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ORIGINAL

Microwave-assisted extraction of antioxidant compounds from sunflower hulls

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Abstract

The objective was to determine the adequate conditions for the microwave-assisted extraction of antioxidant compounds from the seed hull of sunflower hybrids. The existence of genetic and environmental variability in the phenolic content obtained under the selected extraction conditions was also analyzed. The extractions were carried out at 70 °C-20 and 90 °C-10 min, using water as solvent and a power of 600 W. The total phenol, flavonoid and antioxidant activity were evaluated. The microwave extraction process at 90 °C-10 min gave significantly higher values of total phenol (407.13 ± 6.11–512.71 ± 23.54 mg gallic acid·100 g^{-1} hull), flavonoids (210.09 ± 6.15–297.64 ± 5.68 mg catechin·100 g^{-1} hull) and antioxidant activity (76.73 ± 4.40–110.80 ± 3.51 μmol TE·g^{-1} hull) than those obtained at 70 °C-20 min. The cultivation environment also significantly affected the antioxidant yield, with total phenol and flavonoid contents being significantly higher for the hybrids grown in Balcarce than for those from Tandil. A significant interaction between hybrids and cultivation environmental was also observed for the antioxidant activity, indicating that the environmental effects were not similar among hybrids. The results of this study provide valuable information related to giving added value to a residue of the oil industry.

1 Introduction

Sunflower (*Helianthus annuus* L) is one of the most important oilseed crops in the world, grown mainly as a source of edible oil. The seed consists of the kernel (75–80%), where the oil is synthesized and stored, and the hull or pericarp (20–25%) [1, 2]. The components of the hull are represented by the holocellulose fraction (cellulose 31–51%, hemicellulose 13–28%), lignin (20%), protein (4–6%), ash (2–6%) and lipids (5%, of which up to 3% can contain waxes) [2, 3]. Prior to the oil extraction process, the sunflower seeds are partially dehulled until 10–12% of residual hull is obtained, thus reducing the processing of unnecessary raw material, and with a positive impact on the quality of the raw oil and the residual meal. It should be pointed out that the waxes from the seed hulls can crystallize at low temperatures, producing turbidity in the extracted oil [2]. At the same time, an important amount of residue of low specific weight (approximately 0.1 ton/m^3) is produced in this stage of the process. Nowadays oil plants use the seed hulls as fuel, burning the shells in the furnace, with various technical and economic problems, and that is why the industrial use of sunflower hulls is considered a complex problem [2].

Several authors have reported that sunflower seeds are an important source of phenolic compounds (1–4% in weight, flavonoids and phenolic acids), with chlorogenic acid being the predominant compound (80% of the phenolic compounds) [4–6]. Between 0.7–5.4% of phenolic compounds present in the sunflower seed are located in the hull, which makes this industrial residue a potential source of bioactive compounds [7, 8]. From a structural point of view, phenolic compounds

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have an aromatic ring with one or more hydroxyl substituents, and their structures may range from a simple phenolic molecule to a complex high-molecular weight polymer [9]. Phenolic acids, flavonoids and tannins make up the most relevant phenolic groups. Phenolic acids consist of two subgroups: hydroxybenzoic acids (C_6-C_1) and hydroxycinnamic acids (C_6-C_3), and the latter have high antioxidant activity [10]. The predominant phenolic acids in the sunflower seed hulls belong to the hydroxycinnamic group (chlorogenic, caffeic, cynamic, coumaric, ferulic, synaptic and hydroxycinnamic acids) [1]. Flavonoids are compounds of low molecular weight (C_6-C_3-C_6), consisting of two phenyl rings linked via a pyran ring. They constitute the largest subgroup, and to date over 5000 flavonoids have been characterized [10].

Phenolic compounds play an important role in the pigmentation, growth and reproduction of plants, also protecting them against pathogens and insects, among other functions [11]. These compounds also exhibit a range of physiological properties, for example they are anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, and have cardioprotector and vasodilator effects, all associated with their antioxidant activity [2, 9, 10]. That is why research on polyphenolic compounds and their applications has generated much interest for the formulation of functional foods, and also in the pharmaceutical and nutraceutical industries [11]. The quality of the polyphenolic extracts and their antioxidant activity depend both on the properties of the raw material (related to genetic effects, geographical origin, weather conditions, harvest date, storage conditions) [12] and the extraction method [12, 13]. In addition, the antioxidant activity depends on the chemical structure of the phenolic compounds, in particular the number and position of the hydroxyl groups and the nature of the substitutions of the aromatic rings, which is reflected in the capacity to trap free radicals or hydrogen atoms, donate electrons or chelate metallic cations [2, 10].

The chemical nature of phenols is so varied that there is not a uniform or fully satisfactory procedure that is adequate for the extraction of all the polyphenols or a specific group of polyphenolic substances from plants. The efficiency of the extraction of phenolic compounds varies according to the method used and the variables involved in the process [1, 8]. The method used should allow for the complete extraction of the compounds of interest without generating any chemical modification. The extraction of bioactive compounds using conventional techniques, such as solvent extraction (liquid-liquid extraction or solid-liquid extraction) assisted with external factors (for example, mechanical agitation, pressing, or heating systems like Soxhlet), are methods that involve long processing times, low efficiency and high solvent consumption. The current focus on health has stimulated the search for alternative processes to obtain antioxidant compounds, such as microwave-assisted extraction (MAE) [12]. MAE is part of the so-called green technologies because it is a non-destructive method that fulfills the environmental and economic requirements to ensure safe and quality extracts/products. This method uses microwave energy to heat the solvents and the sample, increasing the mass transfer rate of the solute from the matrix of the sample to the solvent. It also has the advantage of being efficient and fast, which leads to less energy and solvent consumption, allowing to obtain extracts of higher purity compared to other conventional techniques [12, 14–17]. Microwave assisted extraction has been reported as a very suitable method for the extraction of phenolic compounds. The efficiency of the MAE can be affected by many process variables such as type and composition of solvent used, particle size, microwave power, irradiation time, extraction temperature and sample-to-solvent ratio. Likewise, the values of these parameters depend by the characteristics of the sample matrix and of the target compounds to be extracted. Some authors such as Krishnaswamy et al. [15], Cao et al. [16], Kumar et al. [17], Dorta et al. [18], Mishra et al. [19], Reis et al. [20], Nguyen et al. [21], Simić et al. [22], Abedi et al. [23], Vu et al. [24], among others, have optimized the microwave-assisted extraction of phenolic compounds in different matrices.

