

Article

Application of Optimized Dry Fractionation Process for Nutritional Enhancement of Different Sunflower Meals

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Abstract: Sunflower meal (SFM), a byproduct of sunflower oil extraction, is a relatively inexpensive protein source with high potential for feed formulations. Dry fractionation methodologies are emerging as ‘green’ and affordable technologies with the potential to additionally enhance the nutritional quality of plant-based raw materials for animal feed, including sunflower meal. Following the optimization of a dry fractionation process in a previous study of the authors, this research aims to validate the defined parameters through the processing of three sunflower meals (SFM1, SFM2, and SFM3) with different characteristics. The dry fractionation process includes two-stage grinding using hammer mill and roll mill and fractionation of sunflower meal by sieving. The process successfully increased the protein content of sunflower meals in the range of 17.5% to 31.2%, reaching levels high enough to be categorized as “high protein” sunflower meals of first quality (42% as is). Exceptionally high fraction yields (76.5–78.9%) were obtained for all three sunflower meals. The lowest energy consumption was recorded for SFM1 (8.44 Wh/kg), while slightly higher values were observed during the processing of SFM2 and SFM3 (9.30 and 9.93 Wh/kg, respectively). Relative amino acid enrichments ranging from 13.35% to 26.64% were achieved, with lysine enrichment ranging from 18.9% to 36% and methionine from 30.6% to 44.1%.

Keywords: sunflower meal; dry fractionation process; protein content; amino acid content



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1. Introduction

Meeting the growing demand for animal-derived proteins remains a critical challenge for the food industry as the global population continues to rise [1,2]. These requirements significantly impact all livestock sectors, which still represent key sources of animal proteins in human nutrition. A crucial role in the production chain is the animal feed industry [3]. The primary task of the animal feed industry is to formulate products that meet the specific nutritional needs of various animal species and categories. To achieve this goal, feed formulations incorporate a variety of plant- or animal-based feedstuffs, selected based on their nutritional profiles to develop optimal feed compositions [4].

Key criteria for selecting potential feed ingredients include protein content, energy value, digestibility, and the presence of anti-nutritional factors [5]. Since animal feed accounts for the largest share of production costs in the animal protein industry, the economic viability and availability of feedstuffs are critical factors in ingredient selection

for final feed formulations [6]. The most expensive and limiting components in animal feed formulations are proteins, which play a vital role in supporting animal growth and development [7,8]. The essentiality of proteins in animal nutrition derives from their amino acid composition since specific amino acids have crucial roles vital to animal health and productivity [9].

Sunflower meal, a by-product of sunflower oil production, is frequently utilized as a feed ingredient in animal feed formulations [10]. Sunflower meal is valued in the feed industry as a high-protein source with few anti-nutritional factors, making it suitable to be implemented in animal nutrition [11]. However, although widely available, protein-rich, and relatively inexpensive, its use in feed formulations for monogastric animals is limited. The primary limitation is its high crude fiber content, originating from the sunflower hull, which monogastric animals, due to the nature of their digestive systems, find difficult to digest [12,13].

To produce more suitable plant-based feed materials, various techniques can be applied, including wet and dry methods as well as enzyme treatments [14]. Dry fractionation methods are proposed as environmentally friendly and cost-effective alternatives, preserving the native functionality of proteins and other components due to their milder processing conditions [15]. Many authors applied dry fractionation processes in order to enhance the nutritional quality of sunflower meals [11,16–20]. Despite the notable increases in protein content achieved in the enhanced feed materials, the proposed processes had several limitations, including low yields of nutritionally enhanced fractions and the relatively high cost of equipment or production lines.

The latest work of Vidosavljević et al. [20] represents the process, including two-stage grinding using a hammer mill and a roll mill and fractionation of sunflower meal by sieving. The process was proven to enhance the protein content of the sunflower meal (the highest protein content was 48.06%, with relative protein enrichment of 33.3%), simultaneously obtaining a high yield of this protein-enriched fraction (fraction yield of 77.22%). Thus, the proposed process for sunflower meal improvement could be considered a simple and economical method, requiring minimal steps and no significant investments for industrial implementation. Given its notable potential, sunflower meals with different nutritional and physical properties were tested to evaluate their suitability for broader industrial applications.

Therefore, within the scope of this study, three different sunflower meals were enriched using the proposed process at pre-defined, optimal parameters, while responses were protein content, fraction yield, and grinding energy consumption of the process. Moreover, the amino-acid profile of protein enriched fraction was determined and compared with conventional protein sources such as soybean and rapeseed meal.

2. Materials and Methods

2.1. Raw Material

A total of 15 bags (30 kg each bag) of sunflower meal (SFM) were obtained for the experimental use, with 5 bags for each of the three types of meals with different characteristics. The first sunflower meal (labeled as SFM1) was delivered from the oil producer companies “Victoria Oil”, Šid, Serbia, while the second and third sunflower meal (labeled as SFM2 and SFM3) was procured from “Dijamant d.o.o.” Zrenjanin, Serbia and “BIMAL SUNCE d.o.o.”, Sombor, Serbia, respectively. All three sunflower meals were produced as a by-product of sunflower oil extraction from sunflower seeds, involving the mechanical pressing of the seeds followed by hexane extraction. Sunflower meals were delivered packed in plastic bags and labeled as sunflower meals of second quality (33% protein content).

