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### Mango and pomegranate peels as ingredients to promote antioxidant activity in cereal bars

#### Cáscaras de mango y granada como ingredientes para promover la actividad antioxidante en barras de cereales

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### Resumen

Las barras de cereal (BC) se destacan como alimentos saludables y funcionales. La incorporación de subproductos del procesamiento de frutas, como cáscaras de mango deshidratadas (CMD) y polvo de cáscara de granada (PCG), en una formulación equilibrada de cereales puede promover la actividad antioxidante. A una formulación de BC (F0) se evaluó el efecto de la adición de: germinados de soya deshidratados (F1) y del 5 al 20% de CMD y PCG (F2-F5). Se determinó el contenido de polifenoles solubles totales de acuerdo con el método colorimétrico de Follin-Ciocalteu, se identificaron los principales compuestos antioxidantes mediante análisis por cromatografía líquida de alta resolución (HPLC-MS) y la actividad antioxidante (TEAC) por ABTS, FRAPP y DPPH. El análisis estadístico se realizó por triplicado los datos se sometieron a análisis de varianza y la prueba de Tukey a una probabilidad del 5%. El contenido de compuestos fenólicos totales fue desde 3.18 a 6.42 mg EAG/g, para el tratamiento F0 y F5 respectivamente. La formulación F5 (6.42 mg EAG/g) duplicó el valor de los compuestos fenólicos, en comparación con la formulación control (F0) (3.1 mg EAG/g). La capacidad antioxidante se incrementó con la adición de los germinados y subproductos. Todos los tratamientos (F1-F5), presentaron valores significativamente más altos de actividad antioxidante en comparación con el tratamiento F0 (control). Sin embargo, a partir de la adición del 10% de subproductos no se presentaron diferencias

estadísticamente significativas en la actividad antioxidante medida con los métodos ABTS (F1:20.5 – F5:48.4  $\mu\text{Mol TE/g}$ ) y DPPH (F1:4.2 – F5:21.7  $\mu\text{Mol TE/g}$ ). Para FRAP (F1:6.9 – F5:72.1  $\mu\text{Mol TE/g}$ ) se presentaron diferencias estadísticamente significativas a partir del tratamiento F3. La actividad antioxidante determinada en las diferentes formulaciones de BC se atribuye al ácido elágico, ácido cafeico, ácido aspártico y otros compuestos identificados por HPLC-MS, con probada acción antioxidante, principalmente el ácido elágico presente en la cáscara de granada. La adición de los subproductos en la formulación BC aumentó el contenido y la actividad de los compuestos antioxidantes, lo que indica que la adición de subproductos como ingrediente en las formulaciones de BC favorece el incremento de compuestos con actividad antioxidante.

**Palabras clave:** Antioxidante, alimentos funcionales, barra de cereal, cáscaras de frutas.

## Abstract

Cereal bars (CB) stand out as healthy and functional foods. The incorporation of fruit processing by-products, such as dehydrated mango peels (DMP) and pomegranate peel powder (PPP), into a balanced cereal formulation, can promote antioxidant activity. To a CB formulation (F0), the effect of the addition of dehydrated soybean sprouts (F1) and 5 to 20% DMP and PPP (F2-F5) was evaluated. The total soluble polyphenol content was determined according to the Folin-Ciocalteu colorimetric method, the main antioxidant compounds were identified by high-performance liquid chromatography analysis (HPLC-MS) and the antioxidant activity (TEAC) by ABTS, FRAPP, and DPPH. Statistical analysis was performed in triplicate and the data were subjected to analysis of variance and Tukey's test at 5% probability. The content of total phenolic compounds ranged from 3.18 to 6.42 mg EAG/g, for treatment F0 and F5 respectively. Formulation F5 (6.42 mg EAG/g) doubled the value of phenolic compounds, compared to the control formulation (F0) (3.1 mg EAG/g). The antioxidant capacity increased with the addition of the sprouts and by-products. All treatments (F1-F5), presented significantly higher values of antioxidant activity compared to the F0 (control) treatment. However, after the addition of 10% of by-products, there were no statistically significant differences in the antioxidant activity measured with the ABTS (F1:20.5 - F5:48.4  $\mu\text{Mol TE/g}$ ) and DPPH (F1:4.2 - F5:21.7  $\mu\text{Mol TE/g}$ ) methods. For FRAP (F1:6.9 - F5:72.1  $\mu\text{Mol TE/g}$ ) there were statistically significant differences from treatment F3 onwards. The antioxidant activity determined in the different CB formulations is attributed to ellagic acid, caffeic acid, aspartic acid, and other compounds identified by HPLC-MS, with proven antioxidant action, mainly ellagic acid present in pomegranate peel. The addition of agroindustrial by-products in the CB formulation increased the content and activity of antioxidant compounds, indicating that the addition of by-products as an ingredient in CB formulations favors the increase of compounds with antioxidant activity.

