UNIVERSITI PUTRA MALAYSIA

OPTIMISATION OF ULTRASONIC- AND ENZYME-ASSISTED METHODS FOR PRODUCTION OF FRUCTOOLIGOSACCHARIDES-RICH YACON [Smallanthus sonchifolius (Poepp.) H. Rob.] SYRUP

FLORENCE CHONG HUEY LIN

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By

FLORENCE CHONG HUEY LIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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OPTIMISATION OF ULTRASONIC- AND ENZYME-ASSISTED METHODS FOR PRODUCTION OF FRUCTOOLIGOSACCHARIDES-RICH YACON [*Smallanthus sonchifolius* (Poepp.) H. Rob.] SYRUP

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March 2019

Chairman: Prof. Faridah Binti Abas, PhD
Faculty: Food Science and Technology

Yacon [*Smallanthus sonchifolius* (Poepp.) H. Rob.] is a highly nutritious tuberous root. It is often extracted conventionally and undergoes multiple harsh processes for long hours causing the deterioration of important compounds such as oligosaccharides and phenolic compounds. Consequently, this research was performed to optimise ultrasonic-assisted and enzyme-assisted methods for the production of fructooligosaccharides (FOS)-rich syrup from yacon. Optimisation was carried out on single response (highest yield (HY), highest antioxidant activity (HA) and highest total soluble solids (HTSS)) and multiple responses for both extraction methods. The saccharide compounds in all the optimised syrups were identified and quantified with LC-Q-TOF-MS and HPLC-RI, respectively. Enzyme-assisted extraction produced the highest yield (HY) syrup using parameters such as low solute to solvent ratio (1:80), high enzyme percentage (1.00%), low temperature (30 °C) and long extraction time (4.0 h). The highest total soluble solids (HTSS) sample using enzyme-assisted extraction was attained with 4.0 h extraction time using 1.00% enzyme percentage and solute to solvent ratio of 1:20 at 30 °C. The optimisation analysis of the highest antioxidant activity (HA) revealed that ultrasonic-assisted extraction resulted in syrup with lower IC_{50} than the enzyme-assisted extraction. The highest antioxidant activity (HA) condition from ultrasonic-assisted extraction was 1:80 solute to solvent ratio and 70% amplitude for 30 min in a continuous mode. The most desirable extraction condition for the ultrasonic-assisted extraction of fructooligosaccharides-rich yacon syrup established in this study was 1:80 solute to solvent ratio, 5.0 min extraction time, 20% amplitude and 10 s pulsed interval. In contrast, the most desirable set of conditions for enzyme-assisted extraction was 1:20 solute to solvent ratio, 1.00% enzyme percentage, 30 °C temperature and 1.0 h extraction time. The physicochemical analysis results from the optimum condition samples revealed that enzyme-assisted extraction was a more effective extraction method. Nevertheless, the duration of the extraction processes was greatly reduced with ultrasonic-assisted extraction. Both the optimum condition syrups contained saccharides compounds such as 1-kestose (GF2), nystose (GF3), sucrose, fructose and glucose. In

contrast, fructofuranosylnystose (GF4) was only identified in the optimum condition of enzyme-assisted extraction yacon syrup. Between the optimum conditions samples, higher FOS content was discovered in the optimum condition of enzyme-assisted extraction syrup at 31%. A greater amount of fructooligosaccharides allowed the optimum condition syrup from enzyme-assisted extraction to have a better capacity at lowering the glucose production rate and it was capable of exhibiting hypocholesterolemic effects. This study deduced that the optimum enzyme-assisted extraction condition is more efficient to extract higher quality of fructooligosaccharides-rich yacon syrup with greater amounts of fructooligosaccharides, antioxidant activity and total soluble solids than the optimum ultrasonic-assisted extraction condition, although it was more economical.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGOPTIMUMAN KAEDAH BERBANTU ULTRASONIK DAN KAEDAH BERBANTU ENZIM UNTUK PENGHASILAN SIRAP YACON [*Smallanthus sonchifolius* (Poepp.) H. Rob.] KAYA FRUKTOOLIGOSAKARIDA

