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EVALUATION OF CHEMICAL COMPOSITION OF SOME *SILPHIUM* L. SPECIES SEEDS AS ALTERNATIVE FOODSTUFF RAW MATERIALS

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The studied seeds of three *Silphium* species (*Silphium perfoliatum* L., *Silphium trifoliatum* L. and *Silphium integrifolium* Michx.) contained (recalculating onto dry matter): up to 33.5% of protein, up to 24.1% of fat, up to 9.58% of water-soluble sugars, and up to 25.4% of cellulose. Ash was up to 6.8% of seeds dry matter. Mean energetic value of the analysed raw materials ranged from 1076 kJ/100 g (*S. trifoliatum*) to about 1530 kJ/ 100 g DM (*S. perfoliatum*). Mineral element content, particularly K (from about 1.6 g/100 g to about 1.8 g/100 g DM), Ca (from about 1.0 g/100 g to about 2.1 g/100 g DM) or Mg (from 651 mg/100 g to 672 mg/100 g DM) as well as that of trace elements, *e.g.* Fe (from about 28 mg/100 g to about 38 mg/100 g DM) in *Silphium* seeds is one of the most important traits affecting their nutritional value. Moreover, the experiment revealed that *S. perfoliatum* was the most valuable plant referring to seed yielding (about 185 g seeds from a single plant, on average).

Glutamic acid (up to 23%) and leucine (7.76%) were found dominating among all amino acids in protein of *Silphium* seeds. An analysis of lipid fraction showed that linoleic (44.4%) and oleic (13.2%) acids were the main fatty acids contained in the oil made from seeds.

The achieved data point out that the analysed raw materials were characterized with a valuable chemical composition and may be used in the food industry.

INTRODUCTION

For thousands of years man has been looking for a meal in the nature, especially in rich plant's world. Those searches are particularly important today when analytical techniques are being developed allowing a detailed determination of the chemical composition of surrounding flora. At present, of about 400 thousand species of plants all over the world, only about 20 thousand are utilised by a man, and about 500 are cultivated. Civilized and industrialized world creates newer threats to people and consequently new diseases. No wonder that man turns back to the nature. Therefore, this century is going to be characterised with a wider search for new plants that could supply foodstuff, raw materials for pharmaceutical industry, and renewable energy sources. Furthermore, investigations upon new cultivated plants are going to be closer associated with natural environment protection, *i.e.* ecological agriculture, biological soil reclamation, and counteracting the greenhouse effect. The above trends affect an increase in farmer's and consumer's interests. Species of *Silphium* L. genus (*Asteraceae*) are one of more interesting plants referring to the problems mentioned. So far only *Silphium perfoliatum* L. has been studied as fodder, medical, melliferous, and recultivation plant. Literature data indicate the presence of: mineral constituents, carbohydrates, proteins and L-ascorbic acid [Duranti *et al.*, 1988; Davidjanc & Abubakirov, 1992; Daniel & Rompf, 1994], terpenoids and essential oil [Bohlmann & Jakupovic, 1979, 1980; Wolski *et al.*, 2000; Kowalski *et al.*, in press], flavonoids [Davidjanc & Abubakirov 1992; El-Sayed *et al.*, 2002], phenolic acids [Kowalski & Wierciński, 2003; Kowalski & Wolski 2003a, 2003b] and oleanosides [Davidjanc & Abubakirov 1992, Kowalski 2002] in leaves, stalks, inflorescences and rhizomes of *Silphium* genus.

A lack of results upon potential usability of *Silphium* made the authors to undertake investigations into this matter. Therefore, the aim of the present study was to evaluate the chemical composition of three species of *Silphium* L. genus: *Silphium perfoliatum* L., *Silphium trifoliatum* L. and *Silphium integrifolium* Michx. referring to their potential utilization as alternative foodstuff raw materials.

MATERIALS AND METHODS

Seeds of three *Silphium* L. species (*S. perfoliatum*, *S. trifoliatum*, and *S. integrifolium*) harvested at wax maturity in September 2002 from the experimental cultivation of the Department of Instrumental Foodstuff Analysis in Kazimierzówka near Lublin, were the experimental material.

Preliminary characteristics of the studied material. Before chemical evaluation, measurements of seed production efficiency were performed for particular species under study: number and weight of seeds in a single infructescence (for 10 infructescences); seed weight from a single stem and whole plant (for 10 plants); and 1000-seed weight (for 10 samples).

