องค์ประกอบทางเคมีของเปลือกถั่วดาวอินคา (*PLUKENTIA VOLUBILIS* L.) และการผลิตโอลิโกแซคคาไรด์ด้วยไซลาเนสทางการค้า

CHEMICALCOMPOSITION OF SACHA INCHI HULLS (*PLUKENTIA VOLUBILIS* L.) AND OLIGOSACCHARIDES PRODUCTION USING COMMERCIAL XYLANASE

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บทคัดย่อ

เปลือกถั่วดาวอินคา (*PlukentiaVolubilis* L.) เป็นชีวมวลที่ได้มาจากการสกัดน้ำมันถั่วดาวอินคา ซึ่งใยอาหารที่เหลืออยู่ส่วนใหญ่จะเป็นเฮมิเซลลูโลสและเซลลูโลสเฮมิเซลลูโลสถือว่าเป็นแหล่งสำหรับผลิตโอลิโกแซคคาไรด์ที่ดี ในการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อวิเคราะห์องค์ประกอบทางเคมีของเปลือกถั่วดาวอินคา และศึกษาผลของความเข้มข้นของเอนไซม์และระยะเวลาในการย่อยของเอนโดไซแลนเนสทางการค้าชนิด Pentopan Mono BG ขั้นแรกทำการวิเคราะห์องค์ประกอบทางเคมีของเปลือกถั่วดาวอินคารวมถึงปริมาณโฮโลเซลลูโลส ลิกนิน ที่ไม่ละลายน้ำและสารแทรก จากนั้นนำเปลือกถั่วดาวอินคาที่ผ่านการพรีทรีตเมนท์ด้วยด่างเพื่อกำจัด Extractives, Non-Extractives และปรับปรุงความสามารถในการย่อยของเอนไซม์ จากนั้นตามด้วยขั้นตอนย่อยของเอนไซม์ โดยจะใช้เอนไซม์ไซแลนเนสทางการค้า จากผลการวิจัยพบว่า เปลือกถั่วดาวอินคาประกอบด้วยโปรตีน (4.10%) ไขมัน (1.55%) เยื่อใย (37.20%) เถ้า (1.44%) ความชื้น (8.51%) และคาร์โบไฮเดรตทั้งหมด (47.20%) นอกจากนี้ยังพบว่า มีโฮโลเซลลูโลสมากถึง 24.12% ในขั้นตอนการผลิตโอลิโกแซคคาไรด์จะนำเปลือกถั่วดาวอินคาที่ผ่านการพรีทรีตเมนท์ด้วยด่าง (2% NaOH, 6 วัน) ย่อยด้วย Pentopan Mono BG (10-150U/g, 50°C, pH 6.0, 2-24 ชั่วโมง) ในระหว่างการย่อยความเข้มข้นของเอนไซม์ที่ 10U/g มีปริมาณน้ำตารีดิวซ์เพิ่มขึ้นเล็กน้อย ในขณะที่ความเข้มข้นของเอนไซม์ที่ 100, 133 และ 150U/g พบว่ามีปริมาณน้ำตารีดิวซ์เพิ่มขึ้นอย่างมาก นอกจากนี้ยังพบว่า ความเข้มข้นของเอนไซม์ที่ 133U/g มีความเหมาะสมที่สุด เนื่องจากให้ปริมาณน้ำตารีดิวซ์ในปริมาณที่ไม่แตกต่างกันอย่างมีนัยสำคัญเมื่อเทียบกับ 150U/g นอกจากนี้จากผลการวิเคราะห์ TLC พบว่า ไม่มีน้ำตาลไซโลสปรากฏในทุกช่วงระยะเวลาในการบ่ม แต่พบไซโลไบโอสเป็นโอลิโกแซคคาไรด์ชนิดหลักที่ผลิตได้ตั้งแต่ 4 ชั่วโมง ดังนั้นการศึกษาครั้งนี้จึงได้พิสูจน์ความเป็นไปได้ในการใช้เปลือกถั่วดาวอินคาเป็นแหล่งสำหรับการผลิตโอลิโกแซคคาไรด์ และควรมีการศึกษาคุณสมบัติพรีไบโอติกของโอลิโกแซคคาไรด์ที่ผลิตในการศึกษานี้ต่อไป

