Sugaring-out-assisted aqueous two-phase extraction of fructooligosaccharides from yacon (Smallanthus sonchifolius)

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Introduction

Fructooligosaccharides (FOS) are a mixture of oligosaccharides, consisting of glucose linked to fructose units by (2→1)-β-glucosidic linkages. They cannot be hydrolyzed by human digestive enzymes, but can be utilized by intestinal bacteria such as bifidobacteria. FOS has received more and more attention as probiotics due to its beneficial features and vital regulatory functions. It has been shown that FOS have a lower heating value, can regulate bowel function, and reduce constipation, regulate of intestinal flora balance, improve immunity, and lower blood sugar response functions. FOS can be extracted from fruits and vegetables such as burdock, onions, chicory root, barley, etc. But the FOS concentrations in these fruits and vegetables are much lower than that in yacon. Yacon [Smallanthus sonchifolius (Poepp. et Endl.) H. Robinson], syn. Polymnia sonchifolia, has high levels of FOS and low level of simple sugars (e.g., glucose, fructose, and sucrose). It has been found that yacon has the highest concentrations of FOS among cultured plants. Therefore, there is a growing interest in extracting and purifying FOS from yacon tuberous roots.

Due to high polarity of FOS, separation and purification of FOS from yacon roots is still a challenge. Raw FOS was obtained by means of hot water extraction or ultrasound-assisted extraction, followed by decolorization and deproteinization. The FOS purification methods include nanofiltration, silica gel absorption, activated charcoal absorption, anion exchange chromatography (AEX), size-exclusion chromatography (SEC), and hydrophilic interaction chromatography. However, these purification methods require high initial equipment investment and hard to scale up.

Salting-out-based aqueous two-phase extraction system has been widely used to extract biomaterials because it provides milder extraction conditions. However, the phase separation usually occurs at high salt concentration, which results in unwanted chemical reactions and equipment corrosion and fouling.

A novel technique sugaring-out-assisted liquid–liquid extraction (SULLE) was recently reported, in which the phase separation of the acetonitrile (ACN)–water mixture can be triggered by the addition of sugars. This new extraction technique has been successfully applied to extract furfural, 5-hydroxymethyl furfural (HMF), ferulic acid, syringic acid, para-coumaric acid, phenolics, and vanillin. In previous experiments, we observed the stable two-phase system when adding acetonitrile into the water extract of yacon roots. It is interesting to find that most of FOS was extracted into the ACN-top phase, due to the high sugar concentration in the water extract of yacon roots, with no extra sugar needed to be added. As a strong electron donor, the sugaring-out acetonitrile molecules extract the highly polarized FOS very easily, which makes the sugaring-out system an efficient extraction method in this application.
This work combines water extraction method with sugaring-out technology to extract and purify FOS from yacon. The effects of sugar concentration in yacon water extract, extraction temperature, and the ACN solution to yacon water extract ratio on the extraction efficiency of FOS from yacon roots.

**Materials and methods**

**Chemicals, reagents and materials**

Fresh yacon roots were obtained in a local supermarket at Haidian, Beijing and stored at 4°C before use. Acetonitrile (A.R.) and sulfuric acid (A.R.) was purchased from Tianjin Xihua Chemical Reagent Co., Ltd., China. Anthrone (A.R.) was purchased from Sionpharm Chemical Regent Beijing Co., Ltd. Distilled and deionized water was used in all experiments.

**Extraction procedure**

Fresh yacon roots were peeled and cut into small pieces (0.2 cm), and 20 g of the yacon roots was thoroughly mixed with water for 20 min at 60°C. After the mixture separated by centrifugation, 4.5 mL of supernatant was transferred into a 30 mL graduated centrifuge, then suitable volume of acetonitrile was added into the tube. The mixture was stirred at an optimized temperature and then centrifuged at 4000 rpm for 10 min to obtain a stable two-phase co-existed system. The volume of each phase was measured after phase separation. All results were repeated for three times.

**Determination of FOS**

The concentrations of FOS in the yacon water extract and in the two phases after separation were analyzed by high-performance liquid chromatography (HPLC 1200 Agilent Technologies) with a refractive index detector. An NH2 column of 4.6 mm × 250 mm, 5 μm (Alltech, USA) was used. A 10 μL sample was eluted isocratically with water-acetonitrile (25/75, v/v). The detection was performed at 30°C with a flow rate of 0.5 mL/min.

The mass ratio of FOS in the top phase was calculated as follows:

\[
W_t = \frac{C_t \times V_t}{m_0},
\]

where \(w_t\) is the mass ratio of FOS in the top phases, \(C_t\) is the concentration of FOS in the top phase, \(V_t\) is the phase volumes of top phase, and \(m_0\) is the total amount of FOS in yacon leaching liquor.

