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Enzymatic production of a soluble-fibre hydrolyzate from carrot pomace and its sugar composition

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Abstract

This study was conducted to determine the sugar composition of soluble dietary fibre from carrot pomace, a by-product from the carrot juice processing industry. Carrot pomace was treated with a mixture of 1% NaOH and 2% acetic acid, and the pretreated sample was hydrolyzed by edible snails crude enzyme. The supernatant portion was then treated with 85% ethanol and separated into alcohol-soluble dietary fibre (ASDF) and alcohol-insoluble dietary fibre (AIDF). AIDF increased from an initial value of 3.3 g/100 g to 41.7 g/100 g carrot pomace after 96 h of reaction. The AIDF contained rhamnose, arabinose, mannose, galactose, glucose and a small amount of xylose. Monosaccharides (glucose, fructose, galactose, arabinose), cellooligosaccharides (cellopentaose, cellotetraose, cellotriose, cellobiose), and galactooligosaccharides (galactotetraose, galactotriose) were detected in the ASDF.

Keywords: Carrot pomace; Soluble dietary fibre; Cellulase

1. Introduction

The benefits of dietary fibre include preventing constipation, lowering the risk of coronary heart disease and colon cancer, attenuating blood glucose levels, reducing serum cholesterol levels, providing weight management, and functioning as a prebiotic (Joanne, Slavin, & Greenberg, 2003; Kritchevsky, 1998; Ohr, 2004). The health benefits of dietary fibres have led to an increased consumption of fibre-rich products and hence encouraged food scientists to search for new sources of fibre as food ingredients (Chau, Chen, & Lee, 2004). Dietary fibre is defined as an indigestible fraction, which contains oligosaccharides and resistant starches, resistant proteins, and associated compounds such as polyphenols (Davidson & McDonald, 1998). Most dietary fibres are components of the cell wall polysaccharides, which are resistant to digestion by the alimentary enzymes of humans (Kotcharian, Kunzek, & Dongowski, 2004). Thus, cell wall materials are of physiological importance as dietary fibre-rich materials. Dietary fibre can be classified as either soluble or insoluble, based on whether it forms a dispersion when mixed with water (soluble fibre), or not (insoluble fibre) (Jiménez-Escrig & Sánchez-Muniz, 2000). Soluble and insoluble fibres share many of the same physical properties. The subject of this report is soluble dietary fibre from carrot pomace. Soluble dietary fibres are often used as food ingredients because of their capacity to form viscous gels with water.

The carrot is an important root vegetable and is often used for juice production. In many countries, a steady increase of carrot juice consumption has been reported (Schieber, Stintzing, & Carle, 2001). Thousands of tons

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of carrot pomace are produced in the process of juice extraction by industry (Chau et al., 2004). Although this agricultural byproduct may be used for animal feed, it is usually discarded as waste material. Because agricultural waste has a high organic matter content, these waste materials pose a serious environmental pollution problem. Many studies have reported that value-added products (organic acids, sugars, and ethanol) can be produced from agricultural byproducts of fruit and vegetable processing (Garg & Hang, 1995; Hang, Lee, & Woodams, 1982; Hang & Woodams, 2001; Martín, Galbe, Wahlbom, Hahn-Hägdrdal, & Jönsson, 2002; Ruengruglikit & Hang, 2003; Stenberg, Galbe, & Zacchi, 2000).

Edible snails have long been recognized as an excellent source of many major cell-wall degradation enzymes, including complex cellulases (endoglucanase, exoglucanase, and β-glucosidase) and β-galactosidase. (Got, Marnay, Jarrige, & Font, 1964; Marshall, 1973; Marshall & Grand, 1976; Strasdine & Whitaker, 1963). Strasdine and Whitaker (1963) reported that the digestive juice of the snail, *Helix pomatina*, contained cellulases and chitinase. β-Mannanases, purified from the edible snail, *Helix lucorum* L., were able to hydrolyze crystalline or amorphous mannan to oligosaccharides of low degrees of polymerization (Flari, Matoub, & Rouland, 1995).

In preliminary studies, we purified cellulase and β-galactosidase from the internal organs of the edible snail, *Helix pomatina*, and monitored the characteristics of these enzymes (Yoon, Kang, Lee, Shin, & Kim, 2002; Yoon & Kim, 2002). The aim of the present study was to investigate the application of the edible snail's enzymes in the production of soluble dietary fibre from carrot pomace as a byproduct of the carrot juice processing industry.

