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Chemical Characterization and Antioxidant Capacity of Flour from Two Soya Bean Cultivars (Glycine Max)

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KEYWORDS

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ABSTRACT:
A comparative study was conducted to evaluate the chemical composition and antioxidant capacity of flour derived from two soybean (*Glycine max*) cultivars: one local variety (Oued Smar, Algeria) and one imported Chinese variety. The chemical composition, including ash, protein, and fat content, was analyzed. Potassium (K^+) and sodium (Na^+) were quantified using a flame photometer. Additionally, polyphenol content and antioxidant activity (FRAP assay) of methanolic extracts from the flours were assessed. The results revealed that the soybean flours contained approximately 8% moisture, over 50% protein, 1.58–1.87 g of fat, and 0.28–0.30 g of ash. Minor differences in mineral content were observed, with potassium levels reaching 489 mg/mL and sodium levels at 20 mg/mL. The total phenolic content of methanolic extracts was around 37 mg GAE/g in both flours. The antioxidant potential (FRAP) of the Chinese variety was slightly higher, likely due to its polyphenol richness. Both soybean flours demonstrated significant protein and phenolic content, with notable antioxidant properties, underscoring their value as functional food ingredients.

Introduction

Soybean (*Glycine max*) is a widely cultivated legume known for its high protein content and nutritional value. It plays a crucial role in food production, particularly as a source of plant-based protein. The increasing demand for functional foods has driven research into soybean-derived products, particularly soybean flour, which can be enriched with bioactive compounds such as polyphenols [1]. These compounds exhibit antioxidant properties that contribute to health benefits, including reducing oxidative stress and preventing chronic diseases [2].

However, the chemical composition and bioactive potential of soybean flour can vary depending on cultivar and environmental conditions [3]. These variations are influenced by genetic factors, agricultural practices, as well as processing and storage conditions [4].

Numerous studies highlight the health benefits of soy consumption. Regular intake of soy-based foods, such as flour, tofu, and soy milk, has been linked to a reduced risk of chronic diseases, particularly cardiovascular conditions [5]. Additionally, soy isoflavones contribute to antioxidant and anti-

inflammatory properties, supporting overall health and disease prevention [6].

In Algeria, soybeans are primarily utilized as livestock feed, but they also hold potential for direct human consumption, particularly in functional foods.

Objectives: The main objective of this study is to evaluate the chemical composition, including protein, lipid, ash, and mineral content, and the antioxidant potential of soybean flours derived from two cultivars: a local variety (Oued Smar) and an imported Chinese variety. The study also aims to investigate the influence of these factors on their nutritional value and potential applications in functional foods, with a focus on their polyphenol content and antioxidant activity.

Materials and Methods

Plant Material and Flour Preparation

Two soybean cultivars were used in this study: the local variety (Oued Smar) supplied by the Technological Institute of Large Cultures in Algeria and an imported Chinese variety. The soybeans underwent several processing steps, including sorting, washing, husking, crushing, boiling, drying, and grinding into flour. The seed coats were removed following a controlled soaking process [7]. The flour was then sieved to ensure uniform texture. The processing of soybeans followed the methodology described in [8].

Chemical Composition Analysis

Moisture content was determined by oven drying at 105°C. Ash content was measured after dry mineralization at 550°C. Protein content was quantified using the Bradford method [9], while lipid content was determined using Soxhlet extraction with petroleum ether [10]. Mineral content (Na$^+$ and K$^+$) was analyzed through flame photometry [11].

Polyphenol Extraction and Antioxidant Activity

Polyphenols were extracted using an 80:20 methanol-water solution through maceration for 24 hours at room temperature [12]. The extraction was repeated three times, filtered, and the solvent was evaporated under vacuum at 40°C using a Rotavapor [13]. The yield of crude extract was calculated as the ratio of dry extract mass to the initial plant material mass.

Total Polyphenol Content

Total polyphenols were quantified using the Folin-Ciocalteu method [14]. This method involves the reduction of phosphotungstic and phosphomolybdic acids during the oxidation of phenols, resulting in blue oxides of tungsten and molybdenum [15]. The intensity of the coloration is proportional to the amount of polyphenols present, which was measured between 725 nm and 750 nm. The total polyphenol content was calculated using a calibration curve with gallic acid and is expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract) [16].

