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ORIGINAL ARTICLE

Rheological, physico-sensory and antioxidant properties of punicic acid rich wheat bread

Aruna Pamisetty[1,3] · K. Ashwath Kumar[2] · D. Indrani[2] · R. P. Singh[1,3]

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Abstract Punicic acid (PA), a predominant fatty acid (85%) in pomegranate seeds, also called as an ω-5 fatty acid, is known to render various health beneficial effects to humans. The objective of this study was to prepare and observe the effect of replacement of wheat flour with 5–12.5% punicic acid rich pomegranate seed powder (PSP, 9XXX fraction) on rheological, physico-sensory and antioxidant properties of bread. The increasing amount of PSP caused decrease in farinograph water absorption capacity, dough stability; amylograph peak viscosity; bread volume and overall quality score, whereas crumb hardness was increased. The combination of additives (CA) showed significant improvement in dough strength, texture and quality of bread with 10% PSP. The total polyphenol content (TPP) and radical scavenging activity (RSA) increased by 10- to 30-fold while PA was increased to 60-fold when compared to control. The recovery of PA from bread samples was in range of 45–60% and further increased by the addition of CA (65%). Hence, 10% PSP bread having 60% recovery of PA can be successfully considered for formulations without altering the rheological and sensory quality of bread. These results reveal that PA rich PSP prepared from a fruit industry by-product can be utilized for preparing antioxidant rich functional bread which also helps in overall improvement of bone health.

Keywords Bread · Rheology · Pomegranate seeds · Punicic acid · Antioxidant activity

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✉ R. P. Singh
singhrp317@gmail.com

1 Department of Biochemistry, CSIR-Central Food Technological Research Institute, Mysuru, Karnataka 570 020, India

2 Flour Milling, Baking and Confectionery Technology, CSIR-Central Food Technological Research Institute, Council of Scientific and Industrial Research (CSIR), Mysuru, Karnataka 570 020, India

3 Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

Introduction

Pomegranate (*Punica granatum*) is a native plant from Iran and belongs to the family of Lythraceae. Polyphenols, flavonoids, anthocyanin, ascorbic acid, ellagic acid, carotenoids, tannins etc. are the main constituents of pomegranate (Jing et al. 2012). Pomegranate seeds along with peel and mesocarp are cosidered as waste by fruit processing industries; but, are treasure of pharmaceutical and nutraceutical active compounds (Khoddami et al. 2014). The seed oil of Pomegranate (PSO) contains punicic acid, a polyunsaturated fatty acid, as a major molecule (Kaufman and Wiesman 2007) which possesses several health benefits owing to its antioxidant properties (Yuan et al. 2015). PSO exhibits the pharmacological activities against serious maladies, such as prostrate cancer (Lansky et al. 2005). Recently, PSO has also been demonstrated for its bone-preserving effects in ovariectomized animals (Saravani et al. 2014). PSO has also been shown to increase the serum insulin-like growth factor type 1 (IGF-1) levels and promotes Peak Bone Mass (PBM) achievement through the activation of IGF-1 signaling in bone (Bachagol et al. 2018) due to increase in the histone acetylation at IGF-1 gene and enhances its secretion from the liver. Hence, due to highly beneficial aspects of seeds as seed oil, it could be exploited for the development of health-beneficial food products. A literature survey reveals that PSO has yet not moved to food industries as a functional ingredient (Aruna et al. 2016). Hence, PA rich pomegranate seed powder could be used in designer food products, like bakery products, which are used by masses, to impart its health beneficial properties. The studies were aimed towards the possible utilization of industrial waste and producing a value added functional food product.

Bread is one of the most common food products and provides 50% of the calories to the entire world. The flour used for making bread is procured by milling of wheat; however, it removes bran and germ part, leading to the loss of essential nutrients viz., dietary fiber, vitamins and minerals (Rosell et al. 2015; El Sheikha 2015). Bread is a staple food and can also be a media for fortification including vitamins and minerals. Gül and Sen (2017) carried out studies on the use of Pomegranate Seed Fraction (PSF) on the rheological and quality aspects of the bread. The authors observed that incorporation of PSF at 5 and 10% level resulted in a decrease in volume and increase in the dietary fiber content of bread. A study conducted by Bourekoua et al. (2018) on addition of pomegranate seed powder (PSP) into gluten-free bread showed a decrease in the lightness and yellowness of crumb and crust, while redness was increased. The Total phenolic content (TPC) was increased with the addition of PSP. However, no reports available on the incorporation of bioactive ingredients, like PA rich fraction (9XXX fraction) in any product and also the quantification of PA in the products.

The present study was aimed to investigate the effect of punicic acid rich pomegranate seed powder fraction (PSP) as a bioactive ingredient in wheat bread, and to determine its effects on the rheological characteristics of dough, physico-sensory and antioxidant properties of bread.

