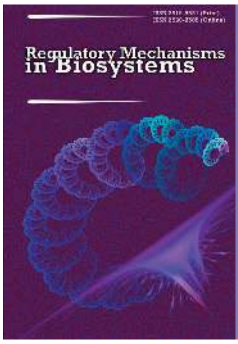

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Biochemical characterization of fruits of *Lycium* spp. in Ukraine

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Fruits of Lycium possess therapeutic properties due to which they are used in traditional and folk medicine and can be used as a kind of functional food. The objective of this study was to evaluate the biochemical characterization of *Lycium* L. (*L. barbarum* L., *L. chinense* Mill. and *L. truncatum* Y. C. Wang) fruits for 16 cultivars and varieties from the collections in the M. M. Gryshko National Botanical Garden of NAS of Ukraine (Kyiv). This study was aimed at determining the concentration of nutrients in the *Lycium* fruits. Individual genotypes of three *Lycium* species: *L. barbarum*, *L.chinense*, and *L. truncatum*, differed in such features as the content of dry matter, sugars, vitamin C, β-carotene, acidity, and tannins in the fruit. Fruits of *Lycium* spp. are a valuable source of nutrients such as vitamin C (4.38–121.0 mg $100g^{-1}$ FW), β-carotene content (1.45–5.52%), and tannin (0.12–1.34%). The sugar content (13.83–20.87%) and acidity of the fruit (0.23–4.62%) meet the consumers' requirements for fresh fruit. The cultivar Amber Sweet (*L. chinense*) had fruits of which the similarities between biochemical characteristics of different studies genotypes were the lowest. The cv. Amber Sweet was characterized by fruit with high sugar content, very high vitamin C content, average acid content, low tannins and β-carotene content, and the lowest dry matter content. Furthermore, a distinctive feature of the other tested genotypes was the yellow colour of the fruit. The data obtained can be used for further selective work.

Keywords: goji berry; cultivars; varieties; fruits; biochemical composition.

Introduction

Some neglected and underutilized species of plants, namely, *Cydonia oblonga* Mill. (Monka et al., 2014), *Sambucus nigra* (Horčinová Sedláčková et al., 2018, 2019), *Cornus mas* L. (Klymenko et al., 2019), *Aronia mitschurinii* A. K. Skvortsov & Maitul. (Vinogradova et al., 2020), containing biologically active compounds with human health-enhancing properties known for hundreds or thousands of years have experienced a renaissance and have aroused interest in the world of science in the last decade (Klymenko et al., 2017; Konarska, 2018). It is also very important to introduce and grow lesser-known and non-traditional plant species, namely, *Pseudocydonia sinensis* (Thouin) C. K. Schneid. (Monka et al., 2014; Grygorieva et al., 2020), *Castanea sativa* Mill. (Grygorieva et al., 2017), *Ziziphus jujuba* Mill. (Ivanišová et al., 2017), and *Elaeagnus multiflora* Thunb., *Mespilus germanica* L., *Diospyros virginiana* L. (Grygorieva et al., 2018a, 2018b, 2018c). Species of the genus *Lycium* L. also belong to this group of plants, since they have long been used in Chinese traditional and folk herbal medicine due to their therapeutic properties.

The genus *Lycium* includes about 92(97) species, of which 35 plant species are used for food or as medicines (Yao et al., 2018). Of these, only the fruits of two species from east Asia, namely *L. barbarum* L. and *L. chinense* Mill. have been consumed in Chinese medicine in the capacity of 'superfood' for over 2000 years (Amagase & Farnsworth, 2011; Yao et al., 2018).

