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Optimization of Acidic Labneh Whey Lactose Hydrolysis with Immobilized Beta-Galactosidase Enzyme from *Kluyveromyces lactis*

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Abstract: Hydrolysis of lactose with β-galactosidase is one of the most promising biotechnological applications in the food industry because of it is use in the production of low lactose dairy products. Acid whey samples were collected from the dairy pilot plant at the University of Jordan, Faculty of Agriculture. β-Galactosidase enzyme from *Kluyveromyces lactis* with initial activity of 3000 LAU/ml was immobilized in a sodium alginate and used for acid whey hydrolysis in fluidized bed reactor. Optimization parameters of lactose hydrolysis were (12 and 24% v/v enzyme concentration), (10 and 20 ml/mint flow rate). The process reaction was done at a temperature of 37°C and 6.5 pH. Lactose hydrolysis of 81% and 85% were obtained during 6 h of incubation for 12 and 24% v/v enzyme concentration at flow rate of 10 ml/min.

Key words: Lactose hydrolysis, acid whey, β-Galactosidase enzyme, fluidized bed reactor, Na-alginate, immobilization

INTRODUCTION

Labneh is a major foodstuff in Jordan and other Middle East countries. It is basically a concentrated yogurt obtained by filtration of normal yogurt and the filtrate is called labneh whey. It is an acidic type of whey, as compared with sweet whey which is a by-product of many cheese processing operations (Yousif *et al*., 1998). The annual production of whey in Jordan is estimated to be 30000 tonnes and more than 66% of that is considered as an acidic type. The bulk of the locally produced whey is disposed of through the sewage system, where due to its high Biological Oxygen Demand (BOD) values, it contributes to the pollution problems of the country (Marwaha and Kennedy, 1988). Acid labneh whey provides of the benefits of sweet whey-namely tenderness, flavor enhancement, crust colour development, as well as special properties due to its acidic nature (Yousif *et al*., 1998).

The average acid whey is composed of (94%) water, (0.4%) fat and (0.8%) ash (4.90%) lactose, (0.75%) protein and 4.5 pH value (Cotton, 1976). Whey contains nutritional components like water-soluble vitamins, Minerals, protein and lactose so that leads to increasing environmental concern and raising the cost of disposed whey. New ways of utilizing cheese whey are being sought, it is possible to use whey solids as food ingredient, to replace non Fat Dry Milk (NFDM) in certain products or to increase nutritional quality of other food products (Kesharvaz and Nakai, 1984). There are many ways to utilize whey in food industry; one possible outlet would be bread. It contributes about 53-65% of the total caloric and protein intake in Jordan. Most of the bread consumed is the Flat-type and French-type (Yousif *et al*., 1997). It was found that the addition of concentrated acidic whey to French type bread improved the internal and external characteristics of the bread and it had a retardation effect on bread staling as well as extend its stability by two days (Yousif *et al*., 1997).

The hydrolysis of lactose in milk products can increase digestibility and may improve functional properties of the lactose containing food, therefore the use of β-galactosidase enzyme (Ec. 3.2.1.23) is one of the most promising applications of enzyme to food industry (Park *et al*., 1979). The optimization conditions for sweet whey have been studied by many investigators. It was shown that the percent of lactose conversion was optimized at 37°C and pH 7 for 24 h by using immobilized β-galactosidase from *Kluyveromyces maxianus* (Diserio *et al*., 2003). In other study Juardo *et al*. (2002) found that the optimum conditions for the whey lactose hydrolysis were achieved at pH 6.6 and temperature 37°C, using of β-galactosidase enzyme from *Kluyveromyces fragilis*.

The objective of this study is to optimize the acidic whey lactose hydrolysis using Immobolized β-galactosidase enzyme in Na-alginate beads.

