DOI: 10.22616/FoodBalt.2019.035

SHORT COMMUNICATION

THE OPTIMIZATION OF ACID WHEY PERMEATE HYDROLYSIS FOR GLUCOSE-GALACTOSE SYRUP PRODUCTION

Ramazon Samadov[1,2]*, Inga Ciprovica[1], Kristine Zolnere[1], Ingmars Cinkmanis[3]

[1] *Department of Food Technology, Faculty of Food Technology, Latvia University of Life Sciences and Technologies, Rigas iela 22, Jelgava, Latvia*

[2] *Faculty of Engineering and Technology, Technological University of Tajikistan, N. Karadoev street 63/3, Dushanbe, Tajikistan, email: saidzodars@gmail.com*

[3] *Department of Chemistry, Faculty of Food Technology, Latvia University of Life Sciences and Technologies, Liela iela 2, Jelgava, Latvia*

Abstract

Whey contains a lot of lactose, which can be easily hydrolysed by commercial enzymes. The aim of the present study was to identify the optimal parameters for the enzymatic hydrolysis of acid whey permeate and glucose-galactose syrup production. Acid whey permeate was hydrolysed using β-galactosidase preparate (NOLA^{TM} Fit 5500, Chr. Hansen, Denmark) with activity 7200 BLU L^{-1}. As the enzyme is strongly inhibited at pH below 4.5, sodium bicarbonate was added to neutralize substrate pH till 6.0–6.3. The hydrolysis was carried out at 40 °C 6 hours. pH and monosaccharides concentration were monitored during the process of hydrolysis. The fermented substrate was concentrated in a vacuum evaporator at 40–60 °C, 4–8 kPa. Glucose-galactose syrup was obtained with 65 and 70% of total solids. Lactose and monosaccharides were determined by HPLC. Fermentation time influenced monosaccharides composition and concentration. After 2 hours of fermentation lactose was completely hydrolysed. Continuing fermentation, the amount of glucose was decreased due to formation of novel oligosaccharides. The study results revealed that the optimal time for acid whey permeate hydrolysis was 2 hours. It should be noted that during the process of hydrolysis the pH of the product increased till 6.5 and such changes are related to cellulase and glucoamylase activity incorporated in the enzyme preparate as well as permeate protein residues hydrolysis. With the increase of syrup total solids, galactose concentration was changed due to galacto-oligosaccharides formation. The degree of sweetness is key factor for the durability of lactose hydrolysis and final syrup concentration.

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

Introduction

Processing and rational application of whey is a topical problem for all countries with a developing dairy industry. Even in Tajikistan, the lack of cost-effective technologies for whey processing for a long time has been the reason that most of whey was considered as a waste stream and thrown into sewages (Кулов, 2017; Бах, 2017). At the same time, this valuable raw material was lost, in addition to significant deterioration in the ecological situation.

The disposal of whey remains a serious problem for the dairy industry. Whey contains between 5 to 6% of total solids, including lactose, which can be recycled (Cote et al., 2004).

In addition, there is a problem associated with the production of sugar, as well as import of this product is expensive in Tajikistan. It is reasonable to hydrolyse lactose into glucose-galactose syrup production, which meaningfully will help the national economics and apply the new product as a sugar substitute. The study on glucose-galactose syrup production from acid whey will help to save food resources in multiply food industry subsectors.

One of the key components of whey – lactose consists of glucose and galactose (Скворцов et al., 2013). Lactose presents in milk of all mammals and exists in two isomeric forms, alpha and beta. The differences are the configuration of the hydroxyl-group. The lactose content in cow's milk varies in range of 4.4 to 5.2%, the average 4.8% (Gänzle et al., 2008; Schaafsma, 2008). Today whey has been processed into beverages, also dehydrated products are produced, as well as fractionation of individual components (fats, lactose, whey proteins, amino acids and peptides) and biological conversion of lactose in order to obtain glucose-galactose and lactulose syrups are practiced (Гавриил, Эдуард, 2013).

Nowadays, utilization of sweet whey has been successfully solved and sweet whey have been developed into multiple food products (Chandrapala et al., 2016); unfortunately, only a few interesting solutions and scientific publications are available for successful processing of acid whey into innovative and value-added food products. Acid whey has a low pH (4.5–4.7), sour taste, high mineral content, including lactates, less protein and lactose compared to sweet whey (Prazeres et al., 2012; Chandrapala et al., 2016). Taking into account the composition of acid whey, it is an opportunity to use whey as a raw material for lactose hydrolysis with the aim to obtain glucose and galactose.

