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LACTOSE HYDROLYSIS IN DIFFERENT SOLIDS CONTENT WHEY AND MILK PERMEATES

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Abstract

Dairy permeates contain almost original amount of lactose, small fraction of other solid compounds and water. For complete lactose hydrolysis and glucose-galactose syrup production, it is essential to ensure the optimal substrate concentration and conditions to improve product quality and save energy, water and material costs. The aim of this study was to investigate β-galactosidase preparates capability using concentrated whey and milk permeates with 20, 30 and 40% (w w^{-1}) of total solids. Commercial β-galactosidase preparates (Ha-Lactase 5200 produced by *Kluyveromyces lactis* and NOLA Fit5500 produced by *Bacillus licheniformis*, Chr.HANSEN; GODO-YNL2 produced by *Kluyveromyces lactis*, Danisco, Denmark) at dosage 500 NLU·L^{-1} (Ha-Lactase 5200, GODO-YNL2) and 500 BLU·L^{-1} (NOLA Fit5500) were used for lactose hydrolysis. The concentration of each permeate was 20, 30 and 40% (w w^{-1}) of total solids. 10% KOH was used to adjust optimal pH for fermentation media. Samples were monitored in incubator for 4 hours at temperature 42.5±0.5 °C. Lactose, glucose and galactose concentrations were determined by HPLC (Shimadzu LC–20 Prominence, USA). Ha-Lactase 5200 preparate was able to increase lactose hydrolysis rate more than 90% in all permeates at 20% and 30% solids concentration. GODO-YNL2 preparate showed the highest conversion of glucose in sweet whey permeate at 20% solids concentration and the lowest conversion of galactose in sweet whey permeate at 30% solids concentration. The results indicated that substrate type and solids concentration have an effect on the relative activity of commercial enzymes. The study results give a greater understanding about permeates suitability for glucose-galactose syrup production.

Keywords: lactose hydrolysis, glucose-galactose syrup, permeate, β-galactosidase

Introduction

In food industry, carbohydrate plays an important role in production, in nutritional value of product and is an important factor of sweet taste formation (Evdokimov et al., 2015). Hydrolysis of lactose into monosaccharides gives several advantages that have been appreciated by the food industry. Final product has higher sweetness and solubility, contains diverse types of monosaccharide at different concentration. Lactose can be enzymatically hydrolysed using β-galactosidase which produced from GRAS microorganisms or chemically hydrolysed with acids and ion-exchange resins (Illanes, 2016). Hydrolysis of lactose at range of 50 to 90% in concentrated whey and permeate syrup increases sweetness and prevents lactose crystallization during evaporation process, planning to reach 60–70% of solids in concentrated whey (Macwan et al., 2016). Products can be used for invention of innovative and low calorie syrups with high sweetness (Rhimi et al., 2010). One of the perspective applications of lactose is glucose-galactose syrup production by enzymatic hydrolysis. Syrup is viscous, thick sugar solution containing approximately 20% of water, 68% of glucose and galactose, 11% of lactose and 1% of minerals (Lindsay et al., 2018). Glucose-galactose syrup characterises with a sweet taste, dark yellow colour, transparency, good solubility (Budriene et al., 2005) and it might be used as sucrose substitute. Milk and whey permeates are valuable sources for enzymatical lactose hydrolyse (Ryan, Walsh, 2016). Industries prefer to hydrolyse lactose using free enzyme however, the immobilization of β-galactosidase begins to draw the attention of producers. The ability to efficiently hydrolyse whey lactose is one of the key factors determining overall process economy (Vasileva et al., 2016). The aim of this study was to investigate β-galactosidase preparates for fermentation capability using concentrated whey and milk permeates with 20, 30 and 40% (w w^{-1}) of total solids.

