

Artículo Científico

# Valorization of oil industry wastes: Extraction of phenolic compounds from different sunflower hull fractions (*Helianthus annuus* L.)

Revalorización de residuo de la industria aceitera:  
Extracción de compuestos fenólicos de distintas fracciones de cáscara de girasol (*Helianthus annuus* L.)

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## Abstract

The recovery of antioxidant compounds present in sunflower hulls, a waste byproduct of the oil extraction process, can be of industrial and environmental interest. The objective of the present work was to determine different operating conditions for the extraction of phenolic compounds from hulls oil sunflower seeds, using water at 90 °C and mechanical agitation. To select the experimental conditions, the hulls of a black hull sunflower hybrid (SPS3120) were analyzed, five particle sizes (>0.84, 0.84 to 0.42, 0.42 to 0.25, 0.25 to 0.149, <0.149 mm), three pH values (5, 7 and 9) and samples with and without oil were evaluated. The selected conditions (pH 9, fractions of particle size  $\leq 0.25$  mm- approximately 24 % of the hull, with a prior removal of the oil) were also applied to other three black-oil hybrids (SyN3840, SyN3950, DK4065) and two striped sunflower hybrids (CF201, PAN7077), evaluating the total phenol content and total flavonoid content in the fractions of particle size  $\leq 0.25$  and  $> 0.25$  mm. By processing approximately 23-24 % of the sunflower hull ( $\leq 0.25$  mm particle-size fraction), it was possible to obtain a minimum of 59 and 62% of total phenols and total flavonoids of the total hull, respectively, under the selected conditions.

**Keywords:** sunflower hulls, phenolic compounds, particle size, oil, pH.

## Resumen

La obtención de compuestos antioxidantes presentes en la cáscara de girasol, residuo del proceso de extracción de aceite, puede ser de interés industrial y ambiental. El objetivo del presente trabajo fue determinar diferentes condiciones de proceso para la extracción de compuestos fenólicos de la cáscara de híbridos de girasol, empleando agua a 90 °C y agitación mecánica. Para la selección de las condiciones experimentales, las cáscaras de un híbrido de girasol de cáscara negra (SPS3120) fueron analizadas, cinco dimensiones de partículas (>0.84; 0.84 a 0.42; 0.42 a 0.25; 0.25 a 0.149 y <0.149 mm), tres valores de pH (5, 7 y 9) y muestras con y sin aceite. Las condiciones seleccionadas (pH 9, fracciones de partículas de tamaño  $\leq 0.25$  mm- aproximadamente el 24 % de la cáscara, previa eliminación del aceite de las mismas) fueron también aplicadas a otros tres híbridos de cáscara negra (SyN3840, SyN3950, DK4065) y dos híbridos de cáscara estriada (CF201, PAN7077), evaluando el contenido de fenoles totales y flavonoides en las fracciones de partículas  $\leq 0.25$  mm y  $> 0.25$  mm. Procesando aproximadamente el 23-24 % de la cáscara de girasol (fracción de partículas  $\leq 0.25$  mm) se logró obtener como mínimo el 59 y el 62 % de los fenoles totales y flavonoides del total de la cáscara, respectivamente, en las condiciones operativas aplicadas.

**Palabras clave:** cáscaras de girasol, compuestos fenólicos, tamaño de partícula, aceite, pH.

## 1. Introduction

The growing demand for processed foods has led as a result to a substantial increase in solid wastes (such as leaves, skins, seeds, pits, stems, pulps, pressed cakes, pomace and other lignocellulosic fractions), generating great economic and environmental challenges to food processing plants (Baiano *et al.*, 2014; Angiolillo *et al.*, 2015; Vats, 2017). Baiano (2014) and Angiolillo *et al.* (2015) have reported about the large potential of these wastes as sources of bioactive compounds (alkaloids, anthocyanins, phytosterols, carotenoids, stilbenes, lignans, coumarins, and mainly polyphenols) with applications in the food industry and for manufacturing pharmaceutical products and cosmetic formulations. Thus, their recovery represents a direct and positive economic and environmental impact for the agri-food sector, adding value by optimizing the use of natural resources with minimum food waste generation (Carciochi *et al.*, 2017).