Oleh

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Yacon [*Smallanthus sonchifolius* (Poepp.) H. Rob.] adalah sejenis akar tuba yang sangat berkhasiat. Ia sering diekstrak dengan cara pengekstrakan konvensional dan proses yang kesat serta jangka masa yang panjang dan mengakibatkan kemerosotan sebatian penting seperti oligosakarida dan sebatian fenolik. Oleh itu, penyelidikan ini dijalankan untuk mengoptimumkan kaedah pengestrakan berbantu ultrasonik dan kaedah pengestrakan berbantu enzim bagi penghasilan sirap yacon kaya fruktooligosakarida (FOS). Pengoptimuman telah dijalankan terhadap tindak balas tunggal (Hasil sirap tertinggi (HY), antioksidan aktiviti tertinggi (HA) and jumlah pepejal larut tertinggi (HTSS)) dan tindak balas berganda bagi kedua-dua kaedah pengekstrakan tersebut. Sebatian sakarida dalam semua sirap yang telah dioptimumkan, masing masing dianalisis dengan LC-Q-TOF-MS dan HPLC-RI. Pengekstrakan berbantu enzim menghasilkan jumlah sirap yang tertinggi (HY) dengan nisbah bahan kepada pelarut yang rendah (1:80), peratusan enzim yang tinggi (1.00%), suhu yang rendah (30 °C) dan masa pengekstrakan yang panjang (4.0 jam). Sampel pepejal terlarut yang tertinggi (HTSS) dari pengekstrakan berbantu enzim boleh dicapai dalam masa pengekstrakan 4.0 jam dengan menggunakan peratusan enzim 1.00% dan nisbah bahan kepada pelarut 1:20 pada suhu 30 °C. Analisis pengoptimuman bagi aktiviti antioksidan yang tertinggi (HA) dalam sirap yacon kaya fruktooligosakarida menunjukkan bahawa pengekstrakan bantuan ultrasonik boleh menyebabkan IC_{50} yang lebih rendah berbanding kepada pengekstrakan berbantu enzim. Keadaan optimum pengekstrakan berbantu ultrasonik untuk aktiviti antioksidan tertinggi (HA) ialah 1:80 nisbah bahan kepada pelarut dan 70% amplitud untuk 30 min dengan mengunakan mod berterusan. Keadaan optimum bagi pengekstrakan sirap yacon kaya fruktooligosakarida dengan kaedah berbantu ultrasonik yang disyorkan dalam kajian ini ialah nisbah bahan kepada pelarut pada 1:80, 5.0 min masa pengekstrakan, 20% amplitud dan 10 s jeda denyutan. Manakala, untuk pengekstrakan berbantu enzim, keadaan optimum yang diperolehi ialah 1:20 nisbah bahan kepada pelarut, 1.00% peratusan enzim, 30 °C dan masa pengekstrakan selama 1.0 jam. Ciri-ciri fizikokimia untuk sampel

daripada keadaan pengekstrakan optimum menunjukkan bahawa pengekstrakan berbantu enzim adalah kaedah yang lebih efektif. Walau bagaimanapun, tempoh untuk proses pengekstrakan telah disingkatkan dengan kaedah pengekstrakan berbantu ultrasonik. Kedua-dua sirap yang telah dioptimum mengandungi sebatian sakarida seperti 1-kestosa (GF2), nystosa (GF3), sukrosa, fruktosa dan glukosa. Sebaliknya, fruktofuranosylnystosa hanya dikenal pasti dalam sirap yacon kaya fruktooligosakarida yang diekstrak daripada keadaan optimum kaedah berbantu enzim. Antara sampel keadaan optimum, kandungan FOS yang tertinggi ditemui dalam sirap yang dihasilkan dari keadaan optimum daripada kaedah berbantu enzim iaitu pada 31%. Kandungan fruktooligosakarida yang lebih tinggi dalam sirap optimum daripada pengekstrakan berbantu enzim menyebabkan kadar pengeluaran glukosa yang lebih rendah dan ia mampu menunjukkan kesan hipokolesterolemik. Kesimpulannya, keadaan optimum kaedah pengekstrakan berbantu enzim adalah lebih efisien untuk mengekstrak sirap yacon kaya fruktooligosakarida yang berkualiti dengan kandungan fruktooligosakarida, jumlah pepejal larut dan aktiviti antioksidan yang lebih tinggi berbanding dengan keadaan optimum kaedah berbantu ultrasonik walaupun ia adalah kaedah yang lebih ekonomik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

