Physical properties and processing of Silphium integrifolium seeds to obtain oil and enriched protein meal

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Abstract

Silphium integrifolium Michx. (Silflower) has been a promising subject for domestication as a perennial oilseed crop. This work was carried out to investigate the seed processing aspect of this effort. Selected physical properties of the seed were evaluated, seed milling to obtain enriched kernel fraction was conducted, and initial characterization of the seed protein was performed. There was wide variation in flat seed length (11.54 to 20.75 mm), width (4.61 to 11.76 mm), and thickness (0.92 to 1.63 mm). The thousand seed weight was 23.8 g but the tapped bulk density was only 189.58 g/L due to the presence of wing around the seed's periphery. The kernel accounted for 56.14% of the seed weight and contained 31.00% oil. An enriched kernel fraction with 79.6% purity was obtained by roller-milling, sifting, and air classification. Linoleic (62.3%) and oleic (19.62%) acids were the major fatty acids in the oil. The defatted enriched kernel fraction contained 63.41% crude protein. Globulin, glutelin, albumin, and prolamin accounted for 55.63%, 19.28%, 16.38%, and 8.71% of the soluble proteins, respectively. At an extraction pH of 9, protein solubility was 62%. Maximum solubility (70%) was obtained at pH 10 while minimum solubility of 9% occurred between pH 4 and 5.5. Aside from the oil, the dehulling of silflower seeds also produced a high-protein defatted meal, which may be used as is or as a starting material for enriching the protein further into a protein isolate.

Physical properties and processing of *Silphium integrifolium* seeds to obtain oil and enriched protein meal*

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Abstract

Silphium integrifolium Michx. (Silflower) has been a promising subject for domestication as a perennial oilseed crop. This work was carried out to investigate the seed processing aspect of this effort. Selected physical properties of the seed were evaluated, seed milling to obtain enriched kernel fraction was conducted, and initial characterization of the seed protein was performed. There was wide variation in flat seed length (11.54 to 20.75 mm), width (4.61 to 11.76 mm), and thickness (0.92 to 1.63 mm). The thousand seed weight was 23.8 g but the tapped bulk density was only 189.58 g/L due to the presence of wing around the seed's periphery. The kernel accounted for 56.14% of the seed weight and contained 31.00% oil. An enriched kernel fraction with 79.6% purity was obtained by roller-milling, sifting, and air classification. Linoleic (62.3%) and oleic (19.62%) acids were the major fatty acids in the oil. The defatted enriched kernel fraction contained 63.41% crude protein. Globulin, glutelin, albumin, and prolamin accounted for 55.63%, 19.28%, 16.38%, and 8.71% of the soluble proteins, respectively. At an extraction pH of 9, protein solubility was 62%. Maximum solubility (70%) was obtained at pH 10 while minimum solubility of 9% occurred between pH 4 and 5.5. Aside from the oil, the dehulling of silflower seeds also produced a high-protein defatted meal, which may be used as is or as a starting material for enriching the protein further into a protein isolate.

KEYWORDS

silphium seeds, silflower seeds, physical properties, oil extraction, seed protein

INTRODUCTION

Silphium integrifolium Michx. (rosinweed, silflower), a native to the central United States, has been the subject of domestication efforts since the early 2000s. The perennial silflower has long taproot that can penetrate deeper into the soil to access water and nutrients below the root zones of most annual crops (Van Tassel et al., 2017; Vilela et al., 2018). Other desirable agronomic traits of silflower include drought tolerance, erect stems, relatively large seeds, and high competitiveness with annual weeds (Van Tassel and DeHaan, 2013). As a close relative of sunflower, silflower seed has potential uses in the food industry both as a grain or for its oil (Kowalski and Wiercinski, 2004; Van Tassel et al., 2014). The seed contains 20.0% crude fat, 33.5% crude protein, and 22.1% cellulose (Kowalski and Wiercinski, 2004). Seed yields ranging from 312 to 1,319 kg/ha have been reported (Van Tassel and DeHaan, 2013; Schiffner et al., 2020).

Like other new crops under development, in addition to seed production and post-harvest handling, processing of silflower seeds need to be investigated. No study has been conducted on the processing of silflower seeds. Information on the physical properties of the seeds is necessary for selecting and designing process equipment needed for cleaning, drying, storage, milling, and oil extraction. The seed's chemical composition will also point to potential products that can be obtained from the seed and its oil. The objectives of this work were to evaluate selected physical properties of silflower seeds, conduct seed processing for oil extraction and production of defatted meal, and perform preliminary characterization of the proteins.

