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Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables

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ABSTRACT

Ultrasound assisted extraction (UAE) was used to extract oligosaccharides from selected fruits (blueberry, nectarine, raspberry, watermelon) and vegetables (garlic, Jerusalem artichoke, leek, scallion, spring garlic and white onion). The individual fractions of the oligosaccharides were analyzed: 1-kestose (GF_2), nystose (GF_3) and 1F-β-fructofuranosylnystose (GF_4) from the fructo-oligosaccharides (FOS), and raffinose and stachyose from the raffinose family oligosaccharides (RFO). Extraction parameters including solvent concentration (35–85% v/v), extraction temperature (25–50 °C) and sonication time (5–15 min) were examined using response surface methodology (RSM). Ethanol concentration of 63% v/v, temperature of 40 °C and extraction time of 10 min gave maximal concentration of the extracted oligosaccharides. The experimental values under optimal conditions were consistent with the predicted values. UAE increased the concentration of extracted oligosaccharides in all fruits and vegetables from 2 to 4-fold compared to conventional extraction. The highest increase of total oligosaccharides extracted by UAE was detected in Jerusalem artichoke, 7.17 ± 0.348 g/100 g FW, compared to 1.62 ± 0.094 g/100 g FW with conventional method.

1. Introduction

Diet rich in fruits and vegetables has been proven to exert a positive effect on preventing the development of a considerable number of chronic diseases such as cancer and cardiovascular diseases. This protective effect has been attributed to the high concentrations of functional compounds [1]. For the effective utilization of ingredients present in consumed foods, gut microflora plays an important role [2]. In this context, prebiotic oligosaccharides are considered to be key compounds. Since the late 1990s and the birth of the prebiotic concept, many scientists have started studying the health properties of prebiotic compounds which resulted in a number of scientific publications describing them in relation to human health [3,4]. The major prebiotic oligosaccharides in the market are fructo-oligosaccharides and galacto-oligosaccharides whose bioactive properties have been evaluated using a range of in vitro and in vivo methods [5–8].

The oligosaccharides are obtained by extraction from natural sources or by chemical or enzymatic synthesis [9–12]. For extraction of low-molecular weight carbohydrates such as oligosaccharides from plant material, the optimal solvent is water [13]. However, water also facilitates interference between carbohydrates and other water-soluble substances such as certain polysaccharides and proteins [14]. Thus, most of the conventional extraction methods for oligosaccharides often use high concentrations of alcohol [15].

The use of ultrasound assisted extraction (UAE) instead of traditional extraction has been increasing and its use has been investigated in the pharmaceutical, chemical and food industries. UAE became a good alternative extraction method when compared to classical extraction methods because of its high efficiency, low energy requirement and low water consumption (no reflux or refrigeration are needed) [16]. The improvement of the extraction process caused by ultrasound is attributed to the disruption of the cell wall, reduction of the particle size and the enhancement of the mass transfer of the cell content to the solvent caused by the collapse of the bubbles produced by cavitation [17]. Therefore, UAE provides increased extraction yield, increased rate of extraction, reduced extraction time and higher processing throughput along with the advantage of usage of reduced temperature and solvent volume which is very advantageous for the extraction of heat labile compounds [18]. The application of UAE in food processing technology is of interest for enhancing extraction of components from plant and animal materials such as phenolic compounds, anthocyanins, aromatic compounds, polysaccharides, oils and functional

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compounds [19–25]. Among all applications, UAE is nowadays considered as the most feasible and economically profitable large-scale application of ultrasound in the food field [26].

The aim of this study was to enhance the extraction of oligosaccharides from selected fruits and vegetables by application of ultrasound assisted method. To optimize the extraction conditions, that is the type and the concentration of solvent, extraction time and ultrasound temperature, response surface methodology (RSM) was used. Ultrasound extraction under optimized conditions was performed to look into the individual fractions of fructo-oligosaccharides (FOS) such as 1-kestose (GF_2), nystose (GF_3), and 1F-β-fructofuranosylnystose (GF_4) as well as raffinose family oligosaccharides (RFO) such as raffinose and stachyose. Furthermore, a comparison between conventional extraction method and ultrasound assisted extraction was conducted. To our knowledge there have been no published data on ultrasound extraction of oligosaccharides and their subsequent analysis for individual fractions.

2. Materials and methods

2.1. Samples and sample preparation

The foods selected for analysis were 4 fruits and 6 vegetables, with the highest content of oligosaccharides according to our previous investigation [27]. Fresh food samples were collected from local green markets (nectarine and watermelon) and grocery stores (Jerusalem artichoke) or directly from producers (blueberry, raspberry, garlic, leek, scallion, spring garlic and white onion). The food samples were transferred to the laboratory and analyzed immediately. Approximately 500 g of each sample was chosen at random. The edible part was cut into small pieces (5–15 mm) and dried at 50 °C in a vacuum oven (Heraeus Instruments vacutherm VT 6025, Hanau, Germany) over period of 12 to 24 h until constant dry mass was reached. Dried samples were crushed with a laboratory grinder to a particle size less than 1 mm before extraction.

