International Food Research Journal 28(3): 517 - 526 (June 2021)

Journal homepage: *http://www.ifrj.upm.edu.my*

Fortification of bread with bee pollen, and its effects on quality attributes and antioxidant activity

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Article history

Received: 16 February 2020
Received in revised form: 15 October 2020
Accepted: 26 October 2020

Keywords

bee pollen,
wheat bread,
nutritional composition,
antioxidant activity,
physical characteristic

Abstract

Bee pollen (BP) is a good source of nourishing substances with preventative effects against metabolic syndrome and subsequent type II diabetes mellitus and cardiovascular diseases. In the present work, the addition of BP changed the characteristics and nutritional values of bread. It was found that the addition of BP significantly increased dietary fibre, thiamine, and riboflavin levels, as well as phenolic contents and antioxidant activity. When blending the wheat flour with 20 - 25% BP, the antioxidant activity of bread increased effectively, while at 20% BP addition level, the specific volume, springiness, hardness, and chewiness were significantly detrimentally affected. Addition of 10% BP was beneficial to water retention, and delayed bread staling. These results suggest that the addition of 10 - 15% BP could effectively improve the nutritional and antioxidant properties of wheat bread with textural and sensory quality being closer to the white bread.

Introduction

Bees harvest pollens and transfer them to the hive; and the pollen pellets are called bee pollen (BP) (Kieliszek *et al.,* 2018). BP is regarded as the "only perfectly complete food" due to its comprehensive and desirable nutritional composition (Kostić *et al.,* 2015). BP is rich in nutrients including protein (10 - 40%), crude fibre (0.3 - 20%), carbohydrates (13 - 55%) (Conte *et al.,* 2018), lipids (1 - 13%) (Li *et al.,* 2017), and abundant of trace elements. There are nine essential amino acids present in BP; among which lysine, proline, and glutamic are the main (Krystyjan *et al.,* 2015). The levels of FA 18:3, FA 18:1, and FA 18:2 among the unsaturated fatty acids in BP are high; in particular, linolenic acid is the essential fatty acid and can be converted into docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the body (Li *et al.,* 2017). BP is particularly rich in vitamins such as nicotinic acid, pantothenic, and riboflavin, and among all plant materials, BP has the highest content of riboflavin (Llnskens and Jorde, 1997). The high-biological activity substances in BP mainly include polyphenolic compounds, and the types and quantities of these phytochemicals depend on the origin of the pollen. BP not only provides comprehensive nutrients to humans, but also has many therapeutic properties such as antioxidant (Almeida *et al.,* 2017), anticancer, immunostimulatory, and anti-atherogenic properties (Rzepecka-Stojko *et al.,* 2017). As a nutraceutical food, BP has attracted increasing attention. However, BP is primarily consumed as an antiseptic additive or parapharmaceutical for chronic prostatitis (Krystyjan *et al.,* 2015). Currently, the use of BP in daily diets is extremely limited. The application of BP in food processing not only can meet people's demands for diversified food, but also improve the nutritional intake. However, there is a lack of thorough studies on the processing and utilisation of BP.

Bread making has been around for more than four thousand years, and bread is still a popular food for most people in the world (Dewettinck *et al.,* 2008). Bread plays an irreplaceable role in global nutrition supply. Traditional white bread is popular with most consumers because of its soft crumb and mild taste (Noort *et al.,* 2017). Nevertheless, white bread is low in some beneficial nutritional components such as dietary fibre, lysine, unsaturated fatty acids, B group vitamins, and polyphenols. Furthermore, consumer tests have shown that people prefer to choose healthy bread with beneficial ingredients. Conte *et al.* (2018) used multifloral BP as a functional ingredient in gluten-free bread, and evaluated its effect on technological and physicochemical properties. However, the effect of addition of BP on nutritional composition and antioxidant potential was not investigated. In that study, the highest level of BP supplementation was only 5%, and it had little effect on improving the nutritional profile of bread.

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DOI: https://doi.org/10.47836/ifrj.28.3.11

The framework of bread is made of a cross-linked gluten network, and starches, lipids, and other molecules are entrapped in the gluten continuous phase (Amigo *et al.,* 2016). During storage of bread products, crumb hardness gradually increases, characteristic flavour is lost, and freshness is decreased (Curti *et al.,* 2011). This non-microbial deterioration is called bread staling. The decline in the edible quality of the bread is responsible for consumer disapproval and massive waste of food resources. The components of bread mainly include gluten, starch, and water (Curti *et al.,* 2014). Enrichment of wheat flour with BP may change the gluten network and affect physicochemical changes during storage of bread. Many references are available to show that water plays an important role in bread staling during storage, and moisture redistribution is a significant mechanism that contributes to the staling effect.

