Theses for doctoral (PhD) dissertation

Study of Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars and ecotypes for green biomass utilisation

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Background and objectives of the doctoral thesis

Jerusalem artichoke (JA) (*Helianthus tuberosus* L.) is an agriculturally annual, botanically perennial North American industrial plant. It has been in and out of the scientific spotlight for over 400 years of its discovery and cultivation.

The national and international literature considers JA has a high biological value, with dual utilization, including the use of tuber and stems that wither at the end of the growing season. It is recommended for crop rotation. Despite its beneficial properties, it is an under-appreciated crop in current national and international agricultural practice, with negligible production volumes. In addition to tubers, much less is known about the values and potential uses of the green leafy stems of JA. This work deals with the green biorefining based on fresh green biomass. The approach integrates biological, chemical, physical and technical knowledge, keeping in mind the concepts of economics, environmental and circular farming. The technological steps in green biorefining can be multi directional, depending on the product being produced. Typically, the first step (primary refining) is wet fractionation to separate the green biomass into fibre-rich press cake and nutrient-rich green juice. In addition to the fibre components, the press cake contains valuable colouring agents, proteins, and various phytochemical compounds. They can be used to produce green fodder pellets and fibre supplements. With further processing, they can be used to produce biogas.

Green juice contains proteins, organic acids, colouring agents, phytochemical components, micro- and microelements. Further processing is recommended due to its moisture content and perishability.

Physical/chemical methods can be used to obtain a product with concentrated proteins that can be stored for long time. In addition, the recoverability of the by-products formed is also worthy of consideration.

Taking this idea further, my research and thesis consider the potential of utilisation of green leafy stems of JA as an industrial crop with high biomass potential, using green biorefinery methodologies. In my work, seven varieties/ecotypes of JA from different geographical locations were studied under extensive conditions. Harvesting of green biomass was carried out twice during the growing season, considering the growth vigour of the plants. The tuber yields at the end of the growing season were also investigated due to the dual utilization of the JA. For evaluability, it was compared with a dedicated plant species of green biorefinery, alfalfa (*Medicago sativa* L. '*Hunor 40*').

The aim of the present work is to investigate the potential of green leafy stalk of JA for value addition using a possible series of steps in green biorefinery. To this the field performance of

JA varieties/ecotypes (germination, growth, harvestable green biomass, regeneration capacity, tuber yield) were investigated. The different fractions (fibre, green juice, brown juice, and leaf protein concentrate (LPC)) were studied following a defined processing technology line for the processing of green biomass. The resulting direct (fibre, green juice) or indirect product candidates (brown juice and LPC) were analysed for their intended industrial use, in accordance with the relevant value-measuring properties (e.g. protein content, amino acid composition), which are important from a holistic, feed and nutritional point of view, using physical/biochemical and analytical (e.g. UPLC-ESI-MS) methods.

2. MATERIAL AND METHOD

2.1. Small plot experimental set-up of Jerusalem artichoke varieties/ecotypes

The small plot experiment was set up at the University of Debrecen, Faculty of Agriculture, Food Science and Environmental Management, Demonstration Garden in 2016 and 2017 under extensive conditions.

The experimental area was 200 m^2. The growing area of one plant was chosen to be 60 x 80 cm (0.48 m^2), with 60 cm as the dead space and 80 cm as the row spacing. Seven Jerusalem artichoke cultivars/ecotypes from different geographical areas were included in the experiment **Table 1**. As a control, a domestically bred alfalfa variety (*'Hunor 40'*) was used in the experiment.

Harvesting of Jerusalem artichoke plants was carried out twice in one growing season. A total of 10 plants were cut back each cultivar.

Table 1. Name and place of origin of the Jerusalem artichoke varieties/ecotypes included in the experiment

Cultivars/ecotypes	Origin
Alba (ecotype)	Hungary, probably Poland
Fuseau (cultivar)	Egypt, French breed
Kalevala (ecotype)	Finland, Helsinki
Kercaszomori (cultivar)	Hungary
Piri (ecotype)	Hungary, Téglás region
Rubik (cultivar)	Hungary, probably Poland
Tápiói sima (cultivar)	Hungary

2.2. Technological steps in green biomass processing

Figure 1 illustrates the fractionation steps based on the Ereky - Pirie method with our protein coagulation modifications.

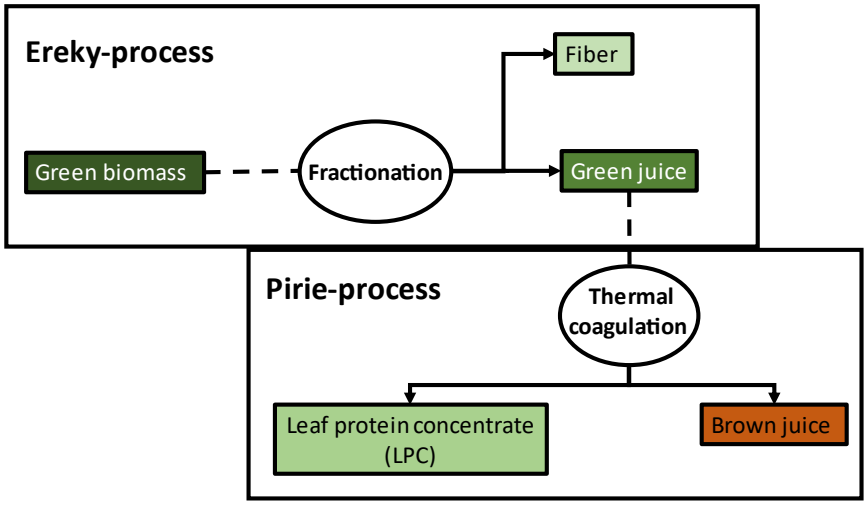


Figure 1 Schematic diagram of how we process green biomass. Source: own editing

After harvesting, 3 x 1 kg of the harvested green biomass was randomly weighed and processed. Fractionation was carried out using a twin screw press. Protein coagulation was carried out by microwave in one step until 80 ± 2°C at 450 watts. The leaf protein concentrate and brown juice fractions were separated by gravity separation.

2.3. Determination of crude protein and amino acid content of green biomass fractions

The determination of the crude protein and amino acid content of the fractions was carried out at the Agricultural Technology Centre, University of Debrecen. The crude protein content was determined according to MSZ EN ISO 5983-2:2009, while the amino acid content was determined according to MSZ EN ISO 13903:2005.

2.4. Amino acid composition/free amino acid content determination by UHPLC

Sample preparation for determination of total amino acid composition:

From each type of sample (green leaf, fibre, green juice, LPC and brown juice) 20-20 mg of powdered, lyophilized sample was measured. The samples were immersed in 10 ml of 6 M hydrochloric acid solution in a Teflon shredding tube and placed in a CEM Mars One (Matthews, USA) microwave shredder at 150°C for one hour at 650 Watts. For the determination of free amino acid content, samples were used directly without hydrolysis. Both types of samples were filtered through a 3kDa Nanosep Omega membrane filter.

Before the UHPLC separation, samples were derivatized with AccQ-Tag Ultra derivatization reagent according to the manufacturer's instructions. The separation of derivatized amino acids

was performed with AccQ-Tag Ultra C18. The flow rate was 0.600 mL/min and the column temperature was 43 ◦C. The results were evaluated (processed) using Waters Empower 3 software (Waters, Milford, MA, USA).

2.5. 1D SDS-PAGE protein expression of different green biomass fractions

The run was carried out in a Bio-Rad Mini-PROTEAN Tetra Veritcal Electrophoresis type instrument.

40-40 mg of lyophilized and powdered samples were weighed on a Sartorius analytical balance. Then, 800 µl of our lysis buffer was added to each sample and the samples were incubated in an ultrasonic water bath for 1.5 h.

The run was carried out in a Bio-Rad Mini-PROTEAN Tetra Veritcal Electrophoresis system. For molecular weight identification, a PageRuler (Prestained Protein Ladder 10-180 kDa) protein marker was used. We started the run at 90 V to concentrate the samples (~15 min), and then adjusted the voltage to 160 V to separate the proteins (45 - 50 min). After staining, a photo of the gel was taken using Bio-Rad Gel Doc XR+ equipment.

2.6. Determination of the total phenolic content of fractions from green biomass

The total phenolic content was determined by spectrophotometric method according to SINGLETON and ROSSI (1965).

A 20 mg sample of solid samples (fibre and LFK) was measured on a Sartorius type analytical balance, and 100 µl sample for brown juice. To this 1 ml of methanol-water solution (80:20) was added. The solid samples were then pipetted. The prepared samples were incubated for 1 hour in an ultrasonic water bath at room temperature. After incubation, the samples were centrifuged at 13,000 rpm for 3 minutes using an Eppendorf Centrifuge 5415 R centrifuge. The supernatant was collected for measurement.

At the time of measurement, 50 µl of the supernatants of the samples were added to a mixture of 1250 µl of Folin-C. reagent and 200 µl of methanol-water (80:20). After waiting for one minute, a further 1000 µl of 0.7M Na_2CO_3 was added. The samples were then incubated in a water bath at 50°C for 5 min. After incubation the absorbance of the samples was measured using a λ=760 nm Ultrospec 2100 pro spectrophotometer (Holliston, USA).

The concentration of the samples was calculated using the equation calculated from the calibration equation ($y=0.164x+0.0001$; $R^2=0.9991$) and expressed in gallic acid equivalent (GAE).

2.7. Qualitative determination of the phytonutrient composition of green biomass fractions by UHPLC-ESI-ORBITRAP-MS/MS analytical system

Sample preparation:

0.5 g lyophilized, milled samples (fibre, brown juice, LPC) of the tested Jerusalem artichoke fractions was measured. Then a mixture of 25 ml methanol-water (80:20) was added. The mixture was stirred at 150 rpm for 2 hours at room temperature. The hydroalcoholic extract was filtered through a 0,22 m PTFE syringe filter.

UPLC settings:

The phytochemical analyses were performed using UHPLC-ESI-ORBITRAP-MS/MS on a Dionex Ultimate 3000RS UHPLC system (Thermo Fisher, Waltham, MA, USA) coupled to a Thermo Q Exactive Orbitrap hybrid mass spectrometer equipped with a Thermo Accucore C18 analytical column (2.1 mm × 100 mm, 2.6 μm particle size). The flow rate was maintained at 0.2 ml/min and the column temperature was set at 25°C ± 1◦C.