คำสำคัญ: เปลือกถั่วดาวอินคา โอลิโกแซคคาไรด์ โฮโลเซลลูโลส ไซแลนเนส ไซโลไบโอส

Abstract

Sacha Inchi (*PlukentiaVolubilis* L.) hull is the biomass derived from the oil extraction, remaining fibers mainly hemicellulose and cellulose. The hemicelluloses were reported to be a good source of oligosaccharides production. The aim of this study was to analyze the chemical composition of SachaInchi hulls and study the effects of enzyme concentration and hydrolysis time of commercial endoxylanase, Pentopan Mono BG. Firstly, the chemical composition of Sacha Inchi hulls including holocellulose content, insoluble lignin, and extractives were analyzed. Then Sacha Inchi hulls were pretreated with alkali solution to remove the extractives, non-extractives and improve the enzymatic hydrolysis ability followed by enzymatic saccharification using commercial xylanase. The results showed that Sacha Inchi hulls consisted of protein (4.10%), fat (1.55%), crude fiber (37.20%), ash (1.44%), moisture (8.51%) and total carbohydrate (47.20%). Among those, the holocellulosewas found to be as much as 24.12%. To produce oligosaccharides, the alkali-pretreated biomass (2% NaOH, 6 days) was hydrolyzed by a commercial xylanase, Pentopan Mono BG (10-150 U/g, 50°C, pH 6.0 and 2-24 h). During hydrolysis with the enzyme content of 10 U/g, the total reducing sugar content was slightly increased while dramatically increased with that of 100, 133 and 150 U/g. The enzyme content of 133 U/g was found to be an optimum content as it gave total reducing sugar contents with no significant difference compared to 150 U/g. The TLC chromatogram was evident that xylose was absent during each incubation time interval and xylobiose was the main oligosaccharide having been produced since 4 h of incubation time. Hence, this study has proved the possibility of using Sacha Inchi hulls as an alternative source for the oligosaccharides production. And the oligosaccharide mixture produced in this study should be further investigated for the prebiotic properties.

Keywords: Sacha Inchi hulls, Oligosaccharides, Holocellulose, Xylanase, Xylobiose

Introduction

Sacha Inchi (*Plukentia volubilis* L.), also known as Inca peanut, is a plant of the Euphorbiaceae family [1]. This plant is widely cultivated in the south of Colombia and thehighlandsof Peru [2]. The Sacha Inchi also has been a part of the diet of various native Tribal because it is rich of oil (35 - 60%) and protein (27%) [3]. In Thailand, Sacha Inchi nowadays iswidely cultivated in the North, Northeast and Central regions. Sacha Inchi used as a new source for edible oil extraction [4] is causing a large amount of by-product such as defatted Sacha Inchi cake as well ashulls yielding around 50% of the raw materials. Nowadays, the wastes from edible oil industry were used for producing various kinds of value-added products because they still contain high nutritious values. For example, lipid extraction markedly increases the proportion of dietary fiber and crude protein contents of rice bran [5]. Previous report has revealed the chemical composition of Sacha Inchi cake and hulls, containing 56.63% and 9.46% of protein and 25.27% and 85.44% of total dietary fiber, respectively [3]. Furthermore, Shridhar et al. [6] reported that Sacha Inchi

composed of albumins, globulins, prolamins and glutelinsaccounted for 43.7%, 27.3%, 3.0% and 31.9% respectively.

To our knowledge, no evidence noting the use of dietary fiber from defatted Sacha Inchi cake and hulls. Many researchers usedagricultural biomass such as wheat straw [7], rice hulls [8], almond shells [9], barley husks [10] and so on as the sources for oligosaccharides production. All of the biomasses are rich in lignocellulosic materials, composing of cellulose, hemicellulose, lignin and other extraneous components [11]. Conversion of cellulose or hemicellulose into oligosaccharides usually requires three main steps, (1) mechanical size reduction of the materials, (2) alkali or acid pretreatment, and (3) enzymatic hydrolysis by cellulase or hemicellulase enzymes. The ultimate goal of the pretreatment process is to improve the enzymatic hydrolysis of carbohydrates (cellulose and hemicellulose) consequently increasing overall bioconversion efficiency for production of the saccharides [12]. For example, Rashid et al. [13] found that pretreatment with 3% NaOH was effective in enhancing the ability of enzymatic hydrolysis of empty fruit bunches. Alkali pretreatment inducing the swelling of cellulose, resulting in increasing of the surface area, increased the accessibility of enzyme binding substrate and altered the lignin structure [14]. Wu et al. [15] evaluated the effect of alkali pretreatment at room temperatures to make cellulose of sweet sorghum bagasse accessible for enzyme. The previously report indicated that xylan was a major constituent of hemicellulose in lignocellulosic biomass such as straw, cob, hull, and bagasse [16]. The enzymatic hydrolysis of xylan may also results in oligomers known as xylooligosaccharides (XOS) [17], which may be used as a functional ingredient in pharmaceutical, including food and feed products. For these reasons, alkali pretreatment followed by enzymatic hydrolysis could be used for effective oligosaccharide production in this study. In this study, Pentopan Mono BG, the commercial xylanase enzyme widely used in XOS production in other biomass was used. The enzyme has endo-xylanase activity of □ 2500 U/g with optimum pH at 5 and optimum temperature at 50°C.