In the present work, our aim was to explore the effects of fortification of bread with different amounts of BP on the nutritional composition, antioxidant activity, and physical and storage characteristics of the bread.

Materials and methods

Materials

Commercial bread flour (Angel Yeast Co., Ltd., Yichang, China), instant yeast, sugar, salt, eggs, and butter for bread making were purchased from a local supermarket in Beijing. The dry rape BP was obtained from Sichuan Kuake Technology Development Co., Ltd. (Sichuan Province, China) without mildew and impurities. Sodium hydroxide, anhydrous ethanol, acetic acid, and petroleum ether were purchased from Beijing Chemical Industry Group Co., Ltd. (Beijing, China). Potassium ferrocyanide, ammonium molybdate, sodium acetate, sodium nitrite, and Folin-Ciocalteu phenol reagent were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Aluminium nitrate and gallic acid were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Rutin, α-amylase, neutral protease, glucoamylase, and diphenylpicrylhydrazyl (DPPH) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). All these chemicals were of analytical grade unless otherwise stated.

Bread preparation

The ingredients for the control bread included bread flour (100 g), yeast (4 g), sugar (20 g), salt (1 g), egg (10 g), butter (10 g), and water (50 mL). BP was added at five different levels as follows: 0% (control), 10% (BP 10%), 15% (BP 15%), 20% (BP 20%), and 25% (BP 25%) on the basis of flour weight. First, yeast, sugar, and salt were blended in warm water (37°C) before the premixed dry ingredients were added. Then, the dissolved ingredients and butter were mixed with the dry powders using a mixer (ACA North America Electrical Appliances Co., Ltd, Zhuhai, China) until the dough were fully developed. The doughs were divided (500 g) and moulded into baking pans. Fermentation processes continued for 60 min in a climate chamber (37°C, 90 - 95% RH). Baking condition are as follows: surface, 180°C; under, 200°C; and continued for 8 min in an electric oven (Midea, Foshan, China). After removing the samples from the oven, the bread samples were placed at room temperature to cool, packed in sealed low-density polyethylene plastic bags, and stored at 25°C for 3, 5, and 7 d.

Nutritional composition of the bread

The proximate nutritional compositions of the BP and breads, including the fat, protein, fibre, ash, moisture, thiamine, and riboflavin levels were analysed according to AOAC methods 935.38, 950.36, 950.37, 930.22, 945.15, 957.17, and 970.65, respectively. The carbohydrate content was calculated by subtracting the contents of ash, fat, fibre, and protein from the dry weight (100%).

Phenolic acid and flavonoid contents

Phenolic acid and flavonoid compounds were extracted according to the method of Nurdianah *et al.* (2016). However, the quantitative determination of flavonoids was based on the recommendation of Zhang *et al.* (2016), while Folin-Ciocalteu method of Yan *et al.* (2019) was employed in phenolic content determination.

Antioxidant activity

The ability of each bread to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined according to a previously described method (Jayaprakasha and Patil, 2007). The method of Lin *et al.* (2009) was used to determine the reducing power. The phosphomolybdenum method was used to evaluate total antioxidant activity as described previously (Jayaprakasha and Patil, 2007).

Specific volume

The specific volume of each bread was calculated by bread volume/weight. The volume of bread was determined by the rapeseed displacement method (Ayadi *et al.,* 2009).

Instrumental texture analysis

Each loaf was cut into slices of 10 mm thickness with a bread knife. The chewiness, hardness, and springiness of the bread samples were measured by a TA-HD plus texture analyser (Stable Micro System, Surrey, UK) equipped with a cylindrical probe (P/36 model). The texture profile analysis was conducted with a trigger power of 5 g, a pre-test speed of 1 mm/s, a test speed of 3 mm/s, a post-test speed of 3 mm/s, and a compression of 50% of the slice thickness.

Proton molecular mobility properties

A low-resolution ^{1}H NMR spectrometer (NMI20-015V-I, Shanghai Electronic Technology Co., Ltd., Shanghai, China) was used to analyse the proton molecular mobility by determining the transverse (T_2) relaxation times. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with a recycle delay of 0.3 s and 3000 data points were used to obtain the T_2 relaxation times. Triplicate analyses were performed.

