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Egg shell as catalyst of lactose isomerisation to lactulose

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Abstract

A feasible way to produce lactulose, employing milk ultrafiltrate as source of lactose and egg shell as catalyst, is proposed as an alternative means for utilising these industrial wastes. Influences of catalyst loadings, lactose concentration and pH on lactose isomerisation were studied. Optimal production of lactulose was reached at 98 °C, employing 6 mg/ml of catalyst loading within 60 min of reaction. Quantities of lactulose of 1.18 g/100 ml and low levels of secondary products (*epi*-lactose, galactose and organic acids) were produced under these conditions of reaction. Methodology to remove coloured by-products from lactulose syrup in a range of 65–92% was established.

Keywords: Lactulose; Egg shell; Lactose isomerisation; Milk ultrafiltrate

1. Introduction

Lactulose (4-*O*-β-D-galactopyranosyl-D-fructose) has interest for both health-care and food industries (Clausen & Mortensen, 1997; Farhadi, Banan, Fields, & Keshavarzian, 2003; Harju, 2001; Horton, 1995; Huchzermeyer & Schumann, 1997; Katsuma et al., 2002; Méndez & Olano, 1979, **?**; Porkka, Salminen, & Salminen, 1988; Voragen, 1998).

Lactulose is generated from lactose (4-*O*-β-D-galactopyranosyl-D-glucose) by treatment in alkaline solution via the Lobry de Bruyn–Alberda van Ekenstein transformation employing either homogeneous or heterogeneous catalysts. Calcium hydroxide (Montgomery & Hudson, 1930), sodium hydroxide (Dendene, Guihard, Nicolas, & Bariou, 1994; De Haar & Pluim, 1991; Deya & Takahashi, 1991; Nagasawa, Tomita, Tamura, Obayashi, & Mizota, 1974; Zokaee, Kaghazchi, Soleimani, & Zare, 2002a; Zokaee, Kaghazchi, Zare, & Soleimani, 2002b), potassium hydroxide and carbonate (Nagasawa et al., 1974), magnesium oxide (Carobbi, Miletti, & Franci, 1985), tertiary amines (Parrish, 1970), borates (Carubelli, 1970; Hicks, 1981; Kozempel & Kurantz, 1994; Kozempel, McAloon, & Roth, 1997; Krumbbolz & Dorscheid, 1991; Mendicino, 1960; Zokaee et al., 2002a; Zokaee et al., 2002b), and sodium aluminate (Carobbi & Innocenti, 1990; Guth & Tumerman, 1970; Tumerman & Guth, 1974; Zokaee et al., 2002a, 2002b) have been employed as homogeneous catalysts to obtain lactulose. Zeolites (Shukula, Verykios, & Mutharasan, 1985) and sepiolites have been proposed as heterogeneous catalysts for lactose isomerisation into lactulose (de la Fuente, Juárez, de Rafael, Villamiel, & Olano, 1999; Troyano, deRafael, Martinez-Castro, & Olano, 1996; Villamiel, Corzo, Foda, Montes, & Olano, 2002). Most of these processes cause a high level of undesirable side products, which are difficult to remove from lactulose syrup. Fig. 1 shows a simplified scheme of lactose alkaline isomerisation. In general, for industrial production, degradation products should be avoided or at least kept to a minimum. The presence of monosaccharides and lactose is especially undesirable for medical purposes needing a clean-up procedure, which is possible but tedious. However, clean-up may be avoided when lactulose is

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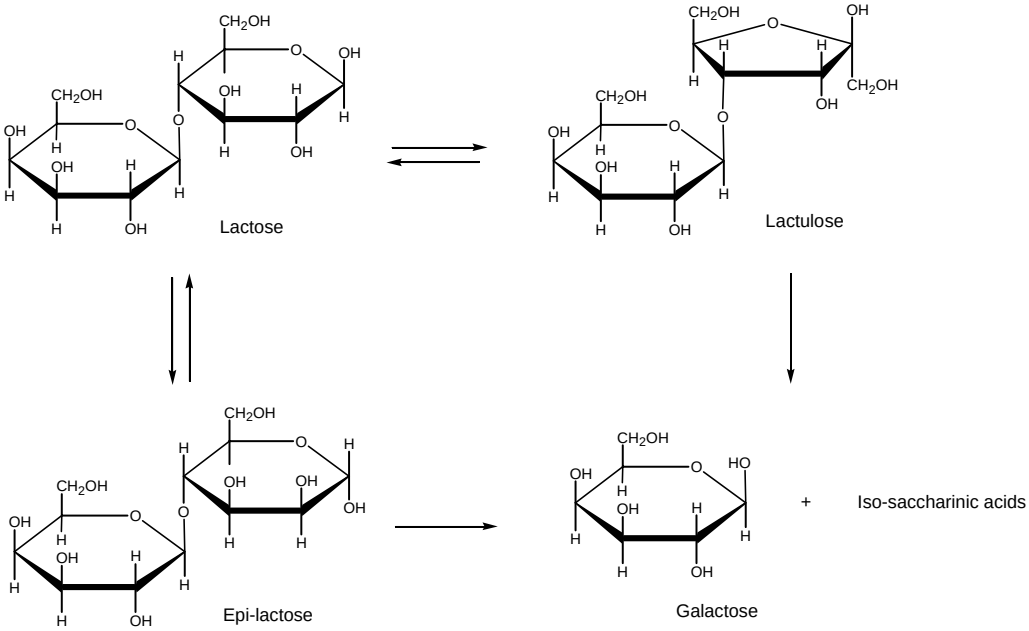

Fig. 1. Simplified model of the alkaline isomerisation of lactose.

employed as a food additive when a food-grade catalyst is used under appropriate conditions.

