J. Dairy Sci. 87:1608–1620
© American Dairy Science Association, 2004.

# Hydrolysis of Lactose in Whey Permeate for Subsequent Fermentation to Ethanol

**A. Coté,[1] W. A. Brown,[1] D. Cameron,[2] and G. P. van Walsum[3]**
[1]Department of Chemical Engineering,
McGill University, Montreal, QC H3A 2B2
[2]Tembec Industries Inc., P.O. Box 5000,
Témiscaming, QC J0Z 3R0
[3]Department of Environmental Studies,
Baylor University, P. O. Box 97266,
Waco, Texas 76798-7266

## ABSTRACT

Fermentation of lactose in whey permeate directly into ethanol has had only limited commercial success, as the yields and alcohol tolerances of the organisms capable of directly fermenting lactose are low. This study proposes an alternative strategy: treat the permeate with acid to liberate monomeric sugars that are readily fermented into ethanol. We identified optimum hydrolysis conditions that yield mostly monomeric sugars and limit formation of fermentation inhibitors such as hydroxymethyl furfural by caramelization reactions. Both lactose solutions and commercial whey permeates were hydrolyzed using inorganic acids and carbonic acid. In all cases, more glucose was consumed by secondary reactions than galactose. Galactose was recovered in approximately stoichiometric proportions. Whey permeate has substantial buffering capacity—even at high partial pressures (>5500 kPa[g]), carbon dioxide had little effect on the pH in whey permeate solutions. The elevated temperatures required for hydrolysis with $CO_2$-generated inhibitory compounds through caramelization reactions. For these reasons, carbon dioxide was not a feasible acidulant. With mineral acids reversion reactions dominated, resulting in a stable amount of glucose released. However, the Maillard browning reactions also appeared to be involved. By applying Hammet's acidity function, kinetic data from all experiments were described by a single line. With concentrated inorganic acids, low reaction temperatures allowed lactose hydrolysis with minimal by-product formation and generated a hexose-rich solution amenable to fermentation.
(**Key words:** lactose hydrolysis, ethanol fermentation, $CO_2$ hydrolysis, carbonic acid)

Received September 23, 2003.
Accepted November 7, 2003.
Corresponding author: W. A. Brown; e-mail: wayne.brown@mcgill.ca.

**Abbreviation key: BOD** = biological oxygen demand, **HMF** = hydroxymethylfurfural, **PC** = principal component, **PCA** = principal component analysis.

## Introduction

The disposal of whey remains a significant problem for the dairy industry. As whey contains 5 to 6% dissolved solids including 3 to 5% lactose, the biological oxygen demand (**BOD**) is high. Generally, whey must be treated prior to discharge into the environment (Marwaha and Kennedy, 1988). Large volumes are produced—in Canada, 3,000,000 tonne of lactoserum are produced annually (CDIC, 2001).

To offset treatment costs, many "valorization" strategies have targeted the main ingredients of the whey, namely lactose and protein (Marwaha and Kennedy, 1988). Whey proteins are almost universally recovered via ultrafiltration and sold as concentrate. The permeate contains the lactose accounting for most of the BOD and dissolved salts, and hence still constitutes a formidable disposal problem.

The disaccharide lactose can be recovered from whey permeate, but world market demand is exceeded by lactose availability. One option that is carried out industrially by several cheese producers is to use whey permeate as an inexpensive feedstock for ethanol production (Marwaha and Kennedy, 1988). With the appropriate yeast strains (e.g., *Kluyveromyces marxianus*) lactose may be fermented directly into ethanol. Compared with the production of ethanol from glucose by traditional *Saccharomyces cerevisiae* strains, organisms fermenting lactose exhibit inferior production rates and yields (Terrel, et al., 1984). Typical ethanol yield from lactose is reported as 80 to 85% of theoretical (Mawson, 1994). Lactose fermenting yeast strains are more sensitive to high ethanol concentrations. Fewer than 10 commercial dairies worldwide ferment lactose in whey permeate directly into ethanol (Mawson, 1994; Murtagh, 1995).

Production of ethanol from lactose would be a more attractive process if the 90 to 93% product yields (Ingledew, 1995) associated with *Saccharomyces cerevisiae* could be realized; however, *S. cerevisiae* is unable to ferment lactose. Genetically modified strains have been developed in which the *lac* genes are added to the wild-type strain. Unfortunately, the benefits of this approach have yet to be realized industrially.

Another approach would be to hydrolyze lactose into its 2 monomeric sugars, glucose and galactose, which are readily and efficiently fermented. There are 2 means by which this can be accomplished. The glycosidic bond of the disaccharide can be hydrolyzed enzymatically with $\beta$-galactosidase. To this end, a number of enzyme sources, reactor configurations, and processes have been proposed and tested (Ovsejevi et al., 1998; Szczodrak, 1999; Splechtna et al., 2002). However, $\beta$-galactosidase enzymes are too expensive for producing a commodity chemical like ethanol.

Fortunately, a more cost-effective method exists, namely acid-catalyzed hydrolysis. The process has been well characterized for solutions of pure glycosides, and for some dairy effluents (Ramsdell and Webb, 1945; Timell, 1964). Acid hydrolysis involves heating with simple reagents, but the process is quite complex from a mechanistic perspective. Monosaccharide products can be further degraded into undesirable chemicals. The number of possible side reactions depends upon, among other things, the permeate composition. As such, evaluation of acid hydrolysis as a means to generate monosaccharides from lactose in whey permeate must be carried out within the context of the intended use of the hydrolysis products.

Early studies on acid catalyzed hydrolysis of whey permeate focused on the hydrolysis of lactose for use as sweet syrup (Coughlin and Nickerson, 1975; De Boer and Robbertsen, 1981). As a food additive, the desirable attributes include light color, sweetness, and acceptable flavor. However, for ethanol production these variables are of little relevance. Instead, factors affecting the physiological state of the fermenting organisms must be known. For instance, acid hydrolysis at high temperatures generates compounds that are inhibitory to yeasts (Larsson et al., 1999). Adding inorganic acids, followed by neutralizing agents, will increase the ionic strength, and increased osmotic pressure may reduce the activity of the yeasts. Previous studies on the hydrolysis of lactose have used inorganic acids. A recent study considered the use of carbonic acid to hydrolyze polymeric sugars (van Walsum, 2001). If carbonic acid could be used for hydrolysis of lactose, the ionic strength of the resulting solution could be minimized.

This study examined the hydrolysis of lactoserum for ethanol production. The hydrolysates generated were also fermented. The results will be disclosed in a separate publication. This study considered lactoserum as a supplemental feedstock for an existing ethanol plant. Tembec Inc. is an integrated forestry products company that operates an alcohol plant in Temiscaming, Quebec (Canada). Hexose sugars contained in spent liquor from acid sulfite pulping are fermented to food and pharmaceutical grade ethanol. Ethanol production could be increased if additional fermentable sugars were available. The effects of the other components in the permeate on the hydrolysis were considered, as was the hydrolysis temperature, acidulant type, and acidulant concentration. Carbon dioxide was tested for its ability to create favorable conditions for lactose hydrolysis, to our knowledge a first in this application.

## MATERIALS AND METHODS

### Substrates

Concentrated whey permeate from cheese making was obtained from Lactancia Parmalat (Victoriaville, QC, Canada) and Saputo (Sainte-Hyacinthe, QC, Canada). The whey contained on the order of 12 to 14% (wt/vol) solids, with a lactose content of between 100 and 125 g/L. Typically, volumes of 4 L were obtained and stored at 4°C until used.

Experiments were also performed with pure lactose solutions. These were prepared by mixing D-lactose (Sigma-Aldrich Canada Ltd., Oakville, ON) with distilled water to obtain lactose concentrations between 0.35 and 0.5 *M*.

