sciendo

10.2478/aucft-2020-0001

COMPARISON OF MORPHOLOGICAL, ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF GOJI FRUITS

– Research paper –

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Abstract: There is a growing public interest in fruits labeled as „superfood" (functional food). A "superfood" should have a high content of bioactive substances with a positive impact on human health. Seven different cultivars of goji berry fruits (*Lycium chinense* Mill.) grown in north-western Poland were evaluated for its physicochemical parameters, antidiabetic and antioxidant activity, and polyphenol content. The length of 1-year-old shoots ranged from 36 cm ('Big Lifeberry') to 82 cm ('Korean Big'). Cultivars from the group of Big were characterized by the biggest fruits (17.3-24.2 mm) with the greatest weight of 100 fruits (96.7-122.1 g). 'Big Lifeberry' contained high amounts of *L*-ascorbic acid (408 mg 1000/g) and provitamin A (190 mg 1000/g) and showed high antidiabetic (α-amylase IC_{50}=33.4 mg/mL; α-glucosidase IC_{50}=9.9 mg/mL) and antioxidant activity (ABTS·+ 6.21 and FRAP 5.58 mmol T/100 g). 'Big Lifeberry' was also characterized by a high total content of polyphenols (43.64 mg 100/g). Furthermore, the nitrite content in all the cultivars tested was at a relatively low level. Among the examined cultivars, the most attractive one concerning the consumers' point of view of the size, weight and high content of health-promoting compounds is 'Big Lifeberry'.

Keywords: *Lycium chinense* Mill., polyphenols, biological activity, antidiabetic activity, harmful substances

INTRODUCTION

An increase in the frequency of the occurrence and the need to prevent civilization diseases has led to intensified research on the use of compounds naturally occurring in plants. To date, many studies have demonstrated that lifestyle changes, exercise, and a well-balanced diet can significantly reduce the risk of human diseases. Therefore, it is good to include fruits with a high content of mineral and bioactive compounds in the diet (Kulczyński and Gramza-Michałowska, 2016).

Lycium chinense Mill. fruit (goji) in recent years has become more popular due to its public acceptance as a "superfood" with highly beneficial nutritive and antioxidant properties. It is a *Solanaceous* defoliated shrubbery, and its fruit is a famous traditional medicine, growing in the Tibetan valleys and in the Himalayas where the areas have not been polluted by civilization or by pesticides for centuries (Kulczyński and Gramza-Michałkowska, 2016). The goji fruits are called "red diamonds" because of their valuable health-promoting properties. The Latin name *Lycium* means "school"- Chinese masters of herbalism believed that goji fruit is, as a school, a source of knowledge. It has been widely used in China for medicinal purposes and as a functional food for more than 2500 years (Burke et al., 2005; Chang and So, 2008). In traditional Chinese medicine, it is believed that their consumption can prolong life and provide energy despite the passage of years-hence their name "fruits of longevity". To confirm such traditional properties, modern research indicates that *L. chinense* extracts have a number of biologic activities, including antioxidant properties (Chang and So, 2008). The extracts also exhibit antiaging

Received: 10.01.2020
Accepted in revised form: 11.04.2020

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effects, neuroprotection, promotion of endurance, increased metabolism, improved control of glucose and other diabetic symptoms, antiglaucoma effect, immunomodulation, antitumor activity, and cytoprotection (Burke et al., 2005; Wojdyło et al., 2018).

Currently, the annual cultivation of goji fruit reaches 95,000 tones and covers an area of about 82,000 ha. They are mainly used in the production of dried fruit and also in the preparation of juices, liqueurs, wines, soup additives, meat, and vegetarian dishes, and tea. Besides, fruit extracts are used as natural, non-toxic dyes for beverages and in cosmetics (Bogacz, 2009).

To date, studies on goji berry plants (*L. chinense*) grown in the field in the northern part of Europe are scarce. Therefore, we have undertaken to compare seven cultivars of goji fruits grown in the climatic conditions of north-western Poland. This research will provide evidence to select shrubs, which are characterized by the most valuable fruit in terms of antidiabetic and antioxidant activity, with the highest content of polyphenols, as well as with the best morphological and physicochemical properties.

MATERIALS AND METHODS

Characteristic of the area of research

The studies were carried out in the Laboratory of Orchard at the Department of Horticulture, West Pomeranian University of Technology in Szczecin in 2015-2018. The research station is located in the north-west part of Poland. According to Heinz and Schreiber's Map of zones, the minimum temperature in this region range from –12 ºC to –15 ºC and in the growing season (April-October) between 1951 and 2012 was 13.7 ºC and rainfall 391 mm (Mijowska et al., 2017, Ochmian et al., 2019). The wheatear during the experimental period significantly differed from the average temperature and rainfall for many years (Table 1). During the experiment, a higher average temperature was found during the growing season, especially in 2018, compared to many years. At the same time, much less rainfall was observed.

The soil in the orchard was an agricultural soil with a natural profile, developed from silt-loam with a considerably lower density of 1.21 mg/m^3, pH 6.9 and the higher water capacity. It also contains much more organic matter – 33.1 g/kg soil. Regardless of the stand, the soils were characterized by low salinity – EC 0.35-0.43 mS/cm, high content of P 78 mg/kg (optimum 20–40 mg/kg), K 164 mg/kg (optimum 50–80 mg/kg) and magnesium 52 mg/kg (optimum 25–40 mg/kg), in comparison to the optimal mineral content of the soil by Sadowski et al. (1990). Every spring, nitrogen fertilization was applied at a rate of 90 kg N per hectare.

The plantation was irrigated annually using a permanently-installed T-Tape drip irrigation line with the emitters performance of 1.5 L per 1 hour (3 L of water on a section of 1 linear meter of the installation). Soil moisture was maintained in the PF 1.8–2.1 range and was determined by contact tensiometers.