METHODOLOGY

Materials

The silflower seeds (2.37 kg) provided by The Land Institute, Salina, KS were harvested from their research plots just south of the city limits of Salina in 2018 and 2019. The seeds were part of the bulk that included improved plots and new, wild introductions, and intermediate improved x wild populations. The crude harvest from the combine was cleaned using Clipper cleaners of various models (A.T. Ferrell Co. Inc., Bluffton, IN) and various screens to separate seed from chaff and then through an indent cylinder with 16 mm indents (Seed Processing Holland, Enkhuizen, The Netherlands) to remove longer stems and sticks. The seeds were stored in resealable polyethylene bags and stored under ambient conditions until processed.

Measurements of selected physical properties

The bulk seed density was determined by weighing the seeds contained in a 1-liter cup (203 Liter Cup, Seedburo Equipment Co., Chicago, IL). For loose bulk density, the cup was filled with seeds flowing freely from a hopper and then leveled using a straight-edged spatula. The tapped bulk density was measured by

shaking and tapping the cup while being filled with seeds and then leveling as described. Seed weight was obtained by weighing 1,000 seeds randomly picked from the bulk sample. The hull and kernel were manually separated from 1,000 seeds. The weights of hulls and kernels were then obtained, and their percentages to the seed weight were calculated. The length, width, and thickness of 20 seeds and kernels were measured using a digital caliper. The true densities of seed, kernel, and hull were obtained by weighing the samples in a 10 cm^3 cup, and the true volumes were determined using an AccuPyc II 1340 gas pycnometer, Micromeritics, Norcross, GA. All determinations were performed in triplicate.

Enriching kernel fraction by milling

The seeds (300 g/ batch) were milled using a 6-inch wide x 6-inch diameter (15.24 cm x 15.24 cm) roller mill with 24 corrugations per inch (9.4 corrugations/cm). The slow and the fast rollers have fixed speeds of 358 and 804 rpm, respectively. Initially, the gap of the rollers was set at 0.02 inch (0.51 mm). The milled seeds were then sifted through a stack of 5-, 12-, 18-, and 25-mesh (4.0, 1.7, 1.0, and 0.7 mm) screens. The fractions on 5- and 12-mesh screens (+5M and +12M) were roller-milled again (with the roller gap reduced after each pass) to recover more kernels still attached to the hulls and screened again. The fractions retained on 18- and 25-mesh screen (+18M and +25M) were aspirated to remove the light material, while the heavier fractions were milled again to pass through the 25-mesh screen. The -25M fractions were combined and defatted with hexane using a Soxhlet extractor. The defatted enriched kernel meal was air-dried in the hood overnight and stored in a capped glass jar under ambient conditions until used.

Proximate composition analyses

Moisture contents were determined gravimetrically according to AOCS Ba 2-38 (AOCS, 2017). Oil contents were determined using a pulsed nuclear magnetic resonance (pNMR) spectrometer (Bruker minispec, Bruker Corporation, Billerica, MA). The spectrometer was calibrated using aliquots of oil extracted from silflower seeds. Crude protein content (Dumas % N x 6.25) of solid samples was determined using LECO 2600 (LECO Corporation, St. Joseph, MI following AOCS Ba 4e-93 (AOCS, 2017). Ash contents were analyzed according to AOAC method 942.05 (AOAC, 2003). Oil, crude protein and ash contents were reported as percent of dry matter. Determinations were done in triplicate for each component. Total carbohydrate content was calculated by the difference (100 – sum of oil, crude protein and ash content).

Fatty acid composition and iodine value

Fatty acid methyl esters (FAME) synthesized from silflower oil were separated using an Agilent Technologies (Palo Alto, CA) 6890N GC fitted with a flame ionization detector and a Supelco (Belefonte, PA) SP2380 30 m × 0.25 mm column. The peaks were identified using a standard mix of saturated FAME (C8 to C30) and mono- and poly-unsaturated C14, C16, C18, C20, C22 and C24 (Nu-Check Prep, Elysian, MN). The details of the FAME preparation and GC parameters are available elsewhere (Chakraborty et al., 2018). The iodine value (IV) of the oil was calculated from the fatty acid composition according to AOCS Cd1-85 (AOCS, 2017).

