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Extraction, quantification and degree of polymerization of yacon (*Smallanthus sonchifolia*) fructans

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Yacon tubers have been a distinguished alternative of fructans, drawing the attention of researchers and food industries. Since fructans are carbohydrate reserves storage can reduce their contents. Additionally, the type of extraction used can provide a higher yield of fructans. Therefore, it was necessary to study yacon storage and its influence on the extraction and quantification of fructans. Thus, the objective of this study was to evaluate three fructan extractions (water 75°C, water 95°C, ethanol 90°C) in yacon with 3 sizes (large, medium, small), stored for 20 days, at room temperature as well as to compare two quantification techniques. The three extractions can be used when fructans are quantified by high performance liquid chromatography (HPLC). For quantification by spectrometry, the best extraction method was ethanol at 90°C. Medium and small-sized tubers presented the highest contents of fructans that large tubers, and storage negatively influenced these contents. Fructan quantification by HPLC was higher than the spectrophotometric technique. All treatments showed a degree of polymerization in the range from 3 to 7, allowing numerous technological applications for fructans present in yacon.

Key words: Fructooligosaccharides, storage, tuber size.

INTRODUCTION

Functional foods represent one of the latest trends in the market. They have compounds which result in benefits to physiological and metabolic activities, in addition to strengthening the immune system, acting in the prevention and reduction of chronic degenerative diseases. Thus, the food industry has a growing interest

in improving the nutritional benefits of products, without compromising their technological properties (Delgado et al., 2010; Castro et al., 2013). Fructans are a polysaccharide group which has received great attention in recent years, mainly inulin and fructooligosaccharides (FOS). Fructans are non-digestible complex carbohy-

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Abbreviations: FOS, Fructooligosaccharides; HPLC, high performance liquid chromatography.

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drates polymers used as functional ingredients, normally incorporated in various food products as dietary fibers. These compounds have been assigned with the ability of reducing the risk of various diseases, causing gastrointestinal benefits, increasing the absorption of calcium and magnesium, and reducing the risk of developing colon cancer and tumors (Sabater-Molina et al., 2009; Delgado et al., 2010; Nair et al., 2010). Additionally, due to their ability to form a stable gel-like network and mimic some of the textural properties of lipids, fructans are used to replace fat in meat products (Castro et al., 2013; Benkeblia, 2013). Furthermore, since they present fiber characteristics, they do not interfere with the sensory properties of the products (Contado, 2009).

Yacon roots present beneficial properties, due to their content of fructans, including inulin and FOS (Castro et al., 2013). The yacon (*Smallanthus sonchifolia*) is a tuberous root that stores large amounts of carbohydrates, especially fructans, and has a sweet taste when consumed fresh (Contado, 2009). Fructans are produced and stored in yacon tubers, when there is a surplus of photoassimilates in the plant. However, they are degraded when the plant needs energy (Capito, 2001). Thus, since fructans are reserve carbohydrates, storage can influence their contents. However, the data about yacon are still divergent and this may be due to various factors, such as harvest time, storage period, ecosystem, climate and soil where the plant is grown (Contado, 2009). The growing interest in plants with a high content of fructans, particularly FOS with a low degree of polymerization ($DP < 12$), encourages research worldwide.

However, there is no analytical method able to quantify all the content existing dietary fiber in foods, especially if FOS and inulin are present in these foods (McCleary, 2003), thus, enzymatic-gravimetric methods, spectrophotometric and high-performance liquid chromatography have been continuously proposed for this quantitation. Additionally, the type of extraction used can provide a higher yield of fructans, therefore, several studies have sought methods to optimize the fructan extracted, but there is no universal methodology, effective and which makes high performance for fructan extraction yacon. Thus, this study evaluated the content, the degree of polymerization and the best fructan extraction method in yacon tubers of different sizes, stored for 20 days at room temperature.