Sensory evaluation

The sensory properties (appearance, colour, flavour, taste, and inner texture) of the bread samples were evaluated with organoleptic assessment tests, using scores ranging from 1 (lowest) to 9 (highest) (Kurek *et al.,* 2017; Conte *et al.,* 2018). The panel composed of 35 students and teachers at College of Food Science and Engineering, Shanxi Agricultural University who have experience and knew well the hedonic scale test system.

Statistical analysis

Statistical analysis of the measured properties was performed with IBM SPSS Statistics 22 software. The least significant difference (LSD) test was used to evaluate significant differences among the different groups. Differences among means were determined at the level of 0.05.

Results and discussion

Nutritional composition

The different nutritional composition of the BP-wheat bread loaves are shown in Table 1. The addition of BP affected the nutritional profile of the bread loaves. BP is mainly composed of protein (27.21%), fat (13.34%), carbohydrate (26.28%), and TDF (19.84%). BP mainly includes glucose, fructose, and sucrose which are beneficial for producing a pleasant flavour, colour, and taste upon baking (Krystyjan *et al.,* 2015). The breads with BP had significantly increased protein as compared to the white bread. BP protein can be used as a promising food ingredient due to its complete essential amino acids (Kaspchak *et al.,* 2017). Enhancement of breads with BP may alleviate the lysine deficiency of white bread.

The total dietary fibre (TDF) content of BP was 19.84 g/100 g, which included soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). The supplemented bread with the smallest amount of BP (10%) resulted in a 6.7-fold increase in IDF, a 2.0-fold increase in SDF, and a 2.6-fold increase in TDF as compared to wheat bread. The enrichment of baked products with high levels of dietary fibre can help to improve the nutritional quality of the products. Dietary fibre is an irreplaceable food ingredient for humans, and a lack of fibre may be associated with the development of some diseases such as obesity, colon cancer, and diabetes (Kurek *et al.,* 2017). However, in one study, breads with additional dietary fibre from

Table 1. Nutritional composition of BP and the breads (fresh weight).

Component	Wheat flour	BP	Wheat bread	BP 10%	BP 15%	BP 20%	BP 25%
Moisture (%)	11.63 ± 0.10^a	7.03 ± 0.10^b	35.62 ± 0.32^c	35.30 ± 0.27^c	35.11 ± 0.55	35.54 ± 0.16	35.06 ± 0.49^c
Ash (%)	1.34 ± 0.04^a	2.90 ± 0.36^b	1.51 ± 0.18^c	1.58 ± 0.20^c	1.59 ± 0.09^c	1.64 ± 0.12^{bc}	1.80 ± 0.09^d
Protein (%)	12.50 ± 0.53^a	27.21 ± 0.45^b	14.68 ± 0.33^c	15.61 ± 0.39^d	16.63 ± 0.42^e	17.28 ± 0.28^f	17.39 ± 0.30^f
Fat (%)	1.42 ± 0.05^a	13.34 ± 0.06^b	9.62 ± 0.11^c	9.70 ± 0.15^d	10.04 ± 0.12^e	10.06 ± 0.09^e	10.11 ± 0.09^e
Carbohydrate (%)	68.16 ± 0.28^a	26.28 ± 0.52^b	34.33 ± 0.38^c	32.07 ± 0.09^d	31.36 ± 0.24^d	28.94 ± 0.30^e	28.02 ± 0.56^e
IDF (%)	0.17 ± 0.01^a	10.71 ± 0.72^b	0.15 ± 0.04^a	1.01 ± 0.02^c	1.13 ± 0.04^d	1.25 ± 0.02^d	1.36 ± 0.03^d
SDF (%)	0.92 ± 0.02^a	9.13 ± 0.55^b	1.06 ± 0.02^a	2.08 ± 0.06^{cd}	2.31 ± 0.06^c	2.60 ± 0.05^d	2.76 ± 0.05^d
TDF (%)	1.09 ± 0.06^a	19.84 ± 1.27^b	1.21 ± 0.06^a	3.09 ± 0.08^c	3.44 ± 0.10^c	3.85 ± 0.07^d	4.12 ± 0.08^e
Thiamine (mg/100 g)	0.05 ± 0.00^a	0.54 ± 0.03^b	0.07 ± 0.01^c	0.11 ± 0.01^d	0.14 ± 0.01^e	0.16 ± 0.02^e	0.22 ± 0.01^f
Riboflavin (mg/100 g)	0.22 ± 0.01^a	1.32 ± 0.05^b	0.40 ± 0.02^c	0.71 ± 0.03^d	0.75 ± 0.03^d	0.93 ± 0.02^e	0.94 ± 0.01^e