Materials

Pomegranate seeds (Mixed variety) were procured from a juice processing industry (Royal Food Stuffs India Pvt. Ltd, Navi Mumbai, India). The commercial wheat flour (*Triticum aestivum*), compressed yeast (Tower brand, AB Mauri, India Pvt Ltd., Chennai, India), hydrogenated fat (Bunge India Pvt Ltd., Mumbai, India); water, salt (common food grade sodium chloride) and sugar were procured from local market in Mysore. Additives, namely fungal alpha amylase (FAA, 30 units/mg) from *Aspergillus oryuzae*; dry gluten powder (DGP, 75% protein) and sodium stearoyl-2-lactylate (SSL) were procured from PD Brothers, Bangalore, India.

Chemicals

Standard Punicic acid was used as described by Pamisetty et al. (2018). Butylated hydroxyanisole (BHA) 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma Chemical Co., Gallic acid (Merck, Mumbai), anhydrous sodium carbonate (99.5%, LR) from Ranbaxy, Folin Ciocalteu phenol reagent (Sigma Chemicals), New Delhi were used for the studies. Other chemicals used were of AR grade.

Methods

Preparation of pomegranate seed fraction

The seeds were cleaned, sun dried and stored at 4 °C. The seeds were ground for 20 s to obtain the pomegranate seed powder (PSP) in laboratory grinder (Panasonic AC-300H). The PSP was successively sieved (Tropenisolation particle siever, Ruetschi, EI-Motoren, CH 5034 Suhr) through a series of sieves (particle size of 1600–118 μ) and the amount of the seed powder of different fractions obtained from different sieves was recorded (Aruna et al. 2018). PSP fraction with particle size of 150 μ (termed as 9XXX fraction) and high powder and oil yield compared with the other fractions, was used in this study for qualitative and quantitative analysis.

Analytical methodologies

Extraction and estimation of punicic acid in pomegranate seed oil

The oil was extracted from PSP in a Soxhlet apparatus for 8 h with hexane. Estimation of punicic acid in oil was done through gas chromatography by making their methyl esters (FAME) with 2M KOH (AOCS Method No: Ce 1–62, Firestone and Reina 1996). Oil sample (100 mg) was mixed with 100 μl of methanolic KOH and kept for 30 min, centrifuged at 3000 g for 15 min and dried with Na_2SO_4. The ensuing FAME samples were analyzed by gas chromatography (Shimadzu GC-2010 Plus-01 gas liquid chromatography system, equipped with FID). The compounds were separated on a column of RTX-2330 Chromosorb (oven temperature 80 °C, column inlet pressure 50 kPa) with the column length 180 × 0.3 cm using flame ionization detector (detector temperature 260 °C, run time-30 min). The carrier gas used was nitrogen with a flow rate of 40 ml/min. The analyses were conducted isothermally at 210 °C. Retention times of respective reference fatty acids were compared with the individual fatty acids.

Antioxidant properties

Determination of radical scavenging activity (RSA)

Determination of RSA in raw materials like PSP, wheat flour along with different samples of bread was carried out, as described by Singh et al. (2002) using DPPH as stable free radical and butylated hydroxyanisole (BHA) as standard, with slight modifications (Venkataramanamma et al. 2016). Various samples were over night extracted with 1:1 (V/V) mixture of ethanol and water and centrifuged at 3000 rpm for 10 min to get the extract. The extract was taken in different aliquots (50, 100 µl) and BHA in 100 µl fixed volume. The DPPH of 0.1 mM solution (100 µM, 5 ml) was added and kept at room temperature in dark for 20 min. The absorbance were measured at 517 nm (Shimazdu, USA) and RSA was expressed as percentage inhibition using the formula

$$\%\mathrm{RSA} = \frac{(OD\ of\ Blank - OD\ of\ Sample)}{OD\ of\ Blank} * 100$$

Determination of polyphenols

The concentration of phenolic compounds in the extracts of PSP, wheat flour and samples of bread was determined by the method of Heinonen et al. (1998). The wheat flour and 9XXX fraction extracts (0.2 ml) were mixed with diluted Folin phenol reagent (1.0 ml) and sodium carbonate solution 7.5% (0.8 ml), and incubated for 30 min at room temperature and the absorbance was measured at 765 nm.

Wheat flour and PSP fraction

Physico-chemical characteristics

The wheat flour and PSP fractions were analyzed for moisture (method 44-15), protein (method 46-10), total ash (method 08-01) and fat (method 30-10) using standard AACC (2000) methods. The dietary fiber was analyzed according to Asp et al. (1983).

Preparation of blends

With the aim of increasing antioxidant activity, PA, protein, dietary fiber in bread, 9XXX PSP were selected for the studies. Different blends were prepared by replacing wheat flour with PSP at 5, 7.5, 10 and 12.5%. Based on the preliminary sensory acceptance, different additives in combination, like gluten powder (2%), fungal alpha amylase with 30 units/mg activity (0.02%) and sodium stearoyl-2-lactylate (0.5%) were used to improve the quality of bread with 10% PSP.

Rheological characteristics of wheat flour

All the blends along with 10% PSP with CA were analysed for rheological characteristics, such as amylograph (method 22-10) and farinograph (method 54-21) by using the AACC (2000) methods.