Egg shell is made up, for the most part, of calcium carbonate with about 39% of elemental Ca and low levels of Al, Pb, Cd and Hg, and may be used as a Ca source in human nutrition (Schaafsma et al., 2000).

High quantities of whey and milk ultrafiltrate are produced during cheese making. Proteins are mainly recovered by ultrafiltration and the remaining permeate, which contains approximately 5% of lactose, may be used as a source of lactose to produce lactulose (Olano, Corzo, Paez, & Martinez-Castro, 1987; Zokaee et al., 2002a; Zokaee et al., 2002b).

In recent last years, recycling industrial by-products and ecological manufacture procedures have excited increasing interest. Both egg shells from egg-breaking operations and milk ultrafiltrate constitute significant waste disposal problems for the food industry, so the development of value-added by-products from this waste is to be welcomed. Therefore, the aim of this investigation was to examine the feasibility of using egg shell as an alternative catalyst for lactulose production from milk ultrafiltrate.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Calcium chloride was supplied by Prolabo (Paris, Francia) and sodium hydroxide by Baker (Deventer, Holland). Reagents employed for GC analysis, including sugar standards (glucose, fructose, lactose, lactulose, and *epi*-lactose), internal standard (β-phenyl-glucoside) and derivatising reagent (*N*-trimethyl-sylil-imidazole), were obtained from Sigma (St. Louis, USA). Lactic acid was also acquired from Sigma. Powder activated carbon was provided by Aldrich (Steinheim, Germany). 18.2 MΩ cm Ultrapure water, with 1–5 ppb TOC and <0.001 EU/ml of pyrogen levels (Milli-Q), was produced in-house, using a Laboratory water purification Milli-Q Synthesis A10 system (Millipore, Bellerica, Massachusetts, USA) and was used throughout.

2.1.2. Egg shell powder

White egg shells were washed with tap water to remove all adhering albumen, dried at 105 °C for 4–24 h and ground in a ball mill (Mixer Mill MM 200, Retsch GmbH & Co. KG, Haan, Germany) at 800 rpm. (13.3 Hz), for 30 min. Egg shell powder obtained, particle size approximately 5 μm, was stored in glass vials in a dry place at room temperature prior to be use (24 h–2 years).

2.1.3. Milk ultrafiltrate

Pasteurised milk acquired at the local market was ultrafiltered, employing a Minitan System provided with a 10 kDa cut-off polysulphonate membrane (Millipore, Bellerica, Massachusetts, USA). Ultrafiltrate was kept in an ice-water bath during filtration, followed by storage at 4–8 °C for not longer than 24 h prior to analysis. Since acidic whey is also an abundant by-product of the milk industries, its usefulness in lactulose production was also checked. Acid whey was simulated, adjusting

the pH of milk ultrafiltrate to 4.5 by adding 85% lactic acid. In addition, these samples (ultrafiltrates with pH 4.5 and 6.8) were employed to evaluate influence of pH on isomerisation of lactose.

2.1.4. Concentrate of milk ultrafiltrate

Ultrafiltrate, obtained as described above, was concentrated up to 5.2- and 7.6-fold, by evaporation at 35 °C under reduced pressure in a rotavapor. Once the desirable concentration was achieved the pH of the samples was brought to the initial pH (6.8) by adding 2 M NaOH.

2.2. Treatments

2.2.1. Heat treatment (isomerisation reaction)

100 ml of milk ultrafiltrate were placed in a 250-ml round-bottom flask provided with an additional necked sampling inlet, treated with egg shell, immersed in a glycerol bath at 125 °C, stirred and refluxed at 98 °C for 150 min. Temperature of the glycerol bath was kept constant.

Initially, the catalyst effect of egg shell on lactose conversion into lactulose was studied by employing milk ultrafiltrate (pH 6.8) and egg shell loadings at final concentrations of 4, 6 and 16 mg/ml. Loadings of catalyst of 4 and 20 mg/ml were used for acidic milk ultrafiltrate. Controls for lactose degradation were carried out without addition of catalyst (egg shell).

Boiling start was considered as zero time of reaction. Samples (10 ml) were taken at 30-minute intervals. Reaction was stopped by cooling in an ice-water bath. Eggshell was removed by centrifugation at 20 °C, 5000*g*, for 10 min. Supernatant was collected, stored at 4–8 °C for not longer than 24 h, and analysed.