Antioxidant Activity (FRAP Assay)

Antioxidant activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, which measures the ability of extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) [17]. Extracts of different concentrations were mixed with a phosphate buffer solution and potassium ferricyanide and incubated at 50°C for 30 minutes. After adding trichloroacetic acid and FeCl$_3$, absorbance was measured at 700 nm. A higher absorbance indicates a greater reducing power. Ascorbic acid was used as a positive control. The relationship between extract concentration and absorbance was used to assess antioxidant activity [18].

Results and Discussion

Soybean Processing Results

Observation on Soaking and Removal of the Seed-Coat

Soaking is a crucial step in soybean processing, aiming to remove anti-nutritional factors such as phytates, lectins, and tannins. This process enhances nutrient assimilation, particularly vitamin B, improves protein bioavailability, and facilitates the breakdown of the seed coat, making grinding easier [19]. Additionally, soaking initiates the germination process, during which seed constituents reorganize to form the embryo, enhancing digestibility for human consumption.

The soaking phase lasts at least one night, as recommended by [20]. In our study, the soaking duration was set at 18 hours following the same methodology. The water-to-seed ratio was maintained at

3:1 to ensure optimal hydration. Preliminary washing was performed to remove residual waste not eliminated during pre-treatment.

Soybean processing involves multiple stages, including soaking, cooking, dehulling, drying, and flour extraction, leading to increased accessibility of its nutrients. According to our findings, the weight of 100 g of dry soybeans increased by 82 g after soaking, reaching a total of 182 g. This significant increase in weight and volume alters the seed's shape, making it more oval.

Dry soybeans initially contain between 10% to 14% moisture, as per the Canadian Grain Commission standards (2001). After soaking, moisture content rises to over 20%, particularly in soaked grains, which can contribute to dry matter losses. The maximum moisture content in soaked soybeans was found to be relatively high. Once soaked, the soybeans were easily dehulled. The removal of the husks is important since they contribute to the characteristic beany taste caused by lipoxygenase activity [21]. At this stage, the seeds became oval and nearly doubled in weight. For 1 kg of seeds and 3 kg of water, we obtained

Table 1: The weight of the seed and its seed coats in (%)

Soybean	Percentage (%)
Graines entières triée	100
Hulled removed	15.22
Dehulled seeds	84.78

The drying process took approximately 51 hours and 40 minutes to ensure that the seeds were sufficiently dried and ready for crushing. A combination of oven drying and air drying proved to be effective in achieving high-quality flour. Table 2 presents the observations recorded during this step.

Table 2: Observations during seed drying

Drying mode	Duration	Observation
Oven	18 h	The seeds become sticky, fragrant
Oven	24 h	Colloidal texture reduced, odor had been perceived
Outdoors	3 h	Seeds slowly turn yellow, attractive odor
Outdoors	6 h 40 mn	Light yellow color, pleasant smell, crumbly texture

After 18 hours of oven drying, the soybeans emitted a sour and unpleasant odor, and they became sticky due to the presence of lipids and the gelatinization of starch within the seeds. This colloidal texture was noted as a significant factor affecting the drying process. Upon returning the seeds to the oven for an additional 24 hours, they regained a softer texture, and the unpleasant odor gradually diminished.

Exposure to ambient air facilitated further moisture evaporation. During this phase, vapors and residual moisture dissipated more efficiently. After three hours of air drying, the seeds displayed uneven rigidity upon touch. Their color changed to a light yellow, and the odor became more appealing, likely due to the degradation of anti-nutritional factors.

A second exposure to open air for an additional 6 hours and 40 minutes resulted in completely dried seeds that emitted a pleasant, cookie-like aroma. At this stage, the seeds were fully dry and ready for milling. Throughout the drying process, a gradual reduction in seed volume was observed, attributed to water evaporation from the soaking phase.

