

Jerusalem Artichoke (*Helianthus Tuberosus* L.) as A potential Chlorophyll Source for Humans and Animals Nutrition

László Kaszás¹, Zoltán Kovács¹, Éva Nagy¹, Nevien Elhawat^{1,2*}, Neama Abdalla³, Éva Domokos-Szabolcsy¹

¹ Agricultural Botantics, Plant Physiology and Biotechnology Department, University of Debrecen, AGTC Böszörményi u. 138, 4032 Debrecen, Hungary (Email: kaszaslacee@gmail.com; zoli.kovacs92@gmail.com; domokosszabolcsy@gmail.com)

² Department of Biological and Environmental Sciences, Faculty of Home Economic, Al-Azhar University, Egypt (nevienelhawat@gmail.com)

³ Plant Biotechnology Department, Genetic Engineering Division, National Research Center, 33-El-Behouth Street, 12622 Dokki, Cairo, Egypt (E-mail: neama_ncr@yahoo.com)

CONSUMPTION of green leafy vegetables is thought to have great potentials on health promoting effects. In addition to different secondary metabolites, photosynthetic pigments have been proved antioxidant, anti-mutagenic, and detoxification activities. The current research was aimed to evaluate the vegetative part of seven ecotypes/varieties of Jerusalem artichoke (i.e., Alba, Fuza, Kalevala, Kercaszomori, Piri, Rubik, and Tápióisima) as a potential source for chlorophyll. Alfalfa (Hunor variety) was applied as a control. Open field experiment was carried out during spring season 2016 at Horticultural Demonstration Garden at the University of Debrecen, Hungary. Pigments were measured in intact leaves, green juice and fiber fraction of different Jerusalem artichoke ecotypes/varieties. Results verified that alfalfa had higher contents of different photosynthetic pigments in both intact leaves and green juice, while fiber fraction of Jerusalem artichoke ecotypes/varieties contained higher photosynthetic pigments contents. Among Jerusalem artichoke varieties, Piri ecotype had highest *chlorophyll a* (6.199 mg g⁻¹ DM), carotenoids (8.865 mg g⁻¹ DM) and xanthophyll (2.946 mg g⁻¹ DM) contents in fiber fraction than other varieties. In green juice fraction, highest contents of carotenoids and xanthophyll (1.752 and 0.709 mg g⁻¹ DM, respectively) were corresponded to Rubic ecotype. Intact leaves of Tápióisima ecotype had the highest contents of *chlorophyll a* and xanthophyll (8.478 and 2.977 mg g⁻¹ DM, respectively) compared to other varieties. While Alba plants had the highest *chlorophyll b* content in both leaves and fiber fraction (2.307 and 3.184 mg g⁻¹ DM, respectively), Fuza ecotype recorded the highest content of chlorophyll b and carotenoids (1.042 and 4.042 mg g⁻¹ DM, respectively) in green juice and leaves, respectively. However, these results revealed that green leaves of Jerusalem artichoke as it is or fractionated as green juice and fiber fractions are potential sources for chlorophyll and they can serve as alternative source for consumption chlorophyll.

Keywords: Jerusalem artichoke, Photosynthetic pigments, Chlorophyll a; Chlorophyll b; Carotenoids, Nutrition.

Introduction

Either by humans or animals, consumption of green leafy vegetables was reported to have beneficial effects on health due to alerting antioxidant, anti-mutagenic, and detoxification activities (Wang and Wink 2016). Large amounts of phytonutrients and nutraceuticals have been identified in green vegetables which helping

the body in maintenance of health and fighting various disease (Mahima et al. 2014). Among phytonutrients, photosynthetic pigments are well known as a main constituent of leaves and stems. The major group of photosynthetic pigments is chlorophylls in plants, with two main different chemical forms, namely chlorophyll 'a' and 'b' (Haraszty 1978).