It is worth mentioning that SFM1 was a new batch of sunflower meal previously used in the authors’ work [20], whereas SFM2 and SFM3 are materials procured specifically for

this experiment. Once the bags were opened prior to the start of the experiment, differences in color, granulation, and the presence of agglomerates were observed, with agglomerates being most prominent in SFM3 (Figure 1). Based on the visual appearance of the sunflower meal and the absence of large agglomerates, it is assumed that SFM2 was probably subjected to coarse grinding after production in order to break up the agglomerates present in the mass.



Figure 1. Visual comparison of all three sunflower meals (SFM1, SFM2, and SFM3).

Additionally, for the purpose of the experiment, one bag of soybean meal (SBM) and one bag of rapeseed meal (RSM) were purchased from a local agricultural supply store.

In order to homogenize SFM samples for further processing, three bags (30 kg each bag) were randomly chosen from each type of SFM and mixed in a twin-shaft paddle mixer (model SLHSJO.2A, Muyang, Yangzhou, China) for 2 min. The homogenized SFM samples were then placed in plastic storage boxes until the beginning of the experimental trials.

2.2. Processing and Dry Fractionation Process

The complete fractionation process of SFMs, including two-stage grinding followed by the sieving of ground SFM, was conducted at the pilot plant for animal feed production of the Institute of Food Technology in Novi Sad (University of Novi Sad, Serbia), and in the milling laboratory of Faculty of Technology (University of Novi Sad, Serbia).

To enhance the protein content of three different types of SFMs, an optimized dry fractionation process was used [20]. In order to disintegrate the agglomerates, which are commonly formed during sunflower oil extraction [17], the SFMs were initially pre-ground using a Hammer mill (ABC Engineering, Pancevo, Serbia), driven by a 2.2 kW electric motor with a rotational speed of 2880 rotation per minute and equipped with 16 hammers assembled in four rows. The rotor was surrounded by a sieve with an opening diameter (SOD) of 2 mm. The SFMs were introduced into the mill using a feeder (model FlexWall® Plus FW40-5, Brabender Technologie KG, Duisburg, Germany), temporarily positioned directly above the loading bin of the hammer mill. The feeding rate (F) was regulated by adjusting the speed of the feeder screw and was maintained at a constant value of 75 kg/h.

The second fine grinding step of pre-ground SFMs was conducted by use of a laboratory roll mill, Variostühl model CEx2 (Miag, Braunschweig, Germany), equipped with smooth rolls (100 mm length and 250 mm diameter). The operating parameters were set as follows: differential—2, roll gap—0.25 mm, feed rate—0.2 kg/cm min, and roll speed—400 rpm.

The fractionation of such grounded SFMs was accomplished using a Bühler laboratory sifter (Model MLU 300, Uzwil, Switzerland). A sieve with an aperture size of 650 µm was used, and the sieving time was set to 3 min. Cleaning the sieves and facilitating particle separation was aided by rubber balls freely moving on the surface of the sieves. Obtained

fractions (fractions with a particle size of less than 650 μm) were weighed, collected into labeled plastic bags, and stored until further analysis.

2.3. Laboratory Analysis

Physical characteristics of starting SFMs and ground SFMs, as well as chemical analysis of the starting SFMs and obtained SFMs fractions (<650 μm) were conducted at the Technological laboratory for feed of the Institute of Food Technology in Novi Sad (University of Novi Sad, Serbia), and in the accredited laboratory “FINSLab” of the Institute of Food Technology in Novi Sad, respectively.

2.3.1. Chemical Analysis

Moisture content, crude fat, and crude ash of the starting SFMs, SBM, and RSM were performed according to the standard method ISO [21–23]. The crude protein content of the starting SFMs obtained SFMs fractions (<650 μm), SBM, and RSM was conducted in accordance with the standard method ISO 5983-1 [24]. The crude fiber content in the starting SFMs, SBM, and RSM was determined according to the Ankom method-America Oil Chemist’s Society Ba 6a-05 [25], using the Ankom 2000 Fiber Analyzer (Ankom Technology, Fairport, NY, USA).

2.3.2. Amino Acid Analysis

Amino acid (AA) analysis was conducted on starting SFMs, obtained SFMs fractions (<650 μm), SBM, and RSM. For each sample, 50 mg was added to 6 M HCl and hydrolyzed for 24 h [26]. After hydrolysis, the samples were cooled to room temperature and dissolved in 10 mL of Loading Buffer (pH 2.2) (Biochrom, Cambridge, UK). The solutions were then filtered using 0.22 μm PTFE filters (Plano, TX, USA) and transferred to vials (Agilent Technologies, Santa Clara, CA, USA). The sampler temperature was maintained at 4 °C.