2. Materials and methods

2.1. Materials

Carrots were purchased from a local market and kept at 4 °C prior to use. The carrot pomace was obtained using a grinder attached to the screw press (Model 210, Hanil, Korea) and dried in an air-oven at 60 °C for 24 h. The moisture content of the dried sample was 3.05 g/100 g. The sample was finely ground to 0.05 mm in size and pretreated with sodium hydroxide-acetic acid as described by Abraham and Kurup (1997). Ten gram of the carrot pomace were pretreated with a mixture of 100 ml of 1% NaOH and 2% acetic acid (1:1) and incubated at 30 °C for 10 h. The pretreated carrot pomace was washed with distilled water, until the wash water pH became neutral, then dried overnight at 80 °C. The dried solid was used as a substrate for the enzymatic hydrolysis experiments.

The cellulase-rich crude enzyme preparation was isolated from edible snails (*Achatina fulica*) as described previously (Yoon et al., 2002; Yoon & Kim, 2002). The internal parts of edible snails (100 g) were homogenized with 500 ml of 10 mM phosphate buffer (pH 6.5), and the homogenate was mixed on a magnetic mixer at 4 °C for 3 h. The crude extract was obtained by centrifuging at 10,400*g* for 30 min. The supernatant was fractionated by salting out with ammonium sulfate (20–80%) and centrifuged at 10,400*g* for 30 min. The precipitate was dissolved in 10 mM phosphate buffer (pH 6.5) and desalted with the same buffer for 24 h. The desalted crude enzyme was concentrated with the Amicon Diaflo system, using Diaflo PM 10 ultrafiltration membranes (MW cutoff; 10,000) by N_2 gas. This concentrated crude enzyme, used in the enzymatic hydrolysis experiments, contained 1250 U/ml of carboxylmethyl cellulase and 560 U/ml of β-galactosidase.

2.2. Enzymatic hydrolysis of the carrot pomace

Enzymatic hydrolysis was conducted in 500 ml Erlenmeyer flasks containing pretreated carrot pomace (2.0% in 10 mM sodium acetate buffer, pH 5.0), to which 0.5 ml of crude enzyme was added. The flasks were agitated at 200 rpm on a shaking incubator for 24 h at 50 °C. The effects of pH and reaction temperature on enzymatic hydrolysis of carrot pomace were investigated using this method, except that the reaction mixtures were adjusted to different pH values (3.0, 4.0, 5.0, 6.0) and different reaction temperatures (30, 40, 50, 60 °C), respectively. The same method was then employed by varying substrate concentration (0.5%, 1.0%, 2.0%, 3.0%) to determine its optimum. Enzymatic hydrolysis is defined as the amount of glucose released as a percent of total sugar. Reducing sugars and total sugar were analyzed with dinitrosalicylic acid reagent (Miller, 1959) and the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Reber, & Smith, 1956), respectively, with glucose as the standard.

In a time study of the production of soluble fibre, 1% pretreated carrot pomace was enzymatically hydrolyzed at 50 °C and pH 5 for a total of 96 h. Samples were taken at 24 h intervals, filtered, and freeze-dried.

2.3. Separation of alcohol-soluble dietary fibre (ASDF) and alcohol-insoluble dietary fibre (AIDF)

ASDF and AIDF were separated according to the method of Chau and Huang (2003) with slight modifications. As shown in Fig. 1, the freeze-dried sample was homogenized in 85% boiling alcohol for 1 min. The suspension was further boiled for another 40 min and filtered. The filtrate was used as ASDF. The residue

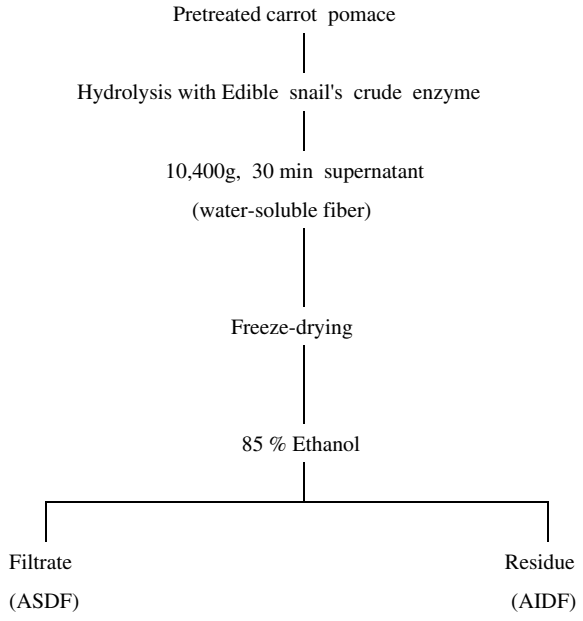

Fig. 1. Scheme for separation of ASDF and AIDF from carrot pomace.

fraction was dissolved in distilled water and used as AIDF. The yields of ASDF and AIDF were determined on a weight basis without correction for protein and starch.