**Key words:** Antioxidant, Cereal bar, Fruit peels, Functional Foods.

## 1. Introduction

Cereal bars (CB) are a multi-ingredient food, primarily cereals (wheat, corn, oats, rice), fruits, nuts, and sugar (sucrose, glucose, and

fructose) (Lobato et al., 2012; Rios et al., 2017), Which have gained popularity due to the health benefits associated with their consumption (Rawat & Darappa, 2015).

Currently, new ingredients such as amaranth, quinoa and chia, which are seeds of broad-leaved plants known as pseudocereals, have been incorporated into formulations due to their similarity in composition and function to true cereals (Alvarez-Jubete et al., 2010), these have a high nutritional and biological value, superior to that of cereals with gluten (wheat, barley, rye, oats, and all their varieties and hybrids) so much for the balanced composition of the amino acids that contain their proteins (globulins and albumin), as for their bioavailability or digestibility (López et al., 2018).

The consumption of these pseudocereals combines the benefits of cereals, fermented products and meat, avoiding the adverse effects of animal protein consumption. Amaranth and quinoa have beneficial effects on health. They help regulate the increase in blood pressure, reduce cholesterol levels in the blood and liver, and have antioxidant activity (Masayo & Katsumi, 2010).

Another ingredient that has been considered with potential to be added in CB formulations are sprouts, due to the amino acid composition of legumes are complementary to the main cereals, so the combined consumption of legumes and cereals increases the protein quality of the food (Gan et al., 2017). Some sprouts such as soybeans have higher contents of raw protein, lipids, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc than ungerminated grains (Price, 1988).

On the other hand, the food processing industry generates large amounts of waste material, which have emerged as an ideal source for the extraction of bioactive compounds. The pomegranate peel (*Punica granatum*) represents 40% of the weight of fresh fruit. According to the variety, it can have dietary fiber contents of between 33.10 and 62 g/100g (Hasnaoui et al., 2014). The

highest content of phenolic compounds is found in the peel (Li et al., 2006). It contains significant amounts of flavonoids (anthocyanins, catechins, and other complexed flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid) (Papaioannou et al., 2020; Smaoui et al., 2019). So it could be used in the development of functional food formulations (Srivastava et al., 2014).

In the industrialization of mango (*Mangifera caesia* Jack ex Wall) a significant amount of waste is generated, equivalent to 36 - 60% of the weight of the fresh fruit, only the peel represents 15 - 20% of the total weight of the fruit (Ajila & Prasada Rao, 2013; García-Magaña et al., 2013; Serna-Cock et al., 2016). Mango peels contain biologically active compounds such as polyphenols, dietary fiber, carotenoids, flavonoids, anthocyanins, vitamins, and enzymes, compounds that, if harnessed, could help mitigate the environmental impacts generated and increase economic gains in agribusiness (Sáyago-Ayerdi et al., 2019; Serna-Cock et al., 2016). According to the study conducted by García-Magaña et al., (2013), the agroindustrial by-products of mango processing could be considered a good source of polyphenols, with the Mexican variety Ataulfo standing out as having the best antioxidant activity and the highest polyphenol content among the mango varieties studied.

Among the main bioactive compounds found in mango peel are soluble dietary fiber (31 - 33 %), insoluble dietary fiber (32.1 - 34 %), polyphenols (55 - 110 mg/g of dry peel) and carotenoids (3092.2 µg /g of dry peel)(Ajila et al., 2007; Banerjee et al., 2017) higher content of polyphenols is found in the peel than in the pulp, especially in ripe fruits (Ueda et al., 2000).