The goji fruits are used in fresh and dried condition and from them are prepared juices, wine, preserves, the substitute of tea made of leaves, processed as tinctures, powders, tablets, consumed in soups, as porridge with rice and added to numerous meat and vegetable dishes (Potterat, 2010; Yao et al., 2018). The *Lycium* fruits contain phenolics, flavonoids, organic acids, and fatty acids (Wang et al., 2010; Mikulic-Petkovsek et al., 2012) and are also high in vitamins such as thiamine, riboflavin, and vitamin C (Donno et al., 2014; Niro et al., 2017), carotenoids (Chang et al., 2015), polysaccharides (Luo et al., 2004; Wang et al., 2010), betaine (Xie et al., 2001; Lee et al., 2014; Qian et al., 2017), and taurine (Potterat, 2010), have high mineral concentrations (Grembecka & Szefer, 2013). With such a rich biochemical composition, goji fruits are very widely used in pharmacology due to their high anti-cancer (Tang et al., 2012; Cumaoglu et al., 2018), anti-hyperglycemic (Potterat, 2010; Wojdyło et al., 2018), anti-inflammatory (Liu et al., 2015; Wang et al., 2017), anti-aging properties (Chang et al., 2015; Wojdyło et al., 2018), and antioxidant properties (Illić et al., 2020). Not only do the fruits contain biologically active substances, but also other parts of plants, especially leaves (Chen et al., 2020; Grygorieva et al., 2020; Szot et al., 2020). The nutritional properties of *Lycium* fruits grown in Ukraine have not been investigated. Therefore, the present study aimed to investigate for the first time, the biochemical composition of the fruits of *Lycium* spp. grown in Ukraine.

Materials and methods

Plants of *Lycium* (Fig. 1) were grown in M. M. Gryshko National Botanical Garden of NAS of Ukraine in Kyiv (NBG) from seeds or cuttings obtained from China, France, Slovak Republic, and other botanical gardens of Ukraine. There 16 genotypes were investigated in an experimental study 2019, including three species (*Lycium barbarum*, *L. chinense*, *L. truncatum*) and 8 cultivars and 8 varieties (LB01–LB03, LC01–LC05). Samples were marked as LB (*L. barbarum*), LC (*L. chinense*).

The dry matter determination was conducted according to the procedure described by Ogbonna et al. (2016). The method is based on the measurement of decrease in mass of air-dry matter. Fixed mass (approximately 5 g) of fresh shredded raw fruit was dried in aluminum containers at 105 °C in an oven till constant weight. Before this procedure, the cleaned containers were dried for 30 min at 105 °C and cooled in a desiccator and then the samples weighed. Results were given in percent. The procedure was conducted with fresh raw material. In this case, 4 g of shredded raw fruit was mixed with distilled water in a volumetric flask (100 mL) and boiled till 70 °C. After cooling down, 1.5 mL of 30% lead acetate was added to the mixture for 12 hours. After this, 1 mL of phosphate buffer was added to the mixture, which was shaken. The obtained solution was left for 30 min. After filtration, 50 mL of extract was mixed with 8 mL of 20% HCl and heated to 70 °C. After cooling, neutralization of plant extracts with 12% NaOH solution was conducted. 3 mL of plant extract was mixed with 6 mL Fehling's reactive solution and boiled for 6 min. Before titration with 0.01 N $KMnO_4$, 10 mL of iron-ammonium alum was added. Results are given in percent (Krishchenko, 1983).

Fig. 1. Fruits of *Lycium* spp. of the NBG collection

The reducing properties of ascorbic acid underlie this method. 2 g of fresh mass was mixed with 50 mL of the acid mixture (1% HCl and 2% oxalic acid) and homogenized. The obtained extracts were put into darkness in 20 min and after this filtered with paper. 10 mL of obtained filtrate was poured into another flask that was titrated by the 0.001 N solution of sodium 2.6-dichlorophenolindophenolate. Results are given in mg/100 g FW (Krishchenko, 1983).

The determination of ß-carotene was conducted in gasoline Galosh extracts. 1 g of absolutely dried raw mass was extracted in 20 mL of gasoline Galosh for 12 hours. Also, 0.2 g of aluminum oxide and 0.05 g of calcium oxide were added to the extracts to connect pigment. After filtration, the density of extracts was measured on a spectrophotometer Unico UV 2800 at the wavelength 440 nm. Absorbance was measured with pure gasoline Galosh. The solution of $K_2Cr_2O_7$ was used as standard, the density determined as 0.00416 mg of carotene in 1 mL (Pleshkov, 1985).

