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Review

Enzymatic production of pectic oligosaccharides from polygalacturonic acid with commercial pectinase preparations

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ABSTRACT

The present study investigates the individual efficiency of six commercial pectinase preparations (Endopolygalacturonase M2, Pectinase, Viscozyme L, Pectinex Ultra SP-L, Pectinase 62L and Macer8 FJ) in catalyzing the liberation of pectic oligosaccharides (POS) from polygalacturonic acid. On the basis of high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) analysis of the enzymatic hydrolysates, products release kinetics revealed a random cleavage pattern and an exo mode of cleavage for all the enzymes except for Endopolygalacturonase M2.

All six enzymes generated oligoGalA with different degree of polymerization (DP); the quantitative composition of oligoGalA depended on the enzyme specificity and the time of enzymatic reaction. Endopolygalacturonase M2 was the best enzyme preparation for production of oligoGalA, with 18% (wt) of digalacturonic acid and 58% (wt) of trigalacturonic acid after 2 h of reaction. Concerning galacturonic acid production, Pectinase 62L was superior to the other enzyme preparations with 47% (wt) after 1 h of reaction.

Keywords: Polygalacturonic acid; Pectic oligosaccharides; Pectolytic enzymes; Degree of polymerization; HPAEC-PAD

Contents

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1. Introduction

Biomass from plant material can be employed as a source of polymeric and oligomeric carbohydrates. In recent years, oligosaccharides have found applications in various fields, notably because of their specific biological activities. The potential of plant cell wall polysaccharides as sources of novel high value-added oligosaccharides has also received special attention. Pectic substances can be employed as functional foods. The conversion of pectic substances by microorganisms or enzymes (Zykwinska et al., 2008; Martínez et al., 2009) constitutes promising biological processes because they allow the production of specific oligosaccharides without any formation of undesirable by-products (Leitão et al., 1995; Cabrera and Van Cutsem, 2005). Most food-grade pectic oligosaccharides (POS) studied to date have been produced by pectinases. Commercial pectinase is a term that generally refers to mixtures of three different enzymatic activities: polygalacturonase (PG), pectin lyase (PL) and pectin esterase (PE). All three activities contribute to the breakdown and modifications of pectic substances from a wide variety of plant materials. Pectinases can be isolated from plants or from microorganisms such as bacteria and fungi (Kaur et al., 2004; Jayani et al., 2005). Most commercial pectinases are produced by *Aspergillus* spp. and are mixtures of pectinolytic enzymes. In spite of the extensive application of pectinases in food industry, commercial pectinase preparations have been investigated. The enzymatic degradation of pectic substances gives galacturonic acid and oligomers (POS).

Galacturonic acid and its derivates can be used in food industry as acidic agents, in chemical industry as washing powder agents and as non-ionic or anionic biodegradable surfactants and in pharmaceutic industry in the production of vitamin C (Molnár et al., 2009; Burana-Osot et al., 2010). POS have also various applications, since they are important signal molecules in plant defences and play roles in plant growth and development processes (Marfà et al., 1991; Ridley et al., 2001; Baldan et al., 2003) and in food industry as potential ingredients (Willats et al., 2006). Moreover, the prebiotic potential of POS has been reported because they selectively increased the populations of beneficial bacteria in human gastrointestinal tract such as bifidobacteria and *Eubacterium rectale* (Manderson et al., 2005). Furthermore, additional functionalities of POS were reported including the repression of lipid accumulation in rats liver, an anti-bacterial activity (Iwasaki et al., 1998), the protection of colonocytes against *Escherichia coli* verocytotoxins (Olano-Martin et al., 2003) and the stimulation of apoptosis of colon cancer cells (Chauhan et al., 2005).

The aim of the present work was to characterize a series of commercial pectinases in order to produce galacturonic acid and pectic oligomers. The enzyme content of six different food-grade commercial pectinase preparations was profiled. Then, the enzymatic hydrolysis of polygalacturonic acid was carried out using the commercial pectinase preparations; a comparison of the production yields by the different pectinase preparations was assessed. The released monomer and oligomers were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) in order to compare the efficiency and specificity of the different commercial enzymes, and thus to establish an activity pattern for each preparation.

2. Materials and methods

2.1. Chemicals

Polygalacturonic acid (PGA), citrus peel pectin (galacturonic acid content approx. 74% and methoxy content approx. 6.7%), D-galacturonic acid monohydrate (AGA), digalacturonic acid (DiAGA) and trigalacturonic acid (TriAGA) were from Sigma Chemical Co., (St. Louis, MO, USA). A standard of saturated oligogalacturonic acids was provided by Prof. P. Van Cutsem (Notre Dame de le Paix University, Namur, Belgium).

All chemicals and reagents were of analytical or HPLC grade.

2.2. Enzymes

All enzyme preparations or pectinases were commercially available. A total of six food grade pectinases from different companies were studied. According to the suppliers, the enzymes were generally produced by *Aspergillus* varieties. Sources, suppliers and experimental conditions for the commercial pectinases used in this work are given in Table 1.

2.3. Enzyme activity measurements

Polygalacturonase (PG) activity was assayed for 10 min with a 0.2% solution of polygalacturonic acid. The number of reducing groups, expressed as galacturonic acid released by enzymatic action was quantified by the 2-cyano-acetamide reagent assay and monitored by the absorbance of resulting colored mixture at 276 nm (Verlent et al., 2005). One unit (U) of PG activity was defined as the amount of enzyme releasing 1 μmol of galacturonic acid per min under assay conditions. Experiment was carried out in duplicate.

Preliminary experiments were conducted to determine optimum pH and temperature of pectinase from *Aspergillus niger* because no information was given by the supplier. The

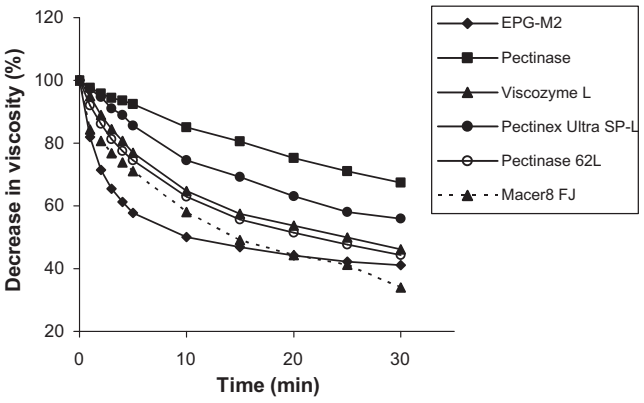

Fig. 1 – Decrease of viscosity catalyzed by commercial pectinase preparations. The results are expressed as means of two tests.