Amino acid measurements were performed using the Biochrom 30 Plus Amino Acid Analyzer System (Biochrom, Cambridge, UK) with EZChrom Elite software (Version 3.3.2 SP2). The separation of amino acids was achieved through ion-exchange chromatography using a column packed with cation-exchange resin. Buffers of varying pH and ionic strength were pumped through the column to achieve separation, with the column temperature precisely controlled and adjusted as needed to produce the required separation. Post-column derivatization was performed using ninhydrin, mixed with the column eluent and passed through a high-temperature reaction coil. In the reaction coil, ninhydrin reacted with the amino acids in the eluate to form colored compounds. The amount of colored compound produced was directly proportional to the quantity of the amino acid present, with detection performed photometrically.

Detection was conducted using a UV detector at 570 nm for all amino acids except proline, which was detected at 440 nm. Identification was achieved by comparing the retention times of amino acid standards with those of the samples, and quantification was based on the peak areas of the detected amino acids relative to calibration curves generated from the standard (Amino Acid Standard Solution, Sigma-Aldrich, St. Louis, MI, USA). The reported values for each analysis represent the average value of two repetitions.

2.3.3. Physical Analysis

The bulk density of the starting and ground SFMs was determined with a bulk density tester (Tonindustry, West and Goslar, Germany). The particle size distribution (PSD) of starting and ground SFMs was analyzed in accordance with the ISO standard [27]. This was achieved by sieving 100 g of material through a series of sieves with apertures of 3550, 2500, 2000, 1250, 1000, 800, 630, 250, 125, and 63 μm , using the Retsch AS 200 Control sieving device (Retsch GmbH, Haan, Germany). The geometric mean diameter (GMD)

and geometric standard deviation (GSD) were calculated to estimate the particle size distribution (PSD), conforming to the A.S.A.E. standard, using the equation provided in a study by Vukmirović et al. [28]. For the purpose of analyzing the flowability of the starting and ground SFMs, the angle of repose measurement method was used [29]. The angle of repose α° determined according to the following equation:

$$\alpha^\circ = \left(\frac{180}{\pi} \right) \times \arctan \frac{h}{D} \quad (1)$$

where α° stands for the angle of repose ($^\circ$), h (cm) is the height of the cone formed by material, while D (cm) is the diameter of the base of the cone (Figure 2).

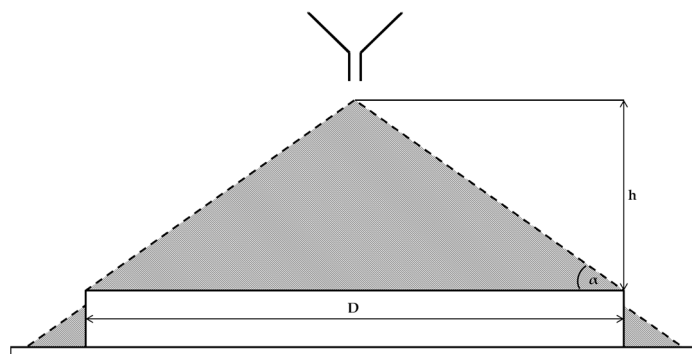


Figure 2. Angle of repose measurement parameters.

Based on the calculated values of the angle of repose, the samples were classified into several categories related to their flowability (Table 1). The reported values for each physical analysis represent the average value of two repetitions.

Table 1. Flowability rating according to the angle of repose [29].

Flowability Rating	Angle of Repose ($^\circ$)
Excellent	25–30 (or less)
Good	31–35
Fair	36–40
Passable	41–45
Poor	46–55
Very poor	56–65
Extremely poor	66–90

2.3.4. Statistical Analysis

One-way ANOVA and Tukey's Honestly Significant Difference test were used to analyze variations in the results. A confidence level of 95% ($p < 0.05$) was used to determine statistical significance. The analysis was performed using the software Statistica 14 [30].

2.4. Process Efficiency Indicators

The calculated parameters applied to evaluate the efficiency of the dry fractionation process were:

- Protein content and relative protein enrichment Δp_e (%);
- Fraction yield y_e (%);
- Total specific grinding energy consumption E_{te} (Wh/kg).

2.4.1. Relative Protein Enrichment

The relative protein enrichment Δp_e (%) of the enriched SFMs fractions is determined using the following formula:

$$\Delta p_e = \frac{p_e - p_s}{p_s} \times 100 \quad (2)$$

where p_s (%) stands for the protein content of starting SFMs, while p_e (%) represents the protein content of the enriched SFMs fractions.

2.4.2. Fraction Yield

The fraction yield y_e (%) of protein-enriched SFMs fractions is calculated using the following equation:

$$y_e = \frac{m_e}{m_s} \times 100 \quad (3)$$

where m_e (g) stands for the mass of the protein-enriched SFM fractions obtained after sieving (fines that passed through the sieve), while m_s (g) represents the mass of the sieved SFM samples.