*Corresponding author e-mail: nevienelhawat@gmail.com

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The chlorophylls as 'green blood' are responsible for the green color of plants and they have essential role in photosynthesis. At the same time physiological impact of them for human health maintenance and prevention of chronic disease have also been attracted interest, recently. The structure of chlorophyll 'a' and 'b' are similar to the structure of hemoglobin, what can be found mostly in mammal's blood. The major difference between the chlorophyll and hemoglobin is their central atom, magnesium or iron (Kospell *et al.* 2005; Rao and Rao 2007).

Beneficial biological activities of natural chlorophylls and their derivatives have been widely investigated (Ferruzzi and Blakeslee 2007). Among other chlorophylls can be attributed positive effects on inflammation, oxidation processes wound healing (Inanc 2011), and control of calcium oxalate crystals formation (Tawashi *et al.* 1980). Chlorophyll and natural or commercial derivatives have demonstrated antioxidant activity, antimutagenic activity, modulation of xenobiotic metabolizing enzymes, and induction of apoptotic events in cancer cell lines *in vitro* and *in vivo* experiments (Ferruzzi and Blakeslee 2007). In addition, they have ability to induce mammalian phase 2 proteins which protect cells against deleterious effect of oxidants and electrophiles (Diwakar Gore *et al.* 2107). Protective effects of chlorophyll and their water-soluble salts (chlorophyllin) against consequence of carcinogen exposure like aflatoxin were also confirmed in animals. Along with this Egner *et al.* (2001) suggested the chlorophyll enriched food as an effective approach to chemoprevention.

Carotenoids as accessory pigments give the yellowish, red and orange colors of plants. It can transmit the light energy from the sun to chlorophylls, but they also have protective role because of their conjugated double bonds with delocalized π -electrons. These bonds can scavenge free oxygen radicals, and reduce oxidative stress in organisms (xanthophyll cycle). Carotenoids can be divided into two groups, the oxygen free carotenes and xanthophylls, which contain oxygen in different forms such as one or more hydroxy or epoxy groups. There are two isomers of the carotene, α -carotene and β -carotene. β -carotene is the precursor of xanthophylls, zeaxanthin, violaxanthin and antheraxanthin (Lichenthaler 1987; Rao and Honglei 2002). In the previous century, Palmer (1915), underlined the photosynthetic pigments importance of nutrition

in poultry and cattle. His work highlighted that the deep yellow colour of laying hens egg yolks and their meat due to xanthophylls (lutein), and the typical colour of the milk of dairy cow duo to carotenes.

Jerusalem artichoke (*Helianthus tuberosus* L.) is a perennial plant, which commonly known as Jerusalem artichoke, or in Hungarian csicsóka. Nowadays growing interest has been directed towards Jerusalem artichoke due to its numerous uses (AbdAlla *et al.* 2014). Along with utilization in a biorefinery context has also been emerged of this plant (Domokos-Szabolcsy *et al.* 2015; Johansson *et al.* 2015). As vegetable, the most valuable part of Jerusalem artichoke is the tuber with high inulin content (Johansson *et al.* 2015). Among monosaccharides fructose and glucose are in smaller amount (Gunnarsson *et al.* 2014). According to Kim *et al.* (2013) the tubers contains 2-3% (dry weight) protein as well, however Johansson *et al.* (2015) wrote 5.3%–10.4%. At the same time Jerusalem artichoke produces huge green biomass which is rich source of biomolecules such as proteins, volatile essential oils (mainly β -bisabolene and 17 other identified volatile compounds) polyacetylenic derivatives sesquiterpene compounds, phenolics, flavonoids and chlorophylls, carotenoids (Chen *et al.* 2013; Helmi *et al.* 2014; Pan *et al.* 2009). These could be important not only for feedstock but for human nutrition/health as well (Duma *et al.* 2014). Despite the valuable green biomass, direct fresh consumption or dried preservation of Jerusalem artichoke is not preferable due to the spines and hairs covered leaves. Ensiling, appears to be the preferable one way to preserve fresh Jerusalem artichoke tops for a long time (Seiler 1993; Razmakh *et al.* 2017). Alternatively, green biomass can be fractionated to soluble green juice and insoluble fiber fractions by pressing. The cell wall deprived green juice is easy to digest food product, with high amount of proteins. Besides proteins, green juice contains valuable carbohydrates, lipids, nutrients, antioxidants and chlorophylls (Fremery *et al.* 1971). Green juice is suitable for further production of leaf protein concentrate (LPC) using heat, solvents, acid and/ or salts precipitation. Because of the pressing process is not entire, significant amount not ruptured or partial ruptured cells remains in the fibre fraction with chlorophylls, cellulose, lignin and other cell wall components (Rawate and Hill 1985). In addition, the LPC contains a high amount of photosynthetic pigments and