Phenolics compounds are capable of capturing free radicals, donate hydrogen atoms or electrons, or quelate metallic cations, and thus inhibit oxidation. Within this group, polyphenols, which include flavonoids, tannins and phenolic acids, are widely distributed in foods of vegetable origin and represent the majority of the antioxidants present in our diet (Hayat *et al.*, 2009). Further, a number of studies have evaluated phenolic compounds (polyphenols) due to their beneficial effects against chronic and acute medical conditions such as cancer, heart and inflammatory diseases (Balasundram *et al.*, 2006; Taha *et al.*, 2012; Baiano *et al.*, 2014).

The phenolic content of foods depends on the characteristics of the raw material related to intrinsic (genus, species, cultivar) and extrinsic factors (agronomic, environmental, handling and storage conditions) (Balasundram *et al.*, 2006). The quality of the polyphenolic extracts, in terms of antioxidant activity, will also depend both on the chemical structure of its phenolic components and on the extraction method (Nkhili *et al.*, 2009; Rodríguez *et al.*, 2019). Therefore, the selection of the appropriate conditions for extraction and the influence of the type of hybrid studied should be

analyzed with great interest (Hayat *et al.*, 2009). Solid-liquid extraction by solvent is the most commonly used technique to obtain phenolic compounds, using water or solvents such as ethanol, methanol, acetone or ethyl acetate, either concentrated or diluted in aqueous mixtures to reach the polarity of the phenolic compound (Oroian *et al.*, 2015). The structural variety of antioxidant compounds is so wide that there is no single experimental condition that allows to extract all phenolic compounds (Rodríguez *et al.*, 2019). Thus in the literature the extraction procedures vary a lot, and include conditions ranging from room temperature to boiling temperature or reflux, as well as different processing times, various solvents and concentrations, varied sample to solvent ratios, pH, particle sizes and number of extraction steps, which directly affect the extraction efficiency (Nkhili *et al.*, 2009; Oroian *et al.*, 2015). As a result, for each vegetable matrix, it is necessary to select the experimental conditions that allow to maximize the yield and antioxidant activity of the phenolic extracts, and at the same time, minimize the environmental impact (Baiano *et al.*, 2014).

Oilseed sunflower seeds consist mainly of the kernel, where the oil is synthesized, and the hull which represents 17 to 31 % d.b. of the seed (De Figueiredo *et al.*, 2015; Menzel *et al.*, 2019). Prior to oil extraction, the sunflower seeds are partially dehulled, until reaching a 10-12 % range of residual hull (De Figueiredo *et al.*, 2011), producing an important amount of residue of low specific weight (approx. 0.1 ton (m<sup>3</sup>)<sup>-1</sup>). Several authors have examined sunflower hulls, reporting that they contain between 0.7-5.4 % of total phenols present in the seed, with chlorogenic acid being the main component (80 % of phenolic compounds) (Pedrosa *et al.*, 2000; Weisz *et al.*, 2009; Szydłowska-Czerniak *et al.*, 2011). Different studies have been conducted to determine the operating conditions for the extraction of phenolic compounds from sunflower hulls. De Leonardis *et al.* (2005) evaluated different solvents at different pH, while Szydłowska-Czerniak *et al.* (2011) analyzed the effect of the polarity of the solvent, temperature and extraction time on the total phenolic content of extracts obtained from sunflower hulls, before and after an enzymatic treatment of the hulls, finding that the total phenolic content increased linearly with extraction temperature and the polarity of the solvent. Taha *et al.* (2012) studied the optimization of the extraction of a phenolic extract from sunflower hulls by analyzing the variables type of solvent, solvent:water ratio and hull:solvent ratio. Zoumpoulakis *et al.* (2017) analyzed the ultrasound- and microwave-assisted extraction, considering as independent variables the nature of the solvent (methanol, aqueous methanol), solvent volume, temperature and operation time. Rodríguez *et al.* (2019) examined the variables time and temperature for the microwave-assisted extraction (600 W) of phenolic compounds from sunflower hulls using water as solvent. None of these studies analyzed the effect of the particle size. In this respect, Menzel *et al.* (2019) evaluated the milling process of hulls from snack sunflower using two sieves (<0.6 mm and <0.2 mm) and up to three milling steps. They were able to mill about 90 % of the raw material to a size <0.6 mm in two milling stages (with a sieving step prior to the second milling, including in this second stage the particles >0.6 mm), thus increasing by approximately 17 % the phenolic extraction, compared to a single-stage milling.

Sunflower hulls also contain about 3-9 % d.b. of lipids, a part of which is wax (Cancalon, 1971; Rodríguez *et al.*, 2017). No studies were found that analyzed the effect of the prior oil extraction from the hull on the phenolic yield. Taking into account this background, the objective of the present work was to determine different operating conditions (particle size, pH, absence/presence of oil) for the extraction of phenolic compounds from hulls oil sunflower seeds, using water at 90 °C and mechanical agitation.