2.2.2. Colour removal

Decolourisation was achieved by adsorption, employing powder activated carbon (Darco D60, 100 mesh), followed by filtration through low protein binding Durapore (PVDF) membrane filters of 0.45 μm pore size (Millex-HV, Millipore, Bellerica, MA, USA). Assays employing levels of activated charcoal in the range of 5–65 mg/ml and standing times of 5–30 minute were performed in order to find out the optimal clean-up conditions.

2.3. Analysis

2.3.1. pH

Measurements of pH were taken before and after thermal treatment at 20 °C, employing a pH-meter MP230 (Mettler-Toledo, Schwerzenbach, Switzerland).

2.3.2. GC analysis

2.3.2.1. Sample preparation. 1 ml of sample was made up to 10 ml with methanol in a volumetric flask to remove proteins and fats. Concentrated samples (P5.6 and P7.2) were prepared in a different way. 100–150 mg of these were exactly weighed, placed in 10 ml volumetric flasks, treated with 1 ml of water and with methanol made up to 10 ml final volume. Mixtures were vigorously stirred, followed by standing for at least 1 h. The supernatant was used for carbohydrate analysis and phenyl-β-D-glucoside was added as internal standard. Previously derivatized equal volumes (1 ml) of supernatant and internal standard were mixed and dried at 38–40 °C in a rotary evaporator.

2.3.2.2. Derivatisation and GC analysis. The dried mixtures were treated with 100 μl of *N*-trimethylsilylimidazole to silylate the carbohydrates; the reaction was completed in 30 minute at 65 °C. Silylated carbohydrates were extracted with 0.1 ml of hexane and 0.2 ml of water. Volumes in the range of 0.2–1 μl of the organic phase containing silyl derivatives were injected into the column.

The trimethylsilyl ethers were separated as has been previously described (Olano, Clavo, & Reglero, 1986) using a 3 m×1 mm id stainless steel column (Chrompack, Middelburg, The Netherlands), packed with 2% OV-17 on nonsilanised 120/140 Volaspher A-2 (Merck, Darmstadt, Germany). Separation was performed at 200 °C for 5 min, followed by an increase up to 270 °C at rate of 15 °C/min and keeping this temperature for 15 min. Temperatures of injector and detector were 300 °C during the analysis. Injections were carried out in split mode 1:30. Data were acquired by means of HP ChemStations (Hewlett–Packard, Wilmington, DE., USA).

2.3.3. Colour measurement

Colour was estimated as the absorbance value measured at 420 nm (Meydav, Saguy, & Kopelman, 1977). Dilution of the samples was only needed for concentrated milk whey ultrafiltrates, which were diluted with water up to the initial concentration values (5.2- and 7.6-folds, respectively).

2.3.4. Statistical analysis

Statistical analysis was done using SPSS 9.0 for Windows programme. (SPSS Inc., Chicago, IL, USA, 1999). Wilcoxon's test was applied to determine differences between two groups of means. One-way analysis of variance (ANOVA) was used to look for differences between means of more than two groups. Where ANOVA indicated differences, the least significant difference (LDS) test ($p < 0.05$) was applied to ascertain which values were different.

3. Results

3.1. Catalytic effect of egg shell

A catalytic effect of egg shell on lactose isomerisation into lactulose was observed. Fig. 2 shows chromatograms of the TMS derivatives of the isomerisation mixture obtained for milk ultrafiltrates treated with 0 and 6 mg/ml of catalyst. Lactose, lactulose, *epi*-lactose and galactose were detected in milk ultrafiltrates heated in the presence of egg shell. A low production of lactulose (3% of the initial lactose content) took place in milk ultrafiltrate heated for 120 min at 98 °C when no egg shell was added. However, by employing an egg shell loading of 6 mg/ml, higher production of lactulose (up to 23.6% of the initial lactose) in a shorter time (60 min) of heating at 98 °C was achieved.

Profiles of consumption of lactose and production of lactulose, *epi*-lactose and galactose at 98 °C and egg shell loadings of 4, 6 and 16 mg/ml are shown in Fig. 3. An increase of lactulose formation with increasing egg shell loading was observed at early stages of reaction; however, similar final levels of lactulose were obtained regardless of catalyst loading. Maximum lactulose formation (1.31 g/100 ml, equivalent to a conversion of the initial lactose of 26.2%) was achieved at 90 min, employing 6 mg/ml of catalyst.

Epimerisation of lactose to *epi*-lactose and formation of galactose were detected (Fig. 3). Progressive increase in galactose concentration with reaction time and catalyst concentration was observed ($p < 0.05$). Galactose content increased up to 0.462 g/100 ml after 120 min of heating at 98 °C when 16 mg/ml of egg shell were added.

Lactose consumption tallied with formation of lactulose, *epi*-lactose and galactose and increased with egg shell loadings. Losses of lactose found for 4, 6 and 16 mg/ml of egg shell were significantly different ($p < 0.05$). Highest decrease of lactose was observed for 16 mg/ml of catalyst.

