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ORIGINAL PAPER

Composition and Physicochemical Characterization of Walnut Flour, a By-product of Oil Extraction

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Abstract
The by-product of walnut oil (*Juglans regia* L.) extraction is a press cake rich in polyunsaturated fatty acids and other bioactive compounds. From this cake, walnut flour is obtained by a milling process. The composition and a physicochemical characterization of walnut flour was performed: proximal composition, mineral content, and fatty acid and amino acid profiles were measured. Besides, antioxidant capacity and water and oil holding capacities were determined. Walnut flour has 55% of lipids with an optimum w6/w3 ratio, a good lysine/arginine ratio, and high levels of antioxidants that contribute to its oxidative stability, the estimated shelf life being 16 months. In regards to interaction with other ingredients, walnut flour retained 258 and 70% (w/w) of water and oil, respectively. Therefore, these results show that walnut flour is a good source of micro- and macronutrients, compared to flours commonly used in breadmaking. Also, walnut flour has good technofunctional properties and thus its incorporation could improve the nutritional and technological characteristics of new bakery products.

Keywords *Juglans regia* L. · Unsaturated fatty acids · Antioxidant capacity · Shelf life

Introduction

Worldwide, the food industry annually generates a large number of by-products, many of which are not used. The volume of by-products generated is such that they may constitute a problem [1]. Food industrial by-products include a wide variety of components such as soy okara, apple pomace, bran, and oil cake, among others.

The production of oil from walnut (*Juglans regia* L.) leads to the obtention of a partially defatted cake. The composition of this oil cake depends on the extraction method [2]. But as it contains polyunsaturated lipids and also a high content of proteins, fiber, minerals, and other bioactive compounds [3]. It has the potential to be used as a supplement in dietary products or as an ingredient of functional foods. Thus, the so-called walnut flour is obtained from the milling of this press cake.

On the other hand, today's lifestyle has led to changes in eating habits, such as diets with a high content of saturated fats, sugars, and salt, which constitute a risk factor for non-transmissible diseases. The modification of these new consumption habits is a demanding challenge and this scenario is still more difficult when there is an allergy or food intolerance, because the diet is necessary to reach a healthy state.

In this context, bakery products appear as an opportunity to develop healthier products of massive consumption because they are versatile and adaptable. These characteristics make them suitable matrices for the development of functional foods. They can incorporate macro- and micronutrients of high nutritional value such as walnut flour, transforming these food matrices into healthier ones.

The use of press cakes in bakery products has been extensively applied to improve their nutritional quality [4–7]. However, there are a few reports about the use of walnut flour (WF). It was used in cakes and bread, modifying color, specific volume, and texture, and improving the antioxidant properties [8, 9]. Finally, WF was used as a low-cost ingredient to produce macarons. The hedonic profile was conserved with a 10% of replacement of almond flour by WF [10]. The scarce use of the WF in bakery products could be related with a lack of knowledge and diffusion of the nutritional and technological properties of this press cake. Thus, the objective of this work is to evaluate the physicochemical characteristics and the nutritional value of WF, a by-product of oil extraction.

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Materials and Methods

Materials

Walnut flour (WF) and walnut oil (Aceites del Desierto S.A., Argentina). WF is a by-product of hydraulic cold oil extraction. WF was packed under vacuum and stored in a chamber at 4 °C in darkness before the tests were done. All the reagents employed were of analytical grade or HPLC grade as required.

Methods

Detailed descriptions of the "Methods" are provided in a file in the Supplementary Material (please refer to the "Supplementary Methods").

Results and Discussion

Proximal Composition

The WF is characterized by a low moisture content (4.5% ± 0.2). The proximal composition of WF in dry basis is: 55.7% lipids, 24.6% proteins, 9.4% dietary fiber content, 2.7% ashes, and the available carbohydrates represent 7.6%, which consist primarily of glucose (0.63%), fructose (0.52%), sucrose (6.09%), and starch (0.36%). The calorie content given by 100 g of WF is 649 kcal, which shows that WF is a dense nutrient mainly due to its high oil content. Despite of depending the proximal composition of the press cake of different variables as genotype, crop year and oil extraction method, comparable WF composition has been informed [2].

Mineral Composition

Table 1 lists the mineral composition of WF and for comparison purposes, the mineral composition of a wheat flour, a rice flour, the recommended nutrient intake (RNI) for males and females (19–65 years old) [11], and the percentage of RNI provided by 100 g of WF. Minerals are classified according to the amounts that are required by the body as macro- or microelements. Calcium and phosphorous are the major constituents of bone. Their contents in WF are higher than the values present in wheat and rice flours [12, 13]. However, the percentage of the calcium RNI covered by a portion of 100 g of WF low (15.2%). In the case of P, 73.7% of the RNI would be covered. The low content of Ca in foods is common, thus the RNI is difficult to reach in the absence of dairy products.

