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Valorization of pomegranate peel from 12 native cultivars from Tunisia: Dietary fiber composition, antioxidant capacity and functional properties

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Abstract

Nowadays, pomegranates (*Punica granatum* L.) are among the most highly demanded fruits, especially in industrialized countries, due to their health and biological effects. Because of this growing demand and knowing that 50% of the processed weight is generated as waste, a large quantity of peel is produced in out-put. The dried powdered peels of 12 cultivars grown in Tunisia, one of the main producers of pomegranates, were used to extract and characterize their dietary fiber, and to assess their functional and antioxidant properties. Total dietary fiber (DF) represented between 33.10 and 62 g/100g depending on the cultivar. The results show that their contents of cellulose, Klason lignin, uronic acid and total neutral sugars (NS) were 16.53-22.71, 20.59-41.86, 13.98-23.31 and 16.88-19.66 g/100g of DF, respectively. Their NS composition was dominated by arabinose and xylose, which represent more than 60% of the NS on average. The ratio of insoluble to soluble DF was around 1, reflecting the balanced composition of pomegranate peel DF. When compared with other agricultural by-products, pomegranate peel powder showed intermediate values for water- and oil-holding capacities: 2.31-3.53 and 2.80-4.05 mL/g, respectively. It has been shown that most of the antioxidants can be extracted, based on the strong soluble antioxidant activity (2018-2649 μmol Trolox/g) compared to the insoluble one (13-23 μmol Trolox/g). Finally, the pomegranate peels of some cultivars have strong effect on the dialysis of glucose, reaching retardations of around 60%. From this work it raises that pomegranate peel could be a good source of dietary fiber, antioxidants and biopolymers and, due to its functional and physiological characteristics, could be considered as a valuable ingredient for food formulation.

Keywords: *Punica granatum* L.; peel; by-products; valorization; functional properties; polyphenols; DPPH; reducing power; IDF/SDF ratio; GDRI

1. Introduction

The *Punica granatum* fruit has shown an explosion of interest during the last decade and has gained a tremendous popularity, because of its numerous health effects. In fact, consumer awareness about the benefits of natural products to health and welfare continue to drive new trends of super-fruit consumption, particularly those with high contents of polyphenols. For the pomegranate, several scientific studies confirmed its biological activities and medicinal effects of its different parts (arils, peels, leaves, and flowers) and products (fresh and fermented juices, enriched extracts, and seed oil)[1-4]. Driven by continuous growing consumer demand, the pomegranate industry showed an incredible growth and is currently the most promising among fruit sectors. [5] Most often the extracted pomegranate juice is concentrated to 65 °Brix, and to produce 1 MT of this concentrated juice, 10 MT of raw material are needed. [6] In fresh weight, the peel and internal membranes of the pomegranate represent 50% on average. This has led to an increase in the waste effluents in many countries, where factories of juice production were installed (USA, Spain, Uzbekistan, Iran, India, and China). This waste, composed mainly of rind and internal membranes of the pomegranate fruit, is considered as one of the most valuable by-products of the food industry. However, most of the previous works published in last few years are practically fully devoted to the extraction, quantification and antioxidant activities of phenolic compounds. [7-9] Only a limited number of studies reported the use of pomegranate peel in food industry application. Kanatt et al. demonstrated the effectiveness of peel extracts to increase the shelf life of chicken and meat products by 2–3 weeks. [10]

Currently, dietary fiber (DF) is considered as a nutrient. [11] Due to its functional properties, especially the capacity of holding water, it plays an important role in the digestion process and is especially beneficial from a physiological point of view. DF helps nutrient movement in the intestine and reduces the incidence of colon cancer. [12] In the context of health

promotion and increasing life expectancy, there is a growing need to look for new sources of fiber for food supplementation. By-products of several vegetables and fruits (pea, apple, sugar beet, soy, citrus…) are an origin of fiber that is incorporated into food formulation. In the unique report, recently published by Viuda-Martos et al., powder from pomegranate bagasses showed promising technological and functional properties that create new options for assessing this by-product. [13]

Based on our experience on pomegranate resources, pomegranate cultivars differ in some appearance characteristics (not published). They can be classified in a first approach into two groups, sweet and sour cultivars. In fact, the blend of juices from both of them leads to pomegranate juices with higher quality and acceptance. [14] The aim of this work is to isolate the fiber fraction from the peel of 12 different Tunisian cultivars, 8 sweet and 4 sour, and to evaluate their chemical composition and functional properties. In addition, other properties such as glucose retardation index and antioxidant capacity have been assayed in vitro in order to add physiological value to this by-product. These characteristics could ultimately promote the use of pomegranate peel as a new ingredient for healthier food formulation.

2. Material and Methods

2.1. Chemicals

4-Morpholinoethanesulfonic acid (MES), protease from *Bacillus licheniformis*, amyloglucosidase solution from Aspergillus niger, tris(hydroxymethyl) aminomethane (Tris), trifluoroacetic acid, 3-phenylphenol, anthrone, Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH• free radical), ferric chloride, 2,2'-dipyridyl (99% minimum purity), Trolox (97% purity), and citric acid were purchased from Sigma-Aldrich Quimica (Madrid, Spain). Trichloroacetic acid (p.a.) was obtained from Merck (Darmstadt, Germany). Amylase thermostable Thermamyl 120 L was from Novo Nordisk Pharma (Madrid, Spain). Na_2CO_3,

sodium hydroxide, glucose anhydrous, and acetic acid were from Panreac Quimica S.A. (Barcelona, Spain). Standards of gallic acid (GA), myoinositol were purchased from Sigma-Aldrich Quimica. Sulfuric acid and acetone were from Sharlau (Barcelona, Spain). Ethanol was purchased from Alcoholes del Sur (Córdoba, Spain).