Plant material

The fruits of seven cultivars of *Lycium chinense* Mill. where used in this study. The research material contained cultivars: No '1', New Big, Sweet Berry, Big Berry, Big Lifeberry, Korean Big, Amber Sweet Goji. Shrubs were planted in the spring of 2010 in the orchard of Department of Horticulture in Szczecin, Poland.

Table 1. Weather conditions during the vegetative season (April-October) in the years 2015-2018 regarding the average growing season during the multi-year period (1951-2012)

	Month							
	IV	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	
year	**average temperature (ºC)**							**Mean**
1951-2012	**8.0**	**13.0**	**16.4**	**18.2**	**17.6**	**13.8**	**9.2**	**13.7**
2015	8.7	12.5	15.6	18.6	21.1	14.1	8.5	14.2
2016	8.8	15.7	18.5	19.0	17.8	16.8	8.6	15.0
2017	6.8	13.5	16.8	17.2	17.9	13.3	11.0	13.8
2018	12.9	17.5	18.1	20.1	19.7	15.2	10.3	16.3
	rainfall (mm)							Total
1951-2012	**39.7**	**62.9**	**48.2**	**69.6**	**74.2**	**58.7**	**37.3**	**391**
2015	29.0	48.0	32.8	62.0	14.7	34.4	22.1	243
2016	20.2	18.9	69.0	50.1	47.8	18.3	55.3	280
2017	42.3	99.2	118.1	182.4	45.4	31.6	95.1	614
2018	24.0	25.4	11.6	92.8	21.4	16.3	20.2	212

Determination of physical parameters
The one-year shoot's length (cm) was measured in autumn on all shrubs covered by the experiment.

Foliage area of one leaf (cm^2)
The foliage area of one leaf was measured using the Delta Image Analysis System (Delta-T Devices LTD, England) scanner connected to the computer.

Determination of Leaf greenness index and colour
Prior to the first harvest of fruits, the leaf greenness index was determined using Chlorophyll Meter SPAD-502 (Minolta, Japan).
The pigment measurement (colour) of the leaf and fruits of the *Lycium chinense* Mill. was analyzed in a transmitted mode evaluated by photocolorimetric method tested in CIE L**a***b** system, as described by Krupa-Małkiewicz et al. (2019). The *a** value showed the place of appearing in the colour gamut, in the range from green to red (+*a** means redness; −*a** means greenness) on the surface of dried fruits of analyzed genotypes. The *b** parameter described the colour in the range from yellow to blue (+*b** means yellow; −*b** means blue) on the surface of dried fruits of tested genotypes. The value of L* parameter ranges from 0 to 100, black to white, respectively.

Determination of Normalized Difference Vegetation Index and Anthocyanins Index
Relative changes in concentration of chlorophyll and anthocyanins on the goji fruits was measured non-destructively spectrometer PA 1101 (Control in Applied Physiology, Berlin-Falkensee, Germany).
The head of the instrument contains five light-emitting diodes (LEDs) in the range of 400–1100 nm remitted from the fruit peel. Relative changes in chlorophyll concentration are expressed as normalized differential vegetation index (NDVI) = (I780-I660)/(I780+I660) and anthocyanins as normalized anthocyanins index (NAI) = (I780-I570)/(I780+I570). Where I is the intensity of the reflected light at a given wavelength (Ochmian et al., 2016).
Fruits from four trees of every cultivar and treatment were used from the lower part of the tree crown for an optimum composite sample, resulting in 120 determinations on each sampling date.

General fruits parameters
The fruit weight was measured with RADWAG WPX 4500 electronic scales (0.01 g accuracy). Diameter and firmness of fruits were measured on fresh berries immediately after the harvest with a FirmTech2 apparatus (BioWorks, USA) of 100 randomly selected berries from three replicate was expressed as a gram-force causing fruit surface to bend 1 mm.

Determination of chemical parameters
Basic measurements
The fruit was collected manually, gently shaking from all the shrubs included in the experiment and measurements were taken on a prepared bulk sample. For juice extraction efficiency berry were homogenized with a blender with pectinase (Rapidaza Super – 0.025 ml/250 g) at 40^{O}C - 3 repetitions of 250 g from each combination. Afterwards, after 60 minutes, the pulp was pressed a laboratory hydraulic press. *L*-ascorbic acid, nitrates V and nitrates III contents were measured with a RQflex 10 requantometer (Merck) and expressed as mg per 100 g berry juice (Piwowarczyk et al. 2020). The soluble solids content was determined with a digital refractometer PAL-1 (Atago, Japan). The acidity was determined by titration of an extract of berry homogenate with 0.1 N NaOH to an end point of pH 8.1 (measured with an Elmetron pH meter) (Mijowska et. al. 2016).

Antidiabetic activity (α-amylase, α-glucosidase)
Extraction mixed fruits were done with 70% ethanol at room temperature for 60 min with constant stirring. After centrifuging at 4000 rpm for 10 min, and filtration, the supernatants were concentrated at 40°C (vacuum evaporator) to remove the ethanol and the aqueous phase was diluted with water. For further analytical and biological activity assays, a gradient of concentrations was prepared via serial dilution of the fruit extracts in pure water.
The activity of the parasite and host extracts was assayed according to the procedure described previously by Podsedek et al. (2014) (*α*-glucosidase) and Nickavar and Yousefian (2011) (*α*-amylase). All samples were assayed in triplicate and the result was expressed as IC_{50}. The amount of the inhibitor (expressed as mg per 1 mL of the reaction mixture under assay conditions) required to inhibit 50% of the enzyme activity is defined as the IC_{50} value. All samples were assayed in triplicate.

