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Research article

Enzymatic preparation of fructooligosaccharides-rich burdock syrup with enhanced antioxidative properties

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ABSTRACT

Background: Burdock (*Arctium lappa* L.) is a fructan-rich plant with prebiotic potential. The aim of this study was to develop an efficient enzymatic route to prepare fructooligosaccharides (FOS)-rich and highly antioxidative syrup using burdock root as a raw material.
Results: Endo-inulinase significantly improved the yield of FOS 2.4-fold while tannase pretreatment further increased the yield of FOS 2.8-fold. Other enzymes, including endo-polygalacturonase, endo-glucanase and endo-xylanase, were able to increase the yield of total soluble sugar by 11.1% (w/w). By this process, a new enzymatic process for burdock syrup was developed and the yield of burdock syrup increased by 25% (w/w), whereas with FOS, total soluble sugars, total soluble protein and total soluble polyphenols were enhanced to 28.8%, 53.3%, 8.9% and 3.3% (w/w), respectively. Additionally, the scavenging abilities of DPPH and hydroxyl radicals, and total antioxidant capacity of the syrup were increased by 23.7%, 51.8% and 35.4%, respectively.
Conclusions: Our results could be applied to the development of efficient extraction of valuable products from agricultural materials using enzyme-mediated methods.
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1. Introduction

Burdock (*Arctium lappa* L.) has long been cultivated and widely consumed as a very popular vegetable for centuries [1,2]. This vegetable, rich in antioxidant component, is among the most popular plants in traditional Chinese Pharmacopeia because of its biological effects, such as antimutagenicity, anticarcinogenicity and antiaging [3,4,5]. In addition to the traditional amino acids, nucleotides and vitamins, most studies on the bioactivities of burdock have focused on the new functional food factors including polyphenols, oligosaccharides and polysaccharides [6,7,8]. Burdock belongs to the Asteraceae, which contains considerable amounts of fructan, a linear chain of a number of β-2,1-linked fructofuranose residues with one terminal β-1,2-linked glucopyranose [9,10,11,12].

Currently, burdock root is commonly used for dieting or is roughly processed into powder or tea after being dried and ground [5,13,14], but the bioactivity of these products is relatively low because the major functional active constituents have not been effectively released. Fructan can be hydrolyzed into fructooligosaccharides (FOS) by controlled enzymatic hydrolysis of endoglycosidases or exogenous enzymes (endo-inulinase) [15,16,17,18]. The degree of polymerization (DP) of FOS is in the range of 3–5 [9,19]. FOS, which exhibits prebiotic activity, low caloric value and low cariogenic properties, has become more attractive as new food ingredients because of its potential to improve the quality and nutritional properties of food [20,21,22,23,24,25]. Therefore, the content of FOS is the main indicator for the quality of burdock products.

Here we reported a novel enzymatic process for preparing a FOS-rich and high antioxidant syrup using burdock root as a raw material. The effects of endogenous and exogenous enzymes

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(endo-inulinase, tannase, endo-polygalacturonase, endo-glucanase and endo-xylanase) on FOS formation and antioxidant activity were investigated.

2. Materials and methods

2.1. Materials and chemicals

Burdock roots purchased from Jiangsu Shunde Fruit and Vegetable Food Company, Jiangsu Province, China, were immediately transported to the laboratory and stored at −20°C until use. Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and gallic acid were purchased from Sigma-Aldrich (Shanghai, China). FOS standards (kestose, nystose and F-fructofuranosylnystose) were bought from Wako Pure Chemical Industries (Osaka, Japan). Kits for the hydroxyl radical scavenging assay and total antioxidant capacity assay was obtained from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). All other reagents were of analytical grade and used without further investigation.