Materials and Methods

Chemicals and materials

Acetonitrile (HPLC grade, ≥99.93% purity), column SUPELCOSIL™ LC-NH_2, (250 mm × 4.6 mm × 5 µm), D-lactose monohydrate (≥99.5% purity), D(+) galactose (≥99% purity), D(+) glucose (≥99.5% purity), KOH (≥85%, pellets) were purchased from Sigma-Aldrich. Sweet whey permeate was donated by SC "Smiltenes piens", but whey and milk permeates were obtained from SC "Tukuma piens".

Enzymes

Three commercial β-galactosidase preparates Ha-Lactase 5200 produced by *Kluyveromyces lactis*, activity 5200 NLU·g^{-1} and NOLA Fit5500 produced by *Bacillus licheniformis*, activity 5500 BLU·g^{-1} (Chr.HANSEN, Denmark) and GODO-YNL2 produced by *Kluyveromyces lactis*, activity 5000 NLU·g^{-1} (Danisco, Denmark) were used in the present study.

Solids concentration

Solids concentration of permeates was performed by Chandrapala et al. (2016) method with some modifications. The concentration of permeates solids was accomplished using pilot scale evaporation FT 22 (Armfield, UK) under vacuum conditions to achieve approximately 20% (w w^{-1}) of solids. Evaporation conditions were as follows: flow rate 8 L h^{-1}, warming steam pressure 1 bar, permeate temperature 78±1 °C, cooling water rate 5 L h^{-1}, and vacuum 0.56 bar. Rotary vacuum evaporator Laborota 4000 efficient (Heidolph, Germany) was used to reach permeates solids concentration 30% (w w^{-1}) and 40% (w w^{-1}).

Refractometer 30GS Mettler (Toledo, Japan) was used for permeates solids determination.

Permeates hydrolysis

Experiments were carried out based on Dutra Rosolen et al. (2015) method with some modification. Commercial enzyme weighted into 100 mL conical flasks and 50 mL permeate added with an appropriate pH. All substrate pH for hydrolysis with Ha-Lactase 5200 enzyme was adjusted till 6.5–6.7, for NOLA Fit5500 enzyme 5.4–5.8 and for GODO-YNL2 enzyme 7.5–7.7. 10% KOH was used to adjust an optimal medium pH for enzymatic hydrolysis. Triplicate experiments were prepared for each type of permeate.

The dosage of commercial enzymes for permeate lactose hydrolysis is summarized in Table 1.

Table 1

Summary of added enzymes (mean values ± SD (n = 3))

Enzyme preparate	Weight, mg	Unit
Ha-Lactase 5200	53.2±2.5	292±13 NLU·g^{-1}
NOLA Fit5500	52.1±2.1	270±11 BLU·g^{-1}
GODO-YNL2	53.6±2.2	268±12 NLU·g^{-1}

Samples were fermented at temperature 42.5±0.5 °C 4 hours. Fermented samples were put in water bath and heated at 80 °C 5 min for enzyme inactivation. Samples were transferred into 50 mL test tubes and placed into freezer at -18 °C for further analyses.

HPLC analysis

Lactose, glucose and galactose was determined according to method which was used in Žolnere et al. (2018) work with some modifications.

Sample preparation: samples were transferred into 2 mL test tube and centrifuged 5 min at 10 000 rpm. Approximately 1.5 mL of filtered sample was placed into sampler vials and sealed for HPLC analysis. HPLC (Prominence HPLC system, Shimadzu LC-20, Torrance, CA, USA) was used for sugar determination, refractive index detector RID-10A; column SUPELCOSIL™ LC-NH2, (25 cm×4.6 mm) 5 µm column; 35 °C temperature; gradient mobile phase acetonitrile: deionized water (80 : 20); volume of the injected sample: 10 µL; total analysis time of up to 15 min; flow rate: 1.0 mL min^{-1}.

Data analysis

Results were expressed as mean ± standard deviation (SD) of three replicates for composition measurements. Statistical analyses were carried out using One-Way ANOVA and Tukey test. Differences were considered statistically significant when $p<0.05$.

