Extraction of free and bound phenolics from glabrous canary seed (Phalaris canariensis L.) hulls

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ABSTRACT The aim was to extract free phenolics using a green solvent (ethanol), and bound phenolics from glabrous canary seed hulls. The efficiency of ethanol 75 % was compared with that of other solvents used in the literature (acetone 70 %; methanol 80 %) in terms of phenolic yield and antioxidant activity. Bound phenolics were extracted with ethyl acetate, prior to hydrolysis with NaOH 2 M. The phenolic extracts obtained were characterized according to extraction yield, total phenols, flavonoides, antioxidant activity, radical DPPH; and phenolic profile, HPLC. Methanol 80 % had lower extraction yield, and acetone 70 % had lower antioxidant activity; whereas ethanol 75 % was the most efficient solvent to obtain free phenolics. Extraction yield of bound phenolics (6411.13 mg/100 g hulls d.b.) doubled the value of free phenolics. Thus, antioxidant activity was four times higher (78.67 % inhibition of radical DPPH). On the other hand, phenolic acids predominated over flavonoids compounds in a rate 26/1. Ferulic acid was the dominant (42.48 mg/100 g hulls), followed by p-coumaric (39.07 mg/100 g hulls). Although the use of glabrous canary seed is new, interest in its processing at industrial level makes canary seed hulls a potential waste material that could be leveraged as a source of bioactive compounds with several applications.

KEYWORDS Phenolic; Antioxidant activity; Ethanol; Canary seed hulls

1. Introduction

Canary seed, as well as wheat, oat, barley and rice, belongs to the grass family of Poaceae (Cogliatti et al., 2014). Canary seed contains an endosperm and a germ covered with two layers called glumelas or hulls (Cogliatti et al., 2014). It is an annual crop grown primarily in America (80.1 % of the world population), followed by Asia (17.8 %) and Oceania (2.1 %), with a production in 2019 of 240,344 tonnes, from which 61.4 % corresponds to Canada, 17.1 % to Argentina, 16.2 % to Thailand, and the remaining 5.3 % to other countries (FAO, 2021).

In the past, canary seed was almost solely used to feed caged birds, alone or mixed with other grains such as millet, sunflower seed and flaxseed (Chen et al., 2016). Its use is limited since the hulls are covered with spicules (or hair), which are small siliceous trichomes that make canaryseed unsafe for human consumption due to potential health risks. It has been linked to esophageal cancer and severe skin irritation (Abdel-Aal et al., 2011a). The reason why birds are not affected is that they dehull the seeds prior to ingestion (Li and Beta, 2012; Chen et al., 2016). In 1997, however, "CDC Maria" was registered in Canada as the first hairless cultivar (glabrous seeds), eliminating trichomes through mutagenesis. Currently, two other varieties have been developed, "CDC Togo" and "CDC Bastia" (Abdel-Aal et al., 2011a; Chen et al., 2016). Since then, Abdel-Aal et al. (2010, 2011b,a), Li et al. (2011), Li and Beta (2012), Cogliatti et al. (2014), Chen et al. (2016) and Abdel-Aal (2021) have been researching on the biological value of glabrous seed and its possible use in the preparation of food for human consumption, animal feeding, medicine, and other industrial applications. The main components of glabrous seed are approximately 60 % starch, 20 % protein, 7 % total dietary fibre, 8 % crude fat, and 2 % ashes (Abdel-Aal et al., 2010; Li and Beta, 2012). It was also found to contain phytochemicals, including phytates, phenols, tannins, carotenoids, and enzyme inhibitors. It is also an important source of vitamins and minerals (Chen et al., 2016).

When compared to other cereal crops, canary seed contains high levels of gluten-free proteins, suitable to elaborate food safe for celiac patients (Abdel-Aal, 2021), as well as high levels of amino acid tryptophan and bioactive peptides that show beneficial health effects in humans (Abdel-Aal et al., 2010; Abdel-Aal, 2021). The use of canary seed flour in a wide variety of foods (bread and tortillas, crackers, muffins, pasta, cereal bars, and spaghetti) has been assessed with promising results, showing that canary seed flour could replace up to 35 % of wheat flour, without affecting the products quality (Cogliatti et al., 2014). Canary seed starch has also generated interest due to the granule's small and uniform size, compared to that of wheat and corn. Hairless canary seed starch is able to form strong and stable gels under refrigerated storage

DOI: 10.52292/j.laar.2024.1268
Received: October 21, 2022.
Accepted: April 22, 2023.
Recommended by Diego Lomonaco.

and freeze-thaw treatments (Abdel-Aal, 2021). These benefits have allowed for its use as thickener, colloidal stabilizers and gelling agents in the food industry, as well as in the pharmaceutical and textile industries (Abdel-Aal et al., 2010). Canary seed oil content is higher than in other cereals such as corn (4-5 %) and it belongs to the oil group "oils low in palmitic acid and high in oleic and linoleic acids" with a high proportion of unsaturated fats (Abdel-Aal et al., 2010; Abdel-Aal, 2021).