Setting up the mass spectrometer:

A Thermo Q Exactive Orbitrap hybrid mass spectrometer (Thermo Fisher, Waltham, MA, USA) was equipped with an ESI source. Samples were measured separately in positive and negative ionization mode. The capillary temperature was 320°C and the spray voltage was 4.0 kV in positive ionization mode and 3.8 kV in negative ionization mode. The resolution was 35 000 for MS1 scans and 17 500 for MS2 scans. The scanned mass interval was 100-1500 m/z. For the tandem MS (MS/MS) scans, the collision energy was set to 30 nominal collision energy units.

2.8. Quantitative analysis of the phytonutrient composition of green biomass fractions

Quantitative determination was made from the compounds identified from qualitative measurements by considering standard compounds, which were: **nicotinamide** (≥98% (HPLC), powder, Sigma-Aldrich, Darmstadt, Germany), **nicotinic acid** (analytical standard, Sigma-Aldrich, Darmstadt, Germany), **biotin** (≥99% (HPLC), lyophilized powder, Sigma-Aldrich, Darmstadt, Germany), **riboflavin** (analiytical standard, Sigma-Aldrich, Darmstadt, Germany), **liquiritigenin** (≥97. 0% (HPLC) powder, Sigma-Aldrich, Darmstadt, Germany), **chlorogenic acid** (analytical standard, powder, Sigma-Aldrich, Darmstadt, Germany), **scopoletin** (analytical standard, Sigma-Aldrich, Darmstadt, Germany), **coumarin** (≥99% (HPLC), Sigma-Aldrich, Darmstadt, Germany).

Sample preparation:

Sample preparation was the same as described in section 2.6.

Measurement procedure:

The mobile phase consisted of methanol (A) and water (B) (both acidified with 0.1% formic acid). The flow rate was 0,2 ml/min. The injection volume was 2 µl.

2.9. Photosynthetic pigment content determination

The photosynthetic pigment content was measured using the method of DUMA et al. (2014) with some modifications.

From each type of sample (leaf, green juice and fibre), 100 mg of lyophilized sample was measured on a Sartorius type analytical scale. It was then incubated in 5 ml 96% ethanol for 1 h in an ultrasonic water bath in the dark. After incubation, the sample was centrifuged at 13,000 rpm for 3 minutes. The supernatants were pipetted into Eppendorf tubes and measured using an Ultrospec 2100 pro spectrophotometer (Holliston, USA) at wavelengths λ= 665, 649, 495, 480 and 440 nm and the photosynthetic pigment content of the samples was determined in mg/g using the following equations.

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

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

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

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

2.10. Statistical evaluation

The statistical evaluation was carried out using the R software package. I calculated the normality, homogeneity, and independence test of the data, followed by ANOVA analysis, then Duncan Posthoc analysis with $p<0.05\%$. The results were plotted in MS Excel graphs.

3. RESULTS

3.1 Comparative field characterisation of Jerusalem artichoke varieties/ecotypes

The general morphological parameters of Jerusalem artichoke plants can affect their productivity. The growth vigour (development of shoot system and foliage) in the open field under extensive conditions has a decisive influence especially on its competitive properties, e.g., its competitiveness against weeds. According to PAS'KO, (1973), these parameters are genetically determined and there is a wide variation between varieties, ecotypes, and clones. However, a high degree of plasticity is also common within the variety/ecotype, even in individual phenophases (**Figure 2**).

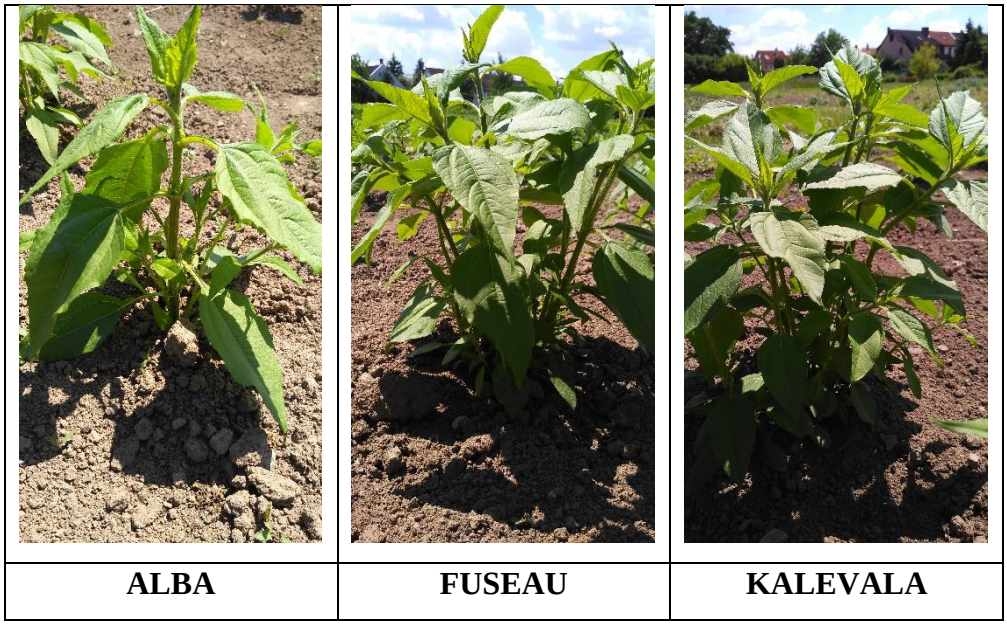


Figure 2. Varieties/ecotypes included in the trial

In 2016, germination was not balanced across varieties/ecotypes. This was also due to storage disease (grey mould rot) on the tubers received, even though all infected tubers were removed and those selected for sowing were treated with a 5% sodium hypochlorite solution beforehand. In contrast, yields were much more balanced in 2017. All varieties/rootstocks performed close to or above the average for the year (93%). This is also related to the fact that the 2017 sowing was done immediately after the tubers were picked in spring, from our self-propagation.

After germination, the growth dynamics of the varieties/ecotypes were monitored during the growing season. In 2016, all Jerusalem artichoke varieties/ecotypes showed intensive growth until the date of the first harvest (27 June). Thereafter, until mid to late August, a more moderate growth dynamics was observed. In the following period we again saw a more intense growth. Comparing the ecotypes, 'Rubik' showed the highest daily vegetative growth rate during the study period, averaging 3.1 cm/day. This value for the other varieties/ecotypes ranged between 2.8-2.9 cm. The average growth of the cultivars/ecotypes ended at a height of around 350 cm by the end of September, except for 'Rubik', where values of around 380 cm were measured. Overall, the growth dynamics of the Jerusalem artichoke ecotypes in 2017, the growing season studied, showed a flatter curve but a more even distribution compared to 2016. Comparing the ecotypes (similar to the previous year), the main stem of the 'Rubik' variety showed the most intense growth in the period studied.

3.2 Comparative evaluation of green biomass production of Jerusalem artichoke varieties/ecotypes

Considering the regeneration capacity of the Jerusalem artichoke cultivars/ecotypes, harvesting was carried out twice during the growing season. The first harvesting was done at plant height of about 150-160 cm from the ground (during the month of June) and the second harvesting was done when the shoots of regenerated plants reached this height again (during the month of August).

In 2016, at the first harvest, the 'Fuseau' variety gave the highest biomass, with an average of 2500 g/ha. On the other hand, the smallest amount was measured for the 'Kercaszomori' variety, with an average of 1100 g/ha. For these varieties, a statistically verifiable difference could be detected. At the second harvest, less biomass was harvested, and no statistical difference could be detected, but the 'Fuseau' and 'Tápiói sima' varieties achieved the highest biomass yields, 898 and 853 g/ha respectively. Looking at the number of shoots at the first harvest, the 'Alba' variety had the highest number of shoots, with an average of 24. 'Rubik' had

the lowest number, with an average of 10/plant. At the second harvest, there were greater differences and clearer distinctions. The varieties 'Kercaszomori' and 'Kalevala' had 14 shoots, while the other varieties had 2-4 shoots.

In the first harvest in 2017, the 'Fuseau' variety had the highest average green biomass weight (1100 g/ha). The other varieties had an average weight of 800-900 g/ha. However, no statistically verifiable differences were found between the varieties studied. In terms of shoot numbers at first harvest, the 'Kalevala' ecotype developed 8 shoots, while the other varieties/ecotypes developed 2-2 shoots. A significant difference was found here. In the second harvest, the 'Rubik' variety performed the best in terms of green biomass yield of the varieties (740 g/ha), which was significantly different from the other varieties/ecotypes. Two varieties, 'Kercaszomori' and 'Tápiói sima' (~350 g/ha), gave the lowest harvestable green biomass. The other cultivars/ecotypes produced almost the same amount of biomass (400-500 g). The largest number of shoots was produced by 'Fuseau' (18 shoots), while the smallest amount was produced by 'Tápiói sima' (12 shoots), in this case also a statistically detectable difference was observed.

Since Jerusalem artichokes are mainly grown for their tubers, we also harvested the tubers at the end of the growing season. This provided information not only on the effect of cutting back the shoots twice on tuber development, but also on the number of tubers for the following year, using the uncut individuals as controls.

In 2016, the total tuber yield under the control plants ranged from 1400 to 600 g/plant, with the number of tubers varying from 34 to 90 per plant variety/ecotype. In 2017, the control plants yielded between 2000 and 4500 g of tubers. The number of tubers varied between 73 and 38 per plant for the control.

In 2016, we obtained values ranging from 21 to 150 g per tuber for the cut back plants, with a range of 2 to 8 tubers. The highest tuber yield of the cut backs was obtained from the 'Fuseau' variety with an average of 157 g/tuber. The smallest yield was obtained from the variety 'Kercaszomori', with 21 g/tuber. In 2017, the yield of tubers from the cut back plants ranged from 340 to 112 g, with several tubers ranging from 11 to 4. For tuber yield, we could detect a statistically proved difference between varieties/ecotypes for the control plants, but no significant difference for the cut individuals. The same phenomenon was observed for the quantitative distribution of tubers.

In conclusion, under the present conditions, the double cutting resulted in a significant reduction in Jerusalem artichoke tuber yields but comparing the number of tubers and their

individual weight, it was found that the small number of tubers was not directly proportional to the individual weight of tubers.

3.3. Results of the green biomass fractionation of Jerusalem artichokes

In line with the objective of the present research work, one important question was whether the green biomass of processed Jerusalem artichokes could potentially be incorporated into the concept of green biorefining. Therefore, following the steps of the "Green Conveyor Belt", the biological/chemical values of the obtained fractions/potential product candidates (including green juice, pressed fibre, leaf protein concentrate, brown juice) (**Figure 3.**) were first investigated in general.