Results seem to indicate that optimal production of lactulose employing milk ultrafiltrate and egg shell, can be achieved by heating at 98 °C for 60 min with an egg shell load of 6 mg/ml. Under these conditions, high levels of lactulose (1.18 g/100 ml) and low levels of *epi*-lactose (0.087 g/100 ml) and galactose (0.117 g/100 ml) were simultaneously reached.

3.2. Influence of lactose concentration

Concentrates of whole whey and ultrafiltrates are common products of the milk industries because removal of water may reduce the risk of fermentation. Therefore, studies employing milk ultrafiltrate concentrated up to 5.2- and 7.6-fold were carried out. Table 1 shows levels of lactulose, *epi*-lactose and galactose produced. Results seem to indicate that both single and concentrated ultrafiltrates may be used to produce lactulose. Similar percentages of conversion were achieved by employing both types of samples.

3.3. Colour removal (lactulose clean up)

Development of colour was observed for all loadings of catalyst studied. Formation of coloured compounds was followed by measuring the absorbance values at 420 nm. In agreement with results described above,

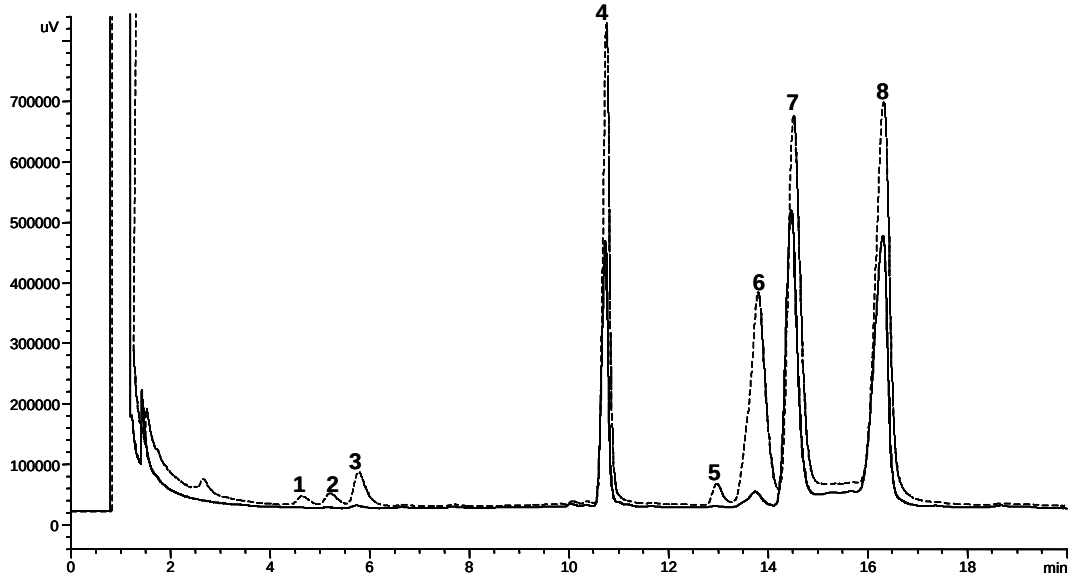

Fig. 2. Trimethylsilyl derivatives of carbohydrates detected in milk ultrafiltrate (pH 6.8) refluxed at 98 °C for 60 min in absence (solid line) and presence (dot line) of shell egg (6 mg/ml). Galactose (1–3), β-phenyl-glucoside (4), *epi*-lactose (5), lactulose (6) and lactose (7–8).

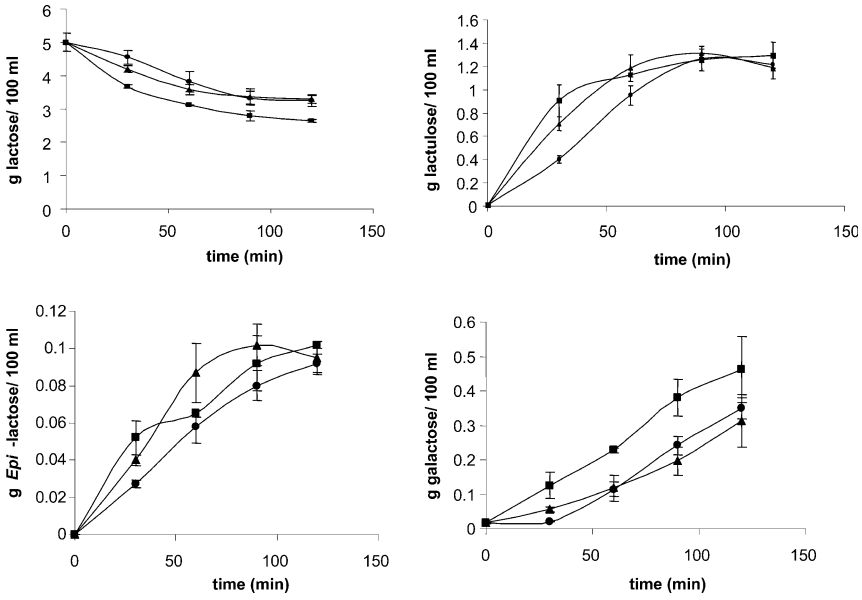

Fig. 3. Catalytic effect of egg shell on lactose isomerisation. Lactose consumption and formation of lactulose, *epi*-lactose and galactose during heating of milk ultrafiltrate, pH 6.8, and egg shell loadings of 4 (black circle), 6 (black triangle) and 16 mg/ml (black square) of milk ultrafiltrate.

