 doi:10.20944/preprints202208.0125.v1

Pomegranate biowastes are rich in valuable bioactive-compounds with potential benefit on human health.

Marra F., Petrovicova, B., Canino F., Maffia A., Mallamaci C., Muscolo A*.

Agriculture Department, Mediterranea University Feo di Vito, Reggio Calabria,

Italy.

***Correspondence**

Adele Muscolo, Agriculture Department,
Mediterranea University Feo di Vito,
89124, Reggio Calabria, Italy.
Email: amuscolo@unirc.it

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Abstract

Pomegranate use is increasing worldwide as it is considered a tasteful healthy food. It is mainly used as fruit, juice and jam. The pomegranate peel represents about 40-50% of the total fruit weight and contains numerous and diverse bioactive substances. The aim of this research was to analyse the pomegranate peel composition of *Wonderful* cultivated in Southern Italy and treated with an innovative physic dry concentration procedure. The aim was to verify how the drying process influenced the bioactive compounds that could be used as nutraceuticals. A comparison with the peel composition of freeze dried *Wonderful* cultivated in Southern Italy, freeze dried *Wonderful* cultivated in South Africa and freeze-dried pomegranate Kullu and Himachal cultivated in India has been done. Results evidenced that in pomegranate peels of *Wonderful* cultivated in Calabria and dried with the innovative process, total phenolic substances, total flavonoids, vitamin C, vitamin E and antioxidant activities, were the highest. Great amounts of single phenolic acids and flavonoids were found in Calabrian *Wonderful* peels dried with the innovative process. Overall, it emerged that the great amount of bioactive and diverse compounds found in Calabrian *Wonderful* pomegranate peel, come from the niche pedoclimatic conditions, and the physic drying innovative methodology.

Keywords: antioxidants; bioactive compounds, nutraceuticals, phenols, pomegranate peels.

1. Introduction

Pomegranate *(Punica granatum;Punicaceae***)** is a tree native to the Middle East, now cultivated worldwide especially in Mediterranean countries, China, South East Asia and other tropical or dry areas [1]. Except its delightful taste, its peel, fresh, seeds, juice and leaves hold a broad gamma of bioactive compounds (phenolics, flavonoids alkaloids, ellagic acid, punicalagin, anthocyanins and tannins) with an anti-oxidant [2], anti-inflammatory [3], anti-microbial [4], anti-cancer [5], anti-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

cardiovascular [6-8] and anti-infective [9] activities. As claimed by in vitro assays, commercial pomegranate juice has three-fold the antioxidant activity of red wine and green tea. In pomegranate predominates anthocyanins over tannins explaining its high reducing activity. Cyanidin-3,5-O-diglucoside and pelargonidin-3,5-O-diglucoside are the most representative anthocyanins in the different genotypes of pomegranate. For its great contents of different phytochemicals with health promoting effects [10, 11], pomegranate fruit is considered the *king of Super Fruits* group [12], and its extracts are also used by pharmaceutic industry for creating supplement in capsules [13]. Pomegranate cultivation covers, worldwide, at about 300,000 ha with a production of 3 million tons of which more than 76% is located in India, Iran, China, Turkey, and USA. The 500 cultivars of pomegranate that have been identified have different physic-chemical characteristics and produce fruits that differ in amount and types of bioactive compounds [14-15]. Fruits are of the best quality at a temperature of 38 °C under a dry climate, thus Mediterranean basin, has the appropriate climatic conditions, representing an ideal areal for a high production of good quality pomegranate fruits. Mediterranean pomegranates are mainly based on local cultivars, and their composition can differ from those of oriental varieties, displaying a large variety of chemical–physical traits and distinct flavour profiles. *Wonderful* is the most widely commercial pomegranate cultivar planted in Mediterranean countries and represents the industry standard variety. In the last decades, over the world, there was an increasing interest in the use of pomegranate and its parts justified by a crescent demand from health careful consumers and pharmaceutical and cosmetic industries [13]. Generally, the edible part of pomegranate is directly consumed as food, or used for the preparation of juices, canned beverages, jams and for the flavouring and colouring of drinks, conversely, pomegranate peel (approximatively 26–30% of the total fruit weight), that currently still represents a waste to be disposed of, is attracting the attention of the scientific community, for its high content of phytochemicals that allowed to individuate it as a new source of bioactive compounds, such as flavonoids, phenolic acids, and tannins with well ascertained antioxidant capacity [16-18]. It has been reported that pomegranate by-products and punicalagins in particular, decrease the level of fats in the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

blood and act as anticancer, antiviral, and anti-inflammatory [15, 19-21]. The scientific community was previously focused on the chemical characterization and health effects of pomegranate as fruit or juice and only few studies were recently focused on the amount and composition of the bioactive compounds present in the pomegranate peel that usually are a mixture, whose synergistic effect can often cause different physiological responses acting on different organ target contemporarily.

Based on the above consideration, the aim of the present study was to analyze the pomegranate peel composition of the variety *Wonderful* cultivated in South Italy and treated with an innovative dry system concentration by Gioia Succhi food industry. The aim was to verify as the innovation dry system affected the chemical composition of the peels and to identify valuable bioactive compounds to be used as an aid in the prevention and management of numerous diseases or metabolic disorder by recycling, reusing and reducing this waste. A comparison with the peel composition of freeze dried *Wonderful* cultivated in South Italy, freeze dried *Wonderful* cultivated in South Africa [15, 22] and freeze-dried Kullu and Himachal [23-24] cultivated in India and already used for pharmaceutical scope has been done.

2. Materials and Methods

2.1. Chemicals

Metaphosphoric acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), NaOH, nitro-blue tetrazolium, dichlorophenol-indophenol (DCPID),2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS•+), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxyl acid(Trolox), phenazine methosulphate, ethanol, gallic acid, ethylene-diamine-tetra acetic acid (EDTA), ferrozine, 2,4,6-tris(2-piridil)-s-triazine (TPTZ) and iron sulphate heptahydrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HPLC grade methanol and acetonitrile (Sigma Aldrich, 99,99%), acetone (Sigma Aldrich, 99,5%), deionised water, formic acid (Carlo Erba, 95 %), hydrochloric acid (Carlo Erba, 37%) were used for sample extraction and HPLC analysis. All

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

chemical standards: gallic acid, protocatechuic acid, procyanidin 1 and 2, syringic acid, *p,m,o* - coumaric acids, pelargonidin, trans-cinnamic acid, bergamottin, cyanidine 3 O- glucoside, catechin, vanillic acid, epicatechin, delphinidin, trans-4-hydroxycinamic acid, sinapinic acid, 3-hydroxycinnamic acid, myricetin, luteolin, punicalagin ,2,5 dihydroxy benzoic acid, caffeic acid, ellagic acid, naringin, apigenin-7-neohesperosside, spiraeoside, quercetin, kaempferol, tocopherol, chlorogenic acid, vicenin 2, eriocitrine, rutin, vitexin, quercitin-3 beta-D glucoside, ferulic acid and apigenin were purchased from Sigma Aldrich Italy. Other chemicals were of analytical grade purchased from Carlo Erba Reagents s.r.l. (Cornaredo, MI, Italy).

2.2. Pomegranate peel preparation and extraction

Fruits of the cultivar *Wonderful* grown in Calabria were washed and hand-peeled. The peel of *Wonderful* mature fruits, has been differently dried: 1) with an innovative process by Gioia succhi a Calabrian food transformation industry that used a physical spry dried innovative process (PDS) that is covered by the industrial secret. 2) stored a -80 °C for two days and then lyophilized with a freeze dry system (Cheimika, SH Top) at -56 °C for 96 h (FD). The dried peels have been both analysed for chemical characteristics.

