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Research Article

Effect of Activated Charcoal on Separation of Fructooligosaccharides from Yacon Root

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ABSTRACT

This study aimed to separate the fructooligosaccharides from yacon root using activated charcoal through size exclusion mechanism. The efficiency achieved by optimizing the effect of activated charcoal, water/ethanol solutions and sample (yacon root powder) in different combinations made using D-Optimal mixture design method (Design expert 6.0). From the design mixture, response parameter in this study was yield of monosaccharides and fructooligosaccharides. The best separation of FOS from yacon root when using charcoal 2.68 g, water/ethanol (90/10) ratio and elutent 70/30 ratio showed good effect. Based on optimized parameters, total FOS recovered from charcoal separation was found (15 g/100 g) in yacon root. Separated FOS from yacon root was freeze-dried; it appeared as whitish and resembled like crystalline matter. SEM analysis of FOS structure in both commercial and yacon root displayed spherical particles were adhered due to hygroscopicity in the image. From this study mixture of sugar was easily separated using activated charcoal that acts as an alternative method from conventional method.

Key words: *Yacon Root, Fructooligosaccharides, Activated Charcoal, D-Optimal Mixture Design, SEM.*

INTRODUCTION

Yacon root was mainly grown in the region of Peru and rich in fructooligosaccharides (FOS) content[1]. Yacon otherwise named as Peruvian ground apple because their taste was like apple and texture of the tuber was resembled like sweet potato. FOS content in yacon contains generally about~ 35-55%[2]. To maintain potential health benefits daily intake of sufficient FOS was using in more than 500 food products as per FDA reports[3]. FOS popularly employed in the functional food or nutraceutical, food industry and pharmaceutical sector due to low calorie natural sweetener, possess fiber content and moreover FOS categorized under prebiotics[4]. FOS found in some source of fruits and vegetables which are considered as Prebiotics due to low degree of polymerization[5].

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Consumption of FOS modulates the function in the body such as immune system, lipid metabolism, intestinal microbiota and calcium absorption[6]. For human consumption FOS compounds were to be separated from mixture of monosaccharides and disaccharides sugars to obtain as pure product. In research studies the fructooligosaccharides separation was achieved through size exclusion chromatography, ion exchange resins, nano-filtration, activated charcoal and yeast treatments[7-13]. From aforementioned statement, activated charcoal was most effective way to separate and purify the FOS due to cheap, ease method, large surface area for absorption of compounds as like recovery of compounds by desorption. Many studies reported that separation of FOS was achieved through activated charcoal. According to Whistler and Durso[14] performed separation of oligosaccharides from honey with aid of celite using different concentration of ethanol; this study aimed to separate the FOS from yacon root using activated charcoal by optimizing the independent process parameters assigned by D-Optimal mixture design method. The obtained FOS compounds were freeze dried and structure was analyzed using SEM to know their functional properties.

MATERIAL AND METHODS

Sample

Commercial yacon root powder was obtained from Holynatural (Gujarat, India). Fructooligosaccharides standard purchased from HiMedia (Mumbai, India). GOD/POD glucose assay kit was procured from (Navasari, India). Ethanol, Whatman filter papers No. 1, activated charcoal and resorcinol from (Merck, Germany). Analytical reagent grade were used for all analysis.

Fractionation of carbohydrates from yacon using activated charcoal

The separation of fructooligosaccharides was followed by two stage process by using charcoal treatment. In first stage process separation of monosaccharide and disaccharides from yacon root, followed by separation and recovery of FOS from charcoal. This follows as:

