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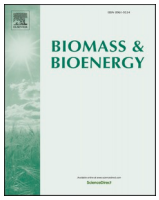

Identification of key parameters and phytochemical composition of fiber and brown juice by-products from green biomass of Jerusalem artichoke (*Helianthus tuberosus* L.)

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ABSTRACT

The investigation within the green biorefinery focused on value-measuring properties of by-products derived from leaf protein concentrate production, particularly from Jerusalem artichokes. Results showed fiber fractions ranged from 17 to 28 % per 1 kg of fresh biomass, while brown juice fractions ranged from 57 to 68 %. Protein content varied from 7 to 14 m/m% for fiber and 0.3–1.4 m/m% for brown juice, with Rubik and Piri varieties exhibiting minimal differences in crude protein content between harvests. Amino acid analysis revealed significant amounts of leucine and lysine in both fractions. Phenolic compounds, notably hydroxy-methoxy flavones, were abundant in brown juice, with Rubik variety showing promising total phenolic content (TPC) and total flavonoid content (TFC). Qualitative determination identified chlorogenic acid and two isomers among cinnamic acid derivatives, suggesting potential medicine candidates. Brown juice fractions, especially from Rubik variety, hold promise for applications such as biostimulants. Further exploration of phenolic components in brown juice could yield valuable insights.

1. Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) as a promising industrial plant is native to North America. While it has often been classified as a perennial plant in botanical descriptions, Verbung et al. [1], Crawley [2], and Koncenková [3] have revised this classification, considering it more accurately as a pseudo-annual plant. This classification implies that at the conclusion of the growing season, the above-ground portion of the plant, along with the root system, withers away, leaving only tubers in the soil. These tubers give rise to genetically identical plants in the subsequent year. Jerusalem artichoke (JA) cultivation primarily centers around its tubers, which are rich in inulin. In addition to their culinary value, these tubers can yield a substantial amount of above-ground biomass, with the potential to reach up to 2.5 kg in fresh weight per plant by the end of the growing season [4]. According to Gunnarsson et al. [5], JA crops can yield between 7 and 16 tons per hectare of dry biomass, owing to their robust tillering and suitability for multiple harvests. Fresh and green shoots of JA can also be employed directly or as silage for animal feed. The entire stalk of the plant can be processed into feed mixtures, although literature indicates that distribution of nutrients and proteins within leaves and stems may not be uniform.

Stalks of JA are rich in essential primary metabolites such as proteins. According to Stauffer [6], protein content in JA leaves can reach up to 20 % by weight, including approximately 5–6% of essential amino acids, such as lysine and methionine. Johansson et al. [7] revealed that protein content in above-ground shoots can reach 23 %. However, processed JA green biomass through green biorefinery exhibited a protein content ranging from 31.6 % to 35.2 % [8].

In addition to its protein content, green leaves are rich in many

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valuable phytochemicals, including pigments, fatty acids, and phenolic compounds. Leafy stems also harbor various carotenoids such as α-, β-, γ-carotene, lutein, lycopene, and zeaxanthin, which may vary broadly according to growth stage, plant organ, and environmental conditions ([9,10]). According to Ersahince and Kara [10], the highest concentration of lutein was recorded during the full flowering stage, amounting to 120.14 mg/kg dry weight, followed in descending order by β-carotene, zeaxanthin, α-carotene, and lycopene. Additionally, leafy stalks contain trace amounts of essential oils ([11,12]). In their study, Radulovic and Đordevic [11] successfully identified 192 essential oil components, with the most prevalent ones being β-bisabolene, α-pinene, kaunan-16-ol, and phenylfurfuran. β-bisabolene was found to be the most abundant component, constituting 22.9–30.5 % of the essential oil composition. Additionally, Bach et al. [13] noted that α-pinene, a monoterpene, ranked as the most abundant component, followed by β-bisabolene. Phenols naturally occur in plants as a defense mechanism against various stressors. However, studies by Petzke et al. [14] and Rohn et al. [15] have revealed that phenolic components can form complexes with proteins, demonstrating the protective role of phenols in safeguarding proteins. Conversely, phenolic compounds can diminish the efficiency of protein utilization within animal organism, leading to effects such as protein precipitation, loss of enzymatic activity, and a slowdown in protein absorption.

In today's context, one of the foremost nutritional challenges is protein deficiency and malnutrition. Addressing this persistent protein shortage is expected to involve the expansion of current agricultural practices into marginal lands. Additionally, green biorefining emerges as a promising solution. Ideal plants for green biorefining are typically perennials (eg. alfalfa) characterized by a substantial volume of green biomass and the ability to regenerate consistently when a continuous supply is required. Another crucial criterion is their adaptability to various climate conditions [16]. Based on literature and our previous experiments Kaszás et al. [8,17,18], Jerusalem artichokes can be included in the list of plants that can be integrated into green biorefineries. Not only because of its high green biomass productivity, but also because of its excellent adaptability to almost any climatic conditions.

According to Domokos-Szabolcsy et al. [16] green biomass can be effectively separated into two distinct fractions: fiber and green juice. Several techniques exist for concentrating proteins within green juice fraction. The most employed methods include thermal, acidic, alkaline, flocculation, and fermentation processes. In the realm of green biorefining, leaf protein concentrate (LPC) serves as the primary product of interest. In addition to the main product, the by-products (fiber, brown juice) also have values [16].

According to Kamm et al. [19] fiber can be used for silage production or can be fermented for further processed products. Also, Clark and Dewarte [20] stated that the fiber fraction can be used for bioethanol and biogas production. In addition, Csatári et al. [21] drew attention to the role of fiber in human and animal nutrition as a source of dietary fiber.

Brown juice contains significant amounts of sugars, oligopeptides, secondary metabolites, and minerals [22]. Due to its composition, brown juice can be used for L-lysine production [23], methane production [24], or as medium for microbes [25]. Results of Barna et al. [26] showed that the brown juice can be used as a biostimulant, which implies the presence of growth regulators.

This work focused on the evaluation of fiber and brown juice fractions obtained from green biorefining of JA biomass through: i) assessing the protein content of fiber and brown juice fractions obtained during the green biorefining process of JA biomass, ii) analyzing the amino acid composition of fiber and brown juice fractions, with a focus on identifying essential amino acids such as lysine and methionine, iii) determining the levels of total polyphenols and flavonoids in fiber and brown juice fractions using colorimetric method and advanced analytical techniques such as UHPLC-ESI-MS, iv) exploring potential applications and value-added uses for fiber and brown juice fractions based on their nutritional and phytochemical profiles, v) contributing to the development of sustainable and economically viable processes for utilizing JA biomass by-products in various industries, including food, pharmaceuticals, and agriculture, and vi) providing valuable insights into the potential health benefits and commercial opportunities associated with these JA biomass-derived fractions.

2. Materials and methods

2.1. Experimental design

In 2016 and 2017, field studies were established at the Horticultural Demonstration Garden located at the University of Debrecen, Hungary (latitude 47° 33′ N; longitude 21° 36′ E). Tubers of seven different JA clones were sourced from various origins as follows: Kercaszomori, Rubik, and Tápiói sima were obtained from the Plant Diversity Centre, Tápiószele, Hungary; Fuseau was imported from Ismailia, Egypt; Kalevala was generously provided from Helsinki, Finland; and Alba and Piri were purchased from a Hungarian local market.

The experimental design was a randomized complete block configuration, featuring six replicates. The total experimental area spanned 8 m in width and 20 m in length, comprising 42 rows, each measuring 3.5 m in length and 0.8 m in width. The in-row spacing was set at 0.6 m, resulting in a planting area of 0.42 m^2 per plant. Cultivation of the JA clones commenced on April 5, 2016 and 2017, using tubers of uniform size (50–60 g). Throughout the cultivation period, germinated tubers received no supplemental irrigation, relying solely on rainwater (rainfed plantation), and no fertilizers were applied. The weather data recorded during the entire experimental duration were as follows: temperature (min. max. avarage), precipitation distribution. The nutritional status of the experimental soil was as follows: total nitrogen (N) content, 555 ± 2 mg/kg; total phosphorus (P) content, 6793 ± 17 mg/kg; total potassium (K) content, 1298 ± 7 mg/kg; and humus content, 1.9 ± 0.02 %.