Results and Discussion

Sweet whey permeate pH in all solids concentration was in range of 5.8–6.1, acid whey permeate 4.4–4.8 and milk permeate 5.5–5.8. According to specification of enzyme preparates for Ha-Lactase 5200 optimal medium pH is range of 6.5 to 8.0, NOLA Fit5500 of 5.4 to 7.0 and for GODO-YNL2 from 7.5 to 8.0 (Žolnere et al., 2018).

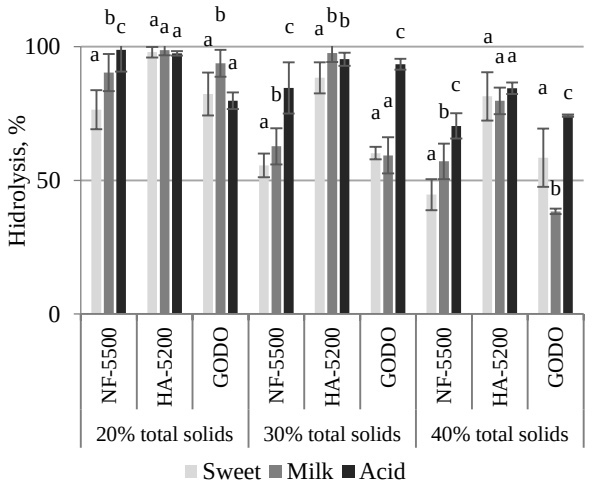

Figure 1. The comparison of analysed commercial enzymes activity in permeates at different solids concentration

*Results indicated with the same letter do not differ significantly ($p>0.05$)

NF-5500 - NOLA Fit5500 enzyme, HA-5200 - Ha-Lactase 5200 enzyme, GODO - GODO-YNL2 enzyme

The highest percentage of hydrolysis (97.5–98.8%) showed Ha-Lactase 5200 enzyme in all permeates at 20% (w w^{-1}) solids concentration. Similar results we noticed at 30% (w w^{-1}) and 40% (w w^{-1}) solids concentration (hydrolyse percentage ranged from 88.3 to 97.6% and 79.7–84.5%, respectively). Other enzymes had showed several results at different solids concentration. The solids concentration can be one of the factor, which influenced NOLA Fit5500 and GODO-YNL2 enzymes activity than for Ha-Lactase 5200 enzyme.

Permeate composition may affect the enzyme activity. Addition of potassium ions during substrate pH adjustment may influence enzyme activity as well. According Jurado et al. (2004) data, metal ions affect the stability and activity of β-galactosidase. The main monovalent ions activator is K^+ for *Kluyveromyces lactis* β-galactosidase but K^+ and Na^+ for *Bacillus licheniformis* β-galactosidase (Juajun et al., 2011; Jurado et al., 2004). For hydrolysis with Ha-Lactase 5200 and GODO-YNL2 was necessary to add 10% KOH to all permeates but for hydrolysis with NOLA Fit5500 it was required to add alkali only for acid whey permeate samples. Hydrolysis results with NOLA Fit5500 enzyme showed that acid whey permeate samples, where 10% KOH was added, performed the highest hydrolyse percentage in all 3 solid concentrations. Its approved K^+ ion positive influence on *Bacillus licheniformis* β-galactosidase activity. Evaluating Ha-Lactase 5200 and GODO-YNL2 enzymes, the lower optimal pH level is 6.5 and 7.0, respectively, which needs to be adjusted. The addition of 10% KOH differs, more alkali was added to optimized GODO-YNL2 enzyme activity in all

permeates samples which leads to increased K^+ ion concentration in substrate and influenced the enzyme activity and lactose hydrolysis capability.

Figure 2 illustrates the amount of glucose and galactose in permeates at 20% (w w^{-1}) solids concentration. Results showed that almost all hydrolyse reactions finished with higher glucose amount but as the exception were acid whey permeate sample with NOLA Fit5500 enzyme (glucose 65.4 g L^{-1}, galactose 70.1 g L^{-1}). Sweet whey and milk permeates hydrolysis results showed that there is no significant difference between galactose amount using different β-galactosidase preparates. Reported by Harju et al., (2012) lactose hydrolysis of 70% in dairy product increases sweetness by an amount corresponding to an addition of approximate 2% sucrose. The hydrolysis of lactose into glucose and galactose increase product sweetness because glucose has higher sweetness level than lactose (Pruksasri, 2015). It's reflected also on results where almost all samples showed higher glucose concentration which means samples sweetness at the end were higher than at the beginning of hydrolysis reaction.