Several studies report the feasibility of incorporating by-products of fruit processing into foods, to increase the nutritional compounds and functional properties of the products (Rios et al., 2017). The market offers a wide variety of cereal CBs focused on the specific nutritional requirements of athletes, children, women or the elderly (Rawat & Darappa, 2015). Some are marketed as a source of protein, fiber or functional as in the case of those added with additives such as prebiotics (Lobato et al., 2012).

Growing consumer awareness of the relationship between nutrition and health (Iñárritu & Vega, 2001; Malcata et al., 2013) has led the industry to develop foods known as functional foods, which resemble conventional foods but contain specific nutraceutical ingredients (Rios et al., 2017). The importance of developing foods that provide significant amounts of natural antioxidants that can provide protection from free radical damage is a challenge for research, given their importance in the treatment of cardiovascular disease, cancer and oxidative stress responsible for DNA, protein and membrane damage (Lee et al., 2012).

Therefore, the objective of this work was to evaluate the effect of the addition of Ataulfo mango (*Mangifera caesia* Jack ex Wall) and pomegranate (*Punica granatum* L) peels on the polyphenol content and antioxidant activity of bars formulated from pseudocereals, and soybean sprouts (*Glycine max.* L).

## 2. Materials and Methods

### 2.1 Raw material

The ingredients for the elaboration of cereal bars (amaranth, inflated quinoa, chia seeds, agave honey) were acquired in the municipal market of the city of Saltillo (Mexico).

The pomegranate peels provided by a juice production company in the city of Monterrey, Mexico. Were washed and immersed in a sodium hypochlorite solution (50 ppm) for 5 min (Torres-León et al., 2018), and dried in a convection oven at 42 °C for 8 h. The dried hulls were pulverized with an electric hammer mill, the ground material was taken to a Rotap (Tyler) for 5 min, and materials with particle sizes smaller than 250 µm were used (Serna-Cock et al., 2015).

The mangoes were washed with potable water and immersed in chlorinated water (100 ppm sodium hypochlorite) for 10 min, dried with a paper towel, the peel was removed with a kitchen knife, previously disinfected with 200 ppm sodium hypochlorite (Serna-Cock et al., 2015). They were then dried at 32 °C for 12 h in an electric food dehydrator (Model 3926TCDB, Excalibur), the dried peels were ground in an electric food processor, to a size of 2 -5 mm.

The soybean sprouts were bought at a local market in the city of Saltillo, this raw material was washed and immersed in a sodium hypochlorite solution (50 ppm) for 5 min, then dried in a forced gas convection oven (HCX PIUS3, Sanso) for 8 h at 37 °C.

All materials were packaged in resealable plastic bags and stored at room temperature until use.

### 2.2 Preparation of cereal bars

Six CB formulations were evaluated: F0 corresponds to the control or base formulation, F1 added with 15% dehydrated soybean sprouts, in formulations F2, F3, F4 and F5 were added 5, 10, 15 and 20% dehydrated mango peels and pomegranate peels powder (relation 50 - 50%), respectively. The formulations evaluated are shown in Table 1. The ratio of dry ingredients

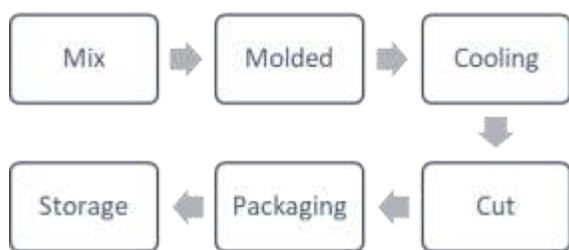
to binder was considered to be 60 - 40 %, respectively (Olivera et al., 2012).

**Table 1** Cereal bar formulations

Ingredients	F0	F1	F2	F3	F4	F5
(g)	Control	Sprouts	5%	10%	15%	20%
Q	15	15	15	15	15	15
A	37.5	37.5	37.5	37.5	37.5	37.5
CH	7.5	7.5	7.5	7.5	7.5	7.5
SS	0	15	15	15	15	15
DMP	0	0	1.9	3.7	5.6	7.5
PPP	0	0	1.9	3.7	5.6	7.5

Quinoa (Q), amaranth (A), chía (CH), soybean sprout (SS); dehydrated mango peels (DMP), pomegranate peel powder (PPP).