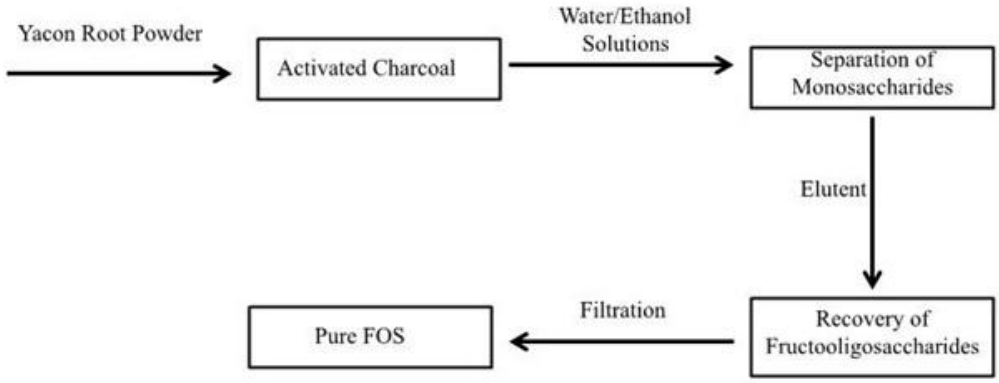


Fig. 1: Schematic process for the separation of fructooligosaccharides from yacon root

Experimental Design

D-Optimal mixture design was a multi-factor design was chosen for the design of experiments in this study. This design used for optimization for extraction and separation efficiency of FOS as response in this study. This could an alternative method when compared to conducting experiments in conventional method. The experimental conditions of independent variables for FOS separation include activated charcoal, sample (yacon root powder), stirring duration and water/ethanol solutions. D-Optimal design displayed for the trials in this study was shown in Table. 1.

Separation of Monosaccharide and Disaccharides

Yacon root containing FOS was extracted using charcoal as per method followed by Morales *et al.*[15]. By following method[15] some modification in that treatment was undergone to optimize the separation technique for FOS to yield the higher recovery of compound. In brief, 2.0 g of yacon root powder was dissolved in water/ethanol (v/v) 100 ml solution was added with activated charcoal in different amounts of (2-8 g) for each treatment respectively. Allowed the mixture to stirring in different duration of (0.5-5 h) and the content was filtered by vacuum filtration consequently by Whatman No. 1 filter paper. The mono and disaccharides were absorbed to the pores of activated charcoal was further washed with 25-30 ml of same water/ethanol (v/v) combination used.

Table 1: D-Optimal mixture design for separation of FOS from yacon root with different combination of independent variables

Run	Formulation Variables		
	Factor 1 A: Charcoal (g)	Factor 2 B: Water (ml)	Factor 3 C: Ethanol (ml)
1	5	85	20.00
2	2	90	20.00
3	2	80	20.00
4	5	82.50	12.50
5	2	90.00	20.00
6	8	85.00	15.00
7	8	80.00	20.00
8	2	80.00	15.00
9	2	80.00	10.00
10	2	90.00	10.00
11	8	90.00	10.00
12	8	90.00	20.00
13	2	85.00	10.00
14	8	90.00	10.00
15	8	90.00	20.00
16	5	90.00	15.00
17	8	80.00	20.00
18	8	80.00	10.00

Recovery of Fructooligosaccharides

The absorbed mono and disaccharides in activated charcoal were removed using higher concentration of ethanol than first step extraction. Different combinations of water/ethanol (v/v) 100 ml solutions were used to desorption the higher saccharides of FOS from charcoal by stirring for 30 min. To recover the FOS from charcoal, filtration procedure was carried out using vacuum filtration consequently by Whatman No. 1 filter paper. The obtained filtrate containing ethanol was removed using (RV- IKA 10 digital) rotary evaporator at 40˚C and concentrate residue was re-dissolved in 5 ml of Milli-Q water. Filtration of the obtained extract was purified using 0.2 µm (Spin-Pure) filter for purity of compound and used for further analysis. The percentage of yield was calculated using

$$\text{Yield (g/100g)} = \frac{\text{Weight of the extract from dried residue}}{\text{Weight of powder taken}}$$

Freeze Drying of Residue

The concentrate residue containing FOS obtained from evaporator of 20 ml was kept in -80˚C at deep freezer and then placed in a freeze drier (Borg LYO 10T) for 24 h.

Color Measurement

The freeze dried powder was allowed to measure the color of FOS compound using HunterLab (ColorFlex EZ) Spectrophotometer. Prior to measuring the instrument were

calibrated with calibration plates, then record the L^* a^* and b^* values.

