J Food Sci Technol (March 2016) 53(3):1717–1721
DOI 10.1007/s13197-015-2085-8

ORIGINAL ARTICLE

Quality characteristics and antioxidant properties of breads incorporated with pomegranate whole fruit bagasse

Soumya Bhol[1] · Divyajyoti Lanka[1] · Sowriappan John Don Bosco[1]

Revised: 4 October 2015 / Accepted: 27 October 2015 / Published online: 18 November 2015

Abstract The aim of this work was to determine the quality characteristics (physical and chemical), total phenols (TPC), and antioxidant activity (DPPH Radical Scavenging method) of pomegranate whole fruit bagasse (PWB) incorporated bread. The pomegranate whole fruit bagasse powders were incorporated into yeast leavened bread at 5 g and 15 g levels (flour basis). The results showed that the mineral content varied and the antioxidant potential of breads was significantly improved. The highest increase in antioxidant potential was measured in the breads made with 15 g *w*/*w* PWB.

Keywords Pomegranate whole fruit bagasse · WD-XRF · Yeast leavened bread · Antioxidant potential

Introduction

Various ingredients include components like vitamins, minerals, phytochemicals, and fibers can be regarded as health promoting. The industrial transformation of vegetables and fruits produces large quantities of co-products rich in bioactive compounds which can be changed into commercial products for making use as intermediate foods ingredients. Different types of dietary fibres obtained from vegetables or fruits co-products, such as pea, apple, sugar beet, soy and citrus fibres, as well as inulin and gums, have been reported to be regularly incorporated into foods for their nutritional or functional properties (Fernández-López 2008).

The edible part of the pomegranate fruit (*Punica granatum* L.) consists of arils and seeds (Viuda-Martos et al. 2010). Generally, the wastes that remain after the juice is extracted from pomegranate are composed mainly of pulp, peel and bagasses. Re-use of this pomegranate bagasses obtained from the pomegranate industries in order to utilize the large quantity of potentially beneficial compounds such as dietary fiber or bioactive compounds mainly phenolic acids and flavonoids which could be used as ingredient in food processing as well as products requiring hydration, viscosity development, and freshness preservation, such as baked foods.

As bakery foods are the major cereal products available to consumers and bread has been the principal food in many countries, many alternatives for wheat flour as a substitution or replacement in yeast leavened bread making are being exploited or thought of in recent times.

The objective of the present study was to determine the effect of pomegranate whole fruit bagasse on total phenolic content (TPC), antioxidant activity and quality characteristics of yeast leavened bread.

Methods and materials

Raw materials

Commercially available refined wheat flour, refined granulated sugar, active dry yeast, refined oil, iodized salt were the basic raw materials for bread preparation. Pomegranate juice extracted waste and peel (bagasse mixture) were collected from a local market of Puducherry, India.

✉ Sowriappan John Don Bosco
boscosjd@gmail.com

Soumya Bhol
soumyabhol01@gmail.com

Divyajyoti Lanka
lankadivyajyothi@gmail.com

[1] Department of Food Science and Technology, Pondicherry University, Puducherry 605 014, India

Preparation of pomegranate whole fruit bagasse powder (PWB)

The bagasse mixture was taken in a piece of muslin cloth, was dipped in water with a constant temperature of 75 °C for 10 min in a beaker for washing. The whole co-product was squeezed to remove the liquid waste after the washing process. The filtered bagasse mixture was then dried at 75 °C for 8 h in hot air oven on a flat stainless steel tray before grinding the dried samples with a grinder mill and sieved into a fine powder.

Preparation of breads

The bread dough formula was: flour (100 g), sugar (5 g/100 g flour), active dry yeast (3 g/100 g flour), fat (10 ml/100 g flour), salt (1.5 g/100 g flour), water (optimum), and pomegranate whole fruit bagasse powder (tested at 5 g/100 g flour and 15 g/100 g flour levels). Breads were prepared by straight dough method of bread preparation (AACC Method 10–10.03). The ingredients were added based on a percentage flour weight. After baking, the bread loaf was kept for cooling for 2 h on a cooling rack.

Bread evaluation

The cooled bread loaf was then weighed on weighing balance to obtain total loaf weight and were then taken up for volume calculation by rapeseed displacement method (Lopez et al. 2004) prior to slicing. The specific volume was calculated by dividing loaf volume by loaf weight.