MATERIALS AND METHODS

The experiment was conducted in the Biochemistry Laboratory, Chemistry Department, and in the Laboratory of Physiology and Genetics of Microorganisms, Biology Department, both at Universidade Federal de Lavras. Yacon tubers were acquired on a commercial farm, located in the municipality of São Joaquim das Bicas, MG, of which 40 kg were washed in running water, with manual brushing, dried, weighed and sorted by weight (wt), to comprise the treatments. The 36 tubers selected were divided into

3 groups of 12 tubers each, according to size, which constituted the treatments: small tubers (with wt below 200 g), medium tubers (weighing between 200 and 499 g) and large tubers (with wt exceeding 500 g), which were stored for 20 days at room temperature (21°C, 52% RH), and the fructan contents of yacon were measured at two times: on the first day of storage (day 1) and on the twentieth day of storage (day 20).

Experimental and statistical design

A completely randomized design (CRD) was used, in a (3 × 2 × 3) factorial scheme, with 3 tuber sizes (small, medium and large), 2 days of storage (days 1 and 20), 3 extraction methods of fructans (water 75°C, water 95°C and ethanol, 90°C), and 3 repetitions of 2 tubers for each treatment. The results of fructan contents were subjected to analysis of variance using the statistical program SANEST, and the means were compared by the Tukey test at 5% probability (Zonta and Machado, 1991).

Extraction I (water at 75°C)

This extract was prepared according to Van Loo et al. (1995). Thus, 50 g of yacon were ground in a blender for 1 min; 250 mL of deionized water was then added and they was incubated in a water bath at 75°C for 1 h. The homogenate was filtered through ordinary filter paper, and the filtrate was stored in glass vials at -18 °C, until the analyses were performed.

Extraction II (water at 95°C)

The preparation of this extract was based on the methodology described by Cabello (2005). A sample of 50 g yacon was triturated in a blender with 200 mL deionized water at 95°C for 1 min. The material was then vacuum filtered with a Buchner funnel containing two quantitative filter papers. After the end of the extract, the residue was washed with 100 mL deionized water at 95°C, for the leaching of adsorbed carbohydrates in the remaining cells. The filtrates were combined, the pH was adjusted to 9.5 to 9.8 with 1 M NaOH, and the extracts were placed in a water bath at 95°C for 2 h. The solution was filtered again and the filtrate was stored in glass vials at -18°C for subsequent analyses.

Extraction III (ethanol at 90°C)

The extracts were performed according to Pollock and Jones (1979). Yacon tubers were peeled and immersed in water at 80°C for 5 min; they were then cooled in water at 20°C. After this bleaching, a sample of 50 g was ground in a blender and centrifuged at 1400 g for 15 min at room temperature. The supernatant was reserved and the sediment was subjected to re-extraction three times consecutively, by the addition of 100 mL of 80% ethanol, followed by heating at 90°C for 15 min, and centrifugation at 1400 g/15 min. The extracts were combined, vacuum filtered with a Buchner funnel and concentrated, in a roto vapor, up to 35 mL. This extract was stored at -18°C until the analyses were conducted.

Spectrophotometric quantification of fructans

The contents of total fructans in the extracts were determined by the quantification of fructose, free and combined, using the anthrone method, modified by Jermyn (1956), therefore, aliquots (0.1 ml) of the test solution were mixed with 1 ml of a 0.02% (w/v)

Table 1. Contents of yacon fructans (mg g⁻¹ fresh weight) with three sizes (large, medium and small), submitted to different types of extraction (water at 75°C, water at 95°C and ethanol at 90°C) evaluated in the first and twenty days storage, at room temperature, assayed by spectrophotometry.

| | Extraction size | Water 75°C | Water 95°C | Ethanol 90°C | Average (size) | Average (time) |
|------|----------------------|---------------------|---------------------|---------------------|--------------------|--------------------|
| T 1 | Large | 67.72 ^{Bb} | 78.78 ^{Aa} | 54.13 ^{Bc} | 66.87 ^A | 61.44 ^A |
| | Medium | 76.78 ^{Aa} | 45.41 ^{Cc} | 56.40 ^{Ab} | 59.53 ^B | |
| | Small | 59.51 ^{Cb} | 67.02 ^{Ba} | 47.21 ^{Cc} | 57.91 ^C | |
| | Average (day 1) | 68.00 ^a | 63.74 ^b | 52.58 ^c | | |
| T 20 | Large | 53.27 ^{Aa} | 45.27 ^{Bb} | 53.26 ^{Ca} | 50.60 ^C | 56.74 ^B |
| | Medium | 44.68 ^{Bc} | 45.27 ^{Bb} | 95.99 ^{Aa} | 60.31 ^A | |
| | Small | 36.32 ^{Cc} | 55.54 ^{Ab} | 86.09 ^{Ba} | 59.31 ^B | |
| | Average (day 20) | 44.76 ^c | 48.69 ^b | 76.78 ^a | | |
| | Average (extraction) | 56.37 ^b | 56.21 ^b | 64.68 ^a | | |

