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Review Article

Pomegranate peel and peel extracts: Chemistry and food features

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Running Title: Food features of pomegranate peel and extract

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Abstract

The present review focuses on the nutritional, functional and anti-infective properties of pomegranate (*Punica granatum* L.) peel (PoP) and peel extract (PoPx) and on their applications as food additives, functional food ingredients or biologically active components in nutraceutical preparations. Due to their well-known ethnomedical relevance and chemical features, the biomolecules available in PoP and PoPx have been proposed, for instance, as substitutes of synthetic food additives, as nutraceuticals and chemopreventive agents. However, because of their astringency and anti-nutritional properties, PoP and PoPx are not yet considered as ingredients of choice in food systems. Indeed, considering the prospects related to both their health promoting activity and chemical features, the nutritional and nutraceutical potential of PoP and PoPx seems to be still underestimated. The present review meticulously covers the wide range of actual and possible applications (food preservatives, stabilizers, supplements, prebiotics and quality enhancers) of PoP and PoPx components in various food products. Given the overall properties of PoP and PoPx, further investigations in toxicological and sensory aspects of PoP and PoPx should be encouraged to fully exploit the health promoting and technical/economic potential of these waste materials as food supplements.

Keywords: Pomegranate Peel; Antioxidant; Prebiotics; Cancer; Cardiovascular diseases; Free Radicals; Lipid Oxidation; SAR; Toxicity

1.0. Introduction

Pomegranate (*Punica granatum* L.) is better known in some countries as the fruit of *Eden* (Al-Quran) for its pleasant taste and excellent health benefiting properties. Over the last decade, pomegranate fruit and fruit extracts have been shown to possess preventive and attenuating activities against numerous chronic and health/life threatening maladies such as cancer (Lansky and Newman 2007; Orgil, Schwartz, Baruch, Matityahu, Mahajna, & Amir, 2014), type 2 diabetes (Banihani, Swedan, & Alguraan, 2013), atherosclerosis and cardiovascular diseases (Rosenblat, Volkova, Coleman & Aviram, M., 2006; Sestili et al., 2007, Aviram et al. 2008; Al-Jarallah et al., 2013; Hamoud et al., 2014). Interestingly, the above nutraceutical properties are not limited to the edible part of pomegranate fruit: in fact the non-edible fractions of fruit and tree (i.e. peel, seeds, flowers, bark, buds and leaves), although considered as waste, contain even higher amounts of specific nutritionally valuable and biologically active components as compared to the edible fruit (Rosenblat, Volkova, Coleman & Aviram 2006; Sestili et al., 2007; Orgil et al., 2014). Indeed, PoP and PoPx, hold significant free radical scavenging, anti-microbial, antiatherogenic and antimutagenic properties and are reported to produce ameliorating effects against many critical maladies (Aviram et al., 2008; Malviya & Hettiarachchy, 2013; Sestili et al. 2007; Zahin, Aqil, & Ahmad, 2010). Unfortunately, functional foods containing PoP or PoPx are not, in general, well accepted by consumers because of their relatively reduced sensory features (Akpinar-Bayizit, Ozcan, & Yilmaz-Ersan, 2012; Syed, Chamcheu, Adhami, & Mukhtar, 2013; Sharma, Prakash, Gupta, Prakash, & Sharma, 2014; Ismail, Akhtar, Riaz, & Ismail, 2014).

Nonetheless, the above health promoting features prompt the food entrepreneurs to focus on PoP and PoPx-containing food preparations including food supplements, neutraceuticals and phenolics enriched diets (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008b; Kanatt, Chander, & Sharma, 2010; Ismail, Sestili, & Akhtar, 2012; Qu, Breksa III, Pan, Ma, & Mchugh, 2012). In addition to their nutraceutical relevance, PoP and PoPx exhibit important technical functions (antioxidant, antimicrobial, colorant and flavoring) and may also act as excellent natural additives for food preservation and quality enhancement. As a consequence, on account of these whole properties the use of peel's fractionated compounds in food and nutraceutical industry is on the rise (Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008b; Kanatt, Chander, & Sharma, 2010; Ismail, Sestili, & Akhtar, 2012; Qu, Breksa III, Pan, Ma, & Mchugh, 2012). In particular, since the peel fraction of pomegranate is a valuable reservoir of diversified polyphenols such as sugar-free mono and oligomeric ellagitannins, it has been frequently utilized as natural antioxidant in various dietary supplements. Currently, to minimize the problem of its bad taste, commercial formulations of PoPx dietary supplements are available as capsules, tablets, and soft gels.

Apart from the established inclusion of PoP in several ayurvedic therapies and the recent tendency to an increased utilization, the food use of PoP and PoPx is still poor and underestimated (Ismail, Sestili, & Akhtar, 2012; Ismail, Akhtar, Riaz, & Ismail, 2014). The aim of the present review is to highlight the importance of PoP, PoPx and their biological fractions as food bulking agents and/or valuable substitutes of common synthetic food additives, providing baseline information on their potential applications with regard to the general issues of food safety, preservation, enrichment and quality enhancement.