Whey hydrolysis can be carried out by two methods: enzymatic and acid hydrolysis (Das et al., 2015). Enzymatic hydrolysis is provided using the commercial enzyme preparate – β-galactosidase which hydrolyses lactose into glucose and galactose. The enzyme is widely distributed, but only a few enzymes have an optimum pH for the operation in acid whey. Enzymatic hydrolysis of lactose is a popular technology for production of glucose-galactose syrup, which is 3 times sweeter than lactose (Ansari, Husain, 2010; Fox, 2011). Generally, glucose-galactose syrup is produced from sweet whey, in turn, it can be obtained also from acid

whey. The production technology of glucose -galactose syrup is important for many countries, including Tajikistan, which allows to get an economically substantial sugar substitute.

The aim of this study was to identify the optimal parameters for the enzymatic hydrolysis of acid whey permeate and glucose-galactose syrup production.

Materials and Methods

Chemicals and materials

Chemicals D-lactose monohydrate, D(+) glucose, D (+) galactose (>98%, HPLC) were purchased from Sigma-Aldrich (Riga, Latvia), as well as $NaHCO_3$ from Voldemars Ltd. (Latvia). The acid whey permeate was gained from JSC Tukuma piens with the following composition: fat – 0.00%, protein – 0.68±0.01%, lactose – 4.09±0.01%, total solids – 5.09±0.01%, solids-no-fat – 4.87±0.01% measured with Milkoscan™ Mars (Foss, Denmark) and pH 4.49±0.30 measured by pH Meter GPH 114 (Germany).

Commercial enzyme

Commercial NOLA ™ Fit5500 (Chr. Hansen, Denmark) β-galactosidase was used in the study. NOLA™ Fit 5500 is a highly purified enzyme gained from *Bacillus licheniformis*. According to the manufacturers' recommendations, the NOLA™ Fit 5500 enzyme is active under acidic conditions (optimum pH 5.0–7.0, temperature 35–50 °C) and enzyme activity is 5500 BLU (bifido lactase units) L^{-1} (NOLA™ Fit 5500 Product Information, 2017).

Technology for glucose-galactose syrup production

pH of the permeate was adjusted to 6.0–6.3 with 10% of $NaHCO_3$ solution. After neutralization of substrate, the acid whey permeate was pasteurized at 80±5 °C 3±1 min for microorganism inactivation. The enzyme with an activity of 7200 BLU was added to L^{-1} of acid whey permeate. After the enzyme addition, permeate was mixed 2 minutes and placed into incubator IN55 (Memmert, Germany), samples were fermented within 6 h. pH was determined every hour.

To obtain glucose-galactose syrup, the fermented solution was evaporated into vacuum-evaporator Heidolph Laborota 4000 efficient (Heidolph Instruments GmbH & Co KG, Germany). The process was conducted at a pressure of 4–8 kPa and temperature at 51±9 °C. The total solids of syrup were adjusted to 65±2% and 70±2%. Concentration of the solids was determined with a refractometer DR301-95 (KRUSS, Germany).

Determination of lactose, glucose and galactose

The changes of lactose, glucose and galactose concentration during fermentation were determined every hour using high performance liquid chromatography (HPLC) LC 20 Prominence (Shimadzu, LC-20, Torrance, CA, USA). The following parameters were set: detector – the index of refraction RID-10A; column – Alltech YMC, 4.6×250.0 mm, 5 μm; temperature 35 °C; isocratic elution mode, mobile phase – A – acetonitrile, B – deionized water (A80 : B20); the volume of the injection sample was 10 μL; total time for analysis – up to 25 minutes; flow rate 1.0 mL min^{-1}. The obtained data was processed using Shimadzu LabSolutions software (LC Solution Version 1.21 SP1) (Zolnere et al., 2018).

Monosaccharide composition and concentration was analysed in syrup with different solids content.

Statistical analysis

The experiments were carried out in three replications. Significant differences among the study results were identified using t-test at the significance level $p<0.05$. Statistical analysis was performed using Windows and Excel programs, version 10.0.

Results and Discussion

The effect of different parameters, such as fermentation time and pH, was studied and results are shown in Figure 1. The recommended medium pH for NOLA™ Fit 5500 enzyme was 5.0–7.0, therefore the acid whey permeate pH was adjusted in the range of 6.23±0.03. During the fermentation time, there was observed the significant increase ($p<0.05$) of substrate pH from 6.23 to 6.37. pH changes can be attributed to the activity of cellulase and glucoamylase, which are incorporated in the enzyme preparate and permeate protein residues hydrolysis (Palmer et al., 2007). Roy and Gupta (2003) concluded that an increase of pH helps to maintain enzyme activity during fermentation as well as at the end of fermentation pH changes become slower.