2.2. Collection of JA green biomass

The aerial parts of JA were gathered twice annually for the purpose of isolating leaf protein. The first harvest took place on June 27, 2016, and 2017, and the second harvest was done on August 8, 2016 and 2017 when the shoot parts had attained a height of about 1.5 m from the soil surface.

2.3. Processing of fresh aerial biomass of JA

To isolate leaf proteins from freshly harvested biomass of JA cultivars, aerial parts were collected at a height of 20 cm above the soil surface in the early morning. They were promptly transported to the laboratory under refrigerated conditions at 4 °C, utilizing ice cubes and an ice box to effectively inhibit the activity of proteases, which can otherwise degrade cellular proteins. For the extraction process, 1 kg of freshly harvested biomass underwent mechanical pressing and pulping, facilitated by a twin-screw juicer (Angel Juicer 7500, Sinpyung Dong, Saha-Gu, Busan, South Korea). This procedure aimed to separate the green juice from the non-digestible fiber present in biomass. The resulting green juice contained chloroplastic and cytoplasmic proteins, pigments, and vitamins. Subsequently, the green juice was subjected to heating at 80 °C to induce protein coagulation according to Kaszás et al. [8]. Following thermal coagulation, the resulting coagulum was separated from a brown-colored liquid, known as brown juice, through cloth filtration (pore size 30 μm). Fiber and brown juice were weighed to determine their fresh mass. Fiber fraction was then subjected to lyophilization using an Alpha 1–4 LSC basic (Martin Christ Ltd., Germany) lyophilizer. Lyophilized fiber, and brown juice were placed into a −20 °C freezer for further analysis.

2.4. Measurements

2.4.1. Crude protein content

The determination of crude protein content in fiber and brown juice fractions was carried out by the Kjeldahl method according to ISO 5983–2:2009 international standard method. Following this digestion process, the total nitrogen (N) content within the digested samples was determined through the titration method. The total protein content of the samples was calculated according to Mariotti et al. [27] and using the following formula:

$$\text{Total crude protein (\%)} = \text{total N content} \times 5.6$$

2.4.2. Quantitative analysis of amino acid composition

2.4.2.1. Sample preparation. A 20 mg lyophilized and powdered fiber sample, or 250 μL of brown juice sample, was precisely measured and introduced into a Teflon digestion tube containing 10 mL of 6 M hydrochloric acid (VWR Ltd., Budapest, Hungary). Subsequently, the tube was purged with inert nitrogen gas to eliminate any traces of oxygen. Following this, the tubes were hermetically sealed and positioned within a CEM Mars One microwave digester (Matthews, USA) set at 150 °C for 60 min, with a power output of 650 Watts. The resultant hydrolysate was subsequently neutralized using an equal volume of 6 M sodium hydroxide (VWR Ltd., Budapest, Hungary). For the free amino acid determination, 250 and 500 μL of AccQ Tag Ultra borate buffer (Waters, Milford, MA, USA) were added to 250 μL brown juice and 20 mg fiber sample, respectively. The process of sample hydrolysis was conducted in triplicate for accuracy. All samples (hydrolyzed and non-hydrolyzed) underwent filtration using a 3 kDa Nanosep Omega-type membrane filter (Fisher Scientific, Göteborg, Sweeden).

Prior to ultra-high performance/pressure chromatography (UHPLC) separation, all samples were derivatized following the manufacturer's instructions, employing the AccQ-Tag Ultra derivatization reagent. This procedure involved the addition of 70 μL of AccQ Tag Ultra borate buffer and 20 μL of AccQ Tag Ultra reagent to 10 μL of the filtered sample, followed by thorough mixing using a vortex. The resulting mixture was then incubated at 55 °C for 10 min.

2.4.2.2. Measurement procedure. The analysis of amino acid composition was conducted utilizing an UHPLC system (Waters, Milford, MA, USA). This analysis employed a pre-column derivatization method.

The mobile phases employed were as follows: A) 100 % AccQ Tag Ultra Eluent A concentrate (Waters, Milford, MA, USA), B) 90:10 HPLC water to AccQ Tag Ultra Eluent B (Waters, Milford, MA, USA), C) 100 % HPLC water (Sigma-Aldrich, Germany), and D) 100 % AccQ Tag Ultra Eluent B (Waters, Milford, MA, USA).

The separation of derivatized amino acids took place on an AccQ-tag Ultra C18 column (1.7 μm; dimensions: 2.1 × 100 mm, Waters, Milford, MA, USA), and it was filtered by an Acquity in-line filter (0.2 μm; dimensions: 2.1 mm, Waters, Milford, MA, USA), which is a proprietary component. The flow rate was set at 0.600 mL/min, and the column temperature was maintained at 43 °C. The entire gradient program developed for the separation process had a total duration of 11 min. The results were subsequently evaluated and processed using Waters Empower 3 software (Waters, Milford, MA, USA). The data obtained in the processing file is expressed in picomoles. The determination of amino acid content in the samples was facilitated through additional calculations.

2.4.3. Spectrophotometric determination of total polyphenols (TPC) and flavonoids (TFC)

Briefly, 20 mg of lyophilized fiber was subjected to extraction with 1 mL 70:30 methanol:distilled water. The samples were then stirred for 2 min and allowed to incubate at room temperature for 30 min within an ultrasound bath. One mL of brown juice was pipetted into Eppendorf tubes. Subsequently, the mixtures and the brown juice underwent centrifugation at 13,000 rpm for 3 min (Eppendorf Centrifuge 5415 R, Enfield, CT, USA). The quantification of total polyphenols in supernatants was performed using the Folin-Ciocalteu reagent method [28]. The absorbance of the resulting color was measured at 760 nm. A standard curve was generated using gallic acid, and the outcomes were expressed as mg of gallic acid equivalents (mg GAE/g sample) on a dry weight basis. Every measurement was conducted five times to ensure consistency and reliability.

The extraction of total flavonoid content followed the same procedure as that for total polyphenolic content. The quantification of total flavonoids in the resulting supernatants was performed using a 10 % aluminum chloride solution, as outlined by Zhishen et al. [29]. The absorbance of the generated color was measured at 415 nm. Rutin served as the standard for generating a calibration curve, and the results were expressed in mg of Rutin Equivalents (mg REV/g sample DW) on a dry basis. To ensure precision and consistency, each measurement was replicated in five.

2.4.4. Qualitative analysis of phytochemicals

2.4.4.1. Sample preparation. For the hydro-alcoholic extracts, 0.5 g of powdered fiber and 0.5 g of powdered brown juice were subjected to extraction using 25 mL of 70 % methanol. The samples were incubated at 150 rpm with constant stirring for 2 h at room temperature. Subsequently, the hydro-alcoholic extracts were filtered through a 0.22 μm PTFE syringe filter.

2.4.4.2. Measurement procedure. Phytochemical analyses were conducted utilizing the UHPLC-ESI-MS (Ultra-High Performance Liquid Chromatography-electrospray ionization/mass spectrometry) technique as Kaszás et al. [17] described. This involved the use of a Dionex Ultimate 3000RS UHPLC system, coupled with a Thermo Q Exactive Orbitrap hybrid mass spectrometer. The analytical column employed was a Thermo Accucore C18 with dimensions of 2.1 × 100 mm and a particle size of 2.6 μm. The flow rate was maintained at 0.2 mL/min, and the column oven temperature was set at 25 ± 1 °C. The mobile phase consisted of methanol (A) and water (B), both acidified with 0.1 % formic acid. The gradient program followed this sequence: 0–3 min, 95 % B; 3–43 min, 0 % B; 43–61 min, 0 % B; 61–62 min, 95 % B; 62–70 min, 95 % B. The injection volume was set at 2 μL.