SEM Analysis

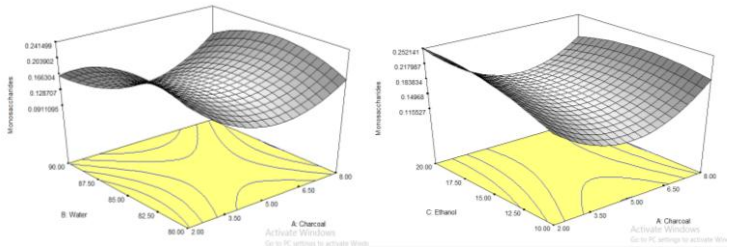
The separated sugars were allowed to predict the structure of FOS from yacon tuber using SEM analysis. FOS was observed under (TESCAN VEGA3, Czech Republic) with an accelerating voltage of 10.0 KV using thermionic emission gun under vacuum, 1×10^{-3}Pa.

Analytical Methods

The separation of monosaccharides (glucose) by activated charcoal was determined by GOD/POD assay kit (Navsari, India) using UV spectrophotometer at 505 nm. Quantitative analysis of recovered fructooligosaccharides was determined by fructan assay as per method followed by Rane *et al.*[16]. This was measured using ease and rapid method by UV spectrophotometer at 490 nm. All analysis was performed in triplicates.

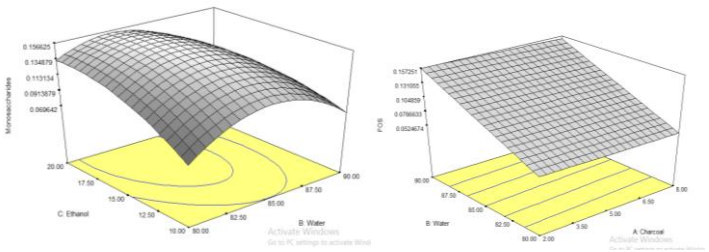
RESULTS AND DISCUSSION

Based on design mixture formulated the separation process were carried out. In figure 2A effect of interaction between charcoal and water for removal of monosaccharides from mixture showed 0.166 mg/ml, when 2g of charcoal and 85% water was used. In figure 2B effect of interaction between charcoal and ethanol displayed good efficiency for separation of monosaccharides 0.252 mg/ml when tried with 2g charcoal and 15% ethanol. In figure 2C elimination of monosaccharides by interacting with water and ethanol showed fair removal of 0.134 mg/ml at 85% water and 20% ethanol was used. This following effect showed that when concentration of ethanol increases, the absorbed monosaccharides will be removed from the charcoal.

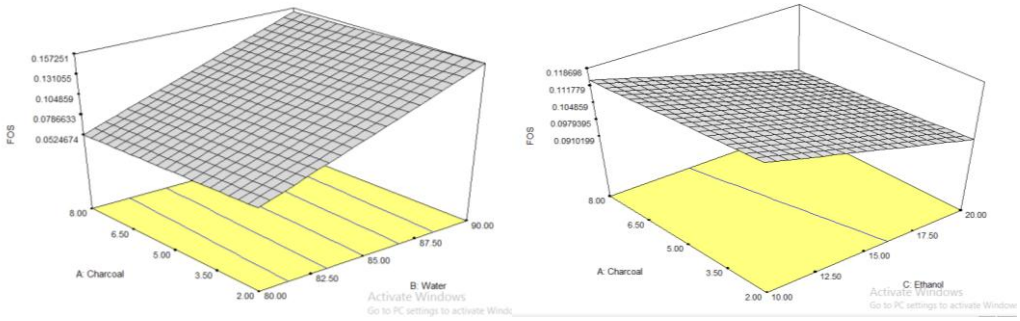


A) **B)**

The next stage of separation was FOS from absorbed activated charcoal using more concentration of water/ethanol (v/v) elutents than first stage of separation process. From the optimal study in figure 2D showed the effect of interaction between charcoal and water. It displayed when water level increases FOS concentration was recovered up to 0.157 mg/ml. Thing to be noticed that charcoal doesn't show much significant effect in the recovery of FOS, because this stage of separation was about to recover the bounded sugar molecules in the pores of charcoal. In figure 2E, same effect showed that inclination of water level leads to more recovery of FOS showed 0.157 mg/ml.