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Evaluation of proximate chemical composition and energetic value. The seeds collected were determined for: dry matter content by drier means, ash after dry digestion at 550°C, cellulose by Kürchner and Hanak's method [Skulmowski, 1974], total protein by Kjeldahl's method according to AOAC-976.06 [2000], and crude fat by means of extraction-gravimetric Soxhlet's method [James, 1995]. Moreover, refraction coefficient and iodine value were estimated for extracted oil [James, 1995]. Seed energetic value was calculated applying mean recalculation coefficients for protein, fat and carbohydrates according to Kunachowicz *et al.* [1998]. These factors are as follows: 1 g of protein – 17 kJ, 1 g of fat – 37 kJ, and 1 g of carbohydrates – 17 kJ.

Evaluation of the contents of mineral components and trace elements. In order to determine the contents of mineral components and trace elements, the material was subjected to wet digestion in a mixture of HNO_3 and $HClO_4$ according to the AOAC 986.15 standard [2000]. The levels of Na, K, Ca, Mg, Zn, Fe, Mn, Cr, Cu were recorded using flame AAS technique applying a "Solaar 939" spectrometer (Unicam); Se content was determined by means of no-flame technique using a "SpectrAA 880Z" spectrometer (Varian).

Isolation and chromatographic analysis of water-soluble sugar fraction. One-gram samples of powdered seeds were treated with 20 mL of distilled water and heated at boiling water bath for 30 min to isolate water-soluble sugars. After filtration, the extract was deproteinized using 2 mL of 30% trichloroacetic acid and adjusted to 100 mL with water. So prepared solution was subjected to an HPLC analysis. Furthermore, to hydrolyze the complex sugars, the material was treated with 0.1 mol/L HCl and heated as above. The hydrolyzate, after filtration, was neutralized with 0.5 mol/L NaOH, deproteinized and adjusted with distilled water to 100 mL. De-proteinized solutions containing monosaccharides (free and products of complex sugar hydrolysis) were centrifuged; the supernatant was filtered through a micro-plate (0.45 μm) made of teflon, and then subjected to a chromatographic determination. Qualitative and quantitative sugar analysis was performed using an HPLC technique applying a "Kromasystem 2000" apparatus (Kontron Systems) with an RI 485 refractometer (Kontron), injection loop 20 μL, sample injector Rheodyne, and column APEX NH_2 RP 5 μ S/M 7073002 (Jones Chromatography) of 200 mm length. The analysis was carried out at ambient temperature. As solution consisting of acetonitrile:water (80:20, v/v) was the mobile phase. Flow rate was 1 mL/min. Sugar identification was made comparing their retention times with those for standards. The content of individual sugars was calculated on the basis of calibration curves prepared as a dependence of area under peaks for standards against their concentration range 1–5 mg/mL.

Isolation and chromatographic analysis of protein amino acids. In order to determine the amino acid composition, seed samples were hydrolyzed in 6 mol/L HCl [Davies & Thomas, 1973]. In addition, separate samples were subjected to oxidation hydrolysis to determine the sulfuric amino acids [Moore, 1963]. Amino acid composition in hydrolyzates (with ninhydrin post-column derivatization) was recorded using an automatic amino acid analyzer AAA T 339 N (Mikrotechna) with UV-Vis (λ = 570 nm) detector, column with strongly acidic cationite (sulfonic styrene--divinyl-benzene) Ostion LG ANB co-polymer (ProChem a Hunt. Vyr., Czech Rep.) of 35 cm length. Sodium citrate

FIGURE 1. *Silphium* seeds equipped in the alas able to fly (marked with arrows).

buffer (pH 2.95, 4.25 and 9.45) was the mobile phase; the flow rate was 0.4 mL/min. Calculations were based on earlier analyses of standard mixture and internal standard addition – norleucine.

Isolation and chromatographic analysis of fatty acids. The contents of fatty acids as methyl esters were determined using a GC technique, after preliminary fat saponification and acid esterification according to norms AOAC-969.33 and 963.22 [2000], applying an internal standard technique (heptadecanoic acid).

A GC 610 Series apparatus (Unicam) with 30 m × 0.32 mm, DB-23 (J&W, USA), film thickness 0.25 μm, carrier gas H_2 (2 mL/min), column temperature – 160°C, injector "split" – 250°C, FID – 250°C, were used in the examination. Calculations were based on earlier analyses of standard mixture and calculation of the individual correction coefficients.