The Thermo Q Exactive Orbitrap hybrid mass spectrometer was equipped with an electrospray ionization (ESI) source. The samples were separately measured in both positive and negative ionization modes. Specific instrument settings were as follows: the capillary temperature was maintained at 320 °C, the spray voltage was set at 4.0 kV in positive ionization mode and 3.8 kV in negative ionization mode. The resolution was set to 35,000 for MS1 scans and 17,500 for MS2 scans, with a scanned mass interval of 100–1500 *m/z*. For MS/MS scans, the collision energy was adjusted to 30 NCE (Normalized Collision Energy). In all cases, the variation between the measured and calculated molecular ion masses was within a margin of less than 5 ppm. Data acquisition and processing were conducted using Thermo Trace Finder 2.1 software, which relied on both proprietary and internet databases (including Metlin, Mass Bank of North America, and *m/z* Cloud). After processing, the results underwent manual verification using Thermo Xcalibur 4.0 software. The identification of compounds present in the extracts was based on a combination of factors including exact molecular mass, isotopic pattern, characteristic fragment ions, and retention time. This identification process drew from our previously published research as well as data available in the literature.

2.5. Statistical analysis

Microsoft Excel was employed to calculate the mean values and standard deviations. The statistical analysis encompassed ANOVA, and the subsequent comparison of means was carried out through Duncan's multiple mean comparator test, all conducted at a significance level of $p < 0.05$. The analysis was executed using the R software package.

3. Results and discussion

3.1. Yield of fiber and brown juice of JA clones

When investigating the incorporation of Jerusalem artichokes into green biorefineries, particular emphasis should be placed on the characterization of the different fractions. For this reason, quantitative and qualitative characterization was an important aspect in the evaluation of the green biomass fractions.

Yields of fiber and brown juice fractions obtained from JA green biomass in the two years are presented in Table 1. In 2016, at the first harvest, the Kalevala variety produced the highest quantity of fiber fraction (242 g/kg fresh biomass), while the Alba variety produced the lowest quantity (166 g/kg fresh biomass). Statistically significant differences were observed for all varieties. However, results revealed that the fiber fractions of the studied varieties represented 15–24 % of the total fresh green biomass.

Regarding fiber yield of the second harvest of 2016, all JA clones showed slightly higher yields, except for Kalevala (Table 1). Fiber fraction represented 17–23 % of the JA green biomass during the second harvest of 2016. The highest yield (230 g/kg fresh biomass) corresponded to the Piri variety, while the lowest yield (177 g/kg fresh biomass) was obtained for the Fuseau variety.

In 2017, the highest fiber yield of the first harvest was 318 g/fresh biomass and corresponded to the Kalevala variety, while the Fuseau variety displayed the lowest yield (266 g/kg fresh biomass). The yield of fiber fraction varied significantly among the seven studied JA clones. Overall, the fiber fraction represented bout 26–32 % of the pressed green biomass. Similar findings were observed in the second harvest in 2017, where significant differences were statistically calculated among the JA clones regarding the fiber fraction yields. The Tápiói sima variety revealed the highest fiber yield (293 g/kg fresh biomass), while the lowest fiber fraction was 206 g/kg fresh biomass and corresponded to the Fuseau variety. Overall, the yield of fiber fraction in 2017 was higher than that recorded in 2016.

Yield of fiber fraction varies according to the plant species; however, in the present study, fiber fraction of JA was 7 % lower than alfalfa and soybean fiber and 10 % lower than broccoli fiber [21].

Brown juice represented that largest fraction obtained from the processed green biomass of JA (Table 1). For instance, processing one kg fresh green biomass of JA produced about 520–740 g brown juice. In 2016, the Fuseau variety recorded the highest brown juice (738 g/kg fresh mass) in the first harvest, while the lowest yield (674 g/kg fresh biomass) corresponded to the Kalevala variety. Differences between the JA clones regarding brown juice yield were significant. In the second harvest of the same year, the Alba and Fuseau varieties exhibited the highest brown juice yield (710/kg fresh biomass), while the lowest yield (664 g/kg fresh biomass) was obtained for the Kercaszomori variety. The seven JA clones did not show significant differences regarding brown juice yield of the second harvest in 2016.

On the other hand, in 2017 first harvest, brown juice yield varied significantly among the JA clones. The Tápiói sima variety revealed the highest brown juice yield (613 g/kg fresh biomass), whereas the lowest recorded brown juice yield was 546 g/kg fresh biomass and corresponded to the Piri. Generally, the brown juice fraction represented about 54–61 % of the processed green biomass. During the second harvest, the Alba variety yielded the highest amount of brown juice (628 g/kg fresh biomass), while the Rubik variety produced the lowest amount (525 g/kg fresh biomass). The difference between the two years can be explained by weather conditions, with 2017 being characterized by dry, droughty weather on several occasions. This could have led to more fiber formation.

Barna et al. [20], who reported that processing one kg alfalfa fresh biomass produced 420–470 g brown juice. Our results showed that the produced brown juice of JA green biomass is 20 % higher than that obtained from alfalfa fresh biomass. Similar findings were reported by Domokos-Szabolcsy et al. [30] for broccoli green biomass, which produced 640 g brown juice per one kg fresh biomass.

3.2. Protein and amino acid contents

Most of the protein accumulates in the leaf protein concentrate during fractionation of the green biomass; however, a small portion of protein was retained in the fiber and brown juice fractions. Fig. 1 shows the crude protein contents of fiber and brown juice fractions within two harvests of two growing seasons, based on the Kjeldahl method. In 2016, similar crude protein contents of the seven JA clones were noticed within the two harvests. Yet, the differences of crude protein contents among the JA clones were statistically significant. The crude protein contents ranged between 11 and 14 m/m% in the first harvest, while in the second harvest it varied between 7 and 13 m/m%.

In 2017, crude protein content in the fiber fraction ranged from 9 to 14 m/m% and 11–13 m/m% in the first and second harvests, respectively. The crude protein content in fiber fraction of in both years did not significantly change.

In agricultural practice, the fiber fraction is mainly used as a fiber supplement in ruminant feed. In addition to minerals, protein intake is also essential in feed; therefore, protein content of fiber fraction is high importance. It is known that the digestion of fiber in the rumen contributes to the quality of milk fat [31,32]).

Similar crude protein content (13 m/m%) was reported in fiber fraction of JA by Rawate and Hill [33], although a different method was used for obtaining fiber fraction. According to FAO data, the protein content of the fiber fraction of JA is about 15 m/m%. According to

Table 1
Fiber and brown juice yields of seven Jerusalem artichoke clones of two harvests in two growing seasons (2016 and 2017) under extensive, rain-fed conditions.