In the same column, means with different lowercase subscripts indicate significant difference ($p < 0.05$).

lentils or carob beans had lower specific volumes than control breads (Turfani *et al.,* 2017). The changes in the physical properties of bread and dough depend on the type of fibre (Krystyjan *et al.,* 2015).

BP is particularly rich in vitamins, and among all plant materials, BP has the highest riboflavin content (Rzepecka-Stojko *et al.,* 2017). The content of thiamine and riboflavin in the breads increased to 157 and 177% of the control level, respectively, upon the addition of 10% BP. The increase in vitamins was connected with the amount of BP in the breads. Therefore, BP is a useful ingredient for the formulation of healthier breads.

Phenolic acid, flavonoid content, and antioxidant activity in the breads

The levels of phenolic acids and flavonoids in BP were 6.56 mg of gallic acid/g and 18.41 mg of rutin/g, respectively. Phenolics were found in the BP-enhanced wheat breads, as expected (Table 2). Substituting 10 - 25% of wheat flour in bread fortified with BP would produce functional BP products. The phenolic acid content in the breads with 10 - 25% BP increased by 5.3 - 7.1-fold as compared to that in control, and a 250 - 472% increase was observed when the flavonoid content in the BP breads was determined. The content of total phenolics in the breads increased along with increased levels of BP addition. BP is characterised by a range of biological activities (antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective activities) (Llnskens and Jorde, 1997). These therapeutic properties are related to the content and type of phenolics (Pascoal *et al.,* 2014), and the phenolic compounds in BP mainly include flavonoids and phenolic acid. Due to having the ability to donate an electron or a hydrogen atom to free radical and transform it into an inoffensive molecule, phenolic compounds have strong antioxidant activities (Lopes *et al.,* 2017). The active constituents are useful in increasing functional value of wheat bread.

Due to its high flavonoid and phenolic acid contents, BP has obvious antioxidation activity. The antioxidant properties were measured as IC_{50} values for comparison. When the ability to scavenge DPPH radicals was measured, the IC_{50} value of BP extract was 0.06 mg/mL, and when the reducing power was evaluated, the IC_{50} value was 0.09 mg/mL. By the phosphomolybdenum method, the IC_{50} value was 0.12 mg/mL (Table 2). With increasing BP levels in bread samples, lower IC_{50} values were measured. The findings provided evidence that the antioxidant activity of bread was enhanced with increasing BP amounts. Krystyjan *et al.* (2015) found that the antioxidant activity of biscuits with 2.5 - 10% BP was 86.4 - 230.4% higher than that of control. These results confirm that there is high correlation between phenolic contents and antioxidant activity.

During storage, limitation of oxidation reactions was expected, especially oxidation reactions of unsaturated fatty acids. Due to their excellent antioxidant activity, BHA, BHT, PG, and TBHQ are commonly used as additives. These additives are used at the milligram level in foods, and if used in excess, there will be safety issues (Lin *et al.,* 2009). BP is a safe and nutritional food ingredient, and BP-enhanced wheat bread has high potential for use as a functional food with powerful antioxidant activity.

Physical characteristics of the bread samples

The physical qualities of the experimental samples were tested on the day of baking in terms of specific volume, proton molecular mobility, hardness, springiness, and chewiness, and the results are shown in Table 3. The specific volumes of the BP-enriched

Table 2. Phenolic acid, flavonoid content, and IC_{50} values of ethanolic extracts from BP and the breads.