2.4.3. Energy Consumption

The specific grinding energy consumption was firstly determined separately for the hammer mill and the roll mill, and their values were summed to determine the total specific energy consumption (Equation (6)). The power required by the hammer mill during grinding was measured using a Network Recorder MC750/UMC750 (Iskra MIS, Kranj, Slovenia). Power readings, P (W), were recorded every 5 s during the grinding process, excluding the first and last 30 s to ensure measurements were recorded only when the mill chamber was fully loaded with material. The average of the recorded power readings was used in subsequent calculations. The specific grinding energy consumption for the hammer mill, E_h (Wh/kg), was determined using the following equation:

$$E_h = \frac{P_h}{F} \quad (4)$$

where P_h (W) represents the average difference in energy consumption of the hammer mill when operating with and without material, while F (kg/h) denotes a feed rate.

A power measurement device, integrated into the laboratory roll mill, was utilized to measure the power consumption during milling operation with and without material. The specific grinding energy consumption of the roll mill, E_r (Wh/kg), was determined using the following equation:

$$E_r = \frac{P_r \times t}{m \times 3600} \quad (5)$$

where P_r (W) represents the average difference in energy consumption of the roll mill when operating with and without material, t (s) denotes the time of grinding recorded by the chronometer, while m (kg) refers to the weight of the ground material.

The total specific grinding energy consumption E_t (Wh/kg), was calculated as the sum of the specific grinding energy consumptions of the hammer E_h (Wh/kg) and roll mill E_r (Wh/kg):

$$E_t = E_h + E_r \quad (6)$$

To determine the total specific grinding energy consumption relative to the protein-enriched fraction obtained after the sieving E_{te} (Wh/kg) (in the following text of the current paper “Energy consumption”), the subsequent equation was used:

$$E_{te} = \frac{E_t \times 100}{y_e} \quad (7)$$

where E_t (Wh/kg) has the same connotation as in the previous Equation (6), while y_e (%) refers to the yield of the protein-enriched fraction obtained after the sieving.

3. Results and Discussion

3.1. Chemical Properties

The chemical composition of starting SFMs (SFM1, SFM2, and SFM3), RSM, and SBM are presented in Table 2. Since sunflower meal is typically traded based on its protein content [31], meeting the required standards for protein levels is crucial for market classification. In the context of this study, it is important to note that, according to Serbian Regulation on animal feed quality [32], the determined protein content of SFM1 and SFM3 corresponds to the level required to classify them as sunflower meal of second quality (33%_(as is)), whereas SFM2 does not meet this standard. Although the aforementioned regulation allows a permissible deviation of 2% in the determined results (Article no. 105, Table 57), which permits a sunflower meal containing 31%_(as is) protein to be classified as a sunflower meal of second quality, SFM2 (32.89%_(dm), equivalent to 30.46%_(as is)), despite being close to the allowable 2% deviation, still does not meet the required protein content and, therefore, cannot be classified as such.

Table 2. Chemical composition of SFM1, SFM2, SFM3, RSM, and SBM.

Chemical Composition	SFM1	SFM2	SFM3	RSM	SBM
Moisture content (%)	7.44	7.40	6.94	10.15	10.26
Crude protein (% _(dm))	35.64	32.89	35.82	35.51	48.04
Crude fiber (% _(dm))	21.70	23.53	22.44	7.51	7.25
Crude fat (% _(dm))	0.93	1.94	2.13	2.84	2.80
Crude ash (% _(dm))	7.44	7.05	6.01	6.51	6.52

SFM1—starting sunflower meal 1; SFM2—starting sunflower meal 2; SFM3—starting sunflower meal 3; RSM—rapeseed meal; SBM—soybean meal.

3.2. Physical Properties

The physical properties of feed play a crucial role in its handling, storage, and processing, as they significantly impact the efficiency of these operations and help minimize material loss [33]. Considering that grinding affects particle size distribution and consequently impacts the bulk density and angle of repose of the feed material, Table 3 summarizes these physical quality characteristics of the starting and ground SFMs. Based on the data, notable differences in GMD between starting sunflower meals SFM1, SFM2, and SFM3 can be indicated, with SFM1 standing out as the coarsest (GMD—1174.6 μm), followed by SFM3 (GMD—828.9 μm), and SFM2 (GMD—530.8 μm) which had the finest particle size distribution. The GMD results, along with the visual appearance of the meals (Figure 1), support the previously mentioned assumption that SFM2 likely underwent coarse grinding after its production. The SFM1 and SFM3 meals had similar values of bulk densities, 468.1 and 460.7 kg/m^3 , respectively, while the lowest bulk density of 404.8 kg/m^3 was recorded for SFM2. Regarding the angle of repose, SFM1 exhibited a slightly lower value (34.9°), which, according to material flowability ratings, classified this meal as a material with “good” flowability. Starting sunflower meals SFM2 and SFM3, with slightly higher angles of repose (37.9° and 38.0°, respectively), fell into the category of materials with “fair” flowability (Table 1).