In relation to phytochemicals, some authors (Abdel-Aal et al., 2011b; Li et al., 2011; Li and Beta, 2012; Chen et al., 2016) have studied the composition of antioxidant compounds (phenolic acids and carotenoids) and other bioactive constituents, both in hairy and hairless (glabrous) varieties. From the phenolic compounds in canary seed, ferulic, caffeic and p-coumaric acids are the main constituents and, as in other cereals, a higher proportion is found in their hulls and covalently bound to cell structural compounds, representing 80 % of the total of phenolics, whereas only 2-3.5 % is found in their free form. Free phenolic acids (soluble) are extracted with organic solvents, while bound phenolic acids (soluble and insoluble) require acid or base hydrolysis to be released from the cell matrix (Abdel-Aal et al., 2011a). Within the organic solvents used to extract free phenolics in cereals where ferulic acid prevails, such as in millet, rice and canaryseed, distilled water or aqueous mixes (ethanol, methanol) and acetone have been used (Chandrasekara and Shahidi, 2011; Li et al., 2011; Alves et al., 2016). Currently, ethanol is one of the most promising solvents used to extract phenolic compounds as it is economical, reusable and non-toxic, and the extracts obtained can be used in the food industry (Vadivel and Brindha, 2015).

Although the use of canaryseed for human consumption is emerging, the high biological value of the dehulled seed (80 % the total seed/groat weight) makes this crop interesting for its processing, although there is little information on its industrial use. Recently, Durruty et al. (2017a) have optimized the dehulling process of canaryseed "CDC Maria". It was reported that the rate of impact of 36.7 m/s and 10 % seed moisture content d.b. constituted the experimental conditions that allowed for maximum values of suitability to dehulling (around 94 %) with a resulting elimination of 98 % of the hull. On the other hand, the hull remains as a potential residue (20 % of the total seed weight) from the use of the grain, apart from being the main source of bioactive compounds of this crop. It has become necessary to study the experimental conditions that allow increasing the extraction efficiency of phenolic compounds in terms of quantity and quality (high antioxidant activity) and simultaneously, decrease the environmental impact through the use of environmentally-friendlier solvents. Therefore, the aim of this work was to extract free phenolics using a green solvent (ethanol), and bound phenolics from glabrous canary seed hulls.

2. Methods

2.1 Sample

Canary seeds from the cultivar "CDC Maria" were obtained from a company settled in Olavarría (Province of Buenos Aires, Argentina). The seeds were cleaned manually and the foreign matter was removed, hulls were obtained using mechanical dehulling of whole grains at 3700 rpm (28.8 m/s), with a pilot-scale centrifugal dehuller (Durruty et al., 2017a). Hulls (moisture 8.11 ± 0.02 d.b. and oil content 0.32 ± 0.04 % d.b.) were preserved in hermetically sealed recipients at 5 °C. Prior to experiments, milling was done using an Ultracomb MO-8100 Mill, China. Hulls were sieved to reach a size particle lower than 0.42 mm (mesh size 40, ASTM, Argentina).

2.2 Characterization of glabrous canary seed grain

Canary seed grains were characterized according to the grain general parameters, such as length, width, thickness, % hull, % groat, ratio hull/groat, average weight of the groat. In relation to its proximal composition, moisture, oil content, crude protein, crude fibre and ashes. Carbohydrate content was estimated by difference, as nitrogen-free extract.

2.3 Extraction of phenolic compounds

Free phenolic and bound compounds were extracted according to the methods of Chen et al. (2016) in canary seed grains of the same cultivar, with minor changes.