2.2. Pomegranate samples

Pomegranate fruits were collected on October 2012 at maturity stage and determined visually based on the color and size of the fruits. They belonged to 12 cultivars: 11 from the National Repository of Pomegranate Resources located at Gabès (Tunisia) (Mars, 2001), namely: Chelfi-2, Andaloussi-1, Gabsi-8, Gabsi-9, Jebali-1, Zaghouani-1 and Zehri-3, which belong to the sweet group and Garoussi-2 Mezzi-1, Mezzi-2, and Mezzi-3, which are sour cultivars. In addition, fruits collected from a private orchard located at Amra (Medenine, Tunisia: 33°26'N 10°24'E) of unknown cultivar and coded AMR, were included.

Harvested pomegranate fruits were transported on the same day to laboratory, washed with tap water and left to dry before being peeled by hand. The non-edible parts of pomegranate fruit, made up of the leathery pith (pericarp) and the internal carpellary membranes (mesocarp), which constitute most of the juice industry and pomegranate farming waste, were cut into small pieces and dried in a ventilated oven at 42 °C until constant weight (3-4 days). We opted for this method because it was shown that it is the most suitable for pomegranate peel drying, i.e. it has the lowest effect on the chemical properties (antioxidant capacity and polyphenolic content). [15] The dried peel pieces were powdered using a grinder mill and passed through a 0.5 mm sieve. The powder of each cultivar was conserved in tightly closed plastic bags and kept in the dark until analyses.

2.3. Dietary Fiber extraction

The amount of DF was determined using the protocol described by Lee et al. with slight modifications. [16] Briefly, three replicates (1 g each) of powder from each cultivar were suspended in 40 mL of MES-Tris buffer and treated with 50 µL of Thermamyl (heat-stable α-amylase) at 100 °C for 15 min and then digested with 100 µL of a 50 mg/mL protease solution (60 °C, 30 min), followed by incubation with 100 µL of amyloglucosidase (60 °C, 1 h) to remove protein and starch. Then, the hydrolysate was passed through the sintered glass crucible (no. 2) using the Fibertec E system (1023 filtration module), and the retained fiber constituted the insoluble dietary fiber (IDF) fraction. Afterwards, four volumes of 96% hot ethanol were added to the filtrate to precipitate the soluble fraction of dietary fiber (SDF), which was rescued after filtration. SDF and IDF were then dried overnight at 105 °C in an air oven, and weighed. The dried fiber was ground in a hammer mill to a particle size of <1 mm and stored at 4 °C until analysis. For total DF correction, ash was determined according to the AOAC method 923.03 by incinerating one of the replicates in a muffle furnace at 550 °C to white ash. Protein contents were calculated from another replicate, analyzed for nitrogen content according to the Kjeldahl method, using a factor of 6.25 to calculate the protein content (AOAC method 955.04).

2.4. Dietary Fiber composition

Neutral sugars (NS) were converted into their alditol acetates and quantified by gas chromatography after the tri-fluoroacetic acid hydrolysis of fiber (121 °C, 1 h). The GC system used was: HP 6890 Plus+ gas chromatograph (Hewlett-Packard, Palo Alto, Ca, USA) fitted with a 30 m × 250 µm × 0.20 mm capillary column (SP-2330, Supelco, Bellefonte, PA, USA). The carrier gas was helium with a constant flow of 2.2 mL/min and a pressure of 21.5 psi (148.24 kPa). The injection was performed in the splitless mode. The oven temperature was held at 50 °C for 2 min after injection, then programmed to 180 °C at 35

°C/min, held at 180 °C for 5 min, and then immediately increased to 220 °C at 5 °C/min and held at 220 °C for 22 min. The total run time was 40.7 min. The injector temperature was 250 °C and the flame ionization detector (FID) was 300 °C. *Myo*-inositol was used as internal standard.

Uronic acids were quantified using the phenyl-phenol method after sulfuric acid hydrolysis as described by Ahmed and Labavitch. [17] Klason lignin (KL) levels were determined gravimetrically as the amount of acid-insoluble material remaining after a two-stage sulfuric acid hydrolysis. Cellulose was quantified from the trifluoroacetic acid insoluble residue after 72% sulfuric acid hydrolysis according to the anthrone method.

2.5. Functional properties

2.5.1. Water holding capacity (WHC)

The WHC was determined using the method described by Jiménez et al. [18] For each sample, 250 mg (in triplicate) were suspended in 15 mL of water. After 24 h of stirring at room temperature, the suspension was centrifuged at 14000g for 1 h. Supernatants were carefully eliminated, and the hydrated fibers were weighed. WHC was expressed as millilitres of water per gram of PomPP.

2.5.2. Solubility

The hydrated pellets resulting from WHC were freeze-dried, and the solubility of pomegranate fiber in water, expressed as a percent, was determined by the difference in weight between before and after the WHC assay.

2.5.3. Oil holding capacity (OHC)

The OHC was determined using the method described by Jimenez et al. [18] OHC was measured by adding 15 mL of sunflower oil (1.0054 g/mL density) to 250 mg (in triplicate) in a 50 mL centrifuge tube. The content was stirred for 24 h at room temperature. Then, the supernatants were carefully eliminated after the centrifugation of tubes (14000g, 1 h) and the oil-embedded powder was weighed. The oil-holding capacity was expressed as milliliters of oil per gram of PomPP.

2.5.4. Glucose dialysis retardation index (GDRI)

The protocol cited by Fuentes-Alventosa et al. was applied to determine the GDRI of the pomegranate peel of the studied cultivars. [19] Briefly, 15 mL of glucose solution (2 g/L) were added to 400 mg of PomP before stirring for 1 h at room temperature. Then, the mix was transferred to previously hydrated dialysis bags 12,000 MWCO, (Sigma Chemical Co.). Samples were placed in a thermostatic water bath (37 °C) and dialyzed against 400 mL of distilled water under continuous shaking for 1 h. At 10, 20, 30, 40, 50 and 60 min, an aliquot of 0.5 mL was collected, and the glucose was quantified spectrophotometrically using the anthrone method. Under the same conditions, bags containing only a glucose solution were used as the control. GDRI was calculated as follows:

$$\text{GDRI} = 100 - \left(\frac{\text{Total glucose diffused, sample}}{\text{Total glucose diffused, control}} \times 100 \right)$$

2.6.Determination of soluble phenols

2.6.1. Extraction of soluble phenols

A quantity of 2.5 grams (in duplicate) of pomegranate peel (PomP) was homogenized in an Ultraturax at top speed for 1 min with 100 mL of 80% EtOH. After filtration, a second

similar extraction was performed using 100 mL of 70% acetone, to finally obtain 200 mL of EtOH-acetone extracts. These extracts were used to determine soluble phenols and soluble antioxidant activities.

