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FRUCTAN CONTENT AND SOLUBLE SUGARS IN SOME ORNAMENTAL PLANTS

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Abstract

The aim of the current study was to evaluate the content of total fructans, as well as the individual sugars in the different vegetal parts of 13 ornamental plants. Six Allium representatives (Allium sphaerocephalon, Allium aflatunence, Allium 'Gladiator', Allium 'Globus', Allium large-flowered mixed and Nectaroscordum siculum Lindl.), two tulip cultivars, two hyacinth cultivars, Lapiedra martinezii Lag., Tanacetum balsamita and Calendula officinalis were used for the analysis. Ultrasound-assisted water extraction was performed to extract fructans and sugars. Thin layer chromatography and high performance liquid chromatography coupled with refractive index detector (HPLC-RID) method were used for analysis of fructans and sugars analysis. Allium representatives showed the highest content of inulin in their bulb. N. siculum bulb showed the highest fructans and inulin content - 24 g/100 g DW and 17 g/100 g DW, respectively. Fructans in tulip cultivars were in the low values - 3.3-3.8 g/100 g DW. Inulin content in L. martinezii bulbs reached 6 g/100 g DW, while in the roots of C. officinalis - 3.5 g/100 g DW. In the leaves of T. balsamita and L. martinezii only glucose, fructose and sucrose were detected.

Key words: *Allium, fructans, inulin, Lapiedra martinezii, tulip.*

INTRODUCTION

Fructans are reserve carbohydrate in plants. They accumulate in the vacuole, and play an important role in plant vegetation, including osmoregulation, cryoprotection and sink regulation, drought and cold resistance (Yoshida, 2021). Fructans can be found in representatives of several families, such as Asteraceae, Liliaceae, Asparagaceae, Boraginaceae, Campanulaceae, and Triticeae. Many annual and perennial plants accumulate fructans as an energy source that ensures the survival of wintering tissues, and degrade fructans for the sprouting or regeneration of tissues in spring (Hendry and Wallace, 1993). Inulin and fructooligosaccharides (FOS) are part of fructans family widely distributed in varieties of plants as plant storage carbohydrates in medicinal plants, fruits, and vegetables (Van Loo et al., 1995). They are used as dietary fiber or as food ingredients in several food products. The occurrence of fructans (especially in storage organs) is widespread in ornamental geophytes, indicating their importance in this group of plants (Hendry & Wallace, 1993).

Allium species contains carbohydrates, as the most abundant class of natural compounds, as the main representatives includes glucose, fructose and sucrose, together with a series of oligosaccharides and the fructans (Darbyshire and Steer, 1990; Kamenetsky and Rabinowitch, 2010). Most of the studies reported values for fructooligosaccharides and inulin in the bulb of Allium representatives (from 65 to 80% of the dry weight) (Van Loo et al., 1995). Fructans were widespread in the underground part of vegetables (e.g. onion, chicory and asparagus), and ornamentals (e.g., tulip, dahlia and hyacinth) (Ranwala and Miller, 2008).

Interest presents some spices used in culinary practice, as *Nectaroscordum siculum* and *Tanacetum balsamita* L.