2.0. Pomegranate peel phytochemistry

PoP – which accounts for about 50% of fruit weight is characterized by the presence of high molecular weight phenolics, ellagitannins, proanthocyanidins, complex polysaccharides, flavonoids and appreciable quantities of microelements that, on the whole, exhibit strong anti-mutagenic, antioxidant, antimicrobial and apoptotic properties (Dikmen, Ozturk, & Ozturk, 2011; Li et al., 2006; Tezcan, Gultekin-Ozguven, Diken, Ozcelik, & Erim, 2009; Prakash, Mathur, Vishwakarma, Vuppu, & Mishra, 2013; Ricci, Giamperi, Bucchini & Fraternale, 2006). The fruit contains a rich variety of flavonoids, constituting nearly 0.2% to 1.0% of the fruit weight; approximately 30% of all fruit anthocyanidins are concentrated in the peel portion. The reciprocal concentration of these compounds depends on the cultivar type and on the various developmental phases of the fruit, and is responsible for the variations in pomegranate peel color (Fischer, Carle, & Kammerer, 2011; Kumari, Dora, Kumar, & Kumar, 2012; Zhao, Yuan, Fang, Yin, & Feng, 2013).

Data from literature indicate that 124 different phytochemicals can be found in pomegranate fruit; among these phytochemicals, high molecular weight polyphenols (e.g. ellagitannins and the pomegranate-peculiar punicalagin) are likely to mediate the protective effects against a wide range of oxidative and inflammatory disorders, including cancer (Heber, 2011). Nearly 48 phenolic compounds (anthocyanins, gallotannins, hydroxycinnamic acids, hydroxybenzoic acids and hydrolysable tannins i.e. ellagitannins, and gallagyl esters) have been identified in PoP and other anatomical parts of the fruit. The whole fruit is rich in large polyphenolic compounds such as punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides) but, interestingly, PoP contains the most promising pool of phenolics (predominantly those from hydrolysable tannins) as compared to their concentration in any other anatomical part of the fruit.

Mounting evidence suggests that hydrolysable polyphenols in PoP, specifically ellagitannins, are the most active antioxidants among the tannins contained therein. These compounds (ellagic acid, punicalagin, punicalin and gallagic acid) have been shown to hold heightened antioxidant and pleiotropic biological activities and notably, to act synergistically together (Seeram & Heber, 2011). Nevertheless *in vivo* studies suggest that the antioxidant properties of dietary absorbed polyphenols are tied to their metabolized compounds, e.g. urolithins (Johanningsmeier & Harris, 2010).

High molecular weight ellagitannins are water soluble plant phenolics that yield different biologically relevant by-products upon hydrolysis. Under normal physiological conditions, orally ingested ellagitannins undergo microbial hydrolysis by gut microflora to relatively smaller compound, i.e. ellagic acid and, upon further bacterial metabolism, urolithins. Ellagitannins' hydrolysis, either through acid, base or microbial activity yields ellagic acid. Punicalagin is unique to pomegranate and is part of a family of ellagitannins which include the minor tannins called punicalin and gallagic acid, which are characterized by a good water solubility.

Hydrolysable tannins are reported to be the first plant polyphenols subjected to analytical research around 200 years ago (Arapitsas, 2012): nonetheless data are still scant to interpret the nutraceutical and food features of a substantial number of PoP polyphenols. Amongst a wide array of PoP isolated fractions of phytochemicals (Table 1) only a few have been thoroughly investigated to date for their efficacy against certain disorders and their potential to be technologically exploited as food additives. Since the major and most studied phenolics of PoP are punicalagin and its metabolites, it would be advisable to study more in depth other PoP compounds to establish their potential role as nutraceutical and food additives.

3.0. Structure Activity Relationship of PoP Phenolics

Ellagitannins are commonly referred to as metabolites of gallotannins. As briefly discussed above, this unique group of phenolics is easily hydrolyzable. Hydrolysis releases hexahydroxydiphenic acid that spontaneously lactonizes to form ellagic acid, a relatively stable monomeric structure of high antioxidant potential (Kaponen, Happonen, Mattila, & Torronen, 2007; Aguilera-Carbo, Augur, Prado-Barragan, Favela- Torres, & Aguilar, 2008).

PoP and the mesocarp of the fruit contain high concentration of hydrolysable tannins i.e. 27 – 172 and 32 – 263 g/Kg respectively, with a prevalence of monomeric phenolics (Fischer, Jaksch, Carle, & Kammerer, 2013). Monomeric hydrolysable tannins (e.g. tellimagrandin I, strinctinin and corilagin) have been reported to possess potent antibacterial activity as compared to oligomeric tannins and dimeric and trimeric procyanidins.

The tendency of either hydrolysable or condensed tannins or of flavonoids to act as antioxidants, or to exhibit antimicrobial features are governed by their chemical structures (Yoshida, Hatano, & Ito, 2000; Heim, Tagliaferro, & Bobilya, 2002). PoP ellagitannins bearing multiple phenolic hydroxyl groups transfer hydroxyl residues to free radicals thus quenching these harmful species. The progressive oxidation by free radicals of native ellagitannins reduces the number of -OH groups, giving rise to by-products such as dehydroellagitannins characterized by a progressively weaker antioxidant activity (Okuda, 1999). Furthermore, adjacent hydroxyl groups, such as catechol -OHs, confer the ability of chelating iron and transition metals, a highly relevant feature in terms of antioxidant capacity (Sestili et al., 2002). Indeed, chelation of iron or of transition metals impedes the propagation of free radicals generated through the Fenton reaction (Sestili et al., 2007). In general, the relative potency of PoPx flavonoids to act as antioxidants is ascribed to the number and configuration of hydroxyl groups. Owing to their planarity, flavanols and flavonols with 3-OH in their structure undergo conjugation and electron dislocation that increase flavonoids phenoxyl radical stability; flavones lacking this feature e.g. luteolin are referred to as weak scavengers of DPPH (Ratty & Das, 1988; Arora, Nair, & Strasburg, 1998; Hirano, Sasamoto, Matsumoto, Itakura, Igarashi, & Kondo, 2001). Substitution of 3-OH group with methyl or glycosyl groups completely abolishes the activity of quercetin and kaempferol against β-carotene oxidation in linoleic acid (Burda & Oleszek, 2001). The presence of a catechol containing B ring in flavones strongly enhances the capacity of inhibiting lipid peroxidation. This configuration in flavonoids is referred to as very effective against various toxic oxygen species: indeed, although luteolin and kaempferol have the same number of hydroxyls, the presence of the catechol B-ring in luteolin makes it much more active in scavenging ROS as compared to kaempferol (Van Acker et al., 1996). Polymerization of flavonoids e.g. procyanidins increases the effectiveness of the compound against free radical scavenging capacity as has been reported for dimers and trimers of procyanidins (Vennat, Bos, Pourrat, & Bastide, 1994).