For deciding the best amount of substitution of refined wheat flour with PWB, sensory evaluation of the products was conducted based on overall external appearance, colour, texture, flavor, taste and overall acceptability. At first a group of 16 members (generally who consume bread or other baked cereal products everyday) were enlisted for the panel formation from the Department of Food Science & Technology, Pondicherry University, Puducherry. Three numbers of coded bread samples were evaluated by the panelists in one session. Seven panelists were selected after the consecutive sessions on the basis of their ability to correctly identify characteristics. Seven panelists, were asked to evaluate the above attributes of the samples and to rate each attributes on a scale from 1 (dislike extremely) to 9 (like extremely) using a 9 point hedonic scale (Meilgaard et al. 1991).

Proximate composition analysis and WD-XRF studies

The bread samples were analyzed for moisture (method 44–16), protein (method 46–10), ash (method 08–01) and fat (method 30–10) using the American Association of Cereal Chemists (AACC 2000) methods. Dietary fiber (method 991.43) was determined according to AOAC (1999) method. All analyses were conducted in triplicate.

For the mineral estimation by WD-XRF (Bruker), the bread samples (crumb only) were ashed (AACC method 08–01) and two grams of these ash samples were mixed with 0.5 g granulated boric acid with a mortar and pestle. The prepared sample was moulded into a 34 mm dia. pellet with the help of a 40 tonnes hydraulic press machine (10 tonnes pressure, 20 min pressing time). The pellets were then inserted in the sample slots of WD-XRF for further analysis (Bhol and Bosco 2014).

Total phenol content

The total phenol content (TPC) was determined using the Folin–Ciocalteu's reagent (Singleton and Rossi 1965). Absorption was measured at 760 nm and compared to a Gallic acid calibration curve. The results were expressed as mg Gallic acid equivalents (GAE)/g sample. Each assay was carried out in triplicate.

Antioxidant activity

Determination of antioxidant activity using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of pomegranate whole fruit bagasse powder incorporated bread was measured in terms of DPPH radical scavenging ability (Brand-Williams et al. 1995). The extraction of antioxidants was conducted as reported above. Five different concentrations of the extract were tested. These solutions (0.1 ml) were added to 3.9 ml of a 6×10^{-5} M methanol DPPH**.** solution. The decrease in absorbance at 515 nm was determined at 25 °C at zero time and every minute for the next 30 min until the recorded curve reached a plateau. The exact initial DPPH**.** concentration (C_{DPPH}) in the reaction was calculated by linear regression from a calibration curve.

For each antioxidant concentration test, the reaction kinetics was plotted. From the graphs (not shown), the percentage of DPPH**.** remaining at the steady state was determined and the values were plotted graphically as the percentage of residual steady state DPPH**.** as a function of the molar ratio of antioxidant to DPPH**.**. Anti-radical activity was defined as the amount of antioxidant (mg dw ml^{-1}) required to lower the initial DPPH**.**concentration by 50 % (efficient concentration EC_{50} (mg dw ml^{-1}/μmol DPPH**.**). For reason of clarity, the results were expressed as $1/EC_{50}$ or radical scavenging activity (RSA).

Statistical analysis

All statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Mean comparisons were statistically analyzed using Duncan's new multiple range tests (DMRT). The significant level was established at $P \leq 0.05$.

Results and discussion

Finding the suitable level of incorporation of pomegranate whole fruit bagasse powder in bread

With increase in PWB incorporation from 0 to 15 g/100 g flour, gritty mouth feel increased for which there was a decrease in the mouth feel and taste results, there was an increase in residue formation in the mouth which also indicated the decreasing score for mouth feel (Table 1). The data also specified that the texture decreases significantly at $P \leq 0.05$ as we increase the level of substitution of PWB in bread from 0 to 15 g/100 g flour which may be attributed to the presence of rough pomegranate whole fruit bagasse powder. According to the overall acceptability score no significant difference could be observed between 5 g PWB/100 g flour and 15 g PWB/100 g flour incorporated bread in comparison to control bread. Hence 15 g PWB/100 g flour incorporation level could be accepted and further increase in PWB level would not give better consumer acceptance (data not shown). The flavor of the bread was influenced more which can be considered to the characteristic combination of fruity flavor of pomegranate pulp and nutty smell of dried arils. Hence incorporation to a level as equal to 15 g PWB/100 g flour was established to have acceptable score and could be determined of providing maximum nutrition with better sensory attributes scoring. The finalized products were now taken up for further comparative analysis tests.