## 2. Materials and methods

### 2.1 Materials

Hulls of four black-hulls sunflower hybrid were evaluated: SPS3120 (Syngenta, 23.3 % d.b. of hull, hull moisture 12.4 % d.b., oil content of the hull 6.4 % d.b.) SyN3840 (Syngenta, 21.3 % d.b. of hull, moisture 11.7 % d.b., oil content of the hull 9.45 % d.b.), SyN3950 (Syngenta, 24.8 % of hull, moisture 9.5 % d.b., oil content of the hull 4.60 % d.b.) and DK4065 (Syngenta, 22.4 % of hull, moisture 12.1 % d.b., oil content of the hull 8.46 % d.b.). Also, hulls of two striped sunflower hybrids, CF201 (Advanta, 21.0 % d.b. of hull, hull moisture 12.1 % d.b., oil content of the hull 7.73% d.b.) and PAN7077 (Pannar, 21.4 % d.b. of hull, hull moisture 11.1 % d.b., oil content of the hull 5.08 % d.b.), were analyzed. All the sunflower hybrids were grown in Balcarce (37°45'S, 58°18'W), province of Buenos (Argentina). Gallic acid and catechin were purchased from Sigma Chemical Co. (St Louis, MO, USA).

### 2.2 Obtention of hulls fractions

The hulls of all sunflower hybrids were obtained by mechanical dehulling of the seeds (the grains were previously cleaned by manually removing the foreign matter) in a centrifugal dehuller with a peripheral speed of 38.8 m/s (De Figueiredo *et al.*, 2015). The particle size analysis was performed by grinding (Ultracomb MO-8100 grinder, China, 20 pulses) and then sieving the samples considering five different particle sizes (>0.84, 0.84 to 0.42, 0.42 to 0.25, 0.25 to 0.149 and <0.149 mm). The material retained in each sieve (ASTM, Argentina) was weighed, calculating the percentage of each fraction. On the other hand, the % hulls of each hybrid (item 2.1) was determined by manual shelling of 10 g of whole seeds. The hulls and kernel obtained were dried in a forced air circulation oven (Dryinghorn DHG-9123A, China) for 3 hours at 130 °C and then weighed. The test was carried out in duplicate, and the results were determined as the ratio between the weight of the hulls and the total weight of the kernels and hulls, expressed as a percentage.

### 2.3 Selection of the conditions for the solid-liquid extraction of phenolic compounds

For the selection of the experimental conditions, hulls of a traditional hybrid of black-hulls sunflower (SPS3120) were used. The extractions were carried out with 2 g of hulls of hybrid SPS3120 by mechanical agitation for 2.65 h, using distilled water at 90 °C as solvent, which were the optimum conditions found by Szydłowska-Czerniak *et al.*, (2011) for the extraction of phenolic compounds from sunflower hulls, with a sample to solvent ratio of 1:20 (Rodríguez *et al.*, 2019). The phenolic extracts were centrifuged (15 min at 3200 g; Thermo Fisher Scientific, Sorvall Legend X1, Germany) and filtered (quantitative filter paper, black ribbon, pore size 28 µm, Brazil). The filtrate was frozen (-18 °C) and later freeze-dried (-50 °C, 26 Pa, 12 h) (Boyikang Laboratory Instruments Inc FD-1A-50, China) to constant weight (dry extracted product).

**2.3.1 Extraction of phenolic compounds by fraction hulls:** The extractions of phenolic compounds were carried out from 2 g of hulls corresponding to each of the five particle sizes analyzed (>0.84, 0.84 to 0.42, 0.42 to 0.25, 0.25 to 0.149 and <0.149 mm). The assay was performed in triplicate.

**2.3.2 Extraction of phenolic compounds by variation of pH:** Using the selected particle size according to the highest total phenolic content, the influence of pH was evaluated, using 0.2 M solutions of NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O and Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O to reach pH values of 5, 7 and 9 (De Leonardis *et al.*, 2005). The pH values of the solutions were confirmed with a pH-meter. The assay was performed in triplicate.

**2.3.3 Extraction of phenolic compounds in the presence/absence of oil:** Subsequently, using all the selected conditions (particle sizes, pH), the effect of the defatting was then analyzed, after the oil extraction in a Soxhlet apparatus for 6 h using n-hexane as solvent. The assay was performed in triplicate.