Based on the analysis of the GMD of the starting and ground SFMs (Table 3), it was evident that grinding, with both mills, significantly reduced particle size in all analyzed SFM samples. Regarding bulk density, grinding of SFM1 and SFM2 with the hammer mill (SFM1:HM and SFM2:HM) resulted in a significant increase in this parameter. However, subsequent grinding with the roll mill (SFM1:RM and SFM2:RM) reduced the bulk density

to a level comparable to the initial samples SFM1 and SFM2. In the case of SFM3, a significant decrease in bulk density was observed only after grinding with the roll mill (SFM3:RM).

The use of a hammer mill reduces particle size, allowing better packing of particles and usually increasing bulk density. However, this was not observed with SFM3. This could be due to the large number of heavy and dense agglomerates present in SFM3 before milling, which increases the overall bulk density of the sample. During the process of hammer milling, most of the agglomerates are disintegrated [17]. This led to a decrease in bulk density which compensated increase in the bulk density produced by reducing particle size in the milled material. As a result, the bulk density of SFM3 (which was 460.7 kg/m³) remained almost the same after hammer milling (SFM3:HM = 456.6 kg/m³).

From Table 3, it can be observed that roll milling resulted in a decrease in the bulk density of all three samples. Although the milling process reduces particle size, which influences the increase in the bulk density, the opposite effect was noted here. This could be attributed to the milling conditions set for the rollers, leading to the flattening of fibrous hull particles present in the sunflower meal [20]. These flattened particles physically occupy more space within the material mass, which is probably a reason for the decreased bulk density of the milled samples by roll mill.

As for the angle of repose, significant changes in these values were recorded following the grinding of sunflower meal samples. However, only the SFM1 meal sample, after grinding with the roll mill (SFM1:RM), changed its flowability category from “good” to “fair”.

Table 3. Physical quality characteristics of the starting and ground SFMs.

Sunflower Meal	GMD (μm)	Bulk Density (kg/m ³)	Flowability Properties	
			Angle of Repose (°)	Flowability Rating
SFM1	1174.6 ± 16.6 ^a	468.1 ± 2.0 ^b	34.9 ± 0.2 ^{a,b}	Good
SFM1:HM	462.2 ± 4.3 ^b	499.5 ± 2.9 ^a	33.0 ± 0.3 ^b	Good
SFM1:RM	333.2 ± 1.2 ^c	460.0 ± 4.4 ^b	35.9 ± 0.1 ^a	Fair
SFM2	530.8 ± 7.8 ^a	404.8 ± 1.5 ^b	37.9 ± 0.1 ^a	Fair
SFM2:HM	431.8 ± 1.7 ^b	431.7 ± 4.4 ^a	35.7 ± 0.1 ^b	Fair
SFM2:RM	353.5 ± 2.1 ^c	405.8 ± 8.4 ^b	38.2 ± 0.3 ^a	Fair
SFM3	828.9 ± 17.8 ^a	460.7 ± 2.6 ^a	38.0 ± 0.1 ^b	Fair
SFM3:HM	406.1 ± 11.1 ^b	456.6 ± 3.5 ^a	39.6 ± 0.1 ^a	Fair
SFM3:RM	364.8 ± 2.7 ^c	444.4 ± 2.6 ^b	40.0 ± 0.1 ^a	Fair

Superscripted letters a, b, and c denote classes from ANOVA. GMD—geometric mean diameter; SFM1—starting sunflower meal 1; SFM1:HM—ground SFM1 after the hammer mill; SFM1:RM—ground SFM1 after the roll mill; SFM2—starting sunflower meal 2; SFM2:HM—ground SFM2 after the hammer mill; SFM2:RM—ground SFM2 after the roll mill; SFM3—starting sunflower meal 3; SFM3:HM—ground SFM3 after the hammer mill; SFM3:RM—ground SFM3 after the roll mill.

3.3. Dry Fractionation Process

Table 4 summarizes the determined values for parameters defined as process efficiency indicators. Protein quantity and quality represent key criteria in the selection of protein sources for animal diets [34], emphasizing the importance of protein enrichment during processing. The optimized dry fractionation process successfully elevated the protein content in the enriched fractions of SFMs to 44.20%_(dm), 43.16%_(dm), and 42.09%_(dm) for EFSFM1, EFSFM2, and EFSFM3, respectively, corresponding to relative protein enrichments of 24.0%, 31.2%, and 17.5%, respectively. Regarding protein content, the determined values for EFSFM1 and EFSFM2 were high enough to meet the criteria, according to the Serbian

Regulation on Animal Feed Quality, for categorization as “high protein” SFMs of first quality (42%_(as is)).

Table 4. Process efficiency indicators (protein content and relative protein enrichment, fraction yield, energy consumption).