2.4. Molecular weight chromatography of AIDF

Polysaccharide composition of AIDF was distinguished on the basis of the molecular weights of the various polysaccharides, estimated by Sephacryl S-200 gel filtration chromatography. The Sephacryl S-200 column (3.0 × 80 cm) was equilibrated with distilled water, and its void volume (V_o) was determined by blue dextran (Mw, 2,000,000) using distilled water as the mobile phase at a flowrate of 0.25 ml/min. Elution volumes (V_e) of standard dextrans of various molecular weight (8800, 39,000, 70,000) were determined. The V_e/V_o ratios were plotted to form a standard curve. The AIDF samples were layered onto the column. The V_e/V_o ratios of detected polysaccharides were used to calculate their individual molecular weights. A sample of each elution volume was subjected to the phenol–sulfuric acid method, both to detect the presence of polysaccharide and to determine total sugar as its glucose equivalent

2.5. Analysis of composition of ASDF and AIDF

The sugar composition of ASDF was determined by HPLC (Waters, model 600E, USA) under the following conditions: Column, Sugar-Pack I; Waters Associates Differential Refractometer RI 410; column temperature, 85 °C; and flow rate, 0.4 ml/min. The cellooligosaccharides and galactooligosaccharides used as standards were purchased from Sigma Chemical Co. (USA).

AIDF was hydrolyzed with sulfuric acid, and derivatized to alditol acetate according to the procedure of Blakeney, Harris, Henry, and Stone (1983). Constituent neutral sugars of AIDF were analyzed by gas chromatography (Hewlett–Packard Model 5890, USA). GC operating conditions were as follows: Capillary column FP™-2380 (30 m × 0.32 mm); detector, FID; column temperature, 200 °C (2 min), then 2 °C/min to 240 °C (2 min); flow rate, 5 ml/min, and carrier gas, N_2.

2.6. Statistical analysis

All experiments were carried out in triplicate, and each sample was analyzed in duplicate. The SAS statistical computer package was used to analyze the experimental data (SAS Institute, Inc., Gary, NC, USA). The tabulated values that have no common superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range test.

3. Results and discussion

3.1. Optimal conditions of enzymatic hydrolysis

Table 1 shows the effect of pH, temperature, and substrate concentration on enzymatic hydrolysis of carrot pomace. The optimal pH and reaction temperature were pH 5.0 and 50 °C at a substrate concentration of 2.0% (Table 1). Substrate concentration had a strong effect on the enzymatic hydrolysis of carrot pomace. The edible snail's enzyme produced significantly more sugar at 1% carrot pomace than at any other substrate concentration at 50 °C and pH 5. The above results show the optimal conditions of enzymatic hydrolysis of carrot pomace to be pH 5, 50 °C and 1% substrate concentration. The optimal pH and temperature of cellulase and β-galactosidase purified from edible snails were reported to be pH 6.0 and 50 °C and pH 3.0 and 60 °C, respectively (Yoon et al., 2002; Yoon & Kim, 2002). The difference in optimal pH and temperature between the edible snail's crude enzyme and these purified enzymes might be due to other carbohydrolases, such as xylanase, as well as cellulase and β-galactosidase, which are contained in edible snail's enzyme system (Flari & Charrier, 1992).

3.2. Yield of soluble fibre

Table 2 is the comparison of the yield of ASDF and AIDF and their sum, i.e., total water-soluble fibre from carrot pomace by the edible snail's crude enzyme. Enzymatic hydrolysis was conducted for 96 h at pH 5.0 and 1% substrate concentration. Starch content was not

Table 1
Effect of pH, temperature and substrate concentration upon 24 h of enzymatic hydrolysis of carrot pomace

