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Use of Exo-polygalacturonase to Improve Extraction Yields of Alginic Acid from Sea Mustard (*Undaria pinnatifida*)

– Research Note –

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Abstract

Exo-polygalacturonase (EPG) from *Rhizopus* sp. was applied to the extraction of alginic acid from sea mustard to increase extraction yield. EPG digestion was examined under distinct conditions within temperatures from 25°C to 50°C, pH 5 to 9, and treatment times from 0 to 36 hr. The optimal conditions for alginic acid extraction with EPG were: pH 7.0 at 30°C for 24 hrs. The EPG hot water extraction yield was 3.4 times higher yield than hot water extraction alone. Using EPG to extract alginic acid from sea mustard should be considered a viable alternative to conventional extraction, with the advantage of reducing hazardous wastes such as strong acid and alkali solutions.

Key words: alginic acid extraction, exo-polygalacturonase, sea mustard

INTRODUCTION

Alginic acid is a linear polysaccharide that is commercially extracted from many strains of marine brown seaweed. Alginic acid is a co-polymer of D-mannuronic acid and L-guluronic acid, in which the uronic acids are arranged in a block fashion in the polymeric chain (1). Alginates have widespread applications in the pharmaceutical and food industries, owing to their ability to form viscous solutions at relatively low concentrations and to form gels with Ca^{2+}. Alginic acid from sea mustard can be extracted with hot water in a sodium carbonate solution, or by alternation of acidic and alkaline treatments (2). The highest industrial extraction yields are obtained utilizing sodium carbonate solutions or acidic and alkaline treatments. However, the strong caustic and acidic chemicals produce hazardous toxic wastes that threaten the environment and damage reactors used in the extraction process. Hot water extraction is safer and produces no toxic chemicals, but is inefficient because of low yields (2). In this study, we evaluated the use of enzymatic digestion of sea mustard, using exo-polygalacturonase (EPG), for increasing the yield of alginic acid from hot water extraction.

EPG, a protopectinase, is normally produced by microorganisms to facilitate plant degradation (3-7). EPG cleaves side chains of neutral sugars of pectins that are linked to cellulose or are residues of homogalacturonan. Applications using EPG include pectin production (8), isolation of single cells from vegetable food material (7,9), and the isolation of protoplasts from plant cells (10). Sea mustard alginic acid is a structural component of the cell wall that is esterified to cellulose or hemicellulose. EPG randomly hydrolyses terminal α-1,4-glucoside bonds of D-guluronic acid, thereby isolating D-guluronic acid. Therefore, the use of EPG may be expected to increase the yields from hot water extraction of alginic acid from sea mustard.

MATERIALS AND METHODS

Materials

Dried sea mustard (*Undaria pinnatifida*) was purchased from a local food market in Seoul, Korea; ground with a hammer mill, and screened through an 80-mesh sieve. EPG (Macerozyme R-10) from *Rhizopus* sp. was obtained from Yakult Co. (Tokyo, Japan). All other chemicals were analytical grade.

Extraction of alginic acid by hot-water solubilization method

The extraction of alginic acid from sea mustard was performed as described by Nishide et al. (2), except that the formaldehyde treatment was replaced by EPG digestion for the enzymatic extraction. For non-enzymatic extraction, 5 g of the ground sea mustard was placed in a stoppered flask containing 50 mL of a 3.7% formaldehyde solution and maintained at 30°C overnight.

For the EPG treatment, 5 g of sea mustard was incubated in 50 mL of various pH buffer solutions containing 50 mg of EPG (3 U/mg of protein). To determine the pH effect

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on the EPG alginic acid extraction, the following buffers were used: 20 mM acetate buffer (pH 5.0), 20 mM sodium phosphate buffer (pH 6.0), 20 mM potassium phosphate buffer (pH 7.0), 20 mM Tris-HCl buffer (pH 8.0), and 20 mM glycine buffer (pH 9.0). The effect of alginic acid extraction with EPG was determined at different temperatures (25, 30, 35, 40, 45 and 50°C) and at different durations of time (0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 hr).