The method of determination of titratable acidity is based on sodium hydroxide titration of free acids in water plant extracts. 5 g of the fresh mass of sample was mixed with approximately 70 mL of distilled water in a volumetric flask (100 mL). Flasks were placed in a water bath at 80 ºC for 15 min with periodic shaking. After cooling, the flasks were filled with water to 100 mL. After filtration, 50 mL of extract was titrated with 0.1 N NaOH in presence of phenolphthalein (two drops). Obtained results were given in percent (Krishchenko, 1983).

For determination of tannin content 5 g of fresh mass was mixed with distilled water in volumetric flasks (100 mL) and put into the water bath for 2 hours at 80 ºC. After the cooling procedure, distilled water was added to flask content till 100 mL and filtered. 10 mL of obtained filtrate was poured into another flask (1 L), and 750 mL of water, and 25 mL of indigo carmine solution were added. The obtained mixture was titrated by 0.1 N $KMnO_4$. At the same time the same procedure was conducted but with 2 g of activated carbon. The difference between sample titration with activated carbon and without corresponds to $KMnO_4$ volume used for oxidation of tannins in this solution (Krishchenko, 1983).

Basic statistical analyses were performed using PAST 2.17 (Norway, 2001); the results are expressed as mean values of three replications ± standard deviation (SD); hierarchical cluster analyses of similarity between phenotypes were computed on the basis of the Bray-Curtis similarity index. Data were analysed with ANOVA test and differences between means compared through the Tukey-Kramer test ($P < 0.05$).

Results

One of the numerous biochemical parameters that demonstrate the value of plant raw material is dry matter content. The data in this study concerning dry matter content ranged between 13.8% (*L. chinense* cv. Amber Sweet) and 20.9% (*L. barbarum* LB03, Fig. 2).

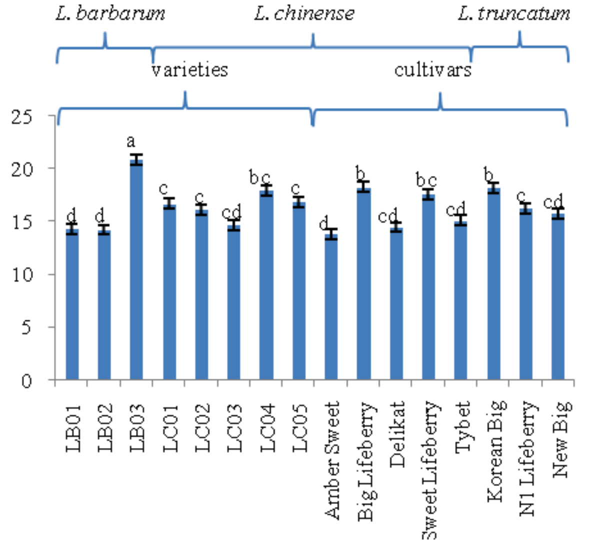

Fig. 2. Content of dry matter of fruits of species of the genus *Lycium* cultivars and varieties (%, means in each column followed by different letters are not significantly different, $P < 0.05$)

The content of sugars, as is known, is an important characteristic of fruit taste. The total sugar contents of the 16 *Lycium* fruits samples were determined from 3.6% (*L. truncatum* cv. New Big) to 11.7% (*L. truncatum* cv. Korean Big, Fig. 3). In this respect, varieties and cultivars of *Lycium* varied greatly.