	Enzymatic hydrolysis (%)
pH	
3	11.5 ± 0.70 [b]
4	12.0 ± 0.20[b]
5	13.5 ± 0.70[a]
6	12.3 ± 0.82[b]
Temperature (°C)	
30	11.3 ± 0.61[bc]
40	12.5 ± 0.62[ab]
50	13.0 ± 0.25[a]
60	9.7 ± 0.62[c]
Substrate concentration (%)	
0.5	15.7 ± 1.15[b]
1.0	20.3 ± 0.58[a]
2.0	14.3 ± 0.66[b]
3.0	9.7 ± 0.57[c]

Means and standard deviations for $n = 3$.
The experimental values that have no common superscript are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 2
Yield of water-soluble fibre from carrot pomace by enzymatic hydrolysis

Time (h)	Yield (g/100 g carrot pomace)		
	ASDF	AIDF	Total
0	14.4 ± 0.14[a]	3.3 ± 0.30[a]	17.7 ± 1.12[a]
24	29.4 ± 2.46[b]	32.3 ± 3.70[b]	61.7 ± 1.22[b]
48	30.1 ± 0.75[b]	36.7 ± 2.22[c]	66.8 ± 1.45[c]
72	30.7 ± 1.35[b]	37.3 ± 1.05[c]	68.0 ± 2.18[c]
96	35.6 ± 2.55[b]	41.7 ± 1.39[d]	77.3 ± 3.89[d]

ASDF, alcohol-soluble dietary fibre.
AIDF, alcohol-insoluble dietary fibre.
Means and standard deviations for $n = 3$.
Yields were determined on a weight basis and were not corrected for protein and starch.
The experimental values that have no common superscript are significantly different ($P < 0.05$) according to Duncan's multiple range test.

measured, because carrot pomace contained 1% starch (Bao & Chang, 1994). The yields of the total water-soluble fibre increases greatly upon 24 h of enzymatic hydrolysis (17.9 g to 61.7 g/100 g carrot pomace) and gradually upon extended hydrolysis up to 77.3 g/100 g carrot pomace at 96 h (Table 2, Total). There is no significant difference in the yield of the ASDF component beyond 24 h. The yield of ASDF after 24 and 96 h of reaction were 29.4 and 35.6 g/100 g of carrot pomace, respectively. The yield of the AIDF component significantly increased as the time of enzymatic reaction was prolonged. For example, the yield of AIDF increased from an initial value of 3.3 g/100 g to 41.7 g/100 g of carrot pomace after 96 h of reaction. Compared with the ASDF content, the relatively higher level of AIDF might be partly due to alcohol precipitates, such as protein and some inorganic substances (Ting, 1970).

Carrot pomace contained 67.4 g of alcohol-insoluble solids and 56.3 g of water-insoluble solids, while total dietary fibre content of the carrot pomace was higher than those of some other agricultural byproducts (Chau et al., 2004). Earlier research suggested that the carrot pomace could be a promising source of functional ingredients in higher-fibre natural food products (Schieber et al., 2001). Bao and Chang (1994) reported that the chemical composition of the carrot pulp, after juice was extracted, was 48.5% dietary fibre, composed of 41.4% AIDF and 7.1% ASDF. In contrast, we report here the enzymatic production of over 70% water-soluble fibre (77.3 g/100 g of carrot pomice). This suggested that edible snail's enzyme was a good enzyme for producing water-soluble fibre from agricultural byproducts.

3.3. Molecular chromatography of AIDF

Fig. 2 shows chromatograms for molecular weights of AIDF estimated by the Sephacryl S-200 column. Dextran standards of Mw 70,000, Mw 39,000, and Mw 8800 were detected in fractions number 58, 67 and 83, respectively. The standard curve of V_e/V_o vs molecular weight was plotted for the standard dextrans. There is a big peak in fractions No. 45–60 ($MW_{app} > 70{,}000$) and small peak in fractions No. 70–80 (MW_{app} 20,000–10,000) at 0 h. As reaction time was extended, degradation of carrot pomace by the edible snail's enzyme increased. The chromatogram for 24 h of enzymatic hydrolysis has a peak in fractions No. 45–60 ($MW_{app} > 70{,}000$), fractions No. 60–75 (MW_{app} 70,000–20,000) and fractions No. <75 ($MW_{app} <$ 20,000). In contrast, samples after 72 and 96 h of enzymatic hydrolysis have smaller peaks before fraction No. 83 ($MW_{app} > 8800$) and bigger peaks after fraction No. 83 ($MW_{app} < 8800$) than those of sample after 24 h. The above results show that high molecular weight polysaccharides were converted to low molecular weight polysaccharides by the edible snail's enzyme as reaction time was prolonged.