JA clones	Fiber (g/kg fresh biomass)				Brown juice (g/kg fresh biomass)			
	2016		2017		2016		2017	
	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest
Alba	166 ± 1.5^{c}	210 ± 13.1^{ab}	279 ± 13.2^{cd}	240 ± 19.0^{a}	720 ± 50.0^{ab}	710 ± 20.0^{a}	549 ± 25.7^{b}	628 ± 14.4^{a}
Fuseau	171 ± 10.0^{c}	177 ± 21.2^{b}	266 ± 32.1^{d}	206 ± 9.1^{b}	738 ± 10.4^{a}	710 ± 45.8^{a}	578 ± 16.1^{ab}	627 ± 12.1^{a}
Kalevala	242 ± 14.6^{a}	182 ± 22.7^{b}	318 ± 12.9^{a}	234 ± 9.9^{ab}	674 ± 10.1^{d}	705 ± 38.8^{a}	576 ± 15.3^{ab}	625 ± 14.2^{a}
Kercaszomori	203 ± 5.5^{b}	224 ± 15.4^{a}	298 ± 20.6^{abc}	231 ± 14.5^{ab}	708 ± 34.2^{abc}	664 ± 13.6^{a}	580 ± 28.0^{ab}	560 ± 30.8^{b}
Piri	200 ± 5.5^{b}	230 ± 11.5^{a}	306 ± 23.1^{ab}	253 ± 18.3^{a}	713 ± 16.0^{abc}	680 ± 18.0^{a}	546 ± 23.1^{b}	558 ± 28.0^{b}
Rubik	205 ± 11.0^{b}	213 ± 16.8^{ab}	285 ± 22.0^{bc}	257 ± 27.0^{a}	691 ± 6.6^{bcd}	671 ± 19.7^{a}	590 ± 30.0^{ab}	525 ± 19.0^{c}
Tápiói sima	201 ± 8.2^{b}	219 ± 28.0^{a}	276 ± 15.3^{cd}	246 ± 19.0^{a}	687 ± 15.6^{cd}	681 ± 30.1^{a}	613 ± 20.2^{a}	610 ± 11.9^{a}
Average	**198 ± 6.8**	**178 ± 18.4**	**289 ± 19.9**	**238 ± 16.7**	**704 ± 14.0**	**688 ± 27.6**	**576 ± 22.8**	**590 ± 18.6**

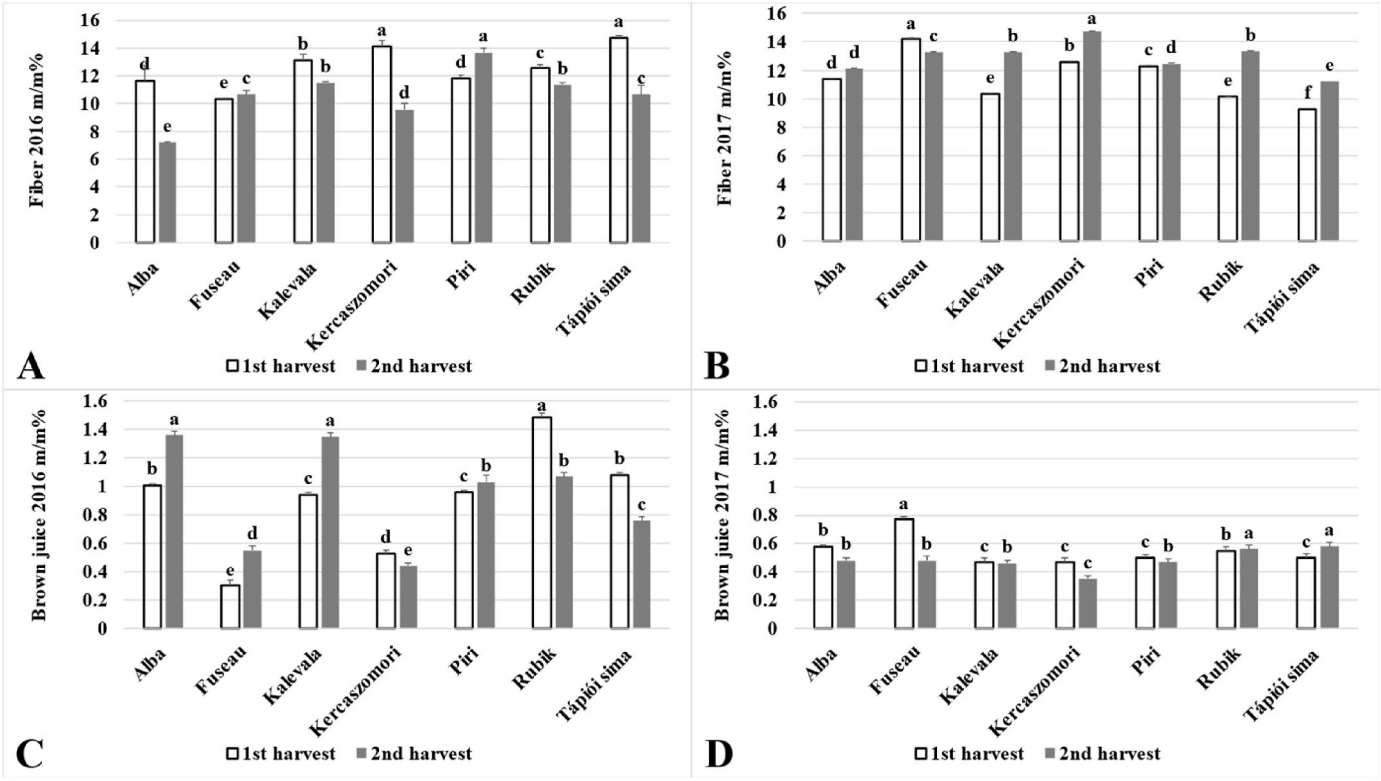

Fig. 1. The crude protein content (m/m%) of the fiber 2016 (A) and 2017 (B) and brown juice 2016 (C) and 2017 (D) fractions obtained from biorefining of seven different Jerusalem artichoke clones under rain-fed conditions (different letters above the columns represent significant difference by Duncan test at $p < 0.05$).

Kamm et al. [19], the fiber fraction produced by green biorefining can also be used to make silage. Zhang et al. [34] reported a crude protein content of 6.66–9.14 m/m% in maize silage, which is lower than that reported in the present study for JA's fiber fraction (Fig. 1A and B). Alnhood and Massimi [35] reported that corn silage has a protein content of 5.56 m/m%, which is lower than the JA fiber fraction, while alfalfa silage displayed higher crude protein content (15.25 m/m%).

The crude protein content of brown juice (Fig. 1C and D) varied between 0.3 and 1.4 m/m%, respectively. Subsequently, 1D SDS PAGE gel electrophoresis was also performed (data not reported) and no band pattern was obtained for the brown juice. This may be related to the presence of oligopeptides, which warrants further investigation. Together with this, the presence of free amino acids (Table 2) may also explain the crude protein content results. Thomsen et al. [23], who investigated brown juice production during green biorefining of white clover (*Trifolium repens*), Italian rye grass (*Lolium multiflorum*) and alfalfa (*Medicago sativa*), documented that the crude protein content of all type of brown juices were around 0.9 m/m%, similar to that of JA. Domokos-Szabolcsy et al. [30] reported a 1.96 m/m% crude protein content in brown juice obtained from broccoli leaves.

The nutritional and feed biological value of proteins is mainly determined by their amino acid composition. Therefore, an analysis of the amino acid profile of fiber and brown juice by-products was performed. The varieties included in the study did not show significant differences in amino acid profile and are summarized in Table 2. The total amino acid composition of the fiber fraction was 148.2 mg/g, while the free amino acid composition was about 100-fold lower (1.542 mg/g). The free amino acid content of the brown juice was 1.007 mg/mL which is about 7.6 % of the total amino acid content (13.2 mg/mL).

Aspartic and glutamic acids were the most abundant in the total amino acid profile of the fiber fraction, recording 16.67 and 16.38 mg/g, respectively, while cysteine and methionine exhibited the lowest contents of 0.65 and 1.06 mg/g, respectively. Asparagine and glutamine were not detected in the total amino acid composition of the fiber fraction. This might be attributed to acid hydrolysis process, where asparagine and glutamine may be converted to aspartic and glutamic acids upon acid hydrolysis [36].

Table 2
Free and total amino acid composition in the fiber and brown juice fractions obtained through green biorefinery of Jerusalem artichoke based on UPLC measurements (LOQ – below limit of quantification).