Regarding the other macro-minerals, WF covers 89.2% of the RNI of Mg, almost 15.5% of potassium RNI, and 2.6% of sodium RNI. Due to the low sodium content, WF could be used for the development of products reduced in salt.

Furthermore, the values of Fe, Mn, Cu, and Zn are greater in WF than in the most popular flours used for breadmaking. The amount of Mn and Cu in 100 g of WF is more than twice the RNI, while in the case of Fe and Zn, it would cover 63.5 and 54.7% of the RNI, respectively. The intake of Fe is of particular concern because of the relation with anemia, since Fe is required for hemoglobin synthesis.

Refined flours have a low level of minerals because most of them are eliminated together with the bran during

Table 1 Mineral composition

Element	mg/kg of WF*	Wheat flour (mg/kg) *[13]	Rice flour (mg/kg) *[12]	Recommended nutrient intakes (RNI)[11]	Percentage of RNI provided by 100 g of WF*
Macroelements					
Ca	1524 ± 43	660	58.1	1000 mg	15.2%
K	7276 ± 257	900	1116.6	4700 mg	15.5%
Mg	2318 ± 43	210	387.6	260 mg	89.2%
Na	619 ± 79	20	17.3	2400 mg	2.6%
Microelements					
P	5157 ± 97	830	1094.8	700 mg	73.7%
Cu	22 ± 1	3.3	2.5	900 µg	243%
Fe	89 ± 8	-	6.9	14 mg	63.5%
Mn	49 ± 0	6	8.4	2.3 mg	212.1%
Mo	≤ dl	0.27	-	45 µg	-
Se	≤ dl	-	-	34 µg	-
Zn	38 ± 1	8	20.4	7 mg	54.7%

Mean ± standard deviation; WF: walnut flour; * dry basis; dl: detection limit. The detection limit for Mo was 0.021 mg/L and for Se 0.008 mg/L milling. Therefore, the use of WF could be a good strategy to improve the nutritional value of gluten and gluten-free bakery products. The incorporation of WF will lead to an increase in the cost of these products or not, depending on the type. In the case of bread probably, its cost would be higher, but in the case of macarons, the use of this oil cake has been studied as a way to obtain a product of similar quality and lower price [10]. In any case, the consumers are increasingly concerned about the nutritional quality of the products they consume, and if the products reach their expectations, they are willing to pay higher prices [14].

Antioxidants

The press cake is rich in the brown skin that covers the kernel which is important to protect fatty acids from oxidation. This action is related to the presence of phenolic compounds [15]. The total phenolic content found for WF (10.9 ± 0.3 mg GAE/g WF) is similar to reported values [16, 17]. Concerning the composition of phenolic compounds, hydrolyzable tannins and flavanols are the major components [18]. The antioxidant activity was estimated by three complementary methods. FRAP determines the capacity of a compound to donate an electron to the ferric-tripyridyltriazine complex and ABTS, and DPPH assays combines the electron transfer and hydrogen atom transfer mechanisms. The antioxidant capacity of WF expressed in terms of $FeSO_4$ and trolox equivalent was 339 ± 2 and 232 ± 8 μmol/g, respectively, and the concentration necessary to decrease to half the initial DPPH concentration was 0.14 ± 0.006 mg WF/mL of extract. The values informed here are comparable with the ones found by other authors in walnut kernels [17, 19]. The antioxidant activity measured is probably related to the total phenolic content from the brown skin. Since the contribution of oil to the antioxidant capacity of walnut is negligible [20]. This shows that WF preserves antioxidant compounds after the walnut is cold-pressed.

Fatty Acid Profile and Oxidative Stability

The fatty acid profile of WF and walnut oil is shown in Table 2. The major fatty acids in WF are linolenic acid, oleic acid, and α-linolenic acid, which together represent almost 90% of lipids. This profile is similar to that found in walnut oil, showing that the press cake has almost the same proportion of fatty acids as walnut kernel. Likewise, in walnut flour the w6/w3 ratio is 4, the same value that has been informed for the walnut kernel. This ratio is considered optimum, while a high w6/w3 is related to cardiovascular disease, cancer, and inflammatory and autoimmune diseases [21].