A study conducted by [22] indicated that after 96 hours of drying, the moisture content of soybean seeds ranged between 13.8% and 14.6%, while the maximum water content was recorded at 48 hours, ranging from 17% to 19%. The same study suggested that seeds with moisture content exceeding 18% should be dried at 32°C, whereas seeds with lower moisture content can be dried at 38°C.

Production Yield on Grain Preparation and Soybean Meal Extraction

For this study, 500 g of soybeans from each variety were used to produce soybean meal [23]. The weight of

the seeds did not vary significantly after soaking and dehulling. The variation in seed weight was evaluated as a percentage. After sorting, 496.4 g of soybeans were obtained, representing 99.28% of the initial weight. This weight loss was attributed to the removal of waste materials [24]. Following washing, the scale displayed the same mass as after sorting.

Soaking caused the seed weight to nearly double due to water absorption by osmosis [25]. The weight of dehulled seeds decreased by 20.16% compared to their weight post-soaking, indicating that the removed seed coat accounted for this percentage. After dehulling, the mass of seeds prepared for processing was 830.62 g, with an additional weight gain of 82% due to soaking. While weight loss from sorting and dehulling was minimal, the overall production yield was estimated at approximately 60.19%.

For flour production, 250 g of soybeans from each cultivar were used, with half of the amount processed into flour. The yield of flour production is depicted in Figure 1. A significant decline in flour yield was observed due to water evaporation during drying. Additionally, the initial quantity of soybeans purchased was reduced. The curve on the graph starts at 250 g and declines to 166.67 g at the end of the drying process. The five successive processing steps resulted in a total loss of 124.67 g.

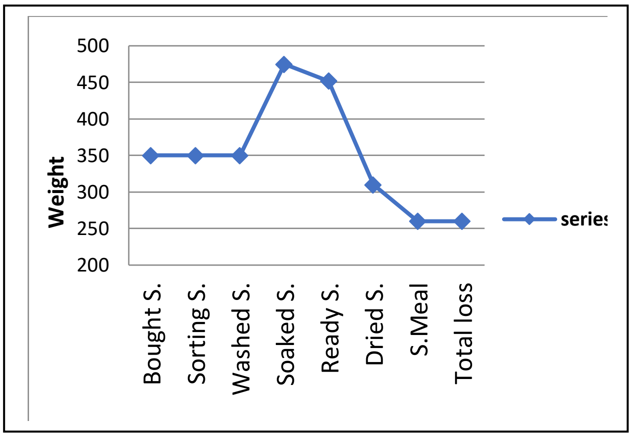

Figure 1: The weight of soybeans during different processing stages

Grinding was performed on the dried seeds to obtain flour. This step introduced additional weight variation. With 250 g of soybeans processed, the method adopted yielded 125.33 g of soybean meal. Consequently, the estimated flour production yield was 50.13%.

The estimation and proportion of on-farm losses during each of the four flour production stages are illustrated in Figure 2. The removal of seed coats accounted for the largest portion of losses during flour production which has been previously discussed [26]. Another substantial loss occurred during the refining of the flour, representing 7.02% of the total loss. The flour produced immediately after grinding still contained impurities. Sieving was necessary to refine the flour and enhance its quality. The removal of coarse particles during this step contributed to further weight reduction.

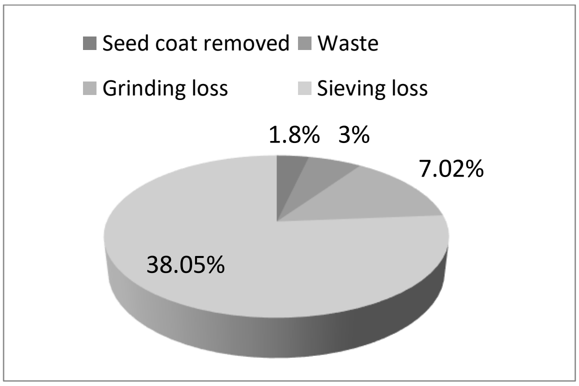

Figure 2: The different percentage losses (%) during the production of soybean flour

Chemical Composition of Soybean Meal

Yield of Methanolic Flour Extracts from Two Soybean Cultivars

The calculation of extraction yield for the two soybean varieties revealed a significant difference between them (Table 3). The Chinese variety exhibited a higher dry extract yield of approximately 35%, whereas the Oued Smar variety recorded a lower value, not exceeding 22%. Since both varieties were subjected to identical experimental conditions, prior studies suggest that the observed yield differences are primarily related to

genetic properties, geographical origin, storage conditions, and harvest duration [27].