Preparation of bread

Effect of incorporation of PSP at different levels on the quality characteristics of bread was studied using the following formulation: wheat flour: 100 g; PSP: 5/7.5/10.0 and 12.5 g; compressed yeast: 2.0 g, salt: 1.0 g; sugar: 3.0 g, hydrogenated fat: 1.0 g and water based on % water absorption in farinograph. All the ingredients were mixed together in a high speed Hobart mixer (Model N-50, Hobart, GmbH, and Offenburg, Germany) with a flat blade for 2 min at 61 rpm and 2 min at 125 rpm. The dough was transferred to humidity chamber for fermentation and it was maintained at 30 °C, 75% relative humidity for 90 min. After first fermentation, the dough was remixed, rounded, again fermented for 25 min, moulded, proofed for 55 min at 30 °C, 85% RH, baked at 220 °C for 25 min, cooled and packed. With the combination of additives (CA) the influence of 10% PSP was also studied on the bread making characteristics as well.

Physical and sensory characteristics

All the bread samples were analyzed for their volume by rapeseed displacement method. Loaf volume of bread was determined by displacement of rapeseed method (Vasudevaiah et al. 2017) using standard volume-measuring apparatus (National Manufacturing Co., Lincoln, and Netherland). The moisture content of the bread samples was analyzed according to AACC method (2000). The texture profile analysis of breads viz., hardness, chewiness, cohesiveness and springiness were analyzed using texture analyzer LR-5 K (Lloyd Instruments Ltd., Hampshire, England). The bread crumb piece of 4 cm square with thickness of 1 inch was subjected to texture analyzer with 5 kg load cell, circular probe with 80 mm diameter, cross head speed of 100 mm/min and compression of 50% of the sample height (Vasudevaiah et al. 2017). Hunter Lab colour measuring system (Labscan XE, Hunter Associates Laboratory Inc., Reston, Virginia, USA) was used to measure the bread crumb colour in terms of lightness (L) and colour (+a: red, −a: green, +b: yellow, −b: blue). A standard white board made from barium sulphate (100% reflectance) was used as a perfectly white object for setting the instrument with illuminant. Bread samples were placed in the sample holder and the reflectance was auto recorded for the wavelength ranging from 360 to 800 nm.

The sensory evaluation of control bread and breads prepared with PSP fraction was carried out by 15 male and female panelists with an age group 35–55 years. The panelists were trained in two sessions, involving 2 h training in each session. Prior to testing, the samples (half bread slice of one inch thickness) were coded with three digit number and each panelist was served with 4 randomly arranged bread samples in a tray with score card consisting of various quality parameters, such as crust colour 1 = dull brown, 10 = golden brown; crust shape: 1 = flat, uneven, 10 = normal; crumb color: 1 = dull white, 10 = greenish white; crumb grain: 1 = very coarse, 10 = very fine; texture: 1 = very firm, 10 = very soft; mouthfeel: 1 = gummy/dry, 10 = no residue in mouth; taste: 1 = astringent taste, 10 = pleasant taste. The overall quality score was taken as the combined score of seven quality attributes according to the method of Sowbhagya et al. (2015).

Chemical characteristics of bread

Extraction of oil

The know amount of bread samples was taken and dried to remove moisture. The dried samples were extracted for oil content using soxhlet method. The oil samples were taken for Fatty Acid Methyl Esters (FAME) preparation and analyzed for the content of punicic acid by gas chromatography, according to the method of Aruna et al. (2018) with minor revisions. The analysis was carried out in similar conditions as mentioned in the GC analysis of punicic acid of PSP. The analysis of samples was carried out in quadruplicate; the punicic acid content in breads was calculated based on the average peak area of the samples.

Bread chemical characteristics

The control and bread with 10% PSP + CA were analyzed for moisture content (method 44-15), protein (method 46-10), total ash (method 08-01), fat (method 30-10) according to the standard methods (Cunniff, AOAC 1996) and dietary fiber (Asp et al. 1983). The RSA and polyphenols were estimated by the method of Venkataramanamma et al. (2016), as described earlier.

Statistical analysis

The data pertaining to chemical and nutritional characteristics of breads were expressed as mean ± standard error and statistically analyzed using Duncan's new multiple range test with different experiment groups appropriate to the completely randomized design as described by Steel and Torrie (1980). The significant level was established at $P \leq 0.05$.

Results and discussion

Yield of 9XXX fraction and quantitative analysis of PA

Fractionation of PSP yielded 9XXX (150 µ) fraction along with other fractions, the yield of the fraction was quite high (38.91% w/w). The 9XXX fraction yielded 39% (w/v) yield of oil at room temperature according to Aruna et al. (2018) and 55–60% in soxhlet extractor with hexane as extraction solvent. TPP content in 9XXX PSP fraction was found to be 2.42 mg/g of GAE. However, the TPP content was found to be significantly low in wheat flour and control bread. The PSP extract showed RSA of 11.91% and 23.39% at 50 ppm and 100 ppm concentration, respectively. 9XXX fraction being high in PA content than other fractions with quantifiable RSA can be used in future study potential.