WF has a high level of unsaturated lipids depending on the shelf life of oxidative stability. Lipids undergo oxidative degradation, leading to the loss of quality parameters such as flavor, aroma, and nutritional value. The oxidative stability of WF is significantly higher than that of walnut

Table 2 Fatty acid profile, oxidative stability indices, kinetic parameters and estimated shelf life at 20 °C of walnut flour and oil

Fatty acid profile			
% Fatty acids (g/100 g of total fatty acids)		Walnut flour	Walnut oil
Palmitic acid (16:0)		7.71 ± 0.00	9.36 ± 1.1
Stearic acid (18:0)		2.22 ± 0.02	1.62 ± 0.1
Oleic acid (18:1)		15.47 ± 0.29	16.04 ± 1.1
Linolenic acid (18:2)		59.80 ± 0.31	59.27 ± 0.7
α-Linolenic acid (18:3)		14.80 ± 0.02	13.71 ± 1.5
∑SFA (%)		9.93	10.98
∑MUFA (%)		15.47	16.04
∑PUFA (%)		74.60	72.98
Ratio: w6/w3		4.04	4.32
Ratio: ∑UFA /∑SFA		9.07	8.11
Oxidative stability			
Oxidative stability index (hours) at	100 °C	17.08^b ± 0.4	4.58 ^{a} ± 0.11
	110 °C	7.10^b ± 0.8	2.06 ^{a} ± 0.00
	115 °C	4.41 ± 0.1	Nd
	120 °C	3.18^b ± 0.1	1.31^a ± 0.01
Activation energy (kJ/mol)		103.7 ^{b} ± 3.3	94.7 ^{a} ± 2.4
Q_{10}		2.28 ^{a} ± 0.08	2.18 ^{a} ± 0.06
Shelf life at 20 °C (months)		16.4 ^{b} ± 1.3	3.2 ^{a} ± 0.1

Mean ± standard deviation; nd: non determined; SFA, MUFA, PUFA: Saturated, monounsaturated, and polyunsaturated fatty acids, respectively; Different letters in the same row indicate significant differences ($p < 0.05$)

Table 3 Amino acid composition of walnut flour protein

Amino acid	Amino acid (mg/ g walnut protein)	Amino acid	Amino acid (mg/g walnut protein)	Chemical score *
Aspartate	172.0 ± 3.3	Valine *	28.5 ± 0.3	0.68
Glutamate	256.0 ± 3.7	Histidine*	17.5 ± 1.5	0.97
Serine	69.2 ± 2.2	Phenylalanine (Phe)	26.3 ± 1.2	-
Methionine (Met)	2.6 ± 0.3	Isoleucine*	64.5 ± 2.4	2.08
Glycine	33.0 ± 2.9	Leucine*	62.7 ± 3.2	1.00
Proline	25.1 ± 1.3	Lysine*	36.2 ± 5.9	0.70
Arginine	104.9 ± 7.5	Threonine*	17.1 ± 5.6	0.63
Alanine	53 ± 4.2	Tryptophan*	9.1 ± 1.8	1.23
Tyrosine (Tyr)	21.0 ± 2.2	Met + Cys*	3.9 ± 0.3	0.15
Cysteine (Cys)	1.3 ± 0.00	Phe + Tyr*	47.3 ± 2.5	1.02

Mean ± standard deviation; *Chemical score corresponds to the amino acids with an asterisk and it was calculated as (mg of essential amino acids (EAA)/ g protein)/(mg of EAA/g FAO requirement protein pattern)

oil (Table 2), which is probably related to the high level of antioxidants present in WF and to the protection that confers the remaining tissue structures. These results show that WF when it is submitted to heating in the presence of continuous airflow, it resists several hours before exhibiting an advanced oxidation state. During baking, the cooking time is generally shorter than one hour, and only the crust is submitted to temperatures higher than 100 °C, while in the crumb, the temperature and the oxygen availability are much lower than in the crust. These is showing that only the crust is more vulnerable to oxidation. Furthermore, several reported works show that when oils or flours with a high content of unsaturated lipids like chia or chestnut, are used for bakery products, the quality of the lipids is conserved or even improve, and they exhibit good sensory preference [22, 23].

Taking into account the Arrhenius model, the activation energy and the temperature acceleration factor (Q_{10}) were calculated. The activation energy is a measure of the temperature sensitivity of the reaction and the Q_{10} factor predicts the increase in oxidation rate with a 10 °C increase in temperature. The activation energy for WF was higher than for the oil, while there were no significant differences in the Q_{10} factor. Finally, the estimated shelf life for WF and walnut oil storage at 20 °C was 16 and 3 months, respectively. These results show that the oil contained in the WF is protected by the antioxidants and probably the remaining cotyledon structures.