Antioxidant activity (ABTS·+, FRAP)
ABTS•+ (2,2'-azobis(3-etylobenzotiazolino-6-sulfonian) compound was used to observe the kinetics of enzyme reactions assay (Arnao et al., 2001). The FRAP (Ferric-Reducing Antioxidant Power) assay was done according to Benzie and Strain (1996). The antioxidant capacity is expressed as Trolox equivalent. Measurements by means of ABTS•+ and FRAP assay involved UV-2401 PC spectrophotometer.

Scopolamine and α-solanine
The scopolamine and α-solanine were identified by comparing band colors after derivatization with Dragendorff and Carr-Price reagents (Kokotkiewicz et al., 2017).

Provitamins A
The content of provitamin A in fruits was determined by high-performance liquid chromatography with UV and fluorescence detection (Knauer K-1001 pump and Knauer K-2001 UV detector; Beckman ODS column (5 μm), dimensions 150×4.6 mm, column temperature 25°C) (EC Commission Regulation 2009).

Polyphenol content
Three replicates of 100 g randomly chosen goji fruits were kept frozen at −65°C until analysis, then prepared according to the methodology of Oszmiański et al. (2018). The content of polyphenols in individual extracts was determined by means of the ultra-performance liquid chromatographyphoto-diode array detector-mass spectrometry (LC-PDA-MS) method. All extractions were carried out in triplicate. Identification of polyphenols of *Lycium chinense* Mill. fruits extracts were carried out using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corporation, Milford, MA) with a mass detector G2 Q-TOF micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation (ESI) source operating in negative and positive modes. The PDA spectra were measured over the wavelength range of 200-600 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards.

Statistical analysis
All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Cracow, Poland). Statistical significance of the differences between means was established by testing homogeneity of variance and normality of distribution followed by ANOVA with Tukey's *post hoc* test, significance was set at $p<0.05$. The results are expressed as means ± SD. To determine the relationship between the cultivars and bioactive compounds the results obtained were subjected to agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method.

RESULTS AND DISCUSSION

Basic physical and chemical properties of goji fruits and shrub
The growth characteristics of the examined cultivars are presented in Table 2. The differences between tested goji genotypes with regard to the height of the one-year-old shoots are significant. The average height of the one-year-old shoots varied between 36 cm and 82 cm. Among all tested cultivars, the 'Korean Big' had taller plants, while the 'Big Lifeberry' was the shortest. It indicates that goji plants had very different growth rates and the ability to adapt. By comparing cultivar Ningqi 6 with the standard Ningqi 1, Wang et al. (2011) observed that cultivar Ningqi 6 is superior in terms of height vigor and productivity. Mencinicopschi and Bălan (2013) and Dzhugalov et al. (2015) also reported the differences in the vigor of goji cultivar. According to Kiełbasa et al. (2005) the size of the leaf blade, which is crucial in photosynthesis, can also indicate the intensity of growth. On analyzing the leaves, it was found that the largest leaf had a cultivar of Amber Sweet Goji with the size varying from 5.38 cm^2 (the biggest) to 2.33 cm^2 (the smallest) – No '1'(Table 2). SPAD chlorophyll meter is used to measure the leaves' green index, which is strongly correlated with the chlorophyll content of the leaves (Pacewicz and Gregorczyk, 2009). Based on these findings, we can assess the condition of the plants (Antal et al., 2013). The highest SPAD value (52.4) was observed in cultivar Sweet Berry, while the lowest (31.2) was in Big Berry (Table 2). The leaves' green index was significantly different but it correlated with the value of the parameter *a** (Table 2). The results of colour determination of the leaves and fruits were also analyzed in the transmitted mode with the photocolorimetric method in the CIE $L^*a^*b^*$ system. The leaves of *Lycium chinense* Mill. were also characterized by a similar value of the green index (Kruczek and Ochmian, 2016).
The value of *a** parameter (colour ranging from green to red) determined on the surface of leaf ranges from -32.9 ('Sweet Berry') to -59.4 ('Big Berry'). The leaves of cultivar Sweet Berry were also darker, which is shown by the lower value of the parameter L^* (41.8). The value of parameter L^* (reaching from 0 to 100, black to white, respectively) is usually used for tracking the colour changes (Ochmian et al., 2019). However, goji leaves of cultivar Amber Sweet Goji are 39% brighter compared to the Sweet Berry leaves (Table 2).

Table 2. Differences in the shrubs' growth and colour of the tested goji fruits (*L. chinense*)

Compounds		Cultivar						
		No '1'	**New Big**	**Sweet Berry**	**Big Berry**	**Big Lifeberry**	**Korean Big**	**Amber Sweet Goji**
One-year shoots length (cm)		61±21bcd	74±17de	47±19ab	68±15cde	36±13a	82±24e	58±15bc
One leaf area (cm^2)		2.33±0.5a	4.05±0.6b	4.73±0.6cd	5.14±0.5de	3.77±0.4b	4.26±0.6bc	5.38±0.7e
Greening index SPAD		38.7±2.7c	35.4±2.3bc	31.2±2.5a	52.4±3.0e	42.5±1.9d	44.1±2.9d	33.7±1.3ab
Color parameters CIE leaves	L*	46.3±2.5c	43.2±3.4a	41.8±3.5a	51.0±2.9d	45.4±2.5b	44.7±2.8abc	58.1±1.8e
	*a**	-38.5±4.5b	-33.1±3.8a	-32.9±4.1a	-59.4±2.7d	-35.3±4.4ab	-35.9±4.8ab	-44.7±2.2c
	*b**	29.7±2.7c	17.5±3.8a	25.8±2.9b	35.3±2.1d	23.8±2.5b	24.8±1.9b	42.0±1.2e
Color parameters CIE fruit	L*	34.7±3.7a	49.1±3.4d	32.6±2.8a	45.8±3.3cd	39.5±4.1b	42.2±2.9bc	77.3±1.9e
	*a**	22.5±2.7ab	34.8±2.4d	31.3±2.5cd	29.7±3.0c	23.4±1.9b	41.2±3.3e	19.8±2.1a
	*b**	25.9±2.9a	44.7±2.5cd	36.8±3.1b	47.9±3.3de	42.3±3.0c	52.0±4.2e	63.4±3.9f
NAI fruit		0.74±0.05bc	0.77±0.04c	0.75±0.05bc	0.81±0.03cd	0.69±0.03b	0.84±0.04d	0.34±0.02a
NDVI fruit		-0.52±0.03ab	-0.53±0.03b	-0.57±0.02b	-0.50±0.04a	-0.64±0.03c	-0.47±0.02a	-0.68±0.03c