## 2.4 Solid-liquid extraction of phenolic compounds from hulls of sunflower hybrids

After selecting the variables of particle size, pH and presence/absence of oil, the extraction of phenolic compounds was carried out using water at 90 °C, by mechanical agitation for 2.65 h, with a sample to solvent ratio of 1:20, from hulls of the three types of black-oil sunflower hybrids and two striped sunflower hybrids. The extracted product, freeze-dried and weighed, was characterized in terms of total phenols and total flavonoids.

**2.4.1 Determination of total phenols:** The effect of the variables of the extraction process (particle size, pH and presence/absence of oil) was evaluated by determining the total phenol content in the obtained extracts using the Folin-Ciocalteu colorimetric method (Rodríguez *et al.*, 2019). The content of total phenols in the hulls of the hybrids of sunflower of black hull and striped hull was evaluated by this technique. The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of hull fraction and of hulls (d.b.).

**2.4.2 Determination of total flavonoids:** The total flavonoid content was determined by the aluminum chloride method in a basic medium, according to the spectrophotometric technique proposed by Molina-Quijada *et al.* (2010). The results were expressed as mg of catechin equivalent (CE) per 100 g of hull fraction and of hulls (d.b.).

## 2.5 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 (Di Rienzo *et al.* 2014). All the tests were carried out in triplicate.

### 3. Results and Discussion

**Table 1** presents the total phenol content of each hull fraction obtained for the hybrid black-hulls sunflower SPS3120. ANOVA analysis allowed to determine statistically significant differences ( $p \leq 0.0001$ ) of total phenol content for the analyzed hull fractions. In general, the total phenol content increased significantly with decreasing particle size, within a range of 389.7 and 1646.2 mg GAE  $100^{-1}$  g hull fraction (d.b.). The two smaller size fractions (0.25-0.149 mm and  $<0.149$  mm) were significantly different from the rest of the fractions and between them, presenting a total phenol concentration that was at least double or triple, respectively, that of the rest. Considering the total phenols extracted from the total sunflower hulls (adding up the fractions, with the content expressed as mg GAE  $100^{-1}$  g hulls d.b.), approximately 51 % of the total phenols obtained from the total hulls was found in the fractions  $\leq 0.25$  mm. It is worth noting that these two fractions comprised approximately 24 % of the hull, which in an industrial process would represent a lower solvent consumption, smaller machines or larger production. Therefore, a particle size smaller than or equal to 0.25 mm (0.25-0.149 mm and  $<0.149$  mm) was selected for this study.

**Table 1.** Selection of particle size for the solid-liquid extraction of phenolic compounds from sunflower hulls (hybrid black-hull SPS3120).

**Tabla 1.** Selección del tamaño de partícula para la extracción sólido-líquido de compuestos fenólicos de cáscaras de girasol (híbrido de cáscara negra SPS3120).

Variables	Levels	Hull yield (%)	Total phenols (mg GAE.100 <sup>-1</sup> g hull fraction d.b.)*	Average of total phenols extracted (mg GAE.100 <sup>-1</sup> g hulls d.b.)
Particle size (mm)	>0.84	7.5 ± 1.2	413.4 ± 17.7 <sup>a</sup>	31.0
	0.84-0.42	35.4 ± 0.6	389.7 ± 7.1 <sup>a</sup>	138.0
	0.42-0.25	33.3 ± 1.5	518.3 ± 19.4 <sup>b</sup>	172.6
	0.25-0.149	13.5 ± 1.0	1387.0 ± 6.2 <sup>c</sup>	187.2
	<0.149	10.3 ± 1.1	1646.2 ± 38.9 <sup>d</sup>	169.6

\*Different letters indicate significant differences ( $p \leq 0.05$ ) between the values of total phenols obtained for each particle size

The process was carried out with a solvent (water) that does not pose any health risk, with easy and flexible handling, and whose efficiency in the extraction of phenolic compounds at 90 °C has been proven by different authors (Paladino, 2008; Szydłowska-Czerniak *et al.*, 2011). The obtained results could be explained by the increase in the interfacial area as the particle size decreased, facilitating the mass transfer and a more efficient solvent-substrate access. The results also suggest the possibility of a non-homogeneous distribution of the phenolic compounds in the structure of the hull, with a larger presence in areas that can break more easily. A similar tendency can be observed when analyzing the data reported by Menzel *et al.* (2019), who evaluated the milling process of hulls from snack sunflowers using two sieves ( $<0.6$  mm and  $<0.2$  mm) and up to three

milling stages. They found that the particles <0.6 mm obtained in the first milling fraction presented a total phenolic content 88 % higher than that of the particles obtained after milling the particles >0.6 mm again, even though they did not report on the size distribution of this second fraction of particles <0.6 mm.