Allium bulgaricum (*Nectaroscordum siculum* Lindl., *Nectaroscordum siculum* ssp. *bulgaricum (Janka) Stearn; Allium ursium* var. Dioscoridis) is a plant from Amaryllidaceae family, subfamily Allioideae, Allium species (Popova et al., 2014). In Bulgaria it is known as samardala or 'Bulgarian honey garlic'used in the preparation of spice mixes and salts, and as a seasoning.
Costmary (*Tanacetum balsamita* L.) known also as balsam herb, costmary, sweet tongue and bible leaf is from Asteraceae family and is a traditional medicinal plant of Iranian origin. The plant is cultivated in many countries and it used as an aromatic plant in Europe and Asia. Fresh and dried costmary leaves have a strong lemony-minty flavor and a sweet, astringent taste and are used as flavorings in soups and meats, especially lamb, sausages and cakes (Derakhshani et al, 2011; Gevrenova et al., 2023; Hassanpouraghdam et al., 2022).
Ranwala & Miller (2008) investigated 30 ornamental geophytes by high-performance anion-exchange chromatography with pulsed amperometric detection and in 25 they found fructans in the storage organs. In tulip bulbs were detected two reserve polysaccharides - starch (the main reserve carbohydrate) and fructan (a secondary reserve material that exists in vacuoles in a hydrated, colloidal state), and soluble sugars (sucrose, glucose, fructose) (Hobson & Davies, 1978; Kamenetsky et al., 2003). Low-temperature treatment of bulbs is accompanied by degradation of polysaccharides in the bulb scales to lower-molecular-weight sugar molecules (Koksal et al., 2010).
Lapiedra martinezii Lag. (Amaryllidaceae) is an autumn-flowering geophyte that grows in coastal and sublittoral environments. It is endemic in the South-west Mediterranean, mainly distributed in some coastal regions of Spain and North Morocco (Vicedo et al., 2021). However, until now there may gaps or unsufficinet data in scientific reports about the presence of fructose and sugar content in many ornamental plants.
In this context, the aim of the current study was to evaluate the content of total fructans, as well as the individual sugars in the different vegetal parts of 13 ornamental plants: six Allium representatives (*Allium sphaerocephalon, Allium aflatunence*, *Allium* 'Gladiator', *Allium* 'Globus', *Allium* large-flowered mixed and *Nectaroscordum siculum* Lindl.), two tulip cultivars, two hyacinth cultivars, *Lapiedra martinezii* Lag., *Tanacetum balsamita* and *Calendula officinalis* L. were evaluated as potential sources of inulin and fructooligosacchides.

MATERIALS AND METHODS

The experimental place and period

The bulbs from samardala of the four populations were collected in May 2017 during the flowering stage from their natural habitats in Bulgaria, as follows: Strandzha mountain (coordinates 41°59'53.76"N/27°49'32.56"E), Black Sea coast (42°44'10.02"N/27°39'8.98"E), Eastern Stara Planina mountain (42°45'36.71"N/27°44'54.10"E), and Central Stara Planina mountain (42°45'51.57"N/25°12'16.53"E). Botanical identification was done by botanist Assoc. Prof. Ina Aneva. Bulb from *Allium sphaerocephalon*, *Allium aflatunence*, hyacinth 'Peter Stuyvesant', hyacinth 'Jan Bos', tulip 'Burgundy', tulip 'Aguila' were purchased from Lidle, Bulgaria with the origin of the bulbs the Netherland during January 2021. *Allium* 'Gladiator', *Allium* 'Globus', *Allium* large-flowered mixed were purchased from online magazine during October 2022 www.semenata.bg.
Lapiedra martinezii Lag. was collected in April 2015 from Santa Pola's Cape, Santa Pola (Spain) from 10 m above sea level. Its botanical identification was done by Jorge Juan-Vicedo (co-author in this study).
The roots of *C. officinalis* and fresh leaves of *T. balsamita* were collected from plant garden during September 2021 (Kostievo village, Plovdiv region).

Plant material

L. martinezii Lag. and samardala plant material were lyophylized and finely ground to powder before analysis. Dry samardala leaves were purchased Decrassin Ltd. (Bulgaria). Bulb from other plants were used fresh as they were purchased. The roots of *C.officinalis* and leaves of *T. balsamita* were dried at room temperature and then ground in laboratory homogenizer.

Reagent and chemicals

All reagents were of analytical grade. Nystose, 1-kestose, sucrose, glucose, fructose and resorcinol were purchased from Sigma-Aldrich (Steinheim, Germany).

Moisture content

The moisture content (%) was analyzed on moisture analyzers balance Kern DAB 100-3 (Germany).

Ultrasound-assisted extraction of fructans and soluble sugars from ornamental plants

The dried plants were finely ground in laboratory homeogenizer to the fine powder. The fresh bulbs were cleared and peeled. Then they were homogenized in blender BN1200AL (Gorenje) and used immediately for analysis. The samples were weighted in a centrifuge tube of 50 mL and extracted with distilled H_2O in solid to liquid ratio 1:10 (w/v) for dry samples and 1:4 (w/v) for fresh samples. The extraction conducted in an ultrasonic bath (IsoLab 621.05.001, Germany, ultrasonic frequency 40 kHz and ultrasonic power 60W) for 20 mins, at 75°C. The obtained extracts were filtered, and the residues were extracted once again under the above mentioned conditions. The both extracts were combined and used for further analysis.