No correlation has been found between the antioxidant activity or the antiproliferative activity on HepG2 cells, and the colour index (i.e. the intensity of red, which is proportional to anthocyanins concentration) of the fruits from different cultivars (Karaaslan, Vardin, Varliklioz, & Yilmaz, 2014): this finding confirms that phenolic acids and flavonoids, rather than anthocyanins, are the predominant compounds influencing pomegranate's bioactivity.

Ellagitannins with galloyl or hexahydroxydiphenoyl groups including casuarinin and corilagin exhibit antiviral properties against herpes simplex virus (HSV); monomer and dimer ellagitannins, and to some extent gallotannins, displayed the higher anti-HSV-I and HSV-2 activities (Fukuchi et al., 1989). The anti-HSV activity is structurally related to the number of hexahydroxybiphenoyl groups and to the abundance and position of the –OH groups, while the importance of the molecular size/weight is still controversial (Quideau et al., 2004). Some ellagitannins and polyphenols contained in both PoP and pomegranate juice have been shown to exert inhibitory activity against various viral strains such as HIV, H5N1 of influenza virus, Hepatitis B and C (Kotwal, 2008), but the structure activity relationships for these effects have not been investigated.

Ellagitannins exert antibacterial effects on a wide number of foodborne pathogens and infectious microorganisms (e.g. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*) (Aguilera-Carbo et al. 2008; Torronen, 2009; Yoshida, Hatano, Ito, & Okuda, 2009) but, to the best of our knowledge, no structure-activity study for the antibacterial effects has been performed.

4.0. PoPx Extraction Optimization and Extracts Stability

Ethanol, water and their mixtures - although exhibiting decreased ability to yield antioxidants as compared to methanol - are of course considered as the food grade solvents for commercial extraction of plant phenolics. Optimization of extraction conditions to yield the maximum level of food grade antioxidant phenolics involves: the type of solvent (ethanol or water) or solvent ratio, the particle size of the plant material, the solvent-solid ratio, extraction temperature and time. Ultrasonic assisted extraction of PoP phenolics coupled with optimal extraction conditions (70% ethanol–water mixture as solvent, temperature of 60 °C and extraction time of 30 min) allows to obtain higher concentration of PoP phenolics (8673.87mg GA/100g), with good extraction yield (45.38%) and ferric reducing antioxidant power (63.37mmol Fe^{2+}/100g) (Tabaraki, Heidarizadi, & Benvidi, 2012). Higher phenolic concentrations and antioxidant capacity can be obtained reducing the particle size of PoP powder: a particle size of ~ 0.2mm increases the surface area of the peel, reduces solvents' transfer rate and increases phenolic yield (Qu, Pan, & Ma, 2010). PoP extracts are sensitive to high pH values and light exposure. After 180 days of storage, PoP extracts stored at low pH (3.5) in dark packaging still retained 67% and 58% of their total soluble phenolic concentration and antioxidant activity, compared with 61% and 43% for high pH (7.0) samples (Qu, Breksa III, Pan, Ma, & Mchugh, 2012).

In nutraceutical products PoP is preferably included as dry extract, which allows a better retention of functionally active components during storage. However the stability of these dried extracts may vary as a function of the following variables: a) the drying conditions, i.e. air or sun drying as compared to freeze drying; b) the moisture content and c) storage temperature. Extracts with higher moisture contents have been reported to express stabilized antioxidant properties at temperature below -33.4˚C while in the case of completely dried extracts storage at $\geq$ 1.2˚C is sufficient to prevent the deterioration of PoPx functional properties (Al-Rawahi, Rahman, Waly, & Guillemin, 2013; Al-Rawahi, Rahman, Guizani, & Essa, 2013). Oppertunities exist for enhancing and stabilizing technological properties of PoPx by microencapsulation. Investigational research is needed in this area to explore stability features of microencapsulated PoPx at various food processing conditions including microwave heating, thermal pasteurization, high hydrostatic pressure, cooking and baking operations.

5.0. PoP and PoPx - a natural class of food additives

Today it is generally accepted that functional foods from plant origin provide a clinically documented health benefit for the prevention, management or treatment of chronic diseases, particularly cardiovascular maladies and cancer. Since pomegranate is one of the most popular functional foods, consumption and marketing of its juice has been rapidly expanding worldwide. Moreover, the utilization of pomegranate juice or juice derivatives as food colorants and flavor enhancers (Al-Maiman & Ahmad, 2002) further increases its production. Pomegranate juice processing industries produce huge waste in the form of peel which has been proposed and evaluated as supplement in animal feed (Shabtay et al., 2008). Considering the excellent antioxidant, anti-inflammatory and anti-infective activity render this inedible part of the fruit nutraceutically more active as compared to pomegranate juice (Rosenblat, Volkova, Coleman & Aviram 2006: Sestili et al., 2007; Lee, Chen, Liang, & Wang, 2010; Mo, Panichayupakaranant, Kaewnopparat, Songkro, & Reanmongkol 2013; Neyrinck et al., 2013; de Silva, Jadhav, Rathnayaka, & Sahoo, 2014). PoP is still underutilized in food systems: sstringency is the key limiting factor in its utilization as food despite its outstanding nutritional and ethnopharmacological potential. The astringent sensation of PoP depends on the formation of tannin - salivary protein complexes. In oral cavity, tannins undergo precipitation when exposed to histatins and proline rich proteins. Development of haze is further strengthened with formation of precipitated tannins and salivary protein, predominantly salivary glycoprotein complexes (Dinnella et al., 2009; Kallithraka et al., 2001).