Amino acid	Fiber (mg/g DW)		Brown juice (mg/mL)	
	Free amino acid	Total amino acid	Free amino acid	Total amino acid
Alanine	0.123	9.84	0.031	0.62
Arginine	0.080	11.01	0.004	0.34
Asparagine	0.049	LOQ	0.027	LOQ
Aspartic acid	0.310	16.67	0.307	2.86
Cysteine	LOQ	0.65	0.001	LOQ
Glycine	0.042	8.57	0.002	0.57
Glutamine	0.091	LOQ	0.136	LOQ
Glutamic acid	0.263	16.38	0.342	4.05
Histidine	LOQ	3.43	0.003	0.19
Isoleucine	0.048	7.46	0.014	0.37
Leucine	0.083	14.58	0.015	0.55
Lysine	0.096	10.04	0.016	0.65
Methionine	0.029	1.06	0.002	LOQ
Phenylalanine	0.068	8.98	0.017	0.48
Proline	0.045	8.11	0.010	0.56
Serine	0.041	7.29	0.018	0.46
Tyrosine	0.052	6.49	0.011	0.34
Threonine	0.065	8.10	0.027	0.57
Valine	0.057	9.54	0.024	0.59
Sum	**1.542**	**148.2**	**1.007**	**13.2**

From a feed perspective, some amino acids are not only essential but can also be limiting. This means that in their absence, life processes slow down or even stop. These amino acids include e.g. lysine, methionine, valine [37]. The essential amino acids with the highest amounts in the

total amino acid content of the fiber fraction were leucine (14.58 mg/g) and lysine (10.04 mg/g), which are also limiting amino acids. They were also predominant among the others when the free amino acid content was measured (0.083 and 0.096 mg/g). The total and free amino acid contents of the first limiting amino acid in poultry feed, valine, were 9.54 and 0.057 mg/g, respectively. The amount of amino acids in a plant is primarily determined by its genotype, but can also be influenced by different environmental conditions, such as soil nitrogen content. The experiments of Zhao [38] and Otálora [39] show how different increasing nitrogen rates affected the amino acid content of the crop. It was found that the ratio of amino acids to each other did not change but their quantity did increase at certain nitrogen levels. It is also true, however, that above a certain dose the amino acid abundance did not increase, on the contrary, it decreased.

3.3. Total polyphenol (TPC) and total flavonoid (TFC) contents

Secondary metabolites are organic compounds that do not play a direct role in the growth, development, or reproduction of living organisms. Unlike primary metabolites, their deficiency does not lead to the rapid death of organisms, but in the long-term they impair survival, fertility, cause aesthetic changes, and may have no consequences. The presence of secondary metabolites is often restricted to certain species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against pests, abiotic stresses and in another interspecific defense. Fig. 2 is illustrating the amount of total phenolic components obtained from the fiber and brown juice fractions from the two years.

In 2016, at the beginning of the growing season, during initial development, an average temperature of 17–22 °C was associated with a favorable effect on plant development. In contrast, in the second period, we had a much higher average air temperature of 25–27 °C. However, the distribution of precipitation was not even, which also appeared as a kind of abiotic stressor, which promoted the production of phenolic components.

During early growing season in 2017, the average temperature was around 25–28 °C, and the distribution of rainfall was also uneven. In the second part of the growing year, air temperatures above 30 °C were not rare, accompanied by an uneven distribution of rainfall, which was exerted by increased stress in the plants.

Examination of the varieties showed that in the first harvest of 2016 the highest TPC (12.75 mg GAE/g DW) was measured in the fiber fraction of the 'Kalevala' variety, while the lowest TPC corresponded to the 'Tápiói sima' variety (3.85 mg GAE/g DW). The high TPC of the Kalevala variety may be ascribed to the fact that the Kalevala variety is originated from Finland, where Hungarian climatic conditions, especially UV radiation, is probably higher than that of Finnish climate, leading to unfavorable growth conditions. At the second harvest, however, all tested JA's clones showed similar values of TPC, except for the Rubik variety, which recorded 12.86 mg GAE/g DW.

Brown juices derived from the Kercaszomori and Fuseau varieties in 2016 revealed the highest TPC values (above 80 mg/mL) in both harvests, while the Alba variety exhibited the lowest TPC value (below 20 mg/mL).

At the first harvest of 2017, the highest TPC corresponded to the brown juice of the Alba variety (61 mg/mL), while the Rubik variety revealed the lowest TPC in brown juice (24.4 mg/mL). In the second harvest, the highest TPC above 92 mg/mL corresponded to the Alba and Piri varieties, whereas the lowest detected TPC was 29.7 mg/mL and corresponded to the Fuseau variety (Fig. 2D).

Within the phenolic components, there is a separated group of flavonoid-type compounds. Fig. 3 illustrates the TFC of the hydro-alcoholic extract of the different JA fractions.

The highest TFC values were 0.21 and 0.19 mg REV/g DW and corresponded to fiber fractions of the Rubik and Kercaszomori varieties. The Fuseau and Alba varieties displayed the lowest fiber TFC values of 0.12 and 0.13 mg REV/g DW. A significant difference was observed between the clones of JA. In the second harvest of the same year (Fig. 3A), no statistically verifiable difference was observed between the varieties. In 2017, in the first harvest, the fiber fraction of the Rubik

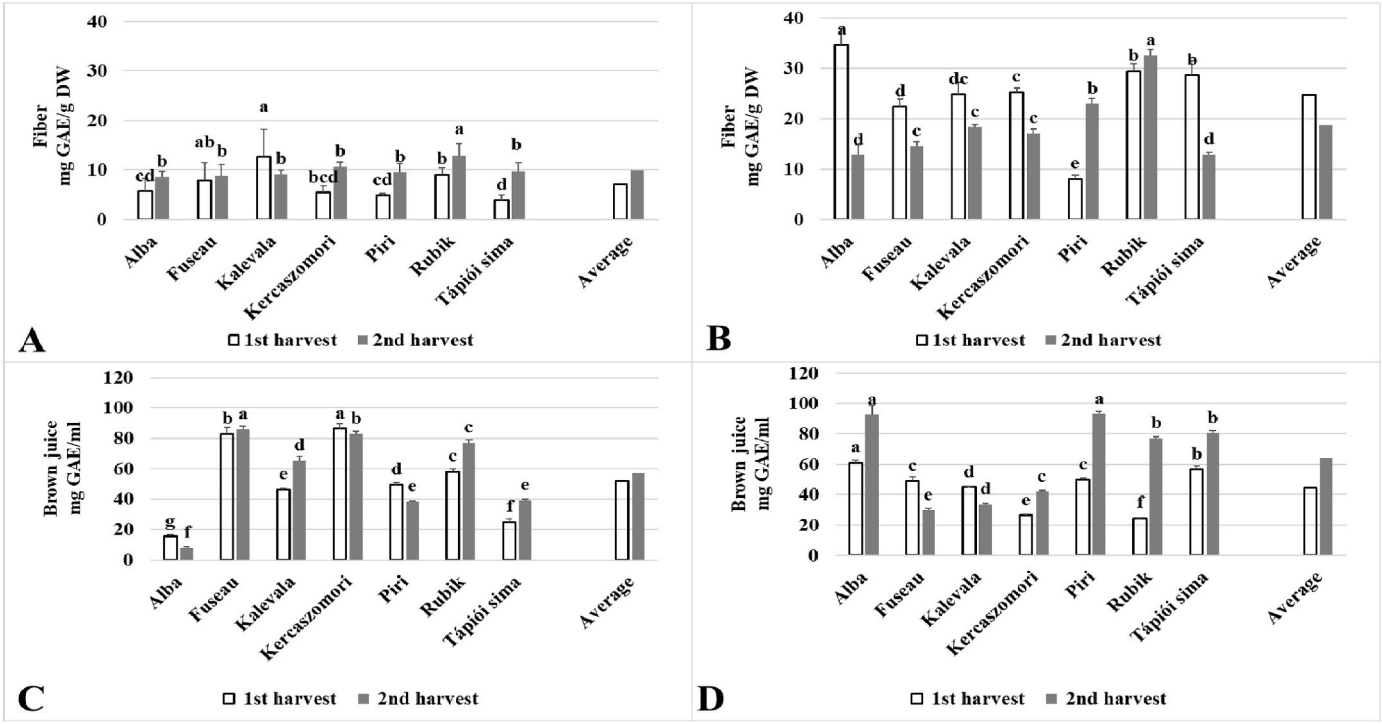

Fig. 2. Total phenolic content of fiber (mg GAE/g DW) and brown juice (mg GAE/mL) fractions obtained from green JA processed biomass (different letters above the columns represent significant difference by Duncan test at $p < 0.05$). A) Fiber fraction from 2016; B) Fiber fraction from 2017; C) Brown juice fraction from 2016; and D) Brown juice fraction from 2017.