Observed Parameters	EFSFM1 *	EFSFM1	EFSFM2	EFSFM3
Protein content after the process (% _(dm))	45.50	44.20	43.16	42.09
Relative protein enrichment (%)	/	24.0	31.2	17.5
Fraction yield (%)	77.9	78.9	77.0	76.5
Energy consumption (Wh/kg)	8.31	8.44	9.30	9.93

EFSFM1, EFSFM2, and EFSFM3—protein enriched fractions of SFM1, SFM2, and SFM3, respectively. * The parameter values of EFSFM1 predicted by the model formed in the previous work of authors [21].

In terms of fraction yield, exceptionally high and similar values were recorded for all three protein-enriched fractions, reaching 78.9%, 77.0%, and 76.5% for EFSFM1, EFSFM2, and EFSFM3, respectively. Regarding energy consumption, the lowest value (8.44 Wh/kg) was recorded during the production of EFSFM1. Slightly higher energy consumption values were observed during the production of EFSFM2 and EFSFM3, at 9.30 and 9.93 Wh/kg, respectively. This trend could partly be a consequence of the calculation method (Equation (7)), where energy consumption is calculated relative to the yield of the enriched fraction, which is slightly lower for SFM2 and SFM3 compared to SFM1. However, achieving high fraction yield values and maintaining low energy consumption are of key importance for broader industrial applications.

The results obtained for all SFMs in this study are comparable to findings from other research on dry fractionation processes applied to sunflower meals. Lević et al. [35] investigated the potential of centrifugal separation for the nutritional improvement of sunflower meals and achieved a maximum relative protein enrichment of 16.8%, with a fraction yield of 43.4%. Similarly, Sredanović et al. [36] utilized centrifugal separation following preliminary grinding and reported a maximum relative protein enrichment of 11.9%, along with a slightly higher fraction yield of 54.8%. Murru and Calvo, in their study [11], applied a combination of milling, sieving, and gravity tables achieving a relative protein enrichment of 17.8% and a significantly higher fraction yield of 65%. These results are in line with those of the present study for EFSFM3, which recorded a relative protein enrichment of 17.5% and an even higher fraction yield of 76.5%. Laudadio et al. [18], by use of an air classification process after the micronization of sunflower meal, also achieved a 17.7% increase in protein content, along with an exceptionally high fraction yield of 87.9%. In comparison, the processing of SFM1 and SFM2 in our study resulted in significantly higher relative protein enrichment (24.0% and 31.2%, respectively), though with slightly lower fraction yields (78.9% and 77.0%, respectively). Banjac et al. [17] and Laguna et al. [19] in their studies using air classification of pre-ground sunflower meal, achieved exceptionally high relative protein enrichment of 41.4% and 67.4%, respectively. However, these results were accompanied by significantly lower fraction yields of 11.5% and 22%, respectively. To the best of the authors' knowledge, the highest relative protein enrichment reported to date (97.1%) was achieved through milling combined with electrostatic separation, while still with a relatively low fraction yield of 18% [19].

In the previous study of authors [20], the dry fractionation process of sunflower meal was optimized, with the optimization set to meet the requirements for maximum flour yield (coefficient of importance (CoI) = 3), minimum energy consumption (CoI = 3), and to provide protein content of the sunflower meal that is not lower than 45.5%_(dm) (CoI = 5). The model proposed the following input parameters: SOD of 2 mm, roll gap of 0.25 mm, feed

rate of 0.2 kg/cm min, and roll speed of 400 rpm, while the predicted values for the desired response ranges were protein content of 45.5%_(dm), fraction yield of 77.9%, and energy consumption of 8.31 Wh/kg (presented as EFSFM1* in Table 4). Since the SFM1 is a new batch of the same sunflower meal used in the aforementioned work, the obtained results for EFSFM1 can be used to compare with the parameter values predicted by the model, serving as a validation of the model's accuracy. In terms of protein content, a slightly lower value was achieved compared to the value predicted by the model (44.20%_(dm) compared to the predicted 45.5%_(dm)), while the fraction yield value was nearly identical to the predicted value (78.9% compared to the predicted 77.9%). Energy consumption, recorded during the process, was 8.44 Wh/kg, which was quite similar to the predicted 8.31 Wh/kg. Considering the heterogeneity of the material characteristic of sunflower meal, as well as the fact that the experiments in the authors' previous research and the current study were performed on different batches of SFM1 and using semi-industrial equipment, it can be stated that these results indicate good agreement between the experimental results and the values predicted by the model, confirming the reliability of the model.