Soluble protein classes

The classic Osborne soluble protein fractions (albumin, globulin, prolamin, glutelin) were extracted sequentially from 10 g of ground, defatted silflower seed by using water, dilute saline, ethanol, and dilute alkali, and adapting the method used previously for milkweed seed (Hojilla-Evangelista, Evangelista and Wu, 2009). The modified method used higher solvent:solid ratios, longer extraction times, and only a single extraction stage per solvent, which led to faster completion of the extraction series. The changed ratio and shaking time for each solvent are as follows: water - 40 mL/g, 30 min; 0.5 M NaCl - 40 mL/g, 30 min; 70% aqueous ethanol - 30 mL/g, 25 min; and 0.1 M NaOH - 30 mL/g, 25 min. Each extract was collected, centrifuged and analyzed for soluble protein content by using the colorimetric Biuret method with bovine serum albumin (BSA) as standard. Protein content of the solid residue obtained after the extraction series was determined as described for proximate composition. Water-extract was freeze-dried, while the other extracts were first dialyzed (Spectra/Por membrane tubing, 34 mm dia., 3.5 kDa molecular weight cut-off (MWCO), Spectrum

Labs, Rancho Dominguez, CA, against deionized water for three days and then freeze-dried. Protein yield (%) was calculated as [(mass extracted × % protein in sample) / (starting mass × % protein in starting sample)] ×100. The determination of soluble protein classes was done in duplicate.

Protein solubility

The protein solubility versus pH profile was generated by following the method of Hojilla-Evangelista, Evangelista, and Wu (2009), which used sample dispersions having 10 mg protein/mL with their pH adjusted to 2.0, 4.0, 5.5, 7.0, 8.5, and 10.0. Soluble protein in the supernatant was determined by the Biuret method using BSA for the standard curve.

Polyacrylamide gel electrophoresis (PAGE)

Molecular weights of polypeptides in defatted silflower, freeze-dried soluble protein fractions, and spent material after sequential extraction of soluble proteins were estimated by gel electrophoresis. Native gel electrophoresis was done according to the method reported previously by Hojilla-Evangelista and Evangelista (2006), with these modifications: 1) sample concentrations were 10 mg/mL (quantity basis), 2) samples dissipated undisturbed in the native sample buffer for 1 h at room temperature before being centrifuged at 11,000 rpm for 3 min, and 3) loading volume was 15 μL.

SDS-PAGE was done on the reduced proteins from the same samples used for native gel electrophoresis following the method of Hojilla-Evangelista et al. (2013), with slight modifications: sample concentrations were 5 mg/mL, protein standard was BioRad Precision Plus Dual Xtra (molecular weight range 2 to 250 kDa) and load volume for samples was 15 μL.

Statistical analyses

Statistical analyses were performed using SAS for Windows v 9.4 (SAS Institute Inc., Cary, NC. Significant differences among the treatments ($p<0.05$) were determined by using analysis of variance (ANOVA) and Duncan's Multiple Range tests on duplicate or triplicate replications.

RESULTS AND DISCUSSION

Physical properties

Silflower seeds are flat, mostly ovate or cordate in shape, with wings on the periphery (Fig. 1a). The seed size varied considerably. The average seed length was 15.55 mm and ranged from 11.54 to 20.75 mm (Table 1). The average seed width was 9.42 mm and varied from 4.61 to 11.76 mm. The wing accounted for about 55% of the seed's length and width. The seed averaged 1.34 mm in thickness and ranged from 0.92 to 1.63 mm. The hull covering the kernel is 0.16 mm thick or 12% of the seed's thickness. Given the wide variation in seed size, seed cleaning and grading can be performed by using a combination of screen sizes and configurations. Cleaning and grading remove inferior seeds and other dockage that will reduce the quality of the final products.

Seed weight is an indicator of seed quality. This is determined from the weight of 100 or 1,000 seeds depending on the size of the seeds. Major crops like corn, soybean, wheat, and others have established standard test weights (bulk density) specified in pounds/bushel (USDA, Agricultural Marketing Service). For silflower, the seed weights and densities may be utilized as indicators of the progress of the crop development. A thousand silflower seeds weighed 23.84 g (Table 1). This is within the range of 100 seed weight of 2.2 to 7.9 g, but well below the 4.4 g and 5.2 g average obtained from two sunflower germplasm studies (Mogali and Virupakshappa, 1994; Sudrik et al., 2014). Seed bulk density varied from 145.27 g/L (loose) to 189.58 g/L (tapped). Tapping reduced the volume by 23.3%. This considerable shrinkage in volume is important in sizing bin capacity for storage or transport. The true density of the seeds, however, is 7.2 and 9.5 times higher than the tapped and loose bulk densities, respectively. With low bulk density and high true density values, the porosity of the bulk seeds ranged from 86.2 to 89.4%. The presence of seed wings is largely responsible for the significant interparticle void volume. From previous work on the likewise perennial milkweed seed, which have the same shape as silflower seeds, dewinging reduced the seed volume by 46% and increased the

bulk density by 63% (Evangelista, 2007). Porosities of flat seeds with no wings have been reported: pumpkin - 65.73%, luffa - 58.23%, and watermelon - 39.14 to 51.68% (Joshni et al., 1993; Aliyu et al., 2017; Araki et al., 2018).