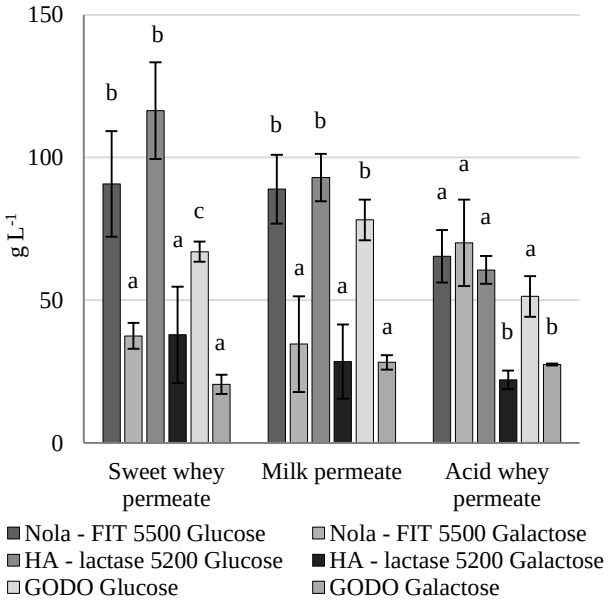

Figure 2. Amount of glucose and galactose in permeates at 20% (w w^{-1}) solids concentration

*Results indicated with the same letter between one type of monosaccharide in certain permeate do not differ significantly ($p>0.05$).

Glucose and galactose amount in permeates at 30% (w w^{-1}) solids concentration (Figure 3) was within results showed in Figure 2. The monosaccharides outcome from all enzyme preparates was quite close. Results indicated that activity of enzyme preparates had affected permeates composition and physical-chemical indices. The hydrolysis of lactose by NOLA Fit5500 enzyme did not show similar outcome results with the hydrolysis of other enzyme preparates. It should be highlighted that in the reaction was used preparates with β-galactosidase from different sources and were used three substrates, which were compared.

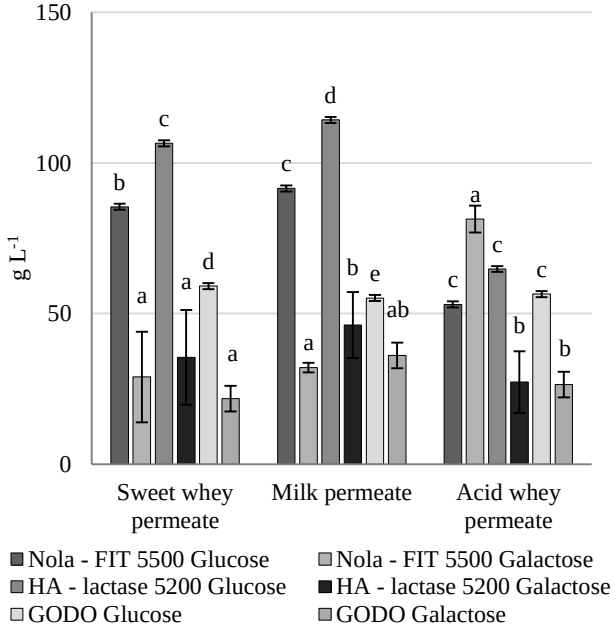

Figure 3. Amount of glucose and galactose in permeates at 30% (w w^{-1}) solids concentration

* Results indicated with the same letter between one type of monosaccharide in certain permeate do not differ significantly ($p>0.05$).