Proteins fulfill diverse biological functions, reflecting their biological value (protein quality), which is determined by their amino acid composition [4]. By removing the hull from the sunflower meal and increasing its protein content, the amino acid content is also increased [37]. The amino acid composition of the starting SFMs, protein-enriched SFMs fractions, RSM, and SBM is presented in Table 5. According to the data, the dry fractionation process achieved relative amino acid enrichment of 21.85%, 26.64%, and 13.35% in the enriched fractions of SFM1 (EFSFM1), SFM2 (EFSFM2), and SFM3 (EFSFM3), respectively. These results of relative amino acid enrichments align with the values of protein enrichment in the same fractions (24.0%, 31.2%, and 17.5%, respectively). The total amino acid content of the starting SFMs (24.62%_(dm), 22.37%_(dm), and 24.50%_(dm) for SFM1, SFM2, and SFM3, respectively) was lower compared to rapeseed meal (25.16%_(dm)) and significantly lower than soybean meal (32.80%_(dm)). After the optimized dry fractionation process, the total amino acid content in all protein-enriched sunflower meal fractions increased to levels (30.00%_(dm), 28.33%_(dm), and 27.77%_(dm) for EFSFM1, EFSFM2, and EFSFM3, respectively) that surpassed rapeseed meal but remained below soybean meal. Additionally, lysine and methionine, which are the first limiting amino acids in typical diets for monogastric animals [38,39], were elevated by 27.9%, 36.0%, and 18.9% in the case of lysine, and 30.6%, 40.8%, and 44.1% in the case of methionine (for EFSFM1, EFSFM2, EFSFM3, respectively). The aforementioned data confirm the efficiency of the process in enhancing the nutritional quality of SFMs with regard to protein and amino acid content.

Table 5. Amino acid composition of starting SFMs, enriched SFMs fractions, RSM, and SBM.

Aminoacid Content (% _(dm))	Starting SFMs and Enriched SFMs Fractions						RSM	SBM
	SFM1	EFSFM1	SFM2	EFSFM2	SFM3	EFSFM3		
Total EAA	10.77 ^d	12.76 ^b	9.71 ^e	12.17 ^{b,c}	10.62 ^d	12.01 ^c	12.30 ^{b,c}	14.85 ^a
Leu	1.47 ^{d,e}	1.57 ^c	1.40 ^e	1.86 ^b	1.53 ^{c,d}	1.78 ^b	1.79 ^b	2.41 ^a
Val	1.59 ^c	1.93 ^a	1.39 ^d	1.77 ^b	1.52 ^c	1.70 ^b	1.73 ^b	1.90 ^a
Thr	1.05 ^e	1.37 ^b	0.94 ^f	1.21 ^d	1.02 ^e	1.20 ^d	1.29 ^c	1.49 ^a
Ile	1.46 ^d	1.84 ^b	1.26 ^e	1.65 ^c	1.40 ^d	1.60 ^c	1.46 ^d	2.02 ^a
Lys	1.04 ^e	1.33 ^c	0.86 ^g	1.17 ^d	0.95 ^f	1.13 ^d	1.66 ^b	2.19 ^a
Met	0.62 ^c	0.81 ^a	0.49 ^d	0.69 ^b	0.59 ^c	0.85 ^a	0.61 ^c	0.56 ^c
His	0.73 ^c	0.92 ^a	0.60 ^d	0.82 ^b	0.67 ^{c,d}	0.82 ^b	0.81 ^b	0.97 ^a
Phe	2.81 ^{c,d}	2.98 ^b	2.75 ^d	2.99 ^b	2.96 ^{b,c}	2.93 ^{b,c}	2.95 ^{b,c}	3.30 ^a

Table 5. Cont.

Aminoacid Content (% _(dm))	Starting SFMs and Enriched SFMs Fractions						RSM	SBM
	SFM1	EFSFM1	SFM2	EFSFM2	SFM3	EFSFM3		
Total NEAA	13.86 ^c	17.24 ^a	12.66 ^d	16.16 ^b	13.88 ^c	15.76 ^b	12.87 ^d	17.95 ^a
Glu	2.91 ^c	3.50 ^a	2.62 ^d	3.34 ^b	2.88 ^c	3.26 ^b	2.74 ^d	3.51 ^a
Asp	2.71 ^e	3.39 ^b	2.38 ^f	3.09 ^c	2.65 ^e	2.95 ^d	2.24 ^g	4.17 ^a
Pro	0.22 ^c	0.35 ^b	0.21 ^c	0.32 ^b	0.24 ^c	0.38 ^b	0.36 ^b	0.60 ^a
Ala	1.23 ^{d,e}	1.53 ^a	1.10 ^f	1.40 ^b	1.19 ^{e,f}	1.34 ^{b,c}	1.31 ^{c,d}	1.56 ^a
Arg	2.36 ^c	3.07 ^a	1.94 ^e	2.80 ^b	2.21 ^d	2.73 ^b	1.83 ^e	2.75 ^b
Gly	2.63 ^d	3.06 ^{a,b}	2.88 ^c	3.12 ^a	2.95 ^{b,c}	3.07 ^{a,b}	2.37 ^e	2.54 ^d
Ser	1.08 ^{d,e}	1.40 ^b	0.95 ^f	1.27 ^c	1.06 ^e	1.23 ^c	1.14 ^d	1.63 ^a
Tyr	0.72 ^{d,e}	0.96 ^b	0.58 ^f	0.81 ^c	0.69 ^e	0.80 ^{c,d}	0.89 ^b	1.20 ^a
Cys	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total AA	24.62 ^d	30.00 ^b	22.37 ^e	28.33 ^c	24.50 ^d	27.77 ^c	25.16 ^d	32.80 ^a
The relative AA enrichment (%)	/	21.85	/	26.64	/	13.35	/	/