3.4. Composition of AIDF

The monosaccharide profiles of the AIDF, which were determined on a weight basis without correction for protein and starch, are shown in Table 3. AIDF is composed of rhamnose, arabinose, mannose, galactose, glucose, and small amount of xylose. The major neutral sugars at 0 h were rhamnose, arabinose, and galactose (13.7, 22.9 and 20.0 mg/g, respectively). High contents of these neutral sugars may be due to breakage between xyloglucan and pectic polysaccharides such as rhamnogalacturonan and arabinogalactan by chemical pretreatment of the carrot pomace (Alberts, Bray, & Lewis,

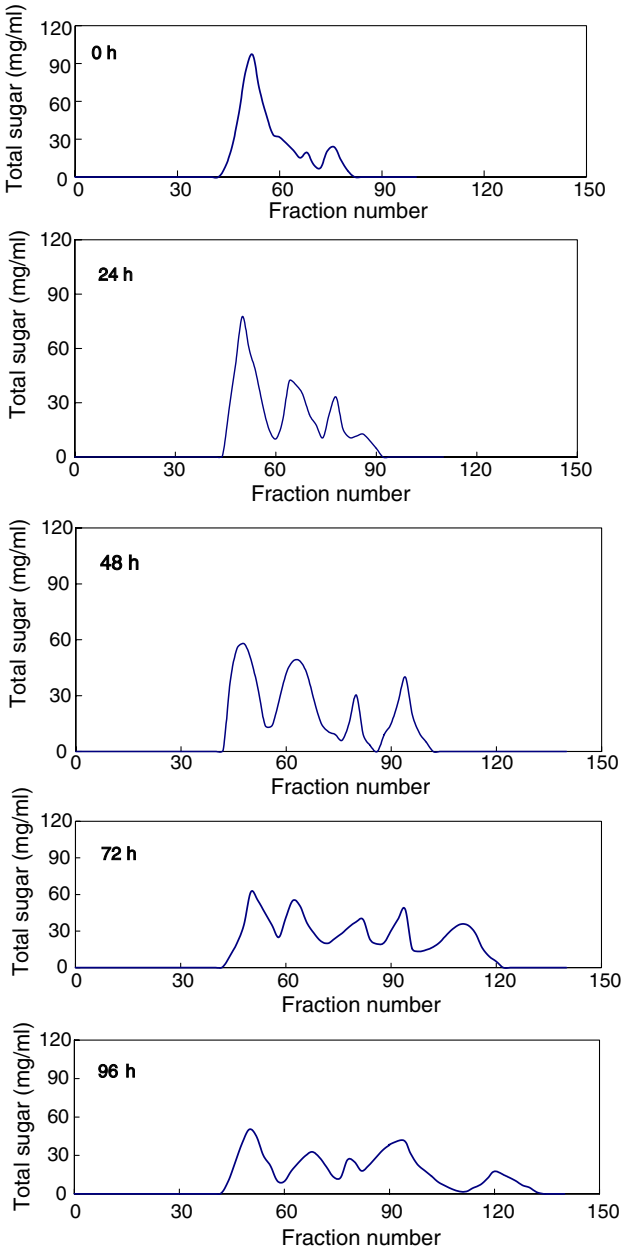

Fig. 2. Gel-permeation profiles of AIDF on Sephacryl S-200: Column size, 3.0 × 80 cm; Flow rate, 3.5 ml/15 min; Elution soln., distilled water; Fraction vol., 3.5 ml.

1983). Contents of rhamnose, arabinose, galactose, and glucose were 4.13, 14.9, 24.9, and 8.65 mg/g, respectively, after 24 h of reaction. As reaction time was prolonged, these contents significantly increased. Contents of rhamnose, galactose, and glucose were 9.55, 34.1, and 15.4 mg/g after 96 h of reaction, respectively. This increase might be due to increased solubility of dietary fibre, because cellulosic substrates are hydrolyzed by cellulase, and cell-wall polysaccharides of carrot pomace were decomposed by β-galactosidase (De Varies, Kester, Poulsen, Benen, & Visser, 2000; Gregg & Saddler, 1996). Amounts of rhamnose and arabinose were solubilized in

Table 3
Neutral sugars of AIDF from carrot pomace by enzymatic hydrolysis

Sugars (mg/g AIDF)	Time (h)				
	0	24	48	72	96
Rhamnose	13.7[a]	4.13[d]	6.33[c]	5.89[c]	9.55[b]
Arabinose	22.9[a]	14.9[c]	19.5[b]	19.0[b]	19.7[b]
Xylose	Nd	0.82	Tr	0.92	Tr
Mannose	4.47[a]	3.18[b]	4.09[a]	4.05[a]	3.33[b]
Galactose	20.0[d]	24.9[c]	29.7[b]	29.2[b]	34.1[a]
Glucose	9.04[d]	8.65[d]	11.3[c]	14.0[b]	15.4[a]

Nd, not detected.
Tr, trace amount (<0.01).
The experimental values that have no common superscript are significantly different ($P < 0.05$) according to Duncan's multiple range test.