Quality characteristics of wheat flour

In this study, the wheat flour used had 11.38% moisture, 0.51% total ash, 1.28% fat, 11.40% protein and 10.38% dry gluten. It exhibited 3.56% dietary fibre, 521 s falling number and 24 ml sedimentation value. The wheat flour resulted in this study was of medium strength and used for making bread with 0–12.5% PSP. The prepared PSP powder had 8.16% moisture, 12.65% protein and 15.61% dietary fiber. These results are in agreement with studies reported by Gül and Sen (2017) on pomegranate seed fractions.

Effect of PSP on rheological characteristics of wheat flour

Farinograph characteristics

The substitution of wheat flour with of PSP on farinograph characteristics is presented in Table 1. From the Table, it can be observed that, the water absorption capacity of the wheat flour decreased with the addition of PSP. The water absorption capacity of control flour was 60.6% which decreased to 57.0% with the addition of 12.5% PSP. This might be due to dilution of gluten (Kumar et al. 2015). The dough development time (DDT, the time required to reach 500 BU consistencies), decreased from 6 min (control) to 5.4, 5.3, 5.2 and 5.1 min for 5, 7.5, 10 and 12.5% of PSP addition, respectively. Similar results were obtained by Gül and Sen (2017) in their study on the effect of pomegranate seed flour incorporation to wheat flour. The strength of the dough as indicated by dough stability, decreased from 5.9 to 5.3 min, respectively for control and 12.5% PSP

Table 1 Effect of PSP fraction on farinograph characteristics of wheat flour

Sample	Water absorption (%)	Dough development time (Min)	Dough stability (Min)	Mixing tolerance index (FU)
Control	60.6 ± 0.12[a]	6 ± 0.23[a]	5.9 ± 0.42[a]	30 ± 0.19[a]
5% PSP	59.5 ± 0.03[a]	5.4 ± 0.42[b]	5.8 ± 0.17[a]	32 ± 0.23[b]
7.5% PSP	59.0 ± 0.15[b]	5.3 ± 0.36[b]	5.6 ± 0.11[b]	32 ± 0.41[b]
10% PSP	58.0 ± 0.16[a]	5.2 ± 0.23[b]	5.5 ± 0.56[b]	33 ± 0.51[b]
12.5% PSP	57.0 ± 0.22[b]	5.1 ± 0.12[b]	5.3 ± 0.63[b]	37 ± 0.45[b]
10% PSP + CA	60.2 ± 0.54[a]	6.3 ± 0.36[a]	7.9 ± 0.23[b]	31 ± 0.14[a]

PSP pomegranate seed powder, *CA* combination of additive

Values are mean ± SD of triplicate analysis. Values not having similar superscripts in the same column are significantly ($P < 0.05$) different

addition. Mixing tolerance index (MTI), which is inversely proportional to dough stability, increased from 30 FU (control) to 37 FU with the addition of 12.5% PSP. Thus, addition of PSP decreased the stability of the dough by dilution of gluten and disrupting the continuity of the gluten matrix. Earlier, Dachana et al. (2010) also reported similar observations on their study on the effect of incorporation of 5, 10 and 15% of dried moringa leaves to wheat flour. The addition of CA to 10% PSP incorporated wheat flour improved it by increasing the water absorption capacity, DDT, stability and decreased MTI values. These results were further substantiated by Indrani et al. (2010) for multigrain incorporated wheat flour up to 15%.

Amylograph characteristics

The effect of PSP incorporation on pasting characteristics of wheat flour showed the increase in pasting temperature (Fig. 1). The increase in pasting temperature of wheat flour could be due to higher resistance of starch granules to swell in the presence of PSP (Kumar et al. 2015). Peak viscosity (PV) is the ability of the starch granules to swell easily before their physical breakdown. PV decreased from 1056 BU (control) to 770 BU for 12.5% PSP incorporation, indicating decreased swelling capacity of wheat flour with the addition of PSP. Hot paste viscosity (the firmness of already broken starch at cooking temperature) and cold paste viscosity (viscosity of cooked paste after cooling to 50 °C) decreased from 848 to 516 BU and 593 to 369 BU, respectively. Breakdown and setback viscosity values also showed similar trend with the addition of PSP and decreased from 255 to 147 BU and 440 to 406 BU, respectively. Similar results of increased pasting temperature and decrease in all other amylograph parameters were also reported by Sindhuja et al. (2005) on amaranth flour incorporation to wheat flour. The authors reported that the decrease in all the amylograph properties may be due to dilution of wheat starch by amaranth starch and also lower quantity of amylopectin content. Incorporation of combination of additives (CA) on 10% PSP incorporated wheat flour showed further decrease in pasting properties, except for setback viscosity. The setback viscosity increased from 318 BU for 10% PSP to 407 BU with combination of additives, indicating influence of additives on the pasting characteristics of PSP wheat flour blend. Earlier, Indrani et al. (2010) also reported that 15% incorporation of multigrain mix decreased the amylograph gelatinization temperature, peak viscosity and setback viscosity.