With an increase in incorporation level there was a lowering in the values of specific volume of bread for both 5 g PWB/100 g flour and 15 g PWB/100 g flour. Specific volume of bread is a vital quality parameter as it is related to dough aerating ability and oven spring. An ideal relation between dough weight and loaf volume yields the most desirable texture in breads (Giannou and Tzia 2007). This explains the specific loaf volume should not be too high or too low as it influences the crumb porous structure. Overly low specific volume is related with a very dense and closed grain structure, while overly high loaf volume gives a sparse grain and porous structure (Sharadanant and Khan 2003).

The effect of pomegranate whole fruit bagasse powder on proximate composition of bread

The data (Table 2) shows that the proximate composition of pomegranate whole fruit bagasse powder incorporated bread vary with increase in the substitution level. The nutritional characteristics of PWB breads in comparison to the control (100 g refined wheat flour) bread increases significantly at $P \leq 0.05$ with respect to ash, protein, dietary fiber and carbohydrates. The increase in the ash content is related to the high content of minerals in pomegranate whole fruit bagasse powder. Supplementing the breads with PWB significantly increased the protein content as compared to the control. The 15 g PWB/100 g flour included bread showed higher dietary fiber percentage in comparison to 5 g PWB/100 g flour included bread, perhaps because of the inclusion of the peel into the bagasse. Lee et al. (1992) suggested that the dietary fiber may prove protection against cardiovascular diseases, diabetes, obesity, colon cancer, and other diverticular diseases.

The effect of pomegranate whole fruit bagasse powder on mineral content of breads

The addition of PWB affected the mineral content of developed breads as compared to the control (Table 3). An increase in the calcium, copper, iron, and sodium content was observed for the higher substitution level of refined wheat flour with PWB, whereas a decrease in the potassium, magnesium, phosphorus, and zinc content was also marked in the same breads. This result suggests that pomegranate whole fruit bagasse powder do not contribute much to the potassium, magnesium, phosphorus and zinc content of the bread, which may have been arose due to processing loss in the PWB.

Table 1 The sensory evaluation and bread volume results for optimization of the pomegranate whole fruit bagasse powder incorporation level in bread making

Sl. no.	Details	Appearance	Texture	Color	Flavor	Taste	Mouth feel	Overall acceptability	Specific volume (cm^3/g bread)
1	0	$7.7^a \pm 0.09$	$7.8^a \pm 0.10$	$7.6^a \pm 0.04$	$7.0^c \pm 0.08$	$7.8^a \pm 0.05$	$7.5^a \pm 0.00$	$8.0^a \pm 0.00$	$2.66^a \pm 0.2$
2	5 %	$7.5^b \pm 0.11$	$7.9^a \pm 0.13$	$7.3^b \pm 0.06$	$7.3^b \pm 0.08$	$7.5^b \pm 0.13$	$7.4^a \pm 0.07$	$7.7^b \pm 0.00$	$2.51^b \pm 0.0$
3	15 %	$7.2^c \pm 0.13$	$7.4^b \pm 0.08$	$7.2^b \pm 0.08$	$7.4^a \pm 0.04$	$7.1^c \pm 0.15$	$6.9^b \pm 0.05$	$7.6^b \pm 0.08$	$2.42^c \pm 0.1$

The sensory results are mean ± std. dev. values of 7 trained panelists' response. Values in the same column with different letters are significantly different at $P \leq 0.05$

Table 2 Proximate characteristics of developed pomegranate whole fruit bagasse powder (PWB) bread

Parameters	Control (Crumb)	5 % PWB (Crumb)	15 % PWB (Crumb)
Ash (g/100 g)	$0.65^{a} \pm 0.02$	$0.67^{a} \pm 0.02$	$0.81^{b} \pm 0.02$
Protein (g/100 g)	$7.80^{a} \pm 0.10$	$8.03^{ab} \pm 0.21$	$8.27^{b} \pm 0.00$
Fat (g/100 g)	$2.20^{a} \pm 0.05$	$2.37^{a} \pm 0.15$	$3.74^{b} \pm 0.18$
Dietary fiber (g/100 g)	$3.20^{a} \pm 0.18$	$4.80^{b} \pm 0.10$	$6.80^{c} \pm 0.15$
Carbohydrate (g/100 g)	$51.00^{c} \pm 0.05$	$46.21^{b} \pm 0.11$	$44.03^{a} \pm 0.21$