All chemical analyses were made in three replications. The results achieved were analysed statistically using variance analysis. Confidence intervals were estimated using Tukey's test at a 5% significance level.

RESULTS AND DISSCUSION

Seeds of three studied species are achenes equipped in two alas able to fly (Figure 1). No morphological differences, besides seed size and different colour, were found among

TABLE 1. Seed characteristics and seed production of three *Silphium* species.

Species	Seed yield (g per shoot)	Seed yield (g per plant)	Weight of 1000 seeds (g)	Weight of seeds in infructescence (mg)	Number of seeds in infructescence
S. perfoliatum	19.02[c]	185.06[c]	21.45[c]	568[c]	31.0[b]
S. trifoliatum	15.54[b]	150.23[b]	16.63[b]	376[b]	28.8[b]
S. integrifolium	4.83[a]	94.02[a]	12.50[a]	272[a]	15.2[a]

a, b, c – values designated with the same letters within columns do not significantly differ at 5% error

TABLE 2. Proximate chemical composition and energetic value of three *Silphium* species seeds.

		Species		
		S. perfoliatum	*S. trifoliatum*	*S. integrifolium*
General chemical composition and energetic value				
Dry matter (% FM)		92.59[a]	92.43[a]	93.74[b]
Ash (% DM)		6.82[b]	6.28[a]	6.63[b]
Total protein (% DM)		29.18[a]	31.66[b]	33.53[c]
Crude fat (% DM)		24.16[c]	10.40[a]	22.05[b]
Total sugar* (% DM)		8.25[b]	9.58[b]	4.29[a]
Cellulose (% DM)		19.46[a]	25.38[c]	22.07[b]
Energetic value (kJ/100 g DM)		1530[c]	1076[a]	1450[b]
The content and composition of water-soluble sugar fraction				
Fructose (% DM)	free	0.20[a]	0.19[a]	1.01[b]
	after hydrolysis	3.84[a]	4.28[a]	3.44[a]
Glucose (% DM)	free	0.35[b]	0.28[b]	0.08[a]
	after hydrolysis	4.41[b]	5.30[b]	0.85[a]
Sucrose (% DM)	free	1.36[a]	1.75[a]	1.57[a]
	after hydrolysis	–	–	–
The contents of mineral and trace elements				
Na (mg/100 g DM)		1.55[a]	1.84[a]	3.37[b]
K (g/100 g DM)		1.80[a]	1.85[a]	1.61[a]
Ca (g/100 g DM)		0.99[a]	1.03[a]	2.08[b]
Mg (mg/100 g DM)		670[a]	651[a]	672[a]
Zn (mg/100 g DM)		5.83[a]	5.64[a]	8.07[b]
Fe (mg/100 g DM)		36.2[b]	38.1[b]	27.7[a]
Mn (mg/100 g DM)		3.51[b]	3.22[a]	4.32[c]
Se (μg/100 g DM)		16.9[a]	21.8[a]	27.2[a]
Cr (mg/100 g DM)		4.59[b]	4.75[b]	1.28[a]
Cu (mg/100 g DM)		2.44[c]	2.29[b]	1.53[a]

* sum of fructose and glucose contents after hydrolysis; a, b, c – values designated with the same letters within line do not significantly differ at 5% error

them. Moreover, seeds of the species studied have soft seed coat and taste similar to that of sunflower seeds.

Data referring to seed production for the species studied and seed characteristics are given in Table 1. *S. perfoliatum* was characterised with the highest seed yield; about 185 g of seeds were achieved, thus it was considered as the most efficient of the three studied species in such a context. Seed yields for other species (*S. trifoliatum* and *S. integrifolium*) accounted for 150 g and 94 g, respectively. The 1000-seed weight was the highest in *S. perfoliatum* and amounted to about 21.5 g; in the case of *S. trifoliatum* and *S. integrifolium* it was about 16.6 g and 12.5 g, respectively.

TABLE 3. Percentage of amino acids in total protein of three *Silphium* species seeds.