The hydrolysis of microbial enzyme *Bacillus licheniformis* properties has not been fully studied therefore, there is a need for numerous studies to obtain a better knowledge of gained results. As it was reported by Božanić et al. (2014) acid whey contains higher amount of the calcium, phosphate, lactic acid and lactate than it is in sweet whey. These could be considered as the main factors, which affects the effectiveness of this particular enzyme activity to hydrolyse lactose in acid whey.

The results in Figure 4 indicated that the amount of glucose and galactose was lower comparing with the results from Figure 2 and 3. It can be approved with the hydrolysis percentage of permeates at 40% (w w^{-1}) solids concentration (see in Figure 1). The cause of low lactose hydrolysis might be that β-galactosidase activity was decreased by the high lactose concentration in 40% (w w^{-1}) solids concentrate permeates and the presence of glucose and galactose. According to Demirhan et al. (2008) study glucose and galactose at concentration 10.32 g L^{-1} and 13.03 g L^{-1}, respectively, start acting as inhibitors slowing down lactose hydrolysis reaction. As an option for complete lactose hydrolysis of permeates at 40% (w w^{-1}) solids concentration would be time extension for reaction.

β-Galactosidase activity may affect several factors, such as temperature, pH, pressure, concentration of substrate and presence of metal ions (Bosso et al., 2016). The study results showed that addition of 10% KOH influenced lactose hydrolysis and new carbohydrates formation.

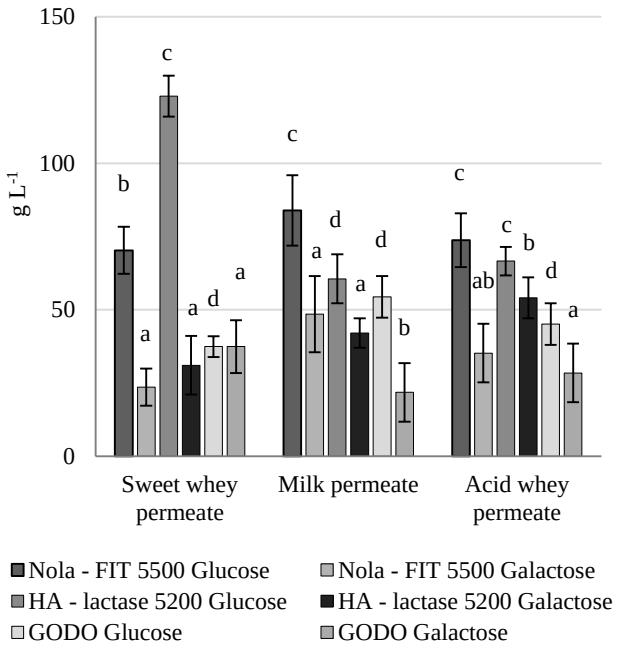

Figure 4. Amount of glucose and galactose in permeates at 40% (w w^{-1}) solids concentration

*Results indicated with the same letter between one type of monosaccharide in certain permeate do not differ significantly ($p>0.05$).

Mariyani et al. (2015) had reported that in the situation when after lactose hydrolysis the galactose concentration was lower than glucose that might be associated to the development of galacto-oligosaccharides. The substrate for hydrolysis containing highly concentrated lactose, β-galactosidase is able to produce galacto-oligosaccharides and affect the final concentration of glucose and galactose. As stated by Suárez et al. (2018), glucose and galactose are being produced equimolar, while lactose concentration decreases slowly and water (as a nucleophile) activity is high. However, when lactose concentration keeps be high, activity of water is low and lactose starts to act as nucleophile and transgalactosylation become more active and starts producing galactooligosaccharides. This statement can explain the relation between glucose and galactose results in this study.

Conclusions

These results provide more accurate information in which substrates the commercial enzyme is able to hydrolyse lactose to a maximal extent. Each substrate at certain concentration has different physical and chemical properties which influence enzyme activity and profile of final outcome. Use of KOH for medium pH control can be evaluated positively because it works as activator for enzyme. Almost all of the results showed that after lactose hydrolysis the dominant monosaccharide was glucose. The obtained results can be used in further research to work on glucose-galactose syrup production technology.

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