Following each enzymatic or non-enzymatic extraction under a given condition, each reaction mixture was diluted with 100 mL of distilled water and hot water extracted by stirring at 100°C for 4 hrs. After filtering through a hemp cloth, the filtrates were dialyzed by cellulose membrane (Avg. flat width 43 mm; Avg. diameter 27 mm; Capacity approx. 175 mL/ft; cutoff size > M.W. 12,000: Sigma Chemical Co.) in distilled water, and filtered through a filter paper (Toyo No.2). The dialyzed inner fluid was concentrated to one-forth of the initial volume using a rotary evaporator (NE-1S, Tokyo Rikakikai Co., Ltd.). Ethanol was then added to make an 80% final concentration. The precipitated alginate gel was obtained by centrifugation at 3,000 × g for 10 min. The pellet was rinsed with ethanol and then with acetone, and centrifuged at 3,000 × g for 5 min. The alginate pellet was dried to powder at 50°C for 12 hrs and weighed. The yield was calculated as the percentage of alginic acid extracted from each 5 g sample. Recovery rate was calculated as the weight ratio of pure alginate extracted to that of the pellet weight. Each experiment was performed in duplicate and the value reported was an average of the two data.

Determination of purity of alginic acid

Purity of extracted alginic acid was determined by the *m*-hydroxydiphenyl method (11), and expressed as % of uronic acid in the total sample.

RESULTS AND DISCUSSION

The effect of temperature on EPG activity was determined from 24 h alginic acid extraction yields at 25, 30, 35, 40, 45 or 50°C at pH 7 (Fig. 1). The highest yield of alginic acid by EPG treatment was obtained at 30°C, which is presumed to the optimal temperature for EPG extraction.

The greatest alginic acid yield from sea mustard was achieved at pH 7.0 after 24 h incubation with EPG (Fig. 2). EPG is stable at pH 5 and 6 (12), but the optimal pHs for pectin extraction with EPG from apple and pear pomace are 7.0 and 7.8, respectively (13,14). The pH of incubation solutions alters the electrical charges of both enzyme and substrate, and profoundly affects the recognition of the active site of enzymes and the separation of products after the reaction. Although we determined that pH 7.0 was

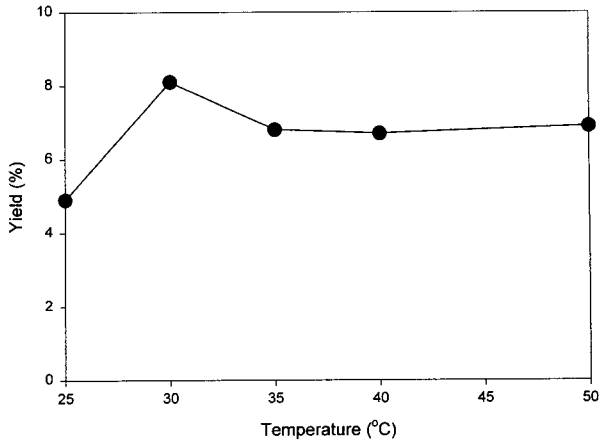

Fig. 1. Temperature-related changes of alginic acid extraction yield with EPG treatment at pH 7.0 for 24 hours.

optimal under the conditions we used, we can not exclude the possibility that different buffers would behave differently and that a different pH would produce maximum yields if a different buffer was used. Therefore, further experiments using a variety of buffers over a broad pH range is needed to determine the optimal pH for EPG extraction.

The maximum yield of alginic acid was obtained after 24 h incubation with EPG (Fig. 3). EPG is a macerating enzyme due to its hydrolytic activity on protopectin in plant cell walls. Pectin extraction from apple and pear pomace by EPG was maximized with incubation times of 60 and 36 hours, respectively (13,14). Although the mechanism is unknown, the hydrolytic activity of EPG on glucoside bonds in sea mustard can be inferred from its effectiveness in alginic acid extraction.