Nguyen et al. [21] and Vu et al. [24] showed that water could be used to effectively recover phenolic compounds and antioxidants from *Phyllanthus amarus* plant and banana peel, respectively, using microware assisted extraction. The solubility of the phenolic compounds increases with the rapid increase in the temperature of the solvent. On the other hand, Li et al. [25] evaluated the effect of extraction temperature in a microwave-assited operation on the antioxidant capacity of the phenolic extracts obtained from tamato, and suggested that a higher microwave temperature and a shorter extraction time are more effective in extracting antioxidative phenolic compounds. Nevertheless, extending the irradiation time with higher microwave temperature may leading to thermal degradation of the phenolics [26]. Microwave power levels are a major factor that affects the recovery of bioactive compounds, obtaining, generally, more phenolic compounds with increasing potency. Nevertheless, the extraction at higher microwave power levels does not always ensure better recovery compared to medium levels of power, probably due to a decrease in the recovery yield due to phenolic degradation [24].

A number of studies about the extraction of antioxidant compounds from sunflower seeds using different solvents (water, ethanol, methanol and/or acetone) have been reported, analyzing various temperatures, times, pH and/or sample/solvent ratios [1, 2, 4–8], but no studies were found in the literature about the microwave-assisted extraction of antioxidant compounds, or comparative studies of the genetic and environmental variability for sunflower seed hulls. The objective of this work was to determine the adequate conditions for the microwave-assisted extraction of antioxidant compounds

(phenols) from the seed hull of sunflower hybrids. The existence of genetic and environmental variability for the phenolic compound yield obtained under the selected extraction conditions and their antioxidant capacity was also analyzed.

2 Materials and methods

2.1 Samples

Samples of hybrids from two different sources were used: A) Sunflower hybrid SPS3120 (Syngenta, black-hull sunflower) and CF201a (Advanta Semillas SAIC, striped-hull sunflower) grown in Balcarce (37°45'S, 58°18'W), province of Buenos Aires (Argentina); and B) Samples of sunflower seeds from the official comparative Yield Trials of Buenos Aires and La Pampa provinces (Argentina, 2012/2013), grown in two environments: Balcarce (37°45'S, 58°18'W) and Tandil (37°14'S, 59°15'W), province of Buenos Aires (Argentina), and hybrids SyN3840, SyN3950 and DK4065 (Syngenta), CF201b (Advanta) and PAN7077 (Pannar). The seed samples were manually cleaned over a light surface to remove the foreign matter. The hulls were obtained by mechanical dehulling of the seeds using a pilot equipment that breaks the hull away from the seed by impact [27]. The hulls were manually separated from the obtained product, they were ground (grinder Ultracomb MO-8100, China) and sieved (mesh 40, ASTM) to obtain a particle size <0.42 mm (according Fanesi et al. [28] and unpublished data). The samples were kept in hermetically-sealed plastic containers at 5 ± 1 °C.

2.2 Physical properties and proximate composition

The physical properties and the proximate composition of the hulls of sources A and B were evaluated. The total hull content was determined by manual dehulling from a sample of 10 g, expressed as dry basis percentage (%, d.b.), and the hull thickness was measured using a micrometer (least count 0.01 mm). As for the proximate composition of the hulls, moisture, crude fiber and ash content were determined according to AOCS recommended practices Ba 2a-38, Ba 6–84 and Ba 5a-49, respectively [29]. Oil and nitrogen content (N) were determined following IUPAC standard method [30] and AOAC method [31], respectively. Protein content was calculated as nitrogen × 6.25.

2.3 Selection of the conditions (temperature-time) for the extraction of phenolic compounds

The extraction of phenolic compounds was carried out with a CEM apparatus Mars 6 (Microwave Enhanced Science, USA), equipped with an MTS-300 probe, which monitors and controls the temperature inside the containers in which the sample is placed. Two experimental temperature conditions were used (70 and 90 °C), with a microwave power of 600 W according Nguyen et al. [21] and Nkhili et al. [32]. The temperatures were selected taking into account that they were below the boiling point of distilled water (100 °C) and above the inactivation temperature of the polyphenolase enzyme (≥65 °C) [25, 33]. One gram of sample was used in the assays, using water as solvent, with a sample/solvent ratio of 1/20. The obtained extracts were centrifuged for 10 min at 3200 g (Thermo IEC, model CL3-R, USA) and filtered with Whatman filter paper N° 4, then they were placed in frozen storage (−18 °C). Before the determination of the phenolic compounds, the extracts were defrosted, filtered with 0.2 μm filters (30 MM SYR Filter PTFE) and centrifuged for 30 min at 15300 g (Eppendorf, model 5702-R, Germany). To determine the process times, a kinetic study of the total phenol extraction was performed using the seed hull of hybrid CF201a (source A). The process times considered were the minimum times needed to reach the maximum extraction of total phenols at each one of the selected temperatures (70 and 90 °C). The sunflower hulls of hybrid CF201a were evaluated with a confocal fluorescence microscope (Zeiss 2011, LSM 700, Heidelberg, Germany) using 20x magnification and a calcein filter to observe the effect of the microwave treatment at the cellular level. Then, for the selection of the adequate temperature-time conditions, the antioxidant compounds were extracted from the samples of source A and the five hybrids grown in Balcarce of source B, determining the contents of total phenols, flavonoids and antioxidant activity of the extracts.