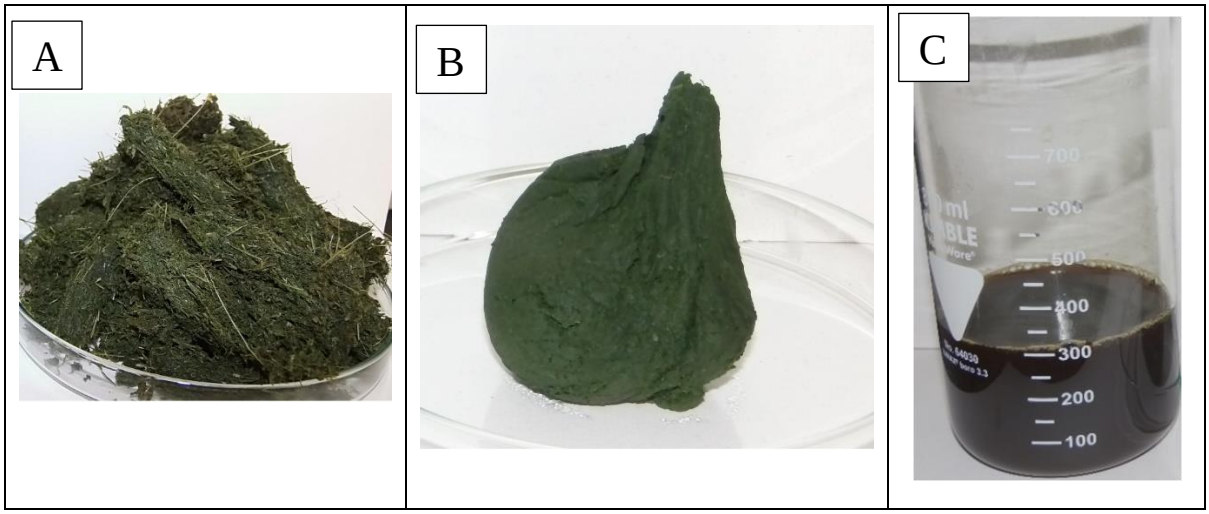


Figure 3. Green biomass fractions of the modified Ereky-Pirie method Fibre (A) Leaf protein concentrate (B) and Brown juice (C)

We also included an alfalfa cultivar, 'Hunor40', as a control in the experiment, as alfalfa is one of the most important forage crops in the integrated field crop production in Hungary. Its value is due to its high green biomass yield (4.5 t/ha, KSH, 2022) and multiple harvestability (up to 4 times per year), as well as its excellent protein content (19-20 m/m% for fresh alfalfa) and amino acid composition, in addition to the atmospheric nitrogen fixation by Rhizobium bacterial strains symbiotic with its roots. It is mainly for these properties that alfalfa was chosen as a control.

Figure 4. summarises the results for the relative weights of the green biomass fractions of Jerusalem artichokes and alfalfa averaged over two years and four harvests.

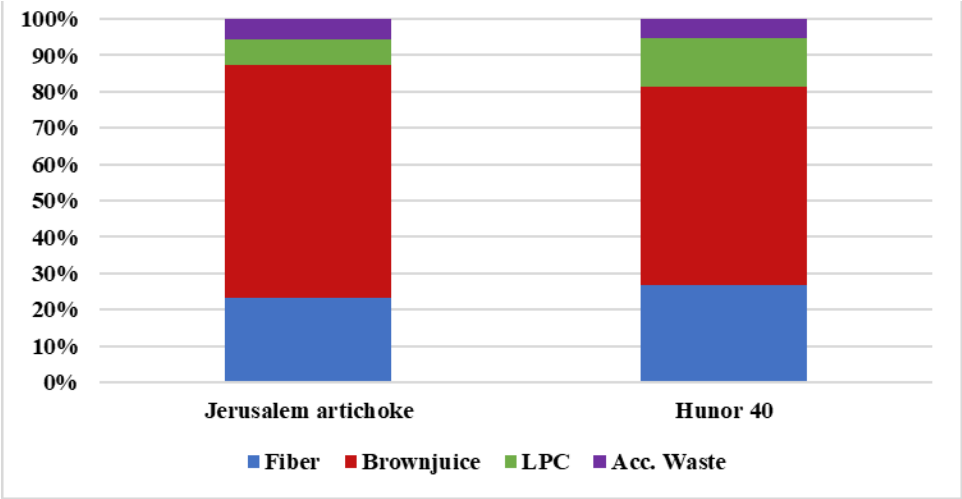


Figure 4. Results for the relative weights of green biomass fractions of Jerusalem artichokes and alfalfa averaged over two years and four harvests.

The figure shows that the fractions obtained from the green biomass of Jerusalem artichokes yielded 23.3% for fibre, while for alfalfa it was 26.7% fibre. The fraction with the highest amount for both plant species was the brown juice fraction. In the case of leaf protein concentrate (LPC), averaged over the two years and all varieties/ecotypes, the relative weight per 1 kg of fresh green biomass was 6.9% for Jerusalem artichoke and 13.6% for alfalfa. During the fractionation of the varieties/ecotypes under laboratory conditions, some losses during dismantling and cleaning of the twin screw press must be considered, which should not be expected under continuous operating conditions. In this case, the accumulated loss of 5,7 % was found for Jerusalem artichokes and 5,1 % for alfalfa.

3.4. Results of comparative tests on quality parameters

Table 2 summarizes the average crude protein results from the extraction of green biomass fractions of Jerusalem artichoke and alfalfa from four harvests in two years, determined by the Kjeldahl method.

Table 2 Crude protein results averaged over two years and four harvests according to the Kjeldahl method

Crude protein content (m/m%)	Leaf	Fiber	Green juice	Brown juice	LPC
Jerusalem artichoke	22,0	11,9	15,3	0,72	29,66
Alfalfa	25,0	12,5	21,9	1,66	41,25

As shown in the table, a 22 m/m% was detected for Jerusalem artichoke leaves. After wet fractionation, an average of 12 m/m% crude protein content was measured in the fibre fraction, similarly for alfalfa it was 12.5 m/m%. The protein content of the fibre fraction is highly

dependent on the pressing method. We used twin-screw pressing, which yields a protein content of around 12 m/m% and can be used as a fibre substitute in dried feed. The protein content of the green juice is mainly due to soluble proteins in the cytoplasm and chloroplasts, which in the case of Jerusalem artichokes average 15.3 m/m%. In contrast, ~22 m/m% crude protein was obtained in the same fraction of alfalfa. For the other two fractions obtained after thermal coagulation, we detected an average of 0.72 m/m% in the brown juice fraction for Jerusalem artichokes and a further 1.66 m/m% in the brown alfalfa fraction. The protein content of brown juice is related to the quality of the filtration technique. The microwave aggregated proteins typically remain in the LFK fraction and are difficult or impossible to solubilise. Depending on the pore size of the filter (textile or membrane), oligopeptide fragments below 5 kDa (**Figure 5**), free amino acids, biogenic amines are typically transferred to brown juice. These add up to the nitrogen content according to Kjeldahl. As an alternative source of feed protein, a dedicated leaf protein concentrate fraction (LPC) for alfalfa contained 41 m/m% crude protein, while for Jerusalem artichokes an average value of ~30 m/m% was obtained, averaged over two years.

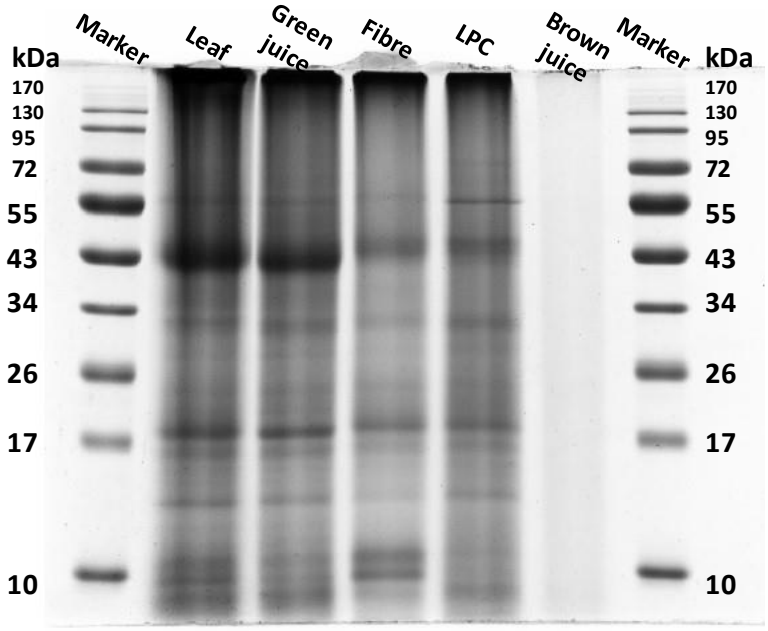


Figure 5 Representative protein expression patterns from green biomass fractions of Jerusalem artichokes by 1D SDS-PAGE.

Looking further at the protein quality of the Jerusalem artichoke fractions, the protein expression pattern that can be separated by molecular weight is illustrated in Figure 5 compared to the intact leaf. The second column shows the protein profile of the intact Jerusalem artichoke leaf, which shows a very similar pattern compared to the green leaf. The fourth column illustrates the fibre fraction. Comparing with green juice and intact leaf, we can see that around ~17 kDa in both leaf and green juice the pattern is similar. This band is no longer visible in the

fibre. In the region between ~10 and 17 kDa, a band is observed in the green juice, but is not present in the fibre fraction. A stronger pattern is visible in the fibre fraction at ~11-12 kDa but is absent in the green juice. The fifth column shows the protein profile of the leaf protein concentrate. In this fraction, proteins are expected in the upper, larger range, but we can see from the expression pattern that the thermal coagulation was not perfect, because proteins are also present in the smaller size range. Looking at the pattern of the brownish fraction, we can see that virtually no proteins of any size were detected there. This can be explained by the low protein content mentioned earlier and by the filtration after thermal coagulation. The average total amino acid composition of the fractions from the different green biomass of Jerusalem artichokes and the free amino acid content of the fractions were determined by pre-column derivatization method using UHPLC **(Table 3**).

Table 3. Results of amino acid and free amino acid content of green biomass fractions of Jerusalem artichoke origin by UHPLC-PDA measurement.