derivatives, as additional value from health aspect. Therefore, the aim of current work was to compare the photosynthetic pigments content of fractionated green biomass of several Jerusalem artichoke ecotypes.

Materials and Methods

Experimental design

The experiment was conducted at the Horticultural Demonstration Garden at the University of Debrecen in spring season 2016. Seven Jerusalem artichoke (*Helianthus tuberosus* L.) ecotypes/varieties were compared and alfalfa (*Medicago sativa* L.) 'Hunor' variety was used as a control (Table 1).

TABLE 1. Names and origins of ecotypes/ varieties of the tested plants.

Plant type	Ecotype/ variety	Origin
Jerusalem artichoke (<i>Helianthus tuberosus</i> L.)	Alba	Hungary, Debrecen region
	Fuza	Egypt
	Kalevala	Finland
	Kercaszomori	Hungary, NÖDIK Pannon seed bank
	Piri	Hungary, Téglás region
	Rubik	Hungary, NÖDIK Pannon seed bank
	Tápióisima	Hungary, NÖDIK Pannon seed bank
Alfalfa (<i>Medicago sativa</i>)	Hunor	Hungary

For the photosynthetic pigments determination, the sample preparation was performed as described by Duma et al. (2014). For leaf pigment extraction 100 mg fresh plant material was weighted and after then grinded in mortar with little amount silica sand and 1-3 mL 96% ethanol was added. The mixture was quantitatively transferred to test tube and brought up to 5mL with 96% ethanol. After vigorous shaking it was incubated in dark place at room temperature for 15 minutes. Last step was centrifugation in 10,000 rpm for 5 minutes (Eppendorf Centrifuge 5415 R). Same preparation procedures were applied for the leaves, green juice and fiber fraction pigment extraction.

Photosynthetic pigments determination

For measuring photosynthetic pigment content *Ultraspec 2100pro* (Biochrom) spectrophotometer was used. The light absorbance was measured on five wavelengths i.e., 665 nm, 649 nm, 440 nm, 480 nm, and 495 nm according to Duma et al (2014). The resulting values were calculated with an appropriate formula as described by Duma et al. (2014) as follow:

Plant sampling and preparation

The green biomass was harvested at the end of June when the young plants were around 1m high. The photosynthetic pigments determination was conducted from the leaves directly, for this, 10 - 10 well-developed leaves from the upper third part of varieties were collected. At the same time, harvested biomass was separated into green juice and fiber using twin screw press machine. The fractionation method followed the Ereky process (Kaszás et al. 2016; Fári and Kralovánszky 2004), which was applied for leaf protein concentrate production. The photosynthetic pigment content was measured from intact leaves, green juice and fiber fractions as well.

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = \frac{13.7 \cdot A_{665} - 5.76 A_{649}}{\text{mass} \cdot 200}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = \frac{25.8 \cdot A_{649} - 7.6 A_{665}}{\text{mass} \cdot 200}$$

$$\text{Carotenoids (mg g}^{-1}\text{)} = \frac{4.7 \cdot A_{480} - 0.263 c_{\text{chla-chlb}}}{\text{mass} \cdot 200}$$

$$\text{Xanthophylls (lutein) (mg g}^{-1}\text{)} = \frac{11.51 \cdot A_{480} - 20.61 A_{495}}{\text{mass} \cdot 200}$$

The final data were corrected based on the dry mass (Table 2). Plant samples were lyophilized using Christ Alfa 1-4 LSC lyophilizer.

