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Optimization of ultrasound-microwave synergistic extraction of prebiotic oligosaccharides from sweet potatoes (*Ipomoea batatas* L.)

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Abstract:

In this study, efficient ultrasound–microwave-assisted extraction (UMAE) of prebiotic oligosaccharides from sweet potatoes (*Ipomoea batatas* L.) was investigated. Response surface methodology was used to optimize the extraction conditions: extraction time, ultrasonic power, and microwave power. The prebiotic effect of extracted oligosaccharides on *Bifidobacterium adolescentis* was also investigated. The results show that the processing conditions of UMAE for optimum the yields of prebiotic oligosaccharides from sweet potatoes (PPOS4 and PPOS5) and corresponding absorbance (OD) are 100 s extraction time, 300 W ultrasonic power, and 200 W microwave power. Under these conditions, the experimental yields of PPOS4 and PPOS5 and the corresponding OD were 1.472%, 5.476%, and 2.966, respectively, which match the predicted values well. Compared with the conventional hot-water extraction (HWE), microwave-assisted extraction (MAE), and ultrasound assisted extraction (UAE) methods, the UMAE procedure exhibited significantly high extraction efficiency ($p < 0.05$). Comparison of SEM images of tissues of the sweet potatoes after extractions indicate microfractures and disruption of cell walls in the potato tissues. These results confirm that UMAE has great potential and efficiency in the extraction of bioactive substances in the food and medicinal industries.

Industrial relevance: Ultrasound–microwave-assisted extraction is a new process technology that combines the ultrasonic and microwave methods. It makes full use of the high-energy effect of microwaves and ultrasonic cavitation. And it overcomes the shortcomings of conventional, ultrasonic, and microwave extractions. Fast, efficient extraction using this method can be realized at low temperature under ambient conditions, enhancing competition of industries to be more ecologic, economic and innovative.

Keywords: ultrasound–microwave; sweet potato; prebiotic oligosaccharide; extraction; response surface methodology

1. Introduction

Purple sweet potato (*Ipomoea batatas* L.), a member of the Convolvulaceae family, is an economically important crop in the tropics, subtropics, and temperate regions around the world (Fan, Han, Gu, & Chen, 2008; Wu, Qu, Jia, Kuang, Wen & Yan, et al., .2015). It is a highly nutritious vegetable, containing numerous healthful bioactive constituents, including dietary carbohydrates, phenolic acids, anthocyanins, and β-carotene (Liu, Mu, Sun, Zhang, & Chen, 2013; Sun, Fan, Wang, Lu, Zhang & Wu, et al., 2015; Zhang, Fan, Zheng, Lu, Wu, Shan, & Hu, 2009). Because of its nutritive value and biological activities, the research community's interest in purple sweet potato has grown in recent years (Ahmed, Akter, Lee, & Eun, 2010; Esatbeyoglu, Rodriguez-Werner, Schlosser, Winterhalter, & Rimbach, 2017).

Gut microflora play an important role in the effective utilization of constituents in foods (Li, Zhang, Yu, Li, Dong, Wang, Gu, & Guo, 2015). Oligosaccharides, in particular, are considered to be compounds that are important to gut microflora. Enrichment of the diet with oligosaccharides has been reported to provide an opportunity for improving the gut microecology in terms of bacterial populations, biochemical profiles, and physiological effects (Gómez, Gullón, Yáñez, Schols, & Alonso, 2016; Yang, Prasad, Xie, Lin, & Jiang, 2011).

Extraction of oligosaccharides is an important step for their application or for further research. Oligosaccharides are mainly obtained by hot-water extraction (HWE), microwave-assisted extraction (MAE), or ultrasound-assisted extraction (UAE) from natural sources. Extraction of oligosaccharides by conventional extraction methods always needs high temperature and extended periods (Liu, Gong, Zhang, Jia, Li, Wang, & Wu, 2014). The main disadvantage of MAE is inhomogeneous heating, which is detrimental to the extraction process (Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2014). For UAE, the solvent temperature is difficult

to control, usually leading to poor repeatability (Afshari, Samavati, & Shahidi, 2015). Ultrasound–microwave-assisted extraction (UMAE) is a new process technology that combines the ultrasonic and microwave methods (Liew, Ngoh, Yusoff & Teoh, 2016;Lu, Zheng, Li, Cao, Zheng, Xiao, Miao & Zheng, 2017) . It makes full use of the high-energy effect of microwaves and ultrasonic cavitation, and it overcomes the shortcomings of conventional, ultrasonic, and microwave extractions. Fast, efficient extraction using this method can be realized at low temperature under ambient conditions, saving energy and time (Perussello, Zhang, Marzocchella, & Tiwari, 2017; (Gambacorta, Trani, Punzi, Fasciano, Leo, & Fracchiolla, et al., 2017; Chanioti, & Tzia, 2018; Chemat, Rombaut, Meullemiestre, Turk, Perino, & Fabiano-Tixier, et al. 2017). UMAE has been used to extract a variety of active compounds from plants, such as lycopene (Zhang & Liu, 2008), vegetable oils (Cravotto, Boffa, Mantegna, Perego, Avogadro, & Cintas, 2008), polysaccharides (Chen, Gu, Huang, Li, Wang, & Tang, 2010), and oligosaccharides (Lu, et al., 2017). However, no study has been devoted to the extraction of oligosaccharides from purple sweet potato.

Although response surface methodology (RSM) has been extensively used in the optimization of the extraction process, such as the yield of products, it has not been studied on the yield and the efficacy of products (such as the efficacy of probiotics). Herein, establishing a high-performance extraction method and optimizing the extraction parameters were necessary to produce oligosaccharides from purple sweet potato. Thus, the aim of this work was to apply UMAE to improve the yield of oligosaccharides and to optimize the UMAE conditions by response surface methodology (RSM). The effects of extraction time, ultrasonic power, and microwave power on the yield of oligosaccharides, as well as the interaction among factors and range of UMAE conditions for optimizing the extraction yield of individual fraction, are discussed. The yields of total oligosaccharides were expressed in terms of their proliferative effect of bifidobacteria

2. Materials and Methods

2.1. Plant material and chemicals

Purple sweet potatoes were from a local company (Zi Xin Purple Potatoes Co., Ltd., Fujian, China). The potatoes were cut into small pieces (5–15 mm size), dried, and then ground with a laboratory grinder (FW-80; Taisite Co., Tianjin, China) to a particle size of less than 1 mm before extraction. *Bifidobacterium adolescentis* used in this study was obtained from Zhuhai Livzon Pharmaceutical Industry Group Company (Guangdong, China). All other chemicals used for extraction were of analytical reagent grade.

2.2. UMAE of oligosaccharides

The extraction of oligosaccharides from purple sweet potatoes was carried out by UMAE according to our previous studies (Lu, et al., 2017), with slight modifications. In brief, the ground samples were mixed with an appropriate amount of distilled water (liquid/solid ratio of 15:1, v/w). The starch was removed from the sample solution on the basis of our previous investigations (Guo, Zeng, Zhang, Lu, Tian, & Zheng, 2015). The starch-depleted test sample solution was then diluted to 150 mL and applied in the response surface design. Subsequently, it was transferred to a heterotype three-port glass vase in the device and subjected to UMAE (XH-300B; Beijing Xianghu, Beijing, China). Upon completion of the reaction, the extracted solution was centrifuged at 4000 rpm for 15 min (L530; Xiang Yi Centrifuge Instrument Co., Ltd. Changsha, China), and the supernatant was concentrated at 65°C under vacuum in a rotary evaporator (Buchi 409; Buchi Corp., New Castle, DE, USA). The concentrated liquid was precipitated with three volumes of 95% ethanol (v/v), and stored overnight at 4°C to precipitate polysaccharides and proteins. After the precipitate was removed, the solution was centrifuged again (L530; Xiang Yi Centrifuge Instrument Co., Ltd., Changsha, China), and the supernatant was then purified using a macroporous resin that was eluted with distilled water. The obtained solution was concentrated and lyophilized (model FD-4C-80; Beijing Fuyikang

Instrument Company, Beijing, China) to obtain oligosaccharides.