2.3.1 Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu assay. A calibration curve was prepared with gallic acid standard (Sigma-Aldrich, Toluca, Mexico) according to the procedure described by Singleton and Rossi [34]. Absorbance was measured at 760 nm using the spectrophotometer model Helios (Thermo Electron Corporation, Cambridge, UK). A simple linear regression of the plot of absorbance versus gallic acid concentration was performed, with a correlation coefficient (R^2) of 0.9921. The results were expressed as mg of gallic acid per 100 g of sunflower hull on dry basis.

2.3.2 Determination of flavonoids

Flavonoids were determined by the aluminum chloride colorimetric method in basic medium, following the spectrophotometric technique proposed by Molina-Quijada et al. [35]. The calibration curve was prepared with the catechin standard (Sigma-Aldrich, Toluca, Mexico) and absorbance was measured at 510 nm. A simple linear regression of the plot of

absorbance versus catechin concentration was performed, with a R^2 of 0.9998. The results were expressed as mg of catechin per 100 g of sunflower hull on dry basis.

2.3.3 Determination of the antioxidant activity

The antioxidant activity was determined by the ORAC assay (Oxygen Radical Absorbance Capacity), which evaluates the ability to inhibit the oxidation of a solution generated by free radicals, according to Prior et al. [36]. The oxidation was measured by placing the free radicals (AAPH) (2,2′-azobis (2-amidinopropane) dihydrochloride), the antioxidant species and another "victim" solution (fluorescein), which is prone to oxidation, on the Varioskan flash equipment (Thermo Scientific, USA). It was also necessary to compare the oxidation of the solution under evaluation with that of another solution with a known antioxidant capacity, and for that purpose a calibration curve using Trolox as standard was prepared. The results were expressed as μmol of Trolox equivalent (TE) per g of sunflower hull on dry basis.

2.4 Determination of the genetic and environmental effect on the antioxidant content

The antioxidant compounds were extracted from the sunflower hulls of source B (grown in Balcarce and Tandil) under the conditions previously selected, quantifying the content of total phenols, flavonoids and the antioxidant activity in the obtained extracts.

2.5 Confocal fluorescence microscopy

To observe the effect of microwave treatment at the cellular level on the sunflower hulls of hybrid, photos were taken with a confocal fluorescence microscope (Zeiss 2011, model LSM 700, Heidelberg, Germany). For the analysis of microscopy the hybrid CF201a (source A) was selected, with microwave-assisted extraction at 70 °C and 90 °C, respectively. The results were compared with the same untreated sample. The technique consisted of making cross-sections in the direction of the fibers of the sunflower husks (approximately 0.5 mm). Then they were placed on a slide, 1–1.5 mL of the 0.01% acidic fuchsin dye was added and allowed to stand for 10 min in the absence of light, then the excess dye was removed and a wash was performed with distilled water avoiding loss of sample. Subsequently, the sample was covered with a coverslip and the evaluation was performed with the confocal fluorescence microscope, with a 20x amplification and a calcein filter.

2.6 Statistical analysis

The results were analyzed by ANOVA, and Tukey's test was used for comparing the means. The statistical analysis was performed with a confidence level of 95% using the InfoStat software [37]. All the tests were carried out in triplicate.

3 Results and discussion

3.1 Physical properties and proximate composition

The physical properties and the proximate composition of the seed hulls of sources A and B are shown in Tables 1 and 2. There were no significant differences ($p > 0.05$) between the hybrids of source A as for their physical properties (hull content and thickness) (Table 1), but there were significant differences in their proximate composition, except for protein

Table 1 Physical properties of the sunflower seed hulls of the hybrids of sources A (grown in Balcarce) and B (grown in Balcarce and Tandil)

			Hull content (%, d.b.)	Hull thickness (mm)	
Source	Origin	Hybrids			Average of hybrids
A	Balcarce	SPS3120	23.31 ± 0.28^{a}	0.33 ± 0.02^{a}	
		CF201	20.63 ± 1.54^{a}	0.27 ± 0.01^{a}	
B	Balcarce 0.28^{a}	SyN3840	21.35 ± 0.57^{a}	0.25 ± 0.004	0.26^{a}
		SyN3950	24.77 ± 0.15^{c}	0.30 ± 0.04	0.36^{b}
		DK4065	22.41 ± 0.54^{ab}	0.29 ± 0.01	0.29^{ab}
		CF201b	21.01 ± 0.17^{a}	0.24 ± 0.003	0.28^{a}
		PAN7077	21.42 ± 0.23^{a}	0.31 ± 0.01	0.32^{ab}
	Tandil 0.33^{b}	SyN3840	20.61 ± 0.09^{a}	0.27 ± 0.01	
		SyN3950	26.94 ± 0.41^{d}	0.42 ± 0.02	
		DK4065	21.86 ± 1.17^{a}	0.30 ± 0.01	
		CF201b	21.68 ± 0.46^{a}	0.32 ± 0.05	
		PAN7077	24.05 ± 0.48^{bc}	0.34 ± 0.02	

d.b., dry basis

Different letters indicate significant differences ($p \leq 0.05$) by columns

Table 2 Proximate composition of the sunflower seed hulls of the hybrids of sources A (grown in Balcarce) and B (grown in Balcarce and Tandil)