### Determination of Lactose, Glucose, and Galactose

The concentrations of lactose, glucose, and galactose were determined using capillary electrophoresis (Dionex CES System I, Dionex, Sunnyvale, CA). A fused silica capillary (CElect FS75, Supelco Inc., Bellefonte, PA) of 75 $\mu$m i.d. and a length of 101 cm was used. The buffer consisted of disodium tetraborate (BDH Inc.), at a concentration of 60 m*M* adjusted to a pH of 9.1. Separation of the analytes was carried out under voltage control at 22 kV, with detection by absorbance at 195 nm. Aqueous standards were made with D-lactose (Sigma- Aldrich Canada Ltd., Oakville, ON), galactose (Fisher Scientific, Montreal, QC) and D-glucose (A&C Chemicals, Montreal, QC), with sucrose (Anachemia, Montreal, QC) added as the internal standard. The 95% confidence interval, quoted as a percentage of the mean, was approximately 4% for

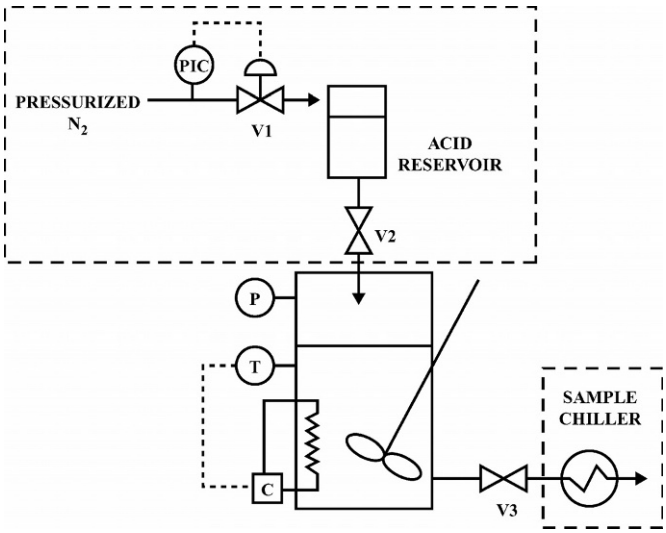


**Figure 1.** Apparatus used for hydrolysis experiments with inorganic acids. Temperature was controlled using a temperature controller (C), an electrical resistance heater, and a thermocouple (T). The reactor pressure (P) was monitored. The pressurized acid delivery system and sample chiller, identified in dashed boxes, were only utilized for experiments at temperatures greater than 95°C. In these cases, the reaction was initiated by adding acid under pressure, by opening valve V2. The nitrogen pressure was regulated (PIC). The chiller consisted of water at 4°C.

lactose (n = 2), 8% for galactose (n = 2), and 10% for glucose (n = 2).

## Hydrolysis Reactors

Depending on the temperature of hydrolysis, and the acidulant used, one of 3 different reactor configurations was used. For temperatures less than 100°C, with inorganic acids, reactions were carried out in an insulated glass reactor equipped with magnetic stirring (Figure 1). The temperature was controlled to within 0.2°C of the set point using a temperature controller, heating tape, and a Teflon-coated thermocouple. The glass reactor was fitted with a rubber stopper to prevent evaporation. The solution to be hydrolyzed was first heated to the desired temperature. The reaction was initiated by adding acid catalyst, either 12 *M* hydrochloric acid or 18 *M* sulphuric acid. No hydrolysis reaction occurred at the natural pH of the solutions, over the temperature range at which this reactor configuration was used. Samples (1 mL) were removed periodically from the reactor using a glass pipette, and immediately treated to neutral pH with sodium hydroxide to quench the reaction.

For reaction temperatures greater than 100°C, a closed vessel was required, since the pressures associated with these conditions were greater than atmospheric. Thus, for these experiments, a closed metal reactor was built (Figure 1). In control experiments, rates of reactions and products formation were found to be independent of the presence or absence of the metal components (data not shown). Agitation, heating, and temperature control were as before. To start the experiment, the liquid to be hydrolyzed was added to the reactor and was brought to the desired temperature. As before, at the natural pH of the solution and the temperatures tested, no measurable reaction occurred (data not shown). The desired amount of acid was then added to the reservoir located at the top of the reactor (Figure 1). The acid reservoir was then pressurized with nitrogen. The reaction was initiated by opening the valve (V2), isolating the reactor from the reservoir, injecting the acid into the reactor. As the system operated under positive pressure, samples were forced periodically from the reactor by opening a sample valve on the reactor. The sample port (V3) was connected to rubber tubing immersed in water at 4°C to halt the reaction of the withdrawn samples (Figure 1).

Experiments using carbonic acid as the acidulant were performed in a 15-mL stainless steel reactor, immersed in a sand bath equipped with temperature control (±1°C). To start the experiment, a 10-mL liquid volume was added to the reactor. The reactor was capped, and pressurized with $CO_2$ to the desired value. To ensure there was no leakage, pressure was measured periodically using a pressure gauge. The reaction was initiated by placing the reactor in the sand bath and allowing 2 min to equilibrate. Initial conditions were taken as being those of the sample withdrawn at 2 min. After the desired time in the sand bath, the reactor was withdrawn and quickly immersed in a 10°C water bath to quench the reaction. To get kinetic data, samples prepared and reacted under the same conditions were withdrawn at different times from the sand bath. The entire reactor was removed from the sand bath and quenched. Samples generated were frozen until analyzed.

## Principal Component Analysis

Due to the number of conditions and variables studied, a principal component analysis (**PCA**) was performed to simplify the analysis of the data. Principal component analysis is a powerful mathematical tool used to identify the "natural" or "latent" variables hidden within a set of data. For details about the PCA technique, see (Eriksson et al., 1999). The Unscrambler v7.6 (CAMO, Corvallis, OR) was used for the calculations. The validation method chosen was the full-cross validation. In this case, when iterating to calculate the latent variables, all the other samples are

**Table 1.** Hydrolysis results of whey permeate and lactose solution using pressurized $CO_2$. The pH was estimated in 2 ways; assuming that all solutions had buffering capacities similar to water ("no buffering"), and based on an empirical estimate of the buffering capacity ("buffered"). In all cases, the effect of $CO_2$ on the pH was estimated using the relationship of Van Walsum. An experiment was performed in which $H_2SO_4$ was added to whey permeate instead of $CO_2$ to provide similar pH to that obtained with $CO_2$, for comparison.

| Medium | Temperature (°C) | $CO_2$ pressure (kPa(g)) | pH | | Apparent rate constant ($min^{-1}$) |
|---|---|---|---|---|---|
| | | | No buffering | Buffered | |
| Whey permeate | 220 | 2758 | 3.92 | 5.04 | 0.4701 |
| | | 4137 | 3.79 | 5.03 | 0.4596 |
| | | 5516 | 3.71 | 5.02 | 0.2471 |
| | 200 | 2758 | 3.84 | 4.98 | — |
| | | 4137 | 3.69 | 4.95 | — |
| | | 5516 | 3.61 | 4.93 | — |
| | 180 | 2758 | 3.77 | 4.97 | 0.0377 |
| | | 4137 | 3.6 | 4.93 | 0.0452 |
| | | 5516 | 3.52 | 4.89 | 0.0476 |
| | 180 | 0.2 m*M* $H_2SO_4$ | 3.7 | 5.00 | 0.0589 |
| Lactose solution | 200 | 5516 | 3.61 | 3.61 | 0.1586 |

used in validation. The data were centered and scaled prior to processing.

## RESULTS

A wide range of hydrolysis conditions was investigated. Acidic conditions were created using either aqueous inorganic acids, or gaseous carbon dioxide. In both cases, experiments were performed using both prepared aqueous lactose solutions and industrial whey permeate. Temperatures between 70 and 220°C were studied. In all figures, the error bars represent the upper and lower 95% confidence lines associated with the calibration curve.