2.3. Quantitative analysis of oligosaccharides from purple sweet potatoes

Oligosaccharides obtained in section 2.2 was separated on an Akta Explorer (GE Healthcare, Uppsala, Sweden) equipped with a size exclusion Bio-Gel P2 column (0.6 × 110 cm, <45 μm filler grain size, BioRad). They were then eluted with deionized water at ambient temperature (12 mL/h flow rate), and the eluate was collected (1 mL/tube) by an automatic fraction collector. All of the constituents were identified and monitored through the phenol–sulfuric acid method. A separation curve was used to combine eluates of similarly patterned oligosaccharides; the eluates were then freeze-dried to powder for further experiments. The examination results indicate that oligosaccharides of various polymerization degrees could be completely separated in the Bio-Gel P2 column. The linear range of quantitative analysis was 0.8375–15 mg/mL. Highly efficient cation exchange chromatography using a refractive index detector (Agilent Technologies, Palo Alto, CA, USA) was performed for the quantitative analysis of oligosaccharides. D-arabinose (Sigma-Aldrich, St. Louis, MO, USA) was added to the test liquid sample as an internal standard.

The process was as follows: (1) Oligosaccharides acquired from section 2.2 were diluted with deionized water to a volume of 100 mL. (2) The test liquid (1 mL) was filtered through a 0.22 μm membrane (Millipore Corporation, Bedford, MA, USA) and infused into an Agilent 1200 series rapid-resolution LC system for quantitative analysis (Fig. 1). The chromatography parameters comprised 20 μL sample volume, an Agilent Hi-plex Na(Octo) column (300 × 7.7 mm, 8 μm filler grain size) as chromatographic column, distilled water as mobile phase, 0.6 mL/min flow rate, and 85°C column temperature.

The mass of oligosaccharides (m_{LOS}) was calculated using Eq. (1):

$$m_{LOS} = \sum_{i=2}^{5} m_i \quad (1)$$

where m_i is the mass of oligosaccharides with polymerization degree of i (g)

2.4. Assessment of the in vitro prebiotic effect of oligosaccharides

Experiments on the prebiotic effect of the oligosaccharides on *B. adolescentis* spp. were carried in batch cultures. Tubes containing the nutrient base medium were supplemented with oligosaccharides, inoculated with *B. adolescentis* spp., and incubated at 37 °C for 48 h under anaerobic conditions. For comparative purposes, additional experiments were performed with media containing glucose (positive control). The quantification of bifidobacteria was performed by UV–vis spectroscopy at 600 nm, and the prebiotic effect of oligosaccharides on bifidobacteria was determined from the optical density (OD). All assays were done in triplicate.

2.5. Experimental design of RSM

Center-combined rotating-response surface design with three independent variables (X_1, extraction time; X_2, ultrasonic power; X_3, microwave power) at three levels (Table 1) was used to explore and optimize the effect of independent variables on the yield of oligosaccharides. The yield (Y_{ij}) of the jth test sample of oligosaccharides was calculated through Eq. (2):

$$Y_{ij}(\%)=\frac{C_i \times n \times V}{W_j}\times 100 \quad (2)$$

where C_i is the constituent concentration of oligosaccharides with i degree of polymerization for the jth test in the sample solution (g/mL), n is the dilution factor of the test sample solution, V is the volume of the test sample solution for the jth test (mL), and W_j is the weight of the potato powder sample used for the jth test (g).

This complete design consisted of 15 experimental points including 12 factorial points, 2 axial points, and 3 center points, and the experiment was carried out in random order. Multiple regressions was used in fitting the second-order polynomial to explain the mathematical relationship between the tests' statistics and process variables, such as the responses of X_1, X_2, and X_3 to Y. The second-order polynomial equation is expressed as

Eq. (3):

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_{jj} x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} x_i x_j + e_i \quad (3)$$

where Y is the response variable; x_i and x_j ($1 < i, j < k$) are the independent coded variables; β_0, β_j, β_{jj}, and β_{ij} are the regression coefficients for the intercept, linearity, square, and interaction, respectively; k is the number of independent parameters; and e_i is the error term.

The response variable (Y) in this study denotes the yield of oligosaccharides (%). Eq. (4) was used:

$$Y(\%) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (4)$$

The statistical significance of the terms in the regression equations was examined by analysis of variance (ANOVA) for every response model.

The adequacy of the model accounting for R^2 and $R_{\mathrm{Adj}}{}^2$ and the absolute deviation *PRESS* was checked using Eqs. (5)–(7) (Montgomery & Myers, 1995):

$$R^2 = 1 - \frac{SS_{Residual}}{SS_{Residual} + SS_{Model}} \quad (5)$$

$$R_{Adj}^2 = 1 - \frac{SS_{Residual} / DF_{Residual}}{(SS_{Residual} + SS_{Model}) / (DF_{Residual} + DF_{Model})} \quad (6)$$

$$PRESS = \sqrt{\sum_{i=1}^{N} (y_{Pred,i} - y_{Exp,i})^2} \quad (7)$$

The degree of precision was calculated as the deviation of the predicted value of the test points relative to the predicted average value. It was defined by Eqs. (8) and (9):

$$\overline{V}(\overline{y}) = \frac{1}{n} \sum_{i=1}^{N} V(\overline{y}) = \frac{P\sigma^2}{n} \quad (8)$$

$$Adequate \quad precision = \frac{Max(\overline{y}) - min(\overline{y})}{\sqrt{\overline{V}(\overline{y})}} \quad (9)$$

In Eqs. (5)–(9), *SS* is the quadratic sum, *DF* is the degrees of freedom of the model,

$y_{exp,i}$ is the response value of the tests, $y_{Pred,i}$ is the predicted response value, $\bar{y}$ is the predicted value, P is the number of the parameters of the model, σ^2 is the residual error of the square mean sum acquired from ANOVA, and n is the number of tests.

2.6 Extraction of sweet potato oligosaccharides by other methods

Sweet potato oligosaccharides were also extracted using hot-water extraction (HWE), microwave-assisted extraction (MAE), and ultrasound assisted extraction (UAE) methods based on the optimized UMAE conditions in section 2.2. Extractions were conducted in triplicate.

2.7 Morphological analysis

The shape and surface characteristics of the samples after extraction (HWE, MAE, UAE, and UMAE) were measured by scanning electron microscopy (SEM) using a Nova NanoSEM 230 (FEI Electron Optics BV, Czech Republic). Each sample was observed at an accelerating potential of 20 kV under high vacuum.

2.8 Statistical analysis

Statistical analysis was performed using the software Design-Expert® 8.0.6 (Stat-Ease Inc., Minneapolis, MN, USA). All experiments were performed in triplicate, and the results obtained were expressed as mean ± SD. The data were analyzed by ANOVA ($p < 0.05$), and the averages were separated by Duncan's multiple-range tests.

3 Results and Discussion

3.1 Effects of single factors on the yield of oligosaccharides

The effects of ultrasonic power, microwave power, and extraction time on the yields of prebiotic oligosaccharides from sweet potatoes (PPOS4 and PPOS5) are depicted in Fig. 2. Final temperature of samples extracted by ultrasound at 100-1500 W was increased from 32.2 to 61.1℃, and that of samples extracted by microwave at 100-300 W was increased from 46.5 to 121.1℃. The yield of PPOS4 increased with increasing ultrasonic power, microwave power, and extraction time in the early stage, and then decreased with

further increases in power and time. The tendency for cavitation increased with the increase in ultrasonic power, thus aiding the release of PPOS4 from the sweet potato (Santos & Capelo, 2007). Microwave power provided localized heating in the plant cells, and it acted as a driving force that disrupts the plant matrix; thus, PPOS4 could diffuse out and dissolve in the solvent (Yan, Liu, Fu, Zu, Chen, & Luo, 2010). However, excessive microwave or ultrasonic power may cause the degradation of the oligosaccharides (Mandal & Mandal, 2010), which leads to a decline in oligosaccharide yield. In contrast to the yield of PPOS4, that of PPOS5 decreased with increasing ultrasonic power, microwave power, and extraction time in this study. PPOS5 could separate more easily from the sweet potato matrix than PPOS4 could. On the other hand, the stability of PPOS5 might be poor; excessive ultrasonic power, microwave power, or extraction time might have thus led to the degradation. According to the above single-factor analysis, the extraction conditions for the optimal yield of PPOS4 and PPOS5 were as follows: 300 W ultrasonic power, 175 W microwave power, and 70 s extraction time.