Table 1
Lactulose, *epi*-lactose and galactose produced in mixtures constituted by single and concentrated ultrafiltrates (5.2- and 7.6-fold) and 4 mg/ml of egg shell heated at 98 °C, pH 6.8

Source of lactose	Lactulose	*Epi*-lactose	Galactose
Single milk ultrafiltrate			
$t=30$	0.388 ± 0.032	0.026 ± 0.002	0.020 ± 0.002
$t=60$	0.914 ± 0.083	0.055 ± 0.009	0.109 ± 0.020
$t=90$	1.22 ± 0.031	0.076 ± 0.008	0.232 ± 0.026
$t=120$	1.17 ± 0.051	0.088 ± 0.005	0.337 ± 0.030
Concentrate, 5.2-fold			
$t=30$	3.41 ± 0.054	0.253 ± 0.001	0.572 ± 0.011
$t=60$	4.13 ± 0.201	0.317 ± 0.017	0.714 ± 0.051
$t=90$	4.39 ± 0.122	0.314 ± 0.045	0.843 ± 0.071
$t=120$	4.80 ± 0.056	0.472 ± 0.017	1.05 ± 0.030
Concentrate, 7.6-fold			
$t=30$	5.33 ± 0.243	0.340 ± 0.015	0.647 ± 0.091
$t=60$	6.08 ± 0.437	0.364 ± 0.014	0.995 ± 0.034
$t=90$	6.82 ± 0.513	0.497 ± 0.037	1.36 ± 0.075
$t=120$	7.15 ± 0.236	0.557 ± 0.024	1.88 ± 0.024

Grammes of sugar produced per 100 ml of ultrafiltrate during heating are given as median values ± RSD ($n=4$).

brown pigment generation depended on catalyst concentration.

Excess of colour is undesirable and it should be removed from the reaction mixture to avoid loss of lactulose. Samples with absorbance values at 420 nm of 1.68 (dark, ultrafiltrate with 4 mg/ml of egg shell and heating for 150 min) and 0.690 (light, ultrafiltrate with 4 mg/ml of egg shell and heating for 90 min), respectively, were treated with different amounts of activated carbon at various times. The decolourisation procedure was very effective. Activated carbon, added at 5, 10 and 15 mg/ml caused a colour reduction, in the range 65–92%, within 5 min of treatment. Remaining colour was no higher than that found in commercial lactulose preparation. Table 2 shows sugar contents before and after decolourisation. Lactulose was completely recovered after decolourisation. No significant differences ($p>0.05$) were found between lactulose values before and after addition of activated carbon. Very high values of recovery were also obtained for lactose, *epi*-lactose and galactose. No relationship between concentration of activated carbon used for decolourisation and sugar recovery was observed. Similar values of recovery were obtained for samples treated with activated carbon, at 5 mg/ml, for 5 min and 30 mg/ml for 15 min.

Table 2
Contents of lactulose, *epi*-lactose and galactose in samples of milk ultrafiltrate or concentrated milk ultrafiltrate (5.2-fold) obtained by heating at 98 °C, 60 min, 4 mg/ml of catalyst, pH 6.8

Source of lactose	Lactulose	*Epi*-lactose	Galactose
Milk ultrafiltrate			
1-A	0.814 ± 0.011	0.054 ± 0.003	0.085 ± 0.003
1-B	0.782 ± 0.027	0.049 ± 0.001	0.078 ± 0.008
2-A	0.850 ± 0.026	0.065 ± 0.005	0.143 ± 0.005
2-B	0.862 ± 0.012	0.060 ± 0.001	0.144 ± 0.004
3-A	0.892 ± 0.015	0.063 ± 0.002	0.131 ± 0.006
3-B	0.969 ± 0.005	0.064 ± 0.001	0.145 ± 0.003
Concentrate, 5.2-fold			
A	4.43 ± 0.219	0.307 ± 0.054	0.868 ± 0.027
C	4.49 ± 0.239	0.283 ± 0.031	0.882 ± 0.031

A, control, without treatment with activated carbon.
B, sample treated with activated carbon (5 mg/ml during 5 min).
C, sample treated with 30 mg/ml during 15 min.
Gramme of sugar produced per 100 ml of permeate are given as median values ± RSD ($n = 4$).

3.4. Influence of pH

Since acid whey milk is also an abundant waste product of the cheese making industries, its usefulness for producing lactulose was evaluated. Acidic ultrafiltrate was simulated by adjusting the pH of the milk ultrafiltrate to 4.5 (adding lactic acid). These studies provided valuable information related to the influence of pH on the isomerisation reaction.