Manual dehulling of the seed also removed the seed coat (Fig. 1b). Like the seeds, the teardrop-shaped kernel also varied widely in size. The kernel length ranged from 5.44 to 8.49 mm, width from 2.81 to 5.63 mm, and thickness from 0.72 to 1.28 mm (Table 1). The kernel accounted for 56.14% of the seed weight. The true density of the kernel (1,198.8 g/L) is lower than that of the hull (1,581.5 g/L). This observation is also true for pumpkin seed kernel (Joshi et al., 1993), and watermelon seed kernel (Teotia and Ramakrisna, 1989). However, because of its fibrous nature, loose silflower hulls are light and can be compressed to reduce their volume.

Proximate composition

Total carbohydrates (43.59%) are the major component of silflower seeds (Table 2) and most of it is contained in the hulls. Crude protein (32.21%) is the second major component of the seed and found mainly in the kernels. This value is comparable to what has been reported (33.53%) previously (Kowalski and Wiercinski, 2004). The seed's oil content (17.69%) is lower than what was reported by Kowalski and Wiercinski (2004), but close to that of soybeans (17.9 to 23.1% at 10% moisture) grown in different geographic locations (Breene et al., 1988). Linoleic acid (62.32%) is the dominant fatty acid in silflower oil and accounted for 99% of the total polyunsaturated fatty acid (PUFA) (Table 3). The second major fatty acid is oleic acid (19.62%) and accounted for 95.3% of the total monounsaturated fatty acid (MUFA). Silflower oil also has considerable amount of saturated fatty acids (SAFA) dominated by palmitic acid (8.57%), with some myristic acid (3.79%) and stearic acid (2.44%) also present. Overall, fatty acid composition is similar to what was reported by Kowalski and Wiercinski (2004) and also within the range of that of sunflower oil.

Another indicator of the degree of unsaturation or the number of double bonds in the oil is given by its iodine value (IV). Highly saturated oils, like coconut oil, have low IV (6 to 11) while highly unsaturated drying oil like linseed oil have high IV (170 to 204) (Gunstone and Harwood, 2007). Silflower oil's IV (132) is the same level as sunflower and soybean oils.

Milling

The silflower seed was milled to remove a fraction of the hulls to obtain an enriched kernel fraction with higher oil content. The initial attempt to remove the seed's wing using a food processor with a blunt blade, a method that worked well in dewinging milkweed seeds (Evangelista, 2007), was not successful for silflower seeds. This could be due to the fibrous nature of the wing. However, a corrugated roller mill with differential speed was able to strip the hull from the kernel. By decreasing the gap of the rollers after each pass of the +5M and +12M fractions, the kernel particle size was reduced while most of the hulls remained intact and stayed on top of the 5- and 12-mesh screens. An enriched kernel fraction with oil content higher than 20%, accounting for 66% of all fractions, was obtained from +18M and finer fractions (Table 4). More hulls were removed from +18M and +25M fractions by air classification and the heavier fractions were milled again to pass through the 25-mesh screen. The combined -25M fractions had an oil content of 24.85% (Table 2). Based on the oil contents of the hulls (1.04%) and the kernel (31.00%), the full-fat kernel-rich fraction has 20.4% hulls, a significant reduction from 43.75% hull in whole seeds (Table 1). After extracting the oil from the kernel-rich fraction, the crude protein content of defatted meal increased to 63.41% (Table 2), just two percentage points short of classifying as protein concentrate (crude protein content from 65 to 89%).