**Table 2** shows the total phenol content for the hull fraction of particle size  $\leq 0.25$  mm of hybrid SPS3120, considering the operating variables pH and presence/absence of oil.

**Table 2.** Selection of the variables pH and presence/absence of oil for the solid-liquid extraction of phenolic compounds from sunflower hulls (hybrid black-hull SPS3120).

**Tabla 2.** Selección de las variables pH y presencia/ausencia de aceite para la extracción sólido-líquido de compuestos fenólicos de cáscaras de girasol (híbrido de cáscara negra SPS3120).

Variables	Levels	Total phenols (mg GAE.100 <sup>-1</sup> g hull fraction d.b.)*
pH**	5	1219.8 ± 51.2 <sup>a</sup>
	7	1422.0 ± 44.3 <sup>b</sup>
	9	1730.9 ± 38.9 <sup>c</sup>
Oil***	W/O	2091.9 ± 48.3 <sup>a#</sup>
	O	1907.2 ± 42.8 <sup>a#</sup>

\*Different letters indicate significant differences ( $p \leq 0.05$ ) between the values of total phenols obtained for each pH level or for the absence/presence of oil;

\*\*Fraction of particle size  $\leq 0.25$  mm;

\*\*\*pH 9, #mg GAE.100<sup>-1</sup> g hull fraction without oil d.b. W/O, hulls without oil; O, hulls with oil

The increase in pH allowed to extract a significantly higher ( $p \leq 0.0034$ ) amount of total phenols, obtaining a maximum of 1730.9 mg GAE 100<sup>-1</sup> g hull fraction (d.b) of size  $\leq 0.25$  mm (equivalent to approximately 412 mg GAE/100 g of hulls d.b.). De Leonardis *et al.* (2005) observed a similar tendency when they studied the influence of pH (5, 7 and 9) on the extraction with water at a lower temperature (25 °C) than used in this work. It is worth noting that the pH increase also results in extracts with higher protein content, which could require a later stage of purification. However, different authors (Rawel *et al.*, 2005; Guimarães Drummond e Silva *et al.*, 2017) have reported on the advantages of the association of proteins and phenolic compounds as potential emulsifiers with antioxidant activity.

The effect of the present/absence of oil was evaluated for the operating conditions sample:solvent ratio of 1:20, particle size  $\leq 0.25$  mm and pH 9. The prior removal of the oil from the hulls allowed to increase the extraction yield of total phenols from 1907.2 to 2091.9 mg GAE 100<sup>-1</sup> g hull fraction. Although from a statistical point of view the increase was not significant, it must be pointed out that the removed oil is another byproduct of this waste from the oil industry. The oil obtained from the sunflower hulls is a potential source of waxes for the pharmaceutical and cosmetic industry, the production of biodegradable films, and foods, among other applications (Carelli *et al.*, 2002). The wax content of the sunflower hybrids used in the present study was determined, obtaining values in a range between 0.64 and 2.08 g wax 100<sup>-1</sup> g hulls d. b. (Rodríguez *et al.*, 2017). Different studies have shown the feasibility of using sunflower waxes as gelling agents, even at low

concentrations, for obtaining organogels, replacing saturated and trans fatty acids of the solid phase in the formulation of products such as margarines (Hwang *et al.*, 2015).

Thus the values selected as the most adequate for the analyzed variables for the extraction of phenolic compounds were: fraction of particle size  $\leq 0.25$  mm (which comprises 24% of the hull), pH 9, with a prior removal of the oil of the hull.

### 3.1 Extraction of phenolic compounds from sunflower hulls under the selected conditions

#### 3.1.1 Black Hulls Sunflower Hybrids

**Table 3** presents the amount of phenolic compounds (total phenolic and total flavonoids) extracted from each hull fraction of the three studied sunflower hybrids. Based on the percentage of each fraction, the concentration of phenolic compounds in relation to total hulls was also calculated.