			Moisture content (%, d.b.)	Oil (%, d.b.)		Crude fiber (%, d.b.)	Protein (%, d.b.)	Ash (%, d.b.)
Source	Origin	Hybrids			Average of hybrids			
A	Balcarce	SPS3120	12.40 ± 0.02^{b}	6.40 ± 0.18^{a}		56.16 ± 0.22^{b}	7.36 ± 0.14^{a}	3.77 ± 0.08^{b}
		CF201	10.95 ± 0.17^{a}	7.87 ± 0.84^{b}		51.45 ± 0.97^{a}	7.27 ± 0.13^{a}	3.37 ± 0.03^{a}
B	Balcarce 7.06^{b}	SyN3840	11.74 ± 0.06^{ab}	9.45 ± 0.65	7.89^{c}	53.27 ± 0.07^{a}	7.04 ± 0.13^{cd}	3.50 ± 0.01^{bc}
		SyN3950	9.48 ± 2.84^{a}	4.60 ± 0.31	3.85^{a}	56.95 ± 0.18^{a}	5.36 ± 0.0004^{a}	3.47 ± 0.05^{b}
		DK4065	12.14 ± 0.36^{ab}	8.46 ± 0.58	7.22^{bc}	53.91 ± 0.07^{b}	6.18 ± 0.33^{ab}	3.70 ± 0.04^{d}
		CF201b	12.10 ± 0.20^{ab}	7.73 ± 0.43	6.41^{b}	53.77 ± 0.87^{c}	8.09 ± 0.26^{e}	3.38 ± 0.04^{b}
		PAN7077	11.07 ± 0.05^{ab}	5.08 ± 0.84	4.27^{a}	57.30 ± 0.34^{a}	6.08 ± 0.39^{ab}	4.11 ± 0.01^{e}
	Tandil 4.79^{a}	SyN3840	12.04 ± 0.01^{ab}	6.33 ± 0.95		56.33 ± 0.36^{b}	6.88 ± 0.13^{bcd}	3.42 ± 0.03^{b}
		SyN3950	14.77 ± 0.39^{b}	3.11 ± 0.50		61.83 ± 0.33^{a}	5.42 ± 0.005^{a}	2.92 ± 0.02^{a}
		DK4065	12.17 ± 0.62^{ab}	5.97 ± 0.87		56.95 ± 0.57^{b}	6.32 ± 0.27^{bc}	3.61 ± 0.02^{cd}
		CF201b	11.97 ± 0.48^{ab}	5.10 ± 0.43		53.02 ± 0.25^{b}	6.64 ± 0.07^{bcd}	3.50 ± 0.04^{bc}
		PAN7077	12.20 ± 0.13^{ab}	3.45 ± 0.22		60.77 ± 0.20^{c}	7.29 ± 0.05^{de}	3.39 ± 0.02^{b}

d.b., dry basis

Different letters indicate significant differences ($p \leq 0.05$) by columns

content (Table 2). Hybrid SPS3120 presented significantly higher values of moisture ($p \leq 0.0066$), crude fiber ($p \leq 0.0320$) and ash content ($p \leq 0.0214$), while hybrid CF201a exhibited higher values in oil content ($p = 0.0143$).

The coefficient of variation for hull content of the group of environments (cultivation areas) and hybrids of source B was of 8.9%, whereas that corresponding to hull thickness was higher (16.8%). The statistical analysis of the variability showed that both hull content and hull thickness of the samples of source B were significantly affected by the type of hybrid ($p \leq 0.0001$ and $p \leq 0.0004$, respectively) and the environment ($p \leq 0.0050$ and $p \leq 0.0006$, respectively). However, a significant hybrid*environment interaction was only detected for hull content ($p \leq 0.0027$). The highest hull content was observed for the hybrid SyN3950, being higher in the sample from Tandil (Table 1). The hybrids grown in Tandil presented higher values for hull thickness. Hybrid SyN3950 presented the maximum thickness, but not significantly differing ($p > 0.05$) from hybrids DK4065 and PAN7077. It is worth noting that a direct correlation was observed between the hull content of the sunflower hybrids (source A and B) and hull thickness ($R^2 = 0.705$).

The moisture content of the seed hulls of source B varied within a range of 9.48 and 12.20% d.b. (Table 2). No significant hybrid*environment interaction was detected for oil content ($p > 0.05$), with a higher oil level being observed for the hybrids grown in Balcarce than those from Tandil. Hybrid SyN3840 exhibited the highest oil content in the hull, not significantly differing from hybrid DK4065, while the hulls of hybrids SyN3950 and PAN7077 presented the lowest oil content. As for the contents of crude fiber, protein and ash, they were significantly affected by the type of hybrid ($p \leq 0.0001$), detecting a

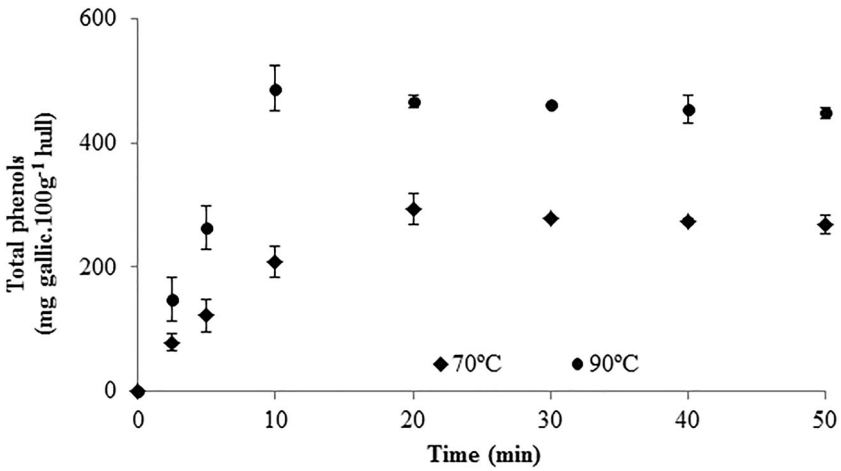


Fig. 1 Kinetics of the extraction of total phenols from sunflower seed hulls of hybrid CF201a (source A), using microwaves at 600 W