2.3.1 Free phenolics (soluble)

The effect of the solvent on the solid-liquid extraction of phenolic compounds by mechanical stirring was analyzed. A gram (1 g) of milled canary seed hulls without oil was weighed. Lipids were washed twice with n-hexane for removal in a ratio hull/solvent 1/10 (w/v), stirring (150 rpm, 15 min) (Shaker M-23, V-VICKIN, Argentina), under dark conditions and at room temperature. Then, the hulls were separated from the micelle (n-hexane+oil) through centrifugation (15,120 × g, 10 min) (Thermo Fisher Scientific, Sorvall Legend X1, Germany).

Aqueous solutions of solvents were used for phenolic extraction, as reported by other authors for "CDC Maria" canary seed and other cereals with prevailing ferulic acid (rice, millet), such as 80 % methanol (v/v) (Abdel-Aal et al., 2011b; Alves et al., 2016; Chen et al., 2016) and 70 % acetone (v/v) (Chandrasekara and Shahidi, 2011; Li et al., 2011; Alves et al., 2016), which were compared with an environmentally-friendlier solvent such as 75 % ethanol (Vadivel and Brindha, 2015). Three consecutive extractions were carried out with a ratio sample/solvent 1/10 (w/v), by stirring (150 rpm, 1 h), under dark conditions and at room temperature (≈ 25 °C). Supernatants were separated through centrifugation (15,120 × g, 10 min) and sieved (pore size 28 µm, J. Prolab, Brazil), combined and evaporated to dryness under vacuum at 40 °C (rotavapor R-114, Büchi, Switzerland). Then, the extracts were frozen and lyophilized (-50 °C, 26 Pa, 12 h) (Boyikang Laboratory Instruments Inc FD-1A-50, China).

2.3.2 Bound Phenolics (insoluble)

Bound phenolics were obtained after extraction of free phenolics. To release bound phenolics, alkaline hydrolysis was carried out with NaOH 2 M, ration hull/solvent 1/20 (w/v) for two hours, by stirring (150 rpm), at room temperature and under dark conditions. Later, pH was adjusted to the mixture with HCl 6 M to reach a value ≤ 2, then centrifuged (15,120 $\times$ g, 10 min) and sieved to separate the solvent from the supernatant. From this, three extractions were done with ethyl acetate, ratio hull/solvent 1/20 (w/v), through stirring (150 rpm, 15 min), at room temperature and under dark conditions. Supernatants were collected and combined, and the resulting mixture was evaporated to dryness under vacuum. Then, the phenolic extracts were frozen and lyophilized (Durruty et al., 2017b).

2.4 Determination of phenolic compounds and antioxidant activity

To determine extract yield, total phenols, flavonoids and antioxidant activity, the extracts obtained were reconstituted in 15 mL of the solvent used to extract free phenolics. Each determination was performed in triplicate, using the spectrophotometer V-1800 (PC), Shangai Mapada Instruments Co., Ltd, (China).

2.4.1 Extract yield

Extract yield was determined gravimetrically through weighing of the lyophilized phenolic extracts in relation to the initial hull mass expressed as mg of phenolic extract per 100 grams of hulls d.b.

2.4.2 Total phenols

A calibration curve was done from a pattern solution of ferulic acid (1600 ppm) (Sigma Aldrich, USA) following the guidelines in Rodríguez et al. (2019). Absorbance was measured at 760 nm and the results were expressed as milligrams of ferulic acid per 100 g of hulls and phenolic extract d.b.

2.4.3 Flavonoids

The method of aluminium trichloride in basic medium was used, following the guidelines of Rodríguez et al. (2019). The calibration curve was done from a pattern solution of catechin (200 ppm) (Sigma Aldrich, USA). Absorbance was measured at 510 nm and the results were expressed as milligrams of catechin per 100 g of hulls and phenolic extract d.b.

2.4.4 Antioxidant activity

The antioxidant activity was evaluated through the radical DPPH (Rodríguez et al., 2019). The DPPH initial absorbance was determined at 517 nm and the resulting after contact with the samples for 30 minutes. The results were expressed as the inhibition percentage of the initial concentration of the radical DPPH.

