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Pectin extraction from pumpkin with the aid of microbial enzymes

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Abstract

An optimised procedure is reported for extraction of pectin from pumpkin pulp, using an enzyme preparation from *Aspergillus awamori*. In contrast to pumpkin pectin obtained previously by digestion with cell-free culture medium from *Bacillus polymyxa*, the material prepared in the present work forms gels with 60 wt% sucrose at pH 3, although the yield is somewhat lower (14% in comparison with 22%). The main action of the enzyme complex from *A. awamori* is to degrade cellulose and other insoluble constituents of the plant tissue, but it also has some pectinesterase activity, which could allow degree of esterification (DE) to be manipulated by varying digestion time. The time used in this investigation (3 h) gave a DE of 53%; reduction in DE at longer times should yield pectin with a higher content of unesterified galacturonate residues, capable of binding lead and other heavy-metal cations. Some possible medicinal and food uses are suggested.

Keywords: Pectin; Pumpkin; Enzymic extraction; *Aspergillus awamori*

1. Introduction

The natural polysaccharide pectin (a basic building material for cell walls of higher plants) possesses a wide spectrum of functional properties. In combination with water and some other substances, it can act as a thickener, gelling agent, stabilizer, emulsifier, cation-binding agent, etc. (Bottger, 1990). One substance having so many separate properties of technological interest makes pectin a biopolymer especially valuable for medicine and food production. Pectin is not only an effective or even necessary additive to form the structure of food products but also has medicinal benefits which include lowering the cholesterol level in blood, removing heavy metal ions from the body, stabilizing blood pressure, and restoring intestinal functions (Voragen, Pilnik, Thibault, Axelos, & Renart, 1995).

The world consumption of pectin constantly grows and has already exceeded 20,000 tons a year. Dried lemon or orange peel and apple pomace are the main raw materials for its production. And although the potential stock of these raw materials enables the main pectin producers (USA, Germany, Denmark) to plan an annual increase of pectin production of approximately 3.8% (Phillips, 2000), searching for new pectin-containing raw materials is an important task of science and industry (May, 1990).

Another urgent task is to improve pectin extraction techniques from raw material. The current technology is based on acidic hydrolysis and has at least two demerits: acidic extraction does not allow pectin to be extracted fully with no damage to its structure and it does not meet environmental safety due to acid usage. An enzyme-hydrolytic technology seems environmentally safe and more effective in terms of pectin yield. Analysis of the scientific and patent literature shows that a number of research centres have been conducting studies to develop a biotechnological method of pectin extraction but these works are of exploratory nature only, they do not yet involve a wide range of researchers, and their results are far from industrial application. However, the achievements of biotechnology encourage optimism, including the problem under consideration.

This manifests itself, in particular, in sound financing of studies during the last 2 years (Phillips, 2000) provided by

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such world-known pectin producers as Hercules (USA), Herbstreit und Fox (Germany), CP Kelco (Denmark) and some others. The interest is obvious: the implementation of a biotechnological method would double (as a minimum) the pectin production and free the processing plants from expensive works to neutralize the acidic components of the traditional technology. Thus, searching for enzymes or enzyme complexes and ways of their application for pectin production is urgent in both theoretical and applied aspects.

The present work was aimed at studying pectin extraction from pumpkin pulp with the aid of some enzyme preparations. It is based on the mechanism of action of enzymic preparations and their efficiency revealed by one of the authors (Rumyntseva, 1993). She used apple pomace as a pectin-containing raw material. However, pumpkin pulp seems more promising for pectin production in Russia (Ptichkina, 2000) and it would be appropriate to verify the hydrolyzing action of the early used enzyme preparation on this non-traditional pectin-containing raw material.

2. Experimental

Air-dry pumpkin pulp was prepared in the laboratory as follows. Pumpkin (*Volzhskaya Grey* variety) was cut and the seeds were removed. The remainder was reduced to 2–4 cm fragments and the juice was pressed out. The fresh pulp obtained was dried in an infrared (IR) drying box made by NITI TESAR Inc. (Saratov, Russian Federation). The drying lasted 5–7 h at a temperature not higher than 65 °C. This mode of drying is mild with respect to pectin—it does not cause decomposition.

At a preliminary stage, six commercial enzyme preparations from *Trichoderma viride*, *Bacillus macerans* and *Aspergillus awamori*, cultured under aerobic conditions in a stirred fermenter, were used. Their catalytic activity with respect to such polysaccharides as xylan, cellulose and pectin (which are present in the vegetable tissue mainly in an insoluble form) and the efficiency of each preparation in the pectin hydrolysis–extraction process was evaluated. The results are presented in Table 1.

The percentage yield of pectin (D) served as the criterion of efficiency:

$$D = 100(m/m_{pp}), \quad (1)$$

Table 1
Composition and activity (UI/g) of enzyme preparations

	Aspergillus awamori	*Basillus macerans*	*Trichoderma viride*
Xylanase	820	150	10000
Cellulase	2000	–	400
β-Glucosidase	780	–	840
Endopolygalacturonase	235	540	480
Pectinesterase	105	37	**28**

Table 2
Characteristics of pumpkin biopectins obtained from enzyme preparations

	Aspergillus awamori	*Basillus macerans*	*Trichoderma viride*
Yield (%)	14.0	10.5	9.8
Moisture content (%)	9.0	9.0	9.2
Ash residue (%)	2.9	3.0	3.0
pH of 1% solution	5.2	5.2	5.1
Polygalacturonate (%)	64	64	63
DE (%)	53	60	65
Molecular mass (kD)	45	43	42
η_{sp}	2.5	2.4	2.2
Gel strength[a] (kPa)	10	10	10

[a] 1 wt% pectin; 60 wt% sucrose; pH 3.0; measured by Valenta method (Pernas, Smidsrød, Larsen, & Haug, 1967).

where m_{pp} denotes the mass of air-dried pumpkin pulp and m denotes the mass of pectin obtained from it.

As shown in Table 2, the enzyme preparation from the microscopic fungus *A. awamori* gave the best results. The technology for making this enzyme preparation was developed at the Institute of Biotechnology, Moscow, and patented (Zyeva, Pavlova, & Rumyantseva, 1993). This preparation is a complex one. Its main action is degradation of cellulose (cellulase, activity 2000 IU/g), but it also has some xylanase (820 IU/g), β-glucosidase (780 IU/g), endopolygalacturonase (235 IU/g), and pectinesterase (105 IU/g) activity.

In order to find optimum conditions of catalysis, the extraction temperature and weight ratio of water to pulp were varied while the preparation dose in the enzyme solution (1 wt%) and the extraction duration (3 h) were kept constant. As a result, a technique of pectin extraction from dried pumpkin pulp with the aid of the complex *A. awamori* enzyme preparation under laboratory conditions has been developed, and is described below.

Pretreatment of raw material: Cut pumpkin pulp is dried by the IR method, and 50 g of the dried material is mixed with distilled water (400 mL) and left to swell at room temperature for 16–17 h.

2.1. Preparation of enzyme solution

The complex enzyme preparation from *A. awamori* (1 g) is diluted with distilled water (100 mL) and mixed using a magnetic stirrer to obtain a homogeneous solution.

2.2. Implementation of hydrolysis

The enzyme solution is poured into the swollen raw material at a weight ratio of 1:10. The concentration of enzyme preparation obtained is 2 mg/mL and its pH is 5.0. The vessel is placed in a thermostat for 3 h for hydrolysis at 45 °C, with periodic stirring.