Bread sample	Phenolic acid content (mg of gallic acid/g)	Flavonoid content (mg of rutin/g)	IC_{50} value (mg extract/mL)		
			Antioxidant activity	Reducing power	Ability to scavenge DPPH radicals
BP	6.56 ± 0.08^{a}	18.41 ± 0.38^{a}	0.12 ± 0.00^{a}	0.09 ± 0.00^{a}	0.06 ± 0.00^{a}
White bread	0.18 ± 0.03^{b}	0.98 ± 0.04^{b}	1.45 ± 0.19^{b}	4.21 ± 0.13^{b}	2.18 ± 0.21^{b}
BP 10%	0.96 ± 0.02^{c}	2.45 ± 0.12^{c}	0.68 ± 0.07^{c}	2.41 ± 0.09^{c}	0.24 ± 0.02^{c}
BP 15%	1.18 ± 0.10^{d}	2.46 ± 0.31^{c}	0.65 ± 0.05^{ce}	1.62 ± 0.04^{d}	0.22 ± 0.04^{c}
BP 20%	1.28 ± 0.05^{d}	2.68 ± 0.47^{c}	0.42 ± 0.02^{d}	1.24 ± 0.08^{e}	0.21 ± 0.01^{c}
BP 25%	1.28 ± 0.03^{d}	4.63 ± 0.62^{d}	0.50 ± 0.03^{de}	1.43 ± 0.03^{f}	0.26 ± 0.02^{c}

In the same column, means with different lowercase subscripts indicate significant difference ($p < 0.05$).

Table 3. Physical characteristics of the bread samples.

Physical characteristic		Bread sample				
		White bread	BP 10%	BP 15%	BP 20%	BP 25%
Specific volume (mL/g)		4.80 ± 0.13[a]	4.45 ± 0.11[a]	4.41 ± 0.21[a]	3.54 ± 0.17[b]	3.41 ± 0.08[b]
Transverse relaxation time (ms)	T_{21}	0.25 ± 0.02[a]	0.21 ± 0.01[b]	0.20 ± 0.00[b]	0.27 ± 0.03[c]	0.19 ± 0.01[b]
	T_{22}	4.64 ± 0.29[a]	3.51 ± 0.07[b]	4.64 ± 0.42[a]	5.34 ± 0.38[c]	4.64 ± 0.15[a]
	T_{23}	132.19 ± 0.86[a]	100.00 ± 1.20[b]	114.98 ± 2.35[c]	132.19 ± 3.01[a]	114.98 ± 1.74[c]
Transverse relaxation peak area (%)	A_{21}	0.69 ± 0.07[a]	0.54 ± 0.05[b]	0.40 ± 0.02[c]	0.60 ± 0.01[a]	0.51 ± 0.00[b]
	A_{22}	89.60 ± 5.21[a]	87.46 ± 3.94[a]	88.86 ± 4.39[a]	89.09 ± 2.05[a]	88.81 ± 3.02[a]
	A_{23}	9.71 ± 0.35[a]	12.50 ± 1.01[b]	10.24 ± 0.74[a]	10.60 ± 0.62[a]	11.17 ± 1.30[c]
Hardness (N)		1127.37 ± 151.76[a]	1184.47 ± 116.49[a]	1105.35 ± 97.58[a]	2415.28 ± 204.70[b]	3045.51 ± 164.82[c]
Springiness (mm)		88.72 ± 2.31[a]	92.21 ± 1.24[a]	88.16 ± 1.91[a]	84.01 ± 2.24[b]	82.50 ± 3.22[b]
Chewiness (mJ)		566.31 ± 42.03[a]	573.99 ± 69.32[a]	514.65 ± 51.11[a]	980.86 ± 85.59[b]	1227.70 ± 101.35[c]

In the same column, means with different lowercase subscripts indicate significant difference ($p < 0.05$).

breads (10 - 25% BP) were lower than that of control, and when more than 20% BP was added, the specific volume was significantly reduced. The specific volume is one of the important physical characteristics related to the appropriate aeration of breads. The standard specific volume of white bread should be 6 cm3/g and not less than 3.5 cm^3/g (Lin *et al.,* 2009). In the present work, all the bread samples met the above criteria. Therefore, BP substitution for wheat flour in the bread formula did not strongly affect the specific volume of white bread, and breads fortified with less than BP 15% had negligible changes.