2.3. Sample extract preparation

The extracts were obtained using the method described in Muscolo et al (2020). Briefly, lyophilized pomegranate peels were extracted at room temperature (22–25 °C) with continuous stirring for 90 min with 15 mL 95% ethanol. The samples were centrifuged (Unicen 21 RT167, Ortoalresa Inc., Madrid, Spain) at 2370×g (4000 rpm) for 15 min and the supernatants were filtered with 1 mm Whatman 185 filter paper (Merck), evaporated to dryness in a rota-vapour (Diagonal condenser RE 400, Stuart Equipment, ST15, Stone, UK) and re-suspended in a final volume of 3.0 mL 95% ethanol.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Lyophilized pomegranate peels were extracted at room temperature with continuous stirring for 60 min with 2.0 mL dH20 (Intercontinental Mod still 3/ES, Bioltecnical Service, s.n.c., Rome, Italy). The samples were then centrifuged at 590×g (2000 rpm) for 10 min and the supernatants were filtered with Whatman 1 filter paper and used for the determination of protein, carbohydrates and ferrous chelating activity.

2.4. Determination of total phenolic compounds, total flavonoids, vitamins A, C and E in pomegranate peel

Total phenol content was determined with the Folin–Ciocalteu reagent according to Muscolo et al. [25]. Briefly, 500 µl of the aqueous extract was mixed with 250 µl of Folin-Ciocalteu reagent and 2 ml of a 20% Na2CO3 aqueous solution, the mixture was filled up to 50 ml with deionised water and placed in the dark for 1 h. The absorbance was measured at 765 nm using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, CA USA). The results were expressed as mg/L of gallic acid equivalents.

Total flavonoid content was determined according to the colorimetric method as reported in Muscolo et al. [25]. The absorbance was measured at 510 nm using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, CA USA). The results were expressed as rutin equivalents (mg/l) using a calibration curve.

Vitamin A was detected as reported in Aremu and Nweze [26]. Absorbance was read at 436 nm and Vitamin A was expressed as Retinol Equivalent (RE).

For vitamin C (ascorbic acid) determination, the method of Davies and Masten [27] was used. Pomegranate powders (0.10 g) were extracted with 10 mL of 3% meta-phosphoric acid - 7.98% acetic acid, centrifuged at 2370×g (4000 rpm) for 10 min and the supernatant was used for the determination of ascorbic acid.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

For vitamin E (α-tocopherol) analysis, pomegranate powder (0.10 g) was extracted with 10 mL of hexane:isopropanol solution (3:2 v/v), with agitation for 5 h, and centrifuged at 1330×g (3000 rpm) for 10 min. The supernatant was used for the determination of vitamin E [28].

2.5. Protein and carbohydrate detection in pomegranate peel

Soluble protein was determined using the Bradford method as reported in Muscolo et al. [25] by using Coomassie Brilliant Blue G-250. The absorbance of each sample was measured at 595 nm using an 1800 UV-Vis Spectrophotometer (Shimadzu, Kyoto, Japan). Bovine serum albumin>99% purity (Sigma) was used as standard, and soluble proteins were estimated as mg BSA/g dw.

The total available carbohydrates were measured using the anthrone method with minor modifications as reported in Muscolo et al. [25]. The amount of available carbohydrates was calculated using a glucose calibration curve (range of 10–100 mg/mL). The results were reported as mg/g dw.

2.6. Determination of antioxidant activities in pomegranate peel

The antioxidant activity against DPPH radical (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was determined with the method reported in Muscolo et al. [25]. The DPPH concentration in the cuvette was chosen to give absorbance values of ∼1.0. Absorbance changes of the violet solution were recorded at 517 nm after 30min of incubation at 37 ∘C. The inhibition I (%) of radical-scavenging activity was calculated as:

$$I\ (\%) = [(A0 - AS)/A0] \times 100,$$

where A0 is the absorbance of the control and AS is the absorbance of the sample after 30 min of incubation. Results were expressed as µmol Trolox/g DW.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

The 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay (ABTS) was carried on according to Muscolo et al [25] by using a solution of 7 mM of ABTS in phosphate buffered saline (PBS). Aliquots of ethanol extracts (25, 50 and 100 µL) were added to 0.5 mL of ABTS+• solution and bring to the final volume of 600 µL with PBS. After 6 min of incubation in the dark at room temperature the absorbance of the samples was measured at 734 nm. Results were expressed as µmol Trolox/g DW

The total antioxidant capacity (TAC) was performed according to Muscolo et al. [25]. Sample absorbance was measured at 695 nm using UV–visible spectrophotometer. Methanol (0.3 ml) in place of extract was used as blank. The antioxidant activity was expressed as µg of α-tocopherol g^{-1} DW on a calibration curve.

2.7. RP-DAD-HPLC identification of phenolic and flavonoid components

Pomegranate peel was finely ground for analysis. By-product samples were subjected to solvent extraction before HPLC–PDA/ESI-MS analysis for determination of the single phenolic and flavonoid compounds. Each sample was extracted in two different ways 0.1 g of previously lyophilised pomegranate peels were dissolved in 10 ml of 1% of HCl in methanol and 0.1g of sample was dissolved in 10 ml of acetone solution: 1% of HCl in methanol (1:1). Each sample was analyzed in six independent replicates [25].

2.8. Statistical analysis

Analysis of variance was carried out for all the data sets. One-way ANOVA with Tukey's Honestly. Significant Difference test were carried out to analyse the effects of treatment/cultivar on each of the various parameters measured. ANOVA and T-test were carried out using SPSS software (IBM Corp.2012). Effects were significant at $p \leq 0.05$. To explore relationships among different treatment/cultivar and chemical parameters datasets were analysed using Principal Component Analysis (PCA).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

3. Results and discussion

Results evidenced that total carbohydrates were contained in major quantity in peels of other cultivars than in peels of *Wonderful*. The lowest carbohydrate content was found in PSD (Fig. 1). Total proteins had an opposite trend, was the lowest in peels of the other cultivars and the highest in PSD (Fig 1 A). Total phenols and total flavonoids, were present in the highest quantity in the Calabrian *Wonderful* spray dried peels and in the lowest amount in the other cultivar peels. Total phenols were more than total flavonoids in all the samples analysed (Fig 1 B). These data evidenced that *Wonderful* cultivar had the majority of total phenols and flavonoids. These data highlighted that the geographic condition in which a determined type of cultivar grow, can drive the synthesis of bio-compounds, sometime shifting the metabolism from primary to secondary one. Data of Ramakrishna and Ravishankar [29] showed how drought conditions increased in different plants and in different part of the plants the amount of total flavonoids and phenolic acids that were used as antioxidants to overcome the stress conditions. Vaneková et al. [30] showed as the environmental factors such as altitude, habitat type, and moreover sunlight exposure, influenced the synthesis of total phenols and flavonoids in seven different cultivars of berries. The distribution of drylands is quite accentuated in the Southern and Mediterranean countries, and Calabria, in particular, is dominated by climate, mainly characterized by dry summers and mild, wet winters conditions that as demonstrated by Fialho et al. [31], right in pomegranates, increase, under stress secondary metabolites, with nutraceutical properties. The data of this research are in line with literature findings and highlighted as the major quantity of total phenols and flavonoids contained in *Wonderful* peel cultivated in Calabria can be the results of the microclimatic conditions which in turn affected metabolism, increasing antioxidants as well as antioxidant activities, and increased also soluble protein amount that can have the double function, to work as osmolyte, or as antioxidative enzymes, as already demonstrated by Kosová et al. [32]. In the spray dried peel, we found the greatest amount of these compounds, evidencing as the innovative