Statistical analysis

Experiments were done in 4 replicates mean values and standard errors were calculated in Microsoft Office Excel 2016. The obtained data was elaborated by ANOVA and Duncan multiple range test at $P < 0.05$, using R Statistics packages.

Results and Discussion

Based on literatures not only the tubers but also areal part of Jerusalem artichoke can be valuable

for animal feeding purpose. Present study focused on the quantification of photosynthetic pigments from Jerusalem artichoke leaves and fractionated green juice and fiber. Control was alfalfa as the most important green biomass for animal feeding.

Photosynthetic pigments in leaves

By comparing the different seven Jerusalem artichoke ecotypes/varieties and applying alfalfa as a control, significant differences were found of photosynthetic pigments in leaves (Fig. 1). The highest amount of *chlorophyll a* was in the intact leaves of Jerusalem artichoke 'Tápióisima' ecotype (8.478 mg g⁻¹ DM), while the alfalfa leaves had 9.423 mg g⁻¹ DM, the other six ecotypes/varieties had almost the same values (6.013-7.800 mg g⁻¹ DM) (Fig. 1). Žnidarčič *et al.* (2011) measured pigment content

of five commonly consumed leafy vegetables; they found similar *chlorophyll a* value, except *Cichoriumintybus* 'Anivip' (238.31 mg/100g) and *Eruca sativa* (261.24 mg/100g) which had higher values. The highest amount of *chlorophyll b* in leaves was found in Alba (2.307 mg g⁻¹ DM) and Tápióisima (1.585 mg g⁻¹ DM) ecotypes. Values of the other five ecotypes were varied between 0.807-1.249 mg g⁻¹ DM, however alfalfa leaves had value of 3.852 mg g⁻¹ DM. Regarding carotenoid content of leaves, data presented in Fig. 1 showed that the highest contents were measured in leaves of Fuza (4.042 mg g⁻¹ DM) and in Tápióisima (3.987 mg g⁻¹ DM), while alfalfa leaves contained 5.361 mg g⁻¹ DM. Almost, similar values of carotenoids were obtained for other ecotypes/varieties (3.278-3.432 mg g⁻¹ DM) expect Alba which had 2.771 mg g⁻¹ DM. Data of xanthophyll content depicted