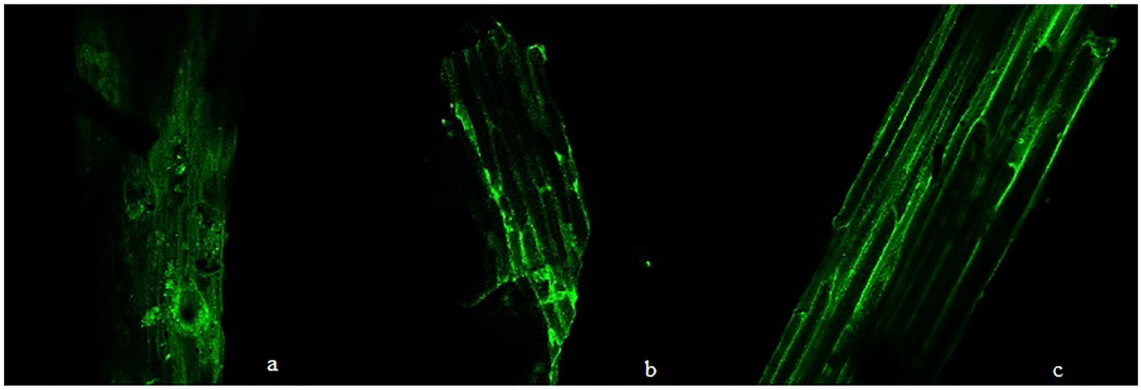


Fig. 2 Images taken on a confocal fluorescence microscope: Untreated sunflower seed hull of hybrid CF201a (source A) a), with microwave-assisted extraction at 70 °C for 20 min b), and at 90 °C for 10 min c)

significant hybrid*environment interaction ($p \leq 0.0001$). In the case of the cultivation area (origin), its effect was observed on crude fiber and ash contents ($p \leq 0.0001$).

3.2 Determination of the extraction conditions. Extraction kinetics - determination of the process time

Figure 1 presents the variation in total phenolic concentration as a function of time for hybrid CF201a (source A), for both process temperatures. The error bars in the figures indicate the standard deviations between the replicates (Figs. 1, 3 and 4). It can be observed that the microwave-assisted extraction at 90 °C gave significantly higher values ($p \leq 0.05$) of total phenols than at 70 °C for each one of the evaluated times. In the kinetic studies carried out at 70 and 90 °C, significant maximum values ($p \leq 0.05$) were obtained at 20 and 10 min of extraction, respectively, and those values were not statistically different from those obtained at longer extraction times. Based on these results, 20 and 10 min were selected as the times to perform MAE at 70 and 90 °C, respectively, of antioxidant compounds from the hulls of different sunflower hybrids.

Additionally, a structural damage in hull of hybrid CF201a (source A), with cellular disorganization and loss of intracellular spaces was observed according to the MAE treatment (Fig. 2). Untreated hull of hybrid CF201a (source A) (Fig. 2a) showed a compact structure, however, the MAE treatment carried out at 70 °C for 20 min (Fig. 2b), caused slight structural changes with modification in the compaction of the cellular structure. The hull did not exhibit any notable modifications in the cell structure compared to the untreated sample. On the other hand, when MAE was carried out at 90 °C for 10 min (Fig. 2c), a thinning and rupture of the cell wall can be observed, favoring the release of antioxidant compounds. The microwave irradiation accelerates cell rupture by the sudden increase in temperature and the increase in internal pressure within the cells, releases bioactive compounds and facilitating their contact with the extraction solvent [38, 39].

3.3 Selection of the time-temperature conditions

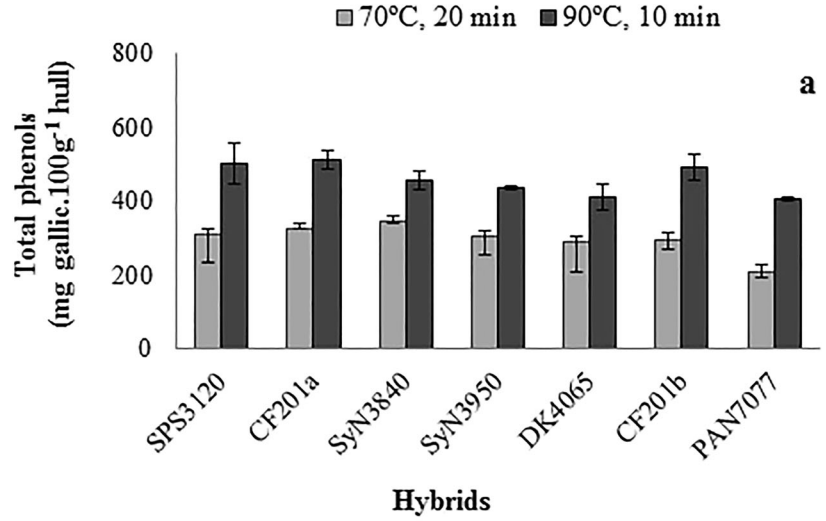
Figure 3 presents the total phenolic content (a, mg of gallic acid·100 g^{-1} of sunflower hull), flavonoid content (b, mg of catechin·100 g^{-1} of sunflower hull) and antioxidant activity (c, μmol TE·g^{-1} sunflower hull) of the hulls of the hybrids grown in Balcarce (two hybrids of source A and five of source B), for MAE at 70 and 90 °C for 20 and 10 min, respectively.