Soluble protein classes

Globulin, the saline-soluble protein, was the major fraction in silflower seed protein and accounted for more than half of the total protein (Table 5). Globulin amount is about 3-fold greater than either water-soluble albumin or alkali-soluble glutelin. The ethanol-soluble fraction (prolamin) was the least at 8.71%. Silflower globulin content compares well with that of sunflower meal protein (40-90%), while its albumin content is also within the range reported for sunflower protein (10-30%) (Gonzalez-Perez and Vereijken, 2007). Glutelin in

sunflower is only a minor fraction (Gonzalez-Perez and Vereijken, 2007) but such is not the case for silflower glutelin, which, at 19%, was the second highest content after the globulins. Sunflower globulin and albumin are reported to have glutamic acid-glutamine, glycine, aspartic acid-asparagine, and arginine as the most abundant amino acids (Baudet and Mosse, 1977), but the albumin is also rich in lysine and sulfur-containing amino acids (cysteine + methionine) (Kortt and Caldwell, 1990; Egorov et al., 1996), which globulin lacks. The prolamin fraction generally has glutamine and proline in the greatest amounts, but is deficient in lysine and tryptophan (Shewry, Napier, and Tatham, 1995). Glutelin peptide sequences were reported to be predominantly glutamine, proline and glycine (high molecular weight) or serine, glutamine, proline, and phenylalanine (low molecular weight) (Wieser, Seilmeier, and Belitz, 1988). The amounts of soluble protein classes in silflower seed do not conform to those found in cereals (28% albumins + globulins, 40% glutelins, 33% prolamins) or high-protein seeds (92% albumins + globulins, 7% glutelins, <1% prolamins) (Nikokyris and Kandylis, 1997).

Protein solubility at different pHs

The plot of protein solubility at different pH extractions (Fig. 2) shows a typical bowl-shaped curve. Protein solubility was highest (70%) at pH 10. The minimum protein solubility ranged from pH 4 to 5.5 where 9-10% of the protein remained in solution. Alkali protein extraction is usually carried out at pH 9 to minimize the production of lysinoalanine, which occurs at high pH values and temperatures (Lusas and Rhee, 1995). Lysinoalanine causes reduced protein nutritional quality and digestibility in rodents and primates (Friedman, 1999). For silflower, the protein solubility at pH 9 is about 62%, which is 11.4% lower than at pH 10.

Gel electrophoresis

The native protein in ground defatted silflower seed showed a thin, defined band that resolved at just above 242 kDa, a dark colored area between 300 and 720 kDa, and a faint band at around 800 kDa (Fig. 3, lane 2). The high molecular weight of the dominant polypeptide (300-720 kDa) may indicate a cross-linked structure. The albumin fraction (lane 3) showed only one band near the 146 kDa marker. The globulin fraction's (lane 4) two obvious major bands resolved at 66 and 146 kDa, but there was one faint band near 50 kDa and four faint bands between 246 and 720 kDa. Silflower albumin has higher molecular weight than sunflower albumin (10-18 kDa), but the globulin is smaller than sunflower globulin (helianthinin, 300-350 kDa) (Gonzalez-Perez and Vereijken, 2007). There was no defined band detected for the prolamin fraction, although a "shadow" is noticeable near the 720 kDa marker (lane 5). The glutelin fraction (lane 6) polypeptide had the lowest molecular weight, as indicated by the dark area that resolved between 10 and 66 kDa. For purification of silflower protein extracts, membrane with MWCO <50 kDA is appropriate for saline-extracted proteins (albumins and globulins) and MWCO <10 kDA for alkali-extracted proteins.

In reducing gel electrophoresis, there were 13 polypeptide bands detected in defatted silflower meal (Fig. 4, lane 2). Their molecular weight range was 13-140 kDa, with the six darkest and/or widest bands resolving around 15-20, 37, and 50-60 kDa. The water-soluble fraction showed one dark band near 15 kDa and eight faint and narrow bands that resolved between 18 and 50 kDa. The NaCl-soluble group (lane 4) showed eight polypeptide bands from 5-75 kDa, with the five most dense bands appearing at the lower molecular weight markers (5, 10, 20, 30, and 40 kDa). The ethanol-soluble (lane 5) and NaOH-soluble (lane 6) fractions showed the least number of polypeptide bands with three each and both sets resolving between 15-30 kDa. In the spent solids (lane 7), only two faint bands near the 20 kDa mark were observed, indicating that sequential solvent extraction removed much of the protein in the defatted silflower meal.

CONCLUSION

The high amount of hulls in silflower seeds makes for a good case for dehulling before oil extraction. Dehulling reduces the volume of material, thus increasing the throughput of the oil extractor. Stripping the hulls from the kernel by using a corrugated roller-mill is a promising method and needs to be optimized further. Grading the seeds by thickness will facilitate the milling process by reducing the number of passes through the rollers with the seeds of similar thickness. By optimizing the dehulling process, it is possible to produce defatted meal well within the range of a protein concentrate. This high protein meal is also a good starting material

for enriching the protein further into a protein isolate.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Roque L. Evangelista: organization, conceptualization, methodology, writing original draft and editing **Milagros P. Hojilla-Evangelista:** conceptualization, methodology, writing original draft and editing. **Steven C. Cermak:** methodology and writing and editing. **David L. VanTassel:** proposed the study, prepared the seed samples, and writing and editing.