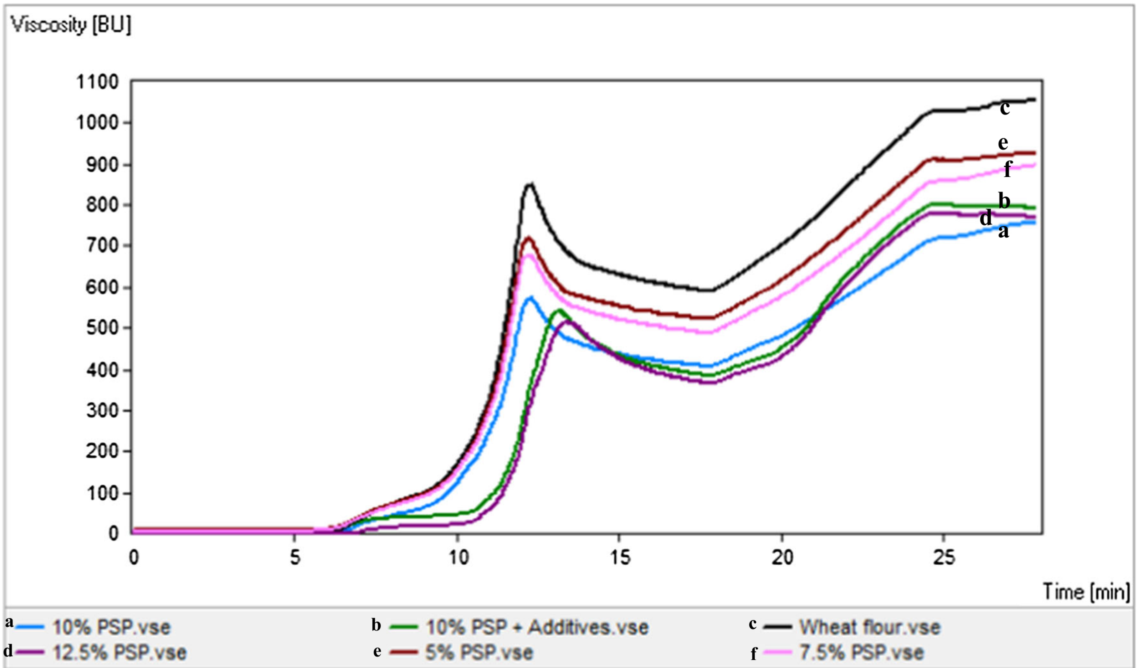
Effect of PSP on bread baking quality

Physical characteristics of bread

With the increase in the addition of PSP from 5 to 12.5%, there was not much change in the bread weight; however, there was a significant decrease in the loaf volume from 580 ml of control to 535 ml. Similar results were found by Osuna et al. (2014) on the replacement of wheat flour by soyabean, flax seed, or wheat bran flour and the studies of Conforti and Davis (2006) who reported decrease in loaf volume by flax and soy flour which could be in a similar fashion as of the decrease in volume of bread with 10% PSP (Supplementary Fig. 1), improvement in the strength of the dough as indicated by the farinograph stability value and also due to the increase in the production of gas during proofing because of FAA present in CA (Indrani et al. 2010).

Colour values of bread

The Hunter colour values of bread samples revealed that as the level of addition of PSP increased crumb colour became darker. The addition of PSP from 5 to 12.5% significantly decreased the lightness (L*) of crumb and increased the redness (a*) value, as shown in Table 2. Similar pattern was observed by Bourekoua et al. (2018), who stated that addition of pomegranate seed powder at



PSP – Pomegranate seed powder; CA – Combination of additive;

Fig. 1 Effect of PSP on amylograph characteristics of wheat flour. *PSP* pomegranate seed powder, *CA* combination of additive

Table 2 Effect of PSP on the physical quality characteristics of bread

Bread	Control	PSP (%)				
		5	7.5	10	12	10 + CA
Weight (g)	133.75 ± 0.77[a]	133.95 ± 1.06[a]	128.1 ± 0.28[a]	134.15 ± 0.63[a]	136.25 ± 1.20[a]	136.65 ± 1.20[a]
Volume (ml)	577.5 ± 3.5[a]	502.5 ± 10.6[b]	560 ± 14.14[a]	562.5 ± 10.6[a]	537.5 ± 3.53[b]	727.5 ± 3.53[b]
L value	75.40 ± 0.23[a]	50.21 ± 0.65[b]	48.31 ± 0.84[b]	42.34 ± 0.32[b]	40.87 ± 0.82[b]	41.63 ± 0.51[b]
a value	0.12 ± 0.06[a]	5.53 ± 0.06[b]	6.02 ± 0.11[b]	6.50 ± 0.05[b]	6.65 ± 0.07[b]	6.65 ± 0.01[b]
b value	12.58 ± 0.31[a]	13.09 ± 0.19[a]	13.82 ± 0.05[b]	13.41 ± 0.17[b]	13.20 ± 0.16[a]	13.13 ± 0.00[a]
Hardness (N)*	20.50 ± 0.43[a]	19.62 ± 1.55[a]	22.60 ± 2.28[b]	24.09 ± 0.41[b]	28.19 ± 0.10[b]	10.03 ± 0.13[b]
Cohesiveness	0.39 ± 0.02[a]	0.30 ± 0.00[b]	0.33 ± 0.00[b]	0.29 ± 0.03[b]	0.27 ± 0.01[b]	0.29 ± 0.01[b]
Springiness (mm)	11.15 ± 0.22[a]	10.42 ± 0.04[b]	10.57 ± 0.56[b]	10.41 ± 0.51[b]	10.05 ± 0.03[b]	10.93 ± 0.55[a]
Gumminess (N)	8.05 ± 0.01[a]	6.03 ± 0.03[b]	7.58 ± 0.06[b]	7.21 ± 0.08[b]	8.09 ± 0.03[a]	2.94 ± 0.30[b]