2.5 Phenolic acids profile

The phenolic acids profile in canary seed hulls was quantified according to the method described by Ross et al. (2009) using a Dionex Ultimate 3000 chromatograph (Thermo Scientific, Germany) with detector of variable wavelength (UV-Visible Hewlett Packard HP 1050 series, Germany). Separation was carried out in a column Hichrom Lichrosorb RP18-5, 5 µm particle size, 250 $\times$ 4.6 mm i.d. column and methanol and formic acid at 0.1 % was used as mobile phase. Elution by linear gradient of 0-41 minutes and isocratic elusion of 41-60 minutes were used with a Flow rate of the mobile phase was 1 mL/min. Calibration curves were done with the standards of caffeic, p-coumaric and ferulic acids at λ of 325 nm in a range of concentrations of 0-200 µg/mL, with retention times of 18.16, 22.90 and 25.40 minutes, respectively. Phenolic extracts were suspended in 2 mL in ethanol/water mixture 50/50 (v/v) and the phenolics acids were expressed as in 100 g of hulls and phenolic extract d.b.

2.6 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) and the Tukey Test was used for mean comparison. Statistical analysis was carried out with a significance level defined at 5 %, using InfoStat Software.

3. Results

3.1 Characterization of Canary seed grain

Table 1 shows the results of "CDC-Maria" canary seed in relation to general parameters of grains and their proximal composition. As for the values obtained in length measurement, these were similar to the ones reported by (Striebeck et al., 2015) for length, width and thickness (4.94 mm, 1.93 mm, 1.48 mm, respectively) in canary seed grains of the same cultivar. The percentage of hulls obtained was lower (35 %) than that reported by (Abdel-Aal et al., 1997). Therefore, it can be estimated that the hull/groat rate obtained by the authors (0.54) would be higher than the one in this work, while the average weight of canary seed grains was similar to that reported by the authors (7 mg) for the "CDC Maria" cultivar. As for proximal composition, canaryseed grain has a high content of proteins compared to other cereals, showing its potential for the food industry (Li and Beta, 2012). The value obtained in this study was similar to the lowest level of the range reported by (Abdel-Aal et al., 2010) for the same cultivar (15.6-25.6 %) and lower than the one reported by (Abdel-Aal, 2021) in canary seed for human consumption. As for the free nitrogen extract, it shows a high content of starch, but slightly lower than that obtained by (Abdel-Aal, 2021) (57.2 %). As for oil content, a lower value than that reported by (Abdel-Aal et al., 2010; Abdel-Aal, 2021) was obtained for the same cultivar (7.6-8.9 % y 7.7 %, respectively). However, oil content was higher than that of other cereals, such as corn (4-5 %) (Kwiatkowski and Cheryan, 2002) and wheat (2.5 %) (Abdel-Aal, 2021). The value of crude fibre obtained was higher than the average value reported by Abdel-Aal et al. (2010); Abdel-Aal (2021) for the same cultivar (6.6 % and 5.9 %, respectively).

3.2 Extraction and quantification of free and bound phenolic compounds

3.2.1 Solvent selection for free phenolic extraction

Table 2 shows yield of free phenolics from canary seed hulls using acetone, methanol and ethanol as solvents.

From these, 75 % ethanol was significantly different

Table 1: Characterization of canary seed whole grains "CDC Maria".

	Properties	Grain "CDC Maria"
General parameters	Length (mm)	4.80±0.18
	Width (mm)	1.95±0.19
	Thickness (mm)	1.41±0.08
	Content of hull (%)	19.82±0.17
	Content of groat (%)	80.18±0.17
	Hull/groat rate	0.25±0.003
	Average weight of the grain (mg)	7.28±0.27
Proximal Composition (% d.b.)	Moisture	10.47±0.02
	Crude protein	15.77±0.21
	Ashes	7.60±0.10
	Oil	6.51±0.04
	Crude fibre	7.00±0.01
	NFE	52.40

Table 2: Comparison of free phenolic extraction solvents in "CDC Maria" canary seed hulls.

Solvents (v/v)	Yield of free phenolics (mg phenolics/100 g hulls d.b.)
Acetone 70 %	2204.89±380.15^{ab}
Methanol 80 %	1697.98±198.42^{a}
Ethanol 75 %	3055.84±203.38^{b}

Different letters on the same column show significant differences ($p \leq 0.05$) among the solvents according to the Tukey Test.

($p \leq 0.0344$) from methanol 80 % due to lower phenolic yield. It is worth mentioning that the other experimental conditions were the same for the three solvents. Therefore, 70 % acetone and 75 % ethanol were selected to continue with the study.