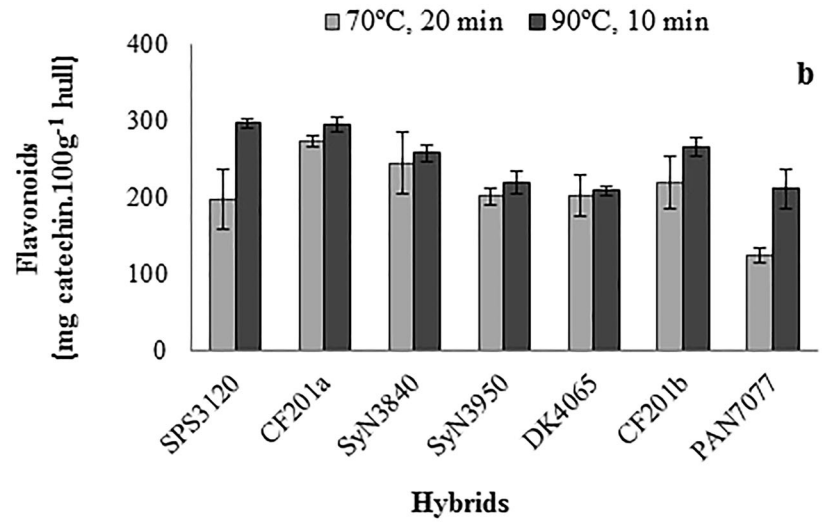
The total phenolic content varied between 211.52 ± 17.45 and 343.78 ± 2.63 mg of gallic acid·100 g^{-1} of sunflower hull when MAE was carried at 70 °C for 20 min, while the total phenol values varied between 407.13 ± 6.11 and 512.71 ± 23.54 mg of gallic acid·100 g^{-1} sunflower hull when MAE was performed at 90 °C for 10 min. It is worth noting that, for all the assays, the extraction of phenolic compounds increased significantly with temperature ($p \leq 0.0001$), with the higher values corresponding to the extraction at 90 °C for 10 min.

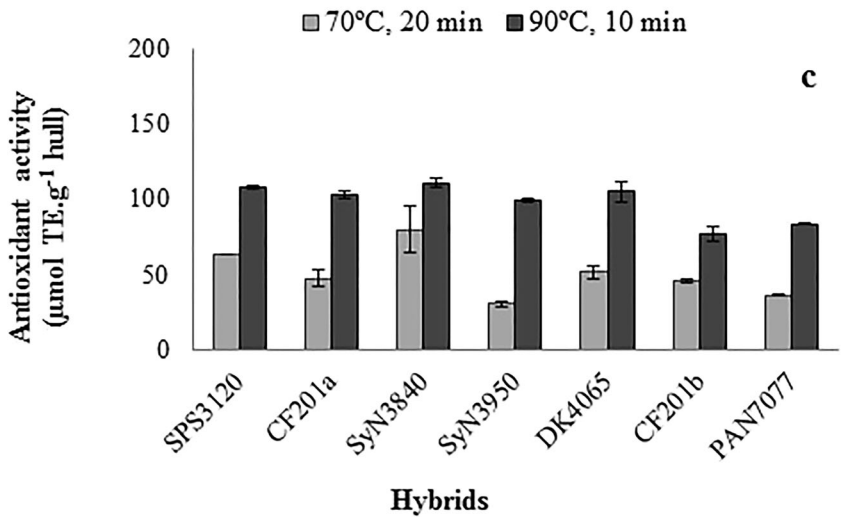
The values of total phenols obtained in this work for MAE at 90 °C and 10 min were higher than those reported by Taha et al. [2] who studied the effect of different variables on the mechanical extraction of total phenols from sunflower seed hulls, achieving the maximum values (312.5 mg gallic acid·100 g^{-1} hull) with 80% methanol and a hull/solvent ratio of 1/30 *w/v*.