Means followed by the same uppercase letter in the columns and lowercase in the rows do not differ by the Tukey test at 5% probability. T1, First day storage; T20, twentieth day storage.

solution of anthrone in 70%(v/v) sulphuric acid and heated for 7.5 min in a boiling water-bath. The absorbance was determined in 625 nm. The reducing sugars were determined according to Nelson (1944), both using fructose as the standard. All analyses were performed in triplicate.

Quantification of fructans by high performance liquid chromatography (HPLC)

Aliquots (1 mL) of the extracts were hydrolysed with 0.5 mL of 0.1 N hydrochloric acid at 100°C for 5 min, and cooled in an ice bath until 25°C. After hydrolysis, 0.5 mL of the extracts were filtered through disposable nylon filters (0.45 µm), HPLC standard. Subsequently, the extract was eluted in a high performance liquid chromatograph (Shimadzu series 10A); using an SCR-101C column (Shimadzu Corporation) with deionized water as the eluent, flow rate of 0.8 mL/min and a refractive index detector. Fructose was used as a standard, according to the method described by Cairns and Pollock (1988).

Degree of polymerization of fructans

Aliquots (0.2 mL) of the extracts were deionized in ion-exchange columns containing anionic resin (Dowex 1 x8- 200) and another column containing cationic resin (Dowex 50 x8- 200). After the elution of the samples in 10 volumes of deionized water, the pH of the eluate was adjusted to 7.0 and the volume was reduced to 1.0 mL, approximately. Fructooligosaccharides were resuspended in deionized water and 5 µL were chromatographed on high-resolution silica gel plates (CCD Alugran SIL G/UV for TLC/ pour CCM of Macherey-Nagel), double developing in butane-2: 2-propanol: water at the ratio 3:12:4 (v/v). Free and bound fructose was revealed by spraying with a urea-phosphoric acid solution (Wise et al., 1955) and GR[®] Raftiline (Embrafarma) used as the inulin standard.

RESULTS AND DISCUSSION

Table 1 shows the contents of fructans (mg g⁻¹) in yacon

of three sizes (large, medium and small), submitted to different types of extraction (water at 75°C, water at 95°C and ethanol at 90°C), during storage for 20 days, quantified by spectrophotometry. It was observed that the type of solvent used influenced the extraction yield, with a higher fructan content (95.99 mg g⁻¹) in the extract prepared with ethanol at 90°C in medium-sized tubers, at 20 days of storage. On the other hand, the lowest fructan content (36.32 mg g⁻¹) was observed in small tubers, extracted with water at 75°C, on the twentieth day of storage. The best extraction was obtained using ethanol at 90°C, and it was not possible to observe a statistically significant difference between the extraction with water at 75°C and water at 95°C, which had 56.37 and 56.21 mg fructans g⁻¹ yacon, respectively. The contents of fructans (mg g⁻¹), quantified by high performance liquid chromatography, in yacon of three sizes (large, medium and small), submitted to different types of extraction (water at 75°C, water at 95°C and ethanol at 90°C) during storage for 20 days are shown in Table 2. When quantified by HPLC, it is possible to observe that there was no significant difference between the three extractions used, obtaining fructan contents between 123.21 and 127.57 mg g⁻¹. However, small tubers had the highest content of fructans in both days of storage. Additionally, it was observed that HPLC allowed the most efficient quantification of yacon fructans, therefore, obtaining higher values (Tables 1 and 2). The amounts of fructan in fresh yacon tubers, described in different studies, had a great variation, and contents between 24 and 101.30 mg g⁻¹ were observed (Capito, 2001; Campos et al., 2012; Castro et al., 2013). In this study, the contents of fructans are in accordance with those of the above-mentioned authors. The high variation observed in the amounts of fructans is probably due to the different