2.2. Enzymes

The enzymes used in this study were endo-inulinase [endo-hydrolysis of (2- > 1)-β-D-fructosidic linkages], tannase (hydrolysis of tannic acid by breaking its ester and depside bonds), endo-polygalacturonase (endo-hydrolysis of α-1,4 glycosidic bonds between galacturonic acid residues), endo-glucanase [endo-hydrolysis of (1- > 4)-linkages in β-D-glucans], and endo-xylanase [endo-hydrolysis of (1- > 4)-linkages in β-D-xylan]. Endo-inulinase (1.36 U/mg), tannase (0.17 U/mg), endo-polygalacturonase (10.65 U/mg), endo-glucanase (3.48 U/mg), and endo-xylanase (0.11 U/mg) were purchased from Jiangsu Ruiyang Biotech Co., Ltd. (Wuxi, China). The protein concentrations were quantified using a BCA Protein Assay kit (Thermo Fisher Scientific Inc., Waltham, MA).

2.3. Enzymatic treatments

2.3.1. The hydrolysis of fructan by endogenous enzymes

Burdock roots were washed thoroughly in running tap water, weighed, peeled and cut into 30 cm sections. The roots were pulped and mixed to ensure a homogeneous suspension and the fructan was hydrolyzed by endogenous enzymes at 50°C for 24 h. Homogeneous suspension (100 μL) was taken at various intervals and centrifuged at 12,000 rpm for 10 min. The contents of individual carbohydrate in each suspension were determined by HPLC.

2.3.2. The hydrolysis of fructan by endo-inulinase

100 g of burdock pulp was inactivated by autoclaving at 121°C for 10 min and cooled at 50°C. 30 U/g FW of endo-inulinase was then added and incubated at 50°C for 12 h. The contents of individual carbohydrates in the hydrolysates were determined by HPLC.

2.3.3. The pretreatment of burdock pulp by tannase

0.5 to 2.5 U/g FW of tannase was added into 100 g of heat pretreated burdock pulp and incubated at 50°C for 4 h, followed by adding 30 U/g FW endo-inulinase. The reaction was then carried out at 50°C for 12 h. The samples were collected and the contents of individual carbohydrates in the hydrolysates were determined by HPLC.

2.3.4. The hydrolysis of polysaccharides by other glycosidases

Endo-polygalacturonase, endo-glucanase and endo-xylanase with dosage of 0.5–2.5 U/g FW was added during the enzymatic hydrolysis of burdock pulp with endo-inulinase. The reaction was carried out at 50°C for 8 h. Total soluble sugars and extractable soluble solids in the hydrolysates were determined as described below.

2.4. Determination of other components of burdock

The fructan content was calculated as the difference between the amount of fructose, sucrose and FOS in the sample before and after hydrolysis with endo-inulinase (30 U/g FW) for 24 h at 50°C. Total soluble sugars were determined by the phenol sulfuric acid assay and expressed as fructose equivalents [6]. Extractable soluble solids were determined by drying the burdock extract in a hot air oven for 12 h at 103°C [26]. Total soluble protein content by the Bradford method was determined using bovine serum albumin as standard [6]. The total soluble phenolic content was determined by Folin–Ciocalteu reagent-based colorimetric assay and expressed as mg gallic acid equivalents [27].

2.5. Measurement of antioxidant activity

The DPPH radical scavenging ability (DRSA) and hydroxyl radical scavenging ability (HRSA) of burdock hydrolysates was determined as previously described [6,27]. The DRSA and HRSA results were expressed as percentage of inhibition in relation to a control test without burdock. The total antioxidant capacity (TAOC) was measured by the method of ferric reducing/antioxidant power assay [28,29].

2.6. HPLC analysis

Quantitative analysis of individual carbohydrates was performed using HPLC (Agilent Technologies, Cheshire, U.K.) with an ELSD detector 2000s (Grace Alltech Associates Inc., Deerfield IL, USA) and a Prevail™ Carbohydrate ES 5u (250 mm × 4.6 mm, Grace Alltech) column. A mixture of water/acetonitrile (35:65, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min. Quantitative measurement of each peak was performed using standard calibration curves of sugar standards as reference. Carbohydrates (glucose, fructose and sucrose) and FOS (kestose, nystose and F-fructofuranosylnystose) contents were expressed as g/100 g burdock roots (fresh weight, FW). The FOS yield and hydrolysis degree were calculated using the following formulas:

$$\text{FOS yield } (\%, \text{w/w}) = \frac{\text{FOS}}{\text{Total sugars}} \times 100$$

$$\text{Hydrolysis degree } (\%, \text{w/w}) = \frac{\text{DP}_{1-5}}{\text{Total sugars}} \times 100$$

Total sugars are the sum of all sugars (fructose, glucose, sucrose, FOS and fructan) in fresh burdock.