C) **D)**



E) **F)**

Fig. 2: In Figure A-C Response surface plots in D-Optimal design displayed the effect of interaction between charcoal, water and ethanol combination for monosaccharides separation; Figure D-F showed effect of interaction between charcoal, water and ethanol combination for the separation of FOS

But in case of figure 2F the process was vice-versa when ethanol used. In briefly, increasing the level of ethanol concentration leads to decreasing the recovery of FOS content. The maximum FOS recovered from the interaction between charcoal and ethanol showed 0.111 mg/ml at 10% ethanol along with charcoal used. The obtained FOS yield was satisfactory by using activated charcoal. By following method of Mores *et al.*[15], separated oligosaccharides from honey sample using two stage separations. In that monosaccharides were first removed, followed by removal of oligosaccharides (maltodextrins) from the honey using water/ethanol (90/10) v/v solutions for monosaccharides removal and higher concentration of water/ethanol (50/50) elutent were used for oligosaccharide removal showed better efficiency in that study.

FOS produced through microbial synthesis was separated using activated charcoal showed less affinity for monosaccharides, but good absorption for FOS[17]. Another study about FOS separation from commercial source using four different ion-exchange resins, from that amberlite and dowex monosphere ionic resins showed good efficiency for separation process[22]. This following study was useful in industrial level.

Response: FOS

ANOVA for Response Surface Linear Model

Analysis of variance table [Partial sum of squares]

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	0.037	3	0.012	4.04	0.0291	significant
A	*7.803E-005*	*1*	*7.803E-005*	*0.025*	*0.8755*	
B	*0.035*	*1*	*0.035*	*11.53*	*0.0043*	
C	*1.875E-003*	*1*	*1.875E-003*	*0.61*	*0.4469*	
Residual	0.043	14	3.062E-003			
Lack of Fit	*0.020*	*10*	*1.972E-003*	*0.34*		
Pure Error	*0.023*	*4*	*5.787E-003*			
Cor Total	0.080	17				

The Model F-value of 4.04 implies the model is significant. There is only a 2.91% chance that a "Model F-Value" this large could occur due to noise.

Values of "Prob > F" less than 0.0500 indicate model terms are significant.
In this case B are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant.

Fig. 3: ANOVA for response of FOS recovery from yacon root obtained through D-Optimal mixture design. From this design F-Value indicated that the model was significant with (*P≤0.05*)

From figure 3. ANOVA for the FOS separation from yacon root showed significant as response for this model. F- value implies that 4.04 in this study and probability showed greater than the F-value which was less than 0.05 indicated that terms assigned in this model were significant.

Scanning Electron Microscopy for FOS

SEM for yacon root powder FOS was showed in figure 2. This represented the smaller spherical particles in image 2A and 2B, where yacon displayed bigger spherical particles in image 2C and 2D. Figure 2 with × 500 magnifications clearly indicated that particles were agglomerates due to high hygroscopicity in both commercial and extracted FOS. In figure 2C and 2D were freeze-dried powder they resembled with more void spaces. From the observation of SEM the particles were tend to appeared as amorphous surfaces due to adherence of particles. Aforementioned statement was agreement with the study found when a smaller particle adhered to the bigger particles showed amorphous surfaces, thereby absence of crystalline form mentioned by Cano-charca *et al.*[19]. A similar study conducted in chicory inulin type fructans showed high hygroscopic when water activity (a_w) of product reached above 0.52 and particles were agglomerated, hence it was difficult to view single particles anymore[20]. Moreover when FOS exposed to environment the color changed from white to yellowish because of hygroscopicity increases with humidity, it displayed that color value b^* for FOS was 1.02±0.03. Similar results were also found in this study, color changed to slight yellowish after exposed to environment and showed b^* value of 1.46±0.04.