Table 2. Contents of yacon fructans (mg g⁻¹ fresh weight) with three sizes (large, medium and small), submitted to different types of extraction (water at 75°C, water at 95°C and ethanol at 90°C) in the first and twenty days storage, at room temperature, assayed by HPLC.

| T | Extraction size | Water 75°C | Water 95°C | Ethanol 90°C | Average (size) | Average (time) |
|------|-------------------------|----------------------|----------------------|----------------------|---------------------|-------------------|
| T 1 | Large | 125.96 ^{Bb} | 109.01 ^{Cc} | 116.43 ^{Bb} | 117.13 ^C | 127.60 |
| | Medium | 117.99 ^{Cc} | 124.17 ^{Ba} | 120.51 ^{Cb} | 120.89 ^B | |
| | Small | 126.07 ^{Ac} | 137.83 ^{Ab} | 170.48 ^{Aa} | 144.79 ^A | |
| | Average (day 1) | 123.34 ^c | 123.67 ^b | 135.80 ^a | | |
| T 20 | Large | 109.00 ^{Cc} | 136.28 ^{Ba} | 130.60 ^{Bb} | 125.29 ^B | 124.93 |
| | Medium | 124.17 ^{Ba} | 123.28 ^{Cb} | 91.03 ^{Cc} | 112.84 ^C | |
| | Small | 137.83 ^{Ab} | 138.15 ^{Aa} | 134.03 ^{Ac} | 136.67 ^A | |
| | Average (day 20) | 123.67 ^b | 132.57 ^a | 118.57 ^c | | |
| | Average (extraction) | 123.50 | 128.12 | 127.57 | | |

Means followed by the same uppercase letter in the columns and lowercase in the rows do not differ by the Tukey test at 5% probability. T1, First day storage; T20, twentieth day storage.

physiological periods of the tuber at harvest, as well as to the cultivar used in the test and the time and type of storage to which they were submitted. At 20th day of storage, the contents of fructans were significantly lower than those found at the beginning of storage, regarding both the spectrophotometric quantification and HPLC (Tables 1 and 2), which reflects the importance of the fructan extraction in early stages after harvest.

Graefe et al. (2004) found a decrease in the concentration of FOS, of about one third of the initial concentration in all cultivars, on the 12th day of storage. Although, the storage used in this study went higher than that reported by Graefe et al. (2004), when comparing the average of the 1st and the 20th day of storage, there was maintaining of approximately 98 and 92% of the total fructan, quantified by HPLC and mass spectrometry, respectively. Changes in the contents of fructose, glucose, sucrose and oligofructans occur during the growth and storage of tuberous yacon roots. A rapid changing process in the chemical composition of carbohydrates starts in the roots immediately after harvest, where polymerized sugars tend to depolymerize, that is, FOS tend to be hydrolysed into simple sugars by the action of the enzyme fructan hydrolase, which converts them into fructose, sucrose and glucose (Santana and Cardoso, 2008), suggesting a reduction in the contents of fructans at the end of storage, as observed in this study. Several factors may influence the contents of oligofructans, in which enzyme systems of biosynthesis and hydrolysis of FOS may be involved (Van Arkel et al., 2012), as well as weather conditions and other factors, such as storage conditions (Narai-

Kanayama et al., 2007; Milella et al., 2011; Fernadéz et al., 2013). Recent studies suggest that high concentrations of FOS can be obtained when yacon roots are subjected to steam cooking, in a microwave oven and boiled yacons (Miyaguchi et al., 2012).

Yacon tubers used in the extraction with ethanol at 90°C were previously subjected to bleaching and, in this extract, significantly higher levels of fructans were observed, when they were quantified by spectrophotometry (Table 1). However, for the quantification by HPLC, this difference was not observed (Table 2). A recent study evaluating the effects of bleaching showed that this treatment is able to reduce the activity of polyphenol oxidase and peroxidase by 84.62 and 83.76%, respectively, besides causing losses in staining levels and in the contents of inulin, glucose and fructose, of 30.64, 39.40 and 15.82%, respectively (Fante et al., 2012), which differs from the behavior observed in yacons, subjected to extraction with ethanol at 90°C, previously bleached. The degree of polymerization of the fructans present in yacon extracts was estimated by reversed-phase thin-layer chromatography (TLC) (Figures 1 and 2) and is shown in Table 3. It is possible to observe, by the technique employed that, all extracts showed the ability to extract fructans with different degrees of polymerization (DP from 3 to 7) and that storage time had no effect on their hydrolysis. The results found in this study are in agreement with Goto et al. (1995) and Fernadéz et al. (2013), who reported that yacon tubers have FOS with DP <10. There are numerous technological applications of FOS with a low DP, including the addition of FOS with