*Means followed by the same letter do not differ significantly at P=0.05 according to Tukey multiple range; values are means ± standard deviation

The colour of the leaf surface is determined by parameter *b** (from yellow to blue colour) which ranged from 17.5 ('New Big') to 42.0 ('Amber Sweet Goji'). A similar value of the parameter *a** was observed for the leaves of *L. chinense* (Kruczek and Ochmian, 2016), while the value of the parameters L^* and *b** was slightly lower in comparison to the goji leaves tested in this study. The appearance and colour of the fruit largely influence the choice of consumers (Chełpiński et al., 2019). The colour of the fruit may encourage consumption by influencing suggestions of certain flavors and/or discourage consumption by warning of their rottenness (Oszmiański and Wojdylo, 2005; Lachowicz et al., 2019). The color of food products may change during processing (Yusufe et al., 2017). The coloration of the fruit peel of *L. chinense* is variable, from the taint and bright yellow-orange to dark orange-red (Kruczek and Ochmian, 2016). Regarding the tested fruit colour, the value of *a** parameter measured was significantly different, which ranged from 19.8 ('Amber Sweet Goji') to 41.2 ('Korean Big') but correlated with the Normalized Anthocyanin Index (NAI) (meaning -1 and +1, redness and red, respectively). Anthocyanin dyes are responsible for the colour of the goji berry fruits. The NAI showed that the highest anthocyanin content was recorded in the fruit of cultivar Korean Big (0.84) and the lowest one was in cultivar Amber Sweet Goji (0.34). According to Kuckenberga et al. (2008) estimating the maturity and quality parameters in 'Golden Delicious' apple fruit can also be a useful Normalized Difference Vegetation Index (NDVI). In the current study, the value of NDVI index in goji fruits was between -0.68 ('Amber Sweet Goji') and -0.47 ('Korean Big'). According to Rutkowski et al. (2008), the different values of NDVI indicate the various stages of the fruit: value higher than -0.4 indicates immature fruit (pre-climacteric), the value between -0.4 and -0.6 characterizes fruits with the onset of ethylene production, and value below -0.8 is representative for fruits with more advanced ripening stage (ripe fruits).

The colour of the surface of the tested goji fruits determined by parameter *b** ranged from 25.9 ('No '1'') to 63.4 ('Amber Sweet Goji'). It was shown that cultivars Amber Sweet Goji and Korean Big contained the highest quantity of red-couloring substances and a smaller quantity of yellow-couloring ones. The value of L^* parameter ranged from 32.6 ('Sweet Berry') to 77.3 ('Amber Sweet Goji'). Similar results and observations were obtained in the case of saskatoon berry where parameter L^* ranged from 32.71 to 49.31 (Lachowicz et al., 2019). However, fruits examined by Kruczek and Ochmian (2016) were darker than in our own research; the value of the L^* parameter ranged from 29.7 to 35.3. Also for highbush blueberry, the L^* parameter was lower (19.2 - 22.9) than in the tested goji fruit (Ochmian et al., 2015).

Firmness

The size, firmness, and resistance of the fruit to mechanical damage are factors that determine the quality of the fruit. They allow the assessment of the

suitability of a given species and cultivar, among others, for mechanical fruit harvesting, transport, and the way they are used. In the case of goji berries, the size of the fruit is not an important parameter, because they are intended mainly for processing (Ochmian et al., 2019). Goji berries are very fragile fruits with a low firmness, which is close to the delicate fruit of blue honeysuckle berries. To reduce the risk of damage, the fruit should be harvested with the stalk (Kruczek and Ochmian, 2016). Among the cultivars tested, Korean Big has the highest weight 122.1 g (100 fruits) with length, firmness and puncture being 21.0 mm, 211 G/mm and 95.1 G/mm, respectively. Because of these characteristics, the fruit of this cultivar was distinguished as 'Korean Big' (Table 3). The physical parameters of the fruit tested in this study were within normal limits or even higher than those reported by Kruczek and Ochmian (2016). This could probably occur due to different environmental conditions and genotypes. On the other hand, Kamchatka berry fruit is characterized by firmness at the level of 165–201 G/mm (Ochmian and Grajkowski, 2007) and cornelian cherry 129–211 G/mm (Ochmian et al., 2019).

Biological activity

Goji berries contain many valuable nutrients, which are characterized by a very high biological activity. They are classified as super-fruits (Mikulic-Petkovsek et al., 2012; Kulczyński et al., 2014; Kruczek and Ochmian, 2016). The content of *L*-ascorbic acid of 'Big Lifeberry' goji varied between 62 mg/1000 g and 408 mg/1000 g (Table 3).