Thin layer chromatography (TLC)

TLC analysis was performed to detect the presence of mono-, di-, fructooligosaccharides (FOS) and inulin in water extracts of studied ornamental plants. The TLC plates silica gel 60 F_{254} were used (Merck, Germany) with mobile phase n-BuOH:i-Pro:H_2O:CH_3COOH (7:5:4:2) (v/v/v/v). The TLC plates were dipped for 20 seconds in diphenylamine-aniline-H_3PO_4–acetone and then dried for 5 min at 120°C. The standards glucose, fructose, sucrose, fructo-oligosacchides from agave (Mexico) and inulin from chicory (Raftiline HPX, Beneo, Orafti, Belgium) were used for TLC analysis (Petkova and Denev, 2013).

Spectrophotometric method for analysis of total fructans

The fructans content was determined spectrophotometrically by resorcinol-thiourea reagent as previously described (Petkova and Denev, 2013). The absorbance was measured at 480 nm against a blank sample prepared with distilled water.

High-performance liquid chromatography with refractive index detection (HPLC-RID) analysis of inulin and sugars

HPLC-RID methods were employed for detection of sugars (glucose, fructose, sucrose), 1- kestose, nystose and inulin in ornamental plant extracts. Analysis was performed on HPLC Shimadzu, with LC-20AD pump, analytical column Shodex® Sugar SP0810 with Pb2+ (300 mm × 8.0 mm i.d.), a guard column (50 × 9.2 mm i.d.) at 85°C, refractive index detector Shimadzu RID-10A at 30°C and software program LC solution version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan). Distilled water was used as a mobile phase with a flow rate 1.0 mL/min (Petkova et al., 2014).

RESULTS AND DISCUSSIONS

The total fructans, inulin and soluble sugars content in different vegetal part of Bulgarian honey garlic, collected from different parts of the country are summerized Table 1. It was found that in leaves of commercial plant leaves the level of fructans is the lowest one and only 1-kestose was detected. Sucrose and glucose were in the highest values in comparison to their content in bulb of *N. siculum*. It is obviously due to the participation of sucrose in photosynthesis. Fructose was detected as the dominant sugar in samardala leaves - 2.4 g/100 g DW. The highest values of total fructans were detected in samardala bulb, as their content varied between 24.5 to 13.6 g/100 g DW depending from sea level. The highest fructans and inulin content was detected in samples collected from Strandzha mountain and Central Stara Planina mountain. Inulin content in this sample was 24.5-23.5 g/100 g DW, while the lowest levels of it were found in samples collected near to Black Sea coast - 7.4 g/100 g DW. From fructooligosacchrides were detected nystose and 1-kestose, while from sugars sucrose, glucose and fructose were presented. Glucose in the bulb was in the lowest levels - 0.1 g/100 g DW, followed by fructose (0.1-0.4 g/100 g DW). Sucrose varied between 0.3-0.8 g/100 g DW, 1-kestose was in the highest values in samples from the mountain regions, as well as nests. The highest values of nystose were found in samardala bulb collected form Strandzha mountain - 7.9 g/ 100 g DW.

Additionally, the detailed fructans profile is shown in the samardala bulb (Figure 1, spot 6) and (Figure 3 a). It is obvious that samardala bulbs contain not only inulin and sugars (Figure 3a), but also many fructoolysosacchrides - 1-kestose, nystose, fructofuranosyl-nystose (GF4) and others (Figure 1).

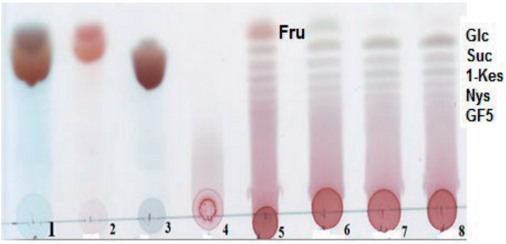

Figure 1. TLC of water extracts from *Allium* sp., where: 1 - glucose; 2 - fructose; 3 - sucrose; 4 - inulin; 5 - fructooligosacchrides from agave, 6 - samardala bulb (*N. siculum*), 7 - *Allium* 'Gladiator' and 8 - *Allium* 'Globus'

This study is the first detailed report about the presence of fructan, fructooligosacchrides and inulin in the bulbs of *N. siculum*. Moreover, the leaves of samardala typically used in culinary as spices showed a similar fraction profile with leaves of *A. schoenoprasum*, as in both 1-kestose were the main representatives of fructooligosacharides (Petkova et al., 2019).