### Hydrolysis with Carbon Dioxide

The feasibility of using carbon dioxide to create acid conditions was examined. Samples were charged with $CO_2$ at pressures of 2758, 4137, and 5516 kPa(g), at room temperature. The hydrolyses were carried out at temperatures between 180 and 220°C. Elevated temperatures were necessary to compensate for the relatively high pH values of the reacting solutions. As a highly pressurized vessel was used for hydrolysis, direct measurement of pH was not possible. Therefore, to compare the rates with carbonic acid to those with mineral acid, it was necessary to estimate the pH of the carbonic acid solutions. Solution pH was estimated at various temperatures and carbon dioxide partial pressures using the empirical relationship developed by van Walsum (2001). Strictly speaking, the equation is valid only for processes carried out in pure water; hence, estimates are truly applicable only for the prepared aqueous solutions of lactose. The pH values of whey permeate were also estimated using this relationship, in the absence of a detailed compositional analysis. The limitations of this assumption will be discussed later. The pH values estimated are shown in Table 1, under the column labeled "No Buffering."

Figure 2 shows a typical profile for the transient sugar concentrations obtained for hydrolysis of lactose in permeate using pressurized $CO_2$. As a control, hydrolysis experiments were also performed without $CO_2$. A maximum in the total hexose sugar concentration occurred after approximately 15 min, hence both monosaccharides are converted into by-products. Although both hexose concentrations achieve maxima, the final galactose concentration is 4 times higher than

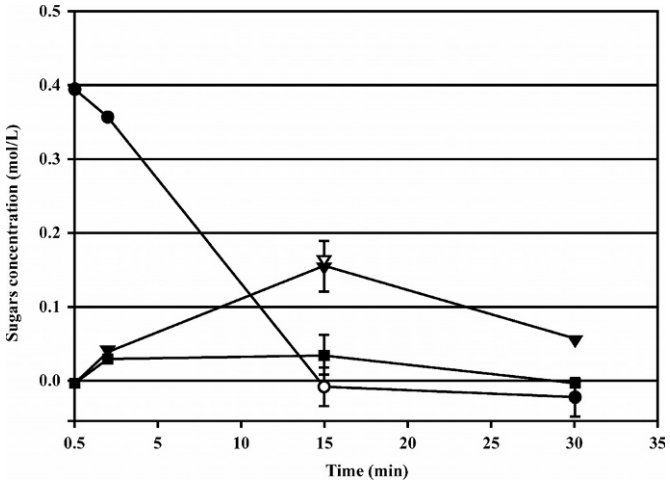


**Figure 2.** Hydrolysis of lactoserum at 200°C in the presence (closed symbols) and absence (open symbols) of carbon dioxide. Data are for a carbon dioxide partial pressure of 2758 kPa(g) $CO_2$. Transient concentrations of lactose (●,○), galactose (▼,▽) and glucose (■,□) are shown. The open square at 15 minutes is hidden by the dark symbol.

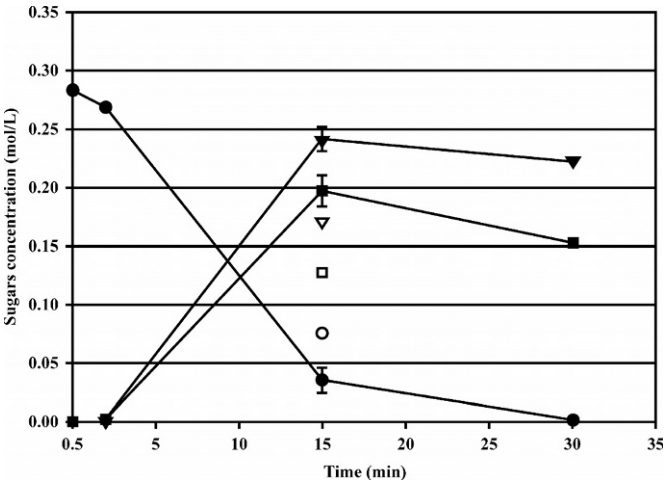


**Figure 3.** Hydrolysis of a lactose solution carried out at 200°C in the presence of $CO_2$ at 5516 kPa(g) (closed symbols) and without $CO_2$ (open symbols). Transient concentrations of lactose (●,○), galactose (▼,▽) and glucose (■,□) are shown.

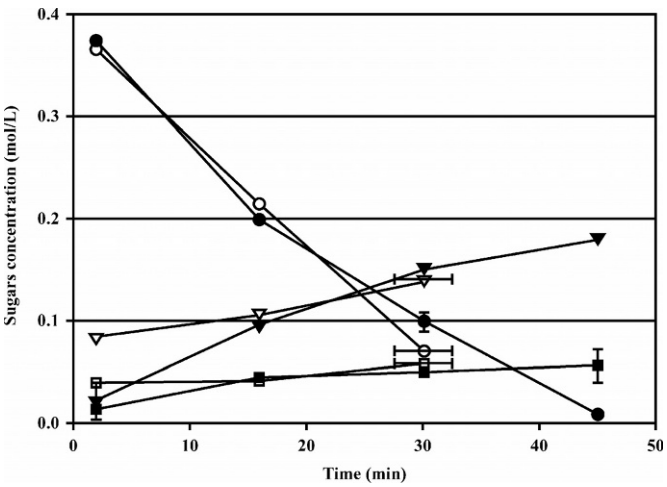


**Figure 4.** Comparison of the transient concentrations of lactose (●,○), galactose (▼,▽) and glucose (■,□) for the hydrolysis of lactoserum at 180°C, catalyzed by 0.2 m*M* sulfuric acid or by carbon dioxide. Open symbols correspond to the $H_2SO_4$ catalyzed experiment. Closed symbols show hydrolysis in the presence of $CO_2$ at a partial pressure of 4137 kPa(g). Horizontal error bars represent the uncertainty in the time measurement at 30 min, due to a problem with the timer.

that of glucose (0.15 vs. 0.04 *M*). Thus, the conversion of galactose into by-products occurs to a lesser extent.

The extent of degradation of hexose sugars into by-products could be estimated qualitatively by observing the solution in the reactor. Over time, the solution became increasingly dark brown, and a precipitate formed. These color changes are associated with the browning of the sugars (Mauron, 1981; Del Pilar Buera et al., 1987b). By inspection, the quantity of precipitate appeared to increase with time. After 30 min, the solution left was black, and had a burnt odor.

Data collected at temperatures of 220 and 180°C also exhibited similar trends for all carbon dioxide partial pressures tested. The maximum amount of galactose produced increased with decreasing temperature, reaching a maximum conversion of 47% at 180°C. The maximum concentration reached for galactose was about twice as great at 200°C compared with 220°C. For the experiment at 220°C, both sugars were completely decomposed after 15 min. It is also apparent that the hydrolysis of the lactose in lactoserum does not require $CO_2$, when carried out at elevated temperatures (Figure 2).

As a control, an experiment was performed using carbon dioxide as an acidulant, but in a solution of lactose and distilled water only (Figure 3). Once again, the initial formation and then decomposition of both hexoses sugars was observed. Despite lower initial lactose concentration in pure lactose solutions than in whey permeate, higher maximal concentrations were obtained for both glucose and galactose in the pure sugar solution than in the whey permeate. At the maximum monosaccharide yield, only 5% of the galactose is lost compared with 22% of the glucose. Again, the hydrolysis of lactose occurred with and without pressurized $CO_2$; however, the rate of hydrolysis increased with pressurized $CO_2$.