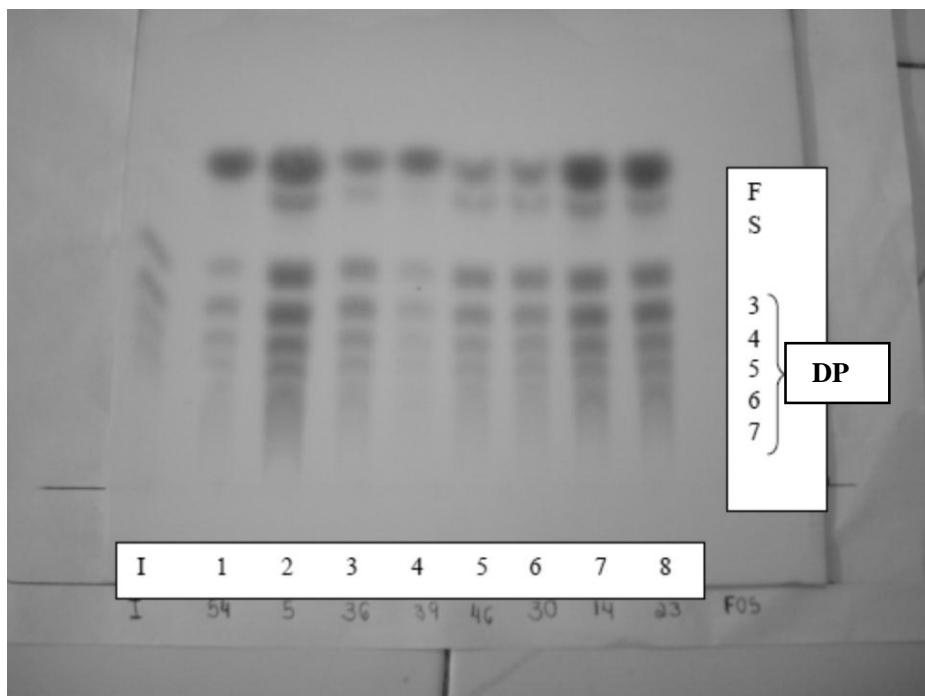


Figure 1. Thin-layer chromatography (TLC). I, inulin; 1, yacon extract, water 75°C, small size, time 1; 2: yacon extract, ethanol 90°C, medium size, time 1; 3, yacon extract, water 75°C, small size, time 20; 4, yacon extract, water 95°C, large size, time 20; 5, yacon extract, water 75°C, large size, time 1; 6, yacon extract, water 75°C, large size, time 20; 7, yacon extract, ethanol 90°C, medium size, time 1; 8, yacon extract, water 95°C, medium size, time 1. F, fructose; S, sucrose; DP, degree of polymerization.