Amino acids	Hydrolized sample AA content					Free AA content				
	Leaf	Green juice	Fiber	LPC	Brown juice	Leaf	Green juice	Fiber	LPC	Brown juice
	aa mg/g sample				aa mg/ml	aa mg/g sample				aa mg/ml
Alanine	14,95	10,57	9,84	14,80	0,62	0,061	0,852	0,123	0,009	0,031
Arginine	14,79	13,44	11,01	16,68	0,34	0,006	0,101	0,080	0,026	0,004
Asparagine	0,86	1,18	LOQ	LOQ	LOQ	0,006	0,315	0,049	0,006	0,027
Aszparaginsav	22,14	16,53	16,67	25,72	2,86	0,083	1,679	0,310	0,067	0,307
Cysteine	0,54	0,55	0,65	0,93	LOQ	LOQ	LOQ	LOQ	LOQ	0,001
Phenylalanine	13,95	7,92	8,98	14,25	0,48	0,033	0,066	0,068	0,006	0,017
Glycine	13,81	8,68	8,57	13,38	0,57	0,116	0,095	0,042	0,002	0,002
Glutamine	LOQ	LOQ	LOQ	LOQ	LOQ	0,006	1,671	0,091	0,022	0,136
Glutamic acid	22,70	19,39	16,38	25,34	4,05	0,001	3,796	0,263	0,067	0,342
Histidine	4,09	3,20	3,43	5,16	0,19	0,005	LOQ	LOQ	LOQ	0,003
Izoleucine	9,52	6,41	7,46	10,99	0,37	0,011	0,077	0,048	0,005	0,014
Leucine	22,44	13,23	14,58	22,54	0,55	0,009	0,087	0,083	0,006	0,015
Lyzine	16,53	10,17	10,04	14,95	0,65	0,003	0,097	0,096	0,007	0,016
Methionine	3,50	0,66	1,06	2,76	LOQ	0,007	LOQ	0,029	LOQ	0,002
Proline	12,00	8,37	8,11	12,68	0,56	0,013	0,135	0,045	0,004	0,010
Serine	15,68	7,56	7,29	11,87	0,46	0,025	0,355	0,041	0,005	0,018
Tyrozine	10,39	9,53	6,49	11,23	0,34	0,031	0,062	0,052	0,005	0,011
Threonine	11,54	7,72	8,10	13,03	0,57	0,009	0,167	0,065	0,007	0,027
Tryptophan	-	-	-	-	-	0,005	0,074	LOQ	0,006	0,006
Valine	12,79	8,53	9,54	14,38	0,59	0,011	0,144	0,057	0,008	0,024
Sum	**222,21**	**153,63**	**148,21**	**230,68**	**13,20**	**0,44**	**9,77**	**1,54**	**0,26**	**1,01**

The total amino acid content of the green biomass fractions of Jerusalem artichoke showed a similar trend to the crude protein content. They show that the total amino acid content of the LPC fraction (which is also the true protein content) is the highest at 230.68 aa mg/g sample. Among the limiting/essential amino acids of feeding relevance, the concentrations of lysine and threonine were 14.95 and 13.03 mg/g, respectively. The LPC fraction typically contains aggregated proteins, as confirmed by the free amino acid content result (0,26 mg/g), which is negligibly small in relation to the total amino acid content. Looking at the results for the fibre fraction, we can see that the total amino acid content is ~15 aa mg/g, while for the free amino acid content we were able to detect 9.77 aa mg/g. Among the essential amino acids, leucine

(14.58 aa mg/g) and lysine (10.04 aa mg/g) were present in the highest abundance in terms of total amino acid content. Brown juice had the lowest amount of both amino acid contents. This was to support the fact that although we measured ~1-1.5 m/m% for the crude protein content of brown juice, it was also evident from **Figure 5** that these were not actual proteins.

In addition to protein, other primary and secondary metabolites in the green biomass of fractionated Jerusalem artichokes may also be of value from a human/animal health perspective. For this reason, the phytonutrient composition of each fraction was investigated by UPLC-ESI-MS/MS coupled analytical technique (**Figure 6**) and the total amount of phenolic components was determined.

For the leaf protein concentrate (LFK) fraction obtained from the first harvest in 2016, the 'Piri' variety showed the highest total phenolic component content (38.8 mg/g GAE), while the 'Fuseau' variety showed the lowest with 18.3 mg/g GAE. A statistically verifiable difference between the Jerusalem artichoke varieties could be detected. In the second harvest of the same year, the 'Kercaszomori' variety gave the highest amount (32.5 mg/g GAE), while the 'Fuseau' variety showed the lowest amount with 13 mg/g GAE. CHEN et al. (2014) showed similar values (23.5 - 30.159 mg/g GAE) in Jerusalem artichoke leaves.

In the leaf protein concentrate (LFK) fraction of the first harvest in 2017, the highest amount was detected in the 'Tapioca smooth' variety (94.02 mg/g GAE), while the lowest was in the 'Fuseau' variety (35.6 mg/g GAE). In the second harvest, the 'Piri' variety showed a truly outstanding result with 252 mg/g GAE. The other varieties showed much lower levels, 159.8 and 40.4 mg/g GAE respectively.

In general, as in the LFK fraction, we measured higher total phenolic component content values in the fibre samples in 2017 than in the 2016 harvests. Comparing the varieties/ecotypes, no trend changes could be detected: the first harvest in 2016 gave significantly the highest value in the fibre fraction in the 'Kalevala' ecotype (12.7 mg/g GAE), the lowest in the 'Tapioca plain' variety (3.8 mg/g GAE). The other varieties/ecotypes showed almost identical values. However, at the second harvest, the highest amount was obtained for the variety 'Rubik' (12.8 mg/g GAE) and the lowest for lucerne (6.5 mg/g GAE).

In 2017, the total phenolic components of the fibre fraction varied between 8 and 35 mg/g GAE. The 'Rubik' variety tended to have high values in both harvests. However, the 'Alba' variety had the highest statistically detectable value in the fibre fraction of the first harvest (34.6 mg/g GAE), while the 'Piri' variety had the lowest (8.06 mg/g GAE). The other Jerusalem artichokes had almost the same values. For the second harvest, the highest yield was obtained by the variety 'Rubik' (32.5 mg/g GAE) and the lowest by 'Alba', with only 12.83 mg/g GAE.

The total phenolic component values of the wet fraction (brown juice) obtained after microwave heat coagulation of the green juice show significant differences (~15 - 86 mg/ml) between the Jerusalem artichoke varieties/rootstocks for the two years of harvest, but the differences do not follow a trend here either. During the first harvest in 2016, the highest amount of total phenolic components was measured in the 'Kercaszomori' variety (86.4 mg/ml GAE), while the lowest amount was measured in the 'Alba' variety (15.6 mg/ml GAE). Compared to the alfalfa (59.4 mg/ml GAE), two Jerusalem artichoke varieties showed higher levels. In the second harvest of the same year, the highest amount of total phenolic components was detected in the variety 'Fuseau' (85.8 mg/ml GAE), and the lowest amount again in the variety 'Alba' (7.9 mg/ml GAE). The 'Kercaszomori' and 'Rubik' varieties showed 82-77 mg/ml GAE at both harvests. However, at the first harvest in 2017, the 'Alba' variety showed the highest amount with 61 mg/ml GAE. The lowest was measured in 'Rubik' with 24.4 mg/ml GAE. Alfalfa (23.1 mg/ml GAE) did not differ from 'Rubik'. At the second harvest, again 'Alba' showed the highest level of 92.6 mg/ml GAE, while 'Fuseau' showed the lowest level of 29.6 mg/ml GAE, less than the alfalfa (28.02 mg/ml GAE), but not statistically different.

A total of eighty-four phytochemicals were identified in the green biomass fractions of Jerusalem artichokes (**Figure 6**). The identification of each component was compared with Metlin, mzCloud, MoNA-MassBank of North America databases based on specific retention time, exact molecular mass, and fragmentation pattern. Negligible differences were observed in the phytochemical profiles of the fractions of the Jerusalem artichoke cultivars/ecotypes included in the study.

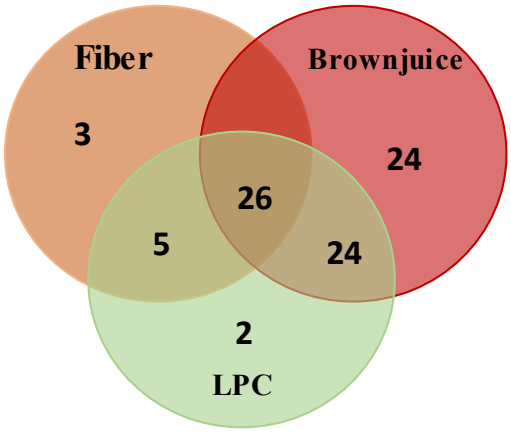


Figure 6. Number of phytochemical components identifiable from green biomass fractions of Jerusalem artichoke varieties/ecotypes determined by UPLC-ESI-MS/MS coupled analytical method.

A significant proportion of the compounds identified were present in all three fractions (Figure 7). A total of 26 phytochemical components with different chemical specificities were

present in all three fractions, such as, Epiafzelechin trimethyl ether, Vanillin, Azelaic acid, γ-Aminobutyric acid (GABA). These secondary metabolites are very commonly found in the plant kingdom. However, there were also some components that were found in only one or possibly two fractions. 24 common components were found in the brown juice and LPC fractions, compared to 5 common compounds in the fibre and LPC fractions. However, we were unable to identify any metabolites that were common only to the brown juice and fibre fractions.

A total of 24 water-soluble compounds were found exclusively in brown juice. In the LPC fraction, there were 2 phytochemical components that were unique to this fraction (quercetin and isoquercetin). However, in the fibre fraction there were 3 compounds unique to this fraction (coumarin, 2-hydroxyhexadecanoic acid, 4-hydroxy-3-methoxycinnamaldehyde).

For ease of reference, the identified compounds were grouped according to their structure into Flavonoid, Non-flavonoid-type compounds, Amino acids, Terpenes, Vitamins and Other metabolites (**Figure 7**).

In the green biomass of fractionated Jerusalem artichokes, several hydroxylated methoxyflavones, such as dimethoxytetrahydroxyflavone, dihydroxymethoxyflavone, and himenoxin and nevadensin, were detected for the first time as flavonoids with proven positive health effects. From the fractions, liquiritigenin, an estrogenic flavanone, as well as butein and kukulkanin B were detected as calcones. However, to our knowledge, this is the first time that isorhamnetin (isorhamnetin-3-O-glucuronide) and isocvercetin (quercetin-3-O-β-d-glucopyranoside), glucuronide derivatives, have been identified in Jerusalem artichokes.