To isolate the effect of carbon dioxide, an experiment was performed in whey permeate at the same pH but with sulfuric acid as an acidulant (Figure 4). A pH of 3.7 was targeted, in the same range as that estimated with $CO_2$ (Table 1). In estimating the amount acid required, we did not account for the buffering capacity of the lactoserum solution. Furthermore, based on estimates of the $pK_a$ values at these temperatures, it was assumed that sulfuric acid liberates only one of its 2 available protons (Hovey and Hepler, 1990). Based on these assumptions, a final concentration of 0.2 m*M* of $H_2SO_4$ was added to provide the desired pH of 3.7. Data from this experiment are similar to those generated using carbon dioxide (Figure 4). Both of the hexose sugars released by hydrolysis react further, as yield was less than stoichiometric. The experiment was not continued sufficiently long to identify maximum yield of hexose sugars.

## Hydrolysis with Inorganic Acids

Both HCl (0.5 and 1 *M*) and $H_2SO_4$ (between 0.15 and 0.5 *M*) were tested for the potential to catalyze the hydrolysis reaction. A typical curve obtained during hydrolysis is shown in Figure 5. As the concentration of lactose decreased, the concentration of galactose

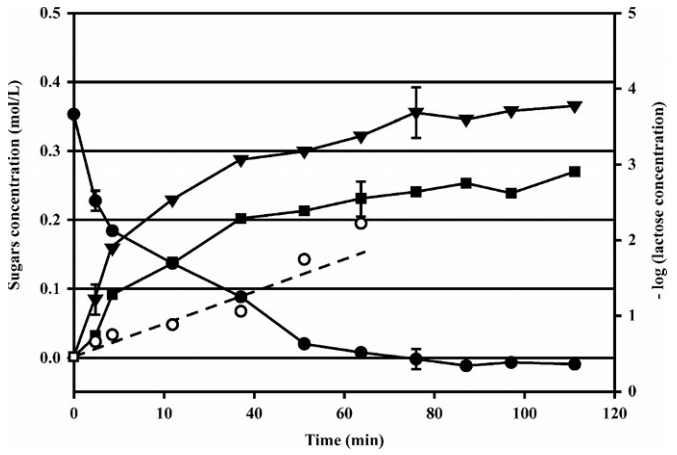


**Figure 5.** Hydrolysis of lactose in whey permeate at 90°C in 1 *M* HCl. [▼] = galactose, [■] = glucose, [●] = lactose. The $\log_{10}$ of the lactose concentration (○) is also shown as a function of time—it supports the assumed reaction order. The dotted line is the regression line from which the apparent rate constant was estimated.

increased in stoichiometric proportion. However, less than one mole of glucose was released per mole of lactose consumed, indicating that side reactions occurred involving glucose. An important variable, considering the product will be later fermented, is the amount of hexose sugars recovered after the hydrolysis. As no galactose is lost in side reactions, the cumulative recovery of glucose from lactose is given by:

$$R = \frac{H_1}{H_2}, \quad (1)$$

where $R$ is the recovery of glucose after hydrolysis, $H_1$ is the concentration of glucose, and $H_2$ is the concentration of galactose at the end of the hydrolysis.

For all conditions, the hydrolysis of lactose went to completion. As the reaction proceeded, a yellowish color developed, changing slowly to a light caramel brown finally to a darker brown, depending on the reaction conditions. The color was much more pronounced for the hydrolysates of whey permeate than for reactions involving prepared lactose solutions. In any case, the color development during the hydrolysis was expected. It is known to be due to browning and caramelization of the sugars (Del Pilar Buera et al., 1987b).

The apparent rate constant ($min^{-1}$) associated with the conversion of lactose was determined assuming first-order kinetics. This assumption is based on the literature (Timell, 1964) and was supported by the data (Figure 5). Over the range of conditions studied, the time of hydrolysis varied between 2.5 min and 25 h. The glucose recovery indicated that between 12 to 32% of the glucose was lost in side reactions.

**Table 2.** Loadings for the first 2 components of a principal component analysis on mineral hydrolysis data. Principal component 1 (PC1) described 62% of the variance, while PC2 described 21%.

| Variable | PC1 | PC2 |
|---|---|---|
| Temperature | –0.464 | 0.289 |
| Acid concentration | 0.526 | –0.159 |
| Acid type | 0.516 | –0.234 |
| Media | –0.441 | –0.35 |
| Glucose recovery | 0.217 | 0.845 |

Principal component analysis was applied to the hydrolysis results. Table 2 shows the loadings associated with principal components (**PC**) 1 and 2, generated by the analysis. These 2 components were able to explain 62 and 21% of the total variance of the data set, respectively. In the PCA technique, 2 variables with similar loadings indicate that they are correlated (Eriksson et al., 1999). Based on the loadings of component 1, the temperature of hydrolysis is correlated with the medium used, and the acid concentration is correlated with the acid type used. The glucose recovery is not strongly correlated with any of the other variables on any of the components. Figure 6 shows the hydrolysis data plotted in terms of the first 2 latent variables, PC1 and PC2. This plot, referred to as a score plot, is very useful in detecting related clusters in the data.

From Figure 6, the data naturally separate into 3 clusters. The groupings are based on the whether whey

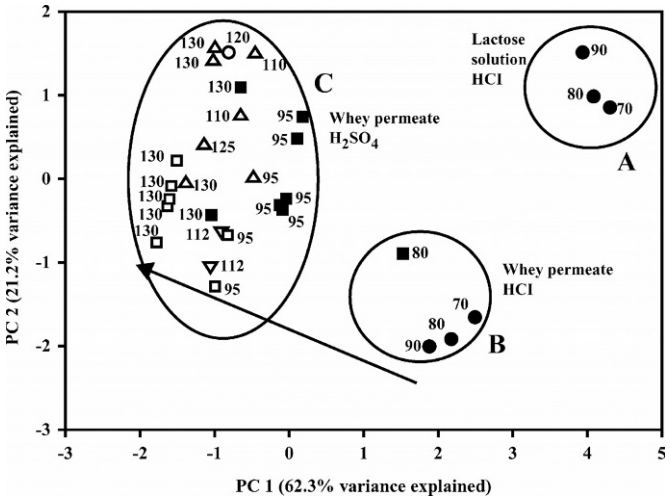


**Figure 6.** Scores associated with the first two principal components for the mineral acid hydrolysis data. The numbers represent the temperature of hydrolysis, in °C. The symbols represent the concentration of acid used for hydrolysis; 1 (●), 0.5 (■), 0.325 (▽), 0.25 (△), 0.21 (○), and 0.15 *M* (□). The type of acid and the lactose containing media is specified next to the clusters.

permeate was used and on the acid type. Specifically, all data resulting from the hydrolysis of prepared lactose solutions grouped together (cluster A). Data associated with lactoserum grouped as a function of acid type. Sulfuric acid hydrolysates appear in cluster C and hydrochloric acid results in cluster B. The hydrolysis of lactoserum clearly has lower values of $R$ compared with the lactose solutions for the same hydrolysis conditions (clusters A and B).

## DISCUSSION

In principle, carbon dioxide can be used to create the acidic conditions needed to catalyze the hydrolysis of lactose. Unlike mineral acids, the acidulant can be stripped out of the aqueous solution following the treatment, making the use of carbon dioxide an attractive option. As a weak acid, dissolved $CO_2$ cannot lower the pH to levels achievable with inorganic acids, and elevated temperatures are required for hydrolysis. However, at these temperatures, the hydrolysis of lactose was observed even without $CO_2$, in prepared lactose solutions (Figure 3). The natural pH of the lactose solution, which was approximately 3.5 at room temperature, was adequate to catalyze the hydrolysis at 200°C. However, the addition of $CO_2$ further decreased the pH of the solution, thus increasing the rate of hydrolysis.

Similarly, hydrolysis of lactose was also observed in whey permeate without the addition of an acidulant of any kind (Figure 2). Under these conditions, a faster rate was measured in the whey permeate compared with the lactose solution. This result was unexpected, as the natural pH of lactoserum (~5.0) is higher than the natural pH of the pure lactose solutions (~3.5). These results imply that the other components in whey (mainly salts, Hobman, 1984) affected the activity of one or more of the components involved in the hydrolysis, with the effect of increasing the observed rates of hydrolysis, compared with the prepared lactose solutions. This effect was mentioned in the literature (Aoyama and Seki, 1999).