The remaining slurry was dried at 100°C overnight. Then, it was suspended in distilled water and homogenized using an Ultraturax at top speed before being lyophilized and powdered. This powder was used to determine the insoluble antioxidant activity.

2.6.2. Determination of soluble phenols

The quantitation of polyphenols in the PomP extracts was performed using the Folin-Ciocalteu reagent. GA was the reference standard and results were expressed as GA equivalents (eq. GA mg/1 g). 80 µL of Na_2CO_3 solution (0.7 M) and 100 µL of Folin-Ciocalteu reagent (0.2 M) were added to 20 µL of extract. The mix was left to stand at room temperature for10 min before reading at 655nm.

2.7. Antioxidant activity

2.7.1. Antiradical activity

2,2'-diphenyl-1-1-picrylhydrazyl (DPPH) was used to measure the antioxidant activity of PomP of different cultivars in terms of radical scavenging ability. [20] First, the extracts were diluted using ethanol (1:9). From this dilution a set of five concentrations was prepared. 195 µL of DPPH methanolic solution (7.6 10^{-2} mg/mL) were added to a volume of 5 µL of each dilution (x3). After 30 min of incubation in the dark, the absorbance was measured at 480 nm. The absorbance of radicals without the antioxidant solution was used as 100%, and the amount of original sample necessary to decrease the absorbance of DPPH by 50% (EC50) was determined graphically and expressed as micromoles of Trolox equivalent per grams of PomPP (µmol Trolox/g). The insoluble antioxidant activity measured on the PomP powder

after phenol extraction was also carried out as described by Fuentes-Alventosa et al. [21] Briefly, between 3 and 20 mg of fibres were transferred to an eppendorf tube (for weights lower than 3 mg, fibres had to be diluted with cellulose as inert material), and the reaction was started by adding 1 mL of the DPPH· reagent. After 30 min of continuous stirring, the samples were centrifuged and the absorbance of the cleared supernatants was measured (in triplicate) at 480 nm. EC50 was calculated as above.

2.7.2. Reducing power

The reducing power of PomPP extracts was assessed using the ferric reducing method (FRP). The working solutions were: 6 mM solution of $FeCl_3$ in citric acid (5 mM) and 5. 10^{-3} $g.mL^{-1}$ solution of *2,2'*-dipyridil dissolved in trichloroacetic acid (12.10^{-3} $g.mL^{-1}$). A mix of 10 µL of extract and 10 µL of $FeCl_3$ solution was allowed to react for 20 min at 50 °C. Then, a volume of 180 µL of dipyridyl was added and left at room temperature for 30 min before reading the absorbance at 490 nm. Blanc reaction was carried out in the same conditions without the antioxidant. The reducing power of each sample was expressed as micromoles of Trolox equivalent per grams of PomPP (µmol Trolox/g) against a Trolox standard curve.

2.8. Statistical analysis

Conventional statistical methods were used to calculate the means and standard deviations of the replicates of assays performed, using Microsoft Office Excel 2003®. In addition, ANOVA was applied to determine homogeneous groups at a significance level of $p<0.05$, using Statgraphics® *Plus* 5.1. To show the inter-cultivar diversity, Principal Component Analysis (PCA) using XLSTAT (version 2013.4.05) was used. The parameters and cultivars studdied were plotted on the PC1 and PC2 plane.

3. Results

3.1. Chemical composition of PomP dietary fiber

The total DF of the pomegranate peel of 12 Tunisian cultivars was extracted by the enzymatic method. Their percentage and chemical composition are presented in Table 1, after correction with ash and proteins, which, on average, account for ~1.5 and ~10 g/100g, respectively (data not shown). PomP DF contents showed high variation among cultivars. The peel of the cultivar Jebali_1 had as much as twice (62.09 % of DM) the DF quantity of AMR peel (33.10 % of DM). This significant variability reflects the genotype effect o DF contents. This conclusion was already observed for other crops, such as date and potato. [22, 23] Furthermore, having the lowest DF content and cultivated under different conditions, the case of AMR cultivar reflects the effect of environmental conditions on DF. Both factors (genotype and environment) have been shown to contribute significantly to the DF content of cereals such as wheat and rye and leguminous like peas. [24-26]

For DF, the IDF to SDF ratio is an important index, especially for certain food applications. It has been shown that an IDF/SDF ratio from 1 to 2.3 is the most advantageous for the beneficial physiological effects associated with DF consumption. [27] Hence, with the IDF/SDF ratio ranging between 0.9 and 1.3, PomP is characterized by a balanced content of insoluble and soluble fibers. Quite higher values (1.5 and 1.7) were obtained for pomegranate bagasses. [13] Due to the different industrial processes that pomegranate fruit undergoes, bagasses and PomP have different compositions. In bagasse, the seeds are also present and they could increase the IDF portion. In addition, the cultivar effect should be considered as an origin of such as differences. Compared to other plant material, more pronounced differences could be observed. For example, in asparagus by-products, the IDF to SDF ratio was >6, [21] in orange peel, the ratio was 3. [28] Lower IDF/SDF values were reported for mango