°	Degree
°C	Degree Celcius
°Bx	Degree of Brix
%	Percentage
±	Plus minus
≤	Less than or equal
<	Less than
>	More than
-	Negative / Not detected
+	Positive / Available
&	and
µL	Microliter
µm	Micrometer
α	Alpha
a_w	Water activity
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
β	Beta
BMI	Body mass index
C	Carbon
Ca	Calcium
cm	Centimeter
DP	Degree of polymerization

DPPH	2,2-diphenyl-1-picrylhydrazyl
et al.	And others
EAE	Enzyme-Assisted Extraction
Eq.	Equation
FFN	Fructofuranosylnystose
FOS	Fructooligosaccharides
g	g-force
g	Gram
GALT	Gut-associated lymphoid tissue
GF_n	Glucose-fructose$_n$
GIP	Glucose-dependant insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GOS	Glucooligosaccharides
GPR	Glucose production rate
h	Hour
HA	Highest Antioxidant
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
HTTS	Highest total soluble solids
HY	Highest yield
IC_{50}	Inhibitory concentration
IgA	Immunoglobulin A
IMF	Intermediate moisture foods
K	Kelvin
kg	Kilogram
Hz	Kilohertz

kj	Kilojoules
L	Litre
LC-MS	Liquid Chromatography-Mass Spectrometry
LDL	Low-Density Lipoprotein
LED	Light-emitting diode
m	Metre
mg	Milligram
mL	Millilitre
mm	Millimeter
mM	Millimole
m/z	Mass to charge ratio
min	Minute
M	Molar
MΩ	Megohm/a unit of resistance
n	n-factorial
nm	Nanometer
NaCl	Sodium chloride
NaOH	Sodium hydroxide
N.D	Non Detectable
NDO	Non Digestible Oligosaccharides
Pas	Pascal
pH	Potential of hydrogen
PO	Phosphates
POD	Peroxidase
PPO	Polyphenol oxidase
ppm	Parts per million

psig	Pounds per square inch gauge
Q-TOF	Quadrupole Time of Flight
R^2	Regression coefficient
RI	Refractive index
RT	Retention time
Rpm	Revolutions per minute
S	Second
SCAC	Sodium Cholate Adsorption Capacity
SCFA	Short chain fatty acids
SD	Standard deviation
TAG	Triacylglyceride
TSS	Total soluble solids
UAE	Ultrasonic-Assisted Extraction
UV	Ultra-violet
UV-VIS	Ultra-violet visible
v/v	Volume/Volume
VLDL	Very low-density lipoprotein
W	Watt
W	Weight
WHO	World Health Organization
w/v	Weight/Volume
w/w	Weight/Weight

CHAPTER 1

INTRODUCTION

1.1 Background

Yacon [*Smallanthus sonchifolius* (Poepp.) H.Rob] is a native crop of the Andean region that belongs to Asteraceae or Compositae family (Douglas *et al.*, 2007; Manrique *et al.*, 2005). It is a perennial plant that has many beneficial effects. However, it was being underestimated in the past (Manrique *et al*., 2005). Yacon has appearance comparable to sweet potato. It is often consumed as fruit by the native. The flesh of yacon is succulent, tender and crunchy alike fresh apple or watermelon. It is juicy and has a sweet taste with a relatively low energy value (Delgado *et al.,* 2013). Its low energy value is because the tuberous root is rich in non-digestible carbohydrate called fructooligosaccharides (FOS) (Campos *et al.*, 2012). Unlike most of the edible tuber roots, it stores carbohydrate in the form of fructans mainly fructooligosaccharides (FOS) and other simple sugar. It is one of the plant material with the highest concentration of FOS (Brochier *et al.*, 2015; Hermann *et al.*, 1998).