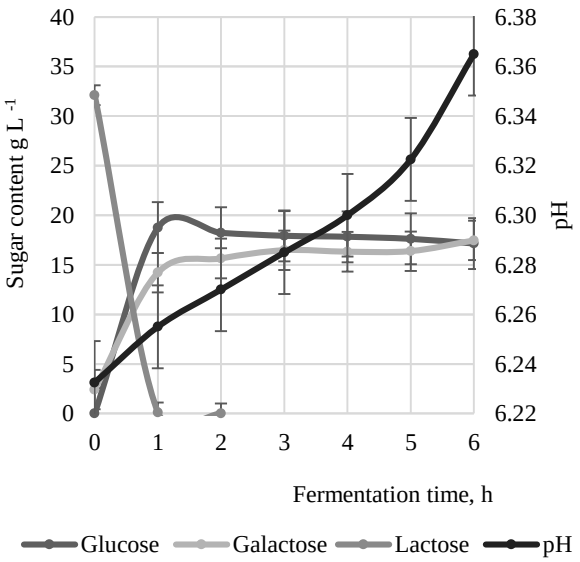

Figure 1. The effect of fermentation process on the monosaccharide concentration and composition, and substrate pH

Analysis of the monosaccharide concentration showed that the optimal fermentation time for the lactose hydrolysis was 2 hours. The duration of the fermentation process affects the concentration of glucose and galactose (Мяло et al., 2013). It should be noted that the

final concentration of glucose and galactose is unequal in substrate at the end of fermentation, which contradicts to the theoretical yield of these monosaccharides. Adding lower amount of the enzyme preparate, the ratio of glucose and galactose increased in towards glucose, whereas with an increased enzyme addition the differences in the monosaccharide concentration decreased. It should be noted that conversion coefficient, which characterizes the lactose transformation into glucose and galactose, ranged from 1.05 to 1.11 (Остроумов, Гаврилов, 2013). Continuing fermentation, the glucose concentration decreased due to the formation of novel oligosaccharides (Cezar et al., 2018).

As shown by several researchers (Bojan et al., 2011; Goderska et al., 2008; Warmerdam et al., 2013), lactose hydrolysis with *Bifidobacterium bifidum* β-galactosidase showed high activity significantly reducing initial lactose amount in substrates. It influences the final monosaccharides concentration, especially higher glucose yield.

Table 1

Comparative analysis of the carbohydrate composition of glucose - galactose syrup

Carbohydrates, g L^{-1}	Experimental syrup*		Commercial syrup* with 65% of total solids**
	65% of total solids	70% of total solids	
Glucose	45±2	43±3	25
Galactose	20±3	24±2	22
Lactose	–	–	12

* Syrups contain at least 1–2% protein.
** Somov et al., 2015

Before vacuum evaporation, heat treatment of substrate was not carried out in order to inactivate the enzyme. The temperature during evaporation process was adjusted at 51±9 °C, under these conditions the enzyme was able to operate.

The glucose-galactose syrup was obtained with total solids concentration of 65±2% and 70±2%. Based on chromatographic analysis, syrups contain 43–45% of glucose and 20–24% of galactose (Table 1). The high concentration of glucose compared to galactose could be explained by its inversion under the action of β-galactosidase in the process of evaporation. With the increase of total solids in syrup, the concentration of glucose decreases and that of galactose increases. Glucose and galactose concentration in experimental syrups was higher compared to commercial syrup obtained by commercial Ha-Lactase 5200 β-galactosidase (Chr. Hansen, Denmark), as well as remaining lactose was found in the commercial syrup (Table 1) (Сомов, 2013). Monosaccharide analysis showed that NOLA™ Fit 5500 β-galactosidase was able completely hydrolyse lactose into glucose and galactose in acid whey (Table 1), as well as provide a higher degree of syrup sweetness.

Conclusions

The study results showed that the optimal time for lactose hydrolysis was 2 hours. Extension of the fermentation process results in decrease of glucose concentration.

As the degree of syrup sweetness is a key factor with regards to syrup quality, it is recommended to concentrate syrup up to 65% total solids.

Acknowledgement

This work was supported by the Latvian State Scholarship (Agreement Nr. 1.-50.3/3912) for study and research.

