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Research Article

Determination of carbohydrates and fructans content in *Cyperus esculentus* L.

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Abstract

The tiger nut contains different active ingredients like oil, tannins, sterols, saponins, alkaloids, vitamins C and E, minerals, and resins. There is a lack of information about carbohydrates content of *Cyperus esculentus* L. Thus, the aim of this study was to determine the content of carbohydrates of tiger nut herb and tubers. The qualitative composition and quantitative content of carbohydrates in tubers and herb of tiger nut (*Cyperus esculentus* L.) were determined by using a GC/MS method. The results of analysis showed that tiger nut herb have free carbohydrates, namely D-saccharose, D-glucose, D-Mannitol, and D-fructose, while tubers have only disaccharide D-saccharose. Free D-saccharose presented in raw materials in the greatest amount, the content in tubers was 63.72 mg/g, in the herb – 9.79 mg/g, respectively. Monosaccharides and their derivatives after hydrolysis presented to D-glucose, D-xylose, D-galactose, D-arabinose in tubers, and D-xylose, D-glucose, D-arabinose, D-galactose, D-Dulcitol, D-Mannitol, D-mannose in the herb of tiger nut. D-glucose dominates in tubers and D-xylose in the herb, their content was 177.26 mg/g and 39.07 mg/g, respectively. The total content of fructans was determined by the spectrophotometric method. Its content was 13.49% in tubers and 8.78% in the herb of tiger nut.

Keywords

Cyperus esculentus L., carbohydrates, fructans, GC/MS, spectrophotometric method, tiger nut

Introduction

Cyperaceae (sedge family) is a family of monocotyledonous plants among which grasses prevail (Bado et al. 2015). The family includes 5,500 species in the world including *Cyperus esculentus* L. (Govaerts et al. 2007; Bado et al. 2015).

Cyperus esculentus L. is a cosmopolitan plant, cultivated in tropical and subtropical areas worldwide and extensively in Africa, Asia, and some European countries for their sweetish tubers (Buzsáki and Béres 2007; El-Naggar 2016). It has other names like tiger nut, yellow nutsedge, chufa, water grass, rush nut, earth almond, nut grass and northern nut grass (Shilenko et al. 1979; Alhassan et al. 2016). *Cyperus esculentus* L. is known in Nigeria as "Ofio" in Yoruba, "Akiausa" in Lgbo and "Ayaya" in Hausa where three varieties (yellow, brown, and black) are cultivated (Umerle et al. 1997; Arafat et al. 2009). The yellow variety is preferred among all three varieties plants because of its properties as an attractive colour, fleshier body and bigger size (Arafat et al. 2009; Bado et al. 2015; Codina-Torrella et al. 2015; Alhassan et al. 2016).

The nuts from *Cyperus esculentus* L. are valued for their highly starch content, carbohydrate, dietary fibre (Umerie and Enebeli 1997; Belewu and Abodunrin 2006). The tiger nut is also very rich in oil, minerals (Calcium, Sodium, Magnesium, Zinc, Potassium and traces of Copper) and

vitamins C and E (Omode et al. 1995; Belewu and Abodunrin 2006). Screening of phytochemical research showed a content of alkaloids, sterols, resins, tannins and saponins in the *Cyperus esculentus* L. (Chukuma et al. 2010).

Cyperus esculentus L. was reported to be also rich in high quality oil about 25.5% of its content. The oil of tiger nut has composition such as olives and also is rich of mineral content (El-Naggar 2016). The oil of tiger nut has high unsaponifiable matter, tocopherols, phytosterols, phospholipids and polyphenols (Sánchez-Zapata et al. 2012; Ezeh et al. 2014; Si-qun et al. 2016). This oil is unsaturated fatty acid which is good for the health of humans (El-Naggar 2016). Recent investigations have demonstrated that *Cyperus esculentus* L. oil contributes to the reduction of cholesterol, it reduces the risk of coronary heart diseases, thrombosis, atherosclerosis and activates blood circulation (Adejuyitan 2011; Sánchez-Zapata et al. 2013; Gambo and Da'u 2014; Alhassan et al. 2016; El-Naggar 2016).