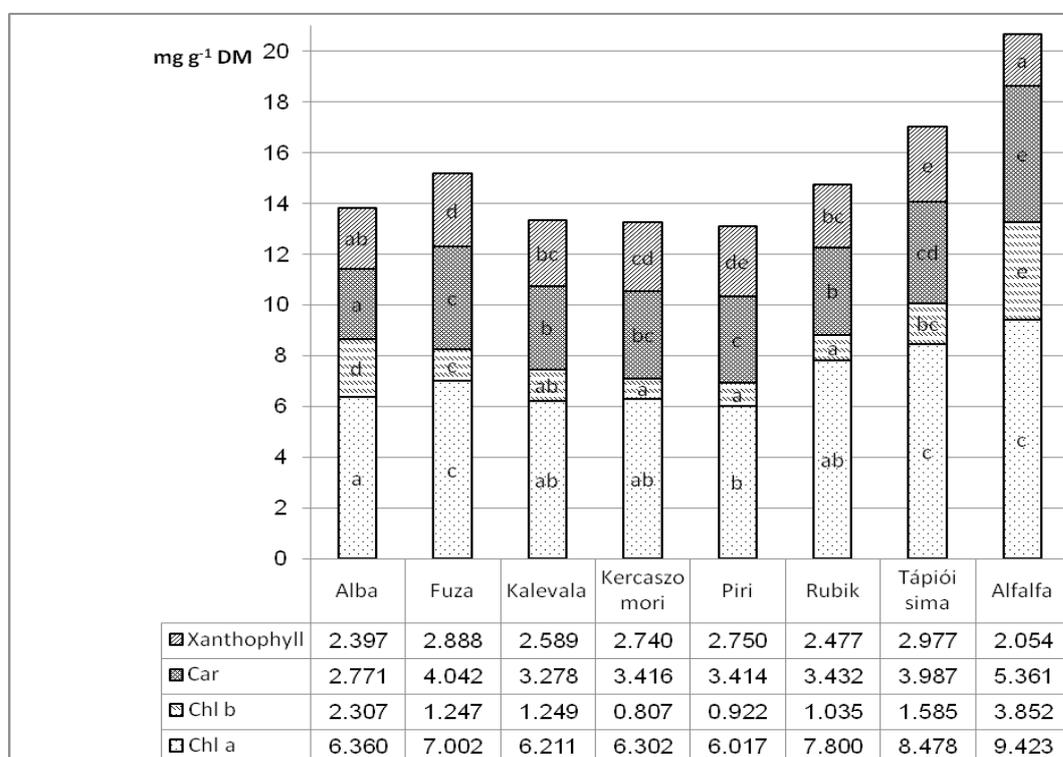


Fig. 1. Photosynthetic pigment content in leaves of Jerusalem artichoke ecotypes/varieties compared to alfalfa as a control (Means in the same column followed by the same letter are not significantly different according to Duncan's test at $P < 0.05$).

in Fig. 1 showed that the higher values in the intact leaves were measured in Tápióisima (2.977 mg g⁻¹ DM) and Fuza (2.888 mg g⁻¹ DM) ecotypes. For other ecotypes/varieties, same values almost were measured and ranged from 2.397 to 2.589 mg g⁻¹ DM.

Photosynthetic pigments in green juice

By examining the *chlorophyll a* content of green *Env. Biodiv. Soil Security* **Vol.2** (2018)

juices (Fig. 2), results proved that fractionated green juice contains lower *chlorophyll a* than intact leaves of tested ecotypes/varieties of Jerusalem artichoke. Among investigated ecotypes/varieties, we saw that the content of *chlorophyll a* in green juices did not follow the tendency of the results in the leaves, expect 'Rubik' ecotype. Highest content was measured in 'Kalevala' (3.067 mg g⁻¹

DM) and ‘Rubik’ (2.668mg g⁻¹ DM) ecotypes, while 5.095 mg g⁻¹ DM was found in leaves of alfalfa. On the other hand, *chlorophyll b* values in green juices extracted from green leaves were the highest in green juice derived from leaves of Fuza (1.042 mg g⁻¹ DM) and Tápióisima (1.030 mg g⁻¹ DM) ecotypes among all studied ecotypes/ varieties of Jerusalem artichoke, while control plant (alfalfa) had 1.725 mg g⁻¹ DM. However,

the low values of Alba (0.949 mg g⁻¹ DM) and Rubik (0.919 mg g⁻¹ DM) are considered not negligible either. By examining the carotenoid content of green juices, data showed that Alba and Rubik had similar values as 1.729 and 1.752mg g⁻¹ DM, respectively. Data of xanthophyll content in green juice fraction showed that among all studied ecotypes/varieties of Jerusalem artichoke the highest content (0.709 mg g⁻¹ DM) was found