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LIST OF FIGURES:

Properties	Properties	Values
Moisture content, %	Moisture content, %	5.59 ± 0.27
1,000 Seed weight, g	1,000 Seed weight, g	23.84 ± 0.40
Bulk density, g/L	Bulk density, g/L	
Loose	Loose	145.27 ± 2.26
Tapped	Tapped	189.58 ± 2.05
True density, g/L	True density, g/L	1,371.4 ± 36.2
Seed dimensions, mm	Length	15.55 ± 1.65
	Width	9.42 ± 1.18
	Thickness	1.34 ± 0.16
Kernel size, mm	Length	6.74 ± 0.67
	Width	4.26 ± 0.65
	Thickness	1.02 ± 0.13
% (weight) kernel	% (weight) kernel	56.14 ± 0.23
% (weight) hull	% (weight) hull	43.75 ± 0.51
True density of kernel, g/L	True density of kernel, g/L	1,198.8 ± 1.8
True density of hull, g/L	True density of hull, g/L	1,581.5 ± 1.3

TABLE 2 Proximate composition of silflower seeds, hull, kernel, and kernel-rich seed meal fractions.

Components (%, dry basis)	Seed	Manually Separated	Manually Separated	Kernel-Rich Seed Meal	Kernel-Rich Seed Meal
		Hull	Kernel	Full fat	Defatted
Oil	17.69 ± 0.13	1.04 ± 0.08	31.00 ± 0.06	24.85 ± 0.10	0.33 ± 0.00
Crude protein	32.21 ± 3.87	5.46 ± 1.67	53.55 ± 1.27	46.38 ± 0.24	63.41 ± 0.21
Ash	5.01 ± 0.24	6.49 ± 0.56	4.30 ± 0.03	5.71 ± 0.07	8.00 ± 0.02
Total carbohydrates	43.59 ± 3.83	87.02 ± 1.15	11.15 ± 1.31	23.06 ± 0.14	28.27 ± 0.19

TABLE 3 Fatty acid composition of oil extracted from silflower seeds.

Fatty acids	Fatty acids	This work	Kowalski and Wiercinski, 2004	Sunflower Oil (O'Brien, 2004)
		%	%	%
Myristic	C14:0	3.79	4.67	<0.2
Myristoleic	C14:1	0	0.04	-
Palmitic	C16:0	8.57	8.99	5.6 -7 .6
Palmitoleic	C16:1	-	-	<0.3
Stearic	C18:0	2.44	2.75	2.7 - 6.5
Oleic	C18:1	19.62	18.73	14.0 - 39.4
Linoleic	C18:2	62.32	63.00	48.3 - 74.0
Linolenic	C18:3	0.35	0.45	<0.2
Arachidic	C20:0	0.28	0.33	0.2 - 0.4
Gondoic	C20:1	0.86	1.04	0 - 0.2
Behenic	C22:0	0.12	-	0.5 - 1.3
Erucic	C22:1	-	-	0 - 0.2
Docosadienoic	C22:2	0.23	-	-

Fatty acids	Fatty acids	This work	Kowalski and Wiercinski, 2004	Sunflower Oil (O'Brien, 2004)
Lignoceric	C24:0	-	-	0.2 - 0.3
Nervonate	C24:1	0.11	-	-
Σ Saturated FA	Σ Saturated FA	15.20	16.74	9.4 - 16.1
Σ Monounsaturated FA	Σ Monounsaturated FA	20.59	19.81	14.3 - 39.8
Σ Polyunsaturated FA	Σ Polyunsaturated FA	62.90	63.45	48.5 - 74.0
Iodine Value	Iodine Value	132*	133*	133

*Calculated from fatty acid composition.

TABLE 4 Milling of silflower seeds to obtain an enriched kernel fraction.

Fraction[a]	Yield, %[b]	Oil content, % as is
+5M	10.27 ± 1.70	5.24 ± 0.80
+12M	23.73 ± 2.83	8.12 ± 1.82
+18M	18.46 ± 2.23	22.27 ± 2.47
+25M	13.81 ± 1.66	25.21 ± 0.06
-25M	33.74 ± 0.56	21.40 ± 0.08

[a] Material was retained (+) on or passed through (-) the screen mesh size (M).

