acid whey, a systematic study is required to establish lactose crystallisation behaviour in the presence of LA and Ca at concentrations present in real acid whey waste streams emphasising impact of pH. Structural modifications of lactose were evident at elevated, more neutral pH in the presence of 1% w/w LA and 0·12% w/w Ca. These structural changes led to changes in the anomeric equilibrium of lactose, which manipulated the water–lactose behaviour and increased the crystallinity. Therefore, altering pH to 6·5 may be the solution to proper industrial processing of acid whey, enhancing the ability of lactose to crystallise properly.

Keywords: Lactose, pH, acid whey.

In general, two whey waste streams originate from dairy processing with notable compositional differences in terms of lactose, minerals, proteins and lactic acid as well as pH levels. Sweet whey, a by-product of the rennet type cheese making, has a pH of 6–6·5, while acid whey, originating from the production of acid coagulated dairy products such as soft cheese and Greek yogurt, has a pH of 4·2–4·5 and contains higher levels of lactose and minerals, lower levels of proteins and, more importantly, more lactic acid (Fischer & Kleinschmidt, 2015). The sweet whey stream is mainly processed downstream by concentrating and spray drying to obtain a whey powder that can be used as a food ingredient in many food products (de Wit, 2001). However, this process has not been applied successfully for acid whey processing due to failure of lactose to crystallise properly which in turns creates problems downstream such as lumping and caking of whey powders.

Proper crystallisation of lactose depends on a number of factors including degree of supersaturation, solution temperature, viscosity, concentration of lactose, presence of minerals and organic acids, and pH (Herrington, 1934). The presence of minerals in lactose solutions may improve or reduce the crystallisation rate depending on their concentration and type (Jelen & Coulter, 1973). Furthermore, the presence of lactic acid (LA) and Ca directly affects the behaviour of lactose, hindering the removal of water surrounding lactose molecules by inducing a formation of a strong hydration layer and thereby inhibiting the overall crystallisation (Wijayasinghe et al. 2015). Furthermore, pH was found to be an important factor in lactose crystallisation due to acceleration of lactose mutarotation under high alkaline conditions (Herrington, 1934). In contrast, Jelen & Coulter (1973) found that changes in pH have minor contributions in the overall lactose crystallisation process and Bhargava & Jelen (1996) found that the changes in pH did not correlate with changes in growth rates of lactose crystals. Due to these contradictory statements, it was vital to establish the effects of the presence of LA and Ca on the kinetics of lactose crystallisation as affected by pH. Thus, a greater understanding of this phenomenon may assist in enabling efficient extraction of lactose from the acid whey waste stream and thus improve its valorisation.

Materials and methods

Materials

Commercial food grade lactose was obtained from Murray Goulburn Co-operative Co. Ltd (Brunswick, Australia). An analytical grade 85% w/w lactic acid (LA) solution and calcium chloride ($CaCl_2$) were obtained from Sigma – Aldrich Pty. Ltd. (Castle Hill, NSW, Australia).

*For correspondence; e-mail: Jayani.Chandrapala@rmit.edu.au

Preparation of solutions

Lactose powder was dissolved in Milli-Q water at 55 °C to obtain a clear 45% w/w lactose solution. Four solutions were made up with necessary amounts of LA and Ca with the use of the LA solution and a 2·4% w/w stock CaCl$_2$ solution. The first solution was prepared with the addition of 0·05% w/w LA plus 0·035% w/w Ca to resemble the sweet whey waste stream. Second, third and fourth solutions were prepared with the addition of 1% w/w LA and 0·12% w/w Ca having pH values of 4·8, 3·0 and 6·5, respectively. The pH adjustments were carried out using 0·1 M HCl and NaOH as necessary. Pure lactose solution was treated as the control.

Concentration

Concentration of the lactose solutions was performed using a rotary evaporator to achieve ∼50 ± 5% w/w total solids. A laboratory refractometer (Atago abbe, Tokyo, Japan) was used for concentration determinations.