Literature values revealed that tiger nut is also known to have carminative, diuretic, aphrodisiac, tonic and stimulant effects, and some healing uses such as treatment of indigestion, flatulence and dysentery (Adejuyitan 2011; Bado et al. 2015). In some societies *Cyperus esculentus* L. is relatively popular as an antidiabetic agent.

Hasan (2007) found that tiger nut tubers had appreciable hypoglycemic and hypolipidemic effects on streptozotocin-induced diabetic rats.

Jing et al. (2020) confirmed that the leaves of *Cyperus esculentus* L. included flavonoids that had various biological activities, such as antibacterial function, promoting blood microcirculation, and anticoagulant effect, antioxidative effects *in vitro* and *in vivo*.

It was observed that there is a lack of experiments providing information on the carbohydrates content of *Cyperus esculentus* L. Thus, the aim of this study was to identify and determine the quantitative content of carbohydrates by gas chromatography/mass spectrometry method (GC/MS) and fructans by spectrophotometric method of tiger nut herb and tubers.

Material and method

Plant materials

Herb and tubers of the tiger nut (*Cyperus esculentus* L.) were collected at the experimental sites of the New Cultures Department of M. M. Hryshko National Botanic Garden of the NAS of Ukraine in Kyiv. The aerial part was harvested during a mass flowering period and tubers were collected in autumn after the death of aerial parts in 2018. The raw material was authenticated by Prof. Dzhamal Rakhmetov. A voucher specimen was deposited in the herbarium at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine. The study plant material was dried using conventional method and stored in paper bags in a dry place (Stoiko and Kurylo 2018).

Standards and chemicals

All reagents were of analytical grade (≥ 99% purity). Standards of polysaccharides including D-mannose (Man), D-fructose (Fru), D-galactose (Gal), D-fucose (Fuc), D-arabinose (Ara), D-xylose (Xyl), L-rhamnose (Rha), D-ribose (Rib), D-glucose (Glc), D-saccharose (Sac), D-sorbitol obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

The 5-Hydroxymethyl-2-furaldehyde (≥ 99% purity) was from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest purity (Fig. 1).

GC/MS determination of carbohydrates

The monosaccharides composition of tiger nut herb and tubers was determined by GC/MS method on gas chromatograph Agilent 6890N with 5973inert mass detector (Agilent Technologies, USA). Samples were analyzed on a capillary column HP-5MS of 30 m in length and an internal diameter of 0.25 mm, a thickness of the stationary phase is 0.25 μm. The first set up oven temperature at 160 °C and held for 8 min, then raised to 240 °C at the rate of 5 °C/min and kept at this point for 6 min. At a constant flow rate of 1.2 sm^3/min was used Helium as the carrier gas. Detection was performed in the SCAN mode at the width range of 38–400 m/z.

Sample Preparation. For the extraction of bonded monosaccharides or monosaccharides after hydrolysis 500 mg of powdered tubers or herb of the tiger nut was placed into the flask and added 5 ml of 2 M trifluoroacetic acid. Hydrolysis was performed under 100 °C for 6 hours. 2 mL of obtained hydrolysate was evaporated and was added 2 mL of an internal standard.

For the extraction of free monosaccharides, 0.5 mg of methanol solution with internal standard (sorbitol) was added to 500 mg of powdered raw materials. The extraction was performed at the ultrasonic water bath at 80 °C for 4 h. Then, to obtain acetylated aldonitriles 2 ml of the extract was evaporated to dryness and was added 0.3 ml of derivatization reagent (32 mg/ml of hydroxylamine hydrochloride in pyridine/methanol (4:1 v/v)). The extract was incubated at 75 °C for 25 min. To the samples was subsequently added 1 ml of acetic anhydride and incubated at 75 °C for 15 min. 2 ml of dichloroethane was added, and the excess of the derivatization reagents was removed by the double extraction with water and 1 M hydrochloric acid. The dichloroethane layer was dried and dissolved in 300 μl of the mixture of ethyl acetate/heptane (1:1 v/v).

Identification of monosaccharides was based on comparing their retention times with retention times of standards of the mass spectral library NIST 02. Quantification was done by using sorbitol added to the sample (Husak et al. 2018; Slobodianiuk et al. 2019).