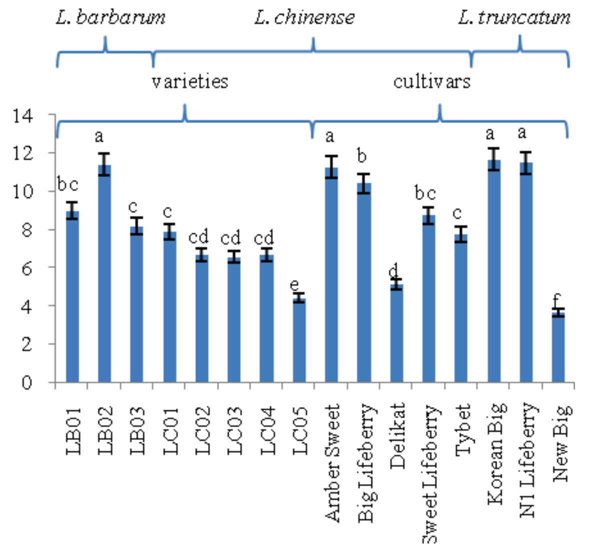

Fig. 3. Total sugar content of fruits of species of the genus *Lycium* cultivars and varieties (%, means in each column followed by different letters are not significantly different, $P < 0.05$)

Significant differences were found in the concentration of vitamin C between the species, cultivars, and varieties in this study. The vitamin C content ranged between 4.38 (*L. truncatum* cv. New Big) and 121.0 $mg \cdot 100 g^{-1}$ (*L. chinense* cv. Amber Sweet, Fig. 4). The fruits of *L. truncatum* had the lowest vitamin C content in comparison with other species.

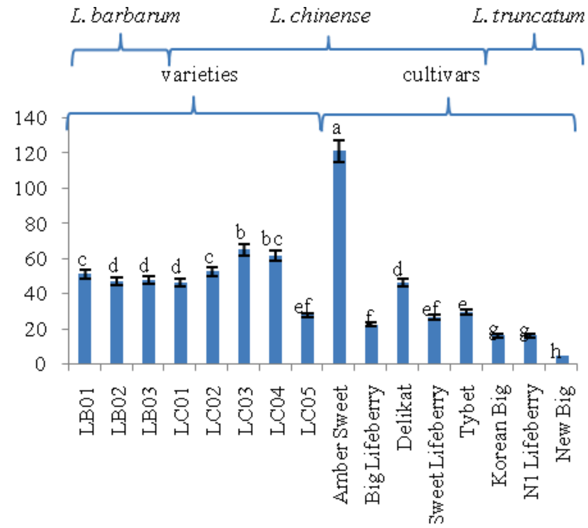

Fig. 4. Vitamin C of fruits of species of the genus *Lycium* cultivars and varieties (mg·100 g^{-1} FW, means in each column followed by different letters are not significantly different, P < 0.05)

Alongside ascorbic acid content, β-carotene is not a less important vitamin with high biological activity. The total β-carotene content of *Lycium* fruits is recorded in Figure 5. The lowest content was observed in *L. chinense* cv. Amber Sweet (1.45 mg·100 g^{-1} FW), while *L. barbarum* (LB02) (5.52 mg·100 g^{-1} FW) exhibited the highest carotene content. The lowest content of this pigment in fruits of *L. chinense* cv. Amber Sweet is explained by the fact that fruits of this variety are yellow.

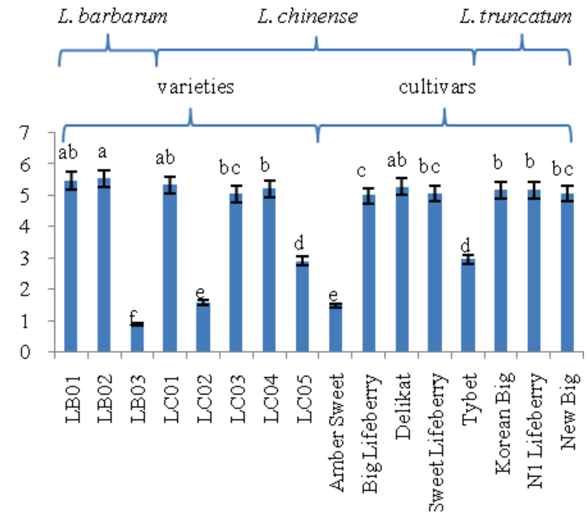

Fig. 5. β-carotene content of fruits of species of the genus *Lycium* cultivars and varieties (mg·100 g^{-1} FW, means in each column followed by different letters are not significantly different, P < 0.05)

The titratable acidity of all fruits ranged 0.23–4.62% (Fig. 6). The fruits of *L. chinensis* (cv. Sweet Lifeberry) were the most acidic with a titratable acidity value of 4.62% while the fruits of *L. barbarum* (LB03) were the least acidic with a titratable acidity value of 0.23%. The titratable acidity values of *L. barbarum* (LB01, LB02, LB03) were characterized by low titratable acidity (<0.8%), which showed that *L. barbarum* genotypes had a better taste than other genotypes tested.