However, notwithstanding the problem of its astringency, nutritional exploitation of PoP is increasingly being considered as of great value. It is well known, for instance, that inadequate supply of certain vital nutrients in regular dietary plans is one of the causes of increased malnutrition-associated morbidity and to some extent maternal as well as infant mortality. By virtue of its composition, PoP could be rationally utilized as a valuable ingredient in food products (Viuda-Martos, Fernandez-Lopez, & Perez-Alvarez, 2010) to easily replete macro- and micro-nutrients such as minerals, vitamins, β-carotene, complex polysaccharides, reducing sugars and fiber (Ullah, Ali, Khan, Khurram, & Hussain, 2012; Viuda-Martos et al., 2012; Ismail, Akhtar, Riaz, & Ismail, 2014) .

5.1. Antioxidant potential of PoP and PoPx

Plant phenolics and derived compounds have been extensively studied for their antioxidant capacity, which depends on their radical scavenging and/or transition metal chelating properties. The antioxidant activity is likely to play a pivotal role in, and contribute to, the health promoting effects ascribed to polyphenols. Thus the presence of natural ingredients such as polyphenols in food products may enhance consumer acceptability because of their beneficial effects but, from a technological point of view, may also increase the food oxidative stability (see also 5.3). Consequently, the use of PoPx as an antioxidant-rich food ingredient seems to be on the rise (Rummun, Somanah, Ramsaha, Bahorun & Neergheen-Bhujun, 2013). To this regard, addition of PoPx to ice creams significantly increased total phenolic level and antioxidant capacity without negatively influencing the content of *Lactobacillus Casei* Shirota, a beneficial probiotic used in the manufacture of various dairy products (Sagdic, Ozturk, Cankurt & Tornuk, 2012); the treatment with PoPx increased the stability of preserved goat fish against lipid oxidation (Paari, Naidu, Kanmani, Statishkumar, Yuvaraj, Pattukumar & Arul, 2012); finally, a very recent study showed that addition of PoP powder up to 2.5% w/w to wheat bread significantly increased its oxidative stability with no effect on innocuousness as assayed with the brine-shrimp larvae assay (Altunkaya, Hedegaard, Brimer, Gokmen, & Skibsted, 2013). Due to its antioxidant and cardiovascular protective properties, PoPx has been incorporated in a functional beverage, i.e. dealcoholized red wine, which provides 82 mg of total ellagitannins following an intake of 500 mL in two servings per day (Tarrega et al., 2013). Functional ice creams supplemented with 0.2 to 0.4% PoPx acquired antioxidant and antidiabetic activities without significant alteration of the sensory properties (Cam, Erdogan, Aslan & Dinc, 2013). Addition of PoPx to jams (Ventura et al., 2013), juices and wines (Wasila et al., 2013) increased their phenolic, flavonoid and thiol concentration with a significant improvement of the free radical scavenging and product stability features.

5.2. PoP and PoPx as dietary supplements

Dietary polyphenols utilization under permissible limits is likely to generate beneficial health effects. Restricted daily consumption of fruits and vegetables both in developed and resource less countries results in decreased dietary intake of plant polyphenols. Pomegranate extracts, either from juice or peels, represent a rich source of phytochemicals; we have already discussed that, although PoP can be considered as a pomegranate industry waste, it contains relatively higher levels of polyphenols as compared to juice or seed and flower fractions of the fruit (Li et al., 2006, Sestili et al., 2007). Hence, commercially speaking pomegranate by-products and PoP bioactive compounds could gain consumers' acceptability if marketed in the form of functional food preparations: this notion seems to have been well understood by food industry as the production of PoP dietary supplements in the form of gels and capsules is continuously growing to accomplish the growing demand for more specific and active fractions of pomegranate. For instance, incorporation of microencapsulated PoPx in ice creams increases the antioxidant activity and α-glucosidase inhibitory properties with least impairment of sensory features, adding significant value to the finished product (Cam, Erdogan, Aslan & Dinc, 2013; Cam, Icyer & Erdogan, 2014).

Furthermore, dietary supplementation of cattle with fresh PoP promoted significant increases in feed intake and alpha-tocopherol plasma concentration, with positive tendency toward increased weight gain of bull calves and can contribute to the economic growth of cattle breeding (Shabtay et al., 2008).