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

methodology used to dry the peels did not denature the bio-compounds but rather concentrated them. Vitamins were contained in greater amount in the *Wonderful* cultivar that in the other cultivars, and were more concentrated in PSD and CFD. Vitamin E was the most abundant in all the pomegranate peel samples, except for the peel of the Indian cultivars. Vitamins have a great role as antioxidants and have important health benefit when intake with diet. The PSD contained a huge amount of vitamins. It was demonstrated that vitamins are enzymatic cofactors and act as antioxidants. Vitamin C increased under stress conditions to protect plants from oxidative stress by acting to detoxify ROS by a direct scavenging or by acting as cofactors in the enzymatic reactions that involved ascorbate peroxidase and glutathione reductase enzymes [33]. Vitamin E that is the most abundant among the vitamins, is a major singlet oxygen scavenger that provides protection against lipid peroxidation [34]. In support of the above findings, the activities of the antioxidant enzymes were greater in PSD than in the other samples. All the activities (DPPH, ABTS and TAC) were mostly expressed in PSD and CFD than in the peels of the other samples. These data evidenced that the innovative dry process didn't affect the biological compounds and the enzymes. Pearson's correlation was used to determine the degree of correlation between selected reference data and variables (Table 3). As expected, TP was positively correlated with all the variables except for CARB. Vitamins and proteins were also positively correlated with all the variables except for carbohydrates. Total flavonoids didn't correlate with the antioxidant activities and CARB. In addition, strong positive correlations between the antioxidant assays were reported at $r = 0.998$, $p = 0.05$, between DPPH and ABTS; at $r = 0.996$, $p = 0.05$, between DPPH and TAC; and at $r = 0.988$, $p = 0.05$, between ABTS and TAC (Table 3). This agrees with our results in Table 1 and suggest that the high phenolic content in peel extracts determine the strong antioxidant activity.

Single phenolic acids were mainly contained in *Wonderful* peels than in the peels of the other cultivars. In PSD the greatest amount of single phenolic acids was detected. Ellagic acid was the more abundant followed in ranking by 2-5 dihydroxy-benzoic, gallic, protocatechuic, *p*-coumaric,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

chlorogenic and ferulic acids. It has been widely demonstrated that ellagic acid (EA) is a potent antioxidant with antimicrobial, anti-inflammatory, neuroprotective, antihepatotoxic, anticholestatic, antifibrogenic, anticarcinogenic, cytotoxic, and antiviral effects [35]. Recently Reis Jordão et al. [36] evidenced that ellagic acid can be a promising alternative treatment for hypertension and cardiovascular disease, and Pei et al. [37] demonstrated that EA can be used to prevent diabetic cardiac dysfunction. The doses of EA generally tested in the prevention health treatments were 30 mg/kg. PSD peel contained a great amount of EA (240 mg/g) suggesting its possible use as nutraceutical supplement for the prevention of numerous diseases. Additionally, gallic, 2-5 dihydroxy-benzoic, protocatechuic and ferulic acids have been found in a number of phytomedicines with diverse biological and pharmacological activities, including radical scavenging, apoptosis of cancer cells, antihyperglycemic, antioxidant effects and cardioprotective activity [38-41]. Among the single flavonoids procyanidin 2, punicalagin, procyanidin 1 and pelargonidin were, in this order, the most abundant compounds in PSD. Conversely, in the freeze-dried Calabrian and South African *Wonderful* peel less amount of single flavonoids than PSD was found but a greater quantity than the Indian cultivars. CFD had a greater amount of punicalagin (65 mg/g), procyanidin 1(1.6) procyanidin 2 (1,6) and delphinidin than SAFD. IC contained only a great amount of procyanidin 2 in respect to CFD and SAFD. PCA analysis confirmed this assertion, evidencing that TP, TF, Vit C and Vit A were mainly correlated with PSD (Fig 4). No correlation between SAFD, and IC were evidenced. Single phenolic acids correlated only with PSD (Fig 5) while single flavonoids were mainly correlated with PSD and in part with CFD. Rutin, luteolin, delphinidin and apigenin were the single flavonoids that were more contained and correlated with CFD (Fig-6)

Procyanidins 1 and 2, were discovered to inhibit human colorectal adenocarcinoma and to improve the survival of chronic disease patients by reducing the complications of cardiovascular disease and metabolic syndrome, improving the overall quality of life [42]. Additionally, punicalagin is a flavonoid with proven antioxidant, hepatoprotective, anti-atherosclerotic and antitumoral activity

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

[43]. An ethical study was performed in 50 subjects (25 treated with supplements and 25 with placebo) to identify clinical features induced by 25 mg pomegranate (*Punica granatum*) dried fruit extract (which in turn contains 3.75 mg procyanidins), 8.75 mg punicalagin-ellagic acid. Results evidenced after 60 days of treatment, that the values for systemic oxidative stress, plasmatic antioxidant capacity, and skin antioxidant power increased significantly [44]. Other authors evidenced that the daily intake of pomegranate juice, rich in flavonoids and phenols decreased the susceptibility of low-density lipoproteins (LDLs) to aggregate and in cultured human coronary artery endothelial cells exposed to high shear stress, down-regulated the expression of redox sensitive genes and increased the functioning of blood endothelial cells [7]. Considering that peels contain much more phenols and flavonoids than juice as already demonstrated by previous study of Derakhshana et al. [45]. and Russo et al [46]. carried out on different cultivars, we can conclude that pomegranate peels represents a resource comparable to the fruit, if not better, that can be used as a source of bio-compounds with high added value in the nutraceutical field to formulate new supplements with beneficial effects on human health.

4. Conclusions

In short, the data evidenced that pomegranate peel is a valuable raw material rich in bioactive compounds. The amount and the type of bioactive compounds depends on the cultivars but also from the area where the plant grows.

Same cultivars grown in different condition can have different variety of phenolic contents and diverse antioxidant activities evidencing as the pedoclimatic variables drive the metabolism of the cultivars increasing or decreasing specific secondary metabolites implicated in physiological adjustment of plants to adapt to stress conditions or changing condition induced by climate. In addition, the new spry drying system developed by Gioia succhi, is determining in the concentration and conservation of the bioactive compounds in respect to freeze dry system that was already

 doi:10.20944/preprints202208.0125.v1

demonstrated, by numerous studies and in diverse cultivars, much relevant than oven dried (40-60 °C).

According to the achieved results, the high antioxidant capacity of pomegranate peel, exalted by the innovative method of drying, highlighted their use as supplement to preserve human health contemporarily from diverse diseases.

 doi:10.20944/preprints202208.0125.v1

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Federica Marra: Formal analysis, **Beatrice Petrovicova**: Methodology, Formal analysis, **Francesco Canino:** Formal analysis, **Angela Maffia:** Statistical analysis**, Carmelo Mallamaci**: Formal analysis, **Adele Muscolo**: Data curation, Writing - original draft, Writing - review & editing.

References

1 Sarkhosh, A.; Zamani, Z.; Fatahi, R.; Ebadi, A. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Sci. Hortic*. **2006,** *111 (1),* 24-29.

2 Derakhshana, Z.; Ferrante, M.; Tadi M.; Ansari F.; Heydarif, A.; Zam W.; Khaddour, A. Anti-virulence effects of aqueous pomegranate peel extract on E. coli urinary tract infection. *Progr. Nutr*. **2019,** *19(1-S),* 98-104.

3 Mastrogiovanni, F.; Mukhopadhya, A.;Lacetera, N.; Ryan, M.T.; Romani, A.; Bernini, R.; Sweeney, T. Anti-Inflammatory Effects of Pomegranate Peel Extracts on In Vitro Human Intestinal Caco-2 Cells and Ex Vivo Porcine Colonic Tissue Explants. *Nutrients* **2019,** *11,* 548; doi:10.3390/nu11030548

4 Alexandre, E.M.C.; Silva, S.: Santos, S.A.O.; Silvestre, A.J.D.; Duarte, M.F.; Saraiva, J.A.; Pintado, M. Antimicrobial activity of pomegranate peel extracts performed by high pressure and enzymatic assisted extraction. *Food Res Int*. **2019,** *115*,167-176. doi: 10.1016/j.foodres.2018.08.044. Epub 2018.