Protein Quality

Table 3 lists the amino acid profile of WF proteins and the chemical score. The chemical score was calculated as (mg of essential amino acids (EAA)/ g protein)/(mg of EAA/g FAO requirement protein pattern). The values for preschool children were adopted as a scoring pattern for all ages [24]. For humans the EAA are leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine. Thus, these amino acids have to be intake with food. In walnut proteins, the more abundant amino acids are aspartate, glutamate, and arginine. This last one is considered a conditionally EAA when the capacity of endogenous amino acid synthesis is exceeded, under conditions of stress and catabolic states [25]. While the limiting amino acids are the sulphurated cysteine and methionine, the protein score being 16%. However, WF contains relatively high levels of Arginine and a Lysine/Arginine ratio equal to 0.345. The low value of this ratio has been related to an antiatherogenic effect mediated by nitric oxide for which arginine is a precursor [26].

Physicochemical Properties

Physicochemical properties of WF were determined with the aim of evaluating how WF could affect a product formulation. WF presented a low water activity value (0.565 ± 0.02) showing that the growth of microorganisms is not favored. With respect to WF color, the following parameters were obtained: L* = 52.9 ± 1.5; a* = 6.2 ± 0.2; b* = 12.8 ± 0.8, and the calculated hue and chroma values were 64.15 and 14.3 ± 0.7, respectively. Lightness and h* values were lower than the ones previously informed by other authors [27]. But

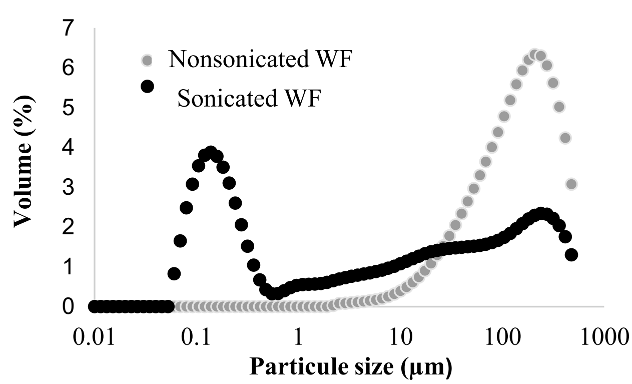

Fig. 1 Volume-weighted distribution of walnut flour (WF)