(0.84), ambarella (1.5) and lime (0.7) by Koubala et al. [29] Balanced IDF/SDF ratios similar to pomegranate peel, have been obtained from onion bagasse and bambangan peels. [30, 31] After isolation, the dietary fibers were hydrolyzed and cellulose, uronic acid, lignin and NS were quantified (Table 1). The contents of PomP DF in cellulose and NS were around 20 g/100 g each. They ranged from 16.53 to 22.71 g/100 g and from 16.88 to 19.66 g/100 g, respectively and showed very little inter-cultivar variation. For uronic acid contents, which varied between 13.98 (Zaghouani_1) and 23.31 g/100 g (Andaloussi_1), a higher inter-cultivar variation was observed. Since this compound is the backbone of pectic polysaccharides, PomP of the cultivars exhibiting high uronic acid values could be considered as a potential source of pectin. In fact, lime peel, which is mostly used for pectin extraction, has a comparable content (25 g/100g). [29] The KL showed the highest variation, since the PomP of cultivar Andaloussi_1 (41.86 g/100g of DF) had as much as twice of the PomP from cultivar Zehri_3 (20.59 g/100g of DF). Peels from cultivars with high KL content could be suitable for prospective applications, such as phenolic resins and adhesives. [32] KL values reflect the degree of lignification of PomP, and seem to be negatively correlated with pomegranate fruit cracking: the cultivars with the lowest KL values presented high percentages of split fruits (data not shown) and *vice versa*.

The NS composition of PomP dietary fiber was dominated by xylose and arabinose (Table 2). They represented >60% of the total carbohydrates in the fiber fraction of PomP, with values ranging between 27.98 (Zehri_3) and 43.10% (Garoussi_2) for the former and between 22.26 (Garoussi_2) and 35.00% (Zehri_3) for the latter. The high levels of xylose and arabinose could be related to the occurrence of relatively high amounts of arabinoxylan in PomP fibers. This is the second most abundant polysaccharide in plants after cellulose and the major hemicellulose in most agricultural by-products. It has drawn considerable interest due to its potential for packaging films and coating food, as well as for its use in biomedical products.

[33] The remaining NS were present in much lower quantities: galactose was the third most present sugar, followed successively by glucose, mannose, rhamnose and fucose. The inter-cultivar variability for these sugars was quite high. As far as we are concerned, the quantification of NS in the fiber fraction is reported for the first time in pomegranate peel. Different profiles of fiber fraction NS of the peels of several exotic fruits have been reported. For example, for ambarella and lime peels, NS were dominated by the presence of xylose and mannose [29] and in mango peel, glucose was the major sugar, followed by arabinose. [34] Glucose also dominated in the fiber fraction of pumpkin peel, [35] whereas in tomato peel, mannose was the major sugar, representing about 40%, while galactose, arabinose, xylose and glucose were equally present. [36]

3.2. Functional properties

3.2.1. WHC,OHC and Solubility

WHC, OHC and solubility are important functional characteristics of fibers that determine their suitability for further application, particularly as food additives and/or ingredients. The results obtained for PomP powder are reported in Table 3. The WHC measures the ability of fiber to immobilize water within its matrix. This ability to trap water is an important characteristic of fibers from both physiological and technological sides, as it influences their metabolic activity along the gut, on one hand, and shows their usefulness as bulking, swelling and/or thickening agents in food formulation, on the other. Pomegranate peels have moderate WHC with 3.53 g/g as the highest value registered for the peel from Andaloussi_1 cultivar. The bagasse resulting from pomegranate juice making had quite higher WHC (4.9 g/g). [13] The difference could be explained because these authors centrifuge the fibers at 3,000g for 20 min, instead of 14,000g for 1 hour, as we did. In addition, the different nature of matrix studied, with higher ratios of IDF/SDF, and/or the drying method applied could also

have an influence on this characteristic. In comparison with other fruits, pomegranate peel displayed similar WHC to ambarella (2.73 g/g) [29] and pear (3.2 g/g) [37] but lower WHC compared to pumpkin peel (27 g/g) [35], passion fruit albedo (13.0 g/g), [38] orange peel (9.63 g/g), [28] apple (6.2 g/g) [37] and lime peel (5.15 g/g). [29]

The solubility is related to the occurrence and structural characteristics of DF components. PomP had a high solubility rate that ranged from 47.87 to 65.00%, with significant differences among cultivars. Similar by-products from other fruits have much lower solubility, such as mango (11.6%)[39] and orange (28.9%). [28] This high solubility in PomP could be related to the low values found for WHC. Taking into account only the insoluble part of the PomP (35-53%), which was in fact responsible for the water retention, the new values of WHC ranged from 4-8 mL/g, similar to those found for other agricultural by-products.

From a technological point of view, OHC is another important property and is related to surface properties, charge density, thickness, hydrophobicity of particles, IDF content, particle size and drying of the fiber matrix. [13] For pomegranate peels, OHC values varied from 2.80 g/g for the AMR cultivar to 4.05 g/g for the cultivar Mezzi_2. Orange peel, which has been successfully used as a fat replacer in ice cream, has an OHC in the same range (3.63 g/g) as pomegranate peel. [28] Thus, pomegranate peel could be useful in the formulation of fiber-enriched foods that require emulsifying properties or even as fat removers in low-calorie products. The PomP values of OHC registered in the present work are significantly lower than those obtained by Viuda-Martos et al. for pomegranate bagasses. [13] This is probably due to differences in composition between bagasses and PomP, and also to experimental conditions, as was mentioned for WHC.

3.2.2. Glucose Dialysis Retardation Index (GDRI)

GDRI is used as a parameter which reflects the effect fibers have on the dialysis of glucose throughout a membrane. It is an *in-vitro* simulation to predict a probable action of fiber on the glucose absorption in the gastrointestinal tract. [40] PomP from different cultivars showed high divergent GDRI (Figure 1). Mezzi_1 and Mezzi_2, Andaloussi_1, Zaghouani-1, and Garoussi_2 were able to delay the glucose diffusion by more than 40% after 60 minutes. The rest of the varieties' GDRI were between 20-30%. Given that all samples were prepared under the same conditions, the above variation could be due to the different PomP fiber composition from different cultivars. It has been stated that GDRI is related to several factors linked to fiber, such as structural and surface properties, content of SDF and uronic acid. [21] Four of the five cultivars showing the highest GDRI values, have the highest total DF content (Table 1), which suggests a correlation between total DF and GDRI. The GDRI exhibited by PomP in the present work, especially by some cultivars, is interestingly high compared to the intermediate values reported for most by-products, such as artichoke fiber (27%), [40] mango peel (21%), [41] carambola pomace (25 %), [42] and asparagus fiber (18-47%). [21] In fact, different parts of the pomegranate tree, including fruit peel, were known to have anti-hyperglycemic activity in ancient Unani and Chinese medicine. [43] The anti-diabetic effects of this fruit species were also demonstrated in several recent studies over last decade. [44]