MATERIALS AND METHODS

Sample collection and preparations: Acid labneh whey samples were collected from the dairy pilot plant at the University of Jordan/Faculty of Agriculture from the labneh production line. Potassium hydroxide (Sigma-Aldrich-St. Louis. Mo USA) was used to neutralize the acidity to the desired pH value (Rajakala and Karthigai, 2006; Wierzbicki and Kosikowski, 1972). pH meter

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(Hanna instruments Hi-8519-Italy) was used to monitor the pH of the treated whey. Whey sample was sterilized by membrane filtration (cellulose nitrate with 0.2 mm pore size and 47 mm diameter, Microfiltration system, U.S.A). Then the sample was introduced into the fluidized bed reactor for lactose hydrolysis.

Chemical analysis of acid whey: Moisture, total solids, ash, titratable acidity and protein were determined following AOAC standard methods (1995). Fat, salt and pH were measured following ISO method (1990). The reported Water activity (^{a}w) was measured using Novasina instrument (Axier Ltd, Type: TH 200, Switzerland); the method given in the manual of the instrument was followed. The temperature was calibrated to reach 25°C, then switching on the instrument for 10-15 min. The sample was placed in plastic plate then introduced in the specified instrument. The instrument was left until the reading of the water activity appears on the screen of the instrument.

Enzyme immobilization: β-galactosidase enzyme (3.2.1.23) from *Kluyveromyces lactis* (Sigma-Aldrich-St. Louis. Mo, USA) was used in the experiment. The enzyme was mixed with an aqueous sodium alginate solution at 3% (w/v) (Sigma-Aldrich St. Louis. Mo, USA). Beads were prepared by dropping the solution using 10 ml sterile syringe into 2% w/v calcium chloride solution (Frutarom-LTD, UK). All operations were carried out under aseptic conditions (Sienkiewicz and Riedel, 1990).

Bioreactor design: The bioreactor used was of Fluidized bed type reactor. It was composed of peristaltic pump (Manostat-Varistaltic pump-advanced model-USA), reactor column (8.3 cm x 20 cm), glass resivioar of 2L capacity fitted with inlet and outlet connected with the pump. The temperature was controlled using water bath (Memmert, Type: WB 14 - Germany). Three-way valve was used for sampling under aseptic conditions (Fig. 1).

Optimization process for lactose hydrolysis: Optimization of different factors for acid whey lactose hydrolysis was carried out on stepwise procedure to attain maximum lactose hydrolysis. The reactor column was packed with 250 ml of Na-alginate beads. Two flow rates (10 and 20 ml/min) and Two enzyme concentrations (12 and 24 v/v) were used in this optimization, Table 1 showed physiochemical properties of the used enzyme. The process of enzyme hydrolysis was done at a temperature of 37°C and at value of 6.5 pH (Table 2). The whey samples were flowed from the packed column under the effect of gravity to the reservoir and was continuously recirculated using peristaltic pump to flow through the column for the determined

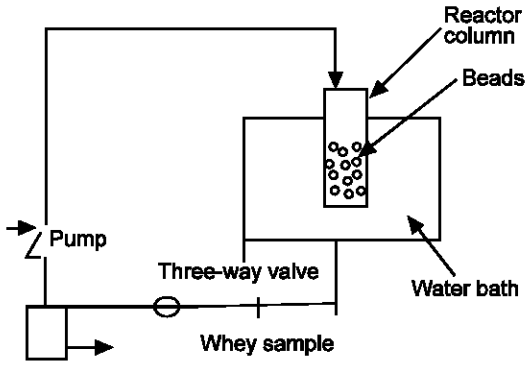

Fig. 1: Flow chart of fluidized bed reactor

Table 1: Physiochemical properties of used enzyme

Property	Lactozyme 3000 L
Physical state	Liquid
Density	1.2
Initial activity (LAU/ml)	3000
Protein concentration (mg/ml)	40

Table 2: Optimization parameters for acid whey lactose hydrolysis

Parameters	Variables
Temperature (°C)	37°C
Lactose concentration (%)	2-4
Enzyme concentration (v/v)	12-24 v/v of Na-alginate
pH	6.5
Flow rate (ml/min)	10 and 20

incubation time of 8 h. The percent of lactose hydrolysis (H %) was measured using the following equation:

H% = [Initial lactose concentration (ppm) – lactose concentration at time interval (ppm) / Initial lactose concentration (ppm)]

The residence time was measured using the following equation:

Residence time = Packed column volume (ml)/flow rate (ml/min) = ml/flow rate (Szczodark, 2000).