Table 3. Physicochemicals parameters and quality of tested goji fruits (*L. chinense)*

	Cultivar						
Compounds	**No '1'**	**New Big**	**Sweet Berry**	**Big Berry**	**Big Lifeberry**	**Korean Big**	**Amber Sweet Goji**
Weight of 100 fruits (g)	62.4±7.3a	96.7±8.3c	73.4±6.5ab	112.6±8.5de	104.5±9.0cd	122.1±7.7e	85.7±6.2b
Fruit diameter (mm)	9.6±1.2ab	8.4±1.1a	9.8±1.3b	11.8±1.0c	11.2±1.5c	10.7±1.3bc	9.8±1.7b
Fruit length (mm)	14.2±2.2a	24.2±2.7e	15.0±1.9ab	17.3±2.1b	18.9±2.3cd	21.0±3.0d	16.1±2.9abc
Puncture (G/mm)	79.2±14.7de	49.5±10.1a	71.3±14.9cd	88.0±11.7ef	68.4±19.3bc	95.1±13.3f	59.3±16.2ab
Firmness (G/mm)	176±21.3b	132±17.3a	174±18.6b	167±20.3b	125±12.2a	211±17.3c	142±18.5a
L-ascorbic acid (mg/1000 g)	323±23e	197±27c	224±18c	279±25d	408±31f	155±19b	62±10a
Provitamin A (mg/1000 g)	153±9e	122±6c	94±4b	137±5d	190±5f	118±4c	35±1a
Nitrates III (mg/1000 g)	1.42±0.11e	0.68±0.07b	1.12±0.09d	0.94±0.10c	0.62±0.06ab	1.05±0.09d	0.53±0.05a
Nitrates V (mg/1000 g)	42.4±5.2b	33.8±3.3a	49.3±5.1b	74.1±3.2c	29.5±2.4a	106.6±3.8d	42.4±2.5b
Extracts (°Bx)	11.4±1.2b	16.4±1.1d	18.4±1.3e	12.5±0.9bc	11.9±0.6b	13.2±0.9c	9.4±0.6a
Total acidity (g/1000 g)	11.1±1.0b	12.4±0.7bc	8.9±0.6a	12.7±1.1c	14.5±1.2d	12.6±1.1bc	13.0±1.3c
antidiabetic activity							
α-amylase IC_{50} (mg/mL)	38.5±2.1b	48.6±1.7d	43.1±1.5c	50.8±2.2de	33.4±1.9a	52.7±1.3e	73.4±1.1f
α-glucosidase IC_{50} (mg/mL)	9.9±0.9c	9.2±0.8c	6.5±0.7a	13.2±0.6d	9.9±0.5c	7.8±0.6b	5.7±0.5a
antioxidant activity							
$ABTS^{\cdot+}$ (mmol Trolox/$100g^{-1}$)	4.11±0.29d	3.45±0.32c	3.88±0.25d	2.29±0.17a	6.21±0.35e	2.95±0.15b	1.89±0.13a
FRAP (mmol Trolox/$100g^{-1}$)	4.28±0.28e	3.01±0.19c	3.46±0.42d	1.84±1.0a	5.58±0.38f	2.61±0.13b	1.66±0.16a
alkaloids							
Scopolamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-solanine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Fruits did not contain steroids (α-solanie) and tylakoides (scopolamine) at the detected level							

Goji berries of the present study showed similar ascorbic acid content compared to other fruits, such as raspberry (31.0–40.0 mg/100 g FW) (Pantelidis et al., 2007), strawberries (46 mg/g FW) (Roberts and Gordon, 2003), oranges (31 mg/g FW) (Roberts and Gordon, 2003), kiwi fruits (29–80 mg/g FW) (Nishiyama et al., 2004), and cornelian cherry (41.7 mg/1000 g FW) (Ochmian et al., 2019). Donno et al. (2015) reported that vitamin C content in goji berries reached 42 mg/100 g, which is typical of citrus fruits. In contrast, the content of provitamin A in the tested goji fruit was 35 mg/1000 g ('Amber Sweet Goji') and 190 mg/1000 g ('Big Lifeberry') (Table 3) and is much higher than the content of provitamin A in those described by Kruczek and Ochmian (2016). However, these values are lower than those for goji varieties described by Wojdyło et al. (2018). Huge variations in provitamin A content are concurrent with other reports (Dong et al., 2012, Wojdyło et al., 2018), suggesting variation in carotenoid content depending on plant origin, subspecies and growth condition. Moreover, environmental factors significantly affect the authenticity and the main active components of *Lycium chinense* Mill. fruits. According to Majchrzak et al. (2000) and Vuong et al. (2002) provitamin A carotenoids, particularly *β*-carotene, provide vitamin A after enzymatic cleavage, and play a key role in human nutrition. *β*-carotene was the dominant provitamin A carotenoid present in the *gac* fruit (175 μg/g edible portion), papaya (12.1 μg/g), mandarin orange (4.65 μg/g) and banana (2.90 μg/g) (Vuong et al., 2002).

The interaction between acid extracts determines the flavor of the fruit. The organic acid content is therefore essential for the taste of goji fruits, as increased sugar and lower acid levels make the fruit taste sweeter (Ochmian et al., 2014). The highest acidity characterized fruits of cultivar Big Lifeberry (14.5 g/1000 g) while they had low overall content of the extract (11.9%) (Table 3). The goji berries from cultivar Sweet Berry had the highest extract content (18.4%) and the lowest acidity (8.9 g 1000/g). Similar results were observed in *L. chinense* 'No 1' (Kruczek and Ochmian, 2016) and in cornelian cherry (Ochmian et al., 2019).

Nitrates III (NO_2) and nitrates V (NO_3) belong to the group of compounds adversely affecting the human body. Nitrates V belong to low-toxic compounds that do not directly threaten health. In contrast, nitrates III are six to ten times more toxic. The current regulations do not specify nitrate content in fruits, but only in a few selected vegetables. According to the EC Commission Regulation of 2006, the daily consumption of nitrates V should not exceed 3.65 mg per kg body weight. However, according to current regulations, these plants can also be considered safe for the consumer. Nitrate V content limits are set only in leafy vegetables in EU legislation. Fresh lettuce may contain up to 5000 mg/1000 g, and in processed foods for feeding infants and young children, it should not exceed 200 mg/1000 g (EU legislation, 2006). In contrast, nitrate III levels should not exceed 0.07 mg per kg of body weight per day. The nitrate III content in all the cultivars tested was at a relatively low level (Table 3). The highest nitrates III content (1.42 mg/1000 g) was obtained in the cultivar No '1', and nitrates V in the Korean Big cultivar (106.6 mg/1000 g). These values were higher in persimmon fruit (Ochmian et al., 2016) cornelian cherry (Ochmian et al., 2019) and highbush blueberry (Ochmian, 2012) while in comparison to grapevine fruit in which the nitrates III content was lower (Ochmian et al., 2013).