3.3. Polyphenol content and antioxidant activity of pomegranate peel

Pomegranate is known to be one of the richest fruits in polyphenols. Particularly, the rind contains high amounts of phenolic compounds, such as punicalagins, ellagic acid isomers and anthocyanins. [7, 12, 45, 46] Studies have also reported that the phenolic content of pomegranate peels was 10 times higher (249.4 mg/g) than that found in the pulp (24.4 mg/g). [47] In the present work, the PomP powder from each cultivar was subjected to extraction with EtOH and acetone. From the ethanol-acetone extracts, soluble polyphenols were measured using the

Folin-Ciocalteu method (Figure 2). All cultivars showed high contents of soluble polyphenolic compounds, with significant inter-cultivar variation. The lowest polyphenolic content was 205 mg eq. GA/g registered in Mezzi_3 and the highest was 276 mg eq. GA/g registered in Garoussi_2. These values are in the same range as the ones reported by Fisher et al. and Ben Nasr et al. who found ~300 g/Kg DM and 217 mg eq. GA/g, respectively. [45, 48] Several authors stated that polyphenol content is a cultivar dependent attribute, allowing it to be used for cultivar differentiation. [46, 49, 50] However, as all secondary metabolites, phenolic compounds are highly affected by environmental conditions, as demonstrated in several cases, including pomegranate fruits. [51]

Owing to its richness in polyphenols, pomegranate peels are known to possess strong antioxidant activity. In accordance with the high content in polyphenols, the pomegranate peel extracts showed very high antioxidant activity (Table 4). The sour cultivar Mezzi_1 showed the highest values of antiradical activity (2696.50 µmol Trolox/g) and reducing power (2079.53 µmol Trolox/g), whereas the lowest values were registered for Zehri_3 (2018.44 µmol Trolox/g) and Gabsi_9 (1604.85 µmol Trolox/g). We also applied the DPPH technique to measure the antioxidant activity of the remaining solid after EtOH-acetone extraction. The insoluble antioxidant activity ranged from 13 to 22.89 µmol Trolox/g. The antioxidant activity is an interesting characteristic of DF because it could behave as a carrier for dietary antioxidants to the gastrointestinal tract. The responsible compounds for the insoluble antioxidant activity could be phenolic compounds structurally linked to fiber. Pectins and arabinoxylans have been clearly identified in the bibliography as the main polymers where phenol-polysaccharide bounds take place [52]. In PomP fiber, both groups of polysaccharides are present in important amounts, as mentioned above (Tables 1 and 2), but more detailed studies are needed to confirm the relationship between pectin/arabinoxylan, phenolic groups and antioxidant activity. Although the insoluble activity was low in PomP, it

is of great physiological significance because phenolic groups linked to fiber structure reach gastrointestinal mucosa and could exhibit their antioxidant activity *in situ* .[53]

This distribution of soluble/insoluble antioxidant activity in PomP shows that the majority of polyphenols could be easily extracted. This could be promising for the added value of this waste material as a source of extracts which are rich in bioactive compounds.

3.4.Principal Component Analysis

To show the variability which exists among the studied cultivars based on the above parameters, a PCA was performed. The contribution of each parameter to the two principal components and the distribution of cultivars on the first plane are shown in Figure 3. This plane absorbs 51.17% of the total variability of the studied parameters. The first axe (F1) was explained positively mainly by mannose, arabinose, solubility and galactose, and negatively by lignin, xylose, insoluble TEAC, soluble FRAP and DPPH activities; while the second principal component (F2) was positively correlated with neutral sugar content, OHC and rhamnose and negatively correlated with total phenolic content and cellulose. On this plane, the cultivar showed a large distribution, reflecting great variability. We can see that the acid-sweet character had no effect on the plotting, and therefore being acid or sweet did not affect any of the studied parameters, particularly the DF characteristics. However, this large distribution supported the cultivar effect on the studied parameters, *i.e.* genotype is likely to play a role in the determination of them. Mezzi_1 and Garoussi_2, on one hand, and Andaloussi_1 and Jebali_1 on the other, which appear closely positioned in the plot, support this fact as these two cultivars have been shown to be closely related genetically based on SSR markers.[54]

4. Conclusion

Being a symbol of super-fruit and famous for its numerous biological effects, the demand for pomegranate (*Punica granatum* L.) thrives in response to its increasing consumption, especially in the juice making industry. This will generate great amounts of waste. The present work is a contribution to the added value of this industrial by-product, constituted mainly by the pomegranate peel and internal membranes. The DF content of PomP varied between 33 and 62% of DM and its NS composition, reported for the first time, was dominated by the presence of arabinose and xylose, likely reflecting the occurrence of arabinoxylans as the major component of fibers. DF from PomP has promising functional properties. Besides the excellent ratio IDF/SDF, its values of WHC, OHC and solubility make it valuable for being added to fiber or antioxidant enriched foods. In addition, its high antioxidant activity and its capacity for glucose retention *in vitro* encourage deeper *in vivo* studies on its physiological functions.

But PomP is not only interesting as a healthier food ingredient, but also as source of added-value compounds. Its content in polyphenols is very high and they are very easily extractable. The solid after phenol extraction is rich in pectins, lignin, and arabinoxylans, which are biopolymers with increasing impact in chemical, food and pharmaceutical industries, especially when coming from natural sources.

The inter-cultivar variability was significant in most of the studied parameters and the diversity among them was great . Therefore, a selection should be necessary in order to choose the variety with the optimal composition according to its ultimate use. Based on these results, and taking into account the low cost and simple drying method of PomP, this by-product presents worthwhile properties, which bestow it with high potential for valorization in food and nutraceutical applications as a source of DF, antioxidants, and biopolymers.

