Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 2466–2470
BIORESOURCE TECHNOLOGY

Microwave-assisted hydrolysis of *Zymomonas mobilis* levan envisaging oligofructan production

Valdemir Cordeiro de Paula [a], Irapuan Oliveira Pinheiro [b], Carlos Edison Lopes [a], Glícia Maria Torres Calazans [a,*]

[a] *Department of Antibiotics, Federal University of Pernambuco, 50.670-901 Recife – PE, Brazil*
[b] *Institute of Biological Sciences, University of Pernambuco, 50.100-130 Recife – PE, Brazil*

Received 4 November 2005; received in revised form 30 April 2007; accepted 30 April 2007
Available online 2 July 2007

Abstract

Levan, a polysaccharide from *Zymomonas mobilis*, was hydrolyzed to obtain oligofructans or fructooligosaccharides with a degree of polymerization varying from 4 to 14. Fructooligosaccharides (FOS) are short chain fructans that beneficially affects the host by selective stimulation of growth and activity of one or a number of bacteria including probiotic bacteria in the colon. The hydrolysis was performed in a microwave oven to shorten the reaction time. The experiments showed that it is possible to maximize selected oligomers by interrupting the hydrolysis at the due time. The results allow one to infer that the procedure may also be useful for production of oligomers from other polysaccharides.

Keywords: Fructooligosaccharides; Hydrolysis; Levan; Microwave; Prebiotics

1. Introduction

Polysaccharides are macromolecules composed of hundreds of monosaccharide units. If the molecular chain contains less than twenty monosaccharides it may be considered an oligosaccharide (Kennedy et al., 1989). Both polysaccharides and oligosaccharides are readily hydrolyzed in acid solutions yielding monosaccharides. However, oligosaccharides may be obtained from polysaccharides by stopping the hydrolysis so as to leave the desired oligomer maximized for subsequent isolation (Li, 1998). This isolation may be performed at an industrial level by continuous chromatography.

Fructooligosaccharides (FOS) are short chain fructans having prebiotic potential. Fructans are oligomers or polymers of fructose. A prebiotic is a nondigestible food ingredient that beneficially affects the host by selective stimulation of growth and activity of one or a number of bacteria including probiotic bacteria in the colon. Probiotic is a food or supplement, containing viable microorganisms that are present in sufficient numbers to actively enhance consumers' health by improving the balance of microflora in the gastrointestinal tract. Probiotic bacteria are mostly represented by genera *Lactobacillus* and *Bifidobacterium* and specific prebiotic substances, such as FOS, that stimulate their development. Lactobacilli and bifidobacteria do not need specific prebiotic substances for growth. However, in the complex ecosystem of the intestinal tract, prebiotics tend to selectively enrich these groups. Many fruit and vegetables contain prebiotic oligosaccharides such as FOS but the levels are too low to have any significant effects. FOS can be commercially produced by hydrolysis of polysaccharides or by enzymatic generation. A number of benefits are attributed to prebiotic intake: protection against colon cancer, resistance to pathogens by increasing bifidobacteria and lactobacilli, better calcium absorption, reduction of

* Corresponding author. Tel.: +55 81 2126 8347; fax: +55 81 2126 8346.
E-mail address: calazans@ufpe.br (G.M.T. Calazans).

0960-8524/$ - see front matter
doi:10.1016/j.biortech.2007.04.062

cholesterol and triglycerides by lactic acid bacteria and immunological effect (Bekers et al., 2002; Manning and Gibson, 2004).

The main interest of food industries in FOS is due to their functional properties. FOS have a number of interesting properties. FOS are free of calories, scarcely hydrolyzed by digestive enzymes and they are not utilized as an energy source in the body, thus they are safe for diabetics; they are noncariogenic; they encourage the growth of bifidobacteria, they discourage the growth of potentially putrefactive microorganisms and they decrease the level of serum cholesterol, phospholipids, and triglyceride (Yun, 1996).

As is often the case with carbohydrate mixtures, it is also very difficult to separate the FOS components from each other (Yun, 1996). Therefore, it is important to develop an economical method for the production of high-content FOS.