Table 1
The main components of fresh burdock root.

Components	Content (g/100 g FW)
Water	84.8 ± 0.8
Total soluble sugars	7.1 ± 0.6
Fructan	3.7 ± 0.4[a]
Total soluble proteins	1.3 ± 0.2
Total soluble polyphenols	0.3 ± 0.0
Others	6.5 ± 0.5

[a] Fructan was calculated as the difference between the amount of fructose, sucrose and FOS in the sample before and after hydrolysis with endo-inulinase (30 U/g FW) for 24 h at 50°C. The data represents the mean ± standard deviation of triplicate determinations.

Table 2
The change of carbohydrates in burdock extracts catalyzed by endogenous enzymes at 50°C.

Time (h)	Carbohydrate content (g/100 FW)					Hydrolysis degree (%, w/w)	FOS yield (%, w/w)
	Fructose	Glucose	Sucrose	FOS	DP_{1-5}		
0	1.3 ± 0.1	0.1 ± 0.0	0.5 ± 0.0	1.6 ± 0.2	3.5 ± 0.4	48.6 ± 2.2	22.2 ± 0.7
6	1.3 ± 0.1	0.1 ± 0.0	0.6 ± 0.1	1.7 ± 0.2	3.7 ± 0.4	51.4 ± 2.3	23.6 ± 0.7
12	1.4 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	1.9 ± 0.2	4.1 ± 0.5	56.9 ± 2.4	26.4 ± 0.7
18	1.4 ± 0.1	0.2 ± 0.0	0.7 ± 0.1	2.0 ± 0.2	4.3 ± 0.5	59.7 ± 2.6	27.8 ± 0.8
24	1.5 ± 0.1	0.2 ± 0.0	0.7 ± 0.1	2.1 ± 0.2	4.5 ± 0.5	62.5 ± 2.6	29.2 ± 0.8

The data represents the mean ± standard deviation of triplicate determinations.

3. Results and discussion

3.1. Composition of fresh burdock root

Table 1 shows the main components of fresh burdock root, including sugars, protein and polyphenols. Fructan, the primarily component of carbohydrate, was approximately 24% (w/w) of the dry weight. Other components were calculated by difference and are assumed to be fiber, lipids and carotene among others.

3.2. Effect of endogenous enzymes on FOS yield of burdock extracts

The contents of monomers and oligomeric hydrolysis products (DP ranges from 1 to 5) from fructan, hydrolyzed by endogenous enzymes, were analyzed after peeling and pulverizing of fresh burdock. The result is summarized in Table 2. The fructan was gradually hydrolyzed and the content of DP_{1-5} increased over 24 h, indicating that there was endogenous fructan hydrolases in the burdock root. After 24 h of incubation, the FOS content increased from 1.6 to 2.1 g/100 g FW and the FOS yield increased from 22.2% to 29.2% (w/w). The hydrolysis degree of fructan by endogenous enzymes treatment after 24 h increased from 48.6% to 62.5% (w/w), indicating that endogenous enzymes of burdock could not effectively hydrolyze all fructan to FOS. Additionally, it was observed that the increasing contents of fructose and sucrose may reduce FOS formation in burdock root. Therefore, the addition of exogenous endo-inulinase is required.