3.2. Optimization of oligosaccharide extraction by RSM

3.2.1 Relevancy analysis

The Pearson coefficient of extraction factors and the yield of oligosaccharides are shown in Table 2. The Pearson coefficient of each of the three extraction factors was 0, indicating that the extraction factors are mutually independent without any relevancy. The Pearson coefficient for ultrasonic power, the PPOS4 and PPOS5 yields, and OD were negative, suggesting a negative correlation between the yield and extraction factors, but it was not significant ($p > 0.05$). The yield of PPOS5 had a significant negative correlation with each extraction factor ($p < 0.01$). However, the PPOS4 yields and OD were positively correlated with the microwave power and extraction time (not significant, $p > 0.05$). The yields of PPOS4 and PPOS5 were positively correlated with the OD,

indicating that the sweet potato oligosaccharides benefit the growth of *B. adolescentis*.

3.2.2 Analysis of disturbing factors

All of the factors in the response curve were consolidated to investigate the disturbing effect of factors on the response value. A higher slope of the single-factor curve represents a more sensitive response value of such factors on the response value (Sayyad, Panda, Javed, & Ali, 2007). During plotting, a single factor in the test range was changed, and the other factors were fixed to examine the deviation of the response value from the datum point. As shown in Fig. 3, the ultrasonic power and microwave power were more sensitive to the PPOS4 yield and OD than was the extraction time; however, extraction time was more sensitive to the yield of PPOS5 than were the other factors. These results indicate that ultrasonic power and microwave power adversely affect the extraction of PPOS5.

3.2.3 Statistical analysis and modeling of extraction of oligosaccharides

The process variables and experimental data for the PPOS4 and PPOS5 yields and OD under different treatment conditions are presented in Table 3. The results of ANOVA, as well as the adequacy and fitness of the models, are summarized in Table 4. According to the model, the effects of all terms on the yield of PPOS4 were statistically significant ($p < 0.05$). For the model for the PPOS5 yield, X_2X_3 and X_2^2 (X_1X_2 excluded) were not significant ($p > 0.05$), and all other terms were statistically significant ($p < 0.05$). For the regression model of the OD, the interaction factors X_1^2 and X_3^2 were insignificant ($p > 0.05$), and the effect of the other terms on the OD was significant ($p < 0.05$).

A significant lack of fit ($p < 0.05$) indicates that the models failed to represent the data in the experimental domain, the points of which were not included in the regression. As shown in Table 4, lack of fit of the three models was not significant ($p > 0.05$), indicating that the model represented the data satisfactorily. The determination coefficient (R^2), modified coefficient of association (R_{Adj}^2), predicted modified coefficient of

association (R_{Pred}^2), and coefficient of variation (CV) were calculated to check the model's adequacy (Table 5).

R^2 of the three models was >0.98, indicating that the test value and predicted value were highly correlated. However, a large value of R^2 did not always imply that the regression model is a good one. R^2 could also increase upon addition of a variable to the model, regardless of whether the additional variable is statistically significant. Thus, R_{Adj}^2 was utilized in parallel to assess the adequacy of the model. If the number of terms or sample capacity was not large enough, then R_{Adj}^2 would be smaller than R^2 (Liu, Lan, & Cheng, 2004). As can be seen in Table 5, R_{Adj}^2 of the three models is slightly smaller than R^2, and the discrepancy between R_{Pred}^2 and R_{Adj}^2 is ≤0.12, suggesting that they are in the reasonable range of fluctuation (Maran, Sivakumar, Sridhar, & Thirugnanasambandham, 2013).

A low CV (<10) for the model indicates that the experimental values are associated with a very high degree of precision and good reliability (Myer & Montgomery, 2002). As shown in Table 5, the CV of the three models is below 5, indicating that the model explains the response adequately. The value of *PRESS* of the three models is <1.5, which also suggests that every point in the trial design could fit the quadratic model rather satisfactorily. The adequacy of precision (*Adeq Precision*) represents the signal-to-noise ratio; a ratio of >4 is desirable (Afshari, et al., 2015; Myer,et al., 2002). Herein, *Adeq Precision* of the three models is >25, which represents the entire extraction process of oligosaccharides. Therefore, the regression equation can be used to describe an actual relationship between every factor and the yield of oligosaccharides and to ascertain the optimum conditions for the extraction procedure.

From the above analysis, the fitted model for the yield (*Y*), which was used to predict the relationships between the independent variables and dependent variables, can be expressed as follows:

$$Y_1\ (\%) = 1.29 + 0.042x_1 + 0.062x_2 - 0.11x_3 + 0.034x_1x_2 - 0.086x_1x_3 - 0.066x_2x_3 - 0.051x_1^2 - 0.13x_2^2 - 0.061x_3^2 \quad (10)$$

$$Y_2\ (\%) = 3.33 - 0.83x_1 - 0.33x_2 - 1.17x_3 - 0.09x_1x_2 - 0.54x_1x_3 + 0.07x_2x_3 + 0.25x_1^2 + 0.36x_2^2 + 0.54x_3^2$$

$$Y_3\ (\%) = 2.3 + 0.15x_1 + 0.23x_2 - 0.14x_3 + 0.20x_1x_2 - 0.24x_1x_3 + 0.05x_2x_3 - 0.019x_1^2 - 0.15x_2^2 - 9.243*10^{-3}*x_3^2$$

where Y_1 (%) and Y_2 (%) represent the yields of PPOS4 and PPOS5, respectively. Y_3 represents OD, x_1 is the extraction time (s), x_2 is the ultrasonic power (W), and x_3 is the microwave power (W)

3.2.4. Analysis of response surface and two-dimensional contour plots

3.2.4.1. Extraction time

As described by Eq. (10), the one-degree terms of extraction time for the yield of PPOS4 are positive, but the quadratic terms are negative in the test's value range. Since the mutual interactions between the extraction time/ultrasonic power ratio and the microwave power were significant, the yield of PPOS4 gradually increased with extraction time, which is evident in the shrinking gap between the contour lines and the elliptical shape of the two-dimensional contour plots. Figures 4A and 5A illustrate that when the ultrasonic power, microwave power, and extraction time reached 450 W, 175 W, and 80–100 s, respectively, the PPOS4 yield was maximal. Because of the significant positive interaction between the extraction time, ultrasonic power, and microwave power, the yield of PPOS4 decreased with the increase in power and time. The contour lines sloped sharply toward the high and low levels of the two factors, and the two-dimensional contour plots became elliptical (Figs. 4A, 4B, 5A, and 5B). As can be observed in Figs. 4A, 4B, 5A, and 5B, we obtained a maximum PPOS4 yield when the microwave power was 175 W, ultrasonic power was 300–420 W, and extraction time was 80–100 s; or when ultrasonic power was 450 W, microwave power was 160–220 W, and extraction time was

80–100 s. The yield of oligosaccharides monotonically increased when the ultrasonic power and microwave power were constant. Similar results can be observed for the effect of extraction time on the OD (Figs. 4E, 4F, 5E, and 5F). This result may be explained by the proliferative effect of PPOS4 on *B. adolescentis*. For the yield of PPOS5, however, only the mutual interactions with the extraction time/microwave power ratio was significant. Both one-degree and quadratic terms of extraction time for the yield of PPOS5 were negative in this study. We obtained a maximum PPOS5 yield when the microwave power was 175 W, ultrasonic power was below 300 W, and extraction time was 90–100 s.

Extraction time was a vital factor that affected the yield of PPOS4 and PPOS5, and it indirectly influenced the OD. As shown in Figs. 4 and 5, the yield of oligosaccharides rapidly increased with extraction time in the initial stage. A plausible explanation of this phenomenon is that the solvent absorbed the microwave energy in the initial stage of extraction, raising the solvent temperature. This change accelerated the dissolution of oligosaccharides in the plant cells and facilitated their entry into the solvent. With longer extraction time, higher temperature could accelerate the molecular movement and change the electroconductivity of the extraction solvent and plant (Yang, Cao, Jiang, Lin, Chen, & Zhu, 2010; Milić, Rajković, Stamenković, & Veljković, 2013). On the other hand, the solvent temperature enhanced the cavitation effect of ultrasound and promoted the formation of cavitation nuclei, resulting in a large burst on the surface of the plant cells, thus facilitating permeation of the solvent into the plant cells (Toma, Vinatoru, Paniwnyk, & Mason, 2001). These two effects accelerated the diffusion of oligosaccharides in the extraction solvent, leading to a maximum yield of oligosaccharides. However, the yield of oligosaccharides was in dynamic equilibrium in the final stage of extraction, which may indicate the complete extraction of oligosaccharides from the plant cells (XuJie, Na, SuYing, ShuGang, & BaoQiu, 2008).