Table 3 shows the comparison in yield results of antioxidant activity and extraction using as solvents acetone 70 % and ethanol 75 %. It is important to mention that even if these solvents are only used to extract free phenolics, they also influence on the extraction of bound phenolics, which occurs after free phenolics extraction. There were no significant differences ($p > 0.05$) in solvents for the extraction yield, both for free and bound phenolics. On the other hand, extraction yield of bound phenolics was significantly higher than that of free phenolics ($p \leq 0.0376$ and $p \leq 0.0447$) both for acetone and ethanol, respectively. Differences of 100 % between both extracts were observed, in agreement with the results reported in the literature, which show that phenolic compounds in canary seed are mostly in their bound form, as in other cereals (Chandrasekara and Shahidi, 2011; Li et al., 2011).

Antioxidant activity varied significantly in solvents ($p \leq 0.0017$ and $p \leq 0.0005$) both for free and bound phenolics, respectively. When 75 % ethanol was used, it was higher in both cases. As for the type of phenolics, antioxidant activity was significantly higher in bound phenolics both for assays with acetone ($p \leq 0.0250$) and with ethanol ($p \leq 0.0002$).

From the results obtained, it is highlighted that phenolics in canary seed hulls are mostly in bound form, and the antioxidant activity was higher when 75 % ethanol was used. This solvent was selected to continue with this study in relation to 70 % acetone. In addition, it can be observed that there is a high positive linear correlation between extraction yield and the antioxidant activity ($p \leq 0.0249$; $R^2 = 0.9751$) of phenolics obtained with 75 % ethanol.

3.2.2 Total phenolic content and flavonoids in bound phenolics

Table 4 shows the results of the characterization of free and bound phenolics obtained with 75 % ethanol in relation to total phenolic and flavonoid content for hulls and phenolic extracts. It is observed that bound phenolics presented significantly higher values than free phenolics, in relation to total phenols and flavonoid content for canary seed hulls ($p \leq 0.0001$ and ≤ 0.0001) and phenolic extracts obtained from them ($p \leq 0.0002$ and $p \leq 0.0053$). Therefore, it is worth mentioning that the values of total phenolics and flavonoids of bound phenolics for hulls were 12 and 4 times higher than those of free phenolics, respectively. Likewise, when the results were expressed for phenolic extracts, values of total phenols and flavonoids of bound phenolics were 6 and 2 times higher in relation with free phenolics. These results agree with those reported by Li et al. (2011) and (Abdel-Aal et al., 2011a), who show that phenolics in canary seed are moslty in their bound form.

In addition, from the total of phenolics (free+bound), it can be observed that the total phenolic content was 26 times higher than that of flavonoids in the results expressed in relation to hulls, and 12 in terms of phenolic extracts. In both cases, the total phenolic ratio was three times higher than flavonoids in bound phenolics compared to free phenolics. Therefore, in "CDC Maria" canary seed hull, bound phenolics prevail compared to free phenolics, mostly formed by phenolic acids and, to a lower extent, by flavonoids. On the other hand, there is a high positive linear correlation between total phenolic content and flavonoids, expressed both in terms of the hull ($p \leq 0.0001$; $R^2 = 0.9998$) and the extracts ($p \leq 0.0525$; $R^2 = 0.9864$), and also between these and the antioxidant activity in relation to the hulls ($p \leq 0.0003$; $R^2 = 0.9991$ and $p \leq 0.0001$; $R^2 = 0.9997$) and to the extracts ($p \leq 0.0004$; $R^2 = 0.9989$ and $p \leq 0.0004$; $R^2 = 0.9879$), respectively.

Total free phenolic content was higher than that reported by Li et al. (2011) in "CDC Maria" canary seed glabro hulls (360-450 mg equivalent of ferulic acid/100 g hulls), using 95 % ethanol acidified with HCl 1 N (85/15 v/v), and by Liyana-Pathirana and Shahidi (2007) in wheat hulls (228-344 mg equivalent of ferulic acid/100 g hulls), using 80 % v/v ethanol. In contrast, the results obtained were lower than those obtained by Vadivel and Brindha (2015) (1,342 mg equivalent of ferulic acid/100 g hulls) in rice hulls. These authors used the same experimental conditions but at 30 °C. However, similar results to those in this study were obtained in terms of the content of total

Table 3: Comparison between phenolic yield and antioxidant activity in "CDC Maria" canary seed hulls using acetone 70 % and ethanol 75 %.