Rates of hydrolysis associated with whey permeate solutions did not increase in the presence of a $CO_2$ atmosphere, compared with those without $CO_2$. This may be due to the buffering capacity of the whey permeate. Since a pressurized apparatus was used for the $CO_2$ hydrolysis experiments, titrations could not be performed at reaction conditions. Therefore pH was estimated using a modeling approach. First, the buffering capacity of the whey permeate was modeled as a 2-component system having pK values of 0.15 and 5.3, respectively. This gave results close to the experimental curve generated in the absence of carbon dioxide (Figure 7, open diamond). The simulation was then expanded to include the effect of $CO_2$ at various pressures and temperatures on the pH. This was accomplished by solving the equations associated with the mass balance, the carbon dioxide equilibria, and the charge balance. The temperature and pressure dependence of the relevant equilibria, and the physical properties of water were also considered (van Walsum, 2001). From this exercise, it was shown that, due to the buffering capacity of the permeate, little change in the pH is expected with $CO_2$ at 5516 kPa(g), at a temperature of 200°C (Figure 7). As a result, the rate of hydrolysis was not affected appreciably. Unfortunately, the model results also indicate that the pH values estimated assuming that whey permeate buffered similarly to water were incorrect (Table 1).

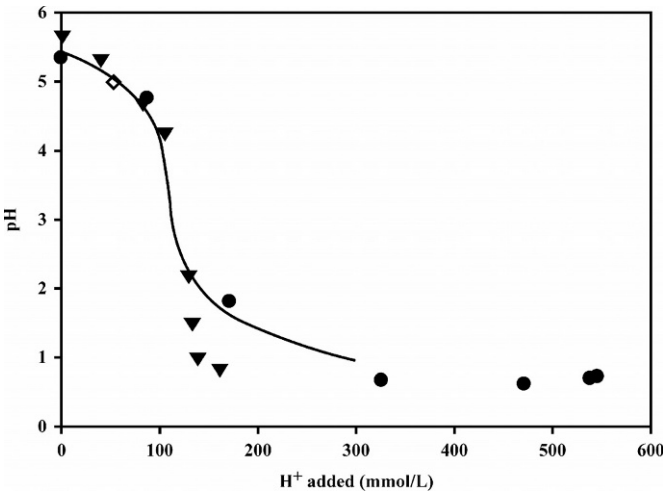


**Figure 7.** Neutralization curve of the whey permeate with $H_2SO_4$ (▼) and HCl (●). The closed symbols represent neutralization data gathered in air, at room temperature. The line represents the equation developed to describe the buffering capacity of the lactoserum. The open symbol represents the estimated pH considering the buffering capacity of the medium in the presence of carbon dioxide at a partial pressure of 5516 kPa(g), and a temperature of 200°C.

The buffering capacity of the whey permeate also explains the similarity between the hydrolysis results obtained using $CO_2$ and sulphuric acid, at room temperature (Figure 4). The natural pH of the lactoserum used during the experiments prior to acid addition was 5.01. To achieve the target pH of 3.7, it was estimated that an acid concentration of 0.2 m$M$ of $H_2SO_4$ was required, not considering the buffering capacity of the whey permeate. Repeat experiments carried out following the hydrolysis experiments indicated that the actual pH in these solutions was 4.97, and not 3.7. This value was close to that estimated using the model for pressurized $CO_2$ (Table 1). In conclusion, due to the buffering capacity of the permeate, the addition of

$CO_2$ or dilute $H_2SO_4$ did not cause a significant change in the pH, resulting in similar rates of reaction to that obtained without acidulant (Figures 2 and 4).

The rate data collected for the hydrolysis of lactose solution and whey permeate using mineral acids at lower temperatures (70 to 130°C) were similar to those reported in the literature (Ramsdell and Webb, 1945; Timell, 1964; BeMiller, 1967, Coughlin and Nickerson, 1975). No literature was found in which free mineral acids were used to catalyze the hydrolysis reaction at the higher temperatures, using both lactose solutions and whey permeate, although studies have been carried out using strong acid cation exchange resin to acidify the solutions at these temperatures (Robbertsen et al., 1978; De Boer and Robbertsen, 1981).

The rate of lactose hydrolysis is a function of the lactose concentration, the temperature, and the amount of acid catalyst present. These dependencies are captured in equation (2), where the apparent rate constant ($k_{APP}$) is a function of the concentration of free protons, and the intrinsic rate constant ($k$). The latter is dependent on temperature only.

$$r = k_{\mathrm{APP}}S = kfn(H^+)S \qquad (2)$$

Here, '$S$' is the concentration of a sugar, in this case lactose.

At low acid concentrations, the rate of hydrolysis is found to depend linearly on the amount of free protons, and thus $fn(H^+) = H^+$. This dependence on catalyst concentration is typical of many catalytic reactions.

At high acid concentrations (≥0.5 $M$), the simple dependence of rate on acid concentration deviates from linearity. Under these conditions, the logarithm of the apparent rate constant varies linearly with the negative of Hammet's acidity function ($-H_0$), defined in equation (3) (Hammett, 1935; Long and Paul, 1957; Timell, 1964). This result is related to the mechanism by which the hydrolysis reaction is known to occur. In the first step, lactose is ionized with a proton to form $SH^+$. The second step of the reaction is rate limiting, and involves the decomposition of the disaccharide into 2 monomeric units. Equation (3) follows if it is assumed that the ionization reaction is reversible and is always at equilibrium.

$$H_0 = -log_{10}\frac{a_{H^+}\gamma_S}{\gamma_{SH^+}} = -log_{10}(h_0) \qquad (3)$$

where $a_i$ and $\gamma_i$ are the activity and activity coefficients of species $i$, respectively. Comparing equations (2) and (3), it is apparent that if $log_{10}(k_{\mathrm{APP}}) \propto -H_0$, then it follows that $k_{APP} \propto h_0$. In dilute solutions, where the activity coefficients are close to unity, $H_0$ is equal to pH (equation (3)), and $h_0$ is then equivalent to $H^+$. Therefore, in general, the intrinsic rate constant can be calculated from measured values of the apparent rate constant for both concentrated and dilute acids using equation (4).

$$k = \frac{k_{\mathrm{APP}}}{h_0} \qquad (4)$$

To apply equations (3), an estimate of $h_0$ is required. Values of $H_0$ for a range of mineral acid concentrations and types can be found in the literature (Timell, 1964). However, these values must be adjusted for the conditions used in this study. As a first approximation, it was assumed that $h_0$ is not a strong function of lactose concentration and temperature. This assumption was made out of necessity, due to the limited data available. For solutions with high pH, $h_0$ can be replaced directly with the proton concentration, as discussed above. This approach is justified for data obtained when $CO_2$ was used as an acidulant, as the pH values were rather high.

For experiments involving strong acids, the nonideal nature of the solutions could not be ignored. It has been shown that the ratio of $\gamma_S$ to $\gamma_{SH+}$ is constant during the hydrolysis of a wide range of carbohydrates under a variety of conditions. Therefore, the ratio of the activity coefficients associated with the ionized and unionized substrate in equation (3) was estimated from $H_0$ values extracted from the literature, and then applied to the current study. The ratio was then multiplied by the proton activity, yielding the desired estimate of $h_0$. As discussed, whey permeate has significant buffering capacity, which influences the pH attained. In these cases, the activity of $H^+$ was estimated from the neutralization curve (pH from Figure 7). Using equation (4), the intrinsic rate constant was then calculated for all data collected using either lactose solution and whey permeate, and for the various acid types and concentrations (Figure 8). As can be seen, all data fall on the same line, suggesting validity of the approach used. The temperature dependence of the intrinsic rate of hydrolysis is described well by an Arrhenius-type relationship. The activation energy was estimated as 38.7 kcal/mol.