5.3. Role of PoP and PoPx in stabilizing unsaturated fatty acids in food systems

Oxidation is a fundamental deteriorative change in foods containing lipid fractions during processing and subsequent storage conditions. Visible onset of lipid oxidation is known to result in negative nutritional and sensorial alteration of foods. Synthetic antioxidants have been industrially used as food additives for more than fifty years as means to prevent peroxidation of fats and oils. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone are effective and common antioxidants preventing oxidation and off-flavor development in fats and oils. However, numerous studies reported safety concerns about the use of these compounds as food additives (Palomba, Sestili & Cantoni, 1998; Iqbal, Haleem, Akhtar, Zia-ul-Haq, & Akbar, 2008; Ahmad, Gokulakrishnan, Giriprasad & Yatoo, 2013). This concern is one of the reasons of the progressive decline of synthetic antioxidants in food systems over the last decade and of the parallel rise in the use of natural antioxidants including ascorbic acid, tocopherols, and plant phenolics-rich extracts (Frost & Sullivan, 2013). With regard to PoPx, it could be conveniently used as natural antioxidant for the stabilization of vegetable oils thereby substituting synthetic antioxidants. e. g. BHT and BHA (Iqbal, Haleem, Akhtar, Zia-ul-Haq, & Akbar, 2008).

Several foods undergo deteriorative changes (i.e. development of off-flavors and accumulation of toxic by-products) during preparation and storage on account of lipid oxidation. Predominant precursors engendering these qualitative alterations in foods include atmospheric oxygen, enzymes, metal ions, ionizing radiations and sunlight. Utilization of PoP powder extracts has been reported to stabilize the food systems against such lipid oxidative changes. By virtue of its high content of hydrolysable tannins i.e. ellagitannins, ellagic acid, punicalin and punicalagin isomers and of the ensuing free radical scavenging properties, PoP has the capacity of efficiently inhibiting lipid oxidation in foods (Li et al., 2006). Several studies and an Indian patent demonstrated that PoP significantly increases the stability of vegetable oils, cooked chicken, beef and goat meat patties against lipid peroxidation (Iqbal, Haleem, Akhtar, Zia-ul-Haq, & Akbar, 2008; Kanatt, Chander, & Sharma, 2010; Kumudavally, Tabassum, Radhakrishna & Bawa, 2009; Naveena, Sen, Kingsly, Singh, & Kondaiah, 2008a; Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008b).

As compared to many natural and synthetic antioxidants such as Vitamin C and BHT, PoPx afforded enhanced antioxidant activity by inhibiting lipid oxidation in cooked chicken patties (Naveena, Sen, Kingsly, Singh, & Kondaiah, 2008a; Naveena, Sen, Vaithiyanathan, Babji, & Kondaiah, 2008b).

It is well known that the presence of higher concentration of TBARS (Thiobarbituric acid reactive substances) is indicative of oxidative damage in meat and meat products. PoPx, in combination with vacuum packaging technology, has been evaluated for its effect on the reduction of TBARS and on the preservation of food organoleptic attributes: supplementing 1% PoPx in goat meat reduced TBARS by 40% as compared to individual vacuum packaging where the reduction rates remained to be 27% (Devatkal, Thorat, & Manjunatha, 2012).

The stability of phenolics-rich extracts and of individual bioactive compounds can be altered by processing and storage conditions, i.e. thermal treatment during sterilization, chilling, refrigeration etc. Importantly PoP liquid extracts are fairly resistant to these conditions and are thus referred to as useful natural additives in various perishable food either thermally processed or stored at chilling or refrigeration temperatures. For instance, Qu et al. (2013) reported that after 180 days, PoP extracts stored at 4 °C retained 67% of the initial total soluble phenolic content and 58% of the original scavenging activity.

5.4. PoP and PoPx as barriers to food spoilage and infections

The emergence of multi-drug resistance foodborne pathogen strains (e.g. *Staphylococcus aureus, Salmonella enteritidis* and *Listeria monocytogens*) is recognized as potential threats to safe food supply leading to higher rates of morbidity and mortality. PoP phenolics and flavonoids have been shown to act as inhibitors against foodborne pathogens. Mechanistically, precipitation of bacterial cell membrane proteins by the reaction of peel phenolics entails bacterial cell lysis. Likewise, phenolic compounds may react with protein sulfhydryl groups and make them unavailable for microbial growth thereby generating phenolic toxicity (Haslan, 1996). Pomegranate polyphenols are hydrophilic in nature and are well extracted with hydrophilic solvents. Contrarily, hydrophobic solvents (i.e. ethyl acetate, chloroform and n-hexane) yield extracts with relatively weaker or no antimicrobial activity (Al-Zoreky, 2009). *In vivo* and *in situ* application of 80% methanolic extract of PoP revealed a potential inhibitory effect for *L. monocytogens, S. aureus, E. coli* and *Yersinia enterocolitica*. The minimum inhibitory concentration (MIC) of the water methanolic extract is 4mg/ml for *S. enteritidis* while 24.7mg dry PoPx/ml was reported to be the minimum bactericidal concentration for *L. monocytogenes* (Al-Zoreky, 2009; Hayrapetyan, Hazeleger, & Beumer, 2012).

Accordingly, strong inhibition of PoPx towards gram positive foodborne pathogens in ready-to-eat meat preparations was reported, suggesting its utilization as a natural food preservative in meat and meat products (Hayrapetyan, Hazeleger, & Beumer, 2012).

Management of microbial infections and diseases in fruits and vegetables has a tremendous contribution to curtail pre- and post-production losses. In emerging trends of organic food production, natural antimicrobials have significantly proven to be reliable alternatives for chemical fungicides, bactericides and pesticides. PoPx naturally enriched with ellagic and gallic acid was validated for its antibacterial properties against *Pseudomonas syringae* – the casual agent for bacterial speck of tomato (Quattrucci, Ovidi, Tiezzi, Vinciguerra, & Balestra, 2013). Hence, a broader scope exists for PoPx in food production to manage food spoilage and infections. In particular, more studies should be directed to explore the potential of PoPx to inhibit the growth of toxicogenic foodborne pathogens and the ensuing production/accumulation of toxins in contaminated food.

