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Enzymatic synthesis of galacto-oligosaccharides from lactose – comparison of different β-galactosidases from *Kluyveromyces lactis, Aspergillus oryzae and Bacillus circulans*

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In a number of publications health-promoting effects for galacto-oligosaccharides (GOS) are described. Bifidogenic and immunostimulating effects as well as protection against bacterial and viral infections are mentioned. Cow milk, however, has only low concentrations of oligosaccharides (MOS). The aim of the current study is to synthesize GOS enzymatically from lactose and to characterize the oligosaccharides in terms of composition and nutritional properties.

For GOS synthesis raw milk was skimmed, milk proteins were separated via ultrafiltration (NMWCO 5000 Dalton) and edible grade lactose was added up to 40% (w/w) in the permeate. After pH settings, β-galactosidases from *K. lactis*, *A. oryzae* or *B. circulans* were added. The reaction was carried out at 40°C and samples were taken hourly. The sugar composition was characterized by high-pH anion-exchange chromatography with pulsed amperiometric detection (HPAEC-PAD) and online electroscopy ion trap mass spectrometry (IT-MS).

Mono-, di-, tri-, tetra- and pentasaccharides were identified in the GOS samples by mass spectrometry. Depending on the applied enzyme distinct differences in the composition of GOS were determined. One main trisaccharide was detected after enzymatic incubation with the β-galactosidases from *K. lactis* (approx. 10%), *A. oryza*e (approx. 15%) and *B. circulans* (approx. 20%), respectively. Due to the different HPAEC retention times of the diverse main trisaccharides, the preferred formation of different glycosidic bound oligosaccharides was identified. This is supported by studies described in the literature [Martínez-Villaluenga et al. JFCA, 21, 540-544 (2008) and Gosling et al. JAFC, 57, 11570-11574 (2009)] where it is stated that β-galactosidases from *A. oryzae* and *K. lactis* preferably generate β1-6GOS and β-galactosidases from *B. circulans* produce GOS predominantly by β1-4bonds.

The highest yields of GOS were achieved by the application of the β-galactosidases from *B. circulans.* Therefore, this enzyme seems to be the most promising for further studies. To evaluate the physiological effectiveness of the GOS samples and to elucidate the structure-activity relationships also anti-inflammatory and bifidogenic investigations are planned.