5 Usha, T.; Middha, S.K.; Sidhalinghamurthy, K.R. Pomegranate Peel and Its Anticancer Activity: A Mechanism-Based Review. **2020;** In: Swamy M. (eds) Plant-derived Bioactives. Springer, Singapore. https://doi.org/10.1007/978-981-15-2361-8_10.

6 Aviram, M.; Rosenblat, M. Pomegranate Protection against Cardiovascular Diseases. Evidence-Evid.-based Complement. *Altern. Med***. 2012**, 382763, 20 doi:10.1155/2012/382763.

7 Wang, D.; Özen, C.; Abu-Reidah, I.M.; Chigurupati, S.; Patra, J.K.; Horbanczuk, J.O.; Jóźwik, A.; Tzvetkov, N.T.; Uhrin, P.; Atanasov, A.G. Vasculoprotective Effects of Pomegranate (Punica granatum L.). *Front Pharmacol*. **2018,** *9*, 544. doi: 10.3389/fphar.2018.00544. PMID: 29881352; PMCID: PMC5977444.

8 Zuraini, N.Z.A., Sekar, M.; Wu, Y.S.; Gan, S.H.; Bonam, S.R.; Mat Rani, N.N.I.; Begum, M.Y.; Lum, P.T.; Subramaniyan, V.; Fuloria, N.K.; Fuloria, S. Promising Nutritional Fruits Against Cardiovascular Diseases: An Overview of Experimental Evidence and Understanding Their Mechanisms of Action. *Vasc Health Risk Manag*. **2021,** *17*, 739–769.

9 Tito, A.; Colantuono, A.; Pirone, L.; Pedone, E.; Intartaglia, D.; Giamundo, G.; Conte, I.; Vitaglione, P.; Apone, F. Pomegranate Peel Extract as an Inhibitor of SARS-CoV-2 Spike Binding to Human ACE2 Receptor (in vitro): A Promising Source of Novel Antiviral Drugs. *Frontiers Chem***.2021,** 9, https://www.frontiersin.org/article/10.3389/fchem.2021.638187

10 Czieczor, L.; Bentkamp, C.; Damerow, L.; Blanke, M. Non-invasive determination of the quality of pomegranate fruit. *Postharvest Biol. Technol.* **2018,** *136*, 74–79. https://doi.org/10.1016/J.POSTHARVBIO.2017.10.008

11 Kumar, N.; Neeraj, D. Study on physico-chemical and antioxidant properties of pomegranate peel. *J Pharmacogn Phytochem*. **2018,** *7*, 2141–2147.

12 Chang, S.K.; Alasalvar, C.; Shahidi, F. Superfruits: Phytochemicals, antioxidant efficacies, and health effects – A comprehensive review, *Crit. Rev. Food Sci. Nutr*. **2019,** *59*, 10, 1580-1604, DOI: 10.1080/10408398.2017.1422111.

13 Karimi, M.; Sadeghi, R.; Kokini, J. Pomegranate as a promising opportunity in medicine and nanotechnology. *Trends Food Sci Technol.* **2017,** *69,* 59–73. https://doi.org/10.1016/J.TIFS.2017.08.019

14 Hmid, I.; Elothmani, D.; Hanine, H.; Oukabli, A.; Mehinagic, E. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum L.*) cultivars grown in Morocco. *Arabian J. Chem*. **2017,** *10*, S2675-S2684.

15 Bassiri-Jahromi, S.; Doostkam, A. Comparative evaluation of bioactive compounds of various cultivars of pomegranate (*Punica granatum*) in different world regions. *AIMS Agric. Food* **2019,** *4(1),* 41-55. doi: 10.3934/agrfood.2019.1.41

16 Lee, C.J.; Chen, L.G.; Liang, W.L.; Wang, C.C. Anti-inflammatory effects of punica granatum Linne in vitro and in vivo. *Food Chem*. **2010,** *118,* 315–322.

17 Panichayupakaranant, P.; Tewtrakul, S.; Yuenyongsawad, S. Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract. *Food Chem.* **2010,** *123,* 400–403. 10.1055/s-0030-1264706

18 Fischer, U.A.; Carle, R.; Kammerer, D.R. Identification and quantification of phenolic compounds from pomegranate (*Punica granatum L.*) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS(n*). Food Chem.* **2010,** *127,* 807–821. 10.1016/j.foodchem.2010.12.156

19 Li, Y.F.; Guo, C.J.; Yang, J.J.; Wei, J.Y.; Xu, J.; Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem*. **2006,** *96,* 254–260.

20 Hossin, F.L.A. Effect of pomegranate (*Punica granatum*) peels and it's extract on obese hypercholesterolemic rats. *Pak. J. Nutr.* **2009,** *8,* 1251–1257. 10.3923/pjn.2009.1251.1257.

21 Lin, L.T.; Chen, T.Y.; Lin, S.C.; Chung, C.Y.; Lin, T.C.; Wang, G.H.; et al. Broad-spectrum antiviral activity of chebulagic acid and punicalagin against viruses that use glycosaminoglycans for entry. *BMC Microbiol.* **2013,** *13,* 187. 10.1186/1471-2180-13-187.

22 Mphahlele, R.R.; Fawole, O.A.; Makunga, N.P. et al. Effect of drying on the bioactive compounds, antioxidant, antibacterial and antityrosinase activities of pomegranate peel. *BMC Complement Altern. Med*. **2016,** *16,* 143 https://doi.org/10.1186/s12906-016-1132-y

23 Bhat, M.; Thakur N.S.; Jindal, N. studies on the effect of drying methods and packaging on quality and shelf life of dried wild pomegranate arils *Asian J. Dairy Food Res.* **2014,** *33 (1),* 18 – 24.

24 Kumar, N.; Pratibha; Neeraj; et al. Effects of drying methods and solvent extraction on quantification of major bioactive compounds in pomegranate peel waste using HPLC. *Sci Rep*. **2022,** *12,* 8000. https://doi.org/10.1038/s41598-022-11881-7

25 Muscolo, A.; Papalia, T.; Settineri, G.; Mallamaci, C.; Panuccio, M. Sulfur Bentonite-organic-based Fertilizers as Tool for Improving Bio-compounds with Antioxidant Activities in Red Onion. *J. Sci. Food Agric*. **2019,** *100,* doi:10.1002/jsfa.10086.

26 Aremu, S.O.; Nweze, CO. Determination of vitamin A content from selected Nigerian fruits using spectrophotometric method. *Bangladesh J Sci Ind Res*. **2017,** *52(2),*153.

27 Davies, S.H R.; Masten, S.J. Spectrophotometric method for ascorbic acid using dichlorophenolindophenol: Elimination of the interference due to iron. *Analytica Chim. Acta* **1991**, *248*, 225–227

28 Prieto, P.; Pineda, M.;Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the determination of Vitamin E. *Anal. Biochem*. **1999,** *269,* 337–341.

29 Ramakrishna, A.; Ravishankar, G.A. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav*. **2011,** *6(11),*1720-31. doi: 10.4161/psb.6.11.17613. Epub 2011 Nov 1. PMID: 22041989; PMCID: PMC3329344.

30 Vaneková, Z.; Vanek, M.; Škvarenina, J.; Nagy, M. The Influence of Local Habitat and Microclimate on the Levels of Secondary Metabolites in Slovak Bilberry (Vaccinium myrtillus L.) Fruits. *Plants* **2020,** *9,* 436. https://doi.org/10.3390/plants9040436

31 Fialho, L.; Ramôa, S.; Parenzan, S.; Guerreiro, I.; Catronga, H.; Soldado, D.; Guerreiro, O; Gonzalez García, V.; Oliveira e Silva, P; Jerónimo, E. Effect of regulated deficit irrigation on pomegranate fruit quality at harvest and during cold storage. *Agric. Water Manage.* **2021,** *251,* 106869, ISSN 0378-3774, https://doi.org/10.1016/j.agwat.2021.106869.