Determination of enzyme activity: Lactase activity on O-nitrophenyl β-galactosidase (Fluka-Biochemika, USA) (ONPG) was determined by adding 25 µl of enzyme solution to 980 µl of substrate solution (860 µl with 50 mM potassium phosphate buffer at pH 6.5 and 120 µl with 25 mM ONPG diluted into 50 mM potassium phosphate buffer at pH 6.5). The added enzyme and substrate was incubated at 37°C. After 5 min, the reaction was stopped by adding 1 ml of 0.5 M sodium carbonate. The absorbance of the nitrophenolate was measured at 420 nm using spectrophotometer (Aleco-SL150 UV-Spectrophotometer). One unit of ONPG activity is defined as 1 µ mol of ONP released per minute under the reaction conditions described above. A standard

curve was established by dissolving various concentrations of O-nitrophneol (Fluka-Biochemika, USA) in the assay solution (Rajakala and Karthigai, 2006; Novalin *et al*., 2005).

Sugar determination: For determination of initial and residual lactose concentration, samples were taken from reactor every one hour time interval. The glucose and lactose amount in these samples were determined using HPLC method. The HPLC system (Knauer Model, Germany) composed of pumping system (Smart pump 1000, U.S.A), software program (Autochro-3000 version 2.0.4). The HPLC column used in the analysis was (Unisol Amide 4.6 x 190 mm column-Agela company, U.S.A) and Refractive Index Detector (RI) (Acmc 900 Younglin-South Korea), with a mobile phase of acetonitrile (Sigma-Aldrich-St. Louis. Mo USA): water (75:25) at flow rate of 1 ml/min. was used. The experiment was carried out in triplicate and the averages were taken (Dimitris *et al*., 2005; Manzanres *et al*., 1993).

Statistical analysis: The Repeated Measure Analysis Model (RMAM) produced by the Statistical Analysis System (SAS) version 7 (SAS® System for Microsoft® Windows® 2001), was used to analyze the data. Differences between the means of treatments were tested using the Least Significant Difference (LSD) test at $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition of acid whey samples: Table 3 shows the chemical composition of the whey sample used in the experimental work. Different chemical analysis were carried out including, moisture, total solid, the pH, ash, acidity, sodium chloride content, lactose, protein and fat percentages. The result obtained indicated that the amount of lactose (3.6%) was relatively high and feasible to be converted into glucose and galactose for producing sweeteners to replace sucrose in different products. This result is comparable to the results obtained by (Cotton, 1976). This relatively high level of lactose could be attributed to the use of a special starter culture with low activity in lactose hydrolysis into lactic acid during labneh production, in addition to that, short fermentation period for the product of yogurt which was converted to labneh after whey drainage. The other parameter of the analysis was with an agreement with previous research reported by (Gernigon *et al*., 2010).

Optimization of lactose hydrolysis process: The maximum whey lactose hydrolysis was obtained at enzyme concentration of 24% v/v during 6 h of incubation which reached about 85% (Table 5). Enzyme concentration of 12% v/v showed lactose hydrolysis value from 76.5-81.7% for the rate of 20 and 10 ml/min respectively (Table 4). These results are in agreement

Table 3: Chemical properties of acid whey samples

Chemical properties	Values
Moisture (%)	93.43±0.38
Dry matter %	6.57±0.27
pH	4.56±0.20
Ash (%)	1.88±0.20
Acidity (%)	0.73±0.10
NaCl (%)	1.30±0.10
Lactose (%)	3.60±0.10
Protein (%)	0.42±0.02
Fat (%)	0.40±0.02

Table 4: Lactose hydrolysis (%) at 12% v/v enzyme concentration at different flow rates

	Lactose hydrolysis (%)	
Incubation time (h)	10 ml/min	20 ml/min
1	40.20a	21.80b
2	47.53a	28.90b
3	55.46a	36.50a
4	75.13a	48.40b
5	77.86a	56.90b
6	81.70a	74.90b
7	81.56a	76.76a
8	81.53a	76.50b