Objectives

The aim of this study was to analyze the chemical composition of Sacha Inchi hull and evaluate the effect of Pentopan Mono BG xylanase enzyme concentration and hydrolysis time for oligosaccharide production.

Methods

Chemical composition analysis of Sacha Inchi hulls

Sacha Inchi hulls were obtained from The Ultimate Bangkok Ltd., Thailand. All chemicals used in this study were analytical grade. The sample was grounded by Hammer mill and then passed through 30 mesh sieving screen. Sacha Inchi hulls powder was dried at 50°C until the moisture content was lower than 10%. Two grams of dried Sacha Inchi hulls powder was used for chemical composition analysis [18]. Holocellulose, insoluble lignin and extractives were determined [19].

Pretreatment of SachaInchi hulls

Dried SachaInchi hulls powder was soaked in 2% NaOH solution at the ratio of 1:1 with continuous stirring for 6 days. After complete pretreatment, the NaOH solution was decanted and then the residues were neutralized by washing with water. The residues were dried at 45°C for 24 h to obtain the pretreated SachaInchi hulls [20].

Oligosaccharides production by xylanase and the determination of saccharide profiles

Hydrolysis of pretreated Sacha Inchi hulls powder was performed with Pentopan Mono BG powder recombinant of *Aspergillusoryzae* from Novozymes, Bagsvaerd, Denmark (endo-xylanase activity 2500 U/g, optimum pH 5, optimum Temp. 50°C). The 6% (w/v) of pretreated Sacha Inchi hulls powder were suspended in 100 mM sodium phosphate buffer (SPB) (pH 6.0). Xylanase was added at 10-150 U/g of enzyme concentration and incubated at 50°C, 170 rpm for 0-24 h.

The hydrolysates were collected every 2 h for determining the total reducing sugar by DNS assay [21] using xylose as a standard. The reaction was stopped by boiling for 30 min. Sugar hydrolysate was analyzed qualitatively by thin layer chromatography (TLC) which was performed on Merck TLC (aluminum sheets 20 x 20 cm) of silica gel 60. The plates were developed once with 1-butanol: acetic acid: water in the ratio of 2:1:1. Spots were visualized by spraying with 10% sulfuric acid in ethanol with 0.2% of orcinol and heating at 110°C comparing with mixed XOS (xylose, xylobiose, xylotriose, xylotetraose, xylopentaoseand xylohexaose) (X_1-X_6) (Wako), arabinose (A_1) (Sigma), 1,3-arabinosyl-xylobiose (A_3X) and 1,2-arabinosyl-xylotriose (A_2XX) standards were purchased from Megazyme. One Unit of xylanase activity is defined as the amount of enzyme required to release one µmole of xylose reducing sugar equivalents per minute from wheat arabinoxylan (5 mg/mL) in sodium phosphate buffer (100 mM), pH 6.0.

Statistical analysis

Data were statistically analysed by ANOVA. Differences in means were analysed using Duncan's multiple RangeTest at 95% confidence level. Statistical analysis was performed using SPSS.

Results

Chemical composition analysis of Sacha Inchi hulls

The proximate analysis results of the Sacha Inchi hulls were given in Table 1. The SachaInchi hulls showed moisture (8.51%), ash (1.44%), fat (1.55%), protein (4.10%), crude fiber (37.20%), and total carbohydrate (47.20%). These results were closed to the report of [3] investigating 1.55% ash, 3.85% fat, and 9.46% protein of Sacha Inchi hulls. The extractives, holocellulose, and insoluble lignin content on dry matter basis for the Sacha Inchi hulls in this study were 15.30%, 24.12%, and 66.30%, respectively.

Table 1 Chemical composition of SachaInchi hulls.