Crystallisation of lactose

Concentrated solutions were subjected to crystallisation using the method adapted by Chandrapala et al. (2016). The next day, lactose crystals were separated using 0·45 μm filter papers and were oven dried at 80 °C for 2 h.

$$\% \text{ Yield} = \frac{\text{Mass of lactose crystal obtained} \; ((g/100 \, g)\text{water})}{\text{Maximum theoratical crystal yield} \; ((g/100 \, g)\text{water})} \times 100$$

Freeze-drying of lactose solutions

All samples were frozen at −20 °C for 24 h and then freeze-dried (Dynavac FD 300, Dynavac Engineering Pty, Australia) for 72 h.

Differential scanning calorimetry (DSC)

Method by Wijayasinghe et al. (2015) was adapted to measure the thermal characteristics of concentrated, freeze dried and lactose crystals using the DSC machine (Mettler Toledo Schwerzenbach, Switzerland).

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of concentrated solutions and lactose crystals were obtained in the range of 600–4000 cm^{-1} using Fourier transform infrared spectroscopy as described by Wijayasinghe et al. (2015).

Statistical analysis

The whole experimental design was replicated two times. When necessary, one-way/two-way ANOVA with a 95% confidence interval was used. The ANOVA data with $P < 0.05$ were considered statistically significant.

Results and discussion

The pure lactose sample resulted in ∼84% yield of lactose crystals (Table 1). In contrast, this yield was significantly reduced to ∼55% in the presence of 1% w/w LA and 0·12% w/w Ca. This was concomitantly followed with the rise of the crystallisation temperature (T_{cr}), up from ∼166 °C, which was determined for the pure lactose solution, to 189 °C in the presence of high concentrations of LA and Ca (Table 1) indicating a delay in crystallisation. In addition, a substantial decline in the enthalpy associated with the loss of water (∼163 J/g) in the same sample as compared to that of pure lactose (∼1110 J/g) was observed (Table 1). Highly hygroscopic LA strongly interacts with water molecules, leading to a formation of a strong hydration layer consisting lactic acid and H$_3$O$^+$ ions around lactose molecules *via* strong H bonds (Wijayasinghe et al. 2015). In addition, Ca^{2+} has the ability to organise the structure of water (Bhargava & Jelen, 1996). Divalent Ca^{2+} ion has a strong electric field which acts as a water structure promoter (Von Hippel & Schleich, 1969). These structural changes of water molecules can directly affect the solubility of lactose and thereby the supersaturation of the lactose solution and thus lead to different behaviour during crystallisation (Mullin, 1979). Furthermore, Ca^{2+} ions strongly interact with four to six layers of water molecules *via* ion-dipole interactions (Reid & Fennema, 2008), which again further restricts the mobility of water molecules. Such an impact on the water mobility was confirmed by decreased intensity of the H bonding region between different hydration states (2800–3500 cm^{-1}) (Raut et al. 2011). In addition, bending vibrations of OH groups of water denoted by a peak at ∼1630 cm^{-1} (Nikanishi, 1962), frequently observed for the pure lactose (Fig. 1), was accompanied with an appearance of another peak at ∼1570 cm^{-1} in the presence of 1% w/w LA and 0·12% w/w Ca which additionally indicated different water behaviour governed by these two solutes.

Reducing the concentration of LA from 1% w/w to 0·05% w/w and Ca from 0·12 w/w to 0·035% w/w decreased the yield of lactose crystals slightly (∼76%) along with a modest decline in the enthalpy associated with the loss of water (∼992 J/g) (Table 1). The diminished strength of the hydration layer consisting of H$_3$O$^+$ and lactic acid minimised the formation of lactic acid-water complexes, decreased the impact of Ca on the solubility of lactose and enhanced attractions between individual lactose molecules and thus consequently improved water mobility, which all may have sequentially resulted in an improved yield. Similarly, FTIR spectra were characterised by the peaks identical to those of the pure lactose.

When pH reduced down to 3, the yield of lactose crystals was reduced slightly (52%) in the presence of 1% w/w LA and 0·12% w/w Ca compared to the same system (55%) without pH adjustment, which had pH of 4·81. At this low pH and in the presence of high concentrations of LA and Ca, lactose was characterised with the lowest dehydration enthalpy (∼151 J/g), indicating retention of an elevated

Table 1. pH, crystal yield, onset, peak and end set temperatures and enthalpy associated with loss of water in concentrated lactose solutions and the crystallisation temperatures of freeze dried lactose samples as affected by the presence of LA and Ca and pH