3.2.4.2. Ultrasonic power

As described in Table 4 and Eq. (10), the mutual interaction between the ultrasonic power and microwave power was significant for the oligosaccharide yield, and its terms were negative. This led to shrinkage of the response curve surface toward the high and low levels of both factors and its elliptical shape (Figs. 4C and 5C). We could obtain a maximum yield of oligosaccharides when the ultrasonic power was in the range of 300–420 W, microwave power was 160–250 W, and the extraction time was 175 s. Similar results can be observed for the effect of extraction time on OD (Figs. 4G and 5G). We could obtain a maximum yield of OD when the ultrasonic power was 300–350 W, the microwave power was 160–250 W, and the extraction time was at 175 s.

Sonication is widely used for the extraction of various substances from plant materials, and it generates a cavitation effect (Quan, Sun, & Qu, 2009; Şahin & Şamlı, 2013). The tendency for cavitation depends on ultrasonic properties, product characteristics, and ambient conditions (Santos, et al., 2007). It increased with the increase in ultrasonic power, and it produced numerous microbubbles. These microbubbles could burst on the surface of plant cells and generate localized heat and pressure (Knorr, Ade-Omowaye, & Heinz, 2002), which destroy the plant cell wall and thereby accelerate the release of components from the cells into the solvent (Zhang, & Liu, 2008). Meanwhile, the increasing ultrasonic power could also promote the penetration of the solvent into the plant matrix, thus raising the yield (Pan, Qu, Ma, Atungulu, & McHugh, 2011). Nevertheless, the numerous microbubbles created by ultrasonic power boosts could also hinder the release of components from the cells, thus impeding the increase in yield of oligosaccharides during extended extraction (Vilkhu, Mawson, Simons, & Bates, 2008).

3.2.4.3. Microwave power

As described above, the mutual interactions between microwave power, ultrasonic

power, and extraction time were significant for the oligosaccharide yield. The extraction efficiency of the oligosaccharides was improved by raising the microwave power from 160 to 250 W (Figs. 4C and 5C). This may be related to the ionic conduction and dipolar rotation effects of microwave energy on the plant materials. The extraction solvent, water, is a polar solvent, which could efficiently absorb microwave energy and lead to efficient heating (Yan, et al., 2010; Abdolmaleki, Mallakpour & Azimi, 2018). The increase in microwave power can enhance the penetration of solvent into the plant matrix and deliver materials efficiently through molecular interaction with the electromagnetic field. It also allows rapid transfer of energy to the solvent and matrix, allowing the dissolved components to be extracted. Moreover, microwave accelerates cell rupture by the sudden rise in temperature and internal pressure inside the cells of the plant matrix, which promotes the destruction of the cell wall matrix and epidermal tissue, and, in turn, the exudation of oligosaccharides within the plant cells into the surrounding solvent (Kratchanova, Pavlova, & Panchev, 2004; Zhang, Yang, & Liu, 2008). However, excessive microwave power hardly affected the interaction among microwave power, extraction solvent, and the sample (Alfaro, Bélanger, Padilla, & Jocelyn Paré, 2003). A higher microwave power could lead to thermal degradation, polymerization, and oxidation of the target compound and thus a gradual decrease in yield (Mandal et al, 2010).

3.2.5. Optimization of extraction parameters and validation of the optimized conditions

The objective of optimization was to determine the UMAE conditions that give the maximum extraction yields for PPOS4 and PPOS5 and corresponding OD. A multi-response approach was applied in the optimization process. This approach were previously developed for every variable between prediction and response (Lu, et al., 2017; Smith, 2005). In order to determine the optimum operating conditions, extraction process parameters within a range (*A*, *B*, and *C*) were selected, and the response *Y* was set as the

maximum. According to the prediction made through optimum theory and software analysis, the optimal extraction conditions and the maximum yields of PPOS4 and PPOS5 and the corresponding OD was as follows: 100 s extraction time, 300.001 W ultrasonic power, and 219.847 W microwave power (Fig. 6). Under these optimal extraction conditions, the theoretically predicted yield of PPOS4 and PPOS5 and the corresponding OD were maintained at 1.472%, 5.476%, and 2.966, respectively. Considering the actual operating conditions, we modified the optimum conditions as follows: 100 s extraction time, 300 W ultrasonic power, and 200 W microwave power.

To validate the predictability of the established model, the optimized parameters were tested in an additional experiment. The average experimental yields of PPOS4 and PPOS5 and the corresponding OD were 1.472%, 5.476%, and 2.966, respectively, which are very close to the predicted theoretical values ($p > 0.05$). Thus, we can see that the response regression model adequately describes the effect of the selected UMAE operating variables on the extraction yields of oligosaccharides.

3.3. Comparison of UMAE with other extraction methods

The quantities of PPOS4 and PPOS5 extracted from purple sweet potatoes using HWE, MAE, and UAE are presented in Table 6. The results indicate that UMAE at the same liquid to material ratio showed significantly high efficiency and shorter time of extraction of PPOS4 and PPOS5 as compared with HWE, MAE, and UAE ($p < 0.05$), proving the great potential of this hybrid technique for energy conservation and efficient (Chemat, Rombaut, Anne-Gaëlle Sicaire, Meullemiestre, Fabiano-Tixier, & Abert-Vian, 2017). Additionally, the MAE process was slightly better than HWE and UAE, probably because of the ionic conduction and water dipole rotation effects of microwave heating (Prakash Maran, et al., 2014). But it may be higher than other extraction methods in terms of energy consumption, from the maximum extraction values, we confirmed that UMAE is an appropriate and effective technique for oligosaccharide extraction from purple sweet

potatoes.

3.4. Morphological analysis

The extraction efficiency was related to physical changes in the cell wall of the plant tissue (Ying, Han, & Li, 2011). The microstructures of purple sweet potato tissue after extraction were observed by SEM. As shown in Fig. 7, the extraction methods significantly affected the physical changes in the potato tissue. The cell wall of the potato tissue (Fig. 7A) was almost intact after HWE; this may be attributed to the slow diffusion of heated solvent through the cell walls of tissues, as well as the subsequent dissolution and washout of targeted compounds. Compared with cell walls subjected to HWE, those treated by UAE, MAE, or UMAE (Fig. 7B–7D) were drastically damaged. The level of cell damage increased in the following order: UAE < MAE < UMAE. Most of the cell walls after UMAE treatment appeared completely disrupted and collapsed as compared with those subjected to UAE or MAE. This difference is attributed to the impact and cavitation due to intense shaking by ultrasound coupled with the heating and expansion due to microwaves (Zhang, Wang, Li, Jiao, Chen, & Mao, 2008). The large instantaneous energy generated by the ultrasound system led to quick dissolution of the oligosaccharides from plant cells into the solvent without a permeation process. On the other hand, water molecules could efficiently absorb microwave energy and lead to efficient heating of the sample (Yan, et al., 2010). Thus, solvent penetration into the inner tissues and a drastic expansion and subsequent severe rupture of the cell walls allowed the release of the compounds into the solvent during UMAE, in meantime to accelerate the release of valuable metabolites, resulting in better yields of prebiotic oligosaccharides (Khadhraoui, Turk, Fabiano-Tixier, Petitcolas, Robinet, & Imbert, et al, 2018). These results are consistent with those of previous studies (Ying, et al., 2011). SEM provided strong evidence of the high efficiencies of oligosaccharide extraction of UMAE.

Ultrasound–microwave-assisted extraction is a new process technology that

combines the ultrasonic and microwave methods. Microwave heats the whole sample very quickly inducing the migration of dissolved molecules. The simultaneous ultrasonic enhance mass transfer, and increase the yield of the target product (Both, Chemat, & Strube, 2014). It is a cost-effective technique for extraction of prebiotic oligosaccharides and a new strategy for process intensification, enhancing competition of industries to be more ecologic, economic and innovative.