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Table 1: Characterization of Pomegranate peel (PomP) total dietary fiber fraction in 12 cultivars

Cultivar	Total DF*#	IDF/SDFratio	Cellulose§	Lignin§	Uronic acid§	Neutral sugars§
AMR	33.10 ± 6.06 a	0.97	17.69 ± 1.20 b	28.67 ± 1.41 d	15.93 ± 0.68 b	16.88 ± 0.58 a
Chelfi_2	47.56 ± 0.09 de	0.92	18.01 ± 1.80 a	35.91 ± 1.87 f	16.28 ± 0.65 b	19.21 ± 0.71 b
Andaloussi_1	40.04 ± 3.81 b	0.93	20.39 ± 1.66 cd	41.86 ± 3.06 g	23.31 ± 0.20 c	18.69 ± 0.62 ab
Gabsi_8	46.41 ± 0.78 cd	1.01	22.71 ± 2.34 e	26.29 ± 0.88 cd	21.17 ± 0.05 d	17.83 ± 0.62 ab
Gabsi_9	45.33 ± 1.77 cd	0.94	18.06 ± 0.81 ab	22.30 ± 0.34 ab	18.14 ± 1.63 c	19.33 ± 0.06 b
Jebali_1	62.09 ± 0.58 g	1.24	20.67 ± 1.82 cd	39.00 ± 0.91 g	19.33 ± 0.70 bc	18.67 ± 0.71 ab
Zaghouani_1	49.97 ± 0.51 def	1.23	20.74 ± 1.08 c	32.37 ± 0.80 e	13.98 ± 0.63 a	19.66 ± 1.16 b
Zehri_3	40.18 ± 1.43 b	1.29	19.64 ± 1.72 c	20.59 ± 0.70 a	22.85 ± 0.66 d	19.33 ± 0.89 b
Garoussi_2♦	54.81 ± 1.84 f	1.28	20.80 ± 1.39 c	28.27 ± 0.68 d	14.66 ± 0.84 a	18.63 ± 0.04 ab
Mezzi_1♦	53.55 ± 1.52 f	1.15	21.19 ± 1.80 d	39.79 ± 0.79 g	17.98 ± 0.95 bc	17.96 ± 0.46 ab
Mezzi_2♦	51.68 ± 1.01 ef	1.08	16.53 ± 0.94 ab	24.45 ± 1.89 bc	17.24 ± 0.16 bc	18.80 ± 1.59 b
Mezzi_3♦	42.00 ± 0.51 bc	1.06	17.43 ± 1.38 ab	24.69 ± 0.48 bc	17.07 ± 0.49 bc	18.73 ± 1.49 ab

**: Total DF values are corrected with ash and proteins*
#: Values are per 100 g of original material, i.e. powder of pomegranate peel
§: Values are per 100 g of extracted dietary fiber
Values with different superscripts in the same row are significantly different ($p<0.05$)
♦*: sour cultivars*

Table 2: Relative neutral sugar percentage of fiber fraction of pomegranate peel

Cultivar	Rhamnose (%)	Fucose (%)	Arabinose (%)	Xylose (%)	Mannose (%)	Galactose (%)	Glucose (%)
AMR	4.67 ± 0.02 ab	1.47 ± 0.06 cd	32.62 ± 0.26 d	30.38 ± 0.34 ab	6.41 ± 0.01 d	14.94 ± 0.02 cd	9.51 ± 0.02 d
Chelfi_2	4.32 ± 0.01 ab	1.30 ± 0.09 b	26.33 ± 1.36 b	35.56 ± 1.69 cd	5.04 ± 0.02 a	14.24 ± 0.50 bc	13.20 ± 0.25 h
Andaloussi_1	4.60 ± 0.26 ab	1.25 ± 0.02 ab	26.16 ± 0.17 b	38.47 ± 0.86 ef	5.77± 0.11 c	13.45 ± 0.06 b	10.30 ± 0.28 ef
Gabsi_8	4.74 ± 0.49 ab	1.48 ± 0.00 cd	32.95 ± 2.51 de	31.28 ± 2.41 b	6.26 ± 0.00 d	16.27 ± 0.82 f	7.01 ± 0.42 b
Gabsi_9	5.73 ± 0.13 d	1.70 ± 0.03 f	30.27 ± 0.32 c	30.68 ± 0.66 b	6.96 ± 0.05 e	15.19 ± 0.04 de	9.47 ± 0.09 d
Jebali_1	4.58 ± 0.11 ab	1.29 ± 0.05 b	29.72 ± 0.91 c	36.30 ± 2.11 cde	5.53 ± 0.27 bc	16.16 ± 0.66 f	6.42 ± 0.11 a
Zaghouani_1	4.57 ± 0.02 ab	1.15 ± 0.00 a	22.33 ± 0.22 a	42.85 ± 0.15 g	5.21 ± 0.07 ab	12.38 ± 0.01 a	11.50 ± 0.04 g
Zehri_3	4.40 ± 0.74 ab	1.43 ± 0.03 c	35.00 ± 1.88 e	27.98 ± 0.58 a	7.14 ± 0.45 e	16.36 ± 0.48 f	7.69 ± 0.62 c
Garoussi_2♦	4.80 ± 0.04 bc	1.24 ± 0.04 ab	22.26 ± 0.41 a	43.10 ± 0.69 g	5.33± 0.04 ab	12.57 ± 0.13 a	10.68 ± 0.02 f
Mezzi_1♦	4.15 ± 0.01 a	1.26 ± 0.02 ab	26.00 ± 0.25 b	38.111± 1.04 def	5.41 ± 0.21 abc	15.20 ± 0.29 de	9.87 ± 0.31 de
Mezzi_2♦	5.34 ± 0.09 cd	1.57 ± 0.02 de	26.50 ± 0.19 b	34.67 ± 0.00 c	6.17 ± 0.01 d	15.93 ± 0.01 ef	9.82 ± 0.07 de
Mezzi_3♦	4.74 ± 0.04 ab	1.60 ± 0.10 ef	24.55 ± 0.35 ab	38.97 ± 0.49 f	6.24 ± 0.07 d	13.74 ± 0.36 b	10.17 ± 0.15 ef