5.5. PoP enhances functional quality of foods

Addition of pomegranate rind powder in raw beef sausages up to 3% has been reported to improve their functional characteristics i.e. water holding capacity of sausages in addition to the high phenolic-associated free radical scavenging activity. Similarly, supplementation (3%) with pomegranate rind powder characteristically improved the quality (hue, chroma, lightness and redness) of cooked meat sausages suggesting the whole fruit bagasse as a potential food ingredient with functional properties (El-Gharably & Ashoush, 2011). Another recent study confirmed the nutritional relevance of pomegranate bagasse showing it to be a potential source of dietary fiber, i.e. total, soluble and insoluble dietary fiber (50.3, 19.9 and 30.4g/100g). The study further explicates pomegranate bagasse powder co-products to be exploited in food products requiring hydration, viscosity development, and freshness, such as baked foods or cooked meat products (Viuda-Martos et al., 2012). The above functional characteristics of PoP (antioxidant capacity, sensory quality, peel colour, mineral and phenolic content) depend on the regional pomegranate cultivar, agro-climatic conditions and ripening and harvest date of the fruits (Borochov-Neori et al., 2009). A better understanding of these variables would help to identify and standardize the conditions associated with the optimal functional characteristics of pomegranate, and would allow to rationally and fully exploit the potential of PoP. Since there are few data regarding this important issue, more focused studies on these topics are needed.

5.6. PoP and PoPx as prebiotics

Prebiotics, food ingredients with no digestibility, are capable of improving selected or randomized growth of colon microbiota. PoPx carry appreciable concentration of ellagitannins which are hydrolyzed by intestinal microflora into punicalagins and ellagic acid which act as prebiotics. As prebiotics, pomegranate extracts inhibit pathogens and promote the growth of beneficial microbiota in human guts. Probiotic lactobacilli were relatively unaffected by pomegranate chemical constituents: ellagic acid slightly reduced the growth of lactobacilli (*Lactobacillus pentosus*, *Lactobacillus ramnosus* and *Lactobacillus acidophilus*) to approximately 10−20% of controls. However, the detected growth inhibition was likely due to a decrease in media quality after tannin complexation with nutritional components rather than tied to specific bactericidal/bacteriostatic effects. Punicalins and gallic acid were not inhibitory, but rather slightly stimulatory, toward the growth of lactobacilli. The effect of pomegranate constituents on the growth of bifidobacteria was species-specific. Selected pomegranate constituents (punicalagins, punicalins, gallic acid and ellagic acid) partially inhibited the growth of *Bifidobacterium animalis lactis* and of *Bifidobacterium bifidum*, while the growth of *Bifidobacterium breve* and *Bifidobacterium infantis* was significantly enhanced. Conversely, pathogens such as *S. aureus*, *Clostridium perfringens, Clostridium clostridioforme, Clostridium ramosum* and *Bacteroides fragilis* were strongly inhibited by ellagitannins and punicalagin (Bialonska, Kasimsetty, Schrader & Ferreira, 2009). Thus, contrarily to pathogens, probiotic growth is relatively unaffected or even enhanced by pomegranate ellagitannins, suggesting that pomegranate products may help regulate pathogens without adverse effects on beneficial bacteria (Bialonska, Kasimsetty, Schrader & Ferreira, 1999).

Gut microbiota is known as an environmental factor to be taken into account when assessing the risk factors related to obesity: interestingly Neyrinck et al. (2012) have recently shown that PoP, by virtue of its prebiotic activity, constitutes a promising food supplement in the control of atherogenic and inflammatory disorders associated with diet-induced obesity.

The mechanisms responsible for the selective bacteriostatic/bactericidal effects are complex: for instance, as to punicalagin and polyphenols, the mechanism of *S. aureus* inhibition seems to be related to the decrease of environmental pH values, while for tannins other mechanisms such as depletion of metal ions and inhibition of enzyme activity are involved. As compared to *in vitro* studies such as that by Bialonska, Kasimsetty, Schrader & Ferreira (1999), these interactions are further complicated in the gut environment, where large variations in the abundance and type of bacterial species present and the quantity and variety of phenolics consumed by the host usually occur. Therefore, the results on individual bacteria species obtained in *in vitro* studies should be verified in studies based on human fecal microbiota.

6.0 Functional and Toxicological Levels of PoP and PoPx

Alike any other plant extract, PoPx might in principle generate toxicity if the consumption or exposure levels exceed threshold limits. Since the utilization of PoP and its extracts in food products for nutraceutical and functional purposes is on the rise, the toxicological/safety issue deserves the highest consideration. Lethal doses or concentrations of PoPx and of some fractionated constituent have been studied *in vitro* and *in vivo* in the past few years. The pioneer study by Vidal et al. (2003) demonstrated that a pomegranate (whole fruit) hydroalcoholic extract (administered I.P. to OF-1 mice) exhibited a good safety profile, with an acute LD50 value of 731.1 mg/Kg b.w., i.e. far higher than the doses used in Cuban folk medicine. Upper levels of pomegranate extracts, PoPx and fractionated compounds (>2000mg/kg b.w.) were evaluated for suspected toxicity in laboratory animals. PoP galactomannan polysaccharides (known to exhibit cytotoxic properties against cancer cells) administered to BALB/c mice did not induce any measurable toxic effect up to 2000mg/kg b.w (Joseph, Aravind, George, Varghese, & Sreelekha 2013). Similar findings have also been reported for ellagic acid and pomegranate extracts (Bhandary, Satheesh, Sharmila, Kumari, & Bhat, 2013). More recently oral administration of pomegranate ethanolic extracts to female rats at a concentration of 2000g/kg b.w did not present any toxicity in the tested animals (Das & Sarma, 2014).