2.2. Conventional extraction of oligosaccharides

The dried and grind samples (200 mg) were extracted with ethanol (85% v/v; 20 mL) as described by Espinosa-Martos et al. [28]. Extractions were performed in screw-capped tubes, at 50 °C in a water bath with constant shaking for 1 h. After cooling at room temperature, the samples were centrifuged at 3000×*g* for 15 min. Ten mL of supernatants were evaporated in a vacuum rotary evaporator at 50 °C until the samples were completely dried. The extracts were redissolved in deionized water (1.5 mL) and passed through 0.45 μm filters (Econofilter, Agilent Technologies, Santa Clara, CA, USA) just before high-performance liquid chromatography (HPLC) analysis.

2.3. Ultrasound assisted extraction of oligosaccharides

The dried and grind samples (200 mg) were extracted with 20 mL ethanol, methanol or acetone (20–96% v/v). Extractions were performed in screw-capped tubes, at different temperatures (20–60 °C) in an ultrasound water bath at 40 kHz (Cole-Parmer 8890, Vernon Hills, Illinois, USA) with constant shaking for 5, 10, 20 or 30 min. After cooling at room temperature, the samples were centrifuged at 3000×*g* for 15 min. Ten mL of supernatants were evaporated in a vacuum rotary evaporator at 50 °C until the samples were completely dried. The extracts were redissolved in deionized water (1.5 mL) and passed through 0.45 μm filters (Econofilter, Agilent Technologies) just before high-performance liquid chromatography (HPLC) analysis.

2.4. Determination of oligosaccharides by high-performance liquid chromatography

Twenty microliters of prepared samples were injected into Agilent Technologies 1200 HPLC fitted with Zorbax carbohydrate analysis column (4.6 × 150 mm, 5 μm particle size), Zorbax NH_2 guard column (4.6 × 12.5 mm) (Agilent, USA) and refractive index (RI) detector. The mobile phase was 75:25 (v/v) acetonitrile/water and the flow rate was 1.4 mL/min. The column temperature was kept at 30 °C. Appropriate dilutions of a solution containing each of the carbohydrates, 1-kestose, nystose, 1F-β-fructofuranosylnystose, raffinose and stachyose, all purchased from Sigma–Aldrich (St. Louis, Missouri, USA) HPLC grade were used as calibration standards.

2.5. Experimental design

2.5.1. Single factor experiments

2.5.1.1. Selection of solvent type. By fixing extraction time (10 min) and extraction temperature (50 °C), samples were extracted with 60% (v/v) acetone, 60% (v/v) ethanol, and 60% (v/v) methanol respectively. The extraction procedure was described in Section 2.3. The solvent type was selected according to the value of extracted oligosaccharides (g/100 g FW).

2.5.1.2. Effect of solvent concentration on extraction of oligosaccharides. Using the best solvent type selected in single factor experiments, Section 2.5.1.1, samples were extracted with solvent ranging from 20% to 96% (v/v) by fixing the extraction time and extraction temperature at 10 min and 50 °C, respectively.

2.5.1.3. Effect of extraction time on extraction of oligosaccharides. Samples were extracted using the best solvent type and the best solvent concentration selected in single factor experiments, Sections 2.5.1.1 and 2.5.1.2, respectively. The extraction procedures were repeated as described in section of single factor experiments by varying the extraction time from 5 to 30 min while setting up the extraction temperature constant at 50 °C.

2.5.1.4. Effect of extraction temperature on extraction of oligosaccharides. Using the best solvent type at its corresponding concentration selected in single factor experiments, Sections 2.5.1.1 and 2.5.1.2, the samples were extracted at various extraction temperatures ranged from 20 to 60 °C at the optimum time determined in single factor experiments, Section 2.5.1.3.

2.5.2. Response surface methodology

The oligosaccharides content was further optimized through the response surface methodology (RSM) approach. Based on the results of the single factor experiments, ranges of the three factors, solvent concentration (X_1), ultrasound time (X_2) and ultrasound temperature (X_3) were determined. The experiments were designed to evaluate the effect of these factors on the yield of oligosaccharides using ultrasound extraction method (Table 1). Predicted response values for the total oligosaccharide concentration were obtained using Box–Behnken Design (BBD). The mathematical model representing the concentration of oligosaccharides as a function of the independent variables within the range studied was expressed as follows:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ii} X_i Y_j \quad (1)$$

where Y is the estimated response; β_0 is a constant; and β_i, β_{ii}, β_{ij} are the linear, quadratic and interactive coefficients of the model,