SECyTARRAGONA 2012
XII REUNIÓN CIENTÍFICA DE LA SOCIEDAD ESPAÑOLA DE CROMATOGRAFÍA Y TÉCNICAS AFINES
Tarragona / 14 - 16 Noviembre 2012
CIAL UAM CSIC

INFLUENCE OF REACTION PRESSURE IN THE ENZYMATIC SYNTHESIS OF POTENTIAL PREBIOTIC OLIGOSACCHARIDES

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INTRODUCTION

- In the present work, we have investigated the production, via enzymatic synthesis, of a bioactive oligosaccharide (2-α-D-glucopyranosyl-lactose) studying the influence of the reaction pressure and comparing the performance to atmospheric conditions.
- This new oligosaccharide may possess high interest due to its potential prebiotic nature. This potential bioactivity is attributed to the formation of the α1-2 linkage in its structure, elucidated by nuclear magnetic resonance (NMR) as Gal β1-4 – [Glu α1-2] – Glu. This type of linkage is characterized by high resistance to the gastro intestinal digestion. Consequently, this compound could effectively stimulate the selective growth of bacteria that are beneficial to the large intestine, mainly bifidobacteria and lactobacilli.

MATERIALS AND METHODS

i. Optimized reaction conditions for the enzymatic synthesis of 2-α-D-glucopyranosyl-lactose in atmospheric conditions (*Díez-Municio et al., 2012. J. Agric. Food Chem. 60, 1945-1953).*

sucrose:lactose 25:25 (g 100 mL^{-1}), 20 mM NaOAc buffer with 0.34 mM $CaCl_2$ pH 5.2, 30 ºC, 0.8 U mL^{-1} dextransucrase from *Leuconostoc mesenteroides* B-512F, 24 h reaction time.

ii. Study of the potential effect of reaction pressure in the high-yield synthesis of the trisaccharide in a high-pressure stirred tank reactor.

Gases used: N_2, CO_2.
Pressure applied: 50, 100 bar.

iii. Chromatographic Analysis: LC-RID *Agilent1220–1260*

NH_2 analytical column (250 x 4.6 mm, 5 µm particle size).
Isocratic elution: ACN:H_2O 75:25; Flow rate 1 mL min^{-1}.
Injection volume: 50 µL (1 mg of total carbohydrates).
Quantitative calibration curves: external standard method.

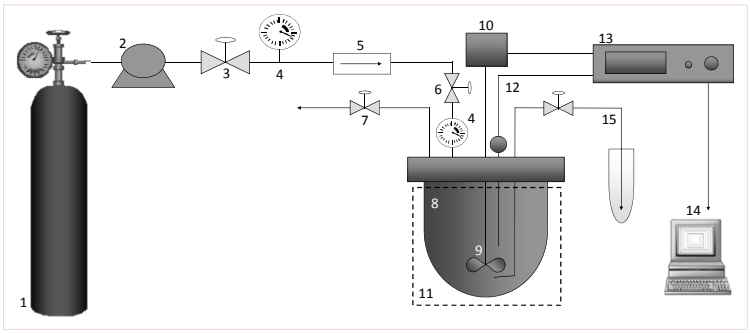

Figure 1. Schematic diagram of the stirred tank reactor used for the synthesis of oligosaccharides under high-pressure conditions. 1. Gas (N_2, CO_2); 2. High-pressure pump; 3. Control valve; 4. Pressure indicator; 5. Valve; 6. On/off pressure inlet valve ; 7. On/off pressure output valve; 8. Reactor vessel (100 mL); 9. Stirrer; 10. Stirring motor; 11. Temperature-controlled bath; 12. Thermocouple; 13. Reactor control system; 14. Computer; 15. Collector tube with check valve.

RESULTS AND DISCUSSION

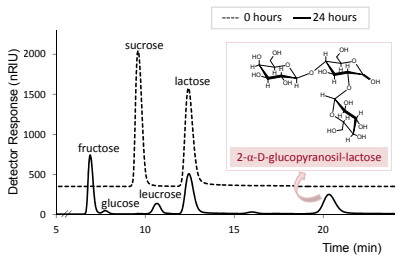

Figure 2. LC-RID profile of transglycosylation reaction based on sucrose:lactose 25:25 (g 100mL^{-1}) catalyzed by dextransucrase from *L. mesenteroides* B-512F (0.8 U mL^{-1}) at 30ºC in 20 mM sodium acetate buffer at pH 5.2, for 0 and 24 hours.

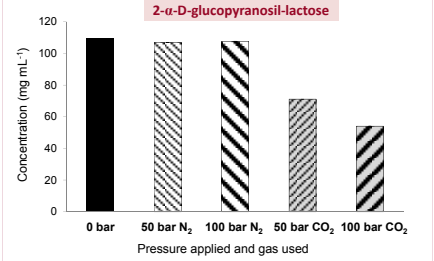

Figure 3. Concentration of 2-α-D-glucopyranosyl-lactose upon transglycosylation reaction (24h) based on sucrose and lactose mixtures catalyzed by dextransucrase from *L. mesenteroides* B-512F, according to the parameters studied, including the gas used to pressurize the system (N_2 or CO_2), as well as the pressure applied.

- **Atmospheric conditions:**
  - ✓ Maximum yield of 2-α-D-glucopyranosyl-lactose was 110 mg mL^{-1} (44%, in weight respect to the initial amount of lactose).
  - ✓ Sucrose was readily consumed by the hydrolytic action of the dextransucrase, resulting in glucose (efficiently transferred) and fructose.
  - ✓ Lactose gradually decreased (loss of 35%) acting as glucose acceptor.
- **High-pressure conditions:**

  N_2 pressurization:
  - ✓ Slight influence of pressure on the final yield of 2-α-D-glucopyranosyl-lactose obtained (regardless of the pressure applied).

  CO_2 pressurization:
  - ✓ The yield of 2-α-D-glucopyranosyl-lactose obtained significantly decreased: Halved with 100 bar and less pronounced with 50 bar. This fact was most probably mainly due to the decrease of pH, produced by dissolving CO_2 in the reaction medium, despite the use of buffer.
  - ✓ To ensure that supercritical CO_2 conditions were reached, it was decided to increase the temperature to 40 C (at 100 bar pressure), but the results were not satisfactory (data not shown). In fact, the yield of 2-α-D-glucopyranosyl-lactose further decreased, possibly due to the deviation of the optimum temperature and also due to the higher dissolution of CO_2 produced with increasing temperature.

CONCLUSIONS

- ❖ Under the experimental conditions used in this work, the production of 2-α-D-glucopyranosyl-lactose via enzymatic synthesis under high-pressure conditions has been achieved.
- ❖ This investigation can be used as a starting point for addressing the synthesis of new bioactive oligosaccharides under pressure conditions as an alternative reaction medium for enzyme-catalyzed reactions.
- ❖ However, for this particular application, the results showed no positive influence of pressure on the final yield of 2-α-D-glucopyranosyl-lactose obtained, according to the parameters studied, including the gas used to pressurize the system (N_2 or CO_2), as well as the pressure applied.

Acknowledgements: The authors gratefully acknowledge financial support from MICINN (project AGL2011-27884 and Consolider Ingenio 2010 FUN-C-FOOD CSD2007-00063). M.D.M. thanks CSIC for her JAE predoctoral fellowship and M.H. thanks MICINN for his "Ramón y Cajal" contract.