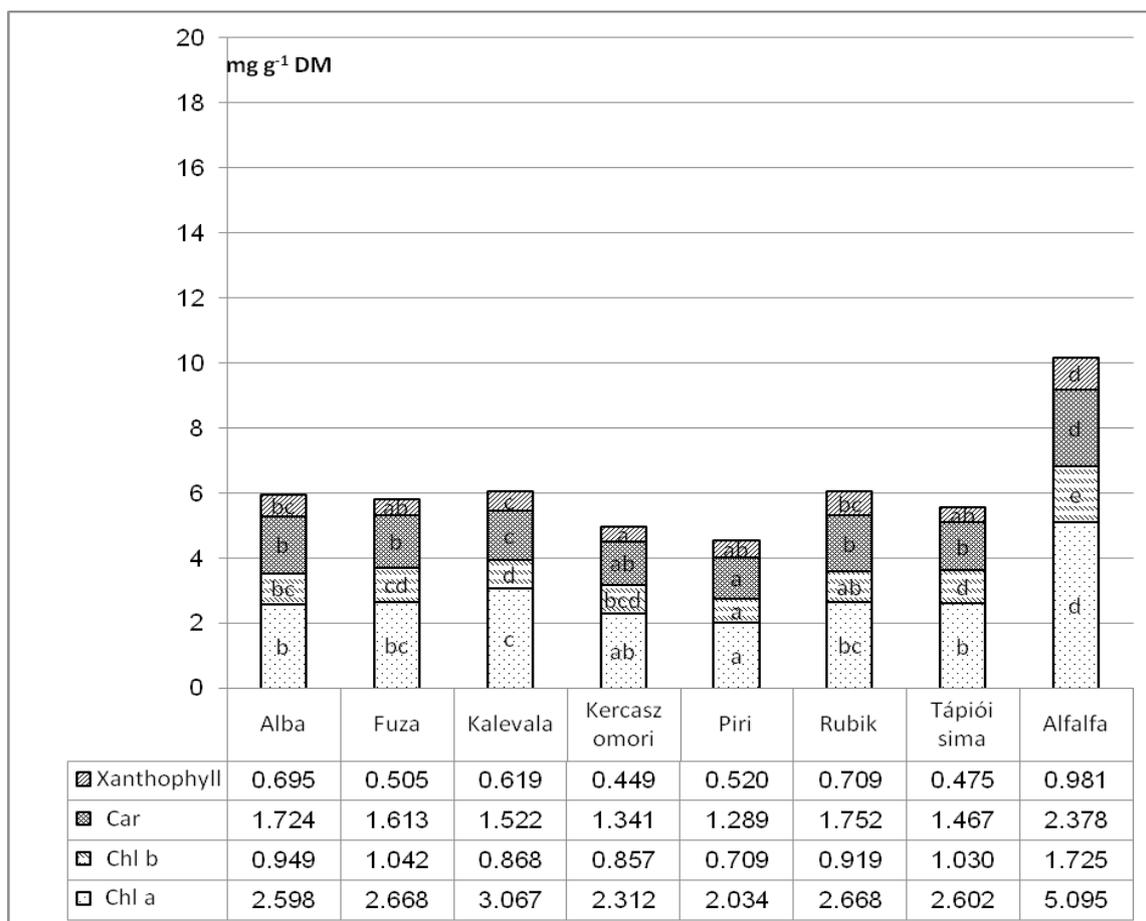


Fig. 2. Photosynthetic pigment content in green juices of Jerusalem artichoke ecotypes/varieties compared to alfalfa as a control (Means in the same column followed by the same letter are not significantly different according to Duncan’s test at $P < 0.05$).

in green juice extracted from leaves of Rubik ecotype followed by Alba ecotype (0.695 mg g⁻¹ DM). However, these results were not far from those derived from green juice of alfalfa leaves which had 0.981 mg g⁻¹ DM (Fig.2).

Photosynthetic pigments in fiber fraction

Regarding fiber fraction, results illustrated that the highest amount of *chlorophyll a* was measured in Alba (5.241 mg g⁻¹ DM) and in Tápióisima (5.312mg g⁻¹ DM) ecotypes, while

the lowest value (1.245 mg g⁻¹ DM) was recorded if fiber fraction of Rubik leaves. However, other ecotypes/ varieties had values ranged from 1.759 to 3.812 mg g⁻¹ DM and alfalfa (control) had 2.351 mg g⁻¹ DM (Fig.3). Content of *chlorophyll b* in fiber fraction was the highest in Alba (2.449 mg g⁻¹ DM) followed by Tápióisima (2.284 mg g⁻¹ DM), while in alfalfa *chlorophyll b* was 1.367 mg g⁻¹ DM. Other ecotypes/varieties showed varied values of *chlorophyll b* but the lowest value (0.475 mg g⁻¹ DM) was corresponded to