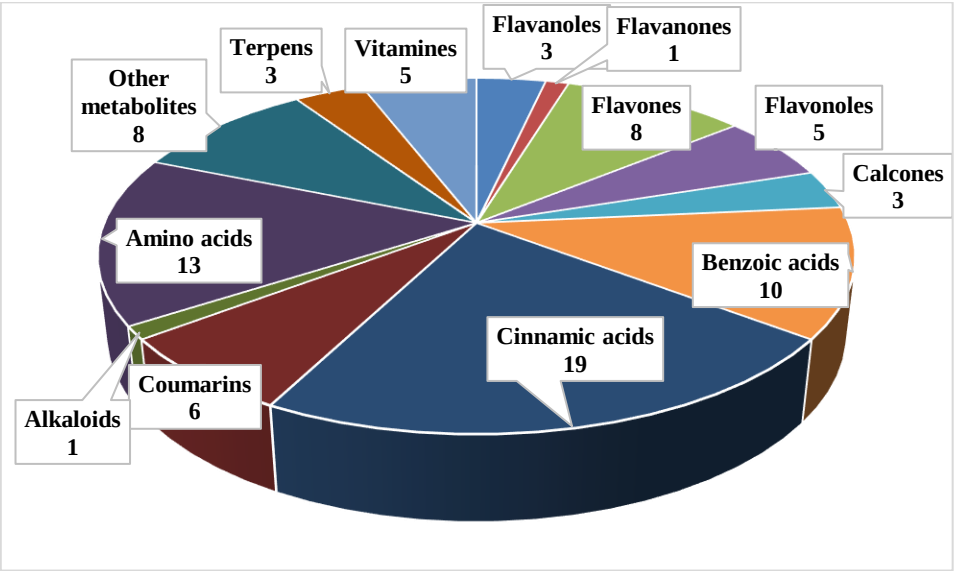


Figure 7. Number of compounds identified by UPLC-ESI-MS from green biomass fractions of Jerusalem artichoke.

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-hydroxycitric acid esters (p-coumaric, caffeic, and ferulic) of quinic acids found in Jerusalem artichoke tubers and shoots (YUAN et al., 2012; OLESZEK et al., 2019; SHOWKAT et al., 2019). Our measurements detected 13 different phenolic acids in fractions from green biomass of Jerusalem artichokes. We identified three structural isomers of caffeoylquinic acid with different retention times. Chlorogenic acid (3-O-caffeoylquinic acid), neochlorogenic acid (5-O-caffeoylquinic acid) and cryptochlorogenic acid (4-O-caffeoylquinic acid) were identified. In the absence of neochlorogenic acid and cryptochlorogenic acid standards, only the relative proportions to each other could be compared by their identical molecular weight and ionisation. The area of the extracted ion chromatograms of the isomers was comparable and, based on this, 3-O-caffeoylquinic acid appeared to be the dominant one (**Figure 8**).

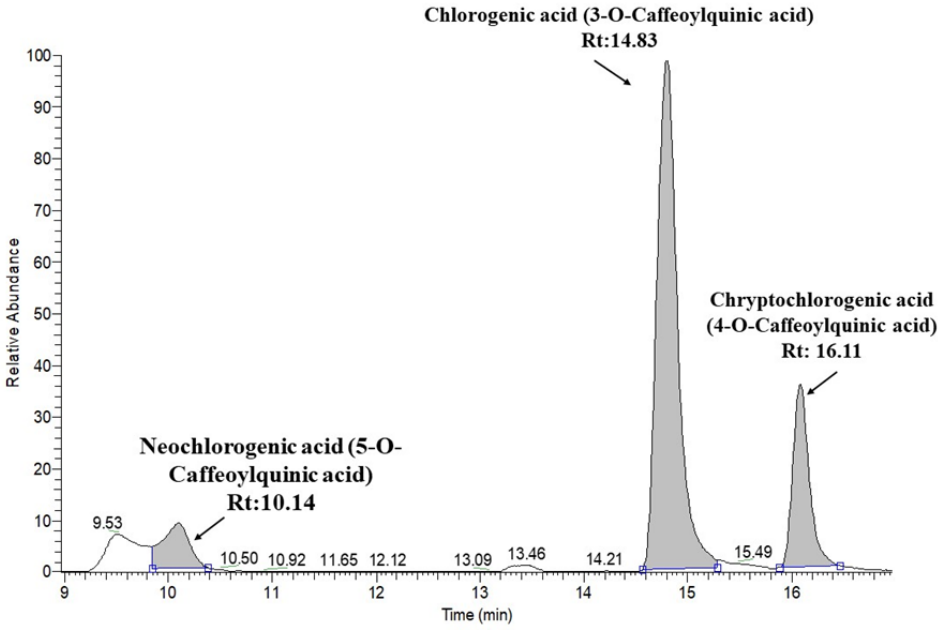


Figure 8. Ratio of the three isomers of chlorogenic acid (caffeoylquinic acid) to each other based on the ion chromatogram of the UPLC-ESI-MS system.

Chlorogenic acid (syn. 3-O-Caffeoylquinic acid) is a major member of the phenolic components. It has recently been studied mainly from a pharmacological and medical point of view. Chlorogenic acid is important from a nutritional biological point of view, as experiments have demonstrated its antibacterial, cardio-, neuroprotective, anti-inflammatory, antipyretic and other effects (NAVEED et al. 2019). Some research has also suggested that it has antihypertensive effects (ZHAO 2011). MEMON et al. (2010) suggest that chlorogenic acid may be a precursor for one of the active ingredients of an HIV antiviral drug. Based on our

quantitative measurements, chlorogenic acid in the LPC fraction of Jerusalem artichokes varied from 1374.6 to 3929.4 μg/g for two years and seven cultivars/ecotypes.

We also found four different coumarin-type compounds. Scopoletin and ayapin have already been described in Jerusalem artichoke tubers and their presence has been suggested in above-ground parts of the plant, as in sunflower (CABELLO-HURTADO et al. 1998). Our measurements confirmed the presence of scopoletin together with isoscopoletin, 6-methylcoumarin and fraxidine. Some of the simple coumarins are known as phytoalexins. However, fraxidine and scopoletin showed strong antiadipogenic activity against preadipocyte cell lines in in vitro assay systems (VENUGOPALA et al. 2013).

Three terpenoid-type compounds were consistently present in all the fractions tested from Jerusalem artichokes (loliolide, dihydroactinidiolide, 7-deoxyloganic acid isomer). Loliolide is a photooxidative or thermally degradable product of carotenoids. Similarly, dihydroactinidiolide, a volatile monoterpenoid, has been identified as a flavour constituent of many plants such as tobacco and tea (SEILER, 1988).

In terms of vitamins, we found vitamin B molecules, nicotinic acid (vitamin B3) and nicotinic amide (vitamin B3 amide), riboflavin (vitamin B2) and biotin (vitamin B7). Quantitative measurements of B vitamins in the LPC fraction of Jerusalem artichokes showed the following trends: the highest abundance of vitamin B2 (23.4-35.5 μg/g), 1.9 μg/g for the two B3 vitamins and 1.45 μg/g for vitamin B7.

In addition, organic acids, malic and citric acids, and plant hormones such as indole acetic acid were also identified in the fractions.

In addition to the compounds identified, it is worth mentioning a group of compounds that could not be detected in any of the Jerusalem artichoke fractions. These are saponins. Some of the saponins have adverse physiological effects due to their haemolytic properties. Several of these were found in the alfalfa fractions (data not reported) but are completely absent in Jerusalem artichokes.

3.5. Crude protein results for green biomass fractions

The crude protein determination of the two harvests of the two years under study was carried out using the Kjeldahl method. The method is commonly used in the determination of crude protein in food and feed.

Table 4 Crude protein content in m/m% (g/100 g sample) of green biomass fractions of Jerusalem artichokes and alfalfa harvested in 2016, based on the Kjeldahl method. Different letters in the respective columns indicate significant difference based on Duncan's test at $p<0.05$.

Variety/ecotype	2016					
	1. harvest			2. harvest		
	Fiber (m/m%)	Brown juice (m/m%)	LPC (m/m%)	Fiber (m/m%)	Brown juice (m/m%)	LPC (m/m%)
Alba	11,67 ±1,09^{c}	1,01 ±0,01^{d}	35,26 ±0,15^{a}	7,19 ±0,07^{f}	1,36 ±0,03^{a}	31,9 ±0,44^{d}
Fuseau	10,36 ±0,04^{d}	0,30 ±0,04^{g}	33,27 ±0,53^{c}	10,86 ±0,28^{cd}	0,55 ±0,03^{e}	35,44 ±0,5^{b}
Kalevala	13,12 ±0,44^{b}	0,94 ±0,02^{e}	33,78 ±0,36^{c}	11,5 ±0,10^{b}	1,35 ±0,03^{a}	33,55 ±0,36^{c}
Kercaszomori	14,12 ±0,42^{a}	0,53 ±0,02^{f}	33,63 ±0,39^{c}	9,58 ±0,47^{e}	0,44 ±0,02^{f}	27,64 ±0,34^{f}
Piri	11,81 ±0,29^{c}	0,96 ±0,01^{e}	34,5 ±0,34^{b}	13,86 ±0,29^{a}	1,03 ±0,05^{b}	32,38 ±0,24^{d}
Rubik	12,54 ±0,28^{bc}	1,49 ±0,03^{a}	30,94 ±0,05^{d}	11,37 ±0,13^{bc}	1,07 ±0,03^{b}	26,46 ±0,37^{g}
Tápiói sima	14,75 ±0,15^{a}	1,08 ±0,02^{c}	30,77 ±0,43^{d}	10,67 ±0,67^{d}	0,76 ±0,03^{d}	30,8 ±0,26^{e}
Hunor 40	12,49 ±0,14^{bc}	1,25 ±0,01^{b}	34,53 ±0,12^{b}	11,81 ±0,21^{b}	0,87 ±0,05^{c}	36,32 ±0,3^{a}

The crude protein content of the Jerusalem artichoke fibre fractions obtained from the first harvest in 2016 (**Table 4**.) was 10-14 m/m%. For alfalfa, it was 12 m/m%. The alfalfa Rust fraction showed no difference compared to Jerusalem artichokes. For the brown juice fraction of Jerusalem artichokes, a crude protein content of 0.3-1.5 m/m% was detected. The leaf protein concentrate (LPC) fraction was highest in the ecotype 'Alba' (35.3 m/m%) and lowest in 'Rubik' and Tápiói sima' (~31 m/m%) in crude protein. Comparing the alfalfa LPC fraction (34%) with the Jerusalem artichokes, we can see that the 'Alba' ecotype had 1% more, which was statistically proven to be more.