FOS in yacon is a short chain carbohydrate with a low degree of polymerization (DP) and molecular weight. It is formed from repeated units of β-(2→1)-D-frutofuranosyl and ends with a terminal sucrose, at an average DP of 4 (Murphy, 2001). The FOS in yacon is mostly made up of 1-kestose (GF2), nystose (GF3) and 1-fructofuranosyl nystose (GF4) (Delgado *et al.*, 2013; Dominguez *et al.*, 2014). The β-(2→1)- glycosidic bond has resistance to the hydrolysis of enzymes in the human digestive system and reaches colon without being digested but rather being selectively fermented by the intestinal microflora which confers health benefits (Wang *et al.*, 2010). According to Alles *et al.* (1996), FOS undergoes complete bacteria fermentation in the large intestines. This prebiotic properties is the rationale for its low caloric value (4.2-6.3 kJ/g). It has low intensity of sweetness, approximately 30-60% of sucrose which is beneficial to diabetic patient as well as healthy human (Murphy, 2001; Ojansivu *et al.*, 2011; Saad *et al.*, 2013). FOS also act as soluble fiber and has been used as sugar and fat replacer in yogurts (Franck, 2002). This suggests that FOS in yacon can be incorporated in the dietary intervention program to improve diet and use as a preventive measure for health problems such as metabolic syndrome like overweight and obesity which can consequently lead to various diseases for instance diabetes and cardiovascular diseases among the Malaysia population.

In concomitant with the above, FOS in yacon also contributes to other health effects including anti-tumor properties (Dominguez *et al*., 2014) and anti-inflammatory effects (Liévin-Le Moal & Servin, 2006; Munjal *et al.*, 2009; Romeo *et al.,* 2010) on colon cancer cells. The end products of fermentation produce short-chain fatty acids (SCFA) that create an acidic environment which promotes hypotriglyceridemic effect (Dominguez *et al.*, 2014; Genta *et al.*, 2005) and enhances mineral solubility and absorptivity to reduce osteoporosis progression (Gudiel-Urbano & Goni, 2002; Wang *et* *al.*, 2010). Studies also demonstrated that daily consumption of yacon syrup had effects on insulin resistance and caused a significant reduction in body weight, waist circumference and body mass index on overweight and obese women suggesting a role in obesity management (Genta *et al.*, 2009). Apart from this, studies had discovered antioxidant in yacon such as tryptophan (Yan *et al.*, 1999), polyphenols compounds, particularly chlorogenic acids and few other caffeic acids derivatives (Takenaka *et al.*, 2003). These compounds were reported to have a distinct role in reducing the risk of cancer and cardiovascular diseases (Cheynier, 2012; Vasconcelos *et al.*, 2015). These compounds also modulated the glucose metabolism and exerted anti-hyperglycemic activity to decrease plasma glucose (Genta *et al.*, 2009).

To date, the bioactive components such as phenolic compounds, prebiotic like inulin or FOS and crude extracts with bioactive compounds of yacon have been extracted using the conventional extraction techniques. The conventional extraction techniques include solid (sample)-liquid (solvent) extraction with continuous heating at high temperature and shaking for long hours or subjecting the yacon sample to food processor for extraction of juice, then centrifugation or filtration to obtain clear juices (Campos *et al.*, 2012; Castro *et al.*, 2012; da Fonseca Contado *et al.*, 2015; Genta *et al.*, 2009; Genta *et al.*, 2005; Lago *et al.*, 2012; Scher *et al.*, 2015; Yan *et al.*, 1999). Sometimes, extraction is repeated for several times on the same sample to garner more extract. In the yacon's industry, syrup is produced using a special evaporator to evaporate and concentrate the juices for syrup (Manrique *et al.*, 2005). In regards to the conventional extraction techniques of yacon tuberous roots, many disadvantages arise including subjecting samples to long hours of heating which may degrade or deteriorate the target components from yacon, requires labour-intensive, long extraction time, high energy consumption and complex purifications procedure (Esclapez *et al.*, 2011; Wang *et al.*, 2013). Consequently, new technologies that are more economical and environment-friendly such as ultrasonic-assisted extraction (UAE) (Entezari *et al.*, 2004; Pourfarzad *et al.*, 2015) and enzyme assisted extraction (EAE) (Puri et al., 2012) have been developed to substitute these conventional extraction methods for the extraction of natural products.