Values are means of three replicates ± standard deviation. Values in the same row with different lower case letters are significantly different at $P \leq 0.05$

The effect of pomegranate whole fruit bagasse powder on antioxidant potential of breads

There are many indirect methods currently in use for measuring the antioxidant activity in food (Roginsky and Lissi 2005). However, the antioxidative ability of food sample after ingestion and digestion is still not fully understood although some results to confirm that inclusion of adequate amounts of natural antioxidants into a main food may influence some criterion of the human immune system. Recently, Seidel et al. (2007) showed that prebiotic breads supplemented with antioxidants enhanced the antioxidative capacity in the blood plasma. In addition, research based mainly on hygienic studies suggests that a regularly high intake of plant-derived food is related to lowered incidence of cancer, coronary heart disease, obesity, hypertension and other chronic diseases. Fruits and vegetables have been widely acknowledged to be rich sources of antioxidants; therefore, these components in the diet may be capable of offering protection against the damaging effects of free radicals (Lindsay 2000).

The total phenolic content (TPC) of the methanolic solutions of pomegranate bagasses is presented in Table 4. The phenolic contents can be used as an important indicators of antioxidant capacity which can be used as a primary screening for any product when intended as a natural source of antioxidants in functional foods. The phenolic contents depend on the cultivar, growing region, climate, maturity, and cultivation practice, storage conditions (Poyrazoglu et al. 2002) and method used to obtain the bagasse powder. These compounds are known for their properties to scavenge free radicals and to inhibit lipid oxidation in vitro (Noda et al. 2002).

In this study, the breads made with PWB were tested for the antioxidant content using DPPH, a stable radical, as the detection agent. The observed activities are shown in Table 4. The addition of PWB significantly increased the antioxidative activity in the breads depending on the supplementation level. The antioxidant activity of control (100 % wheat flour) bread is usually attributed to the presence of Maillard reaction products that are known to posses free-radical scavenging activities (Fan et al. 2006). High antioxidative activity was noted for breads made with 15 % PWB in comparison to 5 % PWB and control (0 %) breads. The DPPH assay measures the potentiality of the extract to donate hydrogen to the DPPH radical, which results in fading of the DPPH solution. The greater the fading action, the higher the antioxidant activity is seen. The DPPH scavenging data suggests that the extract is capable of scavenging free radicals, thus preventing the commencement and proliferation of free-radical-mediated chain reactions. This can be beneficial in the preservation of foodstuffs, where these reactions result in lipid oxidation and ensuing deterioration of the products (Dastmalchi et al. 2008).

Table 3 Effect of pomegranate whole fruit bagasse powder (PWB) on the mineral content[a] of the bread crumb

Parameters	Control	5 % PWB	15 % PWB
Calcium	0.510	3.920	4.080
Copper	0.210	0.350	0.360
Iron	0.120	0.220	0.270
Potassium	2.456	2.279	2.361
Magnesium	0.152	0.111	0.114
Sodium	1.470	1.480	1.580
Phosphorus	0.990	0.870	0.910
Zinc	0.080	0.072	0.080

[a] All values are represented in mg per 100 g sample

Conclusion

Incorporation of Pomegranate whole fruit bagasse powder improved proximate characteristics, mineral content and antioxidative properties though there was no substantial increase in the mineral content of the breads. The mineral content varied depending on the inclusion level of pomegranate whole fruit bagasse powder. Especially marked increase was observed in the calcium content: increases ranging from 3.920–4.080 mg/

Table 4 Total Phenolic Content (TPC) and Radical Scavenging Activity (RSA) of bread made with different levels of pomegranate whole fruit bagasse powder

Substitution level (%)	Total phenolic content (mg GAE/g sample)	Radical scavenging activity (μmol DPPH/mg sample ml^{-1})
0	$0.140^{a} \pm 0.002$	$0.017^{a} \pm 0.006$
5	$0.291^{b} \pm 0.017$	$0.106^{b} \pm 0.035$
15	$0.704^{c} \pm 0.046$	$0.370^{c} \pm 0.012$

Value of TPC and RSA are means of three replicates ± standard deviation. Different lowercase letters within the same column indicate significant differences among the different percentages of pomegranate whole fruit bagasse powder incorporated bread ($P \leq 0.05$)