The elaboration of the CB was carried out following the methodology described by Olivera et al. (2012) with some modifications (Figure 1). The dried ingredients were weighed and mixed in a plastic container until homogenized, the binder (agave honey) was added at a temperature between 25 - 30 °C. The mixture was kneaded until a soft and uniform consistency was obtained and was molded into a cookie tray. A roller was used to compact a thickness of 1 cm. The tray was taken to refrigeration for 4 h. After this time, the bars were cut into 30 g portions, 4 x 13 cm, and were packed in low density polypropylene bags per unit.



**Figure 1.** Flow chart of the process of elaboration of the cereal bars.

### 2.3 Determination of total hydrosoluble polyphenol content and antioxidant activity

#### *Sample preparation and extraction of polyphenols*

The CB samples were diluted in water at a concentration of 0.02 g/mL and kept at 28 °C

for 12 h. The extractions were carried out with water using an ultrasonic bath for 10 min and centrifuging at 5000 g for 10 min (Duarte & Oliveira, 2014; Freitas et al., 2021; Kumar et al., 2021; Wen et al., 2012) the supernatant was used as the extract sample of antioxidant compounds for the determination of total polyphenols and antioxidant activity.

#### *Determination of total hydrosoluble polyphenols*

The phenolic content was estimated by the Folin-Ciocalteu procedure (Goiris et al., 2012). The Folin-Ciocalteu reagent was diluted 10 times in distilled water. Then, 200 µL of sample was added to 1.5 mL of this reagent and allowed to react for 5 min at 28 °C. Then, a solution of sodium bicarbonate (60 g L<sup>-1</sup>) was prepared and 1.5 mL was added to the sample with reagent. The samples were incubated for 90 min at room temperature (28 °C) in the dark. The absorbance was measured at 750 nm (BioTek, EPOCH 2). A standard curve was made with gallic acid in the range of 25 to 150 mg/L. Quantification of total polyphenols in the samples was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g). The samples were analyzed in triplicate.

#### *Determination of antioxidant activity by the ABTS radical method*

The compound ABTS (2,2'-Azino-bis (3-Ethyl Benzothiazolin) -6-Ammonium Sulfonate) brand Sigma-Aldrich® was prepared in a solution of 7 mM concentration and mixed with a solution of potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) concentration 2.45 mM Carlo Erba® brand, to form the cationic radical ABTS. This mixture was made in a 1:1 (v/v) ratio and was left to rest for 16 h in the dark, and then was diluted with Merck® brand analytical grade ethanol to adjust its absorbance at 0.7 ± 0.02 at a wavelength of 734 nm. The reading of the absorbances was carried out in the microplate reader (Bio

Tech, EPOCH 2). Ethanol was used as a reading blank and ABTS-ethanol as a control absorbance.

For the assay, 200  $\mu\text{L}$  of ABTS solution diluted to 10  $\mu\text{L}$  of sample was added. After 5 min of reaction in the dark, a standard Trolox curve was made from 0 to 1190  $\mu\text{moles}$ . The samples were analyzed in triplicate and the results were expressed in antioxidant activity as Trolox equivalent  $\mu\text{mol}$  (TE)/g on dry basis (Re et al., 1999).

#### *Determination of antioxidant activity by the FRAP method*

The FRAP assay was performed following a method previously reported by (Radzki et al., 2016), where 10  $\mu\text{L}$  of sample were added to 290  $\mu\text{L}$  of FRAP reagent (Buffer acetic acid-sodium acetate (pH 3.4), 10 mM TPTZ,  $\text{FeCl}_3$ , in a 10:1:1 ratio), after 30 min of reaction at 37°C, in the dark and the absorbance was determined at a wavelength of 593 nm. This value was compared with the reference curve constructed with Trolox at 1190  $\mu\text{mol}$  and the results were expressed in antioxidant activity as equivalent  $\mu\text{mol}$  of Trolox (TE)/g on a dry basis. The samples were analyzed in triplicate.