Spectrophotometric method determination of fructans

The quantitative content of the fructans was determined on a spectrophotometer Lambda 25 UV Perkin Elmer (USA).

3.00 g of powdered herb or tubers of the tiger nut was extracted 100 ml water at the water bath under reflux at 80 °C for 1 h.

Initial solution. The extract was cooled, filtered and the volume was completed to 100 ml of water.

Tested solution. 2 ml of the initial solution was placed to a 100 ml volumetric flask and 50 ml of 5% hydrochloric acid was added. The obtained solution was hydrolysed at the water bath under reflux for 2 h. 2.0 ml of cooled hydrolysate was placed to a 50 ml volumetric flask and added 5% hydrochloric acid to the mark.

Comparison solution. 2 ml of the initial solution was placed to a 50 ml volumetric flask and added 5% hydrochloric acid to the mark.

The absorbance of the tested solution was measured at a wavelength of 285 nm relative to the compensatory solution.

The total content of fructans in analyzed objects was calculated, as 5-Hydroxymethyl-2-furaldehyde (HMF), and it represents the average of five determinations (Husak et al. 2018).

Statistical processing and data analysis were performed using Statistica v 10.0 program package for Microsoft Office for Windows. The level of significance was set at $* p < 0.05$ for all statistical analyses.

Results and discussion

The GC/MS method was used to determine the qualitative composition and quantitative content of carbohydrates. Free carbohydrates in the herb of the *Cyperus esculentus* L. included D-glucose, D-Fructose, D-Mannitol and D-saccharose (Fig. 1).

In this analyzed material after acidic hydrolysis and derivatization with acetylated aldononitriles D-arabinose, D-xylose, D-mannose, D-glucose, D-galactose, D-Mannitol, D-Dulcitol were identified too (Fig. 2).

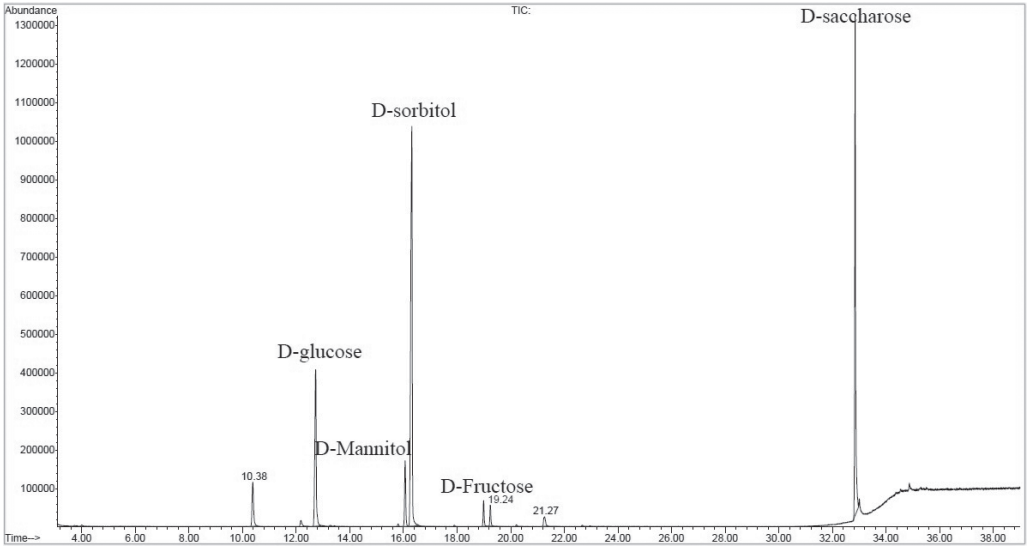

Figure 1. GC/MS chromatogram of free carbohydrates of *Cyperus esculentus* L. herb.