4. Conclusions

In the present study, the conditions for enhanced extraction of prebiotic oligosaccharides from purple sweet potatoes by UMAE were optimized with a three-factor three-level center-combined rotating response surface design. The results show that the processing conditions for UMAE for optimum PPOS4 and PPOS5 yields and corresponding OD are 100 s extraction time, 300 W ultrasonic power, and 200 W microwave power. The yields of PPOS4 and PPOS5 and corresponding OD under such conditions were 1.472%, 5.476%, and 2.966, respectively. Compared with the conventional HWE, MAE, and UAE methods, UMAE exhibited significantly high extraction efficiency. Comparison of SEM images of potato tissues after extractions indicates that UMAE is an efficient extraction method. In addition, the relationship between different extraction conditions and the yield of oligosaccharides, and the relationship between the effect of different oligosaccharides on the bacterial growth of *bifidobacteria* can be found when the response surface method is applied, which is not used in other extraction processes； And analytical techniques can be used directly to avoid sample treatment, derivatization, simultaneous multi-analyte and multi-parameter methods, avoiding toxic reagents. This technique shows simple and fast operation, which conforms to GAC's green chemical principles (Gałuszka A, et al., 2013). These results indicate that UMAE is a rapid, safe, and ecofriendly emerging extraction technology that is highly suitable for applications in the food and medicinal industries.

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Figures and tables captions

Figure 1 High performance liquid chromatography of standard substance (A) and PPOS(B)

Figure 2 Effects of single factor on the extraction yield of PPOS4 and PPOS5

Figure 3 Perturbation plot showing the effect of process variables

Figure 4 Tri-dimensional response surface contour plots showing the experimental factors and their mutual interactions on the yield of oligosaccharides

Figure 5 Two-dimensional contour plots showing the experimental factors and their mutual interactions on the yield of oligosaccharides

Figure 6 Superimposed contour plots for the yield of total oligosaccharides with ultrasonic-microwave assisted extraction process

Figure 7 The picture of sample (after different extractions) by environmental scanning electron microscopy (A：HAE B：MAE C：UAE D：UMAE).

Figure 8 The picture of the steps of procedures

Table 1 Independent variable and level in center-assembled rotating design of response surface

Table 2 Analysis of relevancy between yield of oligosaccharides and extraction factors

Table 3 Results of Response Surface Center Combination Design (not coded)

Table 4 Variance analysis of yield of oligosaccharides

Table 5 Variance analysis of fitted model

Table 6 The yield of PPOS4, PPOS5 extracted from purple sweet potatoes (*Ipomoea batatas L.*) by different methods

Figure 1

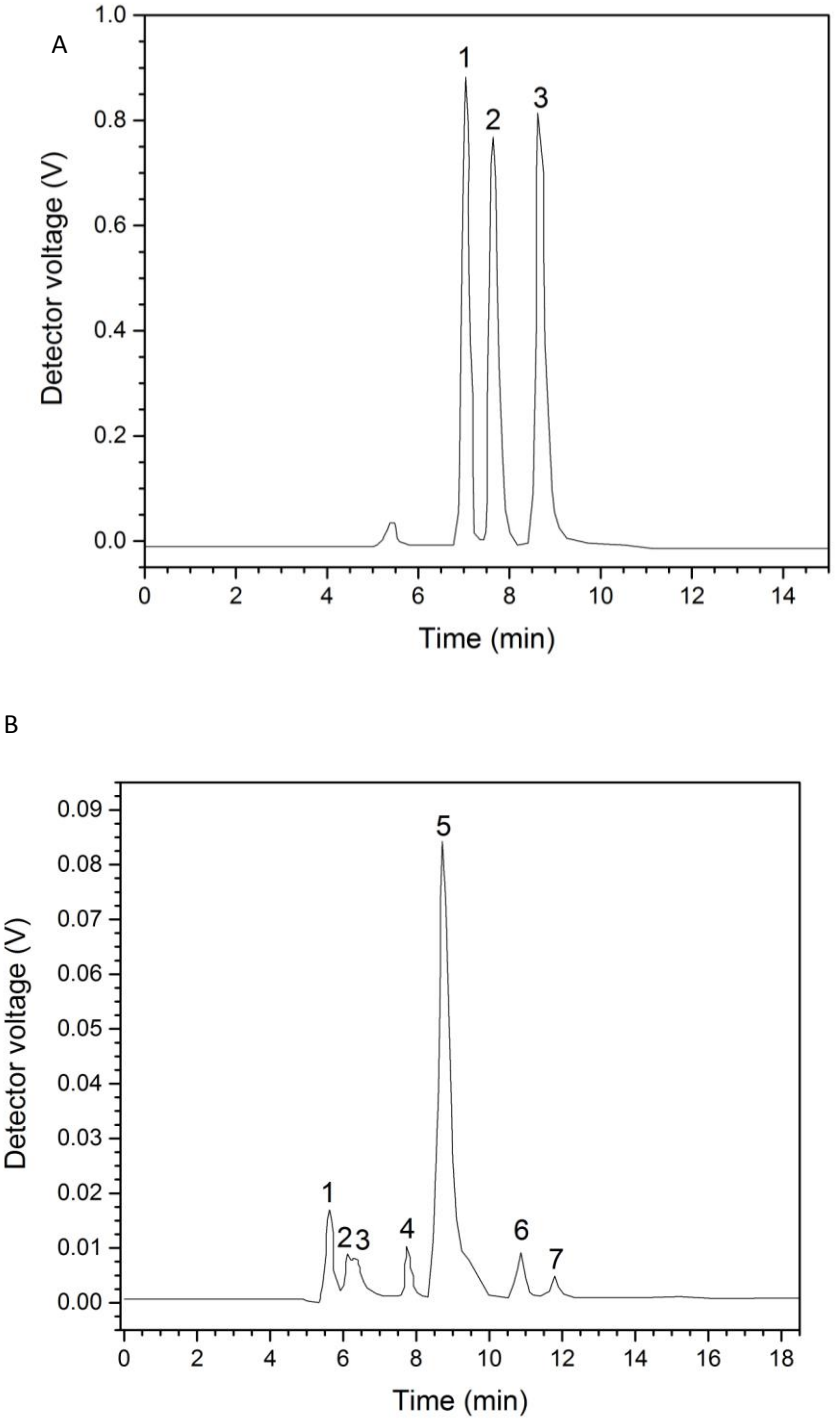
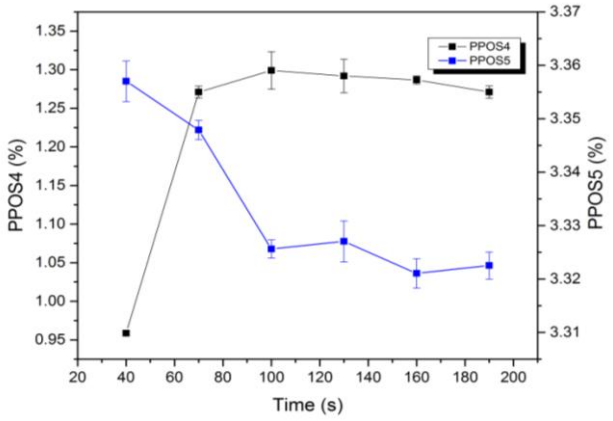
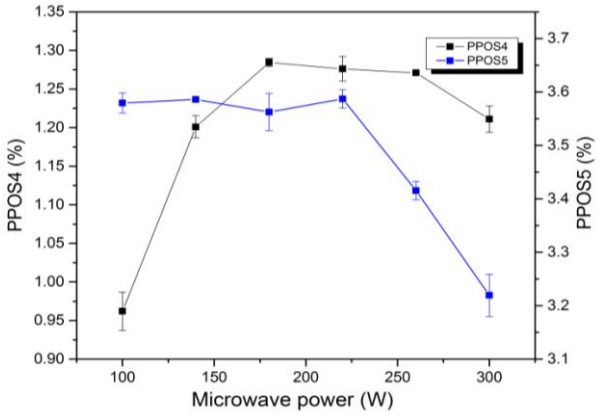