The smaller particles ( $\leq 0.25$  mm) represented a low percentage (approximately between 23-24 %) compared to the larger particles, in agreement with the methodological conditions. The total phenol content determined for the fractions of smaller particle size ( $\leq 0.25$  mm) varied between 504.2 and 1291.9 mg GAE  $100^{-1}$  g hull fraction (d.b.), with the content for each hybrid being significantly higher ( $p \leq 0.05$ ) than that found for the larger size fractions (in the range of 75.9 and 194.8 mg GAE  $100^{-1}$  g hull fraction (d.b.)). The total flavonoid content varied between 423.5 and 830.2 CE  $100^{-1}$  g hull fraction (d.b.) for particle sizes  $\leq 0.25$  mm, and between 66.6 and 75.2 CE  $100^{-1}$  g hull fraction (d.b.) for particle sizes  $> 0.25$  mm, showing that the total flavonoid content was significantly higher ( $p \leq 0.05$ ) when the extractions were carried out with particles  $\leq 0.25$  mm.

**Table 3.** Phenolic compounds extracted from the hull of different sunflower hybrids (black-hull) grown in Argentina (solid-liquid extraction, mechanical agitation).

**Tabla 3.** Extracción de compuestos fenólicos de cáscaras de girasol de diferentes híbridos (cáscara negra) cultivados en Argentina (extracción sólido-líquido, agitación mecánica).

Hybrids	Particle size (mm)	Hull yield (%)	Total phenols	Total flavonoids	Total phenols	Total flavonoids
			*Hull fraction		**Hulls (calculated)	
SyN3840	$\leq 0.25$	23.7	845.7 $\pm$ 26.3 <sup>b</sup>	627.5 $\pm$ 3.8 <sup>b</sup>	200.4	148.7
	$> 0.25$	76.3	181.6 $\pm$ 0.5 <sup>a</sup>	66.6 $\pm$ 4.6 <sup>a</sup>	138.6	50.8
SyN3950	$\leq 0.25$	23.2	1291.9 $\pm$ 76.5 <sup>b</sup>	830.2 $\pm$ 94.9 <sup>b</sup>	299.7	192.6
	$> 0.25$	76.8	194.8 $\pm$ 15.6 <sup>a</sup>	74.7 $\pm$ 4.2 <sup>a</sup>	149.6	57.4
DK4065	$\leq 0.25$	22.8	504.2 $\pm$ 72.3 <sup>b</sup>	423.5 $\pm$ 87.2 <sup>b</sup>	115.0	96.6
	$> 0.25$	77.2	75.9 $\pm$ 13.2 <sup>a</sup>	75.2 $\pm$ 21.6 <sup>a</sup>	58.6	58.1

d.b., dry basis

Different letters indicate significant differences ( $p \leq 0.05$ ) between particles sizes of each hybrid

\*Total phenols (mg GAE. $100^{-1}$  g hull fraction d.b.), \*Total flavonoids (mg CE. $100^{-1}$  g hull fraction d.b.)

\*\*Total phenols (mg GAE. $100^{-1}$  g hulls d.b.), \*\*Total flavonoids (mg CE. $100^{-1}$  g hulls d.b.)



By comparing the fractions of smaller size (particles  $\leq 0.25$  mm), a significant difference was detected between the hybrids, both in total phenolic content and total flavonoid content ( $p \leq 0.0025$  and  $p \leq 0.0276$ , respectively). As for total phenols, all the hybrids differed, with the fraction of hybrid SyN3950 presenting the highest value, followed by SyN3840 and DK4065, in decreasing order. This fraction of SyN3950 was also characterized by a higher total flavonoid yield, but without being significantly different from SyN3840 (Fig. 1A). On the other hand, in the fractions of larger particle size ( $> 0.25$  mm), ANOVA only revealed significant differences between hybrids for total phenolic content ( $p \leq 0.0037$ ), with hybrid DK4065 exhibiting the lowest value. The other two hybrids were not significantly different (Fig. 1B).