In the second harvest of 2016, the crude protein content of the fibre fractions varied between 7.19 and 13.8 m/m%. For the brown juice fraction, most of the Jerusalem artichoke varieties/ecotypes showed values above 1 m/m%. For the LPC fraction, alfalfa had the highest crude protein content (36 m/m%), but the varieties 'Fuseau' and 'Kalevala' were close to this value (35.4 and 33.5 m/m%). TELEK (1983), in his summary work, studied potential protein crop species, 500 species in number. Leaf protein production was carried out by heat denaturation in several steps. His work shows that the Jerusalem artichokes we studied had higher crude protein yields than, for example, Amarant (*Amaranthaceae*) 26.6 m/m%, certain Brassicaceae 17-30 m/m%, mallow (*Malvaceae*) 14 m/m% or sunflower (*Helianthus annuus*) 25.4 m/m% by denaturing green press juice at 82°C. In addition, higher crude protein content

was also detected in most of the legumes we tested; flapweed (*Caesalpinioideae*) ~15 m/m%, buttercup (*Fabaceae*) ~18 m/m%.

At the first harvest in 2017 (**Table 5**), the crude protein content of fibre fractions from Jerusalem artichoke varieties varied between 9-14 m/m%. Compared to alfalfa, one Jerusalem artichoke variety ('Fuseau') had a higher crude protein content (14 m/m%). The brown juice fractions of Jerusalem artichokes could not surpass the crude protein content of alfalfa (1.2 m/m%). For the leaf protein concentrate (LPC) fraction, significantly higher values (49.5 m/m%) were obtained for alfalfa compared to the LPC (20-30 m/m%) values for Jerusalem artichoke.

Table 5 Crude protein content in m/m% (g/100 g sample) of green biomass fractions of Jerusalem artichokes and alfalfa harvested in 2017, based on the Kjeldahl method. Different letters in the respective columns indicate significant difference based on Duncan's test at $p<0.05$.

Variety/ecotype	2017					
	1. harvest			2. harvest		
	Fiber (m/m%)	Brown juice (m/m%)	LPC (m/m%)	Fiber (m/m%)	Brown juice (m/m%)	LPC (m/m%)
Alba	11,35 ±0,06^{e}	0,58 ±0,01^{c}	26,54 ±0,07^{e}	12,12 ±0,04^{f}	0,48 ±0,02^{c}	29,29 ±0,05^{c}
Fuseau	14,19 ±0,06^{a}	0,77 ±0,02^{b}	30,50 ±0,06^{b}	13,18 ±0,02^{c}	0,48 ±0,03^{c}	32,86 ±010^{b}
Kalevala	10,36 ±0,05^{f}	0,47 ±0,03^{d}	24,77 ±0,04^{f}	13,35 ±0,04^{b}	0,46 ±0,02^{c}	28,89 ±0,03^{d}
Kercaszomori	12,58 ±0,05^{c}	0,47 ±0,03^{d}	28,93 ±0,07^{c}	14,71 ±0,03^{a}	0,35 ±0,02^{d}	27,48 ±0,03^{e}
Piri	12,28 ±0,06^{d}	0,50 ±0,02^{d}	27,44 ±0,08^{d}	12,45 ±0,04^{e}	0,47 ±0,02^{b}	27,59 ±0,62^{e}
Rubik	10,15 ±0,04^{g}	0,55 ±0,03^{c}	23,52 ±0,06^{g}	13,33 ±0,04^{b}	0,56 ±0,03^{b}	26,06 ±0,06^{f}
Tápiói sima	9,27 ±0,06^{h}	0,50 ±0,03^{d}	20,49 ±0,03^{h}	11,2 ±0,04^{g}	0,58 ±0,03^{b}	27,45 ±0,07^{e}
Hunor 40	12,99 ±0,12^{b}	1,29 ±0,05^{a}	49,58 ±0,03^{a}	12,84 ±0,04^{d}	1,65 ±0,03^{a}	44,8 ±0,04^{a}

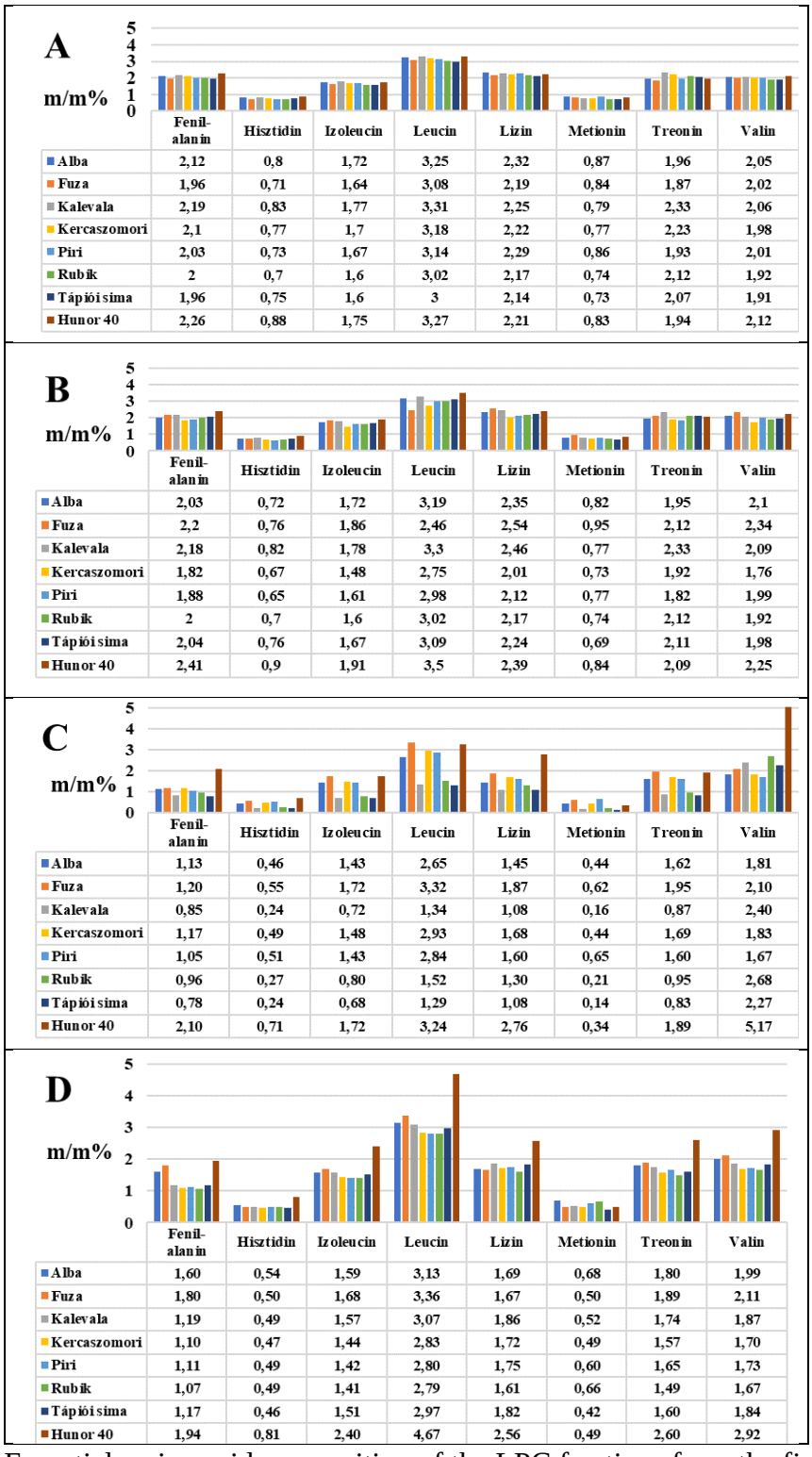
The work of FATHI and TARI (2016) demonstrates that drought stress significantly affects plant physiological processes and key biochemical pathways, with protein synthesis also being disrupted.

At the second harvest, the crude protein content of the fibre fractions of Jerusalem artichokes varied between 11-14 m/m%. The alfalfa had a protein content of 12 m/m%. Among the Jerusalem artichoke varieties, the varieties 'Fuseau', 'Kalevala' and 'Kercaszomori' outperformed alfalfa and this was statistically verified. For the brown juice fraction, alfalfa (1.6 m/m%) had the highest crude protein content. The Jerusalem artichoke varieties showed almost the same amount (0.3-0.5 m/m%). For the leaf protein concentrate fraction, the highest crude protein content was measured in alfalfa (44.8 m/m%). The Jerusalem artichoke cultivars lagged,

with the 'Fuseau' cultivar producing the highest crude protein at 32.8 m/m%. The other cultivars showed crude protein contents of 26-29 m/m%.

3.6. Results on the amino acid composition of green biomass fractions of Jerusalem artichokes

Green biorefining, including leaf protein concentrate, can provide an opportunity to cover an adequate source of protein in a sustainable way, in line with the circular farming approach. An important aspect besides the absolute concentration of proteins is their biological value, which is mainly determined by the ratio of essential/limiting amino acids. In addition, it is advantageous for the chosen crops to be harvested several times a year, which ensures a continuous supply of raw materials for a farm unit. Along these lines, the amino acid composition of the solid fractions (LPC and fibre) obtained from the first and second harvests of the two years under study was analysed and summarised in **Figures 9-10**. The values obtained are expressed per 100 g of dry homogenised sample.