[b] Yield is based on the weight of all fractions.

TABLE 5 Soluble protein classes by sequential extraction.

Fraction	Yield, %	% of total protein extracted
Albumin (Cold water)	10.26 ± 1.76	16.38
Globulin (0.5M NaCl)	34.97 ± 1.00	55.63
Prolamin (70% ethanol)	5.48 ± 0.93	8.71
Glutelin (0.1M NaOH)	12.11 ± 2.98	19.28

Fig. 1. Photograph of (a) silflower seeds and (b) seed kernels (Grid1=1 mm).

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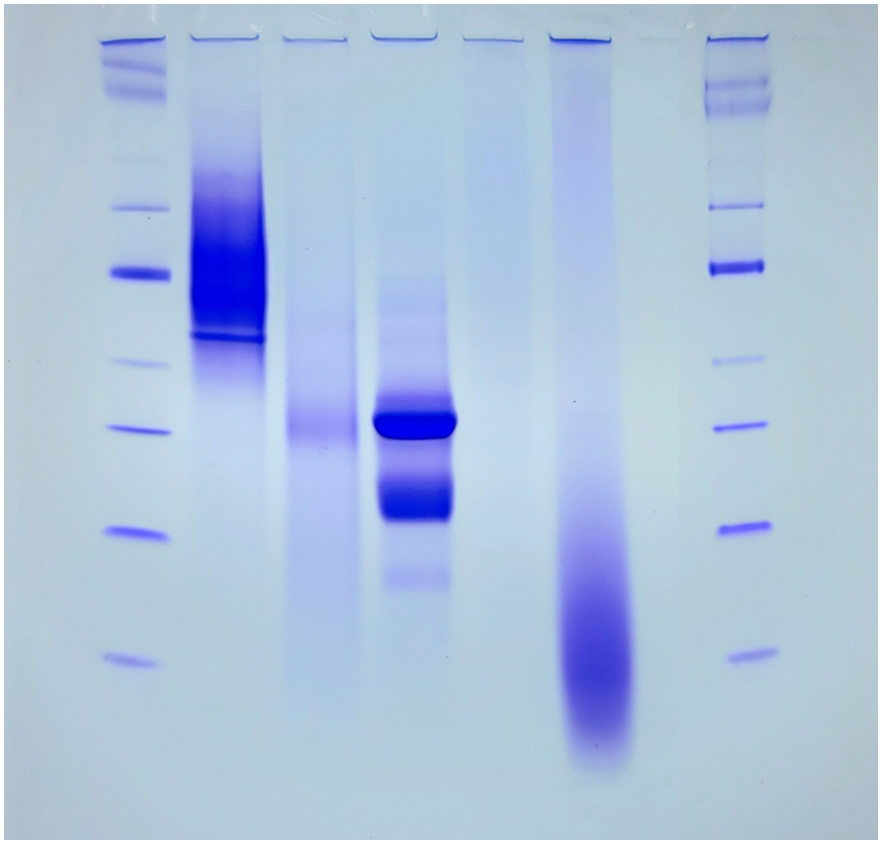
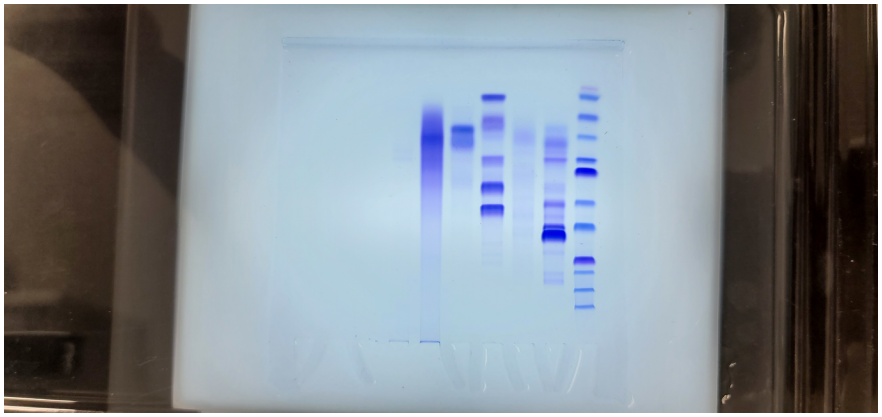
`image5.emf` available at https://authorea.com/users/441153/articles/541721-physical-properties-and-processing-of-silphium-integrifolium-seeds-to-obtain-oil-and-enriched-protein-meal

Fig. 2. Solubility of silflower seed proteins at different pH levels.