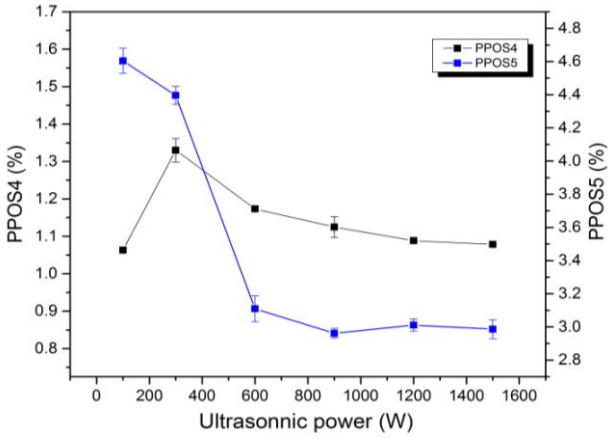
Figure 2



A



B



C

Figure 3

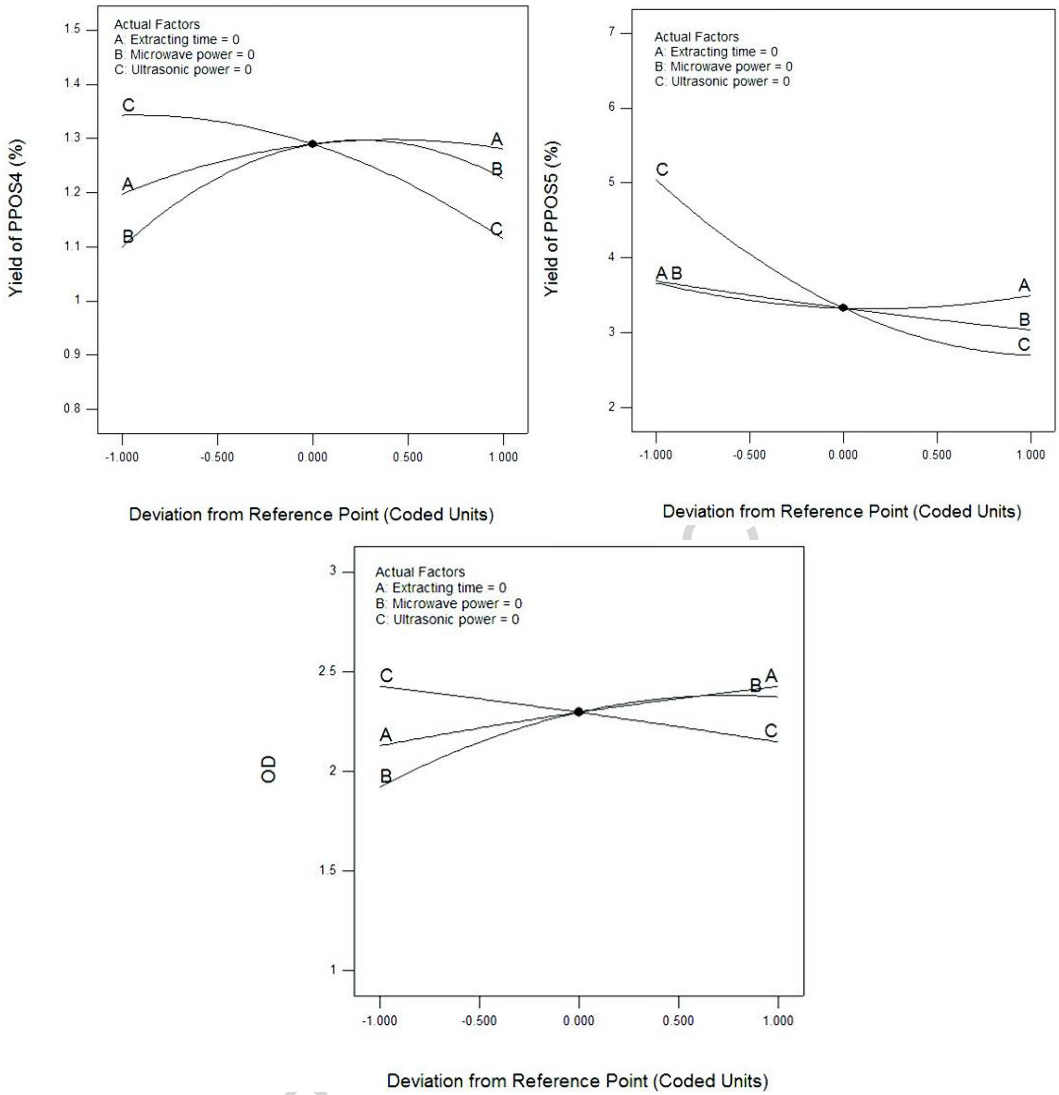


Figure 4

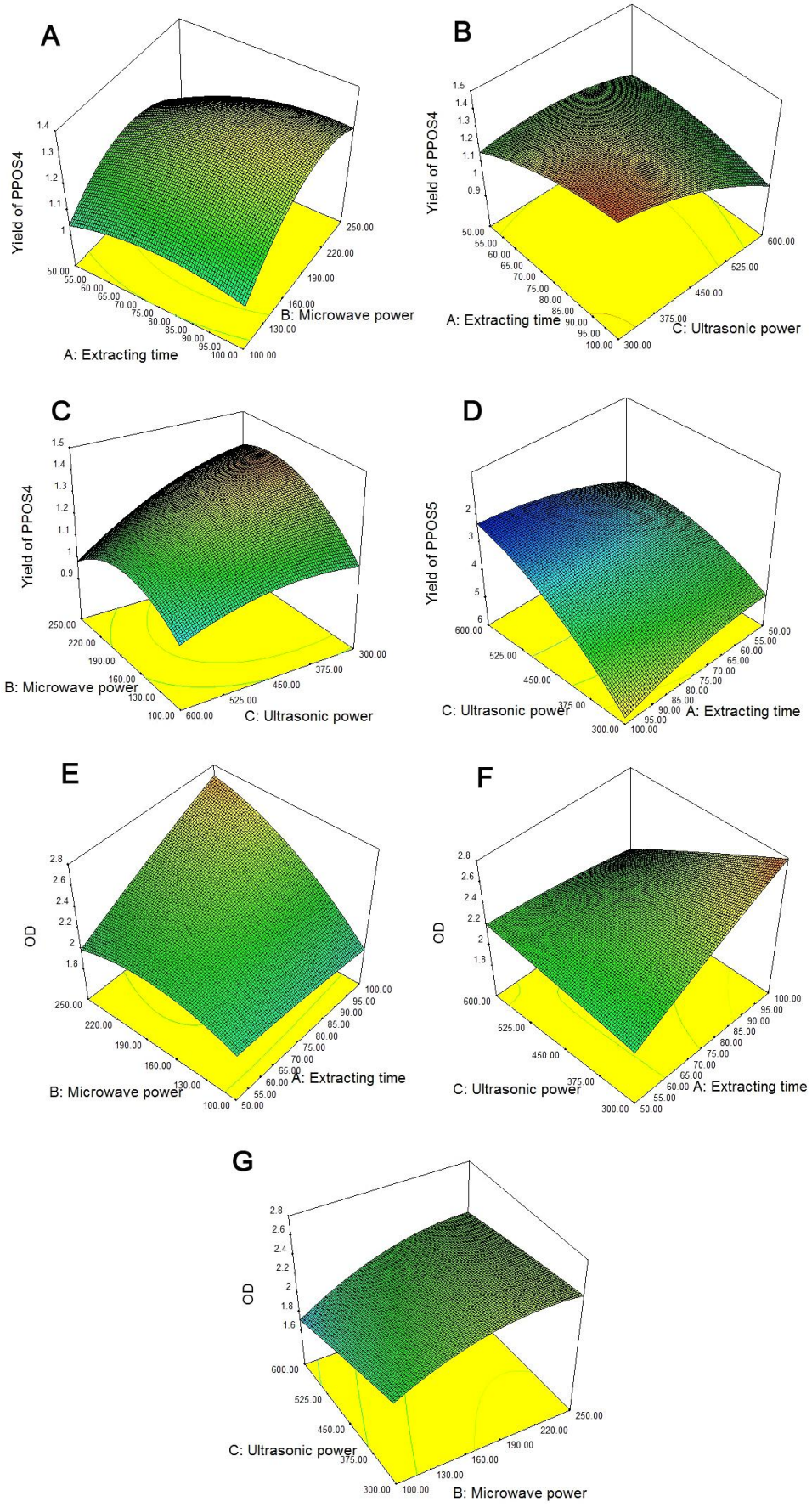


Figure 5

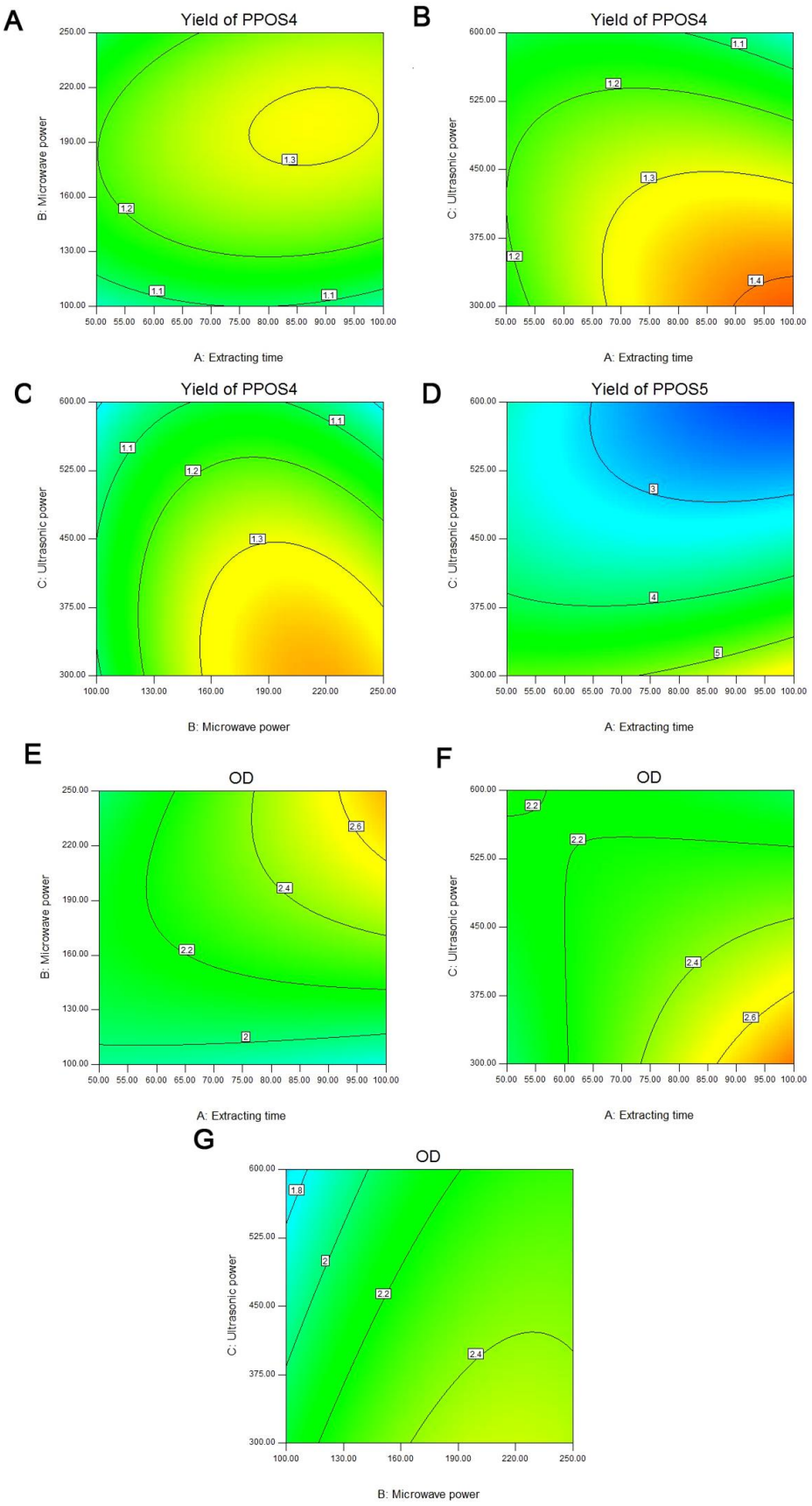


Figure 6

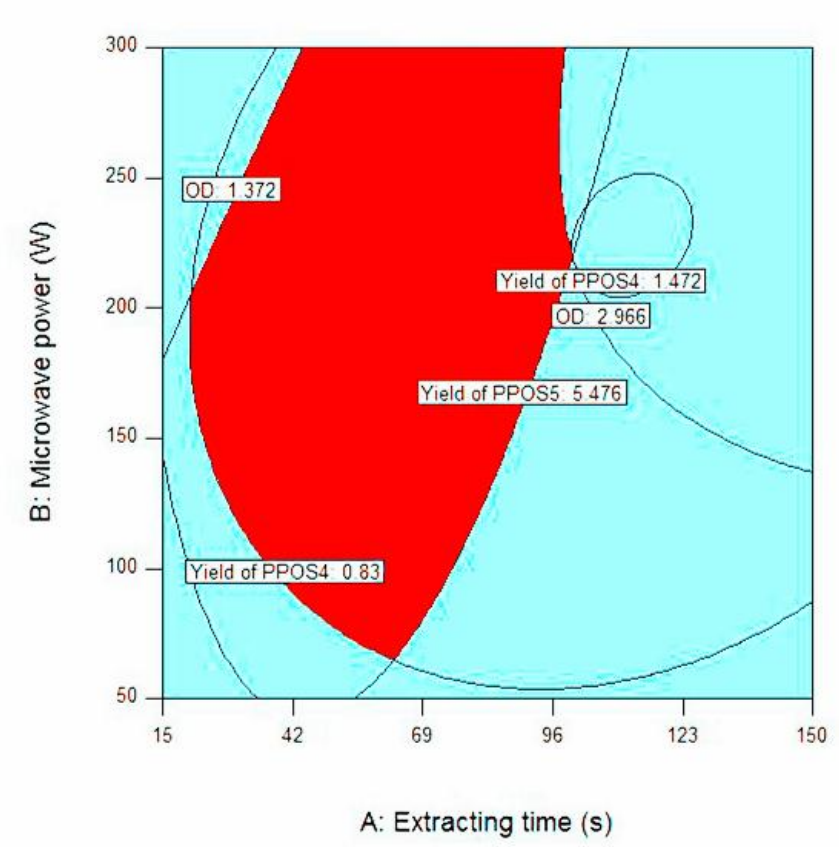