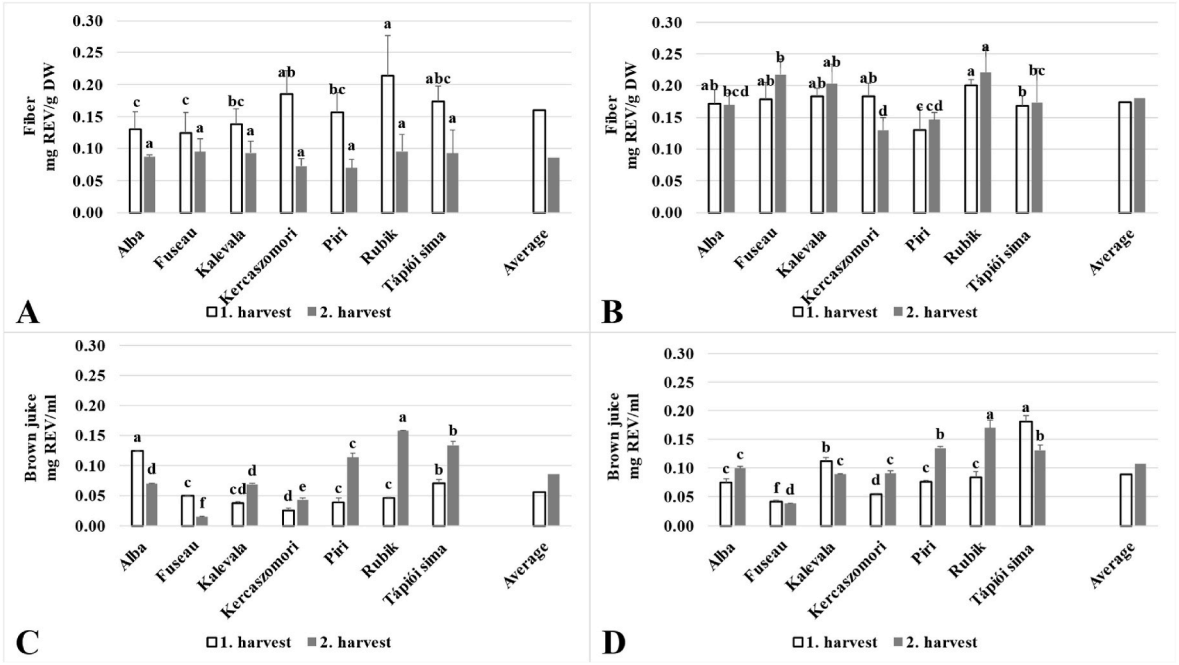

Fig. 3. Total flavonoid content (TFC) of fiber (mg REV/g DW) and brown juice (mg REV/mL) fractions obtained from JA processed biomass (different letters above the columns represent significant difference by Duncan test at $p < 0.05$). A) Fiber fraction from 2016; B) Fiber fraction from 2017; C) Brown juice fraction from 2016′ and D) Brown juice fraction from 2017.

variety displayed the highest TFC value (0.20 mg REV/g DW). In the second harvest during 2017, the highest TFC value also corresponded to the Rubik and Fuseau varieties (0.22 mg REV/g DW), while the lowest TFC was obtained from the Kercaszomori variety (0.13 mg REV/g DW), with statistical differences observed between these varieties.

In 2016, the first harvest, the highest brown juice TFC was 0.12 mg REV/g DW and corresponded to the Alba variety, while the lowest TFC was measured in the brown juice of the Kercaszomori variety (0.03 mg REV/g DW). Significant differences were observed between the varieties during the first harvest. In the second harvest, 2016, (Fig. 3C), the highest TFC was measured in the brown juice of the Rubik variety (0.16 mg REV/g DW), while the lowest TFC corresponded to the Fuseau variety (0.01 mg REV/g DW). The differences among all studied varieties were statistically verifiable. In 2017, first harvest, the highest TFC was 0.18 mg REV/g DW and corresponded to the Tápiói sima variety, the Fuseau variety displayed the lowest TFC value (0.04 mg REV/g DW). It the second harvest, 2017, the highest TFC was measured in the Rubik variety (0.17 mg REV/g DW), whereas the lowest TFC corresponded to the Fuseau variety (0.04 mg REV/g DW) (Fig. 3D). The average TFC in fiber fraction varied between 0.16 and 0.09 mg REV/g DW in 2016 and between 0.17 and 0.18 mg REV/g DW in 2017. In contrast, the brown juice fraction showed a lower amount in 2016 (0.06–0.09 mg/mL) and in 2017 (0.09–0.11 mg/mL). The difference between the two years may be attributed to the variations in weather conditions. In 2017, the temperature was higher than that in 2016, and the UV radiation was also higher (unpublished data). Del Valle et al. (2020) [40] reported that plant can respond to higher UV radiation by changing their physiological parameters, especially their synthesis of UV-absorbing flavonoids constitutes an effective non-enzymatic mechanism to mitigate photoinhibitory and photooxidative damage caused by UV stress. These findings suggest that the choice of JA variety and the timing of harvest can influence the flavonoid content, which could be of importance for applications in the food and pharmaceutical industries.

3.4. Determination of phenolic and other components using UHPLC-ESI-MS

A total of 25 different chemicals were identified in the fiber fraction, and 63 in brown juice with an overlap between the two fractions (Table 3). A total of 70 different compounds have been identified, including coumarins, terpenoids, vitamins, flavonoids, non-flavonoids, and other metabolites. Fig. 4 summarizes the number of compounds identified in the fiber and brown juice fractions.

For clarity, the identified compounds were grouped according to their structure into flavonoid-, non-flavonoid-type compounds, and other metabolites. According to the measurement, the non-flavonoid-type compounds were dominant in every fraction. Negligible differences were observed between the phytochemical profiles of the fractions of the JA clones, and therefore it reported in aggregate (Table 3). The role, structure, and physiological effects of flavonoids have been summarized in numerous works over the past four decades, but their effects and importance for the living organism are still the subject of scientific debate [41].

Flavonoids are widespread secondary metabolites in the plant kingdom. They are mainly found dissolved in the vacuoles of cells. As shown in Table 3, flavanols, flavones, flavanols, and chalcones have been identified among the flavonoid aglycones and glycosyl side-chain compounds. These phytochemicals were mostly present in the brown juice fraction. Only three flavanol type compounds were detected in the fiber fraction. Among the flavonols, kaempferol-3-glucoronide, and astragalin were also identified in the fractions. Chen et al. [42] reported that, in the leaf shoots of JA, the kaempferol glucuronide compounds are the most abundant. The importance of flavonoid-glucuronides is related to their health protective effects, e.g. quercetin-3-O-glucoronide has anti-inflammatory and neuroprotective effects [43].