As shown above, the highest extraction yield (8.1% of alginic acid from sea mustard) was obtained with EPG treatment for 24 hrs at pH 7 and 30°C. In contrast, there was only a 2.4% yield from the non-enzymatic hot water extraction (Table 1). The increased yield with the EPG treat-

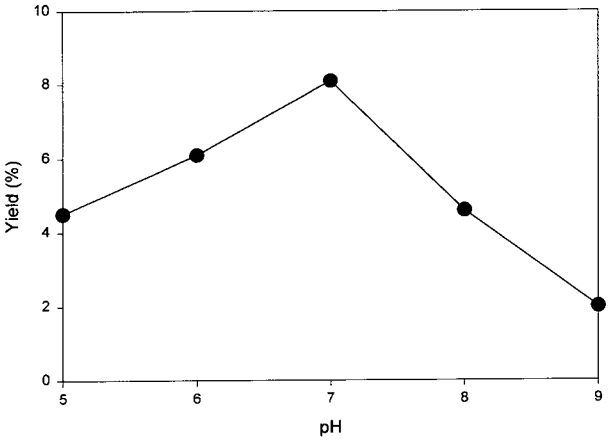

Fig. 2. The pH-related changes of alginic acid extraction yield with EPG treatment at 30°C for 24 hours.

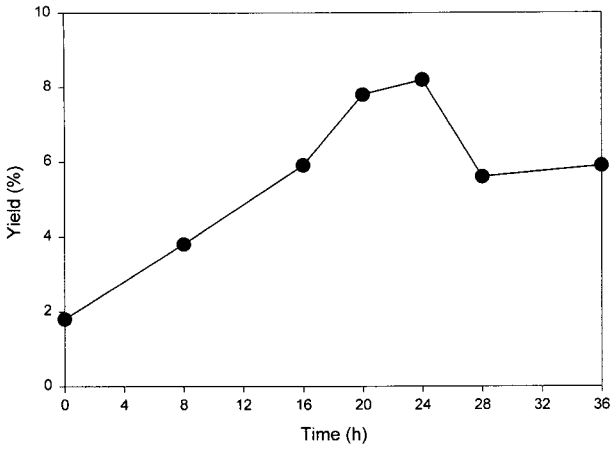

Fig. 3. Time-related changes of alginic acid extraction yield with EPG treatment at 30°C at pH 7.0.

Table 1. Effect of EPG treatment on alginic acid extraction in sea mustard

Treatment	Yield (%)[1)]	Purity (%)	Pure yield (%)[2)]	Recovery (%)[3)]
Hot water without EPG[4)]	2.4	15.5	0.37	1.68
Hot water with EPG	8.1	20.9	1.70	7.73

[1)]The yield was calculated as a percentage of extracted alginic acid amount from sample weight.
[2)]Pure yield = Yield × Purity.
[3)]Recovery means the ratio of the pure alginic acid to the extracted amount from sample weight.
[4)]The hot water extraction method for alginic acid of sea mustard was at pH 7.0, 30°C for 24 hrs.

ment implies that alginic acid, an ester-bound form of cellulose or hemicellulose in the cell wall of sea mustard, is easily released by the enzymatic reaction. This explanation is supported by previous results in which EPG acted as a protopectinase, degrading protopectin to pectin (13); although the optimal conditions were different.

Purity of alginic acid extracts are shown in Table 1. The pellet from the EPG treatment contained 5% more alginic acid than the pellet without enzymatic treatment (Table 1). Recovery rate increased from 1.68 to 7.73% (4.6 times) with EPG treatment. These results suggest that enzymatic hydrolysis may be an effective tool for the extraction of alginate from sea mustard.

In conclusion, we obtained more alginic acid from sea mustard by using EPG in a hot water extraction. Although EPG has been used to extract pectin from apple pomace (13), EPG has not been previously used for the extraction of alginic acid from sea mustard. EPG hot water extraction has the potential to reduce environmentally hazardous wastes produced by conventional extraction methods. Therefore, application of EPG in a hot water extraction system should be considered to be an appropriate alternative method for the extraction of alginic acid from sea mustard.

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