Samples	pH	Yield (%)	Onset loss of water (°C)	Peak loss of water (°C)	Endset loss of water (°C)	Dehydration Enthalpy (J/g⁻¹)	Crystallisation temperature (°C)
Pure lactose	6·63 ± 0·03	84·2 ± 2·1	139·2 ± 1·2	148·7 ± 1·1	155·1 ± 0·9	1109·9 ± 5·8	165·7 ± 1·1
Lactose + 0·05% LA + 0·035% Ca	4·99 ± 0·02	76·4 ± 1·8	134·8 ± 1·5	138·1 ± 0·9	142·5 ± 0·5	992·3 ± 6·2	170·7 ± 1·0
Lactose + 1% LA + 0·12% Ca	4·81 ± 0·03	55·1 ± 1·6	131·4 ± 1·3	144·8 ± 0·6	153·5 ± 0·8	163·0 ± 3·6	189·3 ± 0·8
Lactose + 1% LA + 0·12% Ca – pH 3	2·95 ± 0·03	52·0 ± 1·4	126·9 ± 0·9	129·3 ± 0·5	143·6 ± 1·1	151·4 ± 2·9	179·5 ± 0·4
Lactose + 1% LA + 0·12% Ca – pH 6·5	6·47 ± 0·02	70·8 ± 1·9	146·7 ± 0·7	143·4 ± 1·2	147·2 ± 1·2	667·5 ± 4·7	166·7 ± 0·7

All Values are means of 3 independent observations ($n = 3$); the results are presented as means ± standard deviation (SD).
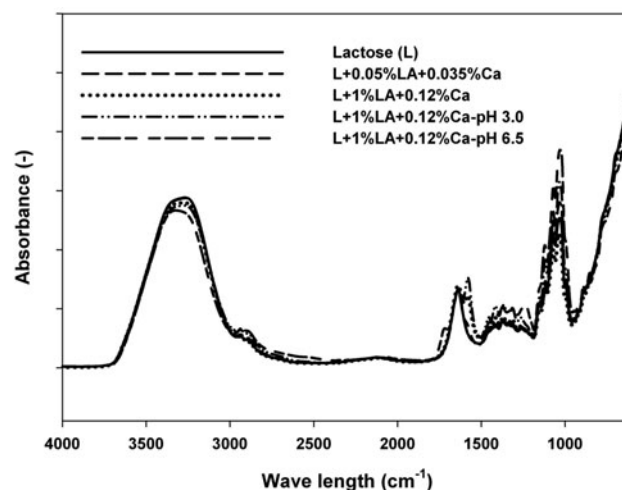

Fig. 1. FTIR spectra of concentrated lactose solutions.

amount of water. FTIR spectra indicated slight changes in the OH bending vibrations of water at pH 3 indicated by increased intensity of a peak at ~1570 cm⁻¹ and decreased intensity of a peak at 1630 cm⁻¹. In addition, under the same conditions FTIR showed changes in C–C/C–H aliphatic, C–CH₃ aliphatic stretching vibrations of LA and C–C/C–O/C–H ring stretching vibrations of lactose (Pamula et al. 2001) denoted by high intensity peaks in a region between 1180 to 1500 cm⁻¹. At this pH, below its pK_a, LA is present largely in its undissociated form, which is very hygroscopic and might have influenced the observed changes.

Increasing pH to 6·5 at the same high concentrations of LA and Ca resulted in significantly increased yield (~71%) and dehydration improved immensely (Table 1). These results indicate that lactose molecules moved more freely and were able to position better to form crystals, while at the same time more water was evaporated at these high concentration of impurities. Even T_{cr} has a similar value to that of the pure lactose (Table 1). In the present study, lactose monohydrate was considered in a 100% crystalline form which was measured by subtracting the heat of crystallisation from the heat of fusion (Wijayasinghe et al. 2015). Following this approach, the freeze dried sample of the pure lactose solution consisted of ~50% crystalline lactose. This crystallinity was substantially reduced in the presence of 1% w/w LA and 0·12% w/w Ca at unadjusted pH as it contained only ~25% crystalline lactose. However, by simply increasing pH of the same solution the crystallinity of the freeze dried sample was substantially improved, up to ~62% crystallinity, greater than that of the pure lactose solution. Complementarily, only one OH bending vibration peak at 1630 cm⁻¹ was evident, which was similar to that of the pure lactose. At high pH, LA appears mostly in the dissociated form of lactate, which has ability to increase the rate of crystallisation (Smart, 1988). The FTIR spectra also contained an intense peak at ~1230 cm⁻¹ which was subdued in the other samples. In the past, this peak was