Means in each row followed by the same letter are not significantly different at 95% confidence

Table 5: Lactose hydrolysis (%) at 24% v/v enzyme concentration at different flow rates

	Lactose hydrolysis (%)	
Incubation time (h)	10 ml/min	20 ml/min
1	47.76a	26.36b
2	56.66a	38.26b
3	62.60a	51.50a
4	79.70a	77.80a
5	83.33a	81.16a
6	85.03a	83.16a
7	85.09a	83.96a
8	84.86a	82.63a

Means in each row followed by the same letter are not significantly different at 95% confidence

with those reported by (Toshiba and Qayyum, 2009a). They reported the 89% of lactose hydrolysis in whey after 3 h, while in milk, the hydrolytic level of lactose reached 79% after 4 h with the use of immobilized β-galactosidase in Ca-alginate-starch beads using a batch process. Also, Toshiba and Qayyum (2009b) reported the value of 86% of lactose hydrolysis in whey using a packed bead column of Ca-alginate beads with an entrapped β-galactosidase enzyme during 4 h of incubation. In addition to that, other researchers reported 87% lactose hydrolysis in milk using packed bed reactor with an immobilized yeast cell in Na-alginate beads after 7 h of incubation (Reeba *et al*., 2010).

The obtained results of an increase in the lactose hydrolysis with an increase in enzyme concentration (24% v/v) compared to (12% v/v) could be due to the increase of enzyme availability which increased the rate

of hydrolysis (Reeba *et al*., 2010). Similar conclusions were reached by Szczodrak (2000), where 71% of lactose hydrolysis was reached at the enzyme concentration of 1 U/g lactose compared to 86% at 5 U/g lactose.

The rate of lactose hydrolysis was measured at two different flow rates (10 and 20 ml/mint) in the packed bed column. Maximum lactose hydrolysis (85%) was obtained at 10 ml/min flow rate rather than at 20 ml/min. This result could be attributed to the residence time (time by which the process fluid passes through the reactor) (Quinn *et al*., 2001). For the first flow rate, the residence time was 25ml/flow rate, where as for the second flow rate, the residence time was 12.5ml/flow rate. So, as the residence time increased by decreasing the flow rate, the availability of enzyme to substrate increased and thus the rate of hydrolysis also increased. These results are in agreement with that reported by Genari *et al*. (2003), where 80% of lactose hydrolysis was achieved at 30 ml/min flow rate used continuous system with recirculation. Also, Quinn *et al*. (2001) reported 90% of lactose hydrolysis at flow rate of 7 ml/min used fluidized reactor with immobilized β-galactosidase on graphite slabs. Furthermore, Szczodrak (2000) found that the lactose hydrolysis at flow rate of 0.7 ml/min was 87.3% compared to 82.6% hydrolysis at 2 ml/min flow rate when used immobilized lactase on silanized porous glass modified by glutaraldehyde binding.

It was seen from the Table 4 and 5 that the rate of hydrolysis was increased gradually up to 6 h of incubation reaching maximum rate of lactose hydrolysis of 85% and then the rate of hydrolysis were remained nearly constant. The stability of lactose hydrolysis values following maximum hydrolysis could be explained to several reasons, such as an increase in the inhibiting concentration of glucose, or exhaustion of an activator, such as lactose, or even some proteolysis of the enzyme (Genari *et al*., 2003). It was reported that a high milk lactose hydrolysis was obtained using a continuous milk (plug flow) system with recirculation compared to the system without recirculation (Genari *et al*., 2003). Moreover, this satisfied the major objective of this research in which cost reduction of lactose conversion was a major target of our research.

Conclusion: This study showed that the 24% v/v enzyme concentration and 10 ml/min flow rate at temperature of 37°C and pH of 6.5 resulted in a maximum lactose hydrolysis. The immobilization of β-galactosidase from *kluyveromyces lactis* on sodium-alginate beads using packed bed reactor system is a convenient and inexpensive method giving an enzyme preparation of a good stability and activity and it is recommended to use this model in the whey hydrolysis with different types acid, sweet, permeate or others in the diary factories.

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