The tannin content of 16 *Lycium* fruits samples is presented in Figure 7. The relatively higher content of tannin was recorded in *Lycium* fruits samples *L. chinensis* cv. Sweet Lifeberry, *L. truncatum* cv. N1 Lifeberry, and LC05 (1.34%, 1.34%, and 1.12%, respectively) while the lowest content of tannin was recorded in samples *L. chinensis* cv. Amber Sweet, *L. chinensis* cv. Tybet, and *L. barbarum* LB01 (0.29%, 0.24%, and 0.12%, respectively). All other samples had tannin content ranging from 0.34% to 0.77%. Correlation analysis was used to explore the relationships between the biochemical characteristics measured for all fruit extracts from *Lycium* cultivars and varieties (Table 1). Strong positive correlation was found only between tannin content and titratable acidity (r = 0.729, P < 0.001). Weak positive correlation was found between β-carotene content and tannin (r = 0.219, P < 0.05), β-carotene and titratable acidity (r = 0.246, P < 0.05), total sugars content and titratable acidity (r = 0.246, P < 0.05), and dry matter and tannins (r = 0.421, P < 0.01).

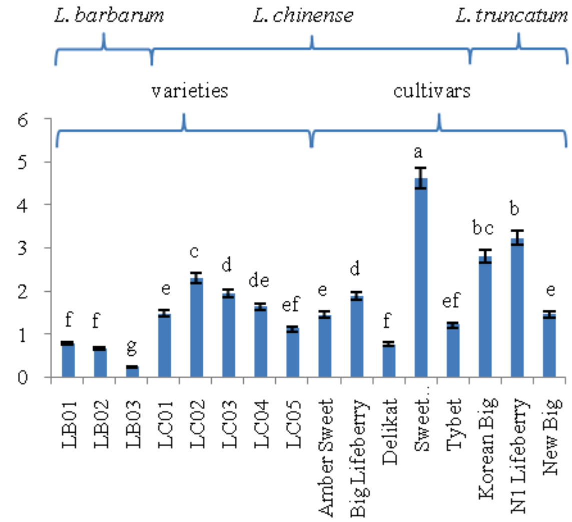

Fig. 6. Titratable acidity content of fruits of species of the genus *Lycium* cultivars and varieties (%, means in each column followed by different letters are not significantly different, P < 0.05)

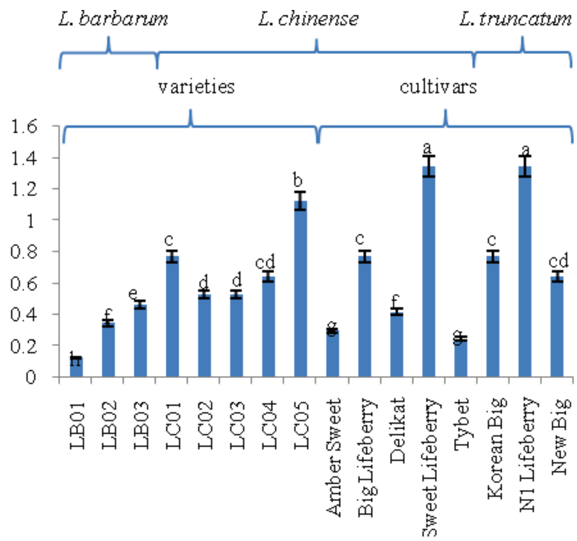

Fig. 7. Tannins content of fruits of species of the genus *Lycium* cultivars and varieties (%, means in each column followed by different letters are not significantly different, P < 0.05)