A number of important reactions are known to compete with the desired hydrolysis. In prepared solutions containing lactose, glucose concentrations obtained from hydrolysates generated with mineral acid were lower than expected, based on stoichiometry. Thus, glucose is involved in side reactions and is consumed as it is liberated from the lactose molecule. The galactose concentrations, being present in stoichiometric amounts, indicate that if this molecule is involved in

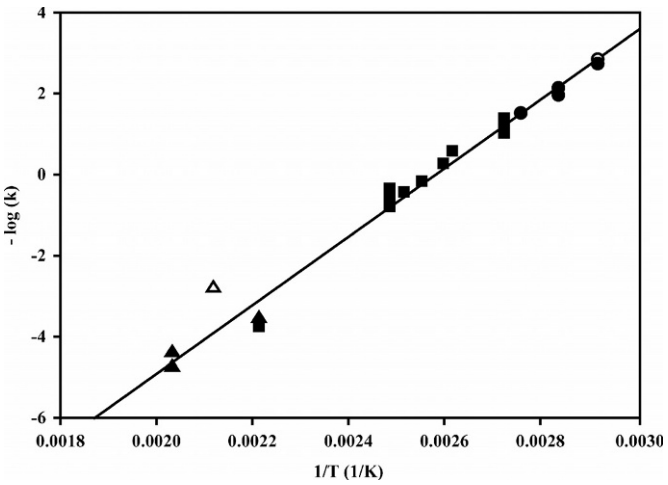


**Figure 8.** Temperature dependency of the intrinsic rate constants for the hydrolysis of whey permeates (closed symbols) and prepared solutions (Open symbols). All conditions are considered. Data points represent results using HCl (●,○), $H_2SO_4$ (■,□), $CO_2$ (▲,△) The rate constants are quoted in units of $min^{-1}$. The line is from regression. In some cases, data points are not visible as they overlap.

side reactions, these processes are negligible compared with those involving glucose.

For prepared lactose solutions containing no amine compounds, according to the literature, 2 classes of reactions involving glucose are important, reversion reactions and caramelization reactions. The reversion reactions occur when the charged intermediate generated in the early stages of the hydrolysis mechanism combines with another monosaccharide in the last step of the reaction, instead of with water. This results in the formation of a disaccharide (Peat et al., 1958; BeMiller, 1967; Huh et al., 1991). When the rates of hydrolysis and reversion are equal, the concentration of reactants and products should remain constant, as observed (Figure 5) and as described in the literature (Moelwyn- Hughes, 1928). Under acidic conditions, a monosaccharide concentration of 0.1 *M* has been shown to be large enough to compete effectively with the solvent (water) in the last step of hydrolysis (Ferrier and Collins, 1972).

The caramelization process is made up of 2 parallel reactions involving glucose, anhydrosugar formation and furan production (Ferrier and Collins, 1972; Del Pilar Buera et al., 1987a, 1987b). If further decomposition of the hexose sugars occurred exclusively by caramelization, the hexose sugar concentration would decrease, eventually reaching zero. This result was not observed under any of the conditions tested in the presence of lactose solutions, using mineral acids. Considering our results, it is reasonable to assume that the loss of glucose is mainly due to reversion, which would cause formation of disaccharides. No attempts were made in the current study to identify these compounds in the product mixture.

The stable, stoichiometric galactose concentrations observed here contradict the results of Huh et al. (1991). They reported that disaccharides containing monomers of galactose are formed during acid hydrolysis of lactose. Our results indicate that only glucose is involved in side reactions to any appreciable extent. However, Huh et al. did not quantify the products. In earlier studies, it was reported that the ease of hydrolysis of polysaccharides is related to the chemical linkage joining the monomeric sugars. In order of decreasing rate of reversion, the effect of the chemical linkage is: $\alpha$-(1-6) < $\beta$-(1-6) < $\beta$-(1-4) < $\beta$-(1-3) < $\beta$-(1-2) (Wolfrom et al., 1963). The most difficult disaccharides to hydrolyze contain the (1-6) linkage, which is formed preferentially (Peat et al., 1958; Huh et al., 1991). In another work, it was found that disaccharides containing glucose are more difficult to hydrolyze than the ones containing galactose (BeMiller, 1967). Based on these reports, it is reasonable to expect that disaccharides containing mainly glucose would be formed preferentially over those containing galactose, explaining the fact that only glucose is disappearing. This hypothesis is supported by the work of Silberman, in which solutions containing galactose or glucose in 1 to 4 *M* HCl were heated at 98°C for 30 to 120 min (Silberman, 1961). No furans were detected, and anhydrosugars were detected only in trace amounts. Both monosaccharides formed reversion products, with glucose having a greater tendency to do so.

Data from the hydrolysis experiments catalyzed using mineral acids were subjected to a PCA. The PCA technique generates a series of "natural," orthogonal variables that best describe the data set. The new variables, PC, are extracted in decreasing order of their importance in describing the variance in the raw data set. Hence, PC1 describes more of the variance in the data than PC2, and so on. When processed in this manner, the data are separated into natural clusters (Figure 6). Data within a given cluster are similar. Interestingly, the data associated with clusters A and B differ only in the source of lactose (Figure 6). Based on the loadings of the original variables on PC2, the clusters differ significantly in the recovery of glucose (Table 2). In all cases, less glucose was recovered in the hydrolysis products derived from the whey permeate. Because the hydrolysis conditions were the same, this indicates that other components present in the lactoserum permeate favored the side reactions. Again, the galactose was present in stoichiometric amounts, indicating that the side reactions involved mainly glucose.

Data associated with the hydrolysis of lactose in the whey permeates are more difficult to interpret, as the solutions are chemically more complex. Even though most of the proteins are removed from the whey during the ultra filtration process, some nitrogen, typically 0.7%, wt/wt, on a solid basis, is present as protein and other nitrogen-containing compounds (Hobman, 1984). The increase in glucose losses in whey permeate compared with the lactose solution can be attributed to Maillard reactions, which occur in the presence of amine compounds (Mauron, 1981). The Maillard reactions occurred in parallel to the glucose reversion reactions in lactose solution, increasing the loss of glucose during the processing of the permeate samples. At the temperatures at which this series of experiments were performed (70 to 90°C), the caramelization reactions do not occur at any appreciable rate and were thus not considered in the analysis (Saeman, 1945).

In the first stage of Maillard reaction, the sugar combines with the amine group through a reversible reaction to form Amadori compounds. The Amadori compounds then react irreversibly through various pathways, depending on the temperature and the pH conditions. In some of these reactions, furans are produced, while in others the products may include reductones, dicarbonyls, among others. These compounds may then react further by a number of pathways, through recombination with the amine groups, to produce stable compounds that are responsible for the aromas and flavors in food. These end products include the *O*-heterocyclics such as dehydrofuranone, maltol dehydropyrone, and the N-heterocyclics such as the thiazoles, the pyrazines, and the pyridines (Mauron, 1981). In addition to reactions involving amino groups, these compounds can also be formed by other mechanisms such as Strecker degradation, involving the oxidative degradation of amino acids by intermediates produced upon breakdown of Amadori compounds. Ultimately, once all the amine groups involved in Maillard reactions have been depleted, sugar losses by the Maillard reactions become unimportant. At this point, decomposition of the monosaccharides is due solely the caramelization browning process. In the case in which dehydration reactions not involving amine groups, such as caramelization, occur to a very small rate or extent, the glucose concentration would be expected to remain relatively stable upon depletion of the amine compounds. This was the situation observed in the hydrolysis results involving lactoserum permeate (Figure 5).