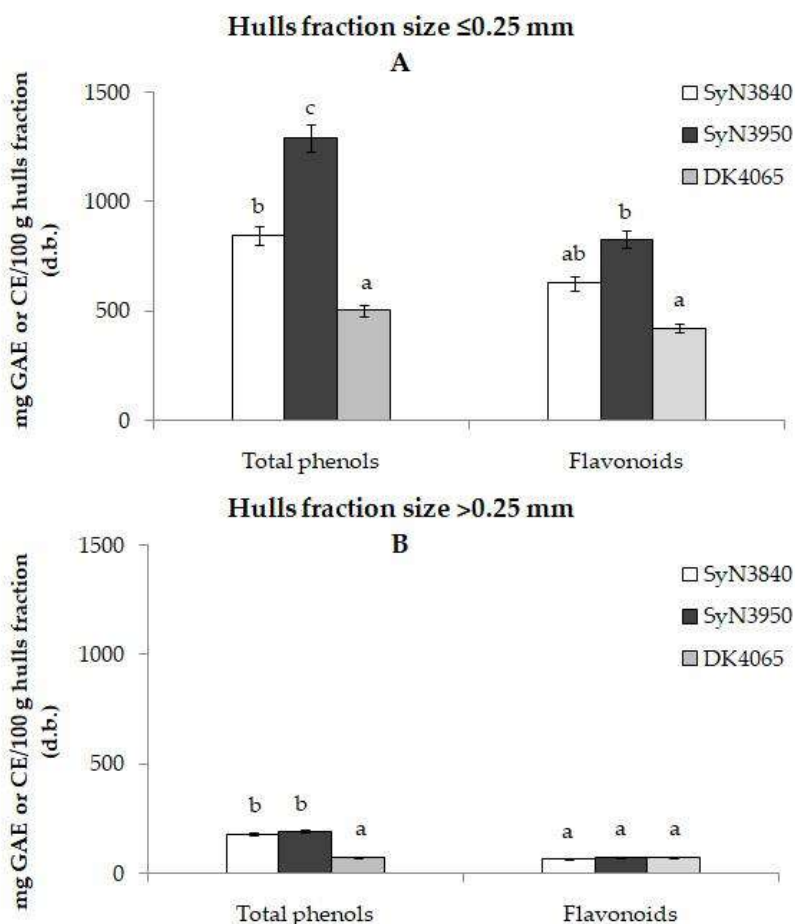


Fig. 1. Total phenols and total flavonoids in sunflower hulls fractions (black-hull). A: Size  $\leq 0.25$  mm, B: Size:  $> 0.25$  mm.

Fig. 1. Fenoles totales y flavonoides totales en fracciones de cáscaras de girasol (cáscara negra). A: Tamaño  $\leq 0.25$  mm, B: Tamaño:  $> 0.25$  mm.

Although the statistical analysis allowed finding significant differences between the hybrids studied. It is worth noting that Rodriguez *et al.* (2019), when evaluating the total phenol and total flavonoid contents in sunflower hulls using a microwave-assisted extraction process (water at 90 °C, 10 min, 600 W, sample:solvent ratio of 1:20, particle size of the hull  $\leq 0.42$  mm), did not observe significant differences between these three hybrids grown in two districts in the Buenos Aires province, Argentina.

By processing the fractions of particle size  $\leq 0.25$  mm by the selected method, it was possible to obtain approximately 59 %, 67 % and 66 % of total phenols from the hulls of hybrids SyN3840, SyN3950 and DK4065, respectively, and approximately 75 %, 77 % and 62 % of total flavonoids, respectively (values calculated based on the last 2 columns of **Table 3**). Therefore, by processing only between 22.8 and 23.7 % of the hull of these hybrids (particle size 0.25mm) by mechanical extraction (agitation), a minimum of 59 % of total phenols and 62 % of total flavonoids was extracted, in relation to total hulls, with a maximum yield of 67 % and 77 %, respectively.

### 3.1.2 Striped Hulls Sunflower Hybrids

Two striped-hull sunflower hybrids, CF201 and PAN7077 were also evaluated. While hybrid CF201 exhibited similar tendencies to those observed for the black oil hybrids (**Table 4**), although with lower relative yield percentages (53 % and 66 % for total phenols and total flavonoids, respectively, obtained from the fraction of particle size  $\leq 0.25$  mm), the hybrid PAN7077 did not present differences in the concentration of total phenols between the fractions, indicating that only 30 % of total phenols were extracted from the smaller size fraction, while the larger proportion of total flavonoids was the same (60 % of total flavonoids of the hull). These results would suggest a differential behavior of the striped sunflower hybrids, with further studies being necessary to examine a larger number of hybrids and evaluate possible relations with morphological differences of the hull (Lindström *et al.*, 2000).

**Table 4.** Phenolic compounds extracted from hulls of two sunflower hybrids (striped hull) grown in Balcarce, Argentina (solid-liquid extraction, mechanical agitation).

**Tabla 4.** Extracción de compuestos fenólicos de cáscaras de dos híbridos de girasol (cáscara estriada) cultivados en Balcarce, Argentina (extracción sólido-líquido, agitación mecánica).