Chemical composition	Content (%w/w) ± S.D.
Moisture	8.51±0.25
Ash	1.44±0.05
Fat	1.55±0.34
Protein	4.10±0.23
Crude fiber	37.20±0.75
Total Carbohydrate	47.20±0.00
Extractives	15.30±0.22
Holocellulose content	24.12±0.40
Insoluble lignin content	66.30±4.95

Oligosaccharides production by xylanase

Soaking with 2% NaOH for 6 days was used to lower the lignin content before xylanase treatment. NaOH induces the swelling of cellulose that not only increases the surface area but also partially decreases the crystallinity of lignocelluloses and the lignin structure is destroyed. Resulting in the lignin dissolution [22] and induces decrease the saponification of intermolecular ester bond crosslink hemicellulose [23]. The yield of the biomass afteralkali pretreatment was 98.2% (data not shown). The production of oligosaccharides was performedusing 6% (w/v) of the pretreated Sacha Inchi hulls hydrolyzing by 10-150 U/g of Pentopan Mono BG at 50°C. Hydrolysis period of 24 h by 133 U/g of the enzyme was considered to be enough for achieving the highest total reducing sugar obtaining 0.68±0.01 mg/mL (Figure 1). During incubation, at the enzyme content of 10 U/g, the total reducing sugar content was slightlyincreased while at 100, 133 and 150 U/g these contents were dramatically increased.

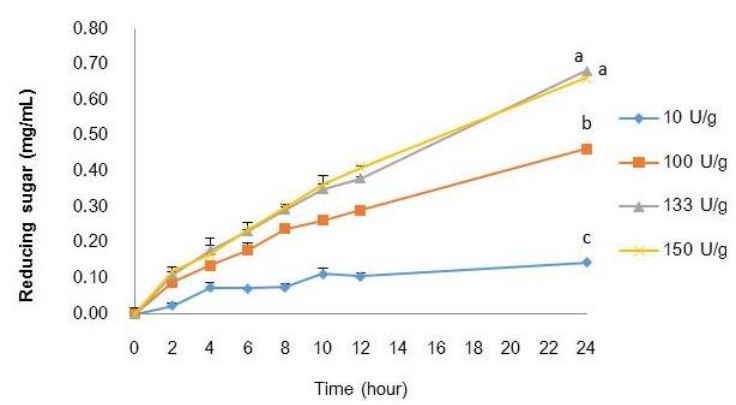
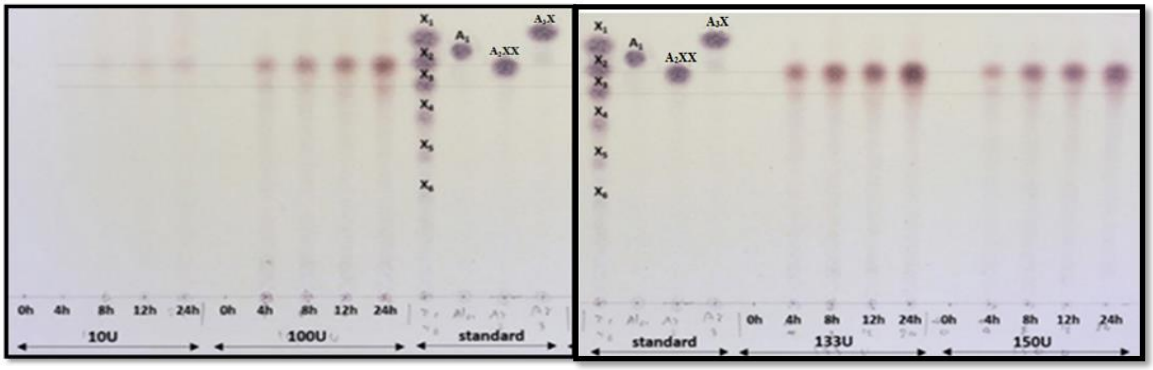


Figure 1. Enzymatic production of oligosaccharides from pretreated Sacha Inchi hulls by Pentopan Mono BG at different enzyme concentration and time at 50°C, pH 6.0.

Values are report as mean; same lower case letters indicate no significant difference ($p>0.05$); n=2

The hydrolysates of Sacha Inchi hulls with Pentopan Mono BG during 2-24 h were determined by TLC carried out with the saccharide standards of X_1-X_6, A_1, A_2XX and A_3X. TLC chromatogram was shown in Figure 2. It was evident that xylose was absent in all incubation time intervals and xylobiose was the main oligosaccharide having been produced since 4 h of hydrolysis.