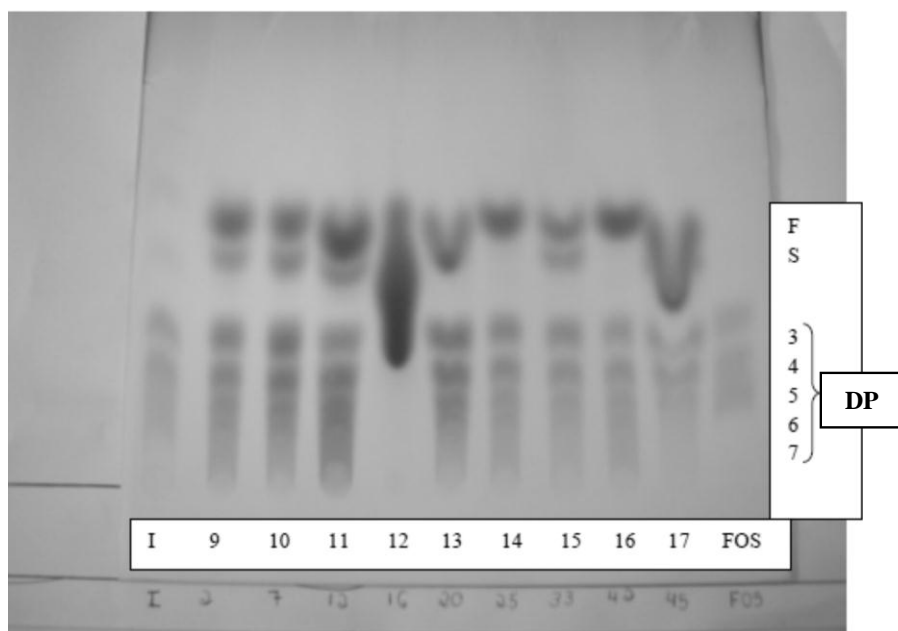


Figure 2. Thin-layer chromatography (TLC). I, inulin; 9, yacon extract, water 75°C, large size, time 1; 10, yacon extract, ethanol 90°C, small size, time 1; 11, yacon extract, ethanol 90°C, large size, time 1; 12, yacon extract, ethanol 90°C, small size, time 20; 13, yacon extract, water 95°C, small size, time 1; 15: yacon extract, water 75°C, medium size, time 20; 16, yacon extract, water 95°C, medium size, time 20; 17, yacon extract, water 95°C, small size, time 20. FOS, Fructooligosaccharide; F, fructose; S, sucrose; DP, degree of polymerization.

Table 3. Identification of thin-layer chromatography (TLC) spots for the degree of polymerization of yacon fructans with three sizes (large, medium and small), submitted to different types of extraction (water at 75 °C, water at 95 °C and ethanol at 90 °C), in the first and twenty days storage, at room temperature.

| T | Extraction | Water 75°C | Water 95°C | Ethanol 90°C |
|------|------------|------------------|-------------------|-------------------|
| | | 5 | 13 | 11 |
| T 1 | Large | F S 3, 4, 5, 6** | F S 3, 4, 5, 6 | F S 3, 4, 5 |
| | | - | 8 | 2 |
| | Medium | - | F S 3, 4, 5, 6, 7 | F S 3, 4, 5, 6, 7 |
| | | 1 | 14 | 10 |
| | Small | F S 3, 4, 5, 6 | F S 3, 4, 5, 6 | F S 3, 4, 5, 6 |
| | | 6 | 4 | 12 |
| T 20 | Large | F S 3, 4, 5, 6 | F S 3, 4, 5, 6 | F S 3, 4, 5 |
| | | 15 | 16 | 7 |
| | Medium | F S 3, 4, 5 | F S 3, 4, 5, 6 | F S 3, 4, 5, 6, 7 |
| | | 3 | 17 | - |
| | Small | F S 3, 4, 5, 6,7 | FS 3, 4, 5 | - |

Bold numbers correspond to the numbering of the extract shown in the TLC plate (Figures 1 and 2).

**Sequence of the degree of polymerization. T1, First day storage; T20, twentieth day storage.

DP between 2 and 8. Their addition in milk products, such as yogurt, is also emphasized, in order to improve their textural properties and sensory acceptance. Additionally, the partial replacement of sucrose by oligofructans in yogurt formulations is also possible (Yi et al., 2010; Castro et al., 2013), which suggests potential applications to FOS present in yacon. The size of fructan molecules ranges according to the plant species and organ studied. Environmental factors and the phenological cycle of the plant may also affect the size of these molecules, since they interfere with the accumulation or use of fructans. However, it is believed that the maximum size of fructan molecules is genetically determined. Comparing the amount of fructans with the degree of polymerization (Tables 1, 2 and 3), it is observed that there is no correlation between the amount of fructans, degree of polymerization and size of the stain obtained on the plate.

Conclusions

Medium and small-sized yacon tubers showed higher contents of fructans than large tubers and storage negatively influenced these contents. The quantification by HPLC was more efficient than the colorimetric. Extraction with water 75°C, water 95°C or ethanol 90°C can be used when fructans are quantified by HPLC; however, for spectrophotometric quantification, the best extraction is with ethanol 90°C. Yacon from all treatments showed a degree of polymerization between 3 and 7, allowing numerous technological applications for fructans present in yacon.

Conflict of interest

The authors declare(s) that they have no conflict interest.

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