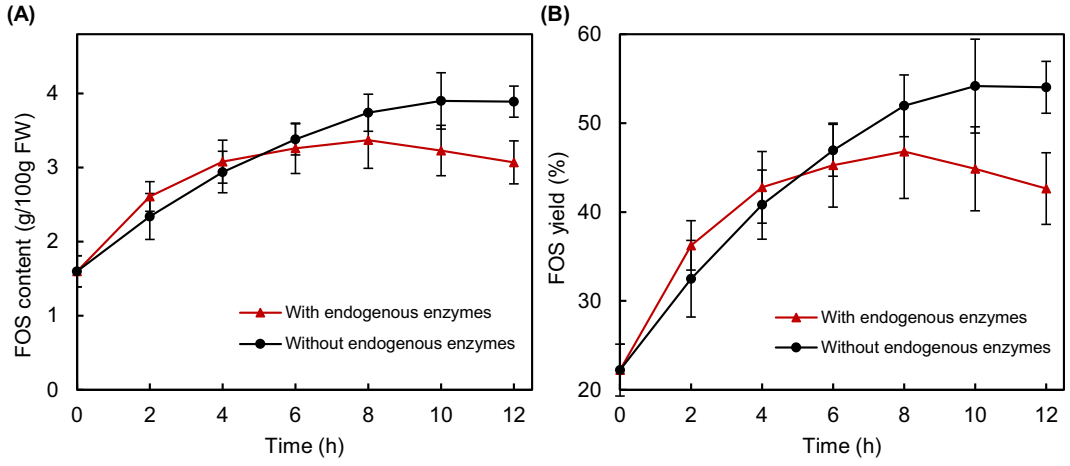

Fig. 1. Effect of endogenous enzymes on FOS content (A) and FOS yield (B) by endo-inulinase hydrolyzing fructan in the burdock pulp. The dosage of endo-inulinase was 30 U/g FW. The reaction was carried out at 50°C for 12 h using the burdock pulp or heat pretreatment of the burdock pulp. The data represents the mean ± standard deviation of triplicate determinations.

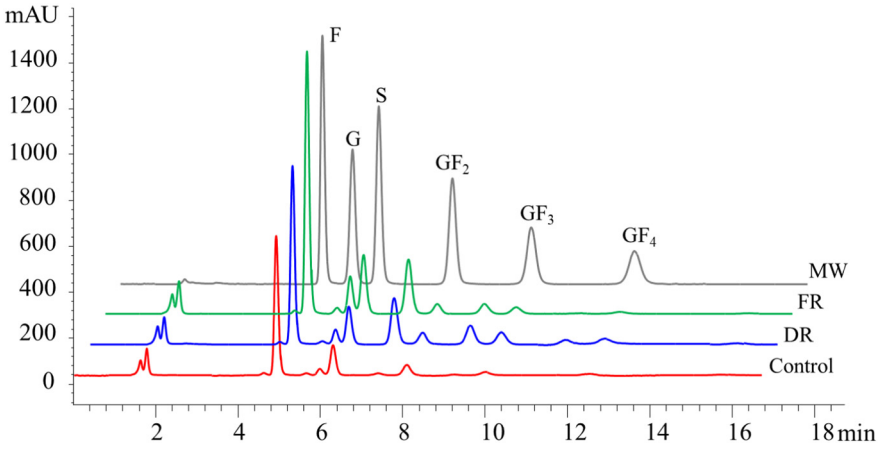

Fig. 2. Sugar profiles of burdock hydrolysates by HPLC analysis. Control, burdock pulp after heat pretreatment; DR, heat pretreated burdock pulp hydrolyzed with endo-inulinase; FR, fresh burdock pulp hydrolyzed with endo-inulinase. MW, reference standards (F, fructose; G, glucose; S, sucrose; GF_2, kestose; GF_3, nystose; GF_4, F-fructofuranosylnystose).