Table 3: Chemical composition and contents of different parameters of the flours of two soybean cultivars

Varieties Parameters	Flour Oued Smar (V1)	Flour de la chine (V2)
Extraction yield (%)	**22**	**35**
Moisture (%)	**8.09**	**8.11**
Ash (g)	**0.30**	**0.28**
Proteins(%)	**50.4**	**55,2**
Fat (%)	**15.8**	**18.7**
Sodium (Na) (mg/ml)	**19.2**	**20**
Potassium (K) (mg/ml)	**481**	**489**
Total Polyphénol (mg Eag/g)	**3.68**	**3.71**

Moisture Content Determination Results

Moisture content is a critical factor influencing the storage stability of soybeans and their derivatives, such as flour. The moisture and dry matter content of processed soybean products were evaluated, with results expressed as percentages. Dry matter content was derived from the measured water content.

According to the results obtained, the highest moisture content was observed in soybean flour derived from the Chinese variety, which recorded 8.11% moisture, yielding 91.89% usable dry matter. Given the low water content in flour, its nutrient density is expected to be high. Previous research indicates that mature soybeans typically contain moisture levels ranging between 13% and 15%, depending on the harvest period and regional climatic conditions [27].

Soybeans contain two types of water: free water and bound water. Free water is removable through drying, whereas bound water is molecularly integrated into the seed and cannot be eliminated by conventional drying techniques. In general, soybeans can be stored without deterioration as long as their moisture content remains below 12% [28].

Crude Ash, Protein, and Fat Content of Flours from Two Soybean Cultivars

The results, expressed as percentages, present the proportions of ash, protein, and fat in the flours obtained from two soybean varieties (Table 3). The ash content was measured after complete incineration of the test samples. Using an average test sample of 5 g of flour, the total ash content in each variety did not exceed 0.3 g, or approximately 6% of the dry matter. This low crude ash content results from production processes that reduce mineral content. A decrease in soybean meal yield influences its raw ash content, as finer flour retains fewer mineral residues.

Regarding protein quantification, soybeans represent a significant source of protein (40-42%) widely used in human nutrition [29]. The agro-food industry utilizes soybean-derived products due to their beneficial health effects. The protein concentration in soybean meal was estimated using a calibration curve of bovine serum albumin ($y = 5.605x + 0.366$, $R = 0.988$) and expressed as a percentage. Analysis revealed that soybean meal is highly proteinaceous, with over 50% of dry matter in flour consisting of protein, making it a viable protein concentrate. Soybean flour, although a semi-finished product unsuitable for raw consumption, contains between 45% and 65% protein . Other studies indicate that protein content can range from 60% to 90% depending on processing methods [30]. Soy protein serves as a base for protein concentrates and isolates, reinforcing its role as a high-quality protein source, particularly in vegetarian diets.

The fat content analysis for 10 g of flour showed 1.58 g (15.8% of dry matter) for the Oued Smar variety and 1.87 g (18.7% of dry matter) for the Chinese variety. Since the flour used was not defatted, it retained a small amount of lipids. During drying, some fat was retained in the soybean product, and its oxidation initially produced an unpleasant odor. The lipid content of soybeans varies significantly depending on variety and cultivation conditions [31].

Composition in Mineral Elements (Potassium and Sodium)

Potassium and sodium concentrations were determined under identical conditions, using an air-propane flame (0.5 kg/cm² – 160 mm of water pressure). The standard curves obtained were linear: Potassium (y = 0.073x + 10.46, R = 0.969) and Sodium (y = 0.067x + 8.755, R = 0.956). Based on these equations, the potassium and sodium concentrations of the samples were calculated. As shown in Table 3, both soybean flours exhibited high potassium concentrations, with slight variations between them. Sodium concentrations were lower, with the highest value recorded in flour from the Chinese variety, which did not exceed 20 mg/mL.