100 g sample were recorded for lower incorporation level (5 g/100 g *w*/*w*) and up to 15 g/100 g *w*/*w* for higher incorporation level. The copper, sodium and iron contents increased less markedly. In general, it was observed that pomegranate whole fruit bagasse powder provided higher increases in the bread minerals (Ca, Cu, Fe, Na) and lowering of bread minerals (K, Mg, P, Zn). Similar observations were made regarding the total phenol content and antioxidative properties (DPPH activity) of the breads. The highest antioxidant potential was recorded in the bread made with 15 g/100 g flour pomegranate whole fruit bagasse powder. High antioxidative potentials were also noted for breads made with 5 g/100 g flour pomegranate whole fruit bagasse powder in comparison to control bread. Bread incorporated with pomegranate whole fruit bagasse powder affected the sensory attributes, as well. The results suggested that both 5 % and 15 % incorporation level of pomegranate whole fruit bagasse powder would interfere with the sensory acceptance of the breads. The present results suggest that the pomegranate whole fruit bagasse powder could be incorporated successfully to bread to a level of 15 % successfully. This ingredient employed several potentially health-promoting effects that include the increase in the mineral content of bread (Ca, Cu, Fe, and Na) and its antioxidative potential.

Acknowledgments The authors would like to thank Pondicherry University, Department of Food Science and Technology for providing lab facilities and Pondicherry University, Central Instrumentation Facility for their helping hand in WD-XRF studies.

References

AACC (2000) Approved methods of the American association of cereal chemists. American Association of Cereal Chemists Inc., St. Paul, MN

AOAC (1999) The official methods of analysis, 16th edn. Association of Official Analytical Chemists, Washington DC

Bhol S, Bosco SJD (2014) Influence of malted finger millet and red kidney bean flour on quality characteristics of developed bread. LWT – Food Sci Technol 55:294–300

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28:25–30

Dastmalchi K, Dorman HJD, Oinonen PP, Darwis Y, Laakso I, Hiltunen R (2008) Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. LWT-Food Sci Technol 41:391–400

Fan L, Zhang S, Yu L, Ma L (2006) Evaluation of antioxidant property and quality of breads containing *auricularia auricular* polysaccharide flour. Food Chem 101:1158–1163

Fernández-López J (2008) Development of functional ingredients: fruits fibre. In: Fernández-López J, Pérez-Alvarez JA (eds) Technological strategies for functional meat products development. Transworld Research, Kerala, pp. 41–57

Giannou V, Tzia C (2007) Frozen dough bread: quality and textural behavior during prolonged storage-prediction of final product characteristics. J Food Eng 79(3):929–934

Lee SC, Prosky L, DeVries JW (1992) Determination of total, soluble, and insoluble dietary fiber in food-enzymatic-gravimetric method, MES-TRIS buffer: collaborative study. J AOAC Int 75:395–416

Lindsay DG (2000) Maximizing the functional benefits of plant foods. Developing functional food products (part III). In: Gibson GR, Williams CM (eds) Functional foods—concept to product. CRC Press, Cambridge, England, pp. 1–6

Lopez ACB, Pereira AJG, Junqueira RG (2004) Flour mixing of rice flour, corn and cassava starch in the production of gluten free white bread. Braz Arch Biol Technol 47(1):63–70

Meilgaard M, Civille GV, Carr BT (1991) Sensory evaluation techniques. CRC Press, Inc., Boca Raton, FL

Noda Y, Kaneyuka T, Mori A, Packer L (2002) Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. J Agric Food Chem 50:166–171

Poyrazoglu E, Gökmen V, Artık N (2002) Organic acids and phenolic compounds in pomegranates (*Punica granatum* L.) grown in Turkey. J Food Comp Anal 15:567–575

Roginsky V, Lissi EA (2005) Review of methods to determine chain-breaking antioxidant activity in food. Food Chem 92:235–254

Seidel C, Boehm V, Vogelsang H, Wagner A, Persin C, Glei M, Pool-Zobel BL, Jahreis G (2007) Influence of prebiotics and antioxidants in bread on the immune system, antioxidative status and antioxidative capacity in male smokers and non-smokers. Brit J Nutr 97:349–356

Sharadanant R, Khan K (2003) Effect of hydrophilic gums on the quality of frozen dough: I. Dough quality. Cereal Chem 80(6):764–772

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic 16:144–158

Viuda-Martos M, Fernández-López J, Pérez-Álvarez JA (2010) Pomegranate and its many functional components as related to human health: a review. Compr Rev Food Sci Food Saf 9:635–654