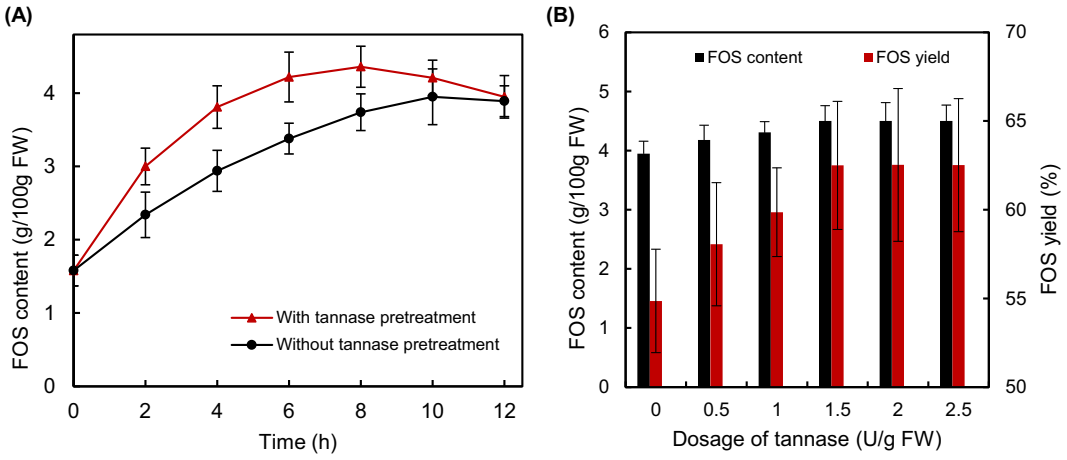

Fig. 3. Effect of tannase pretreatment on the FOS content by endo-inulinase hydrolyzing fructan at 50°C. (A) The burdock pulp was treated with 1 U/g FW of tannase for 4 h, and then catalyzed with 30 U/g FW of endo-inulinase. (B) 0–2.5 U/g FW of tannase were added in the reaction mixture. The data represents the mean ± standard deviation of triplicate determinations.