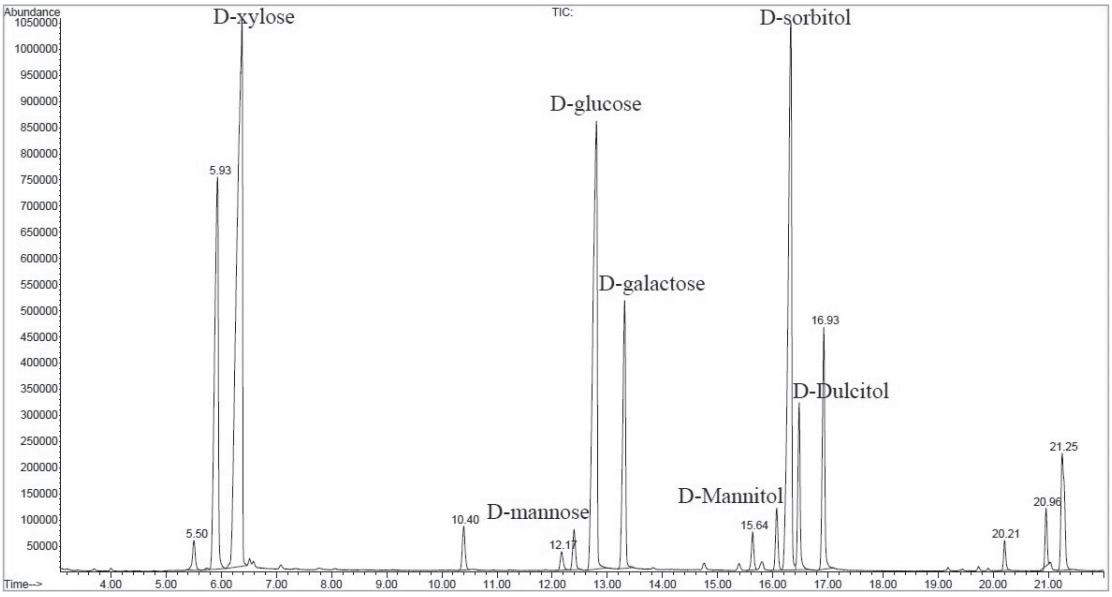

Figure 2. GC/MS chromatogram of monosaccharides and their derivatives after hydrolysis of *Cyperus esculentus* L. herb.

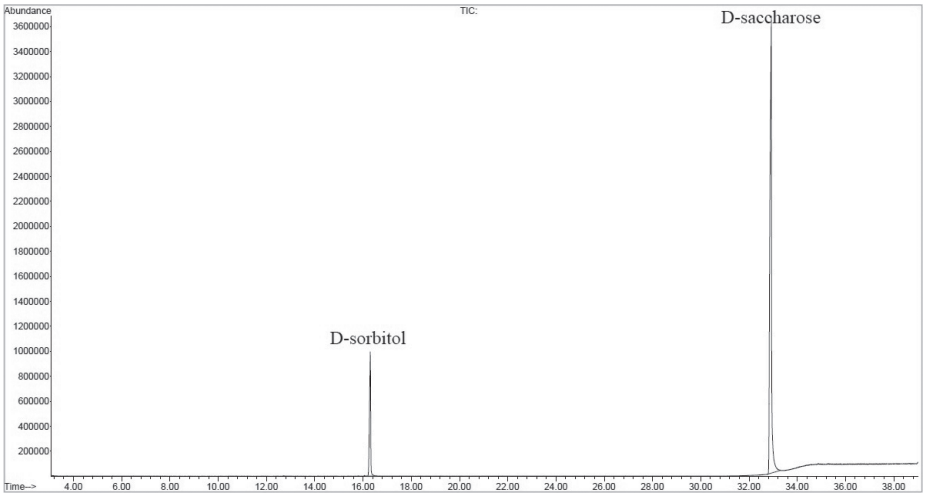

Figure 3. GC/MS chromatogram of free carbohydrates of *Cyperus esculentus* L. tubers.