Value followed by different letters are significantly different ($p<0.05$)
♦*: Sour cultivars*

Table 3: Water holding capacity (WHC), solubility and oil holding capacity (OHC) of pomegranate peel (PomP)

Cultivars	WHC (mL/g)	Solubility(%)	OHC (mL/g)
AMR	2.31 ± 0.06 a	61.16 ± 0.040 e	2.80 ± 0.19 a
Chelfi_2	2.28 ± 0.04 a	47.87 ± 1.15a	3.00 ± 0.31 a
Andaloussi_1	3.53 ± 0.13 f	56.09 ± 1.64 c	3.61 ± 0.19 bcd
Gabsi_8	3.03 ± 0.12 e	57.94 ± 1.61 d	3.70 ± 0.08 bcd
Gabsi_9	2.57 ± 0.11 b	60.04 ± 0.028 e	3.57 ± 0.13 bc
Jebali_1	2.85 ± 0.06 d	52.74 ± 0.052 b	3.58 ± 0.07 bc
Zaghouani_1	2.71 ± 0.05 bcd	53.28 ± 0.02 b	3.90 ± 0.18 de
Zehri_3	2.40 ± 0.12 a	65.00 ± 1.32 f	3.44 ± 0.17 b
Garoussi_2♦	2.76 ± 0.12 cd	53.28 ± 1.74 b	3.47 ± 0.12 b
Mezzi_1♦	2.67 ± 0.12 bc	57.81 ± 0.21 cd	3.60 ± 0.29 bcd
Mezzi_2♦	2.32 ± 0.09 a	61.63 ± 0.32 e	4.05 ± 0.13 e
Mezzi_3♦	2.31 ± 0.05 a	61.61 ± 0.32 e	3.84 ± 0.09 cde

Value followed by different letters are significantly different ($p<0.05$)
♦*: Sour cultivars*

Table 4: Soluble and insoluble antioxidant activities of pomegranate peel (PomP)

Cultivars	Antioxidant activity		
	DPPH EC50 (µmol Trolox/g)		Reducing power (µmol Trolox/g)
	Soluble	Insoluble	
AMR	2313.42 ± 115.51 c	15.73 ± 0.86 ab	1773.04 ± 106.90 bcd
Chelfi_2	2350.90 ± 10.33 cd	22.89 ± 0.48 e	1721.57 ± 204.10 abc
Andaloussi_1	2424.67 ± 115.34 de	20.23 ± 0.64 cde	1777.57 ± 119.24 bcd
Gabsi_8	2435.20 ± 111.71 de	17.59 ± 2.17bc	1695.13 ± 116.55 ab
Gabsi_9	2649.71 ± 240.16 f	19.77 ± 1.02 cd	1604.85 ± 101.91 a
Jebali_1	2296.46 ± 20.05 c	19.00 ± 0.24 cd	1721.86 ± 128.42 abc
Zaghouani_1	2313.50 ± 58.33 c	17.55 ± 1.59 bc	1892.82 ± 152.03 d
Zehri_3	2018.44 ± 29.36 a	13.52 ± 0.51 a	1679.20 ± 81.18 ab
Garoussi_2♦	2522.05 ± 175.62 e	19.60 ± 1.83 cd	1856.15 ± 187.02 cd
Mezzi_1♦	2696.50 ± 31.60 f	20.46 ± 1.69 de	2079.53 ± 74.11 e
Mezzi_2♦	2381.97 ± 12.55 cd	17.55 ± 0.46 bc	1922.31 ± 75.08 de
Mezzi_3♦	2147.01 ± 74.89 b	15.54 ± 1.68 ab	1798.96 ± 203.64 bcd

Value followed by different letters are significantly different ($p<0.05$)
♦*: Sour cultivars*

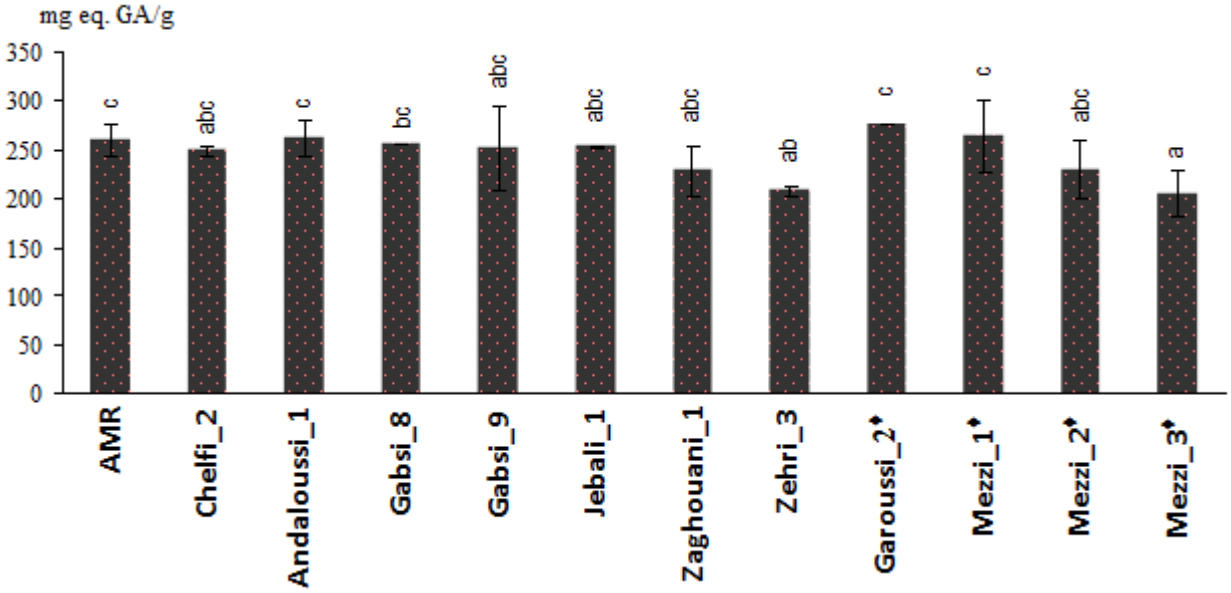

Figure 2: Total phenolic content of pomegranate peel (PomP) of different cultivars. (♦: Sour cultivars)