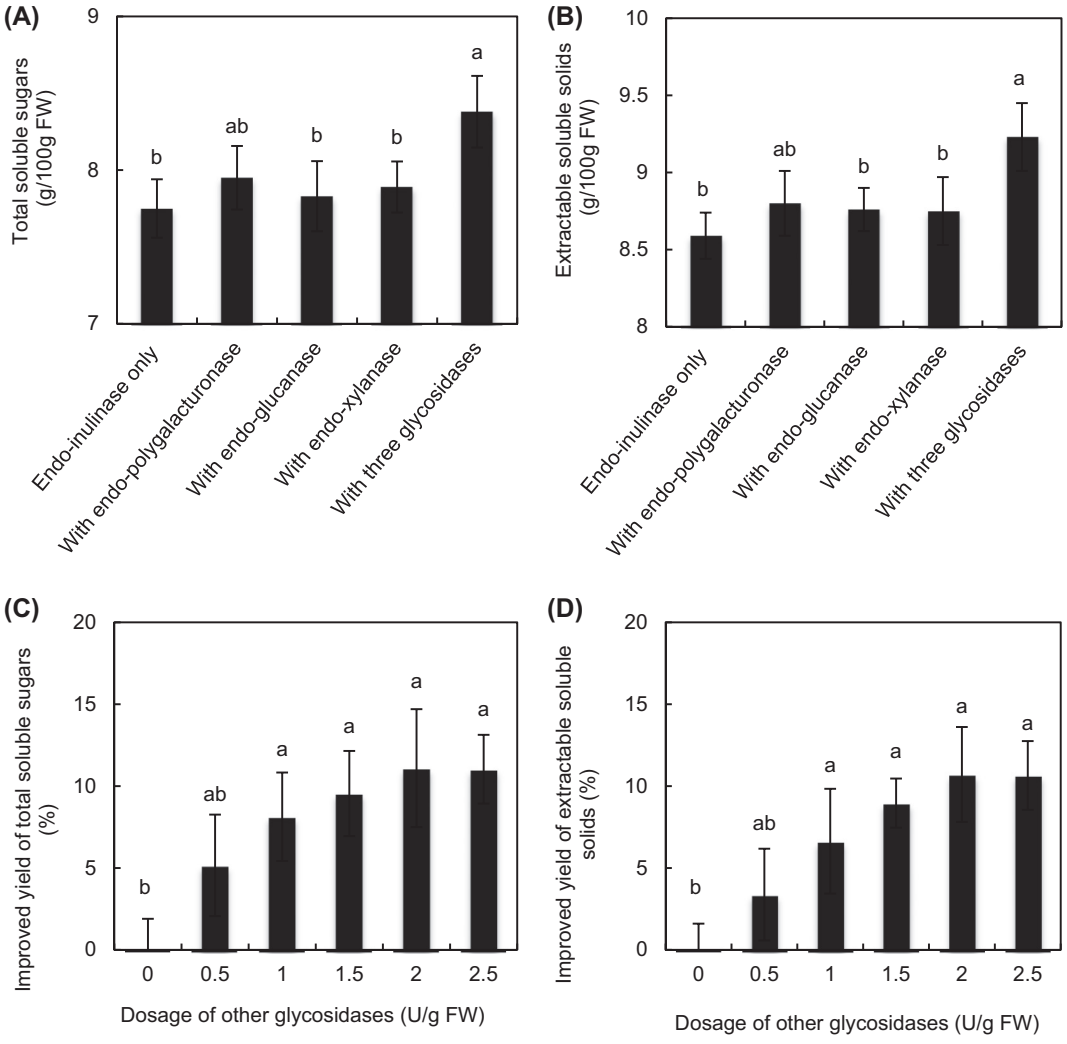

Fig. 4. Effect of other carbohydrate hydrolases on the extraction yields of burdock. Total soluble sugars (A) and extractable soluble solids (B) released in burdock hydrolysates with endo-inulinase combined with one or more other glycosidases (endo-polygalacturonase, endo-glucanase and endo-xylanase). The improved yields of total soluble sugars (C) and extractable soluble solids (D) released in burdock hydrolysates with different dosages of three glycosidases. The experiments were carried out with 1.5 U/g FW of tannase pretreatment for 4 h, followed by simultaneous treatment with 30 U/g of endo-inulinase and 1 U/g of glycosidase(s) (A and B) or with 0–2.5 U/g each of three glycosidases (C and D) for 8 h at 50°C. The data represents the mean ± standard deviation of triplicate determinations. Different letters (a, b, ab on the top of each column) indicated the results were significantly different ($p < 0.05$).

Hydrolysis of fructan to FOS by endo-inulinase with or without endogenous enzymes was examined, as shown in Fig. 1. When the enzymatic reaction was conducted by combined action of endo-inulinase and endogenous enzymes, the maximum content of FOS (3.4 g/100 g FW) after 8 h was lower than that by endo-inulinase alone (3.9 g/100 g FW) after 10 h (Fig. 1A). Comparison of HPLC profiles of the products of fructan hydrolyzed by the two methods revealed that the peaks of fructose and sucrose increased due to the reaction of endogenous enzymes (Fig. 2). These results suggested that burdock has high fructan exohydrolase activity. Fructan exohydrolase can catalyze the hydrolysis of FOSs and fructan at the terminal fructosyl residue and release free fructose and sucrose [30,31,32]. Therefore, the inactivation of endogenous enzymes is necessary for maximizing the FOS content before the hydrolysis of fructan using endo-inulinase, which could significantly improve the FOS yield by 2.4-fold (from 22.2% to 54.2%, w/w) in the hydrolysate (Fig. 1B).

3.3. Effect of tannin on fructan hydrolysis by endo-inulinase

Tannin, one of the most common phenolic compounds found in burdock root [4], may bind with proteins [33]. The protein-tannin aggregation and sedimentation could affect the activity of enzyme. Therefore, tannase was added to the sterilized pulp for 4 h before adding endo-inulinase to hydrolyze the fructan. Tannase pretreatment increased the maximum FOS content from 3.9 to 4.4 (g/100 g FW) (Fig. 3A), with the reaction time shortened from 10 to 8 h as the gallated catechins (tannin) were catalyzed to un-gallated catechins [33]. The gallated catechins are thought to offer more hydroxyl groups for hydrogen bonding with oxygen atoms in the carbonyl groups of proteins causing protein-tannin aggregation [33]. When the dosages of tannase were more than 1.5 U/g FW, the highest FOS content increased to 4.5 (g/100 g FW) and the FOS yield increased by 2.8-fold (from 22.2% to 62.5%, w/w) by adding endo-inulinase after tannase pretreatment (Fig. 3B).