Solvents	Extraction yield (mg phenolics/100 g hulls d.b.)		Antioxidant activity (% of inhibition of DPPH radicals)	
	Free phenolics	Bound phenolics	Free phenolics	Bound phenolics
Acetone 70 %	2204.89±380.15^{a1}	4007.78±328.29^{a2}	12.08±0.49^{a1}	26.53±1.06^{a2}
Ethanol 75 %	3055.84±203.38^{a1}	6411.13±1013.75^{a2}	20.60±0.03^{b1}	78.67±1.18^{b2}

Different letters on the same column show significant differences ($p \leq 0.05$) among solvents according to the Tukey Test.
Different numbers on a same row show significant differences ($p \leq 0.05$) among the types of phenolic extract according to the Tukey Test.

Table 4: Content of free and bound phenolics of the "CDC Maria" canary seed cultivar using ethanol 75 %.

Phenolics	Total phenols		Flavonoids	
	mg equivalent of ferulic acid/100 g hulls d.b.	mg equivalent of ferulic acid/100 g extract d.b.	mg equivalent of catechin/100 g hulls d.b.	mg equivalent of catechin/100 g extract d.b.
Free	645.15±1.10^{a}	10578.33±686.04^{a}	63.97±0.46^{a}	2098.56±154.58^{a}
Bound	7598.71±26.84^{b}	66719.05±235.70^{b}	249.46±1.18^{b}	4136.39±142.19^{b}
Total	8243.86	77297.38	313.43	6234.95

Different letters on the same column show significant differences ($p \leq 0.05$) among phenolics according to the Tukey Test.

bound phenolics in rice hulls (7.972 mg equivalent of ferulic acid/100 g hulls), using also the same experimental conditions. Chandrasekara and Shahidi (2011) also analyzed total free phenolics in two varieties of millet (Kodo and Pearl) through extraction with 70 % (v/v) acetone. In the first case, the values were higher (2.175 mg equivalent of ferulic acid/100 g hulls) and similar in the second case (666 mg equivalent of ferulic acid/100 g hulls) to those shown in Table 4.

Several total phenolic contents are reported in the literature. However, the comparison with other cereals becomes difficult as the results depend directly on the matrix considered, the extraction method and experimental conditions, the method for quantification, standards and basis used to express results. Results expressed in terms of the phenolic extracts obtained were not found. Since there is no information available on the determination of flavonoids in cereal hulls using the method described in this work, comparison with the data obtained was not possible.

3.3 Phenolic acids profile

Table 5 shows the three phenolic acids mostly identified in canary seed hulls according to the literature (Abdel-Aal et al., 2011a; Li et al., 2011). p-coumaric and caffeic acids are observed in free phenolics, while p-coumaric and ferulic acids are present in bound phenolics (Fig. 1). No significant differences ($p > 0.05$) were found either in the results expressed for hulls or phenolic extracts. Likewise, Li et al. (2011) did not find significant differences ($p > 0.05$) in these three acids in bound phenolics of canary seed hulls "CDC Maria". However, significant differences ($p > 0.05$) were observed in the contents of p-coumaric present as free and bound, being significantly higher as bound both in the results expressed for the hulls ($p \leq 0.0010$) and for the extracts ($p \leq 0.0390$). From the total of the phenolics analyzed, bound phenolics are present 123/1 in relation to free phenolics expressed in 100 g of hulls and 80/1 in 100 g of phenolic extract.

Insoluble bound phenolic compounds are predominant in cereals compared to soluble free or bound com-pounds, attaining values of 80-90 % of the total of phenolic acids in brown rice (Zhou and Yu, 2004), 73 % in barley (Andersson et al., 2008), 80 % in wheat, and 90 % in canary seed (Abdel-Aal et al., 2011a). This study showed that for free phenolics, contents of p-coumaric duplicated those reported by Abdel-Aal et al. (2011a) in glabrous canary seed of the same cultivar (0.18 mg/100 g hulls) and in a variety of hairy canary seed (0.17 mg/100 g hulls); they were also higher in wheat (0.07 mg/100 g hulls). The values of caffeic acid were similar to those obtained by Abdel-Aal et al. (2011a) in glabrous canary seed (0.27 mg/100 g hulls) and higher than those obtained in hairy canary seed (0.06 mg/100 g hulls) and wheat (0.1 mg/100 g hulls). However, Abdel-Aal et al. (2011a) also found contents of ferulic acid in glabrous canary seed (0.31 mg/100 g hulls), in hairy canary seed (0.25 mg/100 g hulls) and in wheat (0.71 mg/100 g hulls). Chandrasekara and Shahidi (2011) reported higher values of free phenolic acids in two varieties of millet, in relation to the results obtained in this and other studies. The Kodo variety presented contents of p-coumaric acid (2.57 mg/100 g hulls) and ferulic acid (61.70 mg/100 g hulls), while the Pearl variety presented values of p-coumaric (7.36 mg/100 g hulls) and ferulic acid (22.20 mg/100 g hulls).