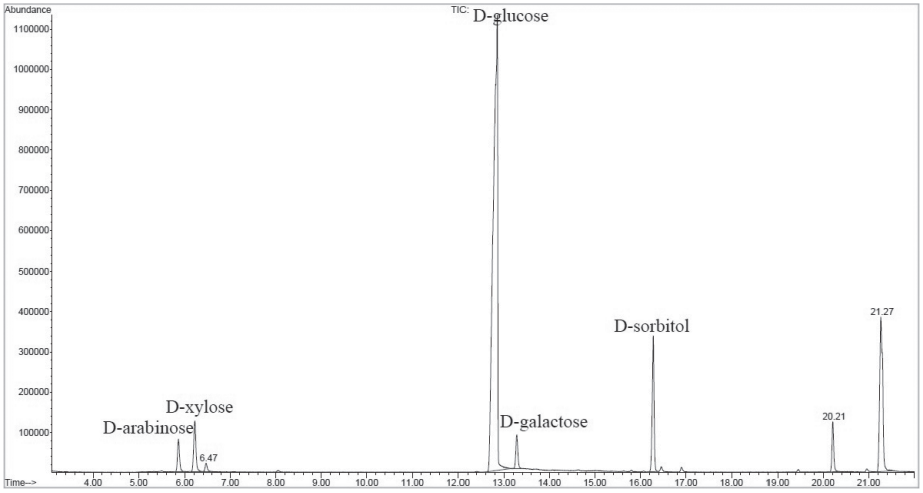

Figure 4. GC/MS chromatogram of monosaccharides and their derivatives after hydrolysis of *Cyperus esculentus* L. tubers.

D-saccharose was determined among free carbohydrates in the tubers of *Cyperus esculentus* L. (Fig. 3).

After hydrolysis of *Cyperus esculentus* L. in the evaluated tubers D-glucose, D-xylose, D-galactose and D-arabinose were identified (Fig. 4).

The quantitative content of carbohydrates is present in Table 1.

Table 1. The content of monosaccharides, their derivatives after hydrolysis and free carbohydrates of *Cyperus esculentus* L.

Carbohydrate	Content in the plant material, mg/g			
	Monosaccharides and their derivatives after hydrolysis		Free carbohydrates	
	herb	tubers	herb	tubers
D-arabinose	18.27	5.93	–	–
D-xylose	39.07	10.25	–	–
D-mannose	1.39	–	–	–
D-glucose	23.54	177.26	5.17	–
D-galactose	9.57	6.12	–	–
D-Fructose	–	–	0.60	–
D-Mannitol	1.90	–	1.73	–
D-sorbitol	internal standard			
D-Dulcitol	4.82	–	–	–
D-saccharose	–	–	9.79	63.72

Free carbohydrate D-saccharose was present in *Cyperus esculentus* L. in the greatest amount. In the herb, its content was 9.79 mg/g, in tubers – 63.72 mg/g respectively. Saccharose is a disaccharide that is formed only by plants. It is an easily assimilated macronutrient that provides a quick source of energy to the body (Khowala et al. 2008). Saccharose is a substrate for fructan synthesis (Vijn and Smeekens 1999). D-glucose presents in *Cyperus esculentus* L. tubers in the greatest amount 177.26 mg/g. Glucose is an energy source for great number of organisms, from bacteria to humans. The brain is the major site of everyday glucose using of 75% (Khowala et al. 2008). Red blood cells that develop and neurons have a big energy demand too. If the concentration of this monosaccharide is lower this deteriorats the processes demanding mental effort (Khowala et al. 2008; Mergenthaler et al. 2013; Slobodianiuk et al. 2019). In the herb of the *Cyperus esculentus* L. predominant ones were D-xylose 39.07 mg/g, D-arabinose 18.27 mg/g D-glucose 23.54 mg/g. Xylose or wood sugar has antifungal and antibacterial properties. This aldopentose is affecting particularly the *Candida species* and gram-negative organisms. Xylose is contained in the

embryos of most eatable plants. In contradistinction to saccharose, xylose promotes the rise of "friendly flora" in the intestines, thus increasing the manufacture and absorption of all foods and strengthening the immune system to help fight off any type of disease. In clinical medical practice, it is used as a diagnostic remedy to assess intestinal absorption (Khowala et al. 2008). Arabinose is obtained by the enzymatic hydrolysis or acid hydrolysis. It is a prevalent component in plant cell walls and is broadly distributed in the plant world. This pentose has the potential to be used as a nutrition additive to support good health and to become better obesity (Yoon et al. 2003).