Some doubt has arisen about the atropine content of the fruits. In 1989, a sample of fruits harvested in India recorded 0.95% of atropine (Harsh, 1989). This finding has no evidence and is in clear contradiction with the widespread consumption of these fruits. Studies did not show the presence of steroids (*α*-solanine) and tropane alkaloids (scopolamine) at detectable levels in goji fruit.

Antidiabetic activity of goji fruits

The inhibition of *α*-amylase in the analyzed goji fruit ranged from 33.4 ('Big Lifeberry') to 73.4 mg/mL IC_{50} ('Amber Sweet Goji'), while the inhibition of *α*-glucosidase in these fruits was between 5.7 ('Amber Sweet Goji') and 13.2 mg/mL IC_{50} ('Big Berry'). The extract obtained from 'Big Lifeberry' goji fruit showed the highest inhibitory activity of *α*-amylase and *α*-glucosidase (Table 3). However, the inhibitory effect obtained was for *α*-amylase IC_{50} 33.4 and *α*-glucosidase IC_{50} 9.9 mg/mL. The inhibition of these enzymes may be efficient in regulating type 2 diabetes by monitoring glucose absorption (Podsedek et al., 2014).

Both *α*-amylase and *α*-glucosidase inhibitory activity in fruits such as pomegranate, strawberry, raspberry, pear, kiwi, plum, lingonberry, black currant, and blueberry extracts has been confirmed in other studies (McDougall et al., 2005; Podsedek et al., 2014; Wojdyło et al., 2018; Ochmian et al., 2019; Lachowicz et al., 2019). Inhibition of these enzymes is particularly helpful in the treatment of non-insulin-dependent diabetes, as it slows down the release of glucose into the bloodstream (Wojdyło et al., 2018).

Antioxidant activity of goji fruits

In order to quantify the antioxidant activity of food samples, the term total antioxidant potential (TAP) is used to describe the ability to neutralize radicals of a mixture of antioxidants (Paradowska et. al., 2016). According to many authors (Wojdyło et al., 2018; Ochmian et al., 2019; Lachowicz et al., 2019), the antioxidant activity of various fruits was determined mainly by genotype (cultivar), fruit maturity, growing conditions, geographical location, climatic conditions, and collection methods. Fruits with high antioxidant activity are of great interest to a potential consumer. Total antioxidant activity in goji berries ranged from 1.89 to 6.21 mmol Trolox/100 g and from 1.66 to 5.58 mmol Trolox/100 g for ABTS·+ and FRAP assays, respectively (Table 3). Cultivar Big Lifeberry showed the lowest and Amber Sweet Goji the highest antioxidant activity. Our data agreed with those reported by Wojdyło et al. (2018) who showed a similar value of antioxidant activity in studied cultivars of goji. According to Donno et al. (2015), goji fruits are rich in a significant amount of phytochemicals and indeed unique health-promoting compounds. This study showed that the analyzed quality and nutraceutical parameters of the fruits of this species are comparable with those of other common fruit species, such as *Ribes nigrum*, *Actinidia deliciosa*, and *Citrus sinensis*.

A cluster analysis conducted using Ward's method (Figure 1) permitted the isolation of three groups having a similar influence on the physical and chemical properties and antidiabetic and antioxidant activities of the goji fruits. Opposing groups (a and c) formed dark-colored fruits of No '1' cultivar and the bright Amber Sweet Goji cultivar..

Polyphenolic identification and compounds of goji fruits

The largest group of phenolic compounds included flavonols, phenolic acid, and the smallest gallotannins (Table 4). Six flavonols identified in goji cultivars were quercetin derivatives, and one isorhamnetin.

Identification of polyphenolic compounds was performed using UPLC-PDA-ESI-MS/MS in negative ion mode. The presence of 14 polyphenolic compounds in goji fruits, including 5 phenolic acids, 1 hydrolyzed tannin and 8 flavonols, was tentatively determined. Compound 1 was initially suggested for the presence of tetragalloyl glucose with a characteristic base fragment at *m/z* 169 which was in line with previous reports for *Lycium barbarum* L. (Pires et al., 2018). Identification of compounds from 2 to 6 indicated the presence of caffeoylquinic acids derivatives with a typical base ion at *m/z* 191. Therefore, compounds 2, 3, 5 and 6 were tentatively referred to as di-*O*-caffeoylquinic acids due to their characteristic main peak at *m/z* 515 (Francescato et al., 2013). Compound 4 was then given as 5-*O*-caffeoylquinic acid and its presence confirmed with an available standard. Of the 8 flavonols, 7 quercetins and 1 isorhamnetins were tentatively described, which were characterized by the presence of base ions at *m/z* 301 and 315, respectively. These compounds were previously identified in *Lycium barbarum* L. as quercetin-3-Soph-7-glucoside (*m/z* 787), quercetin-3-*O*-rutinoside-hexoside (*m/z* 771), quercetin-3-*O*-glucuronide (*m/z* 477), quercetin dihexoside (*m/z* 625), quercetin-3-*O*-rutinoside (*m/z* 609), quercetin-3-*O*-robinobioside (*m/z* 609), oraz isorhamnetin-3-*O*-rutinoside (*m/z* 623) (Francescato et al., 2013; Pires et al., 2018; Tripodo et al., 2018).