Higher level of variability in toxicologically relevant concentration of PoPx administrated intraperitoneally in rats and mice has been reported, probably due to variability in composition of peel biological extracts. A PoP extract evaluated for its antidiarrheal activity, was also screened for its acute toxicity (I.P. administration to rats) and LD50 of 1321 mg/kg b.w was found (Qnais et al., 2007). Later on, a study aimed at investigating the potential adverse effects of a standardized pomegranate extracts in wistar rats following acute and subchronic administration (Patel et al., 2008) reported an oral LD50 > 5g/Kg b.w. in rats and mice, and a no observed-adverse-effect level (NOAEL) of at least 600 mg/kg b.w./day (i.e. the highest dose tested). Plausible toxicological studies of PoPx have been performed on brine shrimps (*Artemia salina*), where a PoP methanolic extract exhibited no significant toxicity (LC50 = 1.42 mg/ml) (Mehru et al., 2008). Another study based on brine shrimps showed that PoP supplementation in bread up to a level of 2.5% was innocuous, but higher concentrations were found to be increasingly toxic (Altunkaya, Hedegaard, Brimer, Gokmen, & Skibsted, 2013). In a further study on *A. salina* doses up to 1000mg per 100ml of a PoPx enriched apple juice were referred as safe (Altunkaya et al., 2013).

As to humans, a study by Heber et al. (2007) demonstrated the safety of pomegranate dietary supplementation: indeed an ellagitannin-enriched polyphenol pomegranate dry extract administered in the form of oral capsules was reported to be safe up to a dosage of 1420mg per day for 4 weeks in normal and obese individuals.

Conclusions

A plethora of literature highlights the ethnopharmacological and nutraceutical features of PoP and PoPx confirming their potential to act as health ameliorating biological ingredients. Relatively fewer reports deal with their possible toxicology, dietary ranges and consumption patterns. Some studies report the ingestion of pomegranate and its peel fractions in the form of pills, capsules and gels as conventional treatment regimens against certain diseases in countries of the developing world. Utilization of PoP and PoPx as effective supplements and food additives in defined concentrations in various organoleptically acceptable food preparations, would open new avenues for scientific research in the realm of food science and nutrition. Incorporation of PoP or its fractionated phytochemicals regardless of their astringency could practically be exploited for health promoting purposes in various food products with slight but acceptable organoleptic modifications. Utilization of pomegranate peel as a reservoir of valuable therapeutic agents that may also act as food preservatives, stabilizers, supplements, probiotics and quality enhancement agents seems to be a pragmatic approach in the prevention of some chronic maladies. However, the efficacy of PoP and PoPx, their nutraceutical role as supplements in food, the stability of their active ingredients under various food processing conditions and organoleptic alterations in finished food products, need to be thoroughly explored to fully exploit the intrinsic value of the waste of this heavenly fruit.