32 Kosová, K.; Vítámvás, P.; Urban, M.O.; Prášil, I.T.; Renaut, J. Plant Abiotic Stress Proteomics: The Major Factors Determining Alterations in Cellular Proteome. *Front Plant Sci.* **2018**, *8,* 9:122. doi: 10.3389/fpls.2018.00122. PMID: 29472941; PMCID: PMC5810178.

33 Laspina, N.V.; Groppa, M.D.; Tomaro, M.D.; Benavides, M.P. Nitric oxide protects sunflower leaves against Cd–induced oxidative stress *Plant Sci.* **2005,** *169,* 323-330.

34 Muñoz, P.; Munné-Bosch, S. Vitamin E in Plants: Biosynthesis, Transport, and Function. *Trends Plant Sci***. 2019**, *24(11),*1040-1051. doi: 10.1016/j.tplants.2019.08.006. Epub 2019 Oct 9. PMID: 31606282.

35 Vattem, D.A.; Shetty, K. Biological functionality of ellagic acid: a review. *J. Food Biochem*. **2005,** *29,* 234 – 266

36 Reis Jordão, J.B.; Paes Porto, H.K.; Lopes, F.M.; Carvalho Batista A.; Lavorenti Rocha M. Protective Effects of Ellagic Acid on Cardiovascular Injuries Caused by Hypertension in Rats. *Planta Med*. **2017,** *83,* 830–836.

37 Pei, S.; Zhao, H.; Chen, L.; He X.; Hua, Q.; Meng X.; Shi, R.; Zhang, J.; Zhang, H.; Liu, R.; Li, D. Preventive Effect of Ellagic Acid on Cardiac Dysfunction in Diabetic Mice through Regulating DNA Hydroxymethylation. *J. Agric Food Chem*. **2022,** *70, (6),* 1902-1910 DOI: 10.1021/acs.jafc.1c07574.

38 Badhani, B.; Sharmaa, N.; Kakkar, R. Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. *RSC Adv*. **2015**, *5*, 27540-27557.

39 Kakkar, S.; Bais, S. A Review on Protocatechuic Acid and Its Pharmacological Potential. *Pharmacology* **2014,** Article ID 952943, 9pp.

40 Sarker, U.; Oba, S. Polyphenol and flavonoid profiles and radical scavenging activity in leafy vegetable Amaranthus gangeticus. *BMC Plant Biol*. **2020,** *20(1),* 499. doi: 10.1186/s12870-020-02700-0. PMID: 33138787; PMCID: PMC7607633.

41 Vázquez-Ruiz, Z.; Toledo, E.; Vitelli-Storelli, F.; Goni, L.; de la O, V.; Bes-Rastrollo, M.; Martínez-González, M.Á. Effect of Dietary Phenolic Compounds on Incidence of Cardiovascular Disease in the SUN Project; 10 Years of Follow-Up. *Antioxidants* **2022,** *11,* 783. https://doi.org/10.3390/antiox11040783

42 Dasiman, R.; Md Nor, N.; Eshak, Z.; Mutalip, S.S.M.; Suwandi, N.R.; HBidin, H. A Review of Procyanidin: Updates on Current Bioactivities and Potential Health Benefits. *Biointerface Res. Appl. Chem*. **2022,** *12,* (5) 5918-5940.

43 Venusova, E.; Kolesarova, A.; Horky, P.; Slama, P. Physiological and Immune Functions of Punicalagin. *Nutrients* **2021,** *13(7),* 2150. doi: 10.3390/nu13072150. PMID: 34201484; PMCID: PMC8308219.

44 Buonocore, D.; Lazzeretti, A.; Tocabens, P.; Nobile, V.; Cestone, E.; Santin, G.; Bottone, M.G.; Marzatico, F. Resveratrol-procyanidin blend: nutraceutical and antiaging efficacy evaluated in a placebo controlled, double-blind study. *Clin Cosmet Investig Dermatol*. **2012**, *5*,159-65. doi: 10.2147/CCID.S36102. Epub 2012 Oct 5. PMID: 23071399; PMCID: PMC3469214.

45 Derakhshan, Z.; Ferrante, M.; Tadi, M.; Ansari, F.; Heydari, A.; Hosseini, M.S.; Conti, G.O.; Sadrabad, E.K. Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food Chem Toxicol.* **2018,** *114*,108-111. doi: 10.1016/j.fct.2018.02.023. Epub 2018 Feb 12. PMID: 29448088.

46 Russo, M.; Fanali, C.; Tripodo, G. et al. Analysis of phenolic compounds in different parts of pomegranate (*Punica granatum*) fruit by HPLC-PDA-ESI/MS and evaluation of their antioxidant activity: application to different Italian varieties. *Anal. Bioanal. Chem*. **2018** *410,* 3507–3520. https://doi.org/10.1007/s00216-018-0854-8.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Figure Captions

Figure 1. Soluble proteins and total carbohydrates (A) and total phenols and total flavonoids (B) in peels of different pomegranate cultivars differently dried. PSD (spry dried *Wonderful* peel, experimental data); CFD (Calabrian *Wonderful* peel freeze dried, experimental data) SAFD (South African *Wonderful* peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data). The experimental data are the mean of six replicates. Soluble protein (mg BSE $*g^{-1}$DW) carbohydrates (mg $*g^{-1}$DW), total phenols (µg TAE $*g^{-1}$DW), total flavonoids (µg quercetin * g^{-1} DW).

Figure 2. Vitamin A, C, E in peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian Wonderful peel freeze dried, experimental data) SAFD (South African Wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data). The experimental data are the mean of six replicates.

Figure 3. Antioxidant activities expressed as 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), total antioxidant capacity (TAC) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), in peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian Wonderful peel freeze dried, experimental data) SAFD (South African Wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data). The experimental data are the mean of six replicates.

Figure 4. Total phenols (TP), total flavonoids (TF), proteins (PRO), vitamin C (VIT C), vitamin A (VIT A), vitamin E (VIT E), total carbohydrates (CARB), soluble proteins, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), total antioxidant capacity (TAC) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS)contained in peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian wonderful peel freeze dried, experimental data) SAFD (South African wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Figure 5. PCA (principal component analysis) diagram of single phenolic acids contained in the peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian wonderful peel freeze dried, experimental data) SAFD (South African wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data).

Figure 6. PCA (principal component analysis) diagram of single flavonoids contained in the peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian wonderful peel freeze dried, experimental data) SAFD (South African wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Table 1 Single phenolic acids contained in the peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian wonderful peel freeze dried, experimental data) SAFD (South African wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data). The experimental data are the mean of six replicates. Different letters in the same row indicate significant differences $p \leq 0.05$.