Amino acid	Species		
	S. perfoliatum	*S. trifoliatum*	*S. integrifolium*
Asp	8.48	8.95	7.93
Thr	3.31	2.81	3.09
Ser	3.27	3.43	3.32
Glu	21.48	21.36	22.98
Pro	2.53	2.44	2.14
Gly	3.65	4.24	3.50
Ala	3.11	3.46	3.00
Val	4.35	4.88	4.88
Ile	3.44	3.32	3.69
Leu	7.39	7.76	7.24
Tyr	2.85	2.98	3.33
Phe	3.80	3.66	4.09
Lys	2.42	3.01	2.46
His	1.70	2.20	2.25
Arg	6.15	7.20	7.27
Cys	2.21	2.53	2.72
Met	2.50	2.36	2.71
Σ	82.67	86.59	86.61

Table 2 presents results of the chemical composition and energetic evaluation of the seeds studied. The results obtained indicate that *S. perfoliatum* seeds were characterised by the highest contents of fat (about 24.2%) and ash (6.82%) among the species studied, and by the highest energetic value (1530 kJ) as well the seeds of *S. integrifolium* demonstrated the highest protein content (about 33.5%) and relatively high fat (about 22.0%), ash (about 6.6%) and cellulose (about 22.1%) levels. Comparing these data with the chemical composition of sunflower (*Helianthus annus* L.) containing about 13% of protein, about 24% of fat, about 2.1% of ash, and about 6% of crude fiber [Kunachowicz *et al.*, 1998], it could be concluded that *Silphium* seeds were not worse in terms of major nutrients than the edible parts of sunflower.

It should be emphasized that the seeds examined contained relatively high amounts of cellulose which is the main component of crude fiber – one of the most important foodstuff constituents when antitumour action is concerned. The presence of crude fiber in a diet stimulates the intestine peristaltic decreases the concentration of bile acids, as well as removes toxins and carcinogenic factors [Zatoński, 1998]. Acetic and propionic acids, as the products of decomposition of soluble crude fiber components, are inhibitors of an enzyme controlling the cholesterol synthesis, which can decrease its concentration in blood serum [Wolski & Dyduch, 2000]. Products containing crude fiber positively influence carbohydrate and lipid metabolism in an organism.

The presence of fructose, glucose and sucrose was confirmed in the tested seeds. The fact of a high increase in fructose and glucose contents after hydrolysis can prove the presence of storage carbohydrates, *i.e.* starch and inulin that is typical of *Asteraceae* family.

Table 2 compiles the contents of mineral and trace elements in the tested seeds. These data are quite differentiated, which is probably affected by the species-specific traits. In general, the content of mineral compounds in *Silphium* seeds is one of important traits influencing their dietetic value. These seeds introduced into a diet can be a supplementary source of mineral elements such as: K (from about

TABLE 4. Composition of fatty acids in the crude fat of three *Silphium* species seeds.

Fatty acid	Species					
	S. perfoliatum		*S. trifoliatum*		*S. integrifolium*	
	g/100 g CF	Percentage participation	g/100 g CF	Percentage participation	g/100 g CF	Percentage participation
14:0	2.68[a]	3.98	2.87[a]	4.42	3.29[b]	4.67
14:1	0.03[a]	0.05	0.03[a]	0.05	0.03[a]	0.04
16:0	4.91[a]	7.29	5.53[b]	8.51	6.33[c]	8.99
16:1	0.05[a]	0.07	0.08[b]	0.12	-	-
18:0	1.70[b]	2.52	1.30[a]	2.00	1.94[c]	2.75
18:1	12.90[b]	19.14	10.26[a]	15.79	13.19[b]	18.73
18:2	44.14[a]	65.49	43.94[a]	67.61	44.38[a]	63.00
18:3	0.07[a]	0.10	0.18[a]	0.28	0.32[b]	0.45
20:0	0.19[a]	0.28	0.20[a]	0.31	0.23[a]	0.33
20:1	0.73[a]	1.08	0.60[a]	0.91	0.73[a]	1.04
Σ	67.40	100.00	64.99	100.00	70.44	100.00

CF – crude fat; a, b, c – values designated with the same letters within line do not significantly differ at 5% error

1.6% to about 1.8%), Ca (from about 1.0% to about 2.1%) or Mg (from about 0.6% to about 0.7%) as well as trace elements, *e.g*. Fe (from about 28 mg to about 38 mg/100g). A comparison of the results achieved in this experiment with those reported for sunflower seeds, *i.e.*: K (about 0.4%), Ca (about 0.7 mg/100g), Mg (about 0.19 mg/100g), and Fe (about 2 mg/100g) [Kunachowicz *et al.*, 1998], proves the surplus of the *Silphium* ones.