A very low level of isomerisation was detected for acid ultrafiltrate (pH 4.5) in the presence of 4 mg/ml of catalyst. Maximum lactulose formation did not exceed 3% (0.14 g/100 ml) of the initial concentration of lactose after 150 min of reaction. Small quantities of *epi*-lactose 0.3% (0.01 g/100 ml) and galactose 1.6% (0.08 g/100 ml) were also formed. Rate of the isomerisation reaction was affected by pH values of milk ultrafiltrates (Fig. 4). Maximum production of lactulose at pH 6.8, employing 4 mg/ml of catalyst, was 25.3% (1.26 g/100 ml) of initial lactose concentration after 90 min of treatment. Under these conditions, *epi*-lactose and galactose reached values of 1.61% (0.08 g/100 ml) and 4.82% (0.24 g/100 ml) of initial lactose concentration, respectively. Lower production of lactulose was achieved at pH 4.5 by employing 20 mg/ml of catalyst, as can be observed in Fig. 4. Formation of lactulose, *epi*-lactose and galactose reached values of 1.01 g/100 ml, 0.06 g/100 ml and 0.13 g/100 ml, respectively, employing this amount of catalyst and heating at 98 °C for 150 min.

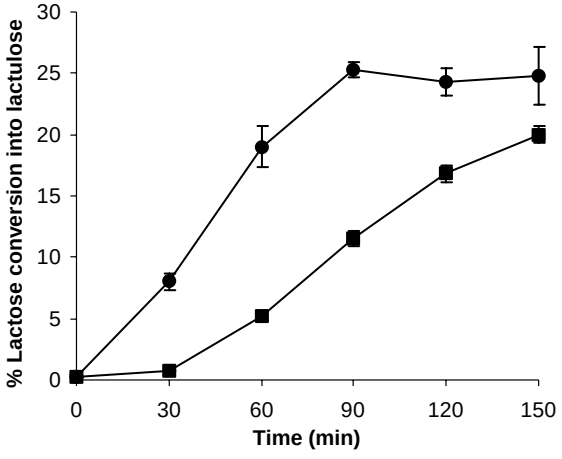

Fig. 4. pH effect on lactose isomerisation. Isomerisation of lactose employing milk ultrafiltrate, 20 mg/ml of shell loading at pH 4.5 (black square). Isomerisation of lactose from milk ultrafiltrate, pH 6.8, employing 4 mg/ml of catalyst (black circle).

4. Discussion

Product distribution indicates that the main reaction routes were isomerisation of lactose, followed by degradation of lactulose as was previously described by Olano and Martinez-Castro (1981) for aqueous lactose solutions and Berg and van Boekel (1994) for milk. Since no glucose or fructose were detected, it may be assumed that the reducing moiety of the disaccharide is first degraded into acidic compounds, followed by its hydrolysis to give rise to galactose and isosaccharinic acids (Méndez & Olano, 1979) which are further degraded into lower molecular weight organic acids (Fig. 1). The decrease of the pH detected during the reaction (data not shown) indicated the formation of acidic compounds. In fact, from the total sugar data obtained at different times of reaction, it may be estimated that production of organic acids was as low as 0.0011, 0.0043 and 0.0204 g/100 ml at 30, 60 and 120 min, respectively. These results support isomerisation of lactose into lactulose as the main reaction which has taken place.

Lactose degradation in milk takes places by means of a very complex reaction network. Previous studies have established that lactose isomerisation and Maillard reac-

tion are the main degradation routes. During milk heating at 110–150 °C for 25 min, lactulose, galactose, formic acid, protein-bound Amadori products, hydroxymethylfurfural (HMF), furfural, and furfurol may be produced by means of these two degradation routes. Lactulose isomerisation and subsequent degradation into galactose, formic acid and C5/C6 compound and Maillard reactions occur, in which lactose interacts with protein-bound lysine, followed also by degradation of the corresponding Amadori compound, mainly to galactose and formic acid (Berg & van Boekel, 1994). In agreement with our results, no glucose has previously been detected in heated milk (Olano, 1989; Patton, 1955; Olano, Calvo, & Carzo, 1989) or model systems constituted by lactose and casein in the same concentration (Berg & van Boekel, 1994).

Previous studies have demonstrated that lactose isomerisation in heated milk is quantitatively much more important (Berg & van Boekel, 1994; Olano, Santa-Maria, Corzo, Calvo, & Martinez-Castro, 1992) than the Maillard reaction. Indeed, the usefulness of whey and whey permeate as adequate media to produce lactulose has already been proved (Olano et al., 1987; Villamiel et al., 2002; Zokaee et al., 2002a, 2002b). Results found by previous authors agree with those here described.

Lactose isomerisation is a base-catalysed reaction and its dependence on the pH has been described (Martinez-Castro & Olano, 1980). Thus, in the case of acid whey (acid milk ultrafiltrates), the formation of lactulose should be lower than in sweet whey, as occurred for the samples here studied.