	PSD	**CFD**	**SAFD**	**IC**
	mg/g SS	mg/g SS	mg/g SS	mg/g SS
Phenolic acids				
Gallic	11.12^{a}	0.2^{c}	1.23^{b}	0.2^{c}
Protocatechuic	7.95^{a}	1.4^{b}	nd	nd
Syringic	0.6	nd	nd	nd
p-coumaric	5.8^{a}	4^{b}	0.086^{c}	0.07^{d}
m-coumaric	4^{a}	0.6^{b}	nd	nd
o-coumaric	0.8^{ab}	nd	1.2^{a}	0.5^{b}
Trans-cinnamic	1.2^{b}	0.4^{c}	2.0^{a}	0.3^{c}
3-hydroxycinnamic	0.6	nd	nd	nd
Trans-4-hydroxycinnamic acid	0.6^{b}	1^{a}	0.3^{b}	0.05^{c}
Sinapic acid	0.7^{a}	0.2^{b}	0.05^{c}	0.01^{d}
2,5 dihydroxy-benzoic acid	16.4^{a}	0.8^{b}	0.4^{b}	0.04^{c}
Vanillic acid	0.8^{b}	2.6^{a}	0.8^{b}	0.2^{c}
Chlorogenic acid	5.6^{a}	6^{a}	1.4^{b}	0.5^{c}
Ferulic acid	4^{a}	0.4^{b}	0.3^{b}	0.02^{c}
Ellagic acid	240^{a}	8^{b}	2^{c}	0.5^{d}

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Table 2 Single flavonoids in peels of different pomegranate cultivars differently dried. PSD (spry dried Wonderful peel, experimental data); CFD (Calabrian wonderful peel freeze dried, experimental data) SAFD (South African wonderful peel freeze-dried, literature data) IC (Indian cultivar peel freeze- dried literature data). The experimental data are the mean of six replicates. Different letters in the same row indicate significant differences $p \leq 0.05$

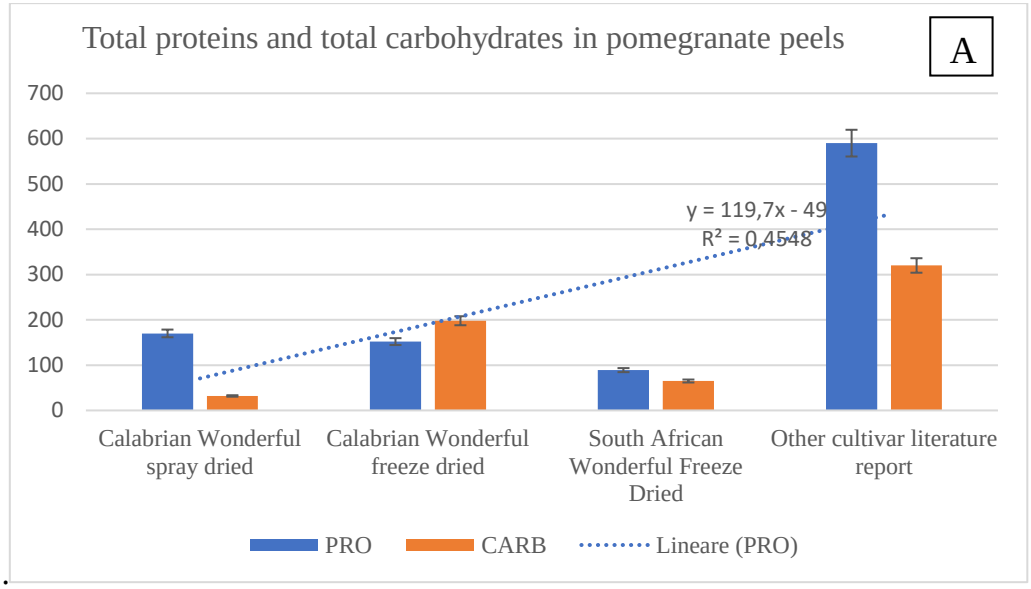
	PSD	**CFD**	**SAFD**	**OCLR**
	mg/g SS	mg/g SS	mg/g SS	mg/g SS
Flavonoids				
Procyanidin 2	178.2^{a}	1.6^{c}	1.2^{d}	8.91^{b}
Pelargonidine	5.8^{a}	nd	nd	nd-
Cyanidine 3 O-glucoside	12.2^{a}	4^{b}	4^{b}	0.15^{c}
Catechin	12^{a}	3^{a}	0.03^{c}	1.4^{b}
Epicatechin	1.369^{a}	nd	0.017^{c}	0.07^{b}
Delphinidin	0.800^{b}	173^{a}	0.39^{c}	0.42^{c}
Myricetin	1.160a	1.4^{a}	nd	nd
Luteolin	nd	1	nd	0.0025
Punicalagin	86^{a}	65^{b}	40	28
Naringin	0.9	nd	nd	nd
Apigenin-7-neohesperosside	0.24^{b}	1.2^{a}	0.34^{b}	nd
Spiraeoside	1.04^{a}	0.6^{b}	0.5^{b}	nd
Quercetin	3^{a}	2^{a}	0.3^{b}	0.02^{c}
Kaempferol	1.2^{a}	0.2^{b}	0.05^{b}	0.1^{b}
Tocopherol	2.4	nd	nd	nd
Procyanidin 1	12.7^{a}	1.6^{b}	1.2^{b}	nd
Vicenin 2	nd	2	nd	nd
Erythrocin	0.92	nd	nd	nd
Rutin	0.26^{b}	3^{a}	0.56^{b}	0.021^{c}
Quercetin-3 beta-D glucoside	1.3^{a}	nd	0.18^{b}	0.05^{c}
Apigenin	2^{a}	2^{a}	0.7^{b}	0.037^{c}

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Table 3. Pearson's correlations (r) between antioxidant compounds, vitamins, antioxidant activities, protein and carbohydrates Values in bold are different from 0 with a significance level alpha=0.05

Variables	TP	TF	VIT A	VIT C	VIT E	DPPH	ABTS	TAC	PRO	CARB
TP	**1**	0,848	**0,954**	0,940	0,883	0,766	0,803	0,727	0,867	-0,649
TF	0,848	**1**	0,787	0,796	0,502	0,314	0,374	0,253	0,488	-0,232
VIT A	**0,954**	0,787	**1**	**0,998**	0,878	0,722	0,750	0,705	0,792	-0,783
VIT C	0,940	0,796	**0,998**	**1**	0,850	0,682	0,710	0,666	0,754	-0,770
VIT E	0,883	0,502	0,878	0,850	**1**	**0,964**	**0,972**	**0,958**	**0,972**	-0,880
DPPH	0,766	0,314	0,722	0,682	**0,964**	**1**	**0,998**	**0,996**	**0,979**	-0,825
ABTS	0,803	0,374	0,750	0,710	**0,972**	**0,998**	**1**	**0,988**	**0,990**	-0,809
TAC	0,727	0,253	0,705	0,666	**0,958**	**0,996**	**0,988**	**1**	**0,958**	-0,859
PRO	0,867	0,488	0,792	0,754	**0,972**	**0,979**	**0,990**	**0,958**	**1**	-0,760
CARB	-0,649	-0,232	-0,783	-0,770	-0,880	-0,825	-0,809	-0,859	-0,760	**1**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1