Superscripted letters a, b, c, d, e, f, and g denote classes from ANOVA. SFM1—starting sunflower meal 1; SFM2—starting sunflower meal 2; SFM3—starting sunflower meal 3; EFSFM1—protein-enriched fraction of SFM1; EFSFM2—protein-enriched fraction of SFM2; EFSFM3—protein-enriched fraction of SFM3; AA—amino acids; EAA—essential amino acids; NEAA—non-essential amino acids; Leu—leucine; Val—valine; Thr—threonine; Ile— isoleucine; Lys—lysine; Met—methionine; His—histidine; Phe—phenylalanine; Glu—glutamic acid; Asp—aspartic acid; Pro—proline; Ala—alanine; Arg—arginine; Gly—glycine; Ser—serine; Tyr—tyrosine; Cys—cysteine.

Among the protein-enriched meals, EFSFM1 has the highest content of total amino acid composition (30%_(dm)). The amino acid composition analysis of EFSFM1 identified the highest contents of glutamic acid (3.50%_(dm)), aspartic acid (3.39%_(dm)), and arginine (3.07%_(dm)). Excluding proline, which was recorded in low concentrations across all analyzed samples, and cysteine, which was not detected in any of the analyzed samples, the least abundant amino acids in EFSFM1 were methionine (0.81%_(dm)), histidine (0.92%_(dm)), and tyrosine (0.96%_(dm)). These results align with the findings on the amino acid composition of sunflower meals reported in the review by Alagawany et al. [10].

Oilseed byproducts RSM and SBM are conventional sources of protein used in feed formulations, with SBM being recognized as the “gold standard” among plant protein sources in the animal feed industry [40,41]. A comparison of the amino acid profiles of EFSFM1 and RSM reveals that EFSFM1 surpasses RSM in total non-essential amino acid content (17.24%_(dm) vs. 12.87%_(dm)), while their total essential amino acid content remains comparable (12.76%_(dm) vs. 12.30%_(dm)). Regarding essential amino acids contents, RSM shows higher levels of leucine (1.57%_(dm) vs. 1.79%_(dm)), and lysine (1.33%_(dm) vs. 1.66%_(dm)), whereas EFSFM1 contains higher amounts of valine (1.93%_(dm) vs. 1.73%_(dm)), threonine (1.37%_(dm) vs. 1.29%_(dm)), isoleucine (1.84%_(dm) vs. 1.46%_(dm)), methionine (0.81%_(dm) vs. 0.61%_(dm)), and histidine (0.92%_(dm) vs. 0.81%_(dm)). No statistically significant difference was observed in the phenylalanine content (2.98%_(dm) vs. 2.95%_(dm)).

In the comparison of EFSFM1 to SBM, the total non-essential amino acid content was comparable (17.24%_(dm) vs. 17.95%_(dm)), while the essential amino acid content favored SBM (12.76%_(dm) vs. 14.85%_(dm)). The SBM had higher content of leucine (1.57%_(dm) vs. 2.41%_(dm)), threonine (1.37%_(dm) vs. 1.49%_(dm)), isoleucine (1.84%_(dm) vs. 2.02%_(dm)), lysine (1.33%_(dm) vs. 2.19%_(dm)), and phenylalanine (2.98%_(dm) vs. 3.30%_(dm)). The EFSFM1 and SBM had comparable levels of valine (1.93%_(dm) vs. 1.90%_(dm)) and histidine (0.92%_(dm) vs. 0.97%_(dm)), while the content of methionine (0.81%_(dm) vs. 0.56%_(dm)) was more abundant in EFSFM1. This is particularly important considering that soybean meal, while rich in

lysine, is deficient in sulfur-containing amino acids such as methionine, which are essential for optimal growth and health in poultry and swine [42].

4. Conclusions

A previously studied dry fractionation process was adapted in this work to enhance sunflower meals. The process was tested on three sunflower meals with different characteristics to evaluate its potential for nutritional improvement and broader applicability. The optimized dry fractionation process demonstrated its capability to nutritionally enhance all three sunflower meals, with SFM1 and SFM2 achieving protein levels high enough to be categorized as “high protein” sunflower meals of first quality (according to the Serbian Regulation on Animal Feed Quality). The optimized dry fractionation process notably increased the total amino acid content in the enriched sunflower meal fractions, exceeding that of rapeseed meal but still remaining below soybean meal. Furthermore, the levels of lysine and methionine, the first limiting amino acids in typical monogastric diets, were significantly enhanced. While the process proved effective across different sunflower meals, further efforts should focus on scaling up the dry fractionation method to industrial applications, ensuring consistency and cost-efficiency. Building on this study’s findings, which demonstrated the efficiency of the process on sunflower meals with protein levels near 33%_(as is), future investigations should explore its application to meals with varying protein levels to validate its adaptability and performance across a broader range of sunflower raw materials.

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