BP addition should change the interactions of water, protein, and starch molecules in breads, and the migration and redistribution of these molecules are usually associated with other physical and chemical properties of breads. ^{1}H NMR techniques are often used to clarify changes occurring in materials over a wide range of molecular relaxation events (Curti *et al.,* 2011). In the present work, according to the degree of binding between water and macromolecular substances, the CPMG pulse sequence successfully detected T_{21}, T_{22}, and T_{23}, thus indicating three different transverse relaxation or water molecular properties in the bread. T_{21} and T_{22} were significantly increased in the breads with 20% BP as compared to control, but there was no significant change in T_{23}. The transverse relaxation times, including T_{21}, T_{22}, and T_{23} were sharply reduced in enriched bread with 10% BP as compared to enriched bread with 20% BP. T_{21} populations (peaking at ~ 0.5 ms) represent water associated with starch or protein by hydrogen bonding. T_{22} populations (peaking at ~ 9 ms) are attributable to water associated with starch and to fast proton exchanges between water and starch; however, polymer matrices restrict water mobility (Chen *et al.,*1997). T_{23} populations (peaking at ~ 280 ms) represent water associated with gelatinised starch or protein, and indicate diffusive exchange of water between protein and starch macromolecules (Wang *et al.,* 2004). The smaller the T_2 value, the more closely water binds to those macromolecules, and the better water retention of the bread. Therefore, the bread with 10% BP had a smaller T_2 value than the other samples, which means that there was a loss of mobility of the gluten-water domain. In the breads with BP addition, based on the transverse relaxation peak area results, the water bound by hydrogen bonds to macromolecules and the water with restricted mobility became freely exchanged water. Luo *et al.* (2017) revealed that inulin could accelerate water migration from the inside to the outside in steamed bread. Overall, the addition of materials with high dietary fibre to breads could change the distribution of water in them.

Crumb hardness, springiness, and chewiness are used to evaluate the quality of bread. As shown in Table 3, the hardness of bread did not change significantly when 10 - 15% BP was added; however, when more than 20% BP was added to the breads, there was a significant increase in hardness. In wholegrain wheat bread, the dietary fibre content is 3.0 - 7.0 g/100 g. In the present work, the addition of BP bread yielded 3.09 - 4.12% of TDF, and the TDF level increased with BP content. The spongy structure likely becomes intertwined with fibres (Ning *et al.,* 2017). The change trend for bread chewiness was the same as that for hardness with gradually increasing BP content. The quality of bread is negatively related to chewiness. The springiness of the breads fortified with 20 - 25% BP significantly decreased as compared to that of control. BP mainly contains protein (27.21%), sugars (26.28%), and fibres (19.84%). The protein from BP, which is non-gluten protein, affects dough rheological properties and bread structure