Rubic ecotype. Alba ecotype had the highest content (2.667 mg g⁻¹ DM) of carotenoids in fiber fraction followed by Tápióisima (2.750 mg g⁻¹ DM), meanwhile control plant (alfalfa) had lower carotenoids content (1.355 mg g⁻¹ DM). The

lowest content among all ecotypes/varieties was denoted for Rubic ecotype which had 0.804 mg g⁻¹ DM. However, other ecotypes had carotenoids contents varied from 1.239 to 1.954 mg g⁻¹ DM. In the case of xanthophyll, these two ecotypes (Alba

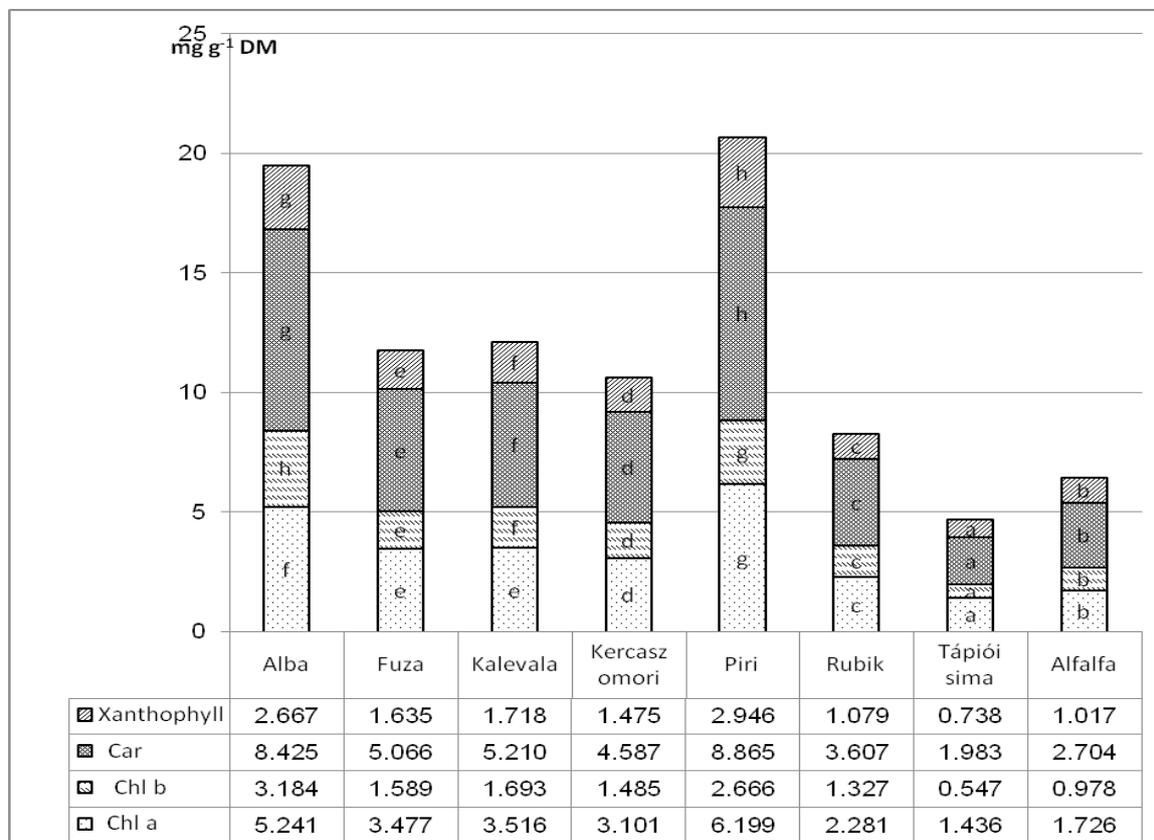


Fig. 3. Photosynthetic pigment content in fibers of Jerusalem artichoke ecotypes/varieties compared to alfalfa as a control (Means in the same column followed by the same letter are not significantly different according to Duncan's test at $P < 0.05$).

and Tápióisima) are also worth mentioning, but Kercaszomori (1.020 mg g⁻¹ DM) also showed good results. In general, the Rubik ecotype did not show any similarity to any of the other Jerusalem artichokes related to xanthophyll content in fiber fraction (Fig.3).