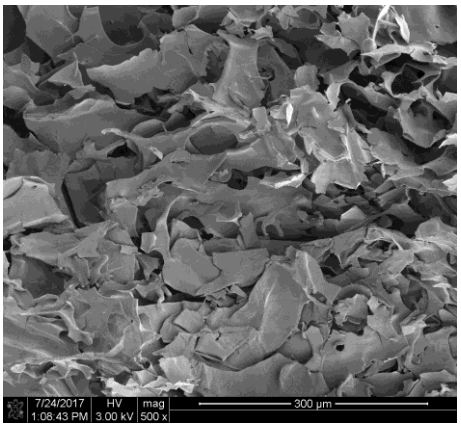
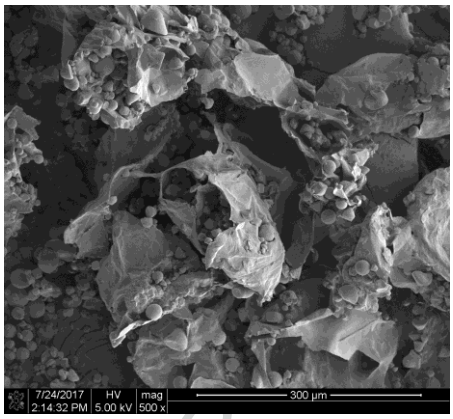


Figure 7



A



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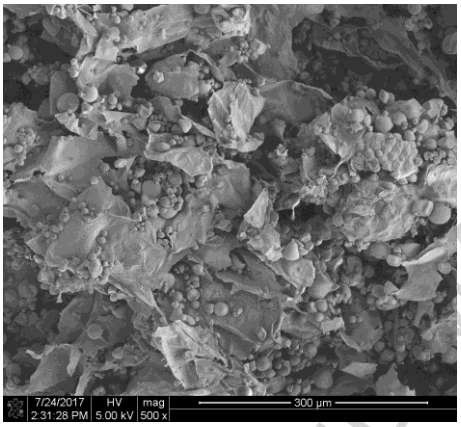

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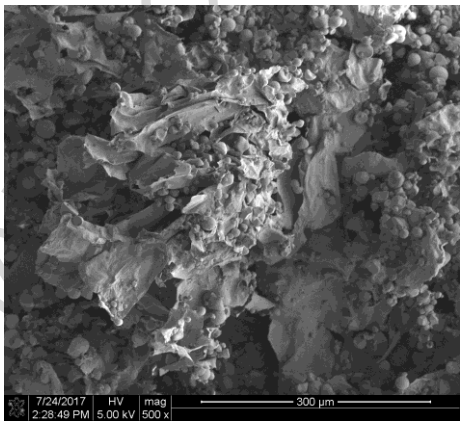