Table 1
Correlation coefficients of a linear relationship between the biochemical characteristics of tested *Lycium* genotypes

Parameter	Dry matter	Total sugar content	Ascorbic acid	β-carotene	Titrable acidity	Tannins
Dry matter	1	0.030	–0.370*	–0.208	0.166	0.421**
Total sugar content	0.030	1	0.159	0.074	0.264*	0.026
Ascorbic acid	–0.370*	0.159	1	–0.406**	–0.288*	–0.505**
β-carotene	–0.208*	0.074	–0.406**	1	0.246*	0.219*
Titrable acidity	0.166	0.264*	–0.288*	0.246*	1	0.729
Tannins	0.421**	0.026	–0.505**	0.219*	0.729***	1

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001.

Hierarchical cluster analysis can be used as useful tools for screening samples to study the similarities between biochemical characteristics of different plants. Hierarchical cluster analysis biochemical characterization was used to evaluate the collected 16 genotypes of Lycium spp (Fig. 8). A comparison clearly shows the different genotypes and grouping and the significant differences between them. The similarity coefficient for different genotypes was in the range 0.55–0.95.

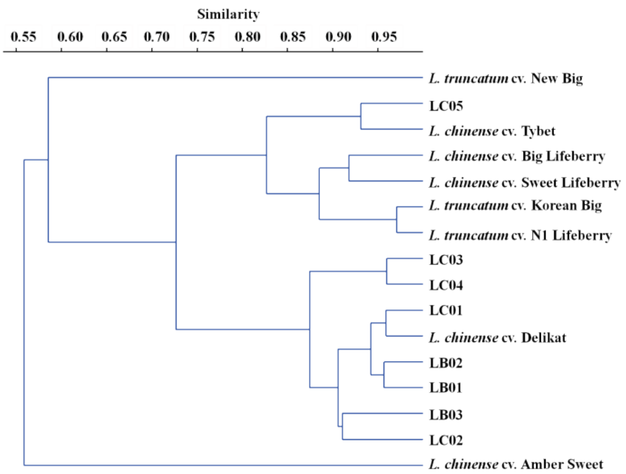

Fig. 8. Dendrogram from hierarchical cluster analysis of the biochemical characterization of 16 genotypes of *Lycium* spp.

The dendrogram that was generated by cluster analysis showed four well-defined groups. The first and fourth clusters include only one sample (*L. truncatum* cv. New Big and *L. chinense* cv. Amber Sweet) with a similarity value of 0.57 and 0.55, respectively. The first cultivar had the lowest total sugar and ascorbic acid content. The second cultivar was distinguished by the highest content of ascorbic acid and the lowest dry matter. The second cluster includes one variety and 5 cultivars, namely, LC05 (*L. chinense*) and Tybet, Big Lifeberry, Sweet Lifeberry (*L. chinense*), Korean Big, N1 Lifeberry (*L. truncatum*) with a similarity value of 0.83. The third cluster was the biggest one and comprised eight samples, namely, LC01-LC04, cv. Delikat (*L. chinense*), LB01-LB03 (*L. barbarum*) with a similarity value of 0.92.

Discussion

The study of the biochemical composition of fruit plants is a very important parameter of raw plant evaluation, especially as a raw food. Fruits contain numerous compounds with different nature, structure, and nutritive value depending on the biochemical composition in its raw state (Fourie, 1996). The most important components of fruits are water, proteins, minerals, sugars, fats, and vitamins. The assessment of different characteristics, including biochemical, of fruits as well as vegetables has a practical value for human health (Wallace et al., 2020). The biochemical composition of fruits *Lycium* depends on many factors, such as the genetic characteristics, the maturity stage of the fruit, the cultivation in which they have been grown, soil conditions, geographic location (Niro et al., 2017). According to the literature, different environmental cultivation also influences the nutritional composition and bioactive compound of the fruits *Lycium* (Liu et al., 2015).