References

1. Ansari S. A., Husain Q. (2010) Lactose hydrolysis by β-galactosidase immobilized on concanavalin A-cellulose in batch and continuous mode. *Journal of Molecular Catalysis B: Enzymatic*, Vol. 63(1–2), p. 68–74.
2. Bojan M., Katarina L., Rajka B., Ljubica T. (2011) Impact of enzymatic hydrolyzed lactose on fermentation and growth of probiotic bacteria in whey. *Food and Agriculture Organization of the United Nations* Vol. 61 (2), p. 154–160.
3. Cezar M., Mano R., Nicolau B., Maria G. (2018) Whey permeate as the raw material in galacto-oligosaccharide synthesis using commercial enzymes. *Food Research International*, Vol. 0–1, p. 1–8.
4. Chandrapala J., Wijayasinghe R., Vasiljevic T. (2016) Lactose crystallization as affected by presence of lactic acid and calcium in model lactose systems. *Journal of Food Engineering*, Vol. 178, p. 181–189.
5. Cote A., Brown W. A., Cameron D., van Walsum G. P. (2004) Hydrolysis of lactose in whey permeate for subsequent fermentation to ethanol. *Journal of Dairy Science*, Vol. 87 (6), p. 1608–1620.
6. Das B., Roy A. P., Bhattacharjee S., Chakraborty S., Bhattacharjee C. (2015) Lactose hydrolysis by β-galactosidase enzyme: Optimization using response surface methodology. *Ecotoxicology and Environmental Safety*, Vol. 121, p. 244–252.
7. Fox P. F. (2011) Lactose and oligosaccharides. **In:** *Encyclopedia of dairy sciences*, London: Elsevier, p. 173–181.
8. Gänzle M. G., Haase G., Jelen P. (2008) Lactose: Crystallization, hydrolysis and value-added derivatives. *International Dairy Journal*, Vol. 18 (7), p. 685–694.
9. Goderska K., Nowak J., Czarnecki Z. (2008) Comparison of the growth of Lactobacillus acidophilus and Bifidobacterium bifidum species in media supplemented with selected saccharides including prebiotics. *Technologia Alimentaria*, Vol. 7 (2), p. 5–20.
10. *NOLA™ Fit5500 Product Information*, [accessed on 06.11.2018.]. Available: https://hjemmeriet.com/se/ChrHansen/Products/NOLA-Fit/PI_GLOB_NOLA_Fit5500_350502_EN.pdf
11. Palmer T., Bonner P. L. (2007) *ENZYMES: Biochemistry, biotechnology and clinical chemistry*, Second Edition. Oxford: Woodhead Publishing. 431 p.
12. Prazeres A. R., Carvalho F., Rivas J. (2012) Cheese whey management: A review. *Journal of Environmental Management*, Vol. 110, p. 48–68.
13. Roy I., Gupta M. N. (2003) Lactose hydrolysis by Lactozym ™ immobilized on cellulose beads in batch and

fluidized bed modes. *Process Biochemistry*, Vol. 39, p. 325–332.
14. Schaafsma, G. (2008) Lactose and lactose derivatives as bioactive ingredients in human nutrition. *International Dairy Journal*, Vol. 18 (5), p. 458–465.
15. Somov V., Evdokimov I., Knyazev S., Perminov S., Kurash Y. (2015) Application of whey-derived syrups in dairy products. *Foods and Raw Materials*, Vol. 3(2), p. 89–95.
16. Zolnere K., Ciprovica I., Ķirse A., Cinkmanis I. (2018) A study of commercial β-galactosidase stability under simulated in vitro gastric conditions. *Agronomy Research*, Vol. 16 (S2), p. 1555–1562.
17. Warmerdam A., Boom R. M., Janssen A. E. M. (2013) β-galactosidase stability at high substrate concentrations. *SpringerPlus*, Vol. 2, p. 1–8.
18. Бах К. Ф., (2017) *Таджикистан. Обзоры результативности экологической деятельности.* Организация Объединенных Наций, Нью-Йорк, p. 451
19. Гавриил Б.Г., Эдуард Ф.К., (2013). Пути рационального использования молочной сыворотки. *Сыроделие и маслоделие,* Vol. 2, p. 10–13.
20. Кулов А. Н., Наджибуллоев А., Будникова Е. В., Исмоилова З., Асматбеков Ф., Ходжаев З. (2017) Статистический ежегодник республики Таджикистан. Агентство по статистике при Президенте Республики Таджикистан, p. 1–365.
21. Мяло С. В., Гаврилова Н. Б., Воронова Т. Д., Щетинин М. П. (2005) Исследование процесса гидролиза молочного сахара энзиматическим и микробиологическим способами. *Ползуновский альманах,* Vol. 1, p. 87–94.
22. Остроумов Л. А., Гаврилов Г. Б., (2013) Биотрансформация лактозы ферментными препаратами β-галактозидазы. *Техника и технология пищевых производств,* Vol. 1, p. 10–13.
23. Скворцов Е. В., Морозова Ю. А., Букуру Л. К., Алимова Ф. К., Канарская З. А. (2014) Эффективность применения β – галактозидазы для гидролиза лактозы молочной сыворотки. *Вестник Казанского технологического университета.* Vol. 13, p. 3–6.
24. Сомов В.С. (2013) *Разработка технологии фруктовых добавок на основе глюкозо-галактозного сиропа, полученного из творожной сыворотки с применением мембранных методов*. [accessed on 14.12.2018.]. Available at: http://tekhnosfera.com/razrabotka-tehnologii-fruktovyh-dobavok-na-osnove-glyukozo-galaktoznogo-siropa-poluchennogo-iz-tvorozhnoy-syvorotki-s-pri