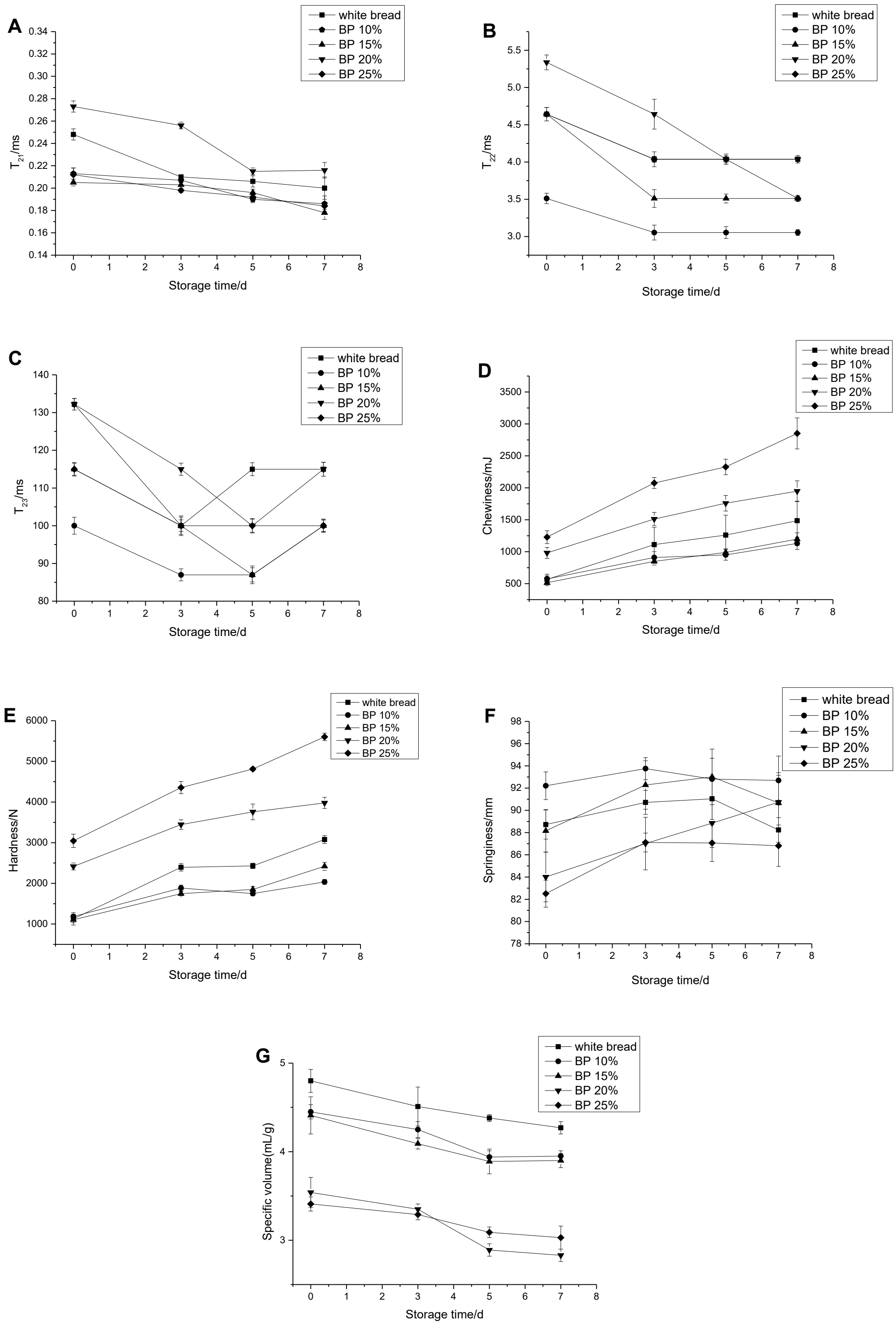

Figure 1. Physical characteristics of bread samples during storage time. (A) = T_{21}; (B) = T_{22}; (C) = T_{23}; (D) = Chewiness; (E) = Hardness; (F) = Springiness; and (G) = Specific volume.

characteristic (Ziobro *et al.,* 2013). According to our previous results (Yan *et al.,* 2019), rape BP contained 147.846 mg/g fructose and 103.100 mg/g glucose. Among varieties of sugar, fructose has the strongest hygroscopicity. With the amount of BP in bread increasing, the gluten quality and loaf volumes were compromised due to the compounds such as fructose competing for water. Additionally, a previous study found that when the amount of fibre added is more than 8 g/100 g of wheat flour, the quality of bread deteriorates (Ning *et al.,* 2017). Due to the complicated composition of BP, the addition of 10 - 15% BP to the bread samples had the least impact on the texture of the products.

Bread staling is a time-dependent process that is attributed to multiple physicochemical reactions occurring simultaneously which results in increased crumb hardness, decreased water absorption capacity, and deterioration of flavour (Curti *et al.,* 2011; Amigo *et al.,* 2016). The specific volume, transverse relaxation, hardness, springiness, and chewiness of the bread samples during storage time are shown in Figure 1. The specific volumes of all bread samples sharply decreased with prolonged storage time. During storage time, the specific volume of the control bread decreased by 0.53 mL/g, but the bread with 10% BP had less of a decline in specific volume. More than 10% BP accelerated the decline in bread specific volume.

Hardness and chewiness significantly increased in all bread samples during storage. The enriched bread with 10% BP underwent the smallest hardness increase during storage among all bread samples, including the control. Similar to bread hardness, chewiness had the smallest increase in the bread with 10% BP. Bonnand-Ducasse *et al.* (2010) revealed that in bread stored for more than one day, the increases in hardness and chewiness values are attributable to starch staling. By day 7, the springiness was higher in the BP-enriched samples than it was on the day of baking. However, there was a decrease in the springiness of the control bread. The presence of BP in the formulation limited the loss of springiness in the bread. The most rapid loss of water occurred in white bread. Water content significantly influences the physical characteristics of breads during storage, such as crumb hardness (Kurek *et al.,* 2017). In conclusion, the substitution of 10% of the wheat flour in the recipe with BP mitigated the deterioration of hardness, springiness, and chewiness during bread storage.

T_2 relaxation times significantly decreased during storage, as shown in Figure 1. Until 7 days of storage, the T_{22} and T_{23} relaxation times had greaterreductions than T_{21}. The result is consistent with a description by Curti *et al.* (2011). The decrease in T relaxation times indicated that there was water loss in the bread, and that proton shift was difficult. The T relaxation times of the breads enriched with different amounts of BP had different change trends.