	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	2,12	0,8	1,72	3,25	2,32	0,87	1,96	2,05
Fuza	1,96	0,71	1,64	3,08	2,19	0,84	1,87	2,02
Kalevala	2,19	0,83	1,77	3,31	2,25	0,79	2,33	2,06
Kercaszomori	2,1	0,77	1,7	3,18	2,22	0,77	2,23	1,98
Piri	2,03	0,73	1,67	3,14	2,29	0,86	1,93	2,01
Rubik	2	0,7	1,6	3,02	2,17	0,74	2,12	1,92
Tápiói sima	1,96	0,75	1,6	3	2,14	0,73	2,07	1,91
Hunor 40	2,26	0,88	1,75	3,27	2,21	0,83	1,94	2,12

	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	2,03	0,72	1,72	3,19	2,35	0,82	1,95	2,1
Fuza	2,2	0,76	1,86	2,46	2,54	0,95	2,12	2,34
Kalevala	2,18	0,82	1,78	3,3	2,46	0,77	2,33	2,09
Kercaszomori	1,82	0,67	1,48	2,75	2,01	0,73	1,92	1,76
Piri	1,88	0,65	1,61	2,98	2,12	0,77	1,82	1,99
Rubik	2	0,7	1,6	3,02	2,17	0,74	2,12	1,92
Tápiói sima	2,04	0,76	1,67	3,09	2,24	0,69	2,11	1,98
Hunor 40	2,41	0,9	1,91	3,5	2,39	0,84	2,09	2,25

	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	1,13	0,46	1,43	2,65	1,45	0,44	1,62	1,81
Fuza	1,20	0,55	1,72	3,32	1,87	0,62	1,95	2,10
Kalevala	0,85	0,24	0,72	1,34	1,08	0,16	0,87	2,40
Kercaszomori	1,17	0,49	1,48	2,93	1,68	0,44	1,69	1,83
Piri	1,05	0,51	1,43	2,84	1,60	0,65	1,60	1,67
Rubik	0,96	0,27	0,80	1,52	1,30	0,21	0,95	2,68
Tápiói sima	0,78	0,24	0,68	1,29	1,08	0,14	0,83	2,27
Hunor 40	2,10	0,71	1,72	3,24	2,76	0,34	1,89	5,17

	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	1,60	0,54	1,59	3,13	1,69	0,68	1,80	1,99
Fuza	1,80	0,50	1,68	3,36	1,67	0,50	1,89	2,11
Kalevala	1,19	0,49	1,57	3,07	1,86	0,52	1,74	1,87
Kercaszomori	1,10	0,47	1,44	2,83	1,72	0,49	1,57	1,70
Piri	1,11	0,49	1,42	2,80	1,75	0,60	1,65	1,73
Rubik	1,07	0,49	1,41	2,79	1,61	0,66	1,49	1,67
Tápiói sima	1,17	0,46	1,51	2,97	1,82	0,42	1,60	1,84
Hunor 40	1,94	0,81	2,40	4,67	2,56	0,49	2,60	2,92

Figure 9. Essential amino acid composition of the LPC fractions from the first (A) and second (B) harvests in 2016 and the first (C) and second (D) harvests in 2017, per 100 g sample.

The ratios of essential amino acids in the leaf protein concentrate fraction were similar between the two harvests studied (**Figure 9/A - D**). In contrast, significant differences can be observed when looking at the absolute amounts of each amino acid.

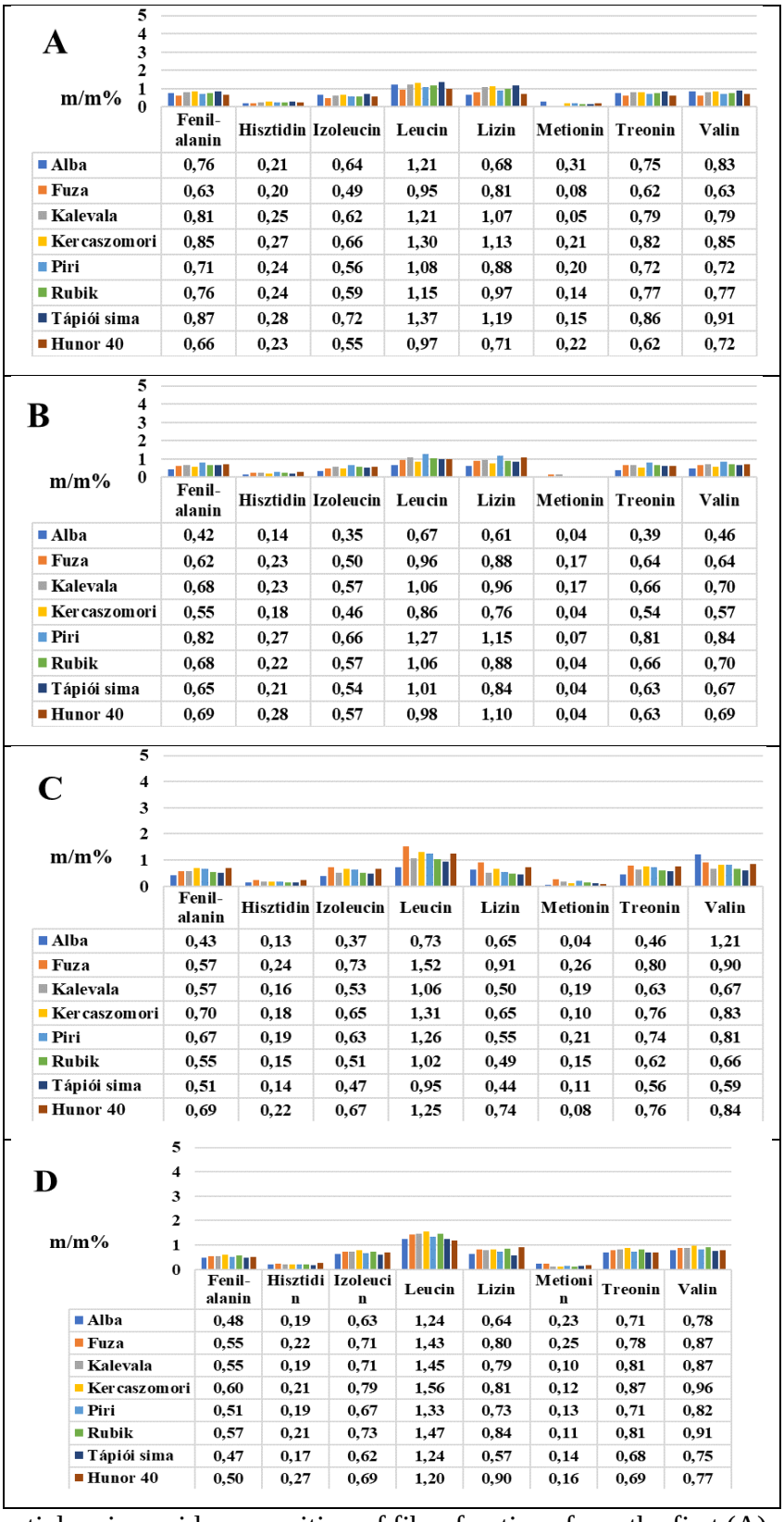
In the leaf protein concentrate fraction, the quantitative distribution of each essential amino acid by variety/ecotype showed small differences between the 2016 harvests (**Figure 9/A and B**). In contrast, in 2017, outliers were observed for several amino acids (e.g., leucine, threonine), which correlated with crude protein content (**Figure 9/C and D**). This was also the case for alfalfa, which had significantly higher prominent amino acid values per dry weight compared to Jerusalem artichokes, irrespective of variety/ecotype. The combination of extensive growing conditions, abiotic factors and soil heterogeneity influenced the protein content and, consequently, the quantitative abundance of amino acids.

For the individual amino acids, in the first harvest of both years, the amino acid leucine was the most abundant (~3 m/m% and 1.2-2.9 m/m%) in the LPC fraction, an amino acid known to constitute about 9% of proteins NYITRAI and PÁL (2013). In terms of proportions, histidine and methionine were the least abundant. These two amino acids are the least abundant in proteins compared to the other proteinogenic essential amino acids. This is also supported by the work of KISBENEDEK and SZABÓ (2015), who found that methionine is present in 1-2% and histidine in 2-3% of plant proteins. Lysine, threonine, and valine amino acids were also present in higher amounts in the LPC fraction. For the 2016 harvests, 2 m/m% or more was measured. In the 2017 harvests, we were able to detect nearly the same amount of the three amino acids, but less than in 2016. This is clearly related to the protein content (**Table 3-4**).

One of the by-products of green biorefining and, within this, the production of leaf protein concentrate is the fibre fraction. This fraction also contains valuable components. For this reason, in green biorefinery practices, the fibre fraction is the basis for product development in several directions, e.g., to produce fibre substitutes for feed applications.

Significant amounts of 10-14% protein in the fibre fraction were still detected in Jerusalem artichoke varieties/ecotypes (**Table 3-4**).

From such an approach, it was interesting to investigate the amino acid composition of the green biomass-derived fibre fractions of two years of harvesting of Jerusalem artichoke varieties/ecotypes, as illustrated in **Figure 10**.



	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	0,76	0,21	0,64	1,21	0,68	0,31	0,75	0,83
Fuza	0,63	0,20	0,49	0,95	0,81	0,08	0,62	0,63
Kalevala	0,81	0,25	0,62	1,21	1,07	0,05	0,79	0,79
Kercaszomori	0,85	0,27	0,66	1,30	1,13	0,21	0,82	0,85
Piri	0,71	0,24	0,56	1,08	0,88	0,20	0,72	0,72
Rubik	0,76	0,24	0,59	1,15	0,97	0,14	0,77	0,77
Tápiói sima	0,87	0,28	0,72	1,37	1,19	0,15	0,86	0,91
Hunor 40	0,66	0,23	0,55	0,97	0,71	0,22	0,62	0,72

	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	0,42	0,14	0,35	0,67	0,61	0,04	0,39	0,46
Fuza	0,62	0,23	0,50	0,96	0,88	0,17	0,64	0,64
Kalevala	0,68	0,23	0,57	1,06	0,96	0,17	0,66	0,70
Kercaszomori	0,55	0,18	0,46	0,86	0,76	0,04	0,54	0,57
Piri	0,82	0,27	0,66	1,27	1,15	0,07	0,81	0,84
Rubik	0,68	0,22	0,57	1,06	0,88	0,04	0,66	0,70
Tápiói sima	0,65	0,21	0,54	1,01	0,84	0,04	0,63	0,67
Hunor 40	0,69	0,28	0,57	0,98	1,10	0,04	0,63	0,69

	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	0,43	0,13	0,37	0,73	0,65	0,04	0,46	1,21
Fuza	0,57	0,24	0,73	1,52	0,91	0,26	0,80	0,90
Kalevala	0,57	0,16	0,53	1,06	0,50	0,19	0,63	0,67
Kercaszomori	0,70	0,18	0,65	1,31	0,65	0,10	0,76	0,83
Piri	0,67	0,19	0,63	1,26	0,55	0,21	0,74	0,81
Rubik	0,55	0,15	0,51	1,02	0,49	0,15	0,62	0,66
Tápiói sima	0,51	0,14	0,47	0,95	0,44	0,11	0,56	0,59
Hunor 40	0,69	0,22	0,67	1,25	0,74	0,08	0,76	0,84

	Fenil-alanin	Hisztidin	Izoleucin	Leucin	Lizin	Metionin	Treonin	Valin
Alba	0,48	0,19	0,63	1,24	0,64	0,23	0,71	0,78
Fuza	0,55	0,22	0,71	1,43	0,80	0,25	0,78	0,87
Kalevala	0,55	0,19	0,71	1,45	0,79	0,10	0,81	0,87
Kercaszomori	0,60	0,21	0,79	1,56	0,81	0,12	0,87	0,96
Piri	0,51	0,19	0,67	1,33	0,73	0,13	0,71	0,82
Rubik	0,57	0,21	0,73	1,47	0,84	0,11	0,81	0,91
Tápiói sima	0,47	0,17	0,62	1,24	0,57	0,14	0,68	0,75
Hunor 40	0,50	0,27	0,69	1,20	0,90	0,16	0,69	0,77

Figure 10 Essential amino acid composition of fibre fractions from the first (A) and second (B) harvests of 2016 and the first (C) and second (D) harvests of 2017.