As is known the antioxidative potential depends on the amount of bioactive components especially polyphenolics. Examined goji cultivars contained on average total phenolic compounds from 18.94 mg/100 g ('Amber Sweet Goji') to 55.46 mg/100 g ('No '1'') (Table 5).

Compounds belonging to gallotannins are essential for human health. The highest content of Tetragalloyl glucose was found in fruits of No '1' cultivar (9.27 mg/100 g). On the other hand, yellow-colored fruits had only 0.34 mg of this compound in their composition. Similar relationships were found in Phenolic acid and Flavonols. In the group of compounds classified as phenolic acid in all cultivars the dominant compounds were Di-O-caffeoylquinic acid and 5-Caffeoylquinic acid. In total they comprised 83% of all phenolic acids. In the flavonols group Quercetin-3-O-robinobioside dominated in most cultivars. According to Wojdyło et al. (2018), the average content of phenols in all goji cultivars grown in Poland was 97.23 mg/100 g, with considerable inter-cultivar differences. In comparison to other fruits, for example, kiwifruit and apple contained small quantities of polyphenolic compounds (70.23–83.40 mg GAE/100 g FW), while a significantly higher polyphenolic content was observed in strawberry (323.39 mg GAE/100 g FW) and blackcurrant (434.43 mg GAE/100 g FW). According to Donno et al. (2015), goji (268.5 mg GAE/100 g FW) was in an intermediate position between orange (158.70 mg GAE/100 g FW) and guava (310.10 mg GAE/100 g FW). The higher the total polyphenol content, the greater is the antioxidant activity (Hernández-Alcántara et al., 2016). Similar to the works of Wang et al. (2011), Donno et al. (2015), and Wojdyło et al. (2018), a dominant phenolic acid

obtained in this study was 5-caffeoylquinic acid. Phenolic acid, especially chlorogenic acid, is a precursor of flavor in fruits and vegetables, and it shows anticarcinogenic, antimutagenic, and antioxidant properties *in vitro*. Chlorogenic acid is poorly absorbed in the human body, and is therefore metabolized mainly by colonic microflora (Sato et al., 2011).

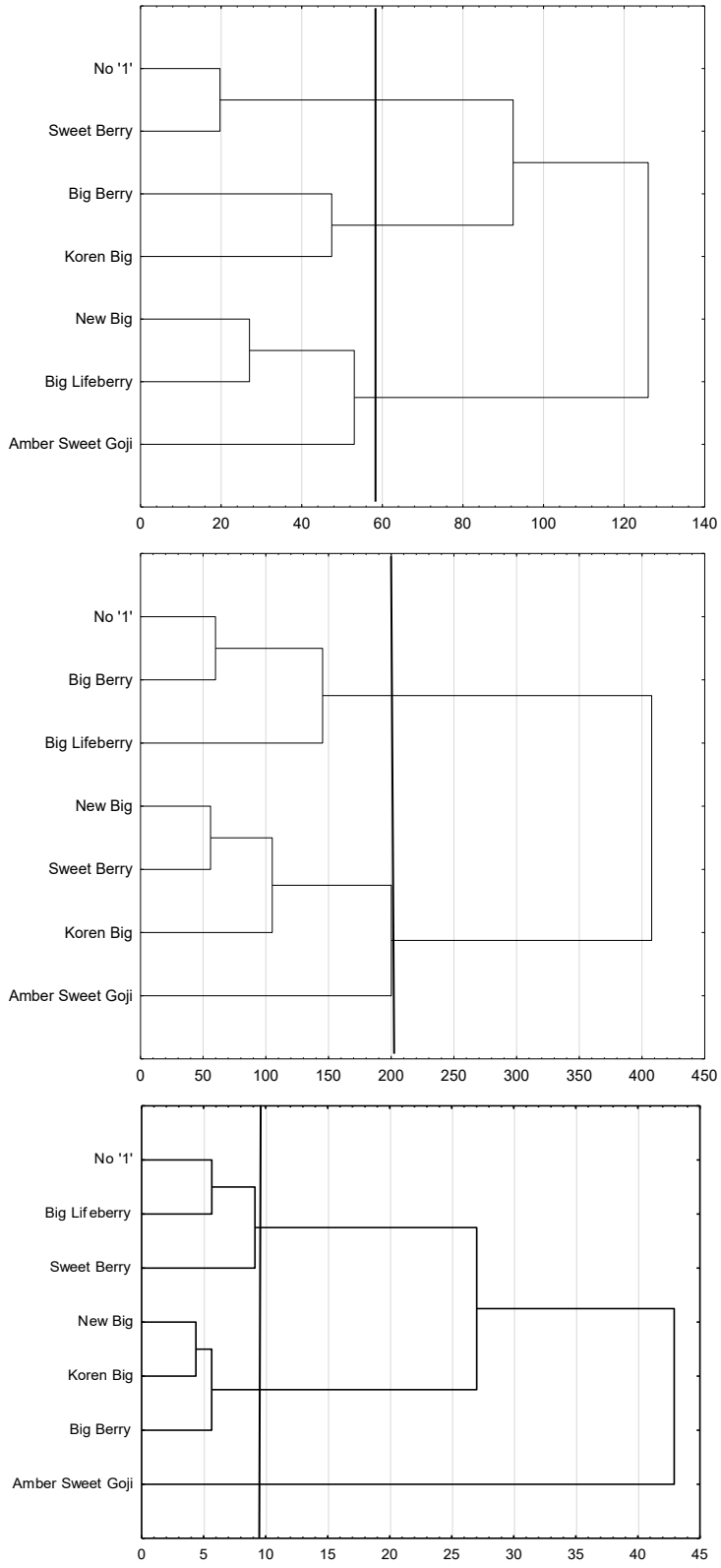


Figure 1. Dendrogram of cluster analysis for the physical composition, chemical composition and bioactive composition of tested goji fruits (*L. chinense*). The vertical line (linkage distance 57, 200, 9) indicate the cut-off used to form the groups