Much attention should be paid to focus on fruits' dry matter concentration, as it relates to maturity, consumer preference in itself, and flavour potential of the fruit (Harker et al., 2009; Palmer et al., 2010). Comparison with other studies showed that dry matter content for *L. chinense* was 11.9% and for *L. barbarum* 22.7–23.3% (Dumont et al., 2020).

The sugars of many fruits and vegetables are important components of taste, play important roles in maintaining fruit commodity quality and determining nutritive value (Ashoor & Knox, 1982; Lu & Zhang, 2000; Gong & Zhang, 2003; Wu et al., 2012; Zhao et al., 2015). The dominant component in the sugar profile is glucose (152.92–284.60 g/kg), fructose (154.20–259.13 g/kg), and lower sucrose content (13.75–36.43 g/kg) as reported by Montesano et al. (2016) in dry fruit of *L. barbarum* marketed in China, and Zheng et al. (2010) in *L. barbarum* and *L. chinense* varieties cultivated in different regions in China. The main composition of *Lycium* sugars are sucrose, fructose, erythrose but the content of different components can change depending on the stage of growth. Content of sucrose decreased with fruit development (Zhao et al., 2015). According to Zheng et al. (2010), after blossom during 24–34 days in fruits increase of glucose and fructose level from 50 to 250 g/kg DW and decrease of sucrose from 50 to 10 g/kg DW was noticed in the fruits. Total sugars (350.4 mmol/kg) (Mikulic-Petkovsek et al., 2012). Goji berries are mainly used in dried condition. Adiletta et al. (2015) found that, regardless of the method of drying goji fruit, the level of fructose decreased, while the content of glucose and trehalose was similar to that in fresh fruit. Genotypes with low content of sugars can be recommended for the creation of special products for diabetics. There is evidence that the sugar content varies with weather and soil conditions (Hecke et al., 2006).

Ascorbic acid is a water-soluble vitamin that plays an important role as a powerful antioxidant and is found in many biological systems. Content of vitamin C is one of the most valuable nutritional quality parameters of many horticultural crops and a necessary component in human vital activity (Diep et al., 2020; Kulaitiene et al., 2020). The content of this vitamin in foodstuffs and beverages is a relevant quality indicator, which can change during the storage process (Pisocschi et al., 2008). Data concerning vitamin C content ranged between 33.15 and 113.8 mg·100 g^{-1}, with an average value of 55. mg·100 g^{-1} (Yossa Nzeuwa et al., 2019). Similar data found in reports by Emine Kocyigit et al. (2017) and Donno et al. (2014), while a much lower value of this parameter was determined by Cheng et al. (2015). As reported by Donno et al. (2014), vitamin C content in different *Lycium* fruit cultivars was on average 48.94 mg·100 g^{-1}, which makes up approximately 60% of the RDA. Numerous studies have reported that *L. barbarum* berries accumulated vitamin C in the range from 30 to 60 mg·100 g^{-1} FW (Vulić et al., 2016; Niro et al., 2017; Kafkaletou et al., 2017, 2018; Montesano et al., 2018) depending on cultivars and growing regions (Donno et al., 2014; Wojdyło et al., 2018; Kulaitiene et al., 2020). It should be noted that some studies represented vitamin C content in some goji berry varieties ten times lower than our results (Wojdyło et al., 2018; Kulaitiene et al., 2020).

Among other biologically active compounds which are contained in goji berries in the amount of 0.03–0.50% are carotenoids that possess health-promoting properties (Kulaitiene et al., 2020). β-carotene is a red-orange pigment that belongs to a group of carotenoids and possess antioxidant and antiradical activity as well as ability to fight cardiovascular diseases and reduce the risk of cancer (Britton et al., 2004; Montesano et al., 2008; Mordente et al., 2011; Fattore et al., 2016). The carotenoids serve as major micronutrients in the human diet (Fraser & Bramley, 2004; Rao & Rao, 2007). In particular, β-carotene and β-cryptoxanthin are important components as predecessors for vitamin A biosynthesis (Weber & Grune, 2012), while lutein and zeaxanthin slow aging-related damage to the retina (Sabour-Pickett et al., 2012). Carotenoids have been shown to be present in large quantities in fruits of *L. barbarum* (Weller & Breithaupt, 2003; Liu et al., 2014; Montesano et al., 2020). Currently, these berries are known as the main source of zeaxanthin dipalmitate, since they represent an average of over 85% of the total carotenoids present in the fruit (Karioti et al., 2014).