The detail data about the presence of fractions and sugars in different plant organs of 12 ornamental plants are shown in Table 2. The biological material have been presented before. From the obtained results it was found that the bulb of *Allium* 'Gladiator', *Allium* "Globus" and *Allium* large flower mix presents a promising source of total fructan 13-11 g/100 g DW. *Allium sphaerocephalon* and *Allium aflatunence* contain more than 60% less fructan than above mentioned Allium species. The total fructan in them is below 5 g/100 g DW, as inulin content is below 3 g/100 g DW (Table 2). In *Allium* 'Gladiator' and *Allium* 'Globus' were detected inulin, sucrose, glucose, fructose, nystose, 1-kestose and other fructooligosacchrides (Figure 1 spots 7 and 8 and Figure 3). Our observation enriches the information and distribution of fructan and inulin. Fructans were present in some *Allium* species. Ranwala and Miller (2008) found that 25 geophytes investigated possessed concentrations of fructans ranged from 23 to 508 mg g^{-1} DW. From them *Allium* species showed the highest concentration of fructans (about half of the DW). *Allium caeruleum*, *Allium christophii*, *Allium hollandicum* 'Purple Sensation', *Allium karataviense*, 'Ivory Queen, *Allium neapolitanum* and *Allium sphaerocephalon* were investigated and fructan content in them varied from 508-439 mg/g DW (Ranwala and Miller, 2008). In these Allium representatives only sucrose, glucose and fructose were detected. In our research *Allium sphaerocephalon* bulbs contained 4.20 ± 0.07 g/100 g DW fructans which are approximately ten times less than the report of Ranwala and Miller (2008). Similar to their report only sugars were detected, therefore this *Allium* did not so much accumulate fructooligosacchides, but mainly high molecular inulin. *Allium* 'Globus' is a hybrid between *Allium karataviense* and possibly *Allium cristophii* (https://www.pacificbulbsociety.org/pbswiki/index.php/Allium_Globus). *Allium* 'Globus' showed 5 times less fraction content than reported by Ranwala and Miller (2008).

It was found that in costmary leaves were detected only sugars, while in leaves of *L. martinezii* similar to samardala leaves contains except glucose, fructose and sucrose, but also 1-kestose (Table 2). According to Derakhshani et al. (2011), the levels of soluble sugars in leaves of *T. balsamita* reached 136-179 mg/g fresh weight. In our study, sugar content in *Tanacetum balsamita* was 1.48 g/ 100 g DW. Marigold (*C. officinalis*) roots and bulbs of *L. martinezii* contained inulin, nystose, 1-kestose, sucrose, glucose and fructose. This is the first report about presence of inulin and sugars in this both plants. Inulin content in bulb of *Lapiedra martinezii* Lag. was 6.82 ± 0.11 g/100 g DW, while its content in marigold roots was twice lower - 3.51±0.12 g/100 g DW. Bulbs of tulip ('Burgundy' and 'Aguila') and hyacinths ('Jan Bos' and 'Peter Stuyvesant') were evaluated for fructans and sugars content. It was found that tulip bulb contains mainly sugars and small amout of inulin (below 3 g/100 g DW) (Table 2). The detailed profile of fructans and soluble sugars content in bulb of different tulip and hyacinth representatives was shown

(Figure 2 and Figure 3). According to Parkin (1899) the inulin in the hyacinth is very similar to that of the garlic. The highest inulin content was found in hyacinth 'Peter Stuyvesant' - 4.00 g/100 g DW, while total fructans in its bulb was 6.04 g/100 g DW. It was reported that in bulbs of narcissus (*Narcissus hybrids* Hort), onion (*Allium victorialis* L.) fructan content reached 19.8%, while in lily, onion, tulip, hyacinth, camash (wild hyacinth) (*Camassia quamash*) was in the range 12-22% (Bagaoutdinova et al., 2001).

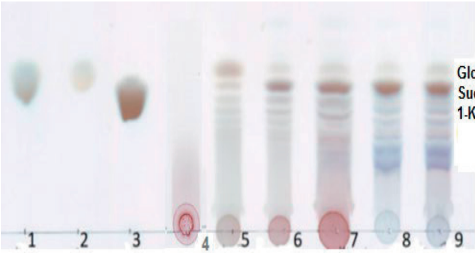

Figure 2. TLC of water extracts from the bulbs of ornamental plants, where: 1 - glucose; 2 - fructose; 3 - sucrose; 4 - inulin; 5 - fructooligosacchrides from agave; 6 - Hyacinth 'Peter Stuyvesant'; 7 - Hyacinth 'Jan Bos'; 8 - tulip 'Burgundy'; 9 - tulip 'Aguila'