Spectrophotometric analysis of fructans is based on the formation of 5-Hydroxymethyl-2-furaldehyde (HMF), through acid hydrolysis of saccharose and fructose while heating with concentrated acids. It was founded that the highest amount of HMF is formed after 2 h from beginning hydrolysis and the maximum absorption is observed at wavelength of 285 nm (Figs 5, 6).

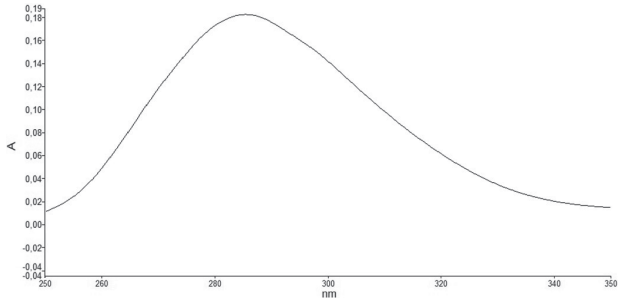

Figure 5. UV spectrum for HMF of the *Cyperus esculentus* L tubers.

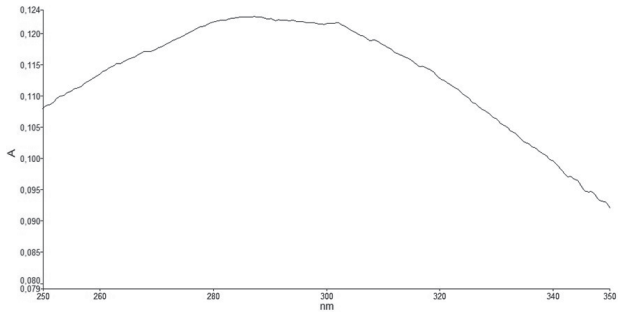

Figure 6. UV spectrum for HMF of the *Cyperus esculentus* L herb.

The results for total content of fructans in the herb and tubers of the *Cyperus esculentus* L. are presented in Table 2.

Table 2. The total content of fructans in *Cyperus esculentus* L.

Raw material	Content in the plant material, % $\bar{x} \pm \Delta \bar{x}$, n = 5, P < 0.05
Tubers	13.49 ± 0.01
Herb	8.78 ± 0.01

Fructans are the main storage carbohydrate of the vegetative tissues of temperate herbs, green algae, bacteria (Peshev and Van den Ende 2014). They are used in the food industry and in medicine (Matvieieva 2010). Fructans are generally regarded as dietary fiber, that counter digestion by peopled enzymes since the majority passes through the stomach and small intestine primarily untouched. Fructans are straight immunomodulatory, antioxidant, indirect prebiotic compounds, and used as a remedy for dysbacteriosis, diabetes, cardiovascular disease (Roberfroid 2005; Matros et al. 2019). The inulin-type compounds decrease the risk of colon carcinogenesis (Roberfroid 2005). The use of fructans in dietary products is relevant for overweight people (Matvieieva 2010).

Conclusion

The carbohydrates qualitative composition and quantitative content of tiger nut herb and tubers were determined by the GC/MS method. Four free carbohydrates as D-saccharose, D-glucose, D-Mannitol, and D-fructose were determined in the herb of study material as the result of the experiment. In this raw material D-xylose, D-arabinose, D-mannose, D-glucose, D-galactose, D-Mannitol and D-Dulcitol were identified after acidic hydrolysis and derivatization with acetylated aldononitriles. The results of the analysis showed that in tiger nut tuber have one free carbohydrate as D-saccharose and four monosaccharides, namely D-glucose, D-galactose, D-arabinose, D-xylose were identified after acidic hydrolysis. In the tiger nut dominant of free monosaccharide – D-saccharose, a content of which was 63.72 mg/g in tubers and 9.79 mg/g in the herb. Among monosaccharides, after hydrolysis D-glucose dominates in tubers and D-xylose in the herb, their content was 177.26 mg/g and 39.07 mg/g, respectively. The total content of fructans was determined by the spectrophotometric method. In tiger nut content of fructans in the herb was 13.49% and in tubers – 8.78%. In conclusion, the results revealed that tiger nut could be used as a food for both the young and old persons due to the high nutrient contents such as the carbohydrates.

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