As for insoluble bound phenolic, the values of p-coumaric were also higher than those reported by Abdel-Aal et al. (2011a) in glabrous canary seed of the same cultivar (21.21 mg/100 g hulls), in a variety hairy canary

Table 5: Content of phenolics mostly present in "CDC Maria" canary seed hulls using ethanol 75 %.

	p-Coumaric	Ferulic	Caffeic	Total
mg/100 g hulls d.b.				
Free	0.42±0.01^{b1}	Traces	0.24±0.07^{a}	0.66
Bound	38.65±1.67^{a2}	42.48±1.72^{a}	Traces	81.13
Total	39.07	42.48	0.24	
mg/100 g phenolic extract d.b.				
Free	66.00±2.00^{b1}	Traces	38.00±7.00^{a}	104.00
Bound	3950.00±1117.23^{a2}	4340.00±1216.22^{a}	Traces	8290.00
Total	4016.00	4340.00	38.00	

Different letters on the same row show significant differences ($p \leq 0.05$) among phenolic acids in the same extract according to the Tukey Test.
Different numbers on a same column show significant differences ($p \leq 0.05$) among the types of phenolic extract according to the Tukey Test.

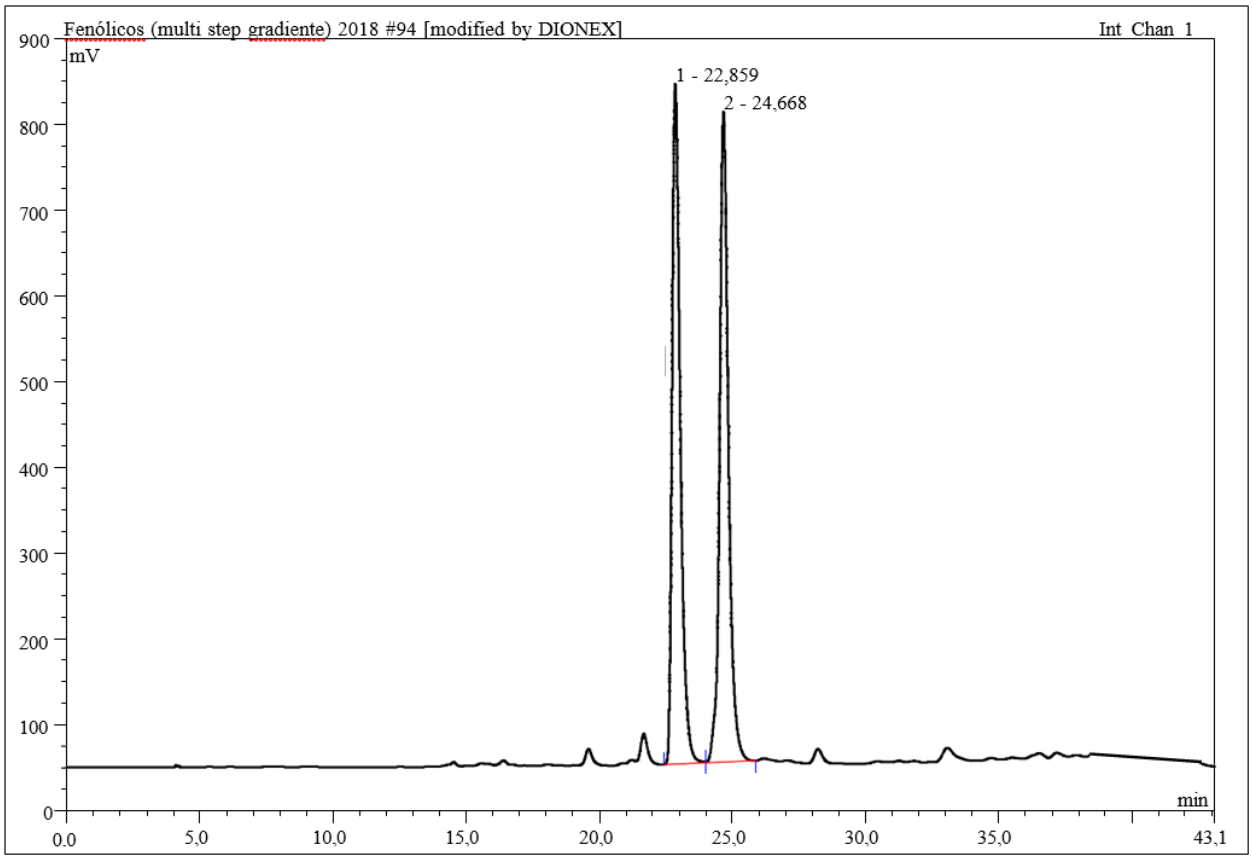

Figure 1: Chromatogram of bound phenolics from glabrous canary seed hulls.

seed (19.31 mg/100 g hulls) and also higher in wheat (7.66 mg/100 g hulls). On the contrary, the values of ferulic acid were lower than those reported by Abdel-Aal et al. (2011a) in glabrous canary seed of the same cultivar (118.86 mg/100 g hulls) and in a variety of hairy canary seed (108.64 mg/100 g hulls), but higher than that of wheat (0.07 mg/100 g hulls). As in glabrous canary seed of the same variety reported by Abdel-Aal et al. (2011a), caffeic acid was only detected in traces. In contrast, Li et al. (2011) found caffeic acid in the same variety of canary seed (30.4-45.2 mg/100 g hulls). Li et al. (2011) reported values of p-coumaric lower that those obtained in this work (11.9-14.2 mg/100 g hulls). However, higher values of ferulic acid (59.3-76.6 mg/100 g hulls) were found for the same variety of canary seed. Kim et al. (2006) found p-coumaric (3.5 mg/100 g hulls), ferulic (191.8 mg/100 g hulls), and caffeic (0.08 mg/100 g hulls) in a commercial variety of red wheat and p-coumaric (3.81 mg/100 g hulls) and ferulic (137.6 mg/100 g hulls) in a commercial variety of white wheat. Awika and Rooney (2004) reported contents of p-coumaric that ranged from 0 to 97 mg/100 g hulls and ferulic ranged from 140 to 217 mg/100 g hulls in

sorghum; while Siebenhandl et al. (2007) reported higher values of p-coumaric (256.5-336.7 mg/100 g hulls) and ferulic (200.2-201.7 mg/100 g hulls) in black barley.