Table 1. Quantity of sugars and fructans in Bulgarian honey garlic or samardala (*Nectaroscordum siculum* Lindl.), g/100 g DW

Samples	Location	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
Samardala leaves	Commercial	4.5±0.1	n.d.	n.d.	0.7±0.1	0.9±0.2	0.8±0.2	2.4±0.2
Samardala bulb 1	Strandzha mountain	24.5±0.2	14.5±0.3	7.9±0.3	1.0±0.1	0.8±0.3	0.1±0.1	0.2±0.1
Samardala bulb 2	Black Sea coast	13.6±0.1	7.4±0.2	1.9±0.3	0.5±0.1	0.3±0.1	0.1±0.1	0.4±0.1
Samardala bulb 3	Eastern Stara Planina mountain	20.1±0.2	12.6±0.4	5.1±0.3	1.0±0.2	0.6±0.1	traces	0.3±0.1
Samardala bulb 4	Central Stara Planina mountain	23.5±0.3	17.1±0.1	4.0±0.3	0.8±0.1	0.5±0.1	traces	0.2±0.1

n.d. = not detectable. Values are means ± SD, n=3

Table 2. Fructans and sugars content in different ornamental plants, g/100 g DW

Plant	Family	Plant organs	Total fructans	Inulin	Nystose	1-Kestose	Sucrose	Glucose	Fructose
Allium sphaerocephalon	Amaryllidaceae	bulb	4.20±0.07	1.95±0.01	n.d.	n.d.	0.18±0.05	0.10±0.02	0.56±0.02
Allium aflatunence	Amaryllidaceae	bulb	4.78±0.17	2.51±0.01	n.d.	0.11±0.01	0.08±0.03	n.d.	0.51±0.06
Allium 'Gladiator'	Amaryllidaceae	bulb	11.21±1.07	7.42±0.05	0.05±0.01	0.12±0.02	0.21±0.05	0.53±0.06	2.23±0.11
Allium 'Globus'	Amaryllidaceae	bulb	10.17±1.417	7.21±0.05	0.02±0.01	0.92±0.02	1.01±0.02	1.65±0.03	2.44±0.08
Allium large flower mix	Amaryllidaceae	bulb	12.42±1.14	6.21±0.12	1.03±0.02	0.92±0.03	1.72±0.02	0.62±0.04	1.52±0.06
Tanacetum balsamita	Asteraceae	leaves	0.47±0.15	n.d.	n.d.	n.d.	1.28±0.12	0.16±0.03	0.04±0.01
Calendula officinalis L.	Asteraceae	roots	9.71±0.45	3.51±0.12	0.51±0.03	0.32±0.02	2.23±0.05	1.21±0.04	2.25±0.12
Lapiedra martinezii Lag.	Amaryllidaceae	bulb	7.84±0.15	6.82±0.11	2.63±0.02	2.72±0.06	4.55±0.02	0.44±0.06	2.43±0.05
		leaves	n.d.	n.d.	n.d.	2.12±0.02	2.96±0.05	2.14±0.07	4.22±0.02
Tulip 'Burgundy'	Liliaceae	bulb	3.39±0.15	1.54±0.12	0.51±0.02	n.d.	2.14±0.05	0.14±0.02	n.d
Tulip 'Aguila'	Liliaceae	bulb	3.89±0.21	2.72±0.09	n.d.	n.d.	3.03±0.02	0.32±0.01	0.11±0.02
Hyacinth 'Jan Bos'	Asparagaceae	bulb	3.73±0.18	2.42±0.11	0.41±0.11	0.22±0.11	0.63±0.11	n.d.	n.d.
Hyacinth 'Peter Stuyvesant'	Asparagaceae	bulb	6.04±0.34	4.00±0.12	n.d.	0.22±0.11	0.32±0.11	0.71±0.11	0.11±0.11