attributed to the OH bending vibrations of lactose molecules, which also means that a change in lactose structure could be predicted and observed with alterations in pH. Lactose molecule has a hemiacetal structure which enables the molecule to interchange between α and β anomers by mutarotation. The ratio between α and β form depends on the compositional and processing conditions such as temperature, concentration, pH and presence of foreign substances (Hartel & Shastry, 1991). Thus, in this particular case, a pH high above pK_a of LA leads to a structural modification of lactose driven by a different rate of mutarotation that results in attainment of a favourable equilibrium of lactose anomeric forms. These changes in anomeric equilibrium of lactose manipulate the water-lactose behaviour and contribute to varying degrees of crystallisation.

Conclusion

Lactose crystallisation behaviour is affected by pH of the system in addition to the concentrations of LA and Ca. Adjusting pH to 6·5 increased lactose crystallinity. This pH adjustment induced structural modifications of lactose changing equilibrium between anomeric forms of lactose and likely manipulated the water–lactose attractions. Consequently mobility of water and lactose molecules was enhanced improving the crystallinity of lactose. Therefore, it appears that pH of acid whey should be increased to alleviate problems associated with processing of this waste stream.

References

Bhargava A & Jelen P 1996 Lactose solubility and crystal growth as affected by mineral impurities. *Journal of Food Science* **61** 180–184

Chandrapala J, Wijayasinghe R & Vasiljevic T 2016 Lactose crystallization as affected by the presence of lactic acid and calcium in model lactose system. *Journal of Food Engineering* **178** 181–189

de Wit JN 2001 *Lecturer's Handbook on Whey and Whey Products.* Belgium: European Whey Products Association

Fischer C & Kleinschmidt T 2015 Synthesis of galacto-oligosaccharides using sweet and acid whey as a substrate. *International Dairy Journal* **51** 15–22

Hartel RW & Shastry AV 1991 Sugar crystallization in food products. *Critical Reviews in Food Science and Nutrition* **30** 49–112

Herrington BL 1934 Some physico-chemical properties of lactose: the influence of salts and acids upon the mutarotation velocity of lactose. *Journal of Dairy Science* **17** 659–670

Jelen P & Coulter ST 1973 Effects of certain salts and other whey substances on the growth of lactose crystals. *Journal of Food Science* **368** 1186–1189

Mullin JW 1979 Crystal growth in pure and impure systems. In *Industrial Crystallization*, pp. 93–103 (Eds SJ Jancic & EJ de Jong). New York: North-Holland Publishing Co.

Nikanishi K 1962 *Infrared Absorption Spectroscopy*. San Francisco: Holden–Day Inc.

Pamula E, Blazewicz M, Paluszkiewicz C & Dobrzynski P 2001 FTIR study of degradation products of aliphatic polyesters-carbon fibre composites. *Journal of Molecular Structure* **556** 69–75

Raut DM, Allada R, Pavan KV, Deshpande G, Patil D, Patil A, Deshmukh A, Sakharkar DM, Bodkel PS & Mahajan DT 2011 Dehydration of lactose monohydrate: analytical and physical characterization. *Der Pharmacia Lettre* **3** 202–212

Reid SD & Fennema RO 2008 Water and ice. In *Fennema's Food Chemistry*, 4th edition, pp. 17–83 (Eds S Damodaran, LK Parkin & RO Fennema). USA: Taylor & Francis group, LLC

Smart JB 1988 Effect of whey components on the rate of crystallisation and solubility of alpha lactose monohydrate. *New Zealand Journal of Dairy Science and Technology* **23** 275–289

Von Hippel PH & Schleich T 1969 The effects of neutral salts on the structure and conformational stability of macromolecules in solution. *Structure and Stability of Biological Macromolecule* **2** 417–574

Wijayasinghe R, Vasiljevic T & Chandrapala J 2015 Water-lactose behaviour as a function of concentration and presence of lactic acid in lactose model system. *Journal of Dairy Science* **98** 8505–8514