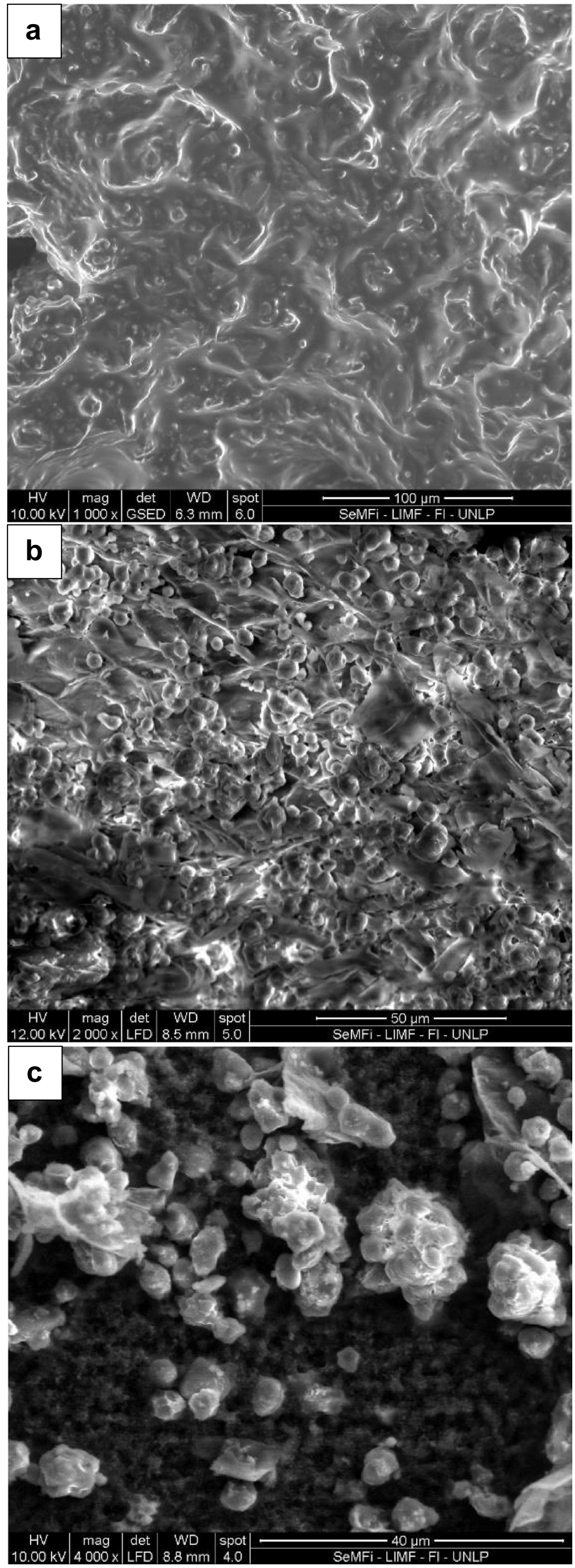

Fig. 2 Electron micrographs of walnut flour (WF). **a**) WF observed in ESEM mode at 1000×; **b**, **c**) defatted WF observed in low vacuum mode at 2000× and at 4000×, respectively

these discrepancies are probably related to the amount of oil in WF. Since most of the pigments are extracted together with oil, the higher the oil content the lower the L* and h* values. This suggests that if WF is added to a bakery product, the color will probably change, which could be desirable in gluten-free ones.

The water holding capacity is related to the ability of a matrix to entrap water. The value found for WF was 2.58 ± 0.1 mL/g WF. The uptake and retention of water by flours is relevant in the breadmaking process because it is related to parameters such as cooking time and the texture after cooking. The oil absorption capacity was 0.72 ± 0.05 g oil/g WF which could be related to a low content of soluble dietary fiber and to the high lipid content present in WF which could saturated all the lipophilic components. In a chia press-cake, the low oil absorption capacity was related with the fact that lipophilic compounds could be modified during the extraction [28].

Nevertheless, the organic molecule absorption capacity was 0.77 ± 0.09 g oil/g WF, and values near 1 have been reported as healthy [29]. The organic molecule absorption capacity is associated with the ability of dietary fiber to interact with carcinogenic and mutagenic substances and with bile acids, leading to a hypocholesterolemic effect [30].

Finally, Fig. 1 shows the volume-weighted distribution of WF with and without sonication treatment. Besides, the D(0.5) which indicates the maximum particle diameter that includes 50% of the population was obtained. The nonsonicated WF exhibited only one peak centered at 264.8 μm (D(0.5)). When WF was submitted to sonication particles deagglomerated, and one defined peak was observed together with two overlapped and broad peaks. Besides, a shift to lower particle sizes was observed. In this case, D(0.5) was 13.4 μm. This value is nearer to the particle diameters of wheat flour [31]. The observed effect caused by sonication is relevant since if the particle size is modified during a process, the technological properties can change.

Microstructure

The walnut (*Juglans regia* L.) cotyledon tissues consist of compactly packed parenchymal cells that are a reservoir of lipids and proteins. The lipids are organized in oil bodies that are covered by protein bodies [32]. This structure is disorganized by the press in the oil extraction and then, by the milling to obtain the flour. Figure 2a corresponds to WF observed by environmental scanning electron microscope (ESEM), the lipids cover the sample preventing the visualization of other components. Furthermore, lipids melt due to the impacting electron beam, and the image is not clear. So, the evaluation of the WF microstructure was performed in defatted WF in low vacuum mode (Fig. 2b, c). As expected, no intact cells were observed. WF is composed primarily of thin layers of cell walls and protein bodies (Fig. 2b). These protein bodies are agglomerated, and there are ghosts of the cytoplasmic network that once surrounded the lipid bodies (Fig. 2c). The small particle size and the absence of intact cells could contribute to the bioaccessibility of WF lipids.

Conclusions

The results of this research indicated that the walnut flour retains most of the properties of walnut (*Juglans regia* L.) and walnut oil, being a good source of micro- and macronutrients, compared to flours commonly used in breadmaking. WF has a very good unsaturated/saturated fatty acid ratio (9.07), a good amino acid profile, and an important content of bioactive compounds. Besides, WF showed good techno-functional properties, *i.e.*, its incorporation could improve the nutritional and technological characteristics of new bakery products. Considering that WF has a high-fat content, oxidative stability is a determining factor in its shelf life. The WF showed to be more stable to oxidation than walnut oil, the estimated shelf life at 20 °C being 16 months.

Abbreviations ABTS: [2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)]; Cys: Cysteine; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; EAA: Essential amino acids; ESEM: Environmental scanning electron microscope; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalents; IC_{50}: Half maximal inhibitory concentration; Met: Methionine; MUFA: Monounsaturated fatty acids; Phe: Phenylalanine; PUFA: Polyunsaturated fatty acids; RNI: Recommended nutrient intake; SFA: Saturated fatty acids; TEAC: Trolox equivalents; Tyr: Tyrosine; WF: Walnut flour

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11130-021-00898-4.

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Data Availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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