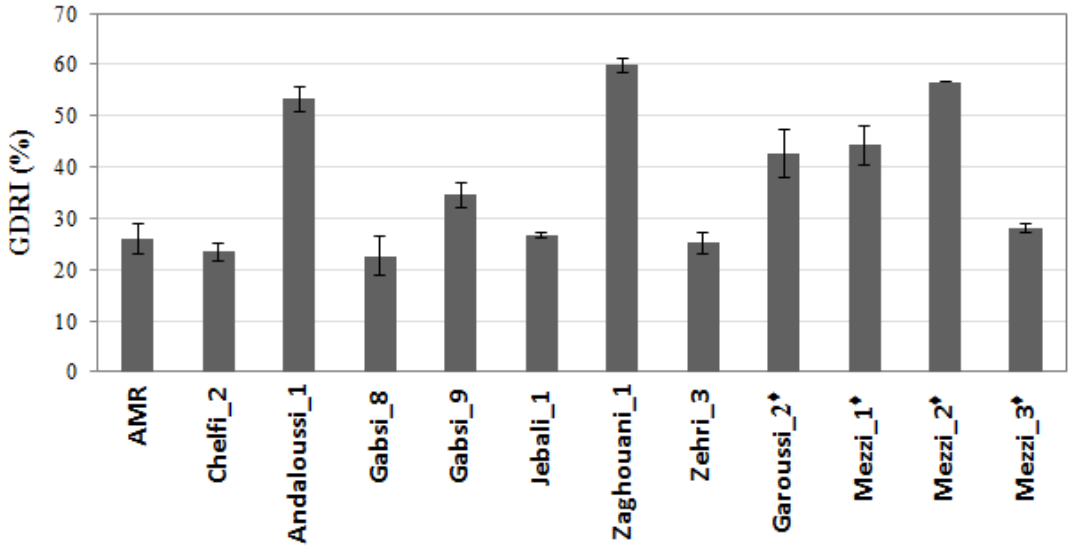

Figure 1: Glucose retardation index (%) of pomegranate peel (PomP) of different cultivars. (♦: Sour cultivars)

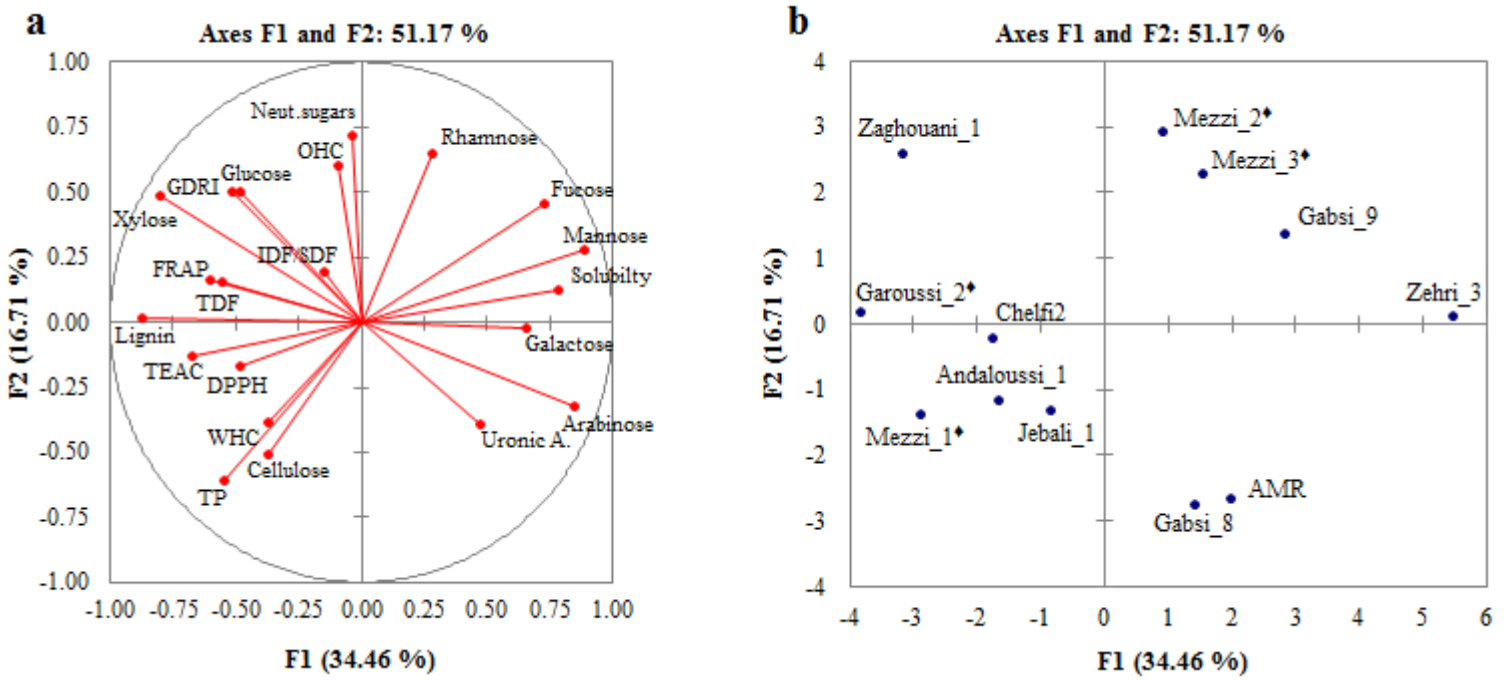

Figure 3: PCA analysis plot (axes 1-2): **a**) contribution of studied parameters and **b**) plot of 12 pomegranate cultivars