n.d. = not detectable, values are means ± SD, n=3

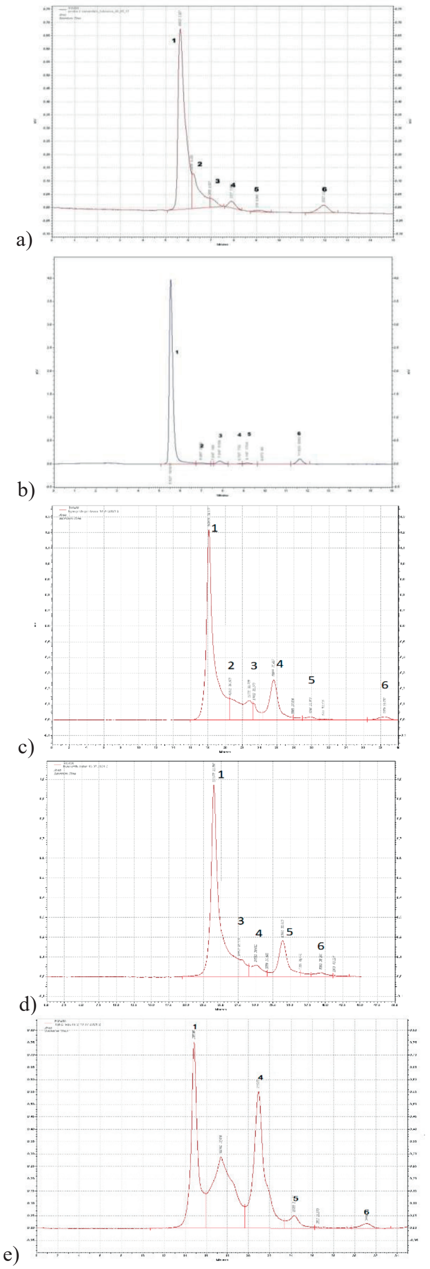

Figure 3. HPLC chromatograms of water extracts from different ornamental plants, as follows: a) samardala (*Nectaroscordum siculum* Lindl.) - bulb 1; b) Allium 'Globus'; c) hyacinth 'Jan Bos'; d) hyacinth 'Peter Stuyvesant'; e) tulip 'Aguila', where: 1 - inulin, 2 - Nystose, 3 - 1-kestose, 4 - Sucrose, 5 - glucose and 6 - fructose

Fructans content in *Hyacinthus orientalis* (‘Carnegie and ‘Pink Pearl’) reached 284 mg/g DW (Ranwala and Miller, 2008). In our studies fructans in bulb of hyacinth 'Jan Bos' and 'Peter Stuyvesant' was 3.73 and 6.04 g/100 g DW. Our data for fructans in hyacinth bulb were lower than reports of some authors (Bagaoutdinova et al., 2001; Ranwala and Miller, 2008), but content of glucose and sucrose were comparable with the study of Ranwala and Miller (2008). However, Enciu et al. (2021) detected only glucose and fructose in the bulbs of hyacinth 'Jan Bos' between 24 and 26%. However, fructans content in tulip bulbs was comparable to reported values for fructans in *Tulipa gesneriana* ‘Apeldoorn’ and ‘Monte Carlo’, *Tulipa tarda* and *Tulipa turkestanica* - 23-54 mg/g DW (Ranwala and Miller, 2008). The lowest inulin and fructans content were detected in bulb of tulip 'Burgundy' - 1.54 and 3.39 g/100 g DW, respectively.

The differences in fructan and inulin values in the bulb of *Allium* species, tulip and hyacinth could be explained by environmental and storage conditions, age of the plant, and time of harvest. In case of hyacinth bulb, their exposure to low temperatures leads to increased glucose, fructose and sugar levels (Koksal, 2010). Onion bulbs cv ‘Hyduro’ decrease fructans content during storage as form 3 weeks fructans content from 39.1 g/100 g DM can decrease to 16 g/100 g DM after storage period 40 weeks (Hansen, 1999).

CONCLUSIONS

In the current study thirteen ornamental plants were investigated for fructans and sugar content. It was found that the leaves of *Tanacetum balsamita* and *Lapiedra martinezii* contains only sugars, as glucose, fructose and sucrose. However, bulbs and roots of other investigated representatives, except sugars contains fructooligosacchides and inulin, as reserve polysaccharides. The highest inulin content was found in bulb of samardala, followed by *Allium* 'Gladiator', *Allium* 'Globus' and *L. martinezii* bulbs. To the best of our knowledge this is the first report for evaluation of carbohydrates profile and fructans content in *N. siculum*, *T. balsamita* and some *Allium* representatives. The present research enrich the information about fructans and inulin content in hyacinth, tulip and some other *Allium* species and reveals the potential of bulbs of samardala, *L. martinezii* and some *Allium* species as a potential source of fructans.

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