Vulić et al. (2016) employed chromatographic analysis to identify and quantify carotenoids, and it was found that the content was 3.52 mg·g^{-1} of methanol extract. As reported Nien-Chen et al. (2011), the carotenoids' content in goji fruit varied from 4.27 to 5.81 mg·g^{-1} dry extract. Li et al. (1997) determined the ß-carotene content in dry goji berry 82.2 mg·g^{-1}. As reported by Merzlyak et al. (2002), a positive correlation between sunlight exposure and the carotenoid concentration in the apple was found. In addition, it has been reported that exposure to high sunlight enhanced biosynthesis increased the carotenoid concentrations (De Azevedo & Rodriguez-Amaya, 2005).

Our results on titratable acidity content were similar to those of other studies. Jatoi et al. (2018) reported that titratable acidity was between 0.90% and 0.93%. In China in mature goji berries, the titratable acidity was between 0.8% and 2.7% (Zhang et al., 2016). In Greece, the majority of goji berry cultivars had titratable acidity between 2.2% and 8.3% (Drogoudi et al., 2017). In another study conducted in Greece, titratable acidity in goji berries ranged between 0.3% and 0.4% (Kafkaletou et al., 2017). The titratable acidity of the fruit is also influenced by the harvest period and the colour of the *Lycium* fruit. *Lycium chinense* fruits contain higher titratable acidity (1.6–1.9%) than *L. barbarum* fruits (0.8–1.4%). But according to the data of Polat et al. (2020), the titratable acidity content of *L. barbarum* fruits was significantly lower (0.48–0.62%) than shown in from the previous study. The titratable acidity of the fruit is also related to the harvest period and the colour of the goji fruit. Thus, as reported by Ilic et al. (2020) in red and yellow goji fruits the titratable acidity was lower (0.70%) than in black fruits (0.89%). The highest titratable acidity was determined in *L. barbarum* fruits in September (0.84%), while the lowest was in June (0.36%) (Çolak et al., 2019).

The weak relationship between accumulation of studied biochemical parameters was obviously affected by different factors. As reported by Matias et al. (2016), in peach cultivars a weak correlation was found between titratable acidity and ascorbic acid. In another study, the total sugar content had a strong correlation with macro- and microelements (Dar et al., 2014).

The content of nutrients of *Lycium* spp. fruits can be useful for wide application in human life. Plant raw materials and different products of these species can compete with other useful fruit plants and be no less effective in various branches.

Conclusions

Thus, the investigated *Lycium* spp. fruits planted in Ukraine are a good source of essential nutrients such as vitamin C, β-carotene content, and tannins. The findings of the present study revealed that 16 *Lycium* germplasms were diverse based on biochemical assessment approaches. The results obtained will serve as a guide for the basis of genotype. Individual genotypes of 3 *Lycium* species: *L. barbarum*, *L. chinense*, and *L. truncatum* differed in investigated features. A strong positive correlation was observed only between tannin content and titratable acidity. The sugar content and titratable acidity of the fruit meet the consumer requirements for fresh fruit. The cultivar Amber Sweet (*L. chinense*) had fruits for which the similarities between biochemical characteristics of the different studied genotypes were the lowest. The cv. Amber Sweet was characterized by fruit with high sugar content, very high vitamin C content, average acid content, low tannins and β-carotene content, and the lowest dry matter content. Furthermore, a distinctive feature of the other tested genotypes was the yellow colour of the fruit,which can be used in further selective work. Also, the obtained data can be used for deep pharmacological study and possible medical purposes.

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