Similar to the results observed in the LPC fraction, the amino acid leucine was the most abundant in the fibre fraction (1.2 m/m% on average), with the least abundance of histidine

(~0.2 m/m%) and methionine (~0.1 m/m%). These can be explained by the amino acid abundances in the previously mentioned proteins.

Furthermore, it can be said that the amino acids lysine, threonine and valine were present in similar proportions in the fibres of the Jerusalem artichoke cultivars/ecotypes as in the LPC fraction but were detected in lower amounts.

In the fibre fractions of the control alfalfa cultivar 'Hunor 40', amino acids were detected in lower amounts than in the Jerusalem artichokes. These proportions are true for all harvests in all years studied.

Observing the 2016 harvests, we can see that essential amino acids were present in almost the same amounts. However, for methionine, we could detect less in the second harvest (**Figure 10/B**) (0.04 m/m%) than in the first (**Figure 10/A**) (~0.15 m/m%) for both Jerusalem artichokes and alfalfa. Its low abundance in proteins, combined with its chemical structure, makes it susceptible to sample preparation by acid hydrolysis. TROVATO et al. (2021). The work of CSAPÓ et al. (2008) confirms that there is no relationship between protein content and the relative proportions of amino acids. In other words, although the protein content is lower in the fibre fraction compared to the LFK fraction, the ratio of amino acids does not change between the two fractions, and no such decrease was observed for methionine in the 2017 harvests.

3.7. Results on photosynthetic pigment content of green biomass fractions of Jerusalem artichokes

Studies have shown that the consumption of green plant parts is beneficial for both animal and human organisms. In addition to phytonutrients, photosynthetic pigments are also present in these plant parts in non-negligible amounts, but these values are species dependent. The two main groups of these pigments are chlorophylls and carotenoids (KOSPELL et al., 2005; WANG and WINK 2016).

The beneficial nutritional biological effects of chlorophylls and their derivatives have been widely studied. Among others, anti-inflammatory and antioxidant effects, and success in the treatment of various epithelial injuries have been achieved (FERRUZZIA and BLAKESLEE 2007). Furthermore, experiments have demonstrated the inhibition of calcium oxalate crystal formation (TAWASHI et al. 1980).

Carotenoids, as photosynthetic accessory pigments, have the primary function of transmitting light energy to chlorophyll molecules and protecting them from intense light energy. They can also act as antioxidants through their conjugated double bonds and are precursors of certain vitamins.

In line with the above, the analysis of the photosynthetic pigment content of some fractions (fibre, green juice) obtained from the green biomass of Jerusalem artichokes and intact leaves was performed by spectrophotometric method. We did not perform measurements from the LPC and brown juice fractions, because microwave heating converts some of the photosynthetic pigments to other derivatives such as pheophytins (their presence was confirmed by hyphenated analytical qualitative measurements, data not reported). Thus, the spectrophotometric method is not suitable for these fractions.

Among the three fractions, we measured the highest photosynthetic pigment content in the leaf fraction and the lowest in the fibre fraction for all parameters tested. In 2016, we measured the highest amount of all pigments in the intact leaf fraction. However, statistically verifiable differences between varieties/ecotypes were not always detected each year. The photosynthetic pigment content of leaves was consistently below average for three cultivars/ecotypes ('Kercaszomori', 'Piri' and 'Rubik').

In 2017, the highest leaf fraction 'chlorophyll a' was found in the cultivar 'Rubik', which was statistically different from the other cultivars/ecotypes. The same trend was also observed for 'chlorophyll b' and 'carotene' and was statistically verifiably different from the other varieties/ecotypes.

In the green juice fraction, lower values were obtained (compared to leaves). For green juices, we also observed a difference between the two years, with higher amounts of pigments detected in 2016 than in 2017.

The fibre fraction showed the lowest levels of pigments. However, compared to the other two fractions (leaves, green juice), no difference was detected in the fibre fraction between the two years.

Comparing the averages of the two harvests with the work of DUMA et al. (2014), we can see that they measured chlorophyll a in the leaves of lettuce (*Lactuca sativa*) at 0.144 mg/g, while for Jerusalem artichoke we could detect more chlorophyll a even in the fibre fraction, with an average of 0.885 mg/g. For spinach (*Spinacia oleracea*), 1.043 mg/g was detected, which is higher than the value we obtained in the fibre fraction, but the average chlorophyll a in the green juice fraction of Jerusalem artichokes was 3.47 mg/g in 2016 and 1.80 mg/g in 2017.

The amount of carotene and xanthophyll was also higher in the fractions we measured than what DUMA et al (2014) measured for leafy greens. Carotene was 0.072 mg/g for lettuce and between 0.2 and 0.3 mg/g for basil and mustard. In contrast, carotene in Jerusalem artichoke leaves was 2.5 mg/g, in green leaves 1.5 mg/g and in fibre 0.5 mg/g. For xanthophylls, values

ranging from 0.03 to 0.39 mg/g were obtained for all plant species. In contrast, an average of 1.9 mg/g was measured in Jerusalem artichoke leaves, 0.65 mg/g in the green juice fraction and 0.26 mg/g in fibre.

The significance of this is that ruminant diets do not always contain adequate amounts of carotenoids. The reason is that in most cases these mixtures undergo a heat treatment where a large part of them decomposes. At the same time, carotenoids are precursors of retinol (vitamin A). Studies have also shown that the consumption of carotenoids improves e.g. fertility (NOZIÉRE et al. 2006).

4. NEW SCIENTIFIC RESULTS OF THE THESIS

1. In the context of the utilization of green biomass of Jerusalem artichoke in green biorefinery, the patent (P1800041/40) obtained based on this work, we have applied microwave coagulation as a first option for thermal coagulation to produce leaf protein concentrate and brown juice.
2. Based on our experimental work comparing varieties, we have found that varieties/ecotypes as influencing factors for green biomass fractionation are less important than harvest dates during the growing season.
3. Following the steps of the "green conveyor belt", we found that a fibre fraction of at least 7.19 m/m% and up to 14.75 m/m% crude protein content can be produced from green biomass of Jerusalem artichokes using twin screw presses, considering the varieties/ecotypes studied. After processing of the green biomass, a brown juice fraction of up to 0.30 m/m% and at least 1.49 m/m% crude protein content was obtained after thermal coagulation and gravity separation. In addition, a leaf protein concentrate (LPC) with a crude protein content of at least 20,49 m/m% and up to 35,26 m/m% was obtained, considering the varieties/ecotypes tested.
4. Based on the amino acid composition of the limiting amino acids, the methionine content of the LPC fraction obtained from green biomass of Jerusalem artichoke (0,44-0,95 m/m%) reached and exceeded the methionine content of the LPC fraction obtained from alfalfa ('*Hunor 40*') (0,34-0,84 m/m%), depending on the Jerusalem artichoke cultivar/ecotype.
5. A total of 84 phytochemical components were identified in the green biomass fractions of Jerusalem artichoke. Negligible differences in the phytochemical composition quality of the fibre, leaf protein concentrate and brown juice fractions of the sampled Jerusalem artichoke cultivars/ecotypes were observed between cultivars/ecotypes.
6. First, we detected a large number of methoxy-hydroxyflavones within the falvonoids, such as dimethoxy-trihydroxyflavone, dimethoxy-tetrahydroxyflavone, dihydroxymethoxyflavone, trihydroxytrimethoxyflavone, and butein, a member of the chalcones, and the glucuronide derivatives isorhamnetin (*isorhamnetin 3-O-glucuronide*) and isocvercetin (*quercetin 3-O-β-d-glucopyranoside*) from fractionated Jerusalem artichoke green biomass, which have not been previously described from fractions of Jerusalem artichoke green biomass origin.

5. PRACTICAL USE OF THE RESULTS

1. For the utilisation of green biomass from Jerusalem artichokes in green biorefineries, a space of 80 × 60 cm can be used, and sufficient quantity and quality of green biomass can be obtained.
2. It was found that under domestic extensive conditions, without irrigation, and considering the regeneration capacity of Jerusalem artichoke, it is safe to harvest green biomass for fractionation purposes twice.
3. We have found that the extensive cultivation of Jerusalem artichokes and their two-fold cutting back does not ensure sufficient quantity and quality of seed tubers for the following year, and that it is necessary to maintain a dedicated stock.
4. From a feed perspective, as a direct source of protein, the protein content of the Jerusalem artichoke leaf protein concentrate fraction is lower than that of alfalfa, but the maximum of 35 m/m% is considered significant, especially when considering that the limiting amino acid methionine is at least as high as or even higher than alfalfa in several ecotypes/varieties ('Alba', 'Fuseau'). In the ongoing operation of decentralised plants, which are emerging as a possibility for green biorefinery, the processing of Jerusalem artichokes can be included alongside alfalfa, if harvesting is timed correctly.
5. Considering the phytochemical composition of leaf protein concentrate and pressed fibre fractions, the presence of a large number of flavonoid and non-flavonoid phenolic components with positive physiological effects can be observed. Furthermore, the antinutritive saponins typically found in alfalfa are not detectable at all. These results confirm the role of leaf protein concentrate (LFK) and pressed fibre fractions as product candidates in animal nutrition. Furthermore, they could be beneficially incorporated into human diets as nutritional supplements.

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In: 4th National Conference of Young Biotechnologists "FIBOK 2020" online conference : abstract book. Ed.: by Tünde Pusztahelyi, Levente Czeglédi, Éva Domokos-Szabolcsy, Tamás Emri, Debreceni Egyetem, Debrecen, 29, 2020. ISBN: 9789634902720

21. Kovács, Z., Fári, M., **Kaszás, L.**, Koroknai, J., Csatári, G., Domokos-Szabolcsy, É.: Obtention of functional leaf protein concentrate (F-LPC) based on Ereky-process: historical survey and preliminary results.
New Biotech. 44, S100-S101, 2018. ISSN: 1871-6784.
DOI: https://doi.org/10.1016/j.nbt.2018.05.978

Informational/educational articles (1)

22. **Kaszás, L.**: Újra fölfedezték a csicsókát.
Kertész. szőlész. 26, 11-12, 2017. ISSN: 0023-0677.

Total IF of journals (all publications): 12,857
Total IF of journals (publications related to the dissertation): 8,922

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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