In a relevant study that supports this claim, various sugars were heated with or without glycine, an amino acid (Del Pilar Buera et al., 1987b). The products of the various side reactions, as measured by color development, reached stable values after a period of time, which varied with the sugar tested. An equilibrium is established with competing side reactions, producing stable final sugar concentration after all nitrogen compounds are depleted. Stable final sugar concentrations were also observed in our study. Galactose does not appear to be involved in Maillard reactions, as none was consumed. However, no literature was found regarding the participation of galactose in Maillard browning.

At the lowest temperatures studied it was suggested that mainly reversion reactions occur and very little caramelization reactions had occurred. However, under more severe conditions, at 130°C in 0.5 *M* $H_2SO_4$ it is very likely that these reactions were important. It is believed that because the hydrolysis reaction was stopped once 100% conversion of the lactose substrate was achieved, a decrease in hexose sugars due to caramelization reactions could not be seen. It is postulated that these reactions would have been observed if the reaction time had been extended. As an example, at the most aggressive conditions tested (130°C and 0.5 *M* $H_2SO_4$), the reaction was stopped after 2.5 to 3 min. Based on the rate data of Seaman (1945), under these experimental conditions less than 9% of the glucose released through hydrolysis would have been lost by caramelization, after 3 min. This value would be difficult to detect with the sugar measurement technique used in this study. However, after 60 min, it is estimated that 16% of the initial glucose is decomposed, and about the same for galactose, for a combined loss of 32% of the lactose products released. This calculated value is close to the 40% of hexoses lost during the hydrolysis of lactose solutions reported by Ramsdell et al. at these conditions (Ramsdell and Webb, 1945). If the presence of salts effectively increases the rate of degradation, an even lower amount of hexoses would have been measured after 60 min. A measurement of HMF (data not shown) confirmed that caramelization reactions occurred.

### Effect of Hydrolysis Temperature

It is known that the rates of the Maillard reactions increase with increasing temperature (Mauron, 1981). For a fixed temperature, the amount of Maillard compounds formed, as measured colorometrically, increases as a function of the square of the reaction time, $t^2$ (Mauron, 1981). The nature of the specific reactions is also a function of temperature, yielding different flavor compounds at different temperatures in food. Cluster B in Figure 6 includes the experiments performed using HCl at temperatures ranging from 70 and 90°C. Within the cluster, the data points increase

in PC2 with decreasing temperature. Decreasing the temperature of hydrolysis had a positive effect on the amount of glucose recovered from hydrolysates of whey permeate. The opposite trend was observed in pure lactose solutions using the same acid type (cluster A). As discussed, while only reversion reactions occur in prepared lactose solutions, the reversion and Maillard reactions compete in the lactoserum permeate. If the Maillard reactions are more temperature sensitive, the downward trend with temperature is dictated by Maillard reactions, which offsets the positive influence of the reversion reactions. This explains the overall negative effect of temperature shown in the data (cluster B).

The PCA analysis for the hydrolysis at higher temperatures (>90°C) in $H_2SO_4$ is shown by cluster C (Figure 6). From these data, the effects temperature and acid type cannot be decoupled, since experiments were performed with lower temperatures (70 to 90°C) and high acid concentration for HCl (cluster B), and high temperatures (95 to 130°C) and low acid concentrations for $H_2SO_4$ (cluster C) (Figure 6). The same conclusion is reached from the study of the PCA loadings (Table 2). Namely, the temperature of hydrolysis and media used are highly correlated on PC1, which is an artifact of the chosen experimental conditions. The glucose recovery does not correlate strongly with any of the variables. This is probably because there is more than one competing side reaction, and the relative impacts depend on temperature and other reaction conditions.

From the literature, lowering the pH inhibits the Maillard reactions, as the reaction pathways are different in acidic solutions than in slightly acidic, neutral, or alkaline solutions (Mauron, 1981; Del Pilar Buera et al., 1987a). This implies that a lower pH should lead to an increase in the glucose recovery ($R$) for reactions carried out in permeate solutions. Our study gives a different trend. For experiments with HCl, increasing the pH decreased the glucose consumed (Figure 6, cluster B). The hydrolysate of lactoserum in 0.5 $M$ HCl and 80°C and has a higher score on PC2 than hydrolysates at 1 $M$ HCl. However, the pH is also known to influence the disaccharide formation that is also catalyzed by acid. In the reversion reaction, a higher acid concentration during hydrolysis favors the combination of the cation of the monosaccharide with another monosaccharide in the last step of the mechanism of hydrolysis (Stanek et al., 1965). This has the effect of decreasing $R$, and could explain the results obtained. Changes in pH have the opposite effect on the Maillard reactions. Thus, the Maillard and reversion reactions compete, making interpretation of the results in the whey permeate difficult.

When looking at the effect of acid along a temperature isotherm for experiments using $H_2SO_4$ (cluster C), the data are scattered. At temperatures between 94 and 106°C, the maximum in $R$ seems to be achieved at the highest acid concentration (0.5 $M$). At higher temperatures, the maximum is obtained with acid concentrations between 0.21 and 0.5 $M$ acid. For all data gathered using $H_2SO_4$ (94 to 130°C), it is obvious that the acid concentration of 0.15 $M$ yields the lowest $R$. The fact that the highest pH (lowest acid concentration) always yielded lower glucose recovery could be related to the increase of browning noted in the literature, with decreasing acidity (Del Pilar Buera et al., 1987a). The opposite trend was seen for HCl at low temperatures (70 to 90°C).

The absence of a trend for the glucose recovery of all data with acid concentration and temperature is difficult to explain. It may be due to the complexity of Maillard reactions, and that the rates and reaction pathways change according to pH, temperature, time, and nature of amino groups present. All these factors affected transient glucose concentrations observed during hydrolysis. The source of the whey permeate also varied with experiments. The difference in solids concentration between batches suggests a different concentration of amine compounds. Whey obtained from the production of different cheese had different compositions of amino acids (Hobman, 1984). In one study, it was noted that during the acid hydrolysis of permeate, the brown color formed was partly dependent on the NPN content (De Boer and Robbertsen, 1981). All of these factors possibly had an effect on the specific Maillard reactions that occurred, and could possibly explain a part of the variation in the results.

### Hydrolysis Using $CO_2$

During the hydrolysis using mineral acids, the yield of galactose was always greater than the yield of glucose (Figure 5). During the hydrolysis using carbonic acid, the same phenomenon could be seen, and the differences between the yields of galactose and glucose were even greater (Figure 2).

The glucose and the galactose concentrations achieved maxima during the hydrolysis of lactose solutions catalyzed by carbon dioxide (Figure 3). Hence glucose and galactose are degraded as they are produced. If the hydrolyses were left for longer, the degradation would have gone to completion. As discussed previously, the presence of reversion reactions results in a constant concentration of the reaction products, while the Maillard reactions occur only in presence of nitrogenous compounds. Therefore, it is postulated that the caramelization reactions are responsible for

the continuous degradation of the 2 monosaccharides. This is not surprising given the high temperatures at which these experiments were carried out. Therefore, the increase in the amount of hexose sugars lost during $CO_2$–catalyzed hydrolyses, compared with those catalyzed by mineral acids, for both whey permeate and lactose solutions, is attributed to the increased rate of the caramelization.

Other factors could also be involved in increasing the loss of the glucose and galactose produced when using $CO_2$ at high temperatures compared with mineral acids at lower temperatures. It is possible that at these higher temperatures enough energy is present to involve the galactose degradation via Maillard reactions, which was not seen at lower temperatures, up to 130°C. The Maillard reaction is greatly influenced by the temperature and pH—at higher pH the Maillard reactions accelerate and different products are formed (Mauron, 1981; Del Pilar Buera et al., 1987b). This could also have caused an increase in the sugars lost compared with lower temperatures and lower pH. The presence of whey components could also have caused an increase in the loss of sugars seen in the whey permeate. It was previously shown that the rate of degradation of sugars at high temperatures is affected by the presence of salts (Aoyama and Seki, 1999).