Table 3 presents data referring to the amino acid composition of total protein. A high content of leucine reaching 7.24% and 7.76% in *S. intergrifolium* and *S. trifoliatum*, respectively, should be emphasised. Glutamic acid (21.36% – *S. trifoliatum*, 21.48% – *S. perfoliatum*, 22.98% – *S. integrifolium*) was the dominating amino acid of *Silphium* protein.

Oil extracted from *Silphium* seeds was characterized with yellow colour similar to that from sunflower. Refraction coefficient and iodine value were as follows: 1.476 and 153 – *S. perfoliatum*; 1.475 and 152 – *S. trifoliatum*; 1.475 and 148 – *S. integrifolium*, respectively. Table 4 lists results of fatty acid contents in lipid fraction extracted from seeds. Linoleic (*cis*, *cis*-9,12-octadecadienoic) and oleic (*cis*-9-octadecenoic) acids dominated in the studied oils similarly as in oil made from sunflower seeds [Kunachowicz *et al.*, 1998].

Summing up, it can be stated that the chemical composition of *Silphium* seeds was determined for the first time. Due to a high content of nutrients, the seeds of three *Silphium* species (*S. perfoliatum*, *S. trifoliatum* and *S. integrifolium*) demonstrate a high potential to be used in the food industry. The seeds tested can also be the source of oil and alternative addition to grainy bakery or various grain mixtures for breakfast.

CONCLUSIONS

1. The experiment revealed that *S. perfoliatum* was characterized with the highest efficiency referring to seed production yielding about 185 g from a single plant, on average (*S. trifoliatum* – 150 g, *S. integrifolium* – 94 g).

2. A high content of major components (mineral substances, protein, fat, cellulose and water-soluble sugars) in the tested seeds of *S. perfoliatum*, *S. trifoliatum* and *S. integrifolium* can be an important criterion determining a positive evaluation of these raw materials as alternative foodstuff sources.

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OCENA SKŁADU CHEMICZNEGO NASION NIEKTÓRYCH GATUNKÓW Z RODZAJU *SILPHIUM* L. JAKO ALTERNATYWNYCH SUROWCÓW SPOŻYWCZYCH

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Przebadane nasiona trzech gatunknw roślin z rodzaju *Silphium* L.: *Silphium perfoliatum* L., *Silphium trifoliatum* L. i *Silphium integrifolium* Michx. zawierały (w przeliczeniu na suchą masę próby): do 33,5% białka, do 24,1% tłuszczu, do 9,58% cukrów rozpuszczalnych w wodzie oraz do 25,4% celulozy (tab. 2). Natomiast oznaczony popiół stanowił do 6,8% suchej masy badanych nasion. Średnia wartość energetyczna analizowanych surowców kształtowała się w granicach: od 1076 kJ/100 g (*S. trifoliatum*) do 1530 kJ/100 g s.m. (*S. perfoliatum*). Zawartość związków mineralnych, a szczegńlnie poziom K (od ok. 1,6 g/100 g do ok. 1,8 g/100 g s.m.), Ca (od ok. 1,0 g/100 g do ok. 2,1 g/100 g s.m.) czy Mg (od 651 mg/100 g do 672 mg/100 g s.m.) oraz pierwiastków śladowych np. Fe (od ok. 28 mg/100 g do ok. 38 mg/100g s.m.) w nasionach *Silphium*, jest jedną z istotniejszych cech wpływających na ich potencjalną wartość odżywczą (tab. 2). Ponadto, doświadczenie wykazało, że *S. perfoliatum* były najbardziej wartościowymi roślinami pod względem plonowania nasion (średnio ok. 185 g nasion z rośliny) (tab. 1).

Stwierdzono, że dominującymi aminokwasami w białku nasion *Silphium* są: kwas glutaminowy (do ok. 23%) oraz leucyna (do 7,76%) (tab. 3). Natomiast analiza frakcji lipidowej wykazała, że kwas linolowy z zawartością (w tłuszczu) do 44,4% oraz kwas oleinowy z zawartością do 13,2% są pod względem ilościowym głównymi kwasami tłuszczowymi w oleju uzyskanym z nasion (tab. 4).

Uzyskane dane wskazują, że poddane analizie surowce charakteryzują się wartościowym składem chemicznym i mogą być wykorzystane przez przemysł spożywczy.