PSP pomegranate seed powder, *CA* combination of additive

L lightness, higher values indicate lighter colour; *a* greenness, *b* yellowness, higher colour intensity is indicated by higher values

Values are mean ± SD of triplicate analysis. Values not having similar superscripts in the same row are significantly ($P < 0.05$) different

*Force at 25% compression measured using texture analyser

10% enhanced the redness value of crumb by 3.2 times than control bread, probably due to dark creamish yellow colour of the pomegranate seeds (Wetzstein et al. 2011). However, the addition of PSP to bread did not alter the yellowness of crust colour. On the other hand, the L* values of the 10% PSP incorporated bread along with the additives were decreased and a* values were increased as shown in Table 2. These changes in the colour values of the bread were satisfactorily acceptable, according to the AACC standard (2000).

Texture profile of bread

In bread crumbs, the hardness was increased with the increasing content of PSP, while chewiness was decreased slightly with increasing PSP content when compared to control bread. The springiness decreased with the addition of 0–12.5% PSP from 11.15 to 10.05 mm. The gumminess of bread was not affected to a large extent by incorporating PSP. The addition of CA considerably improved the textural properties of 10% PSP incorporated bread. The hardness value decreased from 24.09 to 10.03 N while the springiness was increased from 10.41 to 10.93 mm respectively, for 10% PSP bread and 10% PSP + CA incorporated bread. These results are in concurrence with the reports of Mettler and Seibel (1972) with significant improvement in textural properties of whole wheat bread with a mixture of GMS, DATEM and guar gum.

Sensory properties of bread

The data on the effect of PSP on sensory properties of bread are presented in Table 3 indicate that the crust colour of bread changed from golden brown to dark reddish brown colour with the addition of PSP. The crumb colour changed from creamish white to brown colour with the addition of 12.5% PSP. Crumb grains of control bread were very fine, however, the breads prepared with with 5 and 7.5% PSP showed medium finer, breads with 10% PSP showed medium coarse and bread with 12.5% PSP showed coarse grain with thick cell walls. Mouth feel of the bread showed that, there was no residue formation during chewing even at higher level of PSP incorporation. Bourekoua et al. (2018) reported that gluten free bread by PSP powder, the taste of the bread was influenced by the addition of the pomegranate seeds powder at 10% PSP, however, in the present study we observed that even at the higher level addition of PSP, there was no off taste. The overall quality score of bread indicated the combined score of all sensory parameters as 64.5 for control bread which decreased to 49.5 with the addition of 12.5% PSP. However, incorporation of CA showed significant improvement in bread volume, crumb grain and overall quality as showed in Table 3. The crumb grain profile improved from medium coarse to medium finer structure. Overall quality score improved from 56.5 for 10% PSP bread to 61.0 for 10% PSP + CA incorporated bread. Indrani et al. (2010) used combination of additives to enhance the quality of 15% multigrain incorporated bread, and reported that sensory quality of multigrain bread drastically enhanced with combination of emulsifiers like dry gluten, alpha amylase and SSL. Based on the senory, physical and textural properties of bread, the maximum level of incorporation of PSP without affecting the quality of bread was fixed at 10% level.

Chemical composition of bread

The moisture content of control and breads with 5, 7.5, 10 and 12.5% varied from 31.14 to 33.8% indicating not much change in the moisture content with the addition of PSP. There was an increase in all the parameters like protein, ash and fat in bread prepared with 10% PSP + CA when compared with the control bread (Supplementary Table 1). Addition of PSP significantly increased the dietary fiber content in the breads.

Antioxidant properties of bread

Total polyphenols

The major bioactives in the PSP are polyphenols and health beneficial fatty acids, like Punicic acid, which are well known for their beneficial effects, including antioxidant

Table 3 Sensory analysis of the breads prepared with PSP fraction 5/7.5/10/12.5%

Parameter	Control	PSP (%)				
		5	7.5	10	12.5	10% + CA
CRUST**						
Color (10)	9.0 ± 0.56^a	8.0 ± 0.06^b	8.0 ± 0.12^b	7.5 ± 0.25^b	7.5 ± 0.03^b	7.5 ± 0.01^b
Shape (10)	9.0 ± 0.49^a	9.0 ± 0.18^a	8.5 ± 0.03^b	8.5 ± 0.11^b	6.0 ± 0.09^b	9.0 ± 0.14^a
CRUMB**						
Color (10)	9.0 ± 0.55^a	8.0 ± 0.08^b	7.5 ± 0.13^b	7.0 ± 0.14^b	6.0 ± 0.19^b	8.0 ± 0.06^b
Grain (15)	14.0 ± 0.18^a	13.5 ± 0.03^a	13.0 ± 0.12^b	12.0 ± 0.13^b	10.5 ± 0.02^b	12.5 ± 0.09^b
Texture (15)	14.5 ± 0.43^a	13.5 ± 0.19^b	13.5 ± 0.70^b	13.0 ± 0.18^b	12.0 ± 0.03^b	14.5 ± 0.16^a
Mouth feel (10)	9.0 ± 0.19^a	9.0 ± 1.2^a	8.5 ± 0.43^b	8.5 ± 0.19^b	7.5 ± 0.16^b	9.5 ± 0.13^a
Overall quality (70)	64.5 ± 0.08^a	61.0 ± 0.13^a	59 ± 0.05^b	56.5 ± 0.13^b	49.5 ± 0.19^b	61.0 ± 0.23^b