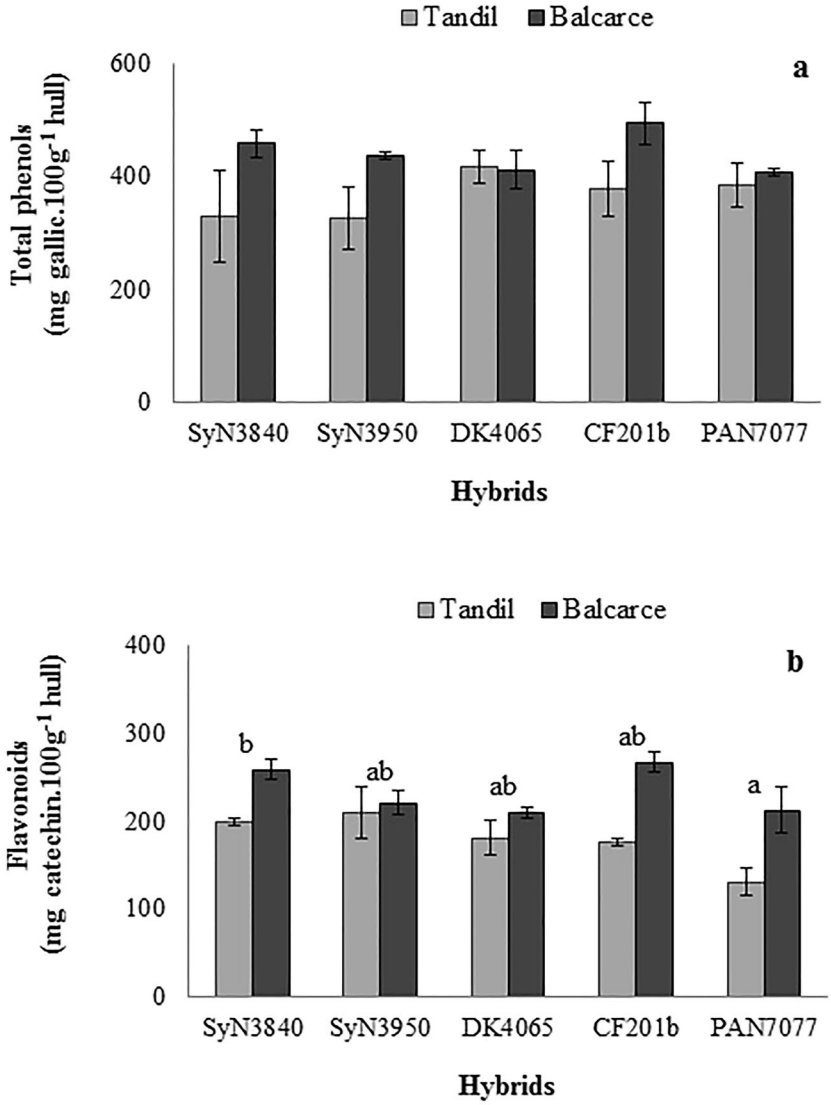
Since Singleton and Rossi [34] developed the colorimetric method to measure total phenolic content using the Folin-Ciocalteu reagent, results have been expressed according to the standard used for the calibration curve. In general, total phenols are expressed in terms of molar equivalents of gallic acid [1, 2, 10], (+) catechin [6, 40], chlorogenic acid [5, 6, 8, 41], ferulic acid [10] or caffeic acid [1]. The gallic acid is the

Fig. 3 Total phenol content (mg of gallic acid.100 g^{-1} of sunflower hull) a), flavonoid content (mg of catechin.100 g^{-1} of sunflower hull) b), and antioxidant activity (μmol TE.g^{-1} sunflower hull) c) of the seed hulls of sunflower hybrids (two hybrids of source A and five hybrids of source B grown in Balcarce), obtained by MAE at 70 and 90 °C for 20 and 10 min, respectively ▶


70°C, 20 min
90°C, 10 min
a
Total phenols (mg gallic.100g-1 hull)
800
600
400
200
0
SPS3120
CF201a
SyN3840
SyN3950
DK4065
CF201b
PAN7077
Hybrids


70°C, 20 min
90°C, 10 min
b
Flavonoids (mg catechin.100g-1 hull)
400
300
200
100
0
SPS3120
CF201a
SyN3840
SyN3950
DK4065
CF201b
PAN7077
Hybrids


70°C, 20 min
90°C, 10 min
c
Antioxidant activity (µmol TE.g-1 hull)
200
150
100
50
0
SPS3120
CF201a
SyN3840
SyN3950
DK4065
CF201b
PAN7077
Hybrids



Different letters indicate significant differences ($p \leq 0.05$) between hybrids.

Different letters indicate significant differences ($p \leq 0.05$) between hybrids and environment interaction.

◀ **Fig. 4** Content of total phenols (mg of gallic acid.100 g^{-1} of sunflower hull) a), flavonoids (mg of catechin.100 g^{-1} of sunflower hull) b), and antioxidant activity (μmol TE.g^{-1} sunflower hull) c) of the seed hulls of sunflower hybrids (source B, grown in Balcarce and Tandil), obtained by MAE at 90 °C for 10 min

most widely used standard due to its satisfactory solubility, adequate stability and low price [42].

The flavonoid content varied between 124.67 ± 8.98 and 273.30 ± 8.01 mg of catechin·100 g^{-1} of sunflower hull when MAE was carried at 70 °C for 20 min, while the flavonoid values ranged between 210.09 ± 6.15 and 297.64 ± 5.68 mg of catechin·100 g^{-1} sunflower hull when MAE was performed at 90 °C for 10 min. Similarly, to what was observed for total phenols, for all the assays the extraction of flavonoid compounds increased significantly with temperature ($p \leq 0.0128$).

On the other hand, the flavonoid content was lower than total phenols in all the analyzed samples since flavonoid compounds are a part of total phenols [10, 43]. It must be noted that flavonoids have a higher antioxidant activity than phenolic acids because they contain multiple hydroxyl groups [44]. Kähkönen and Heinonen [45] determined the flavonoid content, antioxidant activity and the correlation between them for berry samples, finding a directly proportional relationship between antioxidant activity and the presence of flavonoid compounds.

The antioxidant activity varied between 30.53 ± 1.61 and 79.21 ± 15.53 μmol TE· g^{-1} sunflower hull when MAE was carried at 70 °C for 20 min, and it ranged between 76.73 ± 4.40 and 110.80 ± 3.51 μmol TE· g^{-1} sunflower hull when MAE was performed at 90 °C for 10 min. For all the assays, the antioxidant activity of the sunflower hybrids increased significantly with temperature ($p \leq 0.0001$).

The greater extraction efficiency of the treatment at 90 °C can be attributed to the fact that the increase in temperature favors extraction by increasing the solubility of the solute and the diffusion coefficient and to the rupture of the cell [38, 39, 46, 47].