3.4. Effect of multi-glycosidases on the content of total soluble sugar and extractable soluble solids in burdock extracts

Burdock, as a higher plant, also contains pectin, cellulose and hemicellulose [34]. After the tannase pretreatment, the content of total soluble sugar and extractable soluble solids in burdock extracts by simultaneous treatments with endo-inulinase and one or more other glycosidases (endo-polygalacturonase, endo-glucanase and endo-xylanase) were evaluated. Although there were no significant differences between the treatment with and without any other

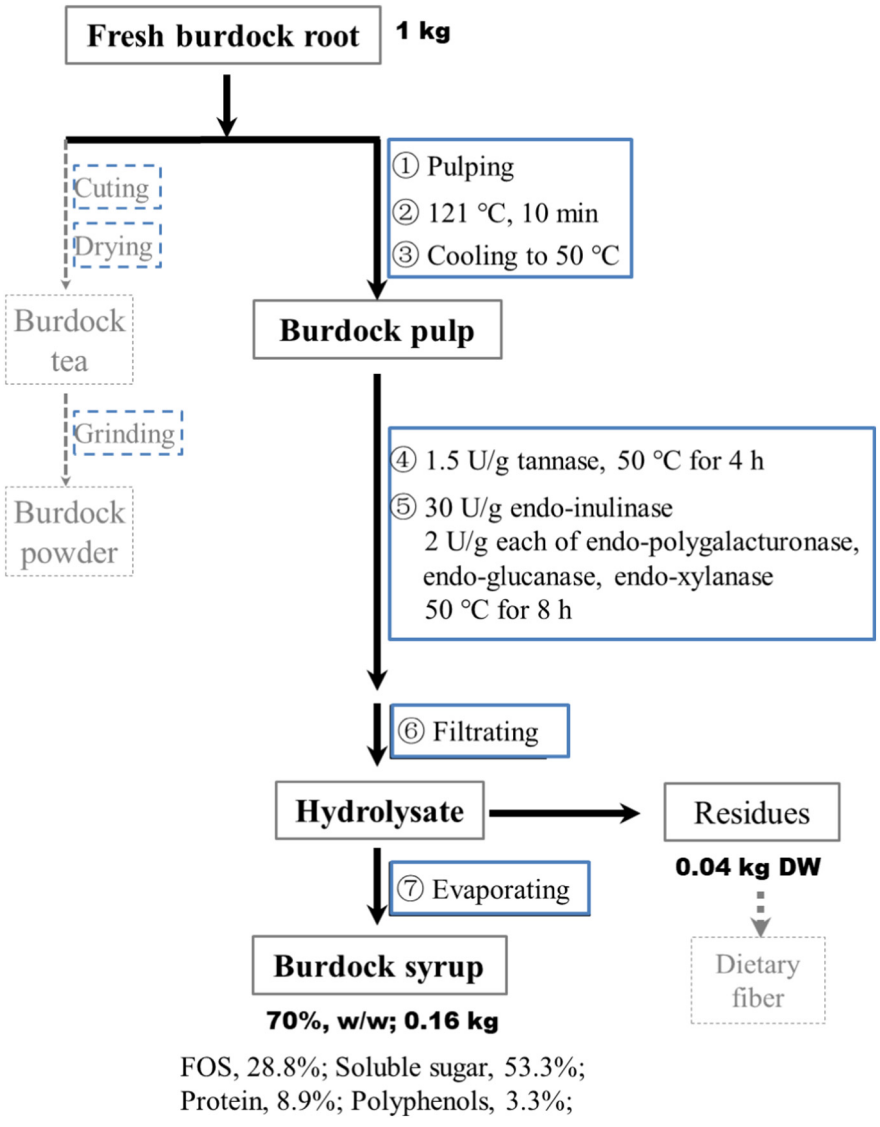

Fig. 5. Schematic diagram for the enzymatic processing of burdock syrup.