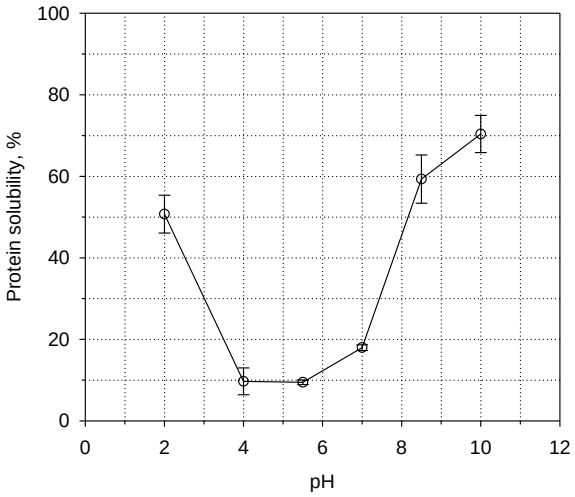
1 - MW standards	5 - Ethanol-soluble protein
2 - Defatted silflower meal	6 - NaOH-soluble protein
3 - Water-soluble protein	7 - Spent solids
4 - NaCl-soluble protein	

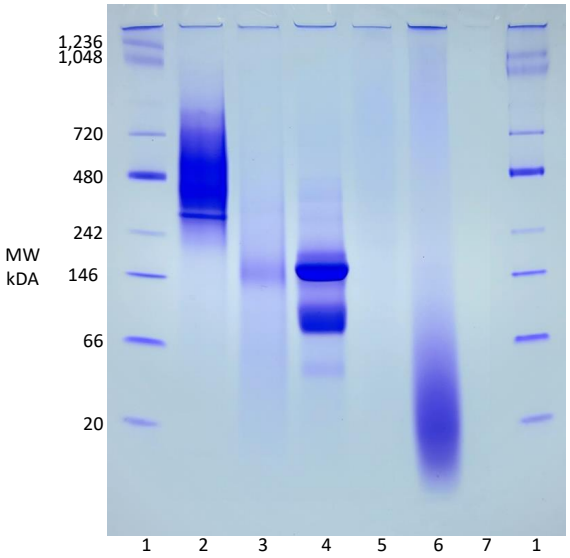
Fig. 3. Native gel electrophoresis band patterns of protein in defatted silflower meal, soluble protein classes and spent solids. Concentration=10 mg sample/mL;15 μL sample load volume.



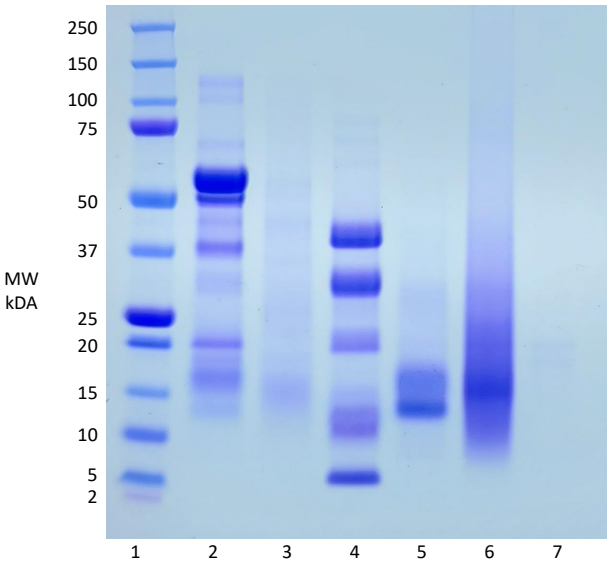
1 - MW standards	5 - Ethanol-soluble protein
2 - Defatted silflower meal	6 - NaOH-soluble protein
3 - Water-soluble protein	7 - Spent solids
4 - NaCl-soluble protein	

Fig. 4. SDS-PAGE band patterns of protein in defatted silflower meal, soluble protein classes and spent solids. Concentration=10 mg sample/mL;15 µL sample load volume.





1 – MW standards	5 – Ethanol-soluble protein
2 – Defatted silflower meal	6 – NaOH-soluble protein
3 – Water-soluble protein	7 – Spent solids
4- NaCl-soluble protein	



1 – MW standards	5 – Ethanol-soluble protein
2 – Defatted silflower meal	6 – NaOH-soluble protein
3 – Water-soluble protein	7 – Spent solids
4- NaCl-soluble protein	