PSP pomegranate seed powder, *CA* combination of additive

Values are mean ± SD of triplicate analysis. Values not having similar superscripts in the same column are significantly ($P < 0.05$) different

**The figures in the parenthesis indicate maximum score for crust and crumb parameters activity. The TPP content of PSP used in the study was 2.86 mg/g of GAE. The TPP content increased significantly with increasing PSP levels in bread from 2.5 to 12.5%. Control bread had 0.41 mg/g GAE of TPP, whereas bread prepared with 10% PSP and in combination of additives was ranged in 1.02 and1.05 mg/g of GAE, the data is presented in Fig. 2a. Addition of PSP up to 10% level caused slight increase in TP content and antioxidant activity of the breads. The RSA of PSP was found to be high as discussed earlier. An addition of 10% PSP in bread increased RSA to 9.6% at 50 ppm, and 18.96% at 100 ppm. Addition of 10% PSP along with additives in bread increased RSA from 0.6 to 19.64% at 100 ppm (Fig. 2b). It can be concluded from the above results that the addition of PSP caused significant increase in RSA thereby enhancing antioxidant potential of bread containing PSP.

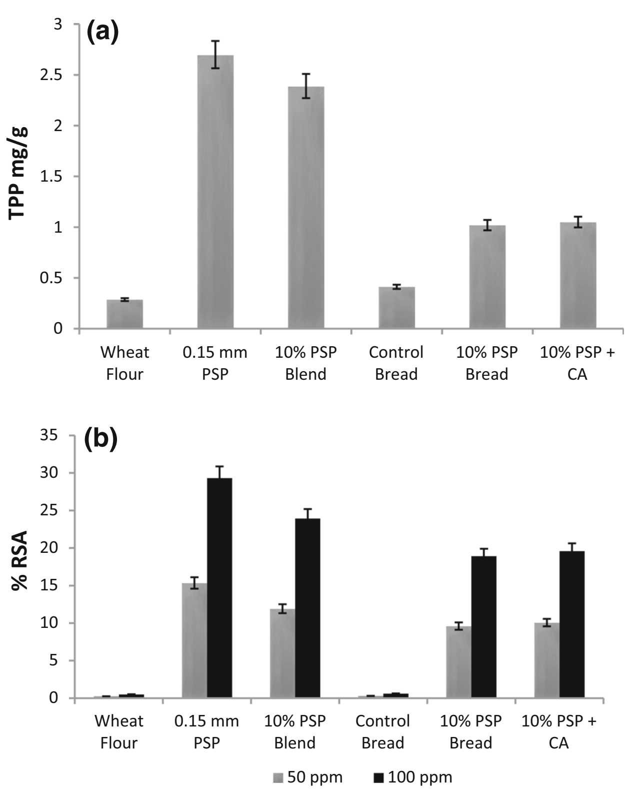


Fig. 2 **a** Antioxidant activity of the wheat flour, control bread, 10% PSP blends, 10% PSP bread and 10% PSP + CA breads. Values are in GAE equivalent mg/g and the standard deviation is indicated. **b** Radical scavenging activity of the wheat flour, control bread, 10% PSP blends, 10% PSP bread and 10% PSP + CA breads incorporated bread. The values are in percentage and the standard deviation is indicated

Estimation of punicic acid in breads

The extracts of 9XXX fraction, PSP blends (0–12.5%), breads prepared with PSP (0–12.5%) and also bread prepared with 10% PSP along with addition of additives were analyzed for their fatty acid composition by gas chromatography. The GC profile of the peaks was analyzed based on the standard punicic acid and is represented in Fig. 3. The blends prepared with PSP fraction has been shown to possess PA peak, whereas in the wheat flour and the control bread, the PA peak was not detectable. Similar results were found in bread prepared with 5–12.5% PSP. The recovery of PA from bread samples was found to be in the range of 45–60% which was further increased by the addition of additives (65%). Hence, the bread prepared with 10% PSP having 60% recovery of PA and does not causes any drastic changes in the rheological and sensory quality of bread could be promoted as a product. From industrial and economical perspective, these findings can be important as these breads can be labeled as ‘PA rich’ and marketed to health conscious consumers while cutting the costs with the addition of PSP which is virtually an industrial waste. Theoretically, intake of 30 g of 10% PSP bread (around three slices) could be able to provide about 1.16 g of PA (64% recovery from the bread). PA is known to get metabolized to CLA (Aruna et al. 2016) to an extent of 76% (Shabbir et al. 2017), and the above consumption of PA rich bread should be able to meet the RDA of CLA (0.5–1 g/day).