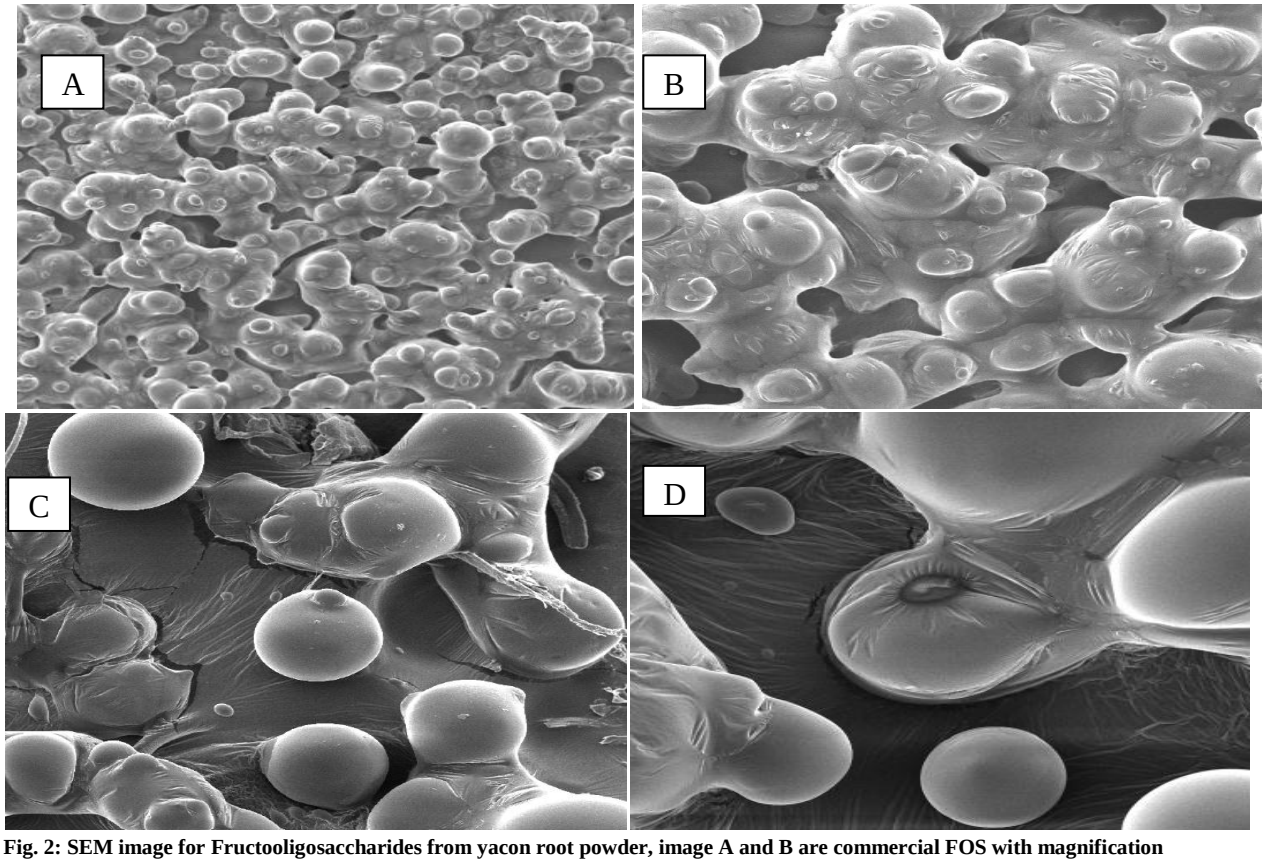


Fig. 2: SEM image for Fructooligosaccharides from yacon root powder, image A and B are commercial FOS with magnification of ×250 and ×500. Image C was yacon FOS under magnification ×500. Image D was yacon FOS with magnification ×1000 to view as single compound for observation after freeze-dried.

SEM results in this study was correlated with another study of spray dried yacon juice powder, allowed to dried at 70˚C, showed the more agglomeration of particles. Due to adherence of particles, they resembled like larger particles that revealed like laminar type

structures that found in Lago *et al.*[19]. In this study FOS structure when viewed clearly, particles were strongly agglomerated due to freeze dried condition.

CONCLUSION

In this study, FOS separation from yacon root was achieved using activated charcoal. D-optimal experiment design have formulated the independent variables for the separation of FOS, based on design the maximum absorbed monosaccharides removed were 0.252 mg/ml at (85/15) water/ethanol (v/v) combination. Then, desorption of FOS recovered displayed best combinations when made using 0.157 mg/ml at (90/10) water/ethanol (v/v) solutions with elutent 70/30 for removal of oligosaccharides. In first stage of separation (monosaccharides) charcoal with ethanol interaction played vital role, while in second stage of separation (oligosaccharides) interaction between ethanol and water showed much significant effect than charcoal was observed due to absorption of oligosaccharides in the previous step. This method employed with low cost and less energy for separation process can find suitable applications in industrial plant to achieve higher economic value.

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