Based on the results of the evaluation of the most adequate conditions (temperature and time) for the MAE of antioxidant compounds from the hull of different sunflower hybrids, comparing values of total phenols, flavonoids and antioxidant activity, the treatment at 90 °C for 10 min was selected to continue the analysis. It is worth noting that, in addition to the advantages of the microwave-assisted extraction [14], the use of water as solvent at 90 °C has been optimized by Szydłowska-Czerniak et al. [8] for the extraction (with and without enzymatic treatment) of phenols from sunflower seed hulls (sample/solvent ratio 1/10), testing different conditions of temperature (30–90 °C), time (1–3 h) and addition of methanol (0–100). The extraction with water reduces the operational and investment costs (given the smaller size of the plant compared to an extraction plant that uses organic solvents), and offers greater operational and environmental safety by reducing the risk of fire or explosion. In addition, it does not present any toxic risk, and allows for greater operational flexibility, because start-ups and shutdowns are safer, facilitating the non-continuous operation [48]. Paladino [49] determined the efficiency of the extraction of total phenols from grape seeds using as solvents 70% methanol, 75% acetone and 20% ethanol (all these solvents at 30 °C) and water at 90 °C, with the latter being the most efficient option to extract the phenolic compounds. Although water at 90 °C can damage the thermolabile phenols, this effect could be compensated by the inactivation of the enzymes that degrade the phenolic compounds, generating extracts that are very active as antioxidants.

3.4 Genetic and environmental influence on the content of antioxidants

Figure 4 presents the content of total phenols (a, mg of gallic acid·100 g^{-1} of sunflower hull), flavonoids (b, mg of catechin·100 g^{-1} of sunflower hull) and antioxidant activity (c, μmol TE·g^{-1} sunflower hull) for the hybrids (source B, grown in Balcarce and Tandil) extracted by MAE (90 °C for 10 min).

The total phenolic content of the extracts obtained by MAE at 90 °C for 10 min varied between 407.13 ± 6.11 and 493.12 ± 36.11 mg of gallic acid·100 g^{-1} sunflower hull for the hybrids grown in Balcarce, and between 325.68 ± 55.90 and 416.78 ± 29.96 mg gallic acid·100 g^{-1} sunflower hull for the same hybrids grown in Tandil. The statistical analysis showed a significant effect of the environment on the phenolic content of the sunflower hulls ($p \leq 0.0026$), but no significant differences were observed between hybrids, or any significant hybrid and environment interaction ($p > 0.05$). The highest concentration of phenolic compounds was obtained for the hybrids grown in Balcarce.

The flavonoid content of the extracts obtained by MAE varied between 210.09 ± 6.15 and 266.30 ± 11.32 mg of catechin·100 g^{-1} sunflower hull for the hybrids grown in Balcarce, and between 130.74 ± 15.87 and 209.62 ± 29.48 mg catechin.100 g^{-1} sunflower hull for the same hybrids grown in Tandil. In contrast to what was observed for total phenols, both sources of variation (hybrid and environment) significantly affected the flavonoid concentration in the sunflower hulls ($p \leq 0.0457$ and $p \leq 0.0006$, respectively). At the same time, the interaction between both sources was not significant ($p > 0.05$). The hybrids PAN7077 and SyN3840 differed significantly in flavonoid content, presenting the lowest and highest values, respectively. For all the assays, the extraction of flavonoid compounds was higher in the hybrids from Balcarce compared to the values obtained from the hybrids grown in Tandil.

The antioxidant activity of the extracts obtained by MAE varied between 76.73 ± 4.40 and 110.801 ± 3.51 μmol TE·g^{-1} sunflower hull for the hybrids grown in Balcarce, and between 57.81 ± 4.90 and 86.92 ± 0.23 μmol TE·g^{-1} sunflower hull for the same hybrids grown in Tandil. The antioxidant activity was significantly affected by the type of hybrid ($p \leq 0.0001$)

and origin ($p \leq 0.0001$), and the hybrid and environment interaction was also significant ($p \leq 0.0357$).

The antioxidant activity can be associated mainly with the presence of chlorogenic acid, which represents 80% of the phenolic compounds in the sunflower hull [4–6, 8]. Chlorogenic acid is part of the family of hydroxycinnamic acids, which exhibit a higher antioxidant activity than the hydroxybenzoic acids possibly associated with the CH=CH-COOH group, which has a greater H-donating ability and radical stabilization than the -COOH group in the hydroxybenzoic acids [10].

4 Conclusions

The yield of antioxidant compounds from sunflower seed hulls obtained by microwave-assisted extraction using water as solvent was affected by the processing temperature and time. The extraction with a microwave treatment at 90 °C for 10 min yielded significantly higher values of phenolic compounds and antioxidant activity than that carried out at 70 °C for 20 min. Future studies using statistical and mathematical techniques and / or artificial neural network (ANN) would be useful for the improvement, simulation and optimization of a microwave-assisted extraction of the antioxidant compounds from sunflower hulls. The genetic and environmental variability of the flavonoid content and antioxidant activity of the seed hulls was also demonstrated, as well as the influence of the environment on the total phenol content. The content of total phenols and flavonoids was significantly higher for the sunflower hybrids grown in Balcarce than for those grown in Tandil. A significant interaction between hybrids and cultivation place was also observed for the antioxidant activity, indicating that the environmental effects were not similar for all the hybrids. Further studies will be necessary to identify and quantify the phenolic compounds responsible for the antioxidant activity. The results of this work provide valuable information related to the microwave-assisted extraction of antioxidant compounds in sunflower hulls, giving added value to a residue of the oil industry, which could find application as ingredients of functional foods, nutraceutical and pharmaceutical products, and in the cosmetic industry.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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