Results indicate that egg shell could be as good a catalyst for conversion of lactose into lactulose as others previously described. Similar yields of isomeric disaccharides were obtained to those found in the literature. However, lower quantities of catalyst were needed and formation of products derived from side-reactions was lower than those generated employing other catalysts. Recently, a comparative study of the three most important catalytic systems for lactose conversion to lactulose has been published (Zokaee et al., 2002a, 2002b). Authors concluded that maximum production of lactulose does not exceed 20% and total by-products reached 5–7% on using sodium hydroxide as catalyst. Yield of production may be improved (up to 68–88%) by using complexing reagents (aluminium and borate) but a large excess of chemical was needed and it is not recommended. Egg shell remains in the solid state after isomerisation and may be easily removed by centrifugation which is an advantage compared with soluble catalysts. Isomerisation, carried out by employing sepiolites, can give yields of lactose conversion into lactulose in the range 20–25% with a catalyst loading of 15 g/l, an amount 3.75-fold higher than that here proposed (de la Fuente et al., 1999; Villamiel et al., 2002). Under optimal conditions (sepiolites submitted to 10 cycles of washing), it was possible to maintain a high yield of lactulose production (25%) and a relatively low degradation, generating levels of *epi*-lactose and galactose of 50 and 150 mg/100 ml, respectively (Villamiel et al., 2002). The method here proposed is simpler and faster because egg shell does not require special treatment before using as catalyst to give a similar or even higher level of lactulose production, allowing it to be recycled as a disposal material from the food industry.

From our results, it can be also concluded that concentrated milk ultrafiltrate, resulting from cheese manufacture, may be directly used for lactulose production. Decolouration worked as expected. Humic acids, i.e., caramel and melanoidins, have been isolated on activated carbon columns from clarified cane juice (Singh, 2000). This procedure reduces colour of the final sugar products without removal of any sugar. Indeed, a similar procedure has been used for decolourisation of fruit juice, jam, syrup and honey and subsequent spectrophotometric analysis of monosaccharides and oligosaccharides in these foods (Caceres, Cardenas, Gallego, & Valcarcel, 2000).

5. Conclusions

Eggshell and milk ultrafiltrate can be employed as raw materials to produce lactulose which may be used as an ingredient in foods, providing an alternative use for these wastes of the food industries. The procedure is simple and a reasonable balance between lactulose production and its degradation is achieved.

References

Berg, H. E., & van Boekel, M. A. J. S. (1994). Degradation of lactose during heating of milk. 1. Reaction pathways. *Netherlands Milk & Dairy Journal, 48*, 157–175.

Caceres, A., Cardenas, S., Gallego, M., & Valcarcel, M. (2000). A continuos spectrophotometric system for the discrimination/determination of monosaccharides and oligosaccharides in foods. *Analytical Chimica Acta, 404*, 121–129.

Carobbi, R., Miletti, S., & Franci, V. (1985). Process for preparing lactulose from lactose, in form of syrup or a crystalline product. US Patent 4536221.

Carobbi, R., & Innocenti, F. (1990). Process for preparing lactulose from lactose by epimerisation with sodium aluminate. European patent 0320670.

Carubelli, R. (1970). Process for lactulose. US patent 3505309.

Clausen, M. R., & Mortensen, P. B. (1997). Lactulose, disaccharides and colonic flora – Clinical consequences. *Drugs, 53*, 930–942.

Dendene, K., Guihard, L., Nicolas, S., & Bariou, B. (1994). Kinetics of lactose isomerisation to lactulose in alkaline-medium. *Journal of Chemical Technology and Biotechnology, 61*, 37–42.

De Haar, W.T., & Pluim, H. (1991). Method of preparing lactulose. European patent 0339749.

de la Fuente, M. A., Juárez, M., de Rafael, D., Villamiel, M., & Olano, A. (1999). Isomerisation of lactose catalysed by alkaline-substituted sepiolites. *Food Chemistry, 66*, 301–306.

Deya, E., & Takahashi, K (1991). Production process of high purity lactulose syrup. US patent 5034064.

Farhadi, A., Banan, A., Fields, J., & Keshavarzian, A. (2003). Intestinal barrier: An interface between health and disease. *Journal of Gastroenterology and Hepatology, 18*, 479–497.

Guth, J.H., Prospect M., & Tumerman, L. (1970). Method of making lactulose. US Patent 3546206.

Harju, M. (2001). Milk sugars and minerals as ingredients. *International Journal of Dairy Technology, 54*, 61–63.

Hicks, K.B. (1981). Ketose sugars from aldose sugars. US patent 4273922.

Horton, B. S. (1995). Commercial utilisation of minor milk components in the health and food industries. *Journal of Dairy Science, 78*, 2584–2589.

Huchzermeyer, H., & Schumann, C. (1997). Lactulose – A multifaceted substance. *Zeitschrift fur Gastroenterologie, 35*, 945–955.

Katsuma, M., Watanabe, S., Kawai, H., Takemura, S., Masuda, Y., & Fukui, M. (2002). Studies on lactulose formulations for colon-specific drug delivery. *International Journal of Pharmaceutics, 249*, 33–43.