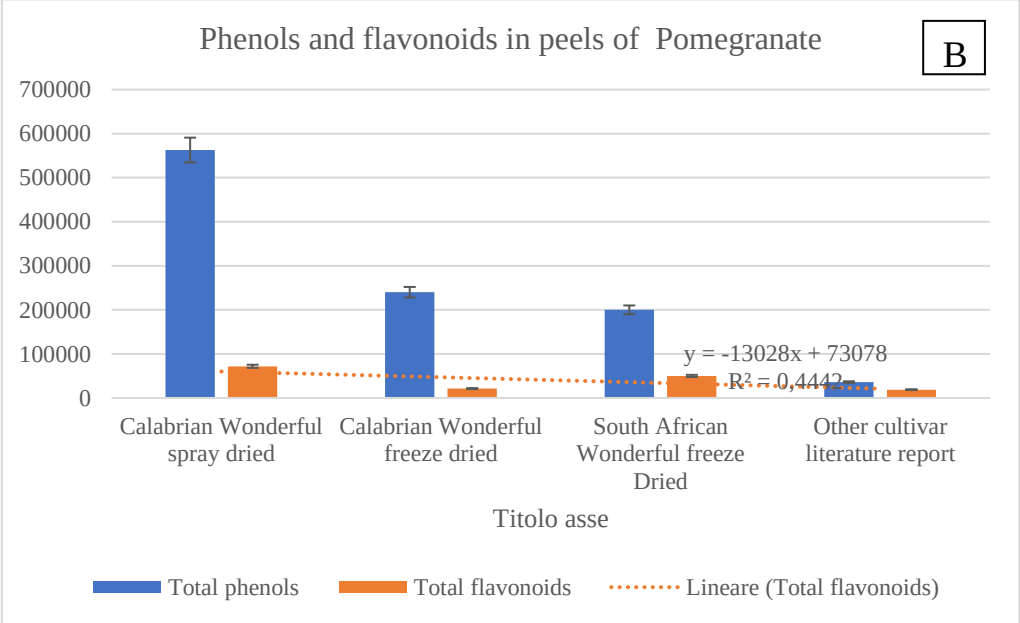


Figure 1.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

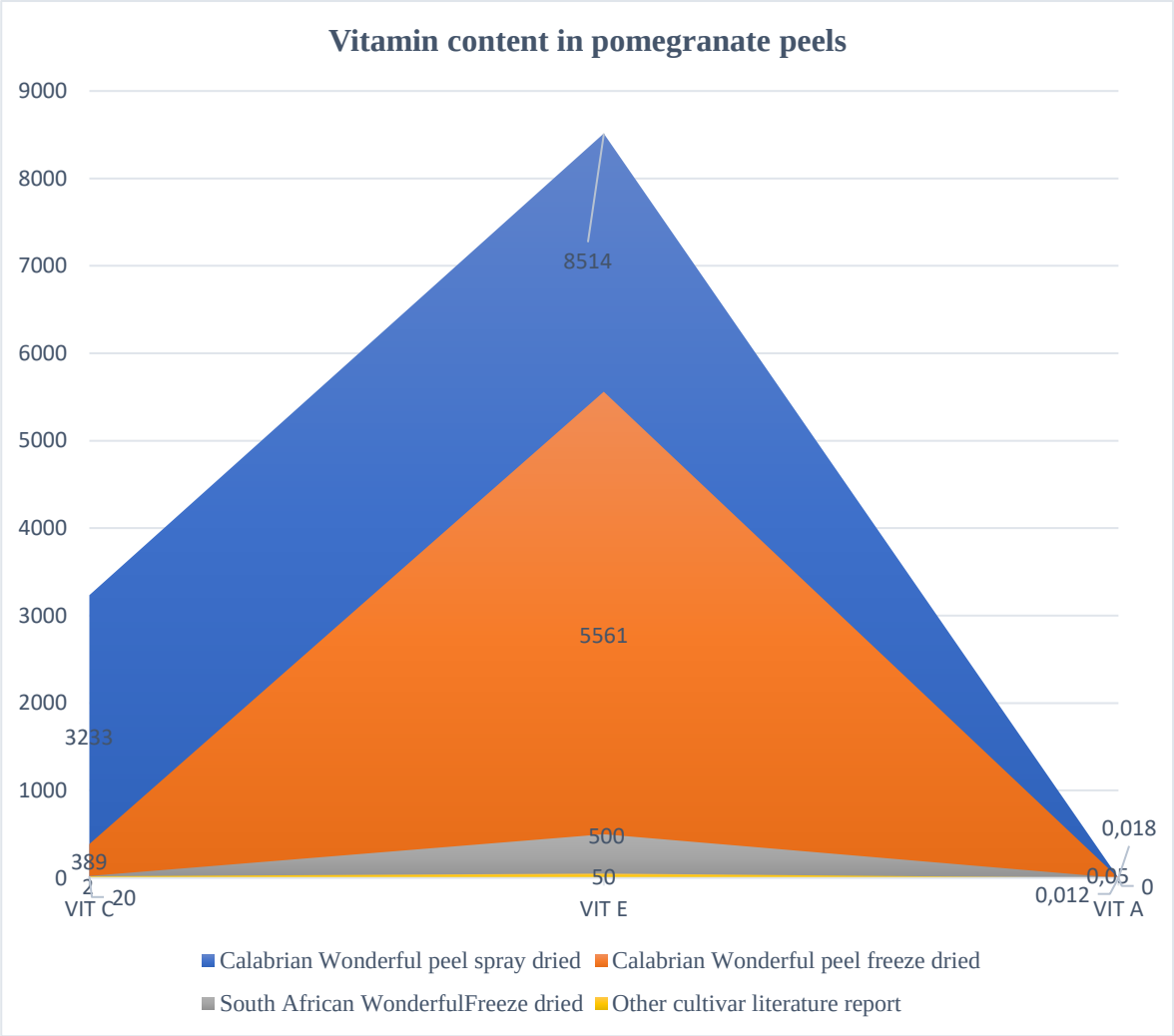


Figure 2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

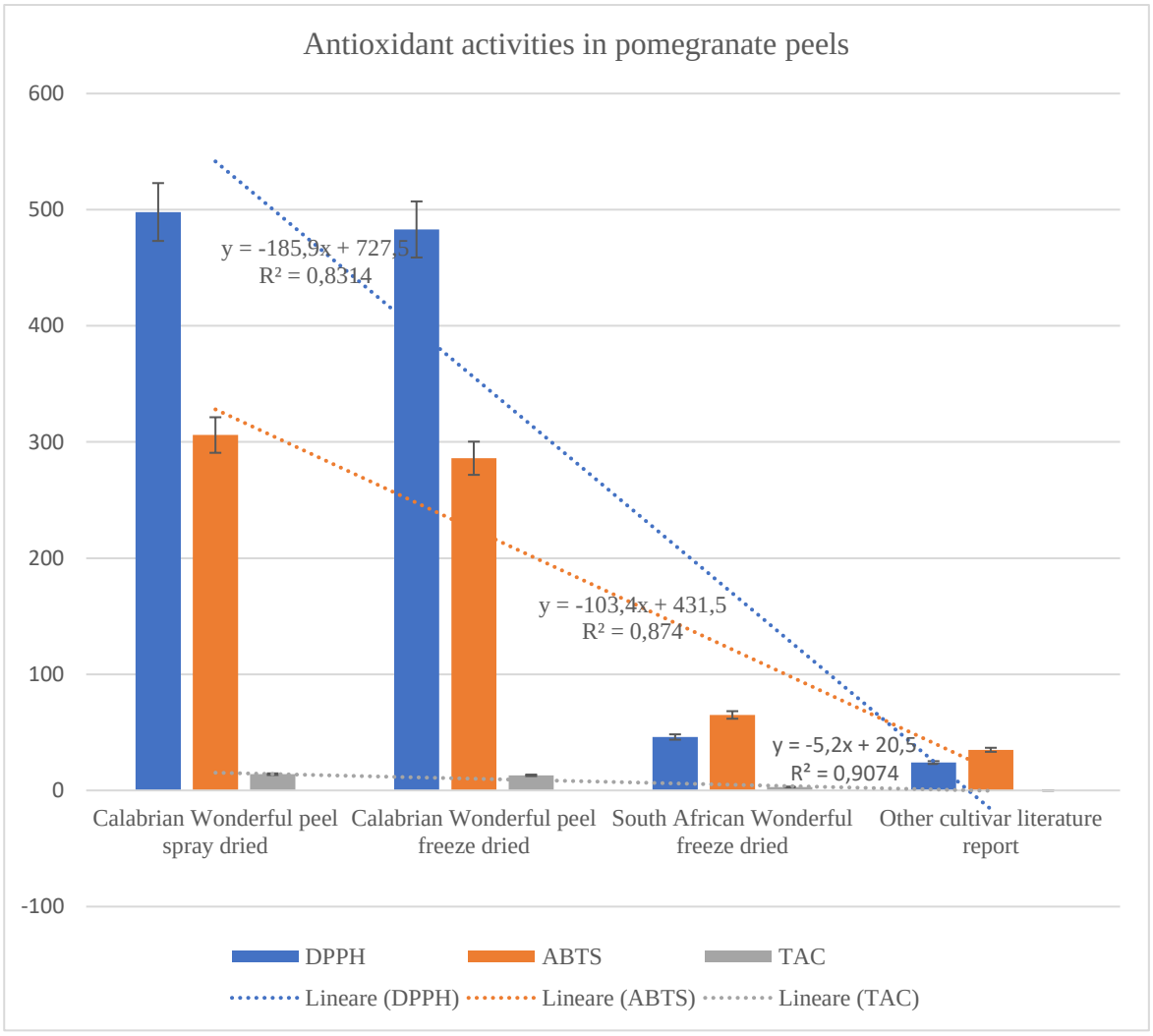


Figure 3.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