**Determination of total sugar concentration in water extract of yacon**

Anthrone colorimetry was adopted to measure the contents of carbohydrate components in yacon leaching liquor and raffinate. The content of sugar except FOS was determined by subtracting the amount of FOS from the total amount of carbohydrates.

The weight ratio of sugar except FOS in the top phase was calculated as follow:

\[
w_{st} = \frac{(m_S - m_F) - (C_S - C_F) \times V_b}{m_S - m_P},
\]

where \(w_{st}\) is the weight ratio of sugar in the top phase except FOS; \(m_S\) and \(m_P\) are the amount of sugar except FOS and FOS in yacon leaching liquor, respectively; \(C_S\) and \(C_F\) are the concentration of sugar except FOS and FOS in the bottom phase; and \(V_b\) is the phase volumes of bottom phase.

**Results and discussion**

**Sugaring-out-induced phase-splitting behavior in system of acetonitrile–water extract of yacon roots and its effect on partition of FOS**

The phase separation induced by sugaring-out in acetonitrile–water mixtures has been investigated. When sugaring concentration in aqueous solution above a critical value, sugaring-out-induced phase-splitting phenomenon will appear. The phase separation behavior is closely related with the sugar concentration in aqueous solution and the temperature. The higher sugar concentration and lower temperature are conducive to the formation of liquid–liquid phase. Dry yacon roots contain 40%–80% of sugar, while fresh ones contain 10%–20% sugar, mainly FOS. Yacon roots also have significant sucrose (~3–11%, dry matter), fructose (~3–22%, dry basis) and glucose (~2–5%, dry matter) contents. Such high sugar concentration allows the formation of ACN–water extract two-phase system without additional sugar. Experiment results indicated that the phase separation phenomena can be observed by controlling the sugar contention in yacon water extract above 50 g/L at room temperature (25°C). ACN–water extract of yacon mixture was triggered into two-phase system at this sugar concentration, with the upper phase rich in ACN and the lower phase rich in water. The sugar contention in yacon water extract can be modulated by concentration. It was surprising to discover that with the formation of sugaring-out two-phase system, most of the FOS can be extracted from the yacon/water slurry to the ACN-rich phase while most of other types of carbohydrate components
remained in the bottom aqueous phase. The abstraction of FOS was closely related to formation of ACN–yacon/water slurry two-phase system.

**Effect of yacon roots to water ratio on sugar concentration**

Sugar concentration is the most critical factor to induce phase separation, the amount of water added in yacon roots decides whether the yacon/water slurry can be separated from ACN phase. Figure 1 shows the sugar concentration in yacon/water slurry when adding different weight ratio of water into the yacon roots. Sugar concentration decreased when less yacon roots were added in the yacon/water slurry. The sugar concentration was only 15.23 g/L when adding 10 wt% yacon roots.

ACN can dissolve in water in any proportion. Additional sugar molecular destroys the hydrogen band between ACN and water molecular and caused phase separation. The higher the sugar concentration, the larger the volume of ACN-rich phase. Figure 2 shows that at a fix temperature, an increase in sugar concentration in yacon/water slurry leads to rich separation of ACN when the sugar concentration was above 50 g/L, the volume of ACN-rich top phase increased with the increase of sugar concentration. However, when the sugar concentration is above 100 g/L, the increase in phase separation is insignificant. Therefore, the volume of sugaring-out middle phase remains unchangeable.

The separation of FOS in ACN–yacon/water slurry is closely related with the sugar concentration in yacon/water slurry. Figure 3 shows the distribution behaviors of carbohydrate as a function of sugar concentration. It can be clearly seen that the mass fraction of FOS in the top phase increases with the increase of the top phase volume when sugar concentration is below 100 g/L, which demonstrates the preferential affinity of FOS towards the ACN-rich phase. When sugar concentration is above 100 g/L, the mass fraction of FOS in the top phase keeps constant. However, the mass fraction of other carbohydrate except FOS was much lower, which was less than 13%, which indicated that the sugaring-out-induced two-phase system has good selectivity in separating FOS and other carbohydrate from water extract of yacon.

The microscopic environments of acetonitrile are different before and after phase separation. High sugar concentration results in the separation of water molecules from the hydrated acetonitrile
molecules. The resulted sugaring-out-dehydrated acetonitrile molecules have better electron-donating ability than the hydrated acetonitrile molecules to interact with FOS. That is the main reason FOS can be extracted into the sugaring-out-induced acetonitrile top phase.