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LIST OF COMPOUNDS

Punicalagin

Punicalin

Casuarinin

Corilagin

Ellagic acid

Pedunculagin

Luteolin

Gallocatechin

Kaempferol

Gallic acid

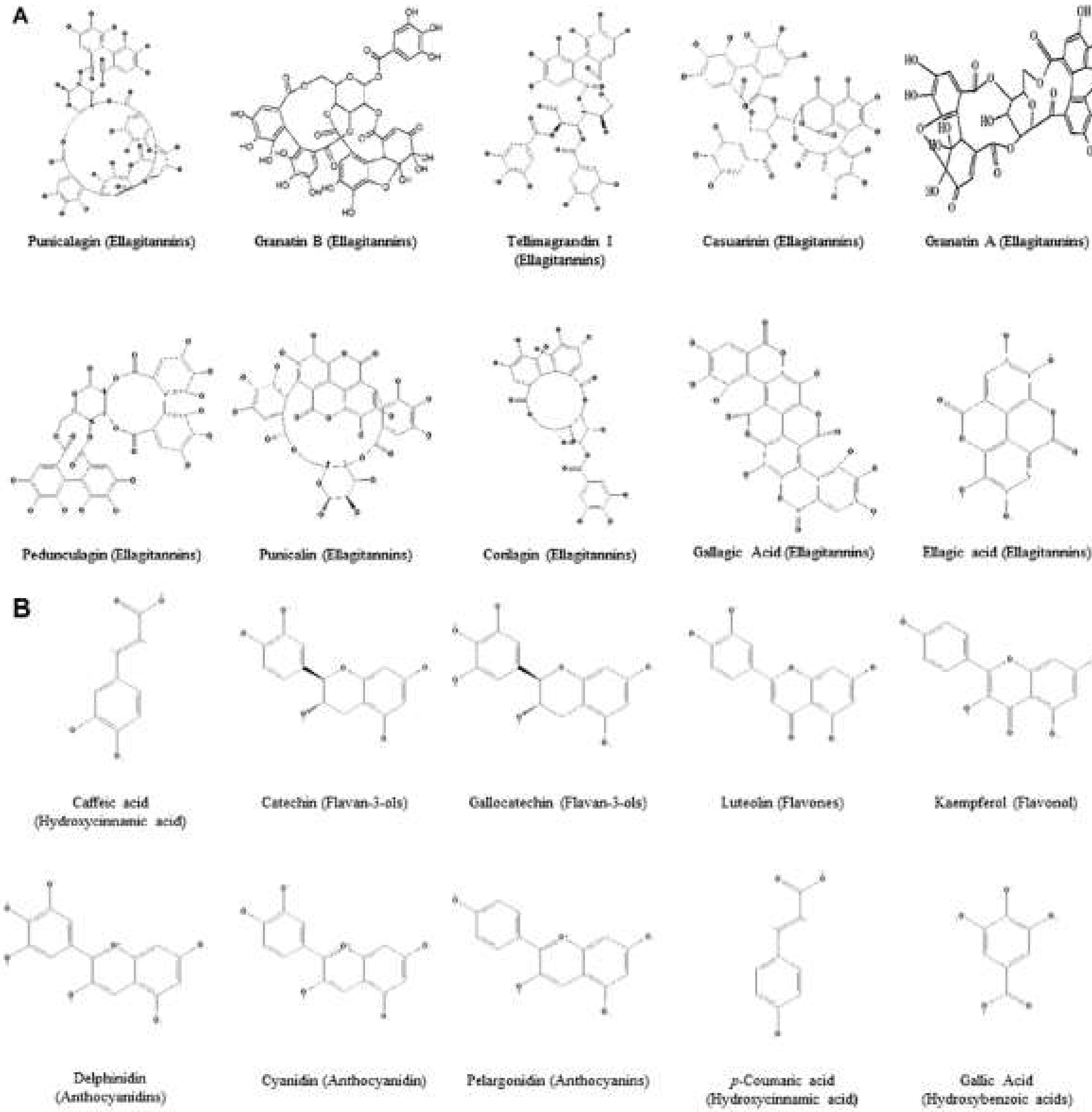
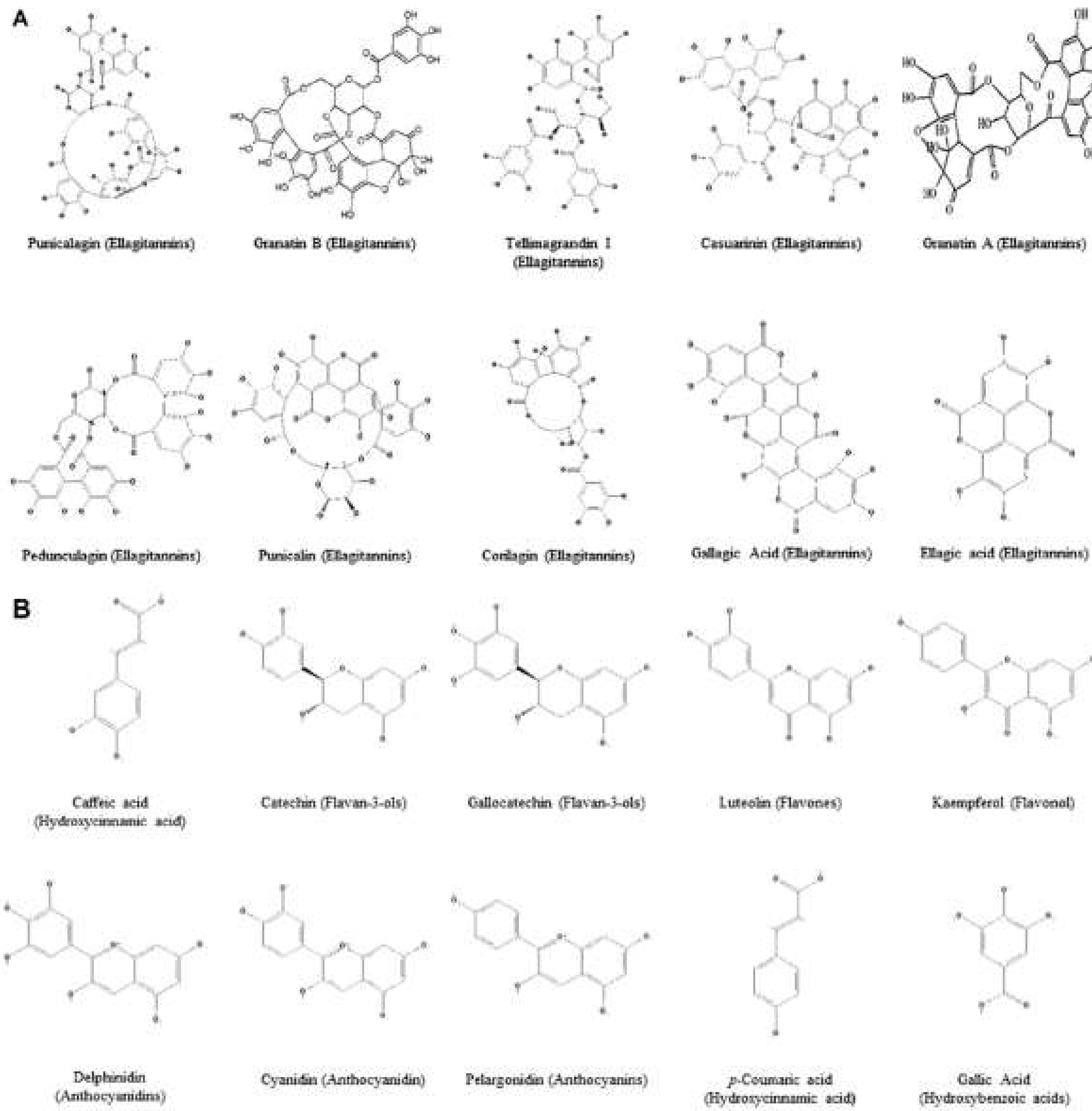


Fig. 1. Chemical structures of selected compounds in pomegranate peel.



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