Conclusion

In today’s worldwide market, customer awareness is increasing towards functional products with health benefits. The present studies were aimed to incorporate Punicic acid rich 9XXX pomegranate seed fraction to bread to impart its beneficial effect which has antioxidants and helps in bone improvement. At 10% level of PSP substitution of wheat flour, dough rheological properties, volume, crumb hardness and sensory scores of breads showed slight decrease as compared to the control group. Beneficial aspects, like PA, TPP and RSA of the breads were increased by adding PSP. Studies concerned with Punicic acid recovery from the bread reveal that the consumption of 30 g of PA enriched bread (approx. 3 slices) could provide the RDA of CLA to an individual. The product could be helpful in the development and maintenance of bone health for children, post menopausal women and pregnant women’s. The studies conducted pave the way for utilization of PSP which is inadvertently produced as a waste form Pomegranate processing industries.

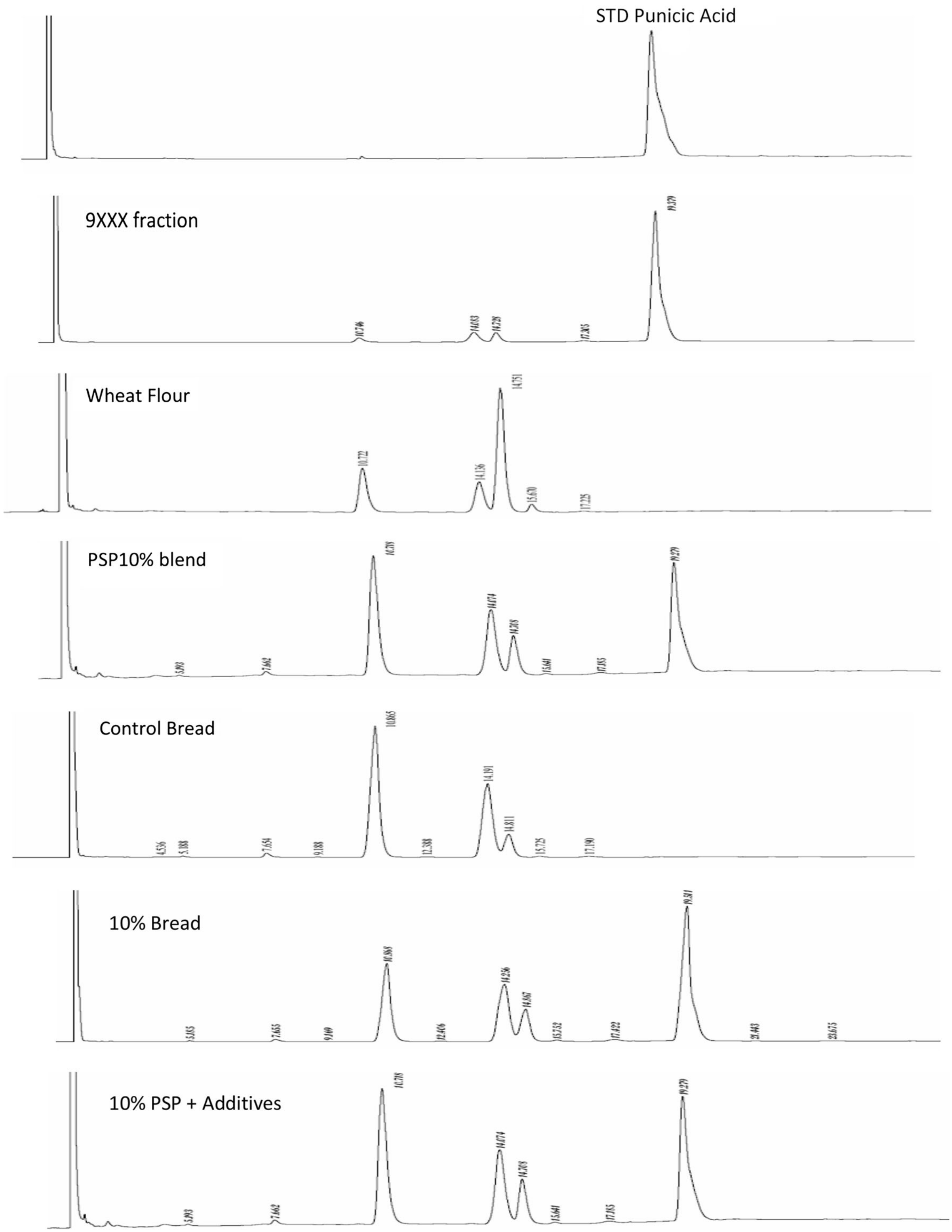


Fig. 3 Gas chromatogram of isomers of punicic acid (PA) in 9XXX fraction, PSP 10% Blend, 10% Bread, 10% PSP + CA additives samples

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Compliance with ethical standards

Conflict of interest All the authors in this manuscript declare that there is no conflict of interest.

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