Property	Hybrid CF201		Hybrid PAN7077		
	$\leq 0.25$	$> 0.25$	$\leq 0.25$	$> 0.25$	
Hull yield (%)	30.8	69.2	24.5	75.5	
Total phenols	*Hull fraction	437.7 $\pm$ 17.8 <sup>b</sup>	171.9 $\pm$ 40.6 <sup>a</sup>	230.6 $\pm$ 24.5 <sup>a</sup>	176.8 $\pm$ 9.1 <sup>a</sup>
Total flavonoids		436.6 $\pm$ 48.9 <sup>b</sup>	99.1 $\pm$ 0.1 <sup>a</sup>	369.5 $\pm$ 2.1 <sup>b</sup>	78.6 $\pm$ 2.1 <sup>a</sup>
Total phenols	**Hulls	134.8	119.0	56.5	133.5
Total flavonoids		134.5	68.6	90.5	59.3

Different letters indicate significant differences ( $p \leq 0.05$ ) between particles sizes of each hybrid;

\*Total phenols (mg GAE.100<sup>-1</sup> g hull fraction d.b.), \*Total flavonoids (mg GAE.100<sup>-1</sup> g hull fraction d.b.);

\*\*Total phenols (mg GAE.100<sup>-1</sup> g hulls d.b.), \*\*Total flavonoids (mg CE.100<sup>-1</sup> g hulls d.b.);

d.b., dry basis

Hemery *et al.* (2011) and Laguna *et al.* (2018) reported that the ultrafine milling with electrostatic sorting or turbo separation are new eco-friendly and energy-efficient technologies that are being studied for the concentration of components of different agricultural resources, such as proteins, cellulose, lignin and polyphenols. Laguna *et al.* (2018) reported that with an adequate combination of these technologies it is possible to recover fractions rich in proteins and phenolic compounds from residual meals of sunflower and canola oil extraction processes. Proteins were separated together with the phenolic compounds, and thus dry fractionation constitutes a pre-purification process, prior to solid-liquid extraction. Taking into account the results obtained in the present work, further studies are desirable to determine the potentiality of applying these technologies to the extraction of phenolic compounds from sunflower hulls with distinguishing features.

The type and amount of phenolic compounds present in the sunflower hulls and in the extracts obtained from the hulls, as well as the antioxidant properties of the extracts, have been reported by various authors (De Leonardis *et al.*, 2005; Weisz *et al.*, 2009; Szydłowska-Czerniak *et al.*, 2011; Taha *et al.*, 2012; Zoumpoulakis *et al.*, 2017; Rodríguez *et al.*, 2019). Taha *et al.* (2012) also found that phenolic extracts of sunflower hulls exhibited antimicrobial activity at different levels against different pathogenic bacteria, as well as anti-carcinogenic activity, which differed between the cell line carcinomas. Rodríguez *et al.* (2019) determined and compared the antioxidant activity of extracts of phenolic compounds (microwave-assisted extraction with water as solvent, 90 °C) from hulls of the sunflower hybrids studied in the present work, grown in two districts in the Buenos Aires province, Argentina. Further studies are necessary to determine the impact of the dry fractionation proposed in this work.

## 4. Conclusions

The extraction of phenolic compounds from hulls of sunflower hybrids by solid-liquid extraction using water at 90 °C, mechanical agitation for 2.65 h and a sample:solvent ratio of 1:20 was evaluated, selecting as the most adequate experimental conditions a particle size  $\leq 0.25$  mm, pH 9, with prior removal of the oil from the hulls. Between 23-24 % of hulls of particle size  $\leq 0.25$  mm and between 76-77 % of size  $> 0.25$  mm were obtained by grinding the hulls of the hybrids under study. It was possible to extract between 59-67 % of total phenols and 62-77 % of total flavonoids from the fraction of smaller size, with respect to total hulls, observing differences in behavior between the hybrids. The present work shows the advantages of combining the grinding process with an adequate classification of the obtained product, which would allow to reduce the material to be extracted, obtaining a good yield of phenolic compounds for their potential application in the formulation of functional foods, or in the pharmaceutical and nutraceutical industries. The differential behavior observed between the hybrids also suggests the need to evaluate and select adequate strategies to process and add value to the hulls of sunflower hybrids with differential structural characteristics.

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## Interest conflict

All the authors declare that they have no conflict of interest and have no competing financial interest for the work covered in this paper.

## Nomenclature

<i>d.b.</i>	dry base
<i>g</i>	relative centrifugal force
<i>GAE</i>	gallic acid equivalent
<i>CE</i>	catechin equivalent

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