Numerous bioactive compounds have been identified from aerial part of JA. The most abundant flavone glycosides are kampferol-3-O-glycoside and quercetin-7-O-glycoside. Glycosides make flavonoids more polar, allowing plants to store them more efficiently in cell vacuoles. Flavonoid glycosides usually contain glucose but may also bind xylose, galactose, and arabinose. In addition, fructose, glucuronic acid

Table 3
Compounds identified by UPLC-ESI-MS coupled analytical system in the Jerusalem artichoke fibre and brown juice fractions (Rt: retention time, $[M+H]^+$: positive ionization, $[M-H]^-$: negative ionization).

		Name	Formula	Rt	[M + H]+	[M − H]-	Fiber	B. juice
Flavonoide type chemicals	Flavanole	Isorhamnetin-di-O-glucoside	C28H32O17	19.35		639.15613		+
		Quercetin-3-O-malonylglucoside-7-O-glucoside	C30H32O20	20.98		711.14087		+
	Flavanone	Liquiritigenin (4′,7-Dihydroxyflavanone)	C15H12O4	30.58		255.06574		+
	Flavone	Apigenin-O-malonylglucoside	C24H22O13	25.21		517.09822		+
		Dimethoxy-tetrahydroxyflavone	C17H14O8	28.39		345.06105		+
		Dihydroxy-methoxyflavone	C16H12O5	29.91		283.06065		+
		Dimethoxy-trihydroxyflavone isomer 1	C17H14O7	30.09		329.06613		+
		Trihydroxy-trimethoxyflavone	C18H16O8	30.37		359.07670		+
		Dimethoxy-trihydroxyflavone isomer 2	C17H14O7	30.38		329.06613		+
		Hymenoxin (5,7,Dihydroxy-3′,4′,6,8-tetramethoxyflavone)	C19H18O8	32.11	375.10800			+
		Nevadensin (5,7-Dihydroxy-4′,6,8-trimethoxyflavone)	C18H16O7	33.91	345.09743			+
	Flavonole	Quercetin-3,4′-di-O-glucoside	C27H30O17	17.56		625.14048		+
		Kaempferol-3,7-di-O-glucoside	C27H30O16	19.02		609.14556		+
		Kaempferol-3-O-glucuronide	C21H18O12	25.16		461.07200	+	
		Astragalin (Kaempferol-3-O-glucoside)	C21H20O11	25.25		447.09274	+	
	Calcone	Epiafzelechin trimethyl ether	C18H20O5	33.33	317.13890		+	+
		Butein (2′,3,4,4′-Tetrahydroxychalcone)	C15H12O5	23.01	273.0763			+
		Kukulkanin B (3′-Methoxy-2′,4,4′-methoxychalcone)	C16H14O5	25.49	287.09195			+
Non falvonoide type chemicals	Benzoic acid	Salicylic acid-2-O-glucoside	C13H16O8	13.53		299.0767		+
		Vanillin	C8H8O3	16.24	153.0552		+	+
		Caffeoylshikimic acid isomer 1	C16H16O8	17.67		335.0767		+
		Caffeoylshikimic acid isomer 2	C16H16O8	17.9		335.0767		+
		Caffeoylshikimic acid isomer 3	C16H16O8	18.49		335.0767		+
		Coumaroylshikimic acid isomer 1	C16H16O7	19.04		319.0818		+
		Coumaroylshikimic acid isomer 2	C16H16O7	19.89		319.0818		+
		Coumaroylshikimic acid isomer 3	C16H16O7	20.37		319.0818		+
		Coumaroylshikimic acid isomer 4	C16H16O7	20.93		319.0818		+
		Coumaroylshikimic acid isomer 5	C16H16O7	22.85		319.0818		+
	Cinnamic acid	Quinic acid	C7H12O6	1.28		191.0556	+	
		Neochlorogenic acid (5-O-Caffeoylquinic acid)	C16H18O9	10.07		353.0873		+
		3-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	12.56		337.0924		+
		3-O-(4-Coumaroyl)quinic acid	C16H18O8	13.21		337.0924		+
		Chlorogenic acid (3-O-Caffeoylquinic acid)	C16H18O9	14.76		353.0873	+	+
		3-O-Feruloylquinic acid	C17H20O9	15.04		367.1029		+
		Chryptochlorogenic acid (4-O-Caffeoylquinic acid)	C16H18O9	16.04		353.0873	+	+
		4-O-(4-Coumaroyl)quinic acid	C16H18O8	16.13		337.0924	+	+
		5-O-(4-Coumaroyl)quinic acid	C16H18O8	17.35		337.0924	+	+
		4-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	18.03		337.0924		+
		5-O-Feruloylquinic acid	C17H20O9	18.42		367.1029		+
		4-O-Feruloylquinic acid	C17H20O9	18.95		367.1029		+
		5-O-(4-Coumaroyl)quinic acid cis isomer	C16H18O8	19.63		337.0924	+	+
		4-Hydroxy-3-methoxycinnamaldehyde (Coniferyl aldehyde)	C10H10O3	20.63	179.0708		+	
		Di-O-caffeoylquinic acid isomer 1	C25H24O12	22.62		515.119	+	+
		Di-O-caffeoylquinic acid isomer 2	C25H24O12	22.78		515.119	+	+
		Salvianolic acid derivative isomer 1	C27H22O12	22.79		537.1033		+
		Salvianolic acid derivative isomer 2	C27H22O12	24.57		537.1033		+
		Di-O-caffeoylquinic acid isomer 3	C25H24O12	24.59		515.119	+	+
	Coumarin	Esculin (Esculetin-6-O-glucoside)	C15H16O9	12.82		341.08726		+
		Fraxidin or Isofraxidin	C11H10O5	18.30		221.04500		+
		Isoscopoletin (6-Hydroxy-7-methoxycoumarin)	C10H8O4	18.35	193.05009			+
		Scopoletin (7-Hydroxy-6-methoxycoumarin)	C10H8O4	19.08	193.05009			+
		6-Methylcoumarin	C10H8O2	19.45	161.06026			+
		Coumarin	C9H6O2	20.40		147.04461	+	
Other detected chemicals	Alkaloids	Kynurenic acid	C10H7NO3	13.75	190.05042			+
	Other metabolites	Malic acid	C4H6O5	1.46		133.0137		+
		Citric acid	C6H8O7	1.76		191.0192	+	+
		3-(Benzoyloxy)-2-hydroxypropylglucuronic acid	C16H20O10	18.17		371.0978		+
		Azelaamic acid (9-Amino-9-oxononanoic acid)	C9H17NO3	19.22		186.113	+	+
		Indole-4-carbaldehyde	C9H7NO	19.67	146.0606		+	+
		Azelaic acid	C9H16O4	25.05		187.097	+	+
		Jasmonic acid	C12H18O3	28.31		209.1178		+
		2-Hydroxyhexadecanoic acid	C16H32O3	45.24		271.2273	+	
	Terpenoids	Loliolide	C11H16O3	20.06	197.11777		+	+
		7-Deoxyloganic acid isomer	C16H24O9	22.39		359.13421	+	+
		Dihydroactinidiolide	C11H16O2	27.18	181.12286		+	
	Vinamins	Nicotinic acid (Niacin)	C6H5NO2	1.57	124.03986		+	+
		Pyridoxine	C8H11NO3	1.60	170.08172			+
		Nicotinamide	C6H6N2O	1.65	123.05584			+
		Biotin	C10H16N2O3S	16.92	245.09599			+
		Riboflavin	C17H20N4O6	19.06	377.14611		+	+

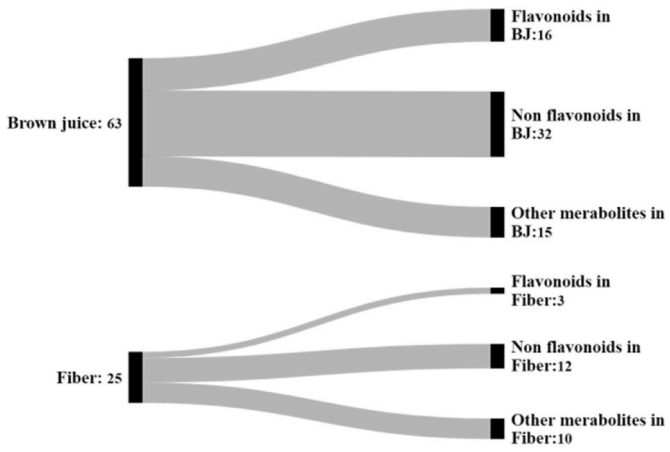

Fig. 4. A summary of identified compounds in the fiber and brown juice (BJ) fractions obtained through green biorefinery of Jerusalem artichoke.

and galacturonic acid are also present [44].