**Effect of temperature on phase separation and FOS abstraction**

It has been confirmed in previous literatures that lower temperature favors the two-phase splitting of acetonitrile–water mixtures.\(^{[24,25]}\) Figure 4 shows the effect of temperature on the volume of ACN-rich top phase. This can be explained by the interaction among ACN, water, and sugar molecules. The intermolecular interaction between ACN and water competes with that between sugar and water. In ACN–sugar–water mixture, the hydrogen bonds between ACN and water molecules were weakened by the addition of sugar. The degree of hydrogen bonding depends on temperature. At lower temperatures, the distance between adjacent ACN and water molecules increases. Thus, sugar molecules will find more chance to replace ACN molecules to associate with water molecules. The dehydrated acetonitrile molecules are pushed out of the water phase and aggregate as a new phase. The abstraction of FOS is closely related with the change in volume of ACN-rich top phase induced by lower temperature. As shown in Fig. 5, lower temperature is conducive to the transfer of FOS from water phase to ACN-rich top phase.

![Figure 4](image1.png)

**Figure 4.** Effect of temperature on volumes of each phase in liquid–liquid phase system. \(V_t\) and \(V_b\) represent the volume of top phase and bottom phase, respectively. Initial volume of water extract and acetonitrile solution was 4.5 mL and 9 mL, respectively.

![Figure 5](image2.png)

**Figure 5.** Effect of temperature on mass ratio of FOS in ACN–top phase. Initial volume of water extract and acetonitrile solution was 4.5 mL and 9 mL, respectively.

![Figure 6](image3.png)

**Figure 6.** Variation of the volume of sugaring-out ACN top phase with \(V_{AN}:V_{AQ}\). Initial volume of water was 3 mL. \([sugar]\) aqu= 100.34 g/L.

**Effect of volume ratio of acetonitrile-to-yacon/ water slurry**

The typical change in the volumes of acetonitrile-rich phase at different \(V_{AN}:V_{AQ}\) was given in Fig. 6. \(V_{AN}\) and \(V_{AQ}\) represent the volumes of added acetonitrile and the volume of yacon/water slurry, respectively. It can be concluded that the volume of acetonitrile-rich phase increases linearly with the increase of \(V_{AN}:V_{AQ}\). The linear increase of the volume of acetonitrile middle phase can be explained by the Setchenov equation: \(\log \frac{S}{S_0} = \frac{-k_sC_s}{w} \), where \(S\) and \(S_0\) are the solubilities of the organic solvent in aqueous sugar solution and pure water, \(k_s\) is the empirical Setchenow constant, and \(C_s\) is the molar concentration of phase-forming agents.\(^{[26]}\)
Since the concentration of carbohydrate ($C_s$) is a given value, $S_0$ is a constant; therefore, the solubility of acetonitrile in aqueous solution ($S$) keeps constant after the phase separation. Consequently, the initial added volume of acetonitrile decides the final volume of the sugaring-out acetonitrile top phase after phase separation, under a fixed sugar concentration and certain acidity of the equilibrating aqueous phase. Figure 7 shows the effect of $V_{AN}:V_{AQ}$ on weight ratio of FOS in ACN-water extract of yacon two-phase system. It is shown that the weight ratio of FOS increases with the increase of $V_{AN}:V_{AQ}$ at a fixed sugar concentration. This is because the increasing $V_{AN}$ brings more acetonitrile molecules into the sugaring-out acetonitrile-rich phase, and therefore results in the increase of FOS weight ratio in acetonitrile-rich phase.

**Conclusions**

Sugaring-out-assisted phase separation occurred after acetonitrile solution addition into the water extract of yacon due to the higher sugar concentration in yacon. This novel sugaring-out-assisted liquid–liquid extraction system of acetonitrile–sugar–water was developed to extract FOS from water extract of yacon. Experimental results indicated that FOS can be concentrated into the sugaring-out acetonitrile-rich top phase of the liquid–liquid extraction system. The abstraction of FOS correlates directly with the phase separation. The increase volumes of acetonitrile top phase caused by the increasing sugar concentration resulted in a corresponding increase in FOS mass fraction in the top phase. Decreasing the system temperature favored FOS transferred into top phase from water extract of yacon. The increase in $V_{AN}:V_{AQ}$ resulted in the increase of FOS mass fraction in acetonitrile-rich phase due to the increased amount of free acetonitrile molecules.

Research on the extraction of FOS in the sugaring-out-assisted two-phase system is still in early stages. Further research is underway to investigate the relationship between selective extraction, phase separation and phase structure in sugaring-out-assisted two-phase extraction system.

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