D

Figure 8

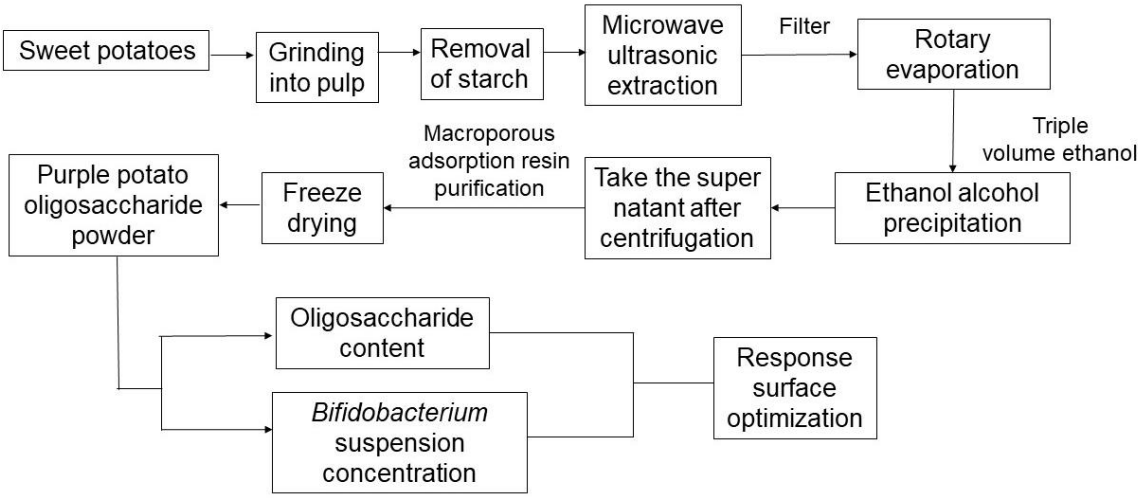

Table 1

Independent variable	Symbol	Coded factor		
		-1	0	1
Extracting time	X_1 (s)	50	75	100
Microwave power	X_2 (W)	100	175	250
Ultrasonic power	X_3 (W)	300	450	600

Table 2

	A:Extracting time	B:Ultrasonic power	C:Microwave power	Yield of PPOS4	Yield of PPOS5	OD
A:Extracting time	1.000	0.000	0.000	0.190	-0.058	0.351
B:Ultrasonic power		1.000	0.000	0.285	-0.229	0.537
C:Microwave power			1.000	-0.524	-0.822	-0.332
Yield of PPOS4				1.000	0.292	0.763
Yield of PPOS5					1.000	0.235
OD						1.000

Table 3

Number	X_1 (s)	X_2 (W)	X_3 (W)	Yield of PPOS4		Yield of PPOS4		OD	
				Test Value	Predicted Value	Test Value	Predicted Value	Test Value	Predicted Value
1	50	100	300	0.95±0.02	0.936	5.12±0.14	5.179	1.89±0.01	1.892
2	100	100	300	1.1±0.03	1.124	6.41±0.07	6.273	2.23±0.05	2.272
3	50	250	300	1.11±0.03	1.124	4.63±0.01	4.559	1.89±0.04	1.852
4	100	250	300	1.48±0.01	1.448	5.13±0.13	5.293	2.98±0.07	3.032
5	50	100	600	1.01±0.02	1.020	3.93±0.02	3.779	2.04±0.02	1.992
6	100	100	600	0.9±0.01	0.864	2.62±0.07	2.713	1.37±0.01	1.412
7	50	250	600	0.99±0.02	0.944	3.29±0.01	3.439	2.18±0.04	2.152
8	100	250	600	0.93±0.04	0.924	2.05±0.04	2.013	2.39±0.03	2.372
9	32.955	175	450	1.07±0.02	1.075	4.17±0.01	4.177	1.92±0.04	1.994
10	117.045	175	450	1.23±0.03	1.216	3.95±0.09	3.898	2.55±0.06	2.499
11	75	48.866	450	0.83±0.02	0.818	3.89±0.04	3.987	1.51±0.03	1.489
12	75	301.134	450	1.01±0.09	1.027	3.01±0.08	2.877	2.21±0.01	2.263
13	75	175	197.731	1.33±0.02	1.302	6.84±0.13	6.825	2.53±0.03	2.509
14	75	175	702.269	0.88±0.01	0.932	2.92±0.04	2.890	1.99±0.05	2.038
15	75	175	450	1.31±0.04	1.290	3.29±0.09	3.330	2.25±0.03	2.300
16	75	175	450	1.26±0.02	1.290	3.38±0.04	3.330	2.34±0.01	2.300
17	75	175	450	1.28±0.03	1.290	3.21±0.03	3.330	2.28±0.04	2.300
18	75	175	450	1.32±0.01	1.290	3.29±0.08	3.330	2.33±0.06	2.300
19	75	175	450	1.29±0.04	1.290	3.42±0.08	3.330	2.35±0.06	2.300
20	75	175	450	1.28±0.02	1.290	3.38±0.07	3.330	2.25±0.04	2.300

Table 4

Source	Sum of squares			Degree of freedom	Mean square			F value			p-Value		
Model	0.64	27.56	2.40	9	0.071	3.06	0.27	61.43	179.96	78.13	< 0.0001	< 0.0001	< 0.0001
X_1	0.024	0.093	0.30	1	0.024	0.093	0.30	20.37	5.49	88.07	0.0011	0.0411	< 0.0001
X_2	0.053	1.46	0.70	1	0.053	1.46	0.70	45.80	85.59	205.67	< 0.0001	< 0.0001	< 0.0001
X_3	0.18	18.73	0.27	1	0.18	18.73	0.27	154.64	1100.54	78.50	< 0.0001	< 0.0001	< 0.0001
X_1X_2	9.113E-003	0.065	0.34	1	9.113E-003	0.065	0.34	7.84	3.81	98.32	0.0188	0.0796	< 0.0001
X_1X_3	0.060	2.35	0.45	1	0.060	2.35	0.45	51.20	138.36	130.91	< 0.0001	< 0.0001	< 0.0001
X_2X_3	0.035	0.039	0.021	1	0.035	0.039	0.021	30.21	2.30	6.04	0.0003	0.1600	0.0338
X_1^2	0.037	0.92	5.281E-003	1	0.037	0.92	5.281E-003	31.78	53.83	1.55	0.0002	< 0.0001	0.2418
X_2^2	0.23	0.019	0.33	1	0.23	0.019	0.33	198.83	1.12	95.46	< 0.0001	0.3138	< 0.0001
X_3^2	0.054	4.23	1.231E-003	1	0.054	4.23	1.231E-003	46.49	248.81	0.36	< 0.0001	< 0.0001	0.5614
Residual	0.012	0.17	0.034	10	1.162E-003	0.017	3.411E-003						
Lack of fit	9.224E-003	0.14	0.025	5	1.845E-003	0.028	5.097E-003	3.84	4.55	2.96	0.0829	0.0610	0.1297
Pure error	2.400E-003	0.031	8.624E-003	5	4.800E-004	6.137E-003	1.725E-003						
Cor total	0.65	27.73	2.43	19									

Table 5

	Yield of PPOS4	Yield of PPOS5	OD
Std. Dev.	0.034	0.13	0.058
Mean	1.13	3.90	2.17
R-Squared	0.9822	0.9939	0.9860
Adj R-Squared	0.9662	0.9883	0.9734
Pred R-Squared	0.8790	0.9550	0.9140
C. V. %	3.03	3.35	2.69
PRESS	0.079	1.25	0.21
Adeq Precision	26.122	52.198	39.115

Table 6

Extraction method	Extraction time/min	Microwave power/W	Ultrasonic power/ W	Temperature/℃	Yield of PPOS4	Yield of PPOS5
HAE	62	-	-	75	0.903	4.391
MAE	3.7	250	-	-	1.247	5.026
UAE	18	-	200	58	1.286	4.824
UMAE	1.67	220	300	-	1.472	5.476

Highlights:

- Compared with the conventional HWE, MAE, and UAE methods, UMAE exhibited significantly high extraction efficiency.
- The optimum conditions of UMAE for prebiotic oligosaccharides are 100 s extraction time, 300 W ultrasonic and 200 W microwave power.
- There were microfractures and disruption of cell walls in the purple sweet potato tissues from SEM images after UMAE procedure.