Ultrasonic-assisted extraction (UAE) method is a green technology that uses ultrasonic waves to induce a phenomenon called cavitation (Zhu *et al.*, 2016). It is an alternative to conventional extraction methods. Currently, this method has been used extensively in the food industry for the extraction of valuable molecules such as antioxidants. There are a few research studies being performed using this extraction method for extracting inulin and fructan among other subjects that have given promising results. For example, direct sonication has been proven to be more efficient for the extraction of fructooligosaccharides from artichoke than conventional extraction (Machado *et al.*, 2015). Moreover, in contrast with water extraction, UAE was reported as a faster extraction method and increase the extraction yield of inulin (fructans) from Burdock root (*Arctium lappa*).

Enzyme-assisted extraction (EAE) method is another emerging eco-friendly non-conventional method for the extraction of bioactive compounds from plants. Phytochemicals in plant matrices are often distributed in the cell cytoplasm and cling in the polysaccharide-lignin network by hydrogen or hydrophobic bonding, which has become a barrier to these intracellular components preventing them from accessing the solvent in a routine extraction process (Azmir *et al.*, 2013; Zhu *et al.*, 2014). Nevertheless, when specific enzymes are applied, the enzymes effectively decompose parts of the plant, such as the cell wall, which can enhance the extraction efficiency (Rosenthal *et al.*, 1996; Singh *et al.*, 1999). EAE is recognised as having a shorter extraction time, increased yield, minimised solvent usage and lowered energy consumption as compared to non-enzymatic extraction methods for compounds including polysaccharides, sugar, soluble fibers and so on (Puri *et al.*, 2012).

1.2 Problem statements

The advancement and revolutions in science and technology has certainly improved and transformed the society in all aspect of life. Current society is more attentive and has demand for a better healthcare and condition such as engaging nutraceutical products and following various dietary interventions to improve health. Hence, natural source like yacon is being explored for its diverse functions such as sweetening agent with nutritional advantages that can improve human physiological functions. The development of various new tools and technology has also gradually obsoleting the conventional extraction methods. The conventional methods used for extraction of yacon syrups have been reported causing disadvantages such as long heating hours which may degrade or deteriorate the target components from yacon, requires labour-intensive, long extraction time, high energy consumption and complex purifications procedure (Esclapez *et al.*, 2011; Wang *et al.*, 2013). Hence, the non-conventional extraction methods with positive impacts and its optimum conditions are studied for optimal production of FOS-rich yacon syrup. Moreover, the insufficient studies regarding the effects of different non-conventional extraction methods on the physiological qualities of FOS-rich yacon syrup also inspired this research study.

1.3 Objectives

In this study, yacon (*S. sonchifolius*) was selected to produce syrup using different extraction methods due to its nutritional advantages and local availability. Therefore, the main goal of the present work was to optimise the extraction methods for FOS-rich yacon syrup and the characterisation of the syrup. The specific objectives of the study are as follow:

1. To optimise the Ultrasonic-assisted extraction (UAE) method (solute to solvent ratio, amplitude, extraction time and pulsed interval) for the production of FOS-rich syrup from yacon with highest yield, total soluble solids, antioxidant activity, ash content and lowest moisture content and water activity.
2. To optimise the Enzyme-assisted extraction (EAE) method (solute to solvent ratio, enzyme percentage, temperature and extraction time) for the production of FOS-rich syrup from yacon with highest yield, total soluble solids, antioxidant activity, ash content and lowest moisture content and water activity.

3. To characterise the yacon syrups obtained from the optimised UAE and EAE methods.

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