The identification of these phenolic acids allowed to understand the high antioxidant activity observed during the DPPH assay in canaryseed hulls, since these three acids belong to the group of hydroxycinnamic acids, which present a high antioxidant activity compared to the group of hydroxybenzoic (Balasundram et al., 2006; Rodríguez et al., 2019). Quantification of phenolic extracts through HPLC determined that the differences in values of antioxidant activity of free and bound phenolics depended on the concentration of phenolic acids and not on the type of phenolic acids present in the extract. In addition to antioxidant activity, these three acids offer beneficial effects for health. p-coumaric acid protects the heart against oxidative stress induced by doxorubicin (Abdel-Wahab, 2003); ferulic acid may offer beneficial effects against cancer, cardiovascular diseases, diabetes and Alzheimer's disease (Zhao and Moghadasian, 2008); and caffeic acid has new and therapeutic effects on hepatocarcinoma cells (Chung et al., 2004).

4. Conclusions

Research on glabrous canary seed shows its high potential as a source of macronutrients of high biological value in the groat (small grains/granules of starch, free-gluten proteins and highly saturated oil) and as a source of bioactive compounds in the hull (phenolic acids, flavonoids and carotenoids), which arouse interest in both healthy food preparation and industrial application. From the results obtained in this study, it can be concluded that phenolics in hulls of canary seed grains "CDC Maria" are mostly bound to structural compounds of cells. Also, it is possible to carry out an extraction method solid-liquid with mechanical assistance using 75 % ethanol as solvent, at 25 °C and with a rate sample/solvent of 1/10 (w/v) to obtain quality phenolic extracts in terms of yield and high antioxidant activity. It is relevant to consider the use of ethanol instead of other solvents used in the literature to obtain phenolics in glabrous canary seed. The advantages are that it is more economical, reusable and non-toxic. Furthermore, the extracts obtained can be used in the food industry. Future research will focus on optimizing the extraction of phenolic compounds of canary seed "CDC Maria" using ethanol and assessing the effect of the solvent concentration and extraction times.

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