Chlorophylls are known to be easily degraded by such as dilute acids, heat and oxygen (Tonucci and Von Elbe 1992). The reason for green color loss during processing is mainly attributed to the conversion of chlorophylls to pheophytins by the influence of pH (Mingues-Mosquera *et al.* 1989). In acid medium, magnesium in the chlorophyll rings is replaced by two hydrogen ions and green chlorophylls are converted to the olive brown pheophytins (Mangos and Berger 1997; Van Bokel 1999; VanBokel 2000). In our current research pH of green juices ranged from 5.53 to

6.54. Formation of pheophytins is initiated by the release of cellular acids and the synthesis of new acids. It is reported that formation of pheophytin in processed vegetables is increased at lower tissue pH values and at higher process temperature (LaBorde and Von Elbe 1990). Likepheophytins, pheophorbides also may occur under the influence of heat or acid (Weemaes *et al.* 1999). The cleavage of phytol chain of chlorophyll by the enzyme chlorophyllase results in formation of chlorophyllide (Heaton and Marangoni 1996). In the presence of acid, chlorophyllides undergo loss of magnesium and form pheophorbides (Heaton and Marangoni 1996; White *et al.* 1963). The chlorophyllase activity also depends on the pH level and ionic content of the medium which thought to be involved in turnover and homeostasis of chlorophyll. The chlorophyllase

also considered the first enzyme in the chlorophyll degradation pathway. The location of the enzyme is the inner envelope membrane of the chloroplast (Matile et al. 1997).

Chlorophyll b degradation is different from *chlorophyll a*, where *chlorophyll b* is degraded by first being converted to *Chlorophyll a* (Ito et al. 1993; Schuermann et al. 1996). The second major problem during the processing is the high amount of polyphenols, which can cover the pigments. The main phenolic acids in *H. tuberosus* leaves are chlorogenic acids (93%) (Chen et al. 2014; Yuan et al. 2012). More broadly, phenolic acids are widely distributed in plants as the secondary metabolites (Mattila and Hellström 2007). According to Chen et al. (2014) the major phenolic compounds are 3-o-caffeoylquinic acid (33%), 4,5-dicaffeoylquinic acid (24%) and 3,5-dicaffeoylquinic acid (21%) of the total phenolics. In addition, the dry matter content may also affect the photosynthetic pigment content. Different fractions yielded the following results: for leaves it represents around 20%, green juices are around 11.5%, fiber dry matter content is 40%.

Conclusion

Alongside high tuber yield, Jerusalem artichoke generated a huge green biomass during its growing season rich in chlorophylls and carotenoids subsequently considerable amounts of chlorophylls and carotenoids for humans and animals consumption can be extracted in cost-effective approach. The recent experiment studied the composition of photosynthetic pigments of seven ecotypes/varieties of Jerusalem artichoke as a potential source for chlorophyll. Alfalfa – the main fodder crop – was applied as a control. Despite, alfalfa plants had the highest contents of chlorophyll a, chlorophyll b, carotenoids and xanthophyll compared to Jerusalem artichoke ecotypes/varieties particularly in intact leaves and green juice extracted by pressing; Jerusalem artichoke ecotypes/varieties had highest contents of photosynthetic pigments in fiber fraction. Also, resulted verified that Piri, Tápíósisima, Alba and Rubic are the best among other studied ecotypes/varieties. It could be concluded that Jerusalem artichoke green biomass (especially leaves) can be a good and rich source of chlorophyll for animal. Along with it spines and fibres free, easy to digest green juice with significant amount of valuable photosynthetic pigments can also be good raw material for further food development involving inhuman nutrition.

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