Our results are slightly lower than values reported in the American food database [32], which indicates an average potassium content of 515 mg per 100 g of soybean flour. The mineral composition of soybean by-products confirms that they are richer in potassium than in sodium. While heat treatment is necessary to reduce certain anti-nutritional factors, it can also affect micronutrient bioavailability . Soybeans contain approximately 4-5% minerals, with low Na^+ levels and high K^+ concentrations Previous studies indicate that soybeans contain around 4.7 mg of sodium and 118 mg of potassium per 100 g [33] Potassium plays a crucial role in muscle excitability and in the metabolism of proteins and glycogen

Estimation of Total Phenolic Compounds

The total phenolic content was determined using the Folin-Ciocalteu assay, a method recognized for its feasibility and reproducibility [34]. This technique was selected due to its high absorbance wavelength (765 nm), which minimizes interference from the sample matrix, often rich in pigments. The total phenolic content of our extracts was estimated using a calibration curve (y = 0.008x - 0.003, R = 0.996), where y represents absorbance and x corresponds to the gallic acid concentration. Results are expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g) (Table 3).

Phenolic compounds, including flavonoids and phenolic acids, are abundant in soy products and play a crucial role as bioactive components in the human diet. As shown in Table 3, total phenolic content varies between samples and cultivars. The methanolic extracts exhibited phenolic contents ranging from 3.71 mg GAE/g in the Chinese variety to 3.68 mg GAE/g in the Oued Smar variety. The high phenolic content in soybean meal may be attributed to the nature of the raw materials and processing methods. Studies suggest that the phenolic content in soybean flour increases under specific conditions [35].

Variability in total phenolic content is influenced by factors such as physiological state at harvest, genetic variation, geographic origin, cultivation methods, post-harvest treatments, and extraction solvents used. Previous research has reported a total phenolic content of 3.27 mg quercetin equivalent/g in soybeans [36].

Evaluation of Antioxidant Activity Using the Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is a widely used, simple, and reproducible method for evaluating antioxidant activity based on iron reduction [37]. Ascorbic acid, a potent antioxidant, was used as a control. The absorbance of the control increased from 1 mg/mL and continued to rise proportionally throughout the experiment.

The results indicate that most extracts exhibited antioxidant activity (Figure 3). The absorbance curves of the various extracts, plotted against their concentrations in the reaction medium, followed a logarithmic trend similar to that of ascorbic acid. This trend suggests a direct relationship between extract concentration and reduction potential.

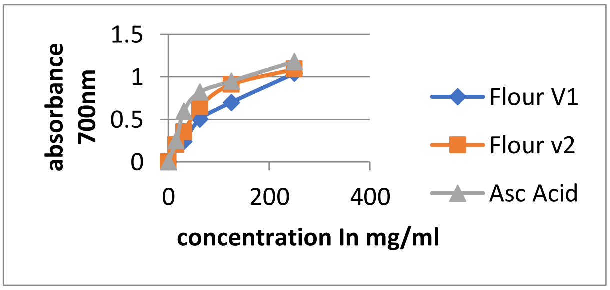

Figure 3: Reducing power of the by-product (Flour) from two varieties used

At a concentration of 1 mg/mL, ascorbic acid showed an absorbance of 0.25. At 4 mg/mL, the flour extract from the Chinese variety exhibited an absorbance of 0.89, significantly higher than that of the Oued Smar variety, which recorded 0.72. The antioxidant activity of soybean meal may be attributed to its high polyphenol content; however, it is important to note that phenolic compounds do not represent the entirety of antioxidant components in an extract [38].

CONCLUSION

This study provides valuable insights into the chemical composition and antioxidant properties of flour derived from two soybean cultivars. Both varieties exhibited high protein and polyphenol content, with notable antioxidant activity, making them suitable for functional food applications. The slight variations between the cultivars highlight the impact of genetic and environmental factors on soybean composition. Future research should explore the bioavailability of these compounds and their potential applications in health-focused food products.

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