Table 4. Identification of polyphenolic compounds in the tested goji fruits (*L. chinense*)

Tentative compouns	**MS [m/z]**	**Tr [min]**
Tetragalloyl glucose	787/169	2.82
Di-*O*-caffeoylquinic acid	515/191	2.53
Di-*O*-caffeoylquinic acid	515/191	3.20
5-caffeoylquinic acid (Chlorogenic acid)	353/191	3.45
Di-*O*-caffeoylquinic acid	515/191	3.89
Di-*O*-caffeoylquinic acid	515/191	4.37
Quercetin-3-Soph-7-glucoside	787/301	2.86
Quercetin-3-*O*-rutinoside-hexoside	771/609/301	3.69
Quercetin-3-*O*-glucuronide	477/301	5.18
Quercetin dihexoside	625/301	5.24
Quercetin-3-*O*-rutinoside	609/301	5.92
Quercetin-3-*O*-robinobioside	609/301	5.97
Quercetin dihexoside	625/301	6.17
Isorhamnetin-3-*O*-rutinoside	623/315	6.86

Table 5. Content of phenolic compounds (mg/100 g) of tested goji fruits (*L. chinense*)

	No '1'	**New Big**	**Sweet Berry**	**Big Berry**	**Big Lifeberry**	**Korean Big**	**Amber Sweet Goji**
Gallotannins				mg/100 g			
Tetragalloyl glucose	9.27±0.24e	4.96±0.32c	2.86±0.17b	7.09±0.33d	6.99±0.35d	5.65±0.17c	0.34±0.02a
Phenolic acid							
Di-*O*-caffeoylquinic acid	13.88±0.22e	7.24±0.17c	4.15±0.29a	10.37±0.30d	10.23±0.29d	8.25±0.22c	5.33±0.19b
Di-*O*-caffeoylquinic acid	2.79±0.09c	1.62±0.10b	0.68±0.05a	2.58±0.17c	2.54±0.13c	1.93±0.12b	0.32±0.04a
5-Caffeoylquinic acid (Chlorogenic acid)	9.46±0.35e	6.48±0.27c	1.86±0.19a	11.15±0.42f	10.99±0.27f	8.00±0.22d	3.24±0.18b
Di-*O*-caffeoylquinic acid	0.39±0.03b	0.44±0.02c	0.59±0.03d	0.30±0.02b	0.30±0.02b	0.400.03bc	0.11±0.01a
Di-*O*-caffeoylquinic acid	1.37±0.13e	1.00±0.09b	0.80±0.05a	1.20±0.09d	1.18±0.11d	1.06±0.08bc	1.12±0.09cd
sum	27.89f	16.78c	8.08a	25.60e	25.24e	19.64d	10.12b
Flavonols							
Quercetin-3-Soph-7-glucoside	3.34±0.15d	2.36±0.11c	3.13±0.17d	1.61±0.12b	1.59±0.09b	2.11±0.15c	0.57±0.04a
Quercetin-3-*O*-rutinoside-hexoside	0.28±0.02b	0.24±0.03a	0.25±0.02a	0.24±0.02a	0.23±0.01a	0.24±0.01a	0.64±0.03c
Quercetin-3-*O*-glucuronide	0.60±0.04c	0.30±0.02b	0.02±0.00a	0.57±0.03c	0.56±0.03c	0.39±0.01b	0.13±0.00a
Quercetin dihexoside	3.82±0.09c	3.17±0.11b	4.46±0.12d	1.91±0.05a	1.88±0.04a	2.75±0.07b	3.98±0.10c
Quercetin-3-*O*-rutinoside	0.27±0.01b	0.41±0.02d	0.66±0.02e	0.17±0.01a	0.17±0.01a	0.33±0.02c	0.98±0.03f
Quercetin-3-*O*-robinobioside	9.60±0.25e	3.66±0.11b	0.61±0.02a	6.73±0.15d	6.64±0.13d	4.66±0.09c	1.13±0.05a
Quercetin dihexoside	0.10±0.00ab	0.12±0.01bc	0.17±0.01c	0.07±0.00a	0.07±0.00a	0.10±0.00ab	0.34±0.02d
Isorhamnetin-3-*O*-rutinoside	0.29±0.01c	0.22±0.01ab	0.17±0.01a	0.26±0.02bc	0.26±0.02bc	0.23±0.02ab	0.71±0.03d
sum	18.01d	10.26b	9.30ab	11.30c	11.14c	10.58bc	7.77a
Total	**55.46D**	**32.22B**	**20.42A**	**44.25C**	**43.64C**	**36.10B**	**18.94A**

* For explanation, see Table 3.

CONCLUSION

Differences in biochemical composition and biological activity of seven goji berry cultivars grown in north-western Poland were shown. These shrubs are highly adapted to the region's climatic conditions. The examined goji shrubs did not show signs of frost damage and the flowers were not damaged by spring frost. Regardless of the weather conditions, the shrubs yielded annually.

Goji berries have a high biological activity spectrum with beneficial effects on health. The cultivars rich in total polyphenols ('No '1'', 'Big Berry', 'Big Lifeberry'), provitamin A, and *L*-ascorbic acid ('Big Lifeberry', 'No '1''), and those that exhibit antidiabetic and antioxidant activity ('Big Lifeberry', 'No '1''), may be considered as a superfood or functional food. In addition, no scopolamine were detected in the tested goji, and the levels of nitrates V and nitrates III were at well below the current standards. Among the tested cultivars, 'Big Lifeberry' fruits for the consumer due to the size, weight, and health-promoting effects were most attractive. The goji fruits of this cultivar, cultivated under the climatic conditions of north-eastern Europe, could be taken into consideration as an important dietary source of natural antioxidants.

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