(a) (b)

Figure 2. TLC chromatogram of enzymatic hydrolysates of pretreated Sacha Inchi hulls by Pentopan Mono BG at 10-100 U/g (a), at 133-150 U/g (b) 50ºC during 4-24 h. X_1-X_6, A_1, A_2XX and A_3X were used as standard.

Conclusions and Discussion

The proximate analysis results of the Sacha Inchi hulls were given in Table 1. The result here is in accordance with the report of [3] investigating Ash (1.55%), Fat (3.85%), and Protein (9.46%) of Sacha Inchi hulls. The holocellulose and insoluble lignin content on dry matter basis for the Sacha Inchi hulls in this study was 24.12%, and 66.30%, respectively. Therefore, this holocellulosemay be consideredas a rich-lignocellulosic raw material [24]. Lignocellulosic materials compose of cellulose, hemicellulose, lignin and other extraneous components [11]. Jeanmarc and Christiane [25] found that wheat bran holocellulose contains 18% of hemicellulose. Most sugar monomers in hemicellulose are reported as xylose and arabinose. Therefore, the oligosaccharides produced by the Hemicellulose may be in the forms of XOS and AXOS. However, the crosslink between hemicelluloses, cellulose and lignin mediating structural stability in the plant cell wall causes the limitations of the enzyme accessibility to thehemicellulose. Many reports showed a variety of alkali reagent having been used to improve enzymatic saccharification of lignocellulosic materials [12]. According to this study, the high insoluble lignin content found in Sacha Inchi hulls should be reduced before oligosaccharides production. For increasing the effectof enzymatic hydrolysis, thepretreatment was required.The total reducing sugar content of the enzyme concentration of 100, 133 and 150 U/g, the contents were dramatically increased. The similar trend of hydrolysis was

observed by Chapla et al. [26] indicating that increasing the xylanase concentration from 5 to 100 U/g increased the production of XOS from wheat straw, rice straw and corncob. The enzyme concentration at 133 U/g was found the most suitable for oligosaccharides production showing no difference with 150 U/g. Moreover, with increasing incubation period, the production of oligosaccharides also increased. Incubating with the enzyme at the concentration of 133 U/g at 4, 8, 12, and 24 h, the total reducing sugar contents were 0.18%, 0.29%, 0.38%, and 0.68%, respectively (Figure 1). Akpinar et al. [27] also observed that 8-24 h was the optimum hydrolysis time during the production of XOS from agricultural wastes using commercial xylanase.

The hydrolysates of Sacha Inchi hulls with Pentopan Mono BG during 2-24 h were determined by TLC (Figure 2). It was evident that xylose was absent in all incubation time intervals. The results were corresponding with the previous study showing no appearance of xylose when using Pentopan mono BG in oligosaccharide production from Riceberry Husk [28]. The presence of monosaccharide reduced the purity of the oligosaccharides because the monosaccharide can generally be absorbed in the upper small intestine [29] showing no prebiotic property. To increase the quality of the final product of prebiotic, it is very important to reduce of monosaccharide present in the medium [30-31]. However, there are some reports on the production of XOS using Pentopan Mono BG with the presence of xylose in the hydrolysates. Mathew et al. [32] found that xylose, xylobiose, and xylotriose including some arabino-xylooligosaccharides (AXOS) were produced by hydrolysis of arabinoxylan by Pentopan Mono BG. These occurrences could depend on the hemicellulose structure of plant varieties. This could be suggested that Pentopan Mono BG contains mainlyendo- xylanase. The enzyme is responsible for the hydrolytic degradation of xylans, a family of natural polysaccharides. The enzyme can be either endo- acting (hydrolyzing bonds in the interior of xylan polymers) without activity of exo-xylanase [33]. In this research, the xylobiose was a main product. This may be associated with the optimum incubation time as long as 24 hours. During this period the long chain oligosaccharidemay be hydrolyzed simultaneously to be the shortest chain oligosaccharide exhibited as xylobiose.

This study showed that Sacha Inchi hulls, by- product of Sacha Inchi oil industry can be an alternative source of oligosaccharide production. The chemical composition exhibited that Sacha Inchi hulls are a rich- lignocellulosic material. Commercial Pentopan Mono BG xylanase can produce short chain oligosaccharides mainly xylobiose from alkali-pretreated Sacha Inchi hulls. The enzyme concentration and time influenced the amount of total reducing sugar content.The increasingin enzyme concentration was not highly advantageous. A hydrolysis period of 24 h was enough for achieving the highest production of xylobiose at 133 U/g of enzyme concentration. The short chain oligosaccharides from Sacha Inchi hulls should be further investigated for the prebiotic properties.

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