Other important phenolic compounds are phenolic acids, coumarins, xanthones, chalcones, stilbenes, lignin, and lignans. Phenolic acids can be subdivided into benzoic acid derivatives and cinnamic acid derivatives. Coumarins are phenolic acid derivatives, which are lactones of hydroxy-cinnamic acids. In plants, these are often found in free and bound forms [44].

Within the flavonoids, the flavone compounds were all hydroxylated methoxyflavones, such as the two isomers of dimethoxy trihydroxyflavone, dimethoxy-tetrahydroxyflavone, dihydroxymethoxyflavone, and trihydroxytrimethoxyflavone. The significance of this is that the flavone hydroxyl groups are known to have free radical scavenging ability, but their bioavailability is less than that of hydroxyflavones, undergoing rapid enzymatic sulfurization and glucoronation in the small intestine and liver, thereby rapidly losing their activity. When methoxyl groups are attached to hydroxyl groups, they become more stable and retain their function for longer period [45].

Mersereau et al. [46] cited in their work that the liquiritigenin is an estrogenic secondary plant metabolite that is a selective β-estrogen receptor antagonist and may be helpful for women suffering from menopausal symptoms.

Yuan et al. [47] reported that the chlorogenic acid, which is pharmacologically important, is present in higher amounts than phenolic acid. In their work, they also pointed out that several components have been identified in JA tubers, but lack of information is available on the leafy stalks. Yuan et al. [47] also reported high concentrations of 3-O-caffeoylquinic acid and 1,5-dicaffeoylquinic acid in JA leaves. Kapusta et al. [48] used the UHPLC-ESI-MS to characterize phenolic compounds in JA tubers, and they found seven of them, including caffeoylquinic acid isomers namely neo-chlorogenic acid, chlorogenic acid, and crypto-chlorogenic acid and four isomeric di-caffeoylquinic acids.

Chlorogenic acid (3-O-caffeoylquinic acid), neo-chlorogenic acid (5-O-caffeoylquinic acid) and crypto-chlorogenic acid (4-O-caffeoylquinic acid) were identified in leaf protein concentrate obtained JA green biomass [17]. From the relative proportions of the three isomers, it was found that chlorogenic acid dominates over the other two isomers. However, according to Liang and Kitts [49], 5-O-caffeoylquinic acid is the predominant isomer in fruits and vegetables. Chen et al. [50] have found six phenolic acids in JA leaves. Among them caffeic acid, 3, 4-dicaffeoylquinic acid and 1,5-dicaffeoylquinic acid played a dominant role. Chen et al. [42] detected 3,5-dicaffeoylquinic acid in the leaf stalk of JA as the most dominant compound, also 3,4-; 1,5-; and 4, 5-dicaffeoylquinic acid were identified. In addition, p-coumarylquinic acid, ferulic acid and, for the first time, caffeoyl glucopyranose were found in JA leaves. They also quantified kaempferol and quercetin glycosides. Caffeoylquinic acid isomers are known for several biological roles, including antioxidant and antibacterial effects, liver, and heart protection, anti-inflammatory and antipyretic, neuroprotective, anti-obesity, antiviral and anti-hypertension effects, and central nervous system stimulation [34].

Yuan and Yang [51] identified another group of secondary metabolites (sesquiterpene lactones) in the leaves of JA. Furthermore, it has been recognized that the production of different phenolic components in plants is also associated with adaptation to environmental factors.

Phenolic compounds and their subgroup phenolic acids are an important subgroup of the Asteraceae family. The most studied phenolic acids are the mono-, di-, and tri-hydroxy citric acid esters of quinic acids (p-coumaric, caffeic, and ferulic acids) found in the tubers and shoots of JA [52]. Our measurements revealed 13 different phenolic acids in fractions obtained from green biomass of JA (Table 3).

Among the coumarins, scopoletin and ayapin have been detected in JA tubers and their presence has been suggested in above-ground parts as well [53]. Our measurements confirmed the presence of scopoletin, isoscopoletin, 6-methylcoumarin, and fraxidine in fractions obtained from green biomass of JA. Some of the simple coumarins are known as phytoalexins. However, fraxidine and scopoletin showed strong anti-adipogenic activity against preadipocyte cell lines in in vitro assay systems [54].

Three terpenoid-type compounds were presented in all tested fractions of JA. Loliolide is a photooxidative or thermally degradable product of carotenoids. Similarly, we identified dihydroactinidinediol, a volatile monoterpenoid that is a flavor constituent of many plants such as tobacco and tea [55]. According to YUAN et al. [51], heat treatment induces the formation of dihydroactinidinediol from β-carotene. Studies have demonstrated that loliolide inhibits growth and germination, but is also phytotoxic, repels leaf-cutting ants, and has antitumor and antimicrobial effects in both animals and microorganisms [52]. Furthermore, in terms of vitamins, we identified vitamin B molecules such as nicotinic acid and riboflavin, in addition to organic acids, malic and citric acids, and plant hormones in the fractions.

4. Conclusion

Within the green biorefinery, an investigation of the value-measuring properties of the by-products obtained during the production of leaf protein concentrate showed that the fiber fractions from Jerusalem artichokes were distributed between 17 and 28 % per 1 kg of fresh biomass, while the brown juice fraction was produced in the range of 57–68 %. The protein content of the resulting fractions ranged from 7 to 14 m/m% for fiber and 0.3–1.4 m/m% for brown juice. No clearly outstanding ecotype was found, but the crude protein content of Rubik and Piri varieties showed the smallest differences between harvests.

In terms of amino acid content, leucine and lysine were detected in both fractions in outstanding amounts for essential and limiting amino acids. In terms of phenolic components, they were the most abundant in the brown juice. Some trends in the TPC results for the fiber fraction can be detected, but these are related to harvests rather than varieties. Looking at the TPC and TFC values together, the Rubik variety stands out. Further investigation of the brown juice fraction obtained from this variety could be beneficial, e.g. in the field of biostimulants. The qualitative determination of phenolic components highlighted the presence of several hydroxy-methoxy flavones. According to the literature, these substances have anti-inflammatory properties which could be used as natural substances in the pharmaceutical industry. In the qualitative determination of phenolic components, more phenolic components were found in the brown juice than in the fiber fraction. Among the cinnamic acid derivatives, chlorogenic acid and two isomers were detected, which some researchers suggest could be a promising drug candidate.

CRediT authorship contribution statement

László Kaszás: Writing – original draft, Software, Methodology,

Data curation, Conceptualization. **Zoltán Kovács:** Visualization, Investigation, Conceptualization. **Judit Koroknai:** Resources, Data curation. **Nevien Elhawat:** Investigation, Data curation. **Miklós Fári:** Resources, Conceptualization. **Zoltán Cziáky:** Software, Data curation. **Tarek Alshaal:** Validation, Methodology, Conceptualization. **Éva Domokos-Szabolcsy:** Validation, Supervision, Methodology, Investigation, Conceptualization.

Data availability

The data that has been used is confidential.

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