Kozempel, M., & Kurantz, M. (1994). The isomerisation kinetics of lactose to lactulose in the presence of borate. *Journal of Chemical Technology and Biotechnology, 54*, 25–29.

Kozempel, M., McAloon, A., & Roth, L. (1997). Simulated scale-up and cost estimate of a process for alkaline isomerisation of lactose to lactulose using boric acid as complexation. *Journal of Chemical Technology and Biotechnology, 68*, 229–235.

Krumbbolz, R.E., & Dorscheid, M.G. (1991). Method of manufacturing lactulose. European patent 0375040.

Martinez-Castro, I., & Olano, A. (1980). Influence of thermal processing on carbohydrate composition of milk. Formation of epilactose. *Milchwissenschaft, 35*, 5–8.

Mendicino, J. F. (1960). Effect of borate on the alcali-catalyzed isomerisation of sugars. *Journal of the American Chemical Society, 20*, 4975–4979.

Méndez, A., & Olano, A. (1979). A review on some chemical properties and applications in infant nutrition and medicine. *Dairy Science Abstract, 41*, 531–535.

Meydav, S., Saguy, I., & Kopelman, I. S. (1977). Browning determination in citrus products. *Journal of Agricultural and Food Chemistry, 25*, 602–604.

Montgomery, E. M., & Hudson, C. S. (1930). Relation between rotary power and structure in the sugar group. XXVII. Synthesis of a new disaccharide ketose (lactulose) from lactose. *Journal of America Chemical Society, 52*, 2101–2106.

Nagasawa, T., Tomita, M., Tamura, Y., Obayashi, T., & Mizota, T. (1974). Process for preparing a lactulose syrup. US Patent 3814174.

Olano, A., & Martinez-Castro, I. (1981). Formation of lactulose and epilactose from lactose in basic media. A quantitative study. *Milchwiss, 36*(9), 533–536.

Olano, A., Clavo, M. M., & Reglero, G. (1986). Analysis of free carbohydrates in milk using micropacked columns. *Chromatographia, 21*, 538–540.

Olano, A., Corzo, N., Paez, M. I., & Martinez-Castro, I. (1987). Isomerisation of lactose during heat-treatment of liquid and freeze-dried simulated milk ultrafiltrates – Effect of pH and calcium. *Milchwissenschaft Milk Science International, 42*, 628–630.

Olano, A., Calvo, M. M., & Carzo, N. (1989). Changes in the carbohydrate fraction of milk during heating. *Food Chemistry, 31*, 259–265.

Olano, A., Santa-Maria, G., Corzo, N., Calvo, M. M., & Martinez-Castro, I. (1992). Determination of free carbohydrates and Amadori compounds formed at the early stages of non-enzymatic browning. *Food Chemistry, 43*, 351–358.

Parrish, F.W. (1970). Isomerisation of glucose, maltose and lactose with amino compounds. US patent 3514327.

Patton, S. (1955). Browning and associated changes in milk and its products: A review. *Journal of Dairy Science, 38*, 457–478.

Porkka, L., Salminen, E., & Salminen, S. (1988). The effects of lactulose sweetened yoghurt on the rate gastric emptying and intestinal transit in healthy human volunteers. *Erhnarungswiss, 27*, 150–154.

Schaafsma, A., Pakan, I., Hofstede, G. J. H., Muskiet, F. A. J., Van Der Veer, E., & De Vries, P. J. F. (2000). Mineral, amino acid and hormonal composition of chicken eggshell powder and the evaluation of its use in human nutrition. *Poultry Science, 79*, 1833–1838.

Shukula, R., Verykios, X. E., & Mutharasan, R. (1985). Isomerisation and hydrolysis reactions of important disaccharides over inorganic heterogeneous catalyst. *Carbohydrate Research, 143*, 97–106.

Singh, K. (2000). Electrochemical and viscous properties of humic acid isolated from clarified cane juice. *International Sugar Journal, 102*, 355–362.

Troyano, E., deRafael, D., Martinez-Castro, I., & Olano, A. (1996). Isomerisation of lactose over natural sepiolite. *Journal of Chemical Technology and Biotechnology, 65*, 111–114.

Tumerman, L., & Guth, H. (1974). Method for manufacture of ketose sugars. US patent 3822249.

Villamiel, M., Corzo, N., Foda, M. I., Montes, F., & Olano, A. (2002). Lactulose formation catalysed by alkaline-substituted sepiolites in milk permeate. *Food Chemistry, 76*, 7–11.

Voragen, A. G. J. (1998). Technological aspects of functional food-related carbohydrates. *Trends in Food Science and Technology, 9*, 328–335.

Zokaee, F., Kaghazchi, T., Soleimani, M., & Zare, A. (2002a). Isomerisation of lactose to lactulose using sweet cheese whey ultrafiltrate. *Journal of the Chinese Institute of Chemical Engineers, 33*, 307–313.

Zokaee, F., Kaghazchi, T., Zare, A., & Soleimani, M. (2002b). Isomerisation of lactose to lactulose – Study and comparison of three catalytic systems. *Process Biochemistry, 37*, 629–635.