## CONCLUSIONS

Hydrolysis of lactose solutions and whey permeate is feasible using mineral acid. Hammet's acidity function combined with Arrhenius temperature dependency described well the rate dependence on acid concentration, acid type, and reaction temperature. Although the amount of glucose released is less than that of galactose, acid hydrolysis appears to be a valid pretreatment to generate fermentable sugar monomers from lactose. The reversion reaction was shown to be the main competing reaction, and the caramelization reaction producing inhibitory compounds for the yeast (HMF) did not occur at any appreciable rate.

The hydrolysis of lactose with and without carbonic acid takes place at very high temperatures (180 to 220°C). However, an important amount of hexose sugars are lost in side reactions due to caramelization. Lactose hydrolysis with $CO_2$ at high temperature is not a suitable pretreatment for fermentation.

## ACKNOWLEDGMENTS

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), and Tembec Inc. The authors would also like to thank Saputo and Lactancia-Parmalat for supplying the lactoserum samples

## REFERENCES

Aoyama, M., and K. Seki. 1999. Acid steaming for solubilization of bamboo grass xylan. Bioresource Technol. 69:91–94.

BeMiller, J. N. 1967. Acid-catalysed hydrolysis of glycosides. Adv. Carbohyd. Chem. 22:25–108.

CDIC. 2001. Subject: Canada 1999–2000 Milk utilization per class—Utilisation du lait par classe. Accessed July 2, 2002.

Coughlin, J. R., and T. A. Nickerson. 1975. Acid-catalyzed hydrolysis of lactose in whey and aqueous solutions. J. Dairy Sci. 58:169–174.

De Boer, R., and T. Robbertsen. 1981. A purified, hydrolysed lactose syrup made from ultrafiltration permeate. Neth. Milk Dairy J. 35:95–111.

Del Pilar Buera, M., J. Chrife, S. L. Resnik, and R. D. Lozano. 1987a. Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to caramelization of various single sugars. J. Food. Sci. 52:1059–1062,1073.

Del Pilar Buera, M., J. Chrife, S. L. Resnik, and G. Wetzler. 1987b. Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. J. Food. Sci. 52:1063–1067.

Eriksson, L., E. Johansson, N. Kettaneh-Wold, and S. Wold. 1999. Introduction to multi- and megavariate data analysis using projection methods (PCA & PLS). Umetrics, Umea, Sweden.

Ferrier, R. J., and P. M. Collins. 1972. Monosaccharide Chemistry. Penguin Books Ltd., Harmondsworth, Middlesex, UK.

Hammett, L. P. 1935. Reaction rates and indicator acidities. Chem. Rev. 16:67–79.

Hobman, P. G. 1984. Review of processes and products for utilization of lactose in deproteinated milk serum. J. Dairy Sci. 67:2630–2653.

Hovey, J. K., and L. G. Hepler. 1990. Thermodynamics of sulphuric acid: Apparent and partial molar heat capacities and volumes of aqueous $HSO_4^-$ from 10–55°C and calculation of the second dissociation constant to 350°C. J. Chem. Soc. Faraday T. 86:2831–2839.

Huh, K. T., T. Toba, and S. Adachi. 1991. Oligosaccharide structures formed during acid hydrolysis of lactose. Food Chem. 39:39–49.

Ingledew, M. 1995. The biochemistry of alcohol production. Chapter 7 in The Alcohol Textbook. T. P Lyons, D. R. Kelsall, and J.E. Murtagh, eds. Nottingham University Press, Nottingham, UK.

Larsson, S., E. Palmqvist, B. Hähn-Hägerdal, C. Tengborg, K. Stenberg, G. Zacchi, and N.-O. Nilvebrant. 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb. Tech. 24:151–159.

Long, F. A., and M. A. Paul. 1957. Application of the $H_0$ acidity function to kinetics and mechanisms of acid catalysis. Chem. Rev. 57:935–957.

Marwaha, S. S., and J. F. Kennedy. 1988. Review: Whey pollution problem and potential utilization. Int. J. Food Sci. Technol. 23:323–336.

Mauron, J. 1981. The Maillard reaction in food: A critical review from the nutritional standpoint. Prog. Food Nutr. Sci. 5:5–35.

Mawson, A. J. 1994. Bioconversions for whey utilisation and waste abatement. Bioresource Technol. 47:195–203.

Moelwyn-Hughes, E. 1928. The reactivity of glucose in presence of hydrochloric acid Part I. T. Faraday Soc. 24:321–330.

Murtagh, J. E. 1995. Whey as a feedstock for alcohol production. Chapter 4 in The Alcohol Textbook eds T.P Lyons, D.R. Kelsall, and J.E. Murtagh, Nottingham University Press, Nottingham, UK.

Ovsejevi, K., V. Graz, and F. Batista-Viera. 1998. $\beta$-Galactosidase from *Kluyveromyces lactis* immobilized on to thiosulfinate/thiolsulfonate supports for lactose hydrolysis in milk and dairy by-products. Biotechnol. Tech. 12:143–148.

Peat, S., W. J. Whelan, T. E. Edwards, and O. Owen. 1958. Quantitative aspects of the acid reversion of glucose. J. Chem. Soc.586–592.

Porro, D., E. Martegani, B. M. Ranzi, and L. Alberghina. 1992. Developments of high cell density culture of engineered *Saccharomyces cerevisiae* able to grow on lactose. Biotechnol. Lett. 14:1085–1088.

Ramsdell, G. A., and B. H. Webb. 1945. The acid hydrolysis of lactose and the preparation of hydrolyzed lactose syrup. J. Dairy Sci. 28:677–686.

Robbertsen, T., R. De Boer, and L. Radema. 1978. Some experiences with the preparation of an acid-hydrolyzed lactose syrup from UF-permeate. Pages 950–951 in Proc. 20th Int. Dairy Congress, Paris, France.

Saeman, J. F. 1945. Kinetics of wood saccharification: Hydrolysis of cellulose and decomposition of sugars in dilute acid at high temperature. Ind. Eng. Chem. 37:43–52.

Silberman, H. C. 1961. Reactions of sugars in the presence of acids: a paper chromatographic study. J. Org. Chem. 26:1967–1969.

Splechtna, B., I. Petzelbauer, B. Kuhn, K. D. Kulbe, and B. Nidetzky. 2002. Hydrolysis of lactose by $\beta$-glycosidase CelB from hyperthermophilic archaeon *Pyrococcus furiosus*. Appl. Biochem. Biotechnol. 98–100:473–488.

Stanek, J., M. Cerný, and J. Pacák. 1965. The Oligosaccharides. Pub. House of the Czechoslovak Academy of Sciences, Prague, The Czech Republic.

Szczodrak, J. 1999. Hydrolysis of lactose in whey permeate by immobilised b- galactosidase from *Penicillium notatum*. Acta Biotechnol. 19:235–250.

Terrel, S. C., A. Bernard, and R. B. Bailey. 1984. Ethanol from whey: Continuous fermentation with a catabolite repression-resistant Saccharomyces cerevisiae mutant. Appl. Environ. Microbiol. 48:577–580.

Timell, T. E. 1964. The acid hydrolysis of glycosides. I. General conditions and the effect of the nature of the aglycone. Can. J. Chem. 42:1456–1471.

van Walsum, G. P. 2001. Severity function describing the hydrolysis of xylan using carbonic acid. Appl. Biochem. Biotech. 91–93:317–329.

Wolfrom, M. L., A. Thompson, and C. E. Timberlake. 1963. Comparative hydrolysis rates of the reducing disaccharides of D-glucopyranose. Cereal Chem. 40:82–86.