AIDF and were characterized by a high pectic polysaccharide content (Redgwell et al., 2003). Chau et al. (2004) reported that some major sugars of AIDF from carrot pomace were uronic acid, glucose, galactose, arabinose, and mannose. In general, the most abundant monosaccharide in dietary fibre (nonstarch polysaccharide) of vegetables and fruits was glucose, which is derived from cellulose (Sánchez-Castillo et al., 1995). It was previously suggested that AIDF was mainly composed of pectic polysaccharides and hemicellulose, followed by cellulose (Schneeman, 1986).

3.5. Composition of ASDF

Table 4 shows the composition of oligosaccharides and sugars produced by enzymatic hydrolysis of carrot pomace by edible snail's crude enzyme. Some cellooligosaccharides (cellopentaose, cellotetraose, cellotriose, cellobiose), galactooligosaccharides (galactotetraose, galactotriose), and monosaccharides (glucose, fructose, galactose, arabinose) were detected. Galactooligosaccharides were not detected at 0 h and increased by

Table 4
Contents of the mono and oligosaccharides of ASDF from carrot pomace by enzymatic hydrolysis

Sugars (mg/g ASDF)	Time (h)				
	0	24	48	72	96
Glucose	2.01[a]	1.71[a]	4.28[b]	4.70[b]	4.92[b]
Galactose	0.11[a]	0.52[c]	0.33[b]	0.42[b]	0.67[d]
Fructose	0.19[a]	0.26[b]	0.22[b]	0.19[a]	0.20[b]
Arabinose	Nd	0.41[a]	0.79[b]	1.01[c]	1.11[c]
Cellopentaose	1.01[a]	24.56[b]	26.27[c]	25.61[c]	24.44[b]
Cellotetraose	0.44[a]	3.32[b]	3.40[b]	3.56[b]	4.62[c]
Cellotriose	0.27[a]	0.90[b]	1.53[c]	1.32[c]	1.07[b]
Cellobiose	1.12[a]	0.72[b]	0.87[b]	0.75[b]	0.42[c]
Galactotetraose	Nd	Tr	0.11[a]	0.23[b]	0.29[b]
Galactotriose	Nd	Tr	0.34[a]	0.36[a]	0.64[b]
Total	5.66[a]	32.40[b]	38.14[c]	38.15[c]	38.38[c]

Nd, not detected.
Tr, trace.
The experimental values that have no common superscript are significantly different ($P < 0.05$) according to Duncan's multiple range test.

only a small amount after 96 h of reaction. Cellooligosaccharides, except for cellobiose, were significantly increased after 48 h of reaction compared with 0 h of reaction but did not increase after 48 h of reaction time, except for cellotetraose. Total yield of oligosaccharides and sugars reached maximum values after 48 h of reaction, and there was not a significant increase after 96 h of reaction. Kanda, Wakabayashi, and Nisiazwa (1980) reported that endo-cellulase, purified from *Irpex lacteus*, produced a series of cellooligosaccharides (cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose) from sodium carboxymethyl cellulose, as well as from water-insoluble cellulose.

4. Conclusion

To produce fibre-rich aqueous extracts from pretreated carrot pomace by edible snail's crude enzyme, optimal conditions of enzymatic hydrolysis were pH 5, 50 °C, and 1% substrate concentration. After 96 h of enzymatic hydrolysis, the edible snail's crude enzyme produced 77.3 g of water-soluble fibre/100 g of carrot pomace. These results suggest that crude enzyme could be utilized for the exploitation of carrot pomace and other agricultural byproducts as sources of soluble dietary fibre and oligosaccharides by enzymatic hydrolysis. We need to undertake further investigations of the characteristics, functional properties, and in vitro physiological effects of water-soluble fibre from carrot pomace to monitor its application possibilities as a fibre-rich functional food ingredient.

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