FOA26

SYNTHESIS OF OLIGOSACCHARIDES DERIVED FROM STACHYOSE HYDROLYSIS BY PECTINEX ULTRA SP-L

A. Montilla, A. I. Ruiz Matute,A. Olano, N. Corzo
Instituto de Investigación en Ciencias de la Alimentación CIAL (CSIC-UAM).
Nicolás Cabrera 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain,
nieves.corzo@csic.es

α-Galactosides are derivatives of sucrose that consist of galactose residues linked by α-(1-6) linkages to the glucose moiety. These oligosaccharides are found abundantly in grain legumes and their consumption is associated with the production of flatulence. However, they might be used as a prebiotic growth substrate for intestinal bacteria because they pass undigested to the colon [1]. Several studies provide convincing evidence that α-galactosides have beneficial effects on the survival of different bifidobacteria strains [2,3]. Raffinose and stachyose are industrially available in large amounts as a byproduct from the production of soy protein isolate, and they seem to be a promising raw material to manufacture of new oligosaccharides. The relatively inexpensive commercial enzyme preparation Pectinex Ultra SP-L (Pectinex), produced by *Aspergillus aculeatus*, has been shown to contain fructosyltransferase activity [4] and therefore, it could be used as a catalyst in the large-scale production of stachyose-derived oligosaccharides with improved prebiotic properties. The objective of this work has been to investigate in more detail the fructosyltransferase activity from *A. aculeatus* of the commercial enzymatic preparation Pectinex during stachyose hydrolysis.

Enzymatic synthesis of oligosaccharides from stachyose using Pectinex was carried out under different reaction conditions such as temperature (50, 60, and 70 ºC), pH (3.5, 4.5, 5.5, 6.5, and 7.5), stachyose concentration (100, 300, and 600 g/L), enzyme concentration (17, 34, and 78 U/mL), and time up to 24 h. Purification of the reaction mixtures was performed following the method described by Morales et al. [5]. The enriched fraction was characterized by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Mass spectra were obtained over the *m/z* range 100-1500. Analysis of carbohydrates was performed by gas chromatography (GC) using a flame ionization as detector (FID). The trimethylsilyl oximes of mono-, di-, and oligosaccharides were resolved using a 8 m × 0.25 mm × 0.25 μm film fused silica capillary column coated with CP-SIL 5CB (methyl silicone). The oven temperature was programmed from 150 to 165 ºC at 3 ºC/min, then at 5 ºC/min to 340 ºC and held at this temperature for 10 min. Quantitative analysis was carried out by the internal standard method. The amount of remaining stachyose and the yield of oligosaccharides in the reaction mixtures were expressed as weight percentage of total carbohydrate content.

Different oligosaccharides from DP2 to DP8 were formed during stachyose hydrolysis by fructosyltransferase activity of *A. aculeatus*. Galactosyl-melibiose (DP3) was synthesized as a result of fructosidase activity, whereas fructosyl-stachyose (DP5) and difructosyl-stachyose (DP6) were formed as a consequence of the fructosyltransferase activity of the enzyme preparation. Temperature, pH, substrate and enzyme concentrations were varied to select the optimum conditions for tri- or oligosaccharide synthesis. The optimal reaction conditions for the synthesis of penta- and hexasaccharides were 60 ºC, pH 5.5, 600 mg/mL of stachyose, and 34 U/mL of enzyme during 3h. However, to obtain the maximum yield of galactosyl-melibiose (67%), the assays should be carried out at 60 ºC and pH 5.5, using 100 mg/mL of stachyose and 34 U/mL of enzyme during 24 h since it was a very stable to hydrolysis.

In conclusion, stachyose could be used as a substrate for the enzymatic synthesis of new oligosaccharides that may open new opportunities in the development of future prebiotics.

[1] L.AM. Van den Broek, et al, Lait 85 (2005) 125.
[2] I. Trojanova, E. Vlkova, V. Rada, M. Marcunek.Folia Microbiol. 51 (2006) 320.
[3] C. Martínez-Villaluenga, R. Gómez. Int. Dairy J. 17 (2007) 116.
[4] A. Montilla, N. Corzo, A. Olano, M.L. Jimeno, J. Agric. Food Chem. 57 (2009) 5007.
[5] V. Morales, M.L Sanz, A. Olano, N. Corzo, Chromatographia 64 (2006) 233.

Acknowledgments: this work has been financed under a R+D program of the Spanish Ministry of Science and Innovation, Consolider Ingenio 2010 (FUN-C-FOOD): CSD 2007-00063; R + D program of the Comunidad de Madrid, project ALIBIRD 2009/AGR-1469 ;R + D program of the Comunidad de Castilla-La Mancha POII10-0178-4685 and IBEROFUN P109AC0302 (CYTED).