#### *Determination of antioxidant activity by the DPPH method*

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) solution (Sigma-Aldrich®) was prepared by dissolving 0.6 mM DPPH in 80% methanol and the ability of the samples to trap the DPPH radical was evaluated. The solution was diluted with 80% methanol until obtaining a  $0.70 \pm 2$  (517 nm) in a spectrophotometer (BioTek, EPOCH 2). The absorbance of the reaction of 30  $\mu\text{L}$  of sample and 270  $\mu\text{L}$  of DPPH solution was measured at 517 nm after 30 min incubation in the dark. A blank was also measured with methanol and the radical solution. A standard curve of 0-1990  $\mu\text{mol}$  of Trolox dissolved in 80% methanol was used as the antioxidant

standard. All determinations were made in triplicate. The antioxidant capacity was expressed in equivalent  $\mu\text{mol}$  of Trolox (TE) / g CB on a dry basis (Villarino et al., 2014).

#### *Identification of polyphenolic compounds*

The identification of polyphenolic compounds was determined following the methodology described by (Mendez-Flores et al., 2018). High performance liquid chromatography (HPLC) analysis was performed on a Varian HPLC system including an autosampler (Varian ProStar 410, USA), a ternary pump (VarianProStar 230I, USA) and a PDA detector (Varian ProStar 330, USA). For the mass spectrometry analysis, a Varian 500 / MS (USA) instrument with an electrospray ionization (ESI) trap was used. Samples were injected (5  $\mu\text{L}$ ) onto a Denali C18 column (150 mm \* 2.1 mm, 3  $\mu\text{m}$ , Grace, USA). The column temperature was 30 °C. The eluents were formic acid (0.2%, v / v; solvent A) and acetonitrile (solvent B). The applied gradient was: initial, 3% of B; 0-5 min, 9% B linear; 5-15 min, 16% B linear; 15-45 min, 50% B linear. The column was washed and reconditioned. The flow used was 0.2 mL / min, measurements were made at 245, 280, 320 and 550 nm.

All experiments were performed in the negative mode  $[\text{M}-\text{H}]^{-1}$ . Nitrogen was used as the nebulizer gas and helium as the buffer gas. The ion source parameters were: spray voltage 5.0 kV, capillary voltage and temperature of 90.0 V and 350 °C, respectively. Data were collected and processed using MS Workstation software (V6.9). Samples were analyzed first in the full scan mode acquired in the m/z 50-2000 range.

### 3. Results and Discussion

#### *Polyphenol content and antioxidant activity*

The content of phenolic compounds can be used as an indicator of antioxidant capacity (Viuda-Martos et al., 2011), since phenolic compounds are the main contributors to antioxidant capacity in some foods (Floegel et al., 2011). Table 2. shows the polyphenol content for the different formulations evaluated, with a range between 3.18 and 6.42 mg EAG / g. The polyphenol content in the F5 formulation (20% by-products) was two times higher than the obtained in the control formulation. These results are consistent with studies that report the content of CB polyphenols, using quinoa, oat bran and pineapple peel; in which values of 0.26 mg GAE/g and 2 mg GAE/g were obtained for oat bran and quinoa, respectively. The highest polyphenol contents were presented in the combination of the ingredients, for the mixture of quinoa - pineapple peel showed a content of 7.1 mg GAE/g, while the mixture quinoa-oat bran was 3.1 mg GAE/g. These results indicated that the greater increases in polyphenols occurred with the addition of fruit residues (Márquez Villacorta & Pretell Vázquez, 2018).

For the control formulation (F0) with pseudocereals and the F1 formulation with the addition of dehydrated soybean sprouts, there were no statistically significant differences ( $p < 0.05$ ), with values of 3.18 and 3.58 mg GAE/g, respectively.

Pseudocereals such as quinoa and amaranth have antioxidant contents of 1.11 and 0.51 mg GAE/g, respectively. This content is higher than that reported for traditional cereals such as wheat, rice, barley, and millet, whose content ranged from 0.16 and 0.36 mg GAE/g (Masayo & Katsumi, 2010). Therefore, the antioxidant content in the formulation can be attributed to these pseudo-cereals.