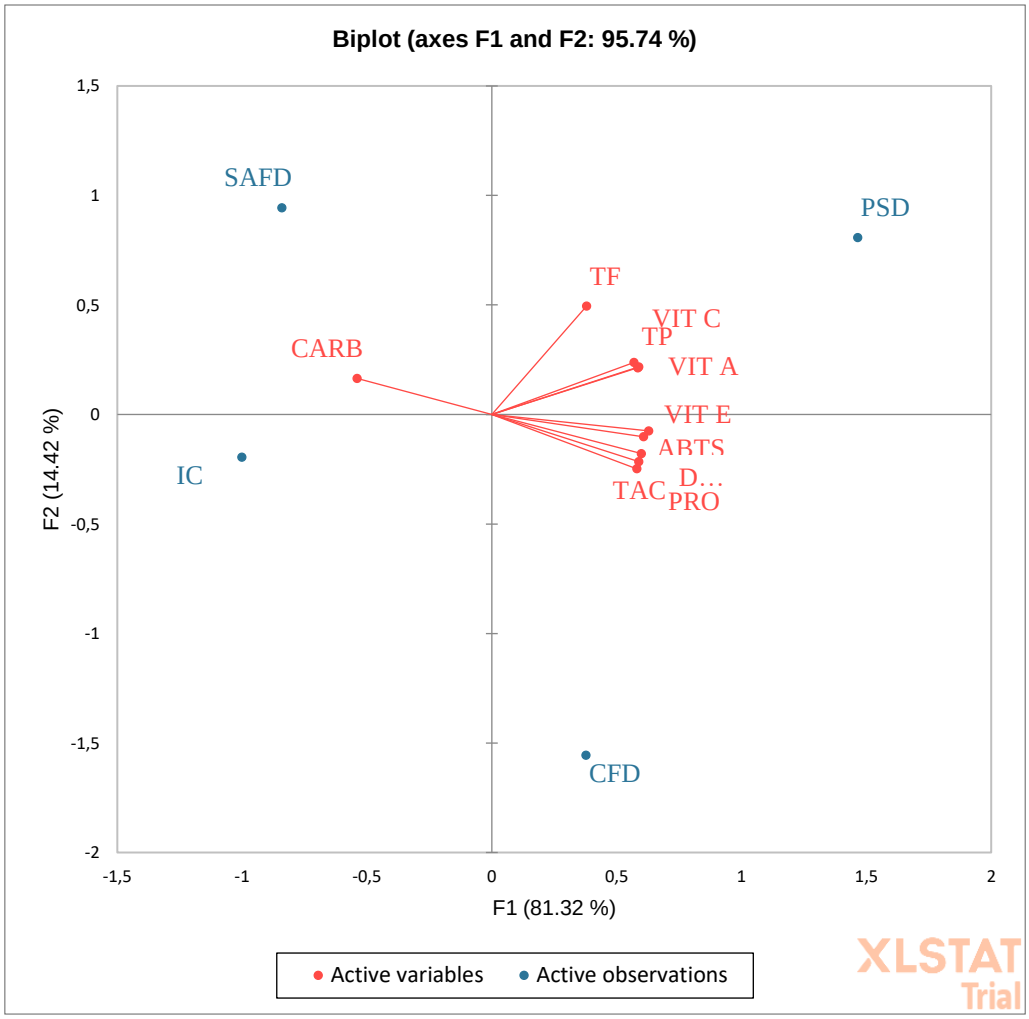


Figure 4.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

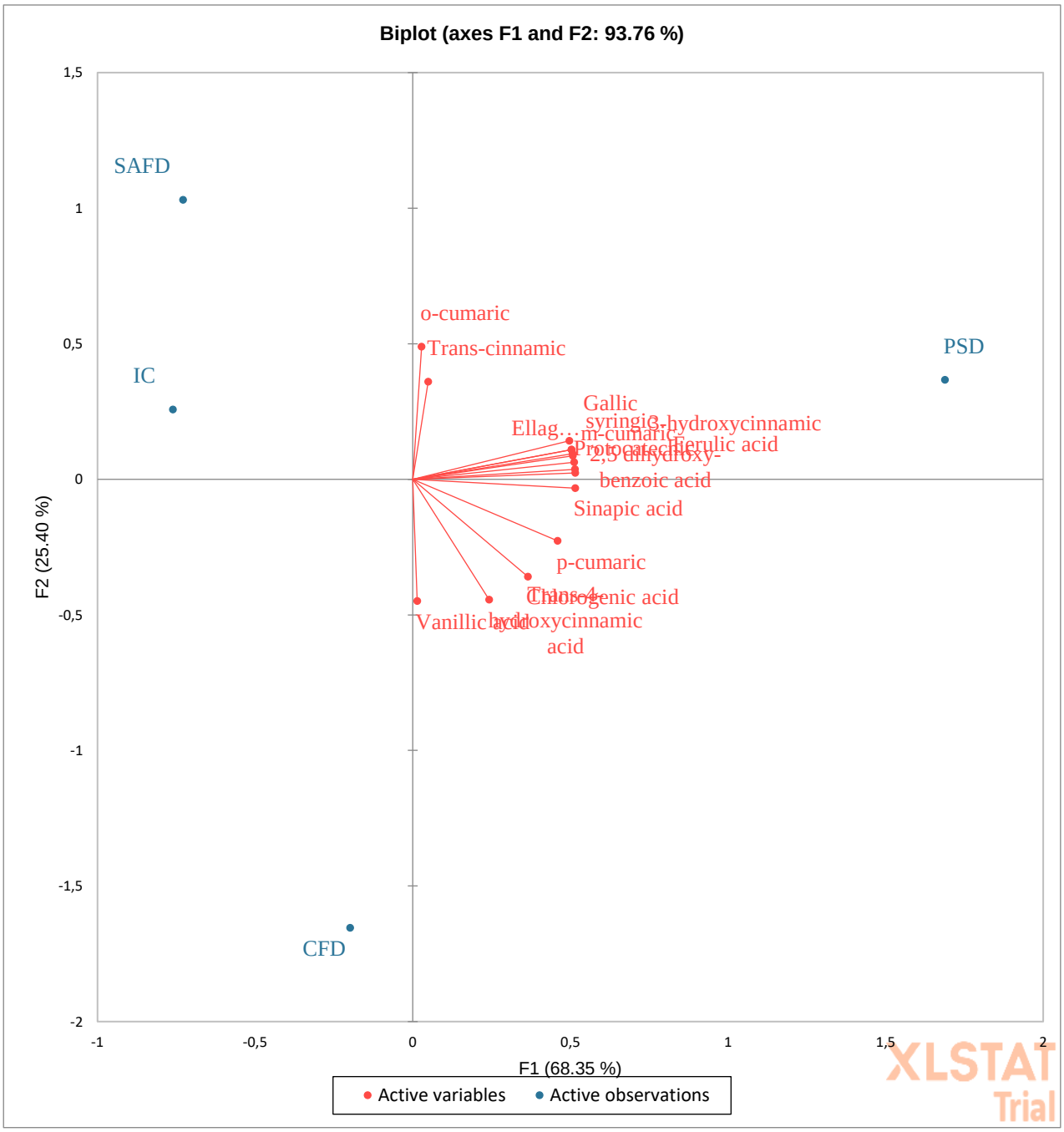


Figure 5.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 August 2022 doi:10.20944/preprints202208.0125.v1

Figure 6.