Our interest in fructooligosaccharides mainly arises from their possible use in pharmaceutical applications as an antitumourous means since experiments have pointed towards low molecular weight levans as having higher antitumourous activities (Calazans et al., 1997, 2000). The levan molecular weight distribution curve shows that fractions with low molecular weights are produced in small quantities during the fermentation process. Therefore, it is necessary to develop an efficient way to increase the amount of FOS produced.

The FOS utilized in this paper was produced by *Z. mobilis*, a Gram-negative, facultative anaerobic bacterium that ferments glucose, fructose, or sucrose as carbon sources (Viikari, 1988). These carbohydrates are metabolized via the same biochemical route, the Entner-Doudoroff pathway. *Z. mobilis* are rods 2–6 μm in length and 1–1.5 μm in width, flagellated but lack spores or capsules.

Recently the microwave has become a useful tool for use in organic and inorganic synthesis, in analytical chemical laboratories and in many chemical manipulations during the process of standardizing procedures (Corsaro et al., 2004; Das, 2004; Lindström et al., 2001; Jöergensen and Thestrup, 1995; Morales-Rubio et al., 1993; Singh et al., 2003). The main advantage of applying microwave approaches is the time saving it achieves. The heating effect is due mainly to dielectric polarization.

In this paper a microwave oven was used to accelerate the levan acid hydrolysis envisaging the production of oligofructans for antitumour uses. The experiments were directed to measuring the influence of hydrolysis time on levan hydrolysis at specific conditions of pH and microwave oven potency.

2. Methods

2.1. Levan

Levan ($M_w = 769{,}500$ and $M_n = 50{,}100$) produced by *Z. mobilis* ZAG-12 in sucrose medium was used in all experiments. The purity of levan was approximately 100%.

2.2. Standards

As qualitative standard oligomer a light corn syrup – ALO – 3038 and the standard fructose n° 374 both acquired from Phenomenex (Torrance, CA/USA) were used.

2.3. Hydrolysis

A 20 g/L levan solution was prepared and the pH was adjusted to 2.5 using 0.1 M hydrochloric acid. The hydrolysis was performed in a microwave oven working at 650 Watts that correspond to 60% of the nominal potency. The hydrolysis was performed in an open 250 mL beaker at atmospheric pressure. The working volume was 30 mL of levan solution. The initial temperature was around 30 °C. The beaker was placed into another 600 mL beaker with 200 mL of water to avoid temperatures above 100 °C for safety reasons. The maximum temperature in the hydrolysis was 87 °C. The evolution of temperature during the hydrolysis was not measured. The samples were cooled and neutralized using diluted NaOH (2.5 M, approximately 50 μL) solution to interrupt the reaction at the due time. To eliminate chloride content of the solution the levan was resuspended in distilled water and precipitated twice by ethanol addition. The samples were obtained by evaporation of 200 mL of hydroalcoholic solution followed by recuperation in 4 mL of water. Due to these procedures the concentrations were multiplied by 2.5.

2.4. Oligomer analysis

The separation technique for oligomer analysis was size exclusion (or gel fíltration) chromatography (SEC). The hydrolized samples were analyzed by HPLC (Agilent 1100 series) using refractive index detector, the mobile phase was deionised water and the column used was Phenomenex Rezex-RS0 Oligosaccharide (12 μm × 10 mm × 200 mm, USA).

2.5. Fructose analysis

The fructose was analyzed using deionised water as the mobile phase and in the same equipment the column used was Beckman U-Spherogel Carbohydrate (10 μm × 6.5 mm × 300 mm, USA). The oven temperature was 80 °C in both cases.

3. Results and discussion

The chromatogram of the standard mixture revealed various peaks associated to different degrees of polymerization (DP). These peaks correspond to the monosaccharide and oligosaccharides present in the standard mixture with DP varying from 4 to 14. The chromatographic retention times for each oligomer varied from 17 to 45 min. In Fig. 1 the chromatograms of the standard oligomer (light corn