The treatments with the addition of agroindustrial by-products (F2-F4) from 5 to 15% did not present statistically significant differences among themselves, with polyphenol content between 4.28 and 6.4 mg GAE/g, however, the treatment with the addition of 20% (F5) was statistically different from the other treatments ( $p > 0.05$ ), this corresponds to the highest content of polyphenols.

These results can be attributed to the addition of mango peels and pomegranate peel powder. Some studies report values between 55 and 110 mg GAE/g of dry peel of different varieties of mango, with a highest phenolic content (68.13 mg GAE/g) for Ataulfo variety among others mango cultivars (García-Magaña et al., 2013; Palafox-Carlos et al., 2012).

For its part, pomegranate peels are recognized for having a high concentration of polyphenolic compounds (Torres-Leon et al., 2018; Venkitasamy et al., 2019), such as punicalagin (69.67 mg/g), punicalin (30.41 mg/g), ellagic acid (23.83 mg / g) and gallic acid (10.46 mg/g) which were some of the main phenolic compounds identified (Grabež et al., 2020).

The addition of flour from mango peels between 25% and 75% increased the content of polyphenols in food products between 5 - 6.78 mg GAE/g (León and Sarmiento et al., 2015). It was found that the antioxidant activity of the food is directly proportional to the percentage of addition of the by-product flour (Segura López & Vargas Urquijo, 2017).

#### *Antioxidant activity (ABTS, DPPH and FRAP)*

The different levels of antioxidant activity measured in the CB obtained from the tests, reflects a relative difference in the capacity of the antioxidant compounds in the extracts to extinguish the aqueous peroxide radicals and

reduce ABTS +, the free radical of DPPH and the ferric iron in systems *in vitro* (Thaipong et al., 2006).

The values ET ( $\mu\text{M/g}$ ) by ABTS in the CB developed in the present work were between 9.59 and 48.43  $\mu\text{M/g}$ , values higher than those reported for different fruits such as mango, strawberry, açai, and grape of 2 -13, 0-12, 4-9 and 2-9  $\mu\text{M/g}$ , respectively (Kuskoski et al., 2005). This is possible because designed CB is a food composed of ingredients that have individually been considered as a source of antioxidants, such as pomegranate peels, mango peels and pseudo-cereals (Ajila et al., 2010; He et al., 2021; Palafox-Carlos et al., 2012).

**Table 2.** Polyphenol content and antioxidant activity of cereal bar formulations.

Formulation	Polyphenols	ABTS	DPPH	FRAP
(Treatment)	mg EAG/g	$\mu\text{Mol TE/g}$	$\mu\text{Mol TE/g}$	$\mu\text{Mol TE/g}$
F0	3.18 $\pm$ 0.01 <sup>a</sup>	9.59 $\pm$ 1.5 <sup>a</sup>	3.22 $\pm$ 1.1 <sup>a</sup>	3.71 $\pm$ 0.3 <sup>a</sup>
F1	3.58 $\pm$ 0.01 <sup>a</sup>	20.56 $\pm$ 2.7 <sup>ab</sup>	4.18 $\pm$ 1.5 <sup>ab</sup>	6.99 $\pm$ 0.1 <sup>a</sup>
F2	4.28 $\pm$ 0.03 <sup>ab</sup>	30.71 $\pm$ 5.2 <sup>bc</sup>	8.98 $\pm$ 1.4 <sup>b</sup>	13.82 $\pm$ 2.0 <sup>a</sup>
F3	4.86 $\pm$ 0.01 <sup>ab</sup>	40.33 $\pm$ 0.2 <sup>c</sup>	18.74 $\pm$ 2.3 <sup>c</sup>	24.84 $\pm$ 1.2 <sup>b</sup>
F4	5.10 $\pm$ 0.02 <sup>ab</sup>	43.33 $\pm$ 3.7 <sup>c</sup>	19.59 $\pm$ 0.7 <sup>c</sup>	51.65 $\pm$ 8.9 <sup>c</sup>
F5	6.42 $\pm$ 0.05 <sup>bc</sup>	48.43 $\pm$ 0.9 <sup>c</sup>	21.73 $\pm$ 0.7 <sup>c</sup>	72.12 $\pm$ 2.3 <sup>d</sup>

F0: control cereal bar (without soybean sprouts, without by-products); F1: 15% soybean sprouts (without by-products); F2: 5% by-products; F3: 10% by-products; F4: 15% by-products; F5: 20% by-products. † Values expressed in dry base. Mean  $\pm$  standard deviation (n = 3 repetitions). Different letters in the same row indicate significant differences by Tukey's test (P < 0.05).