glycosidase ($p > 0.05$), the content of total soluble sugars markedly increased using simultaneous treatments of all four glycosidases ($p < 0.05$) (Fig. 4A). Additionally, the content of extractable soluble solids also significantly improved ($p < 0.05$) (Fig. 4B). When the dosages of other glycosidases were more than 2 U/g FW, the highest content of total soluble sugars and extractable soluble solids increased by 11.1% and 10.7% (w/w), respectively (Fig. 4C and Fig. 4D). These results showed that the mixed use of multi-glycosidases facilitated the release of soluble sugar hydrolyzed polysaccharide. Therefore, the multi-glycosidases treatment could be considered in the enzymatic process of burdock root.

3.5. Development of an efficient enzymatic process for preparation of FOS-rich burdock syrup

Given the content of FOS and extractable soluble solids can be accelerated using the aforementioned results, a highly efficient enzymatic processes could be divided into three steps: a) inactivation of endogenous enzyme; b) tannase pretreatment; c) endo-inulinase and other three glycosidases simultaneous treatments. After the enzymatic reaction, the hydrolysate was condensed into burdock syrup and the experimental procedure is schematically described in Fig. 5. Based on this procedure, heat-inactivated burdock immediately after homogenate was used as a control sample, the preparation of FOS-rich burdock syrup (70%, w/w) was developed and the result is shown in Table 3. The enzymatic reaction significantly improved the yield of burdock syrup by 25% (w/w). In the prepared syrup, FOS content reached to, which was about 54.0% (28.8%/53.3%) of total soluble sugars. Additionally, enzymatic treatment could significantly improve the antioxidant activity of burdock syrup, increasing the DRSA and HRSA inhibition ratio and TAOC by 23.7%, 51.8% and 35.4%, respectively. Previously it was shown that the hydrolytic products (epicatechin, epigallocatechin and gallic acid) of tannin hydrolyzed by tannase had higher antioxidant activity than the substrates (epicatechin gallate and epigallocatechin gallate) [26,35].

4. Conclusions

Our results have shown that an enzyme-mediated preparation of burdock extracts by combining tannase pretreatment with endo-inulinase and other three glycosidases simultaneous treatments was feasible. This high-efficiency enzymatic process significantly improved the yield of burdock syrup, and particularly increased FOS content and antioxidant activity in burdock syrup. Furthermore, this enzymatic treatment of an agricultural crop could serve as a model applicable to other agricultural materials.

Table 3
Comparison of burdock extract prepared with and without enzymatic process.

Evaluation index	Preparation technology		Increased degree (%)
	Enzymatic	No-enzymatic	
Burdock syrup yield (%, w/w)[a]	16.0 ± 1.3	12.8 ± 0.8	25.0
Total soluble sugars (%, w/w)[b]	53.3 ± 7.5	51.8 ± 2.5	2.9
FOS (%, w/w)	28.8 ± 3.7	11.8 ± 1.7	143.2
Total soluble polyphenols (%, w/w)[b]	3.3 ± 0.4	2.4 ± 0.4	37.5
Antioxidant activities			
DRSA inhibition ratio (%)	91.4 ± 1.8	73.9 ± 1.6	23.7
HRSA inhibition ratio (%)	90.0 ± 2.2	59.3 ± 1.1	51.8
TAOC (U/g)	2209 ± 79	1631 ± 59	35.4
Total soluble protein (%, w/w)[b]	8.9 ± 0.8	8.1 ± 0.5	9.9

The data represents the mean ± standard deviation of triplicate determinations.
[a] The syrup yield was calculated as the percentage of the amount of 70% (w/w) syrup from 100 g of fresh burdock root.
[b] The contents in 70% (w/w) of burdock syrup.

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Conflict of Interest Statement

The authors declare that a Chinese invention patent based on the related enzyme and its mutants as well as method for preparation of fructooligosaccharides-rich and highly antioxidative products from burdock root is under application.

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