In all the tests a positive correlation was found between the amount of by-product added in the formulation and the antioxidant activity, with formulation F5 with addition of 20% of by-product the one that presented the highest activity value compared to the other formulations. Nevertheless, the statistical analysis indicated that in the antioxidant activity there are no statistically significant differences between the formulations F3 to F5 for the ABTS and DPPH methods, while

for FRAP this occurs between the formulations from F0 to F2.

#### Identification of polyphenolic compounds

Through HPLC analysis of the cereal bar samples the presence of various compounds was identified. Table 3 shows the main compounds responsible for the antioxidant activity identified in the different treatments.

The main compounds identified in the formulations have been reported to be present in pomegranate peels, rich in phenols, especially ellagitannins, which are derived from ellagic acid (punicalagin and punicalin), hydroxybenzoic acids and flavonoids (Papaioannou et al., 2020).

*Ellagic acid* is a phenolic compound, known to be a potent antioxidant, anticarcinogen, antiviral and antibacterial, found in fruits, vegetables, berries, black tea and red wine (Jiménez-García et al., 2018).

*Apigenin 7-O-glucoside* is a phenolic compound, derived from epigenina (Argentieri et al., 2020). According to research by Wang et al. (2018), apigenin-7-O-glucoside did not exhibit antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl radical scavenging tests (DPPH) or in ferrous ion chelation tests (FRAP), while It did show ABTS radical scavenging capacity, which may partly explain the antioxidant activity presented in cereal bars, where the determination by ABTS showed higher values than by the DPPH and FRAP methods.

*Cafeoil glucose* is a derivative of caffeic acid (Błaszczak et al., 2020), with antioxidant potential, classified as hydroxycinnamic acid (Alshwyeh, 2020; Marasca et al., 2020). Present in some fruits such as pomegranate, strawberries, raspberries, blueberries, and blackberries (Patras et al., 2018).

There is no consensus on the recommended intake of phenolic compounds (Marques et al., 2015), however, the daily intake of phenolic antioxidants has been associated with reducing the risk of developing diseases such as atherosclerosis, cardiovascular disease, different types of cancer, infections, Alzheimer's disease among others (Fruhirth, 2007). An assessment of the trend of regular dietary polyphenol intake of adults in the United States (2007-2016), reported that dietary polyphenol intake was highest in

adults over 40, women, non-Hispanic white adults, and college graduates, with foods and beverages contributing 99 percent. 8% of the polyphenol intake is coffee (39.6%), beans (9.8%), and tea (7.6%), and the main classes of polyphenols consumed were phenolic acids and flavonoids (Huang et al., 2020).

**Table 3.** Identification of polyphenol compounds present in cereal bars.

Identified compound	Function
Ellagic acid	Antioxidant, anticancer, antiviral and antibacterial <sup>49, 42</sup>
Apigenin 7-O-glucoside	Antioxidant, antispasmodic, anti-inflammatory and anticancer <sup>50</sup>
Caffeoyl glucose	Antioxidant, antibacterial, anticancer and antiviral <sup>51-52</sup>
Caffeoyl aspartic acid	Antioxidant, antibacterial, anticancer <sup>51</sup>

#### 4. Conclusions

The incorporation of up to 20% of mango and pomegranate peels in a cereal bar formulation can double the content of phenolic compounds present, increasing the nutritional contribution not only of fiber, but also by the content of compounds with antioxidant activity present. The main compounds identified in the formulation have been widely reported by powerful antioxidant activity. The incorporation of these agroindustrial by-products directly contributes to close the cycle in a circular economy where the residues are reduced to zero. These by-products can be considered an economic and sustainable source of antioxidants and their incorporation in cereal bars is a viable alternative for their use, decreasing the environmental impact.

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