Table 4. Consumer preference of the attributes of the BP-wheat composite bread loaves.

Sample	Storage time (d)	Appearance	Colour	Taste	Flavour	Inner texture
White bread	0	6.0 ± 0.1^{a}	6.5 ± 0.2^{a}	6.8 ± 0.2^{a}	6.5 ± 0.1^{a}	6.7 ± 0.2^{a}
	3	6.0 ± 0.2^{a}	5.2 ± 0.1^{b}	5.0 ± 0.1^{b}	5.2 ± 0.0^{b}	5.5 ± 0.2^{b}
	5	5.3 ± 0.1^{b}	5.5 ± 0.1^{c}	5.5 ± 0.2^{c}	5.0 ± 0.0^{bc}	5.7 ± 0.1^{c}
	7	4.5 ± 0.0^{c}	5.7 ± 0.2^{d}	4.7 ± 0.1^{d}	4.5 ± 0.0^{d}	5.7 ± 0.2^{c}
BP 10%	0	6.0 ± 0.3^{a}	6.0 ± 0.2^{e}	6.0 ± 0.2^{e}	5.5 ± 0.1^{e}	6.0 ± 0.3^{d}
	3	6.0 ± 0.4^{a}	5.7 ± 0.2^{d}	5.2 ± 0.1^{b}	5.0 ± 0.1^{bc}	5.2 ± 0.2^{e}
	5	5.0 ± 0.0^{d}	5.8 ± 0.1^{d}	5.5 ± 0.1^{c}	5.2 ± 0.0^{b}	5.7 ± 0.2^{c}
	7	5.7 ± 0.2^{e}	6.0 ± 0.3^{e}	4.7 ± 0.1^{d}	4.8 ± 0.0^{c}	5.3 ± 0.1^{b}
BP 15%	0	5.7 ± 0.2^{e}	5.5 ± 0.2^{cd}	5.5 ± 0.1^{c}	5.3 ± 0.1^{b}	5.8 ± 0.1^{c}
	3	5.5 ± 0.1^{f}	5.5 ± 0.1^{cd}	5.8 ± 0.2^{f}	5.7 ± 0.1^{f}	5.8 ± 0.2^{c}
	5	5.5 ± 0.1^{bf}	5.8 ± 0.2^{d}	5.2 ± 0.0^{b}	5.7 ± 0.2^{f}	6.0 ± 0.2^{d}
	7	5.8 ± 0.2^{e}	5.5 ± 0.1^{c}	4.8 ± 0.0^{d}	5.2 ± 0.1^{b}	5.2 ± 0.1^{e}
BP 20%	0	5.5 ± 0.1^{f}	5.7 ± 0.2^{d}	5.5 ± 0.1^{c}	5.3 ± 0.0^{b}	5.3 ± 0.1^{be}
	3	5.8 ± 0.2^{e}	5.7 ± 0.2^{d}	5.8 ± 0.2^{f}	5.3 ± 0.1^{b}	5.5 ± 0.2^{b}
	5	6.0 ± 0.3^{a}	5.8 ± 0.2^{d}	5.2 ± 0.1^{b}	5.2 ± 0.1^{b}	5.5 ± 0.2^{b}
	7	4.8 ± 0.0^{g}	4.8 ± 0.1^{f}	4.3 ± 0.0^{g}	4.7 ± 0.1^{c}	5.0 ± 0.1^{f}
BP 25%	0	5.2 ± 0.1^{bd}	5.5 ± 0.2^{c}	6.0 ± 0.3^{e}	5.2 ± 0.1^{b}	5.3 ± 0.1^{be}
	3	5.0 ± 0.1^{d}	5.3 ± 0.2^{b}	4.8 ± 0.1^{d}	4.7 ± 0.1^{c}	4.7 ± 0.1^{g}
	5	5.0 ± 0.2^{d}	4.7 ± 0.1^{f}	4.7 ± 0.0^{d}	4.7 ± 0.1^{c}	4.0 ± 0.0^{h}
	7	4.2 ± 0.0^{h}	4.2 ± 0.1^{g}	4.3 ± 0.1^{g}	4.3 ± 0.0^{g}	3.5 ± 0.0^{i}

In the same column, means with different lowercase subscripts indicate significant difference ($p < 0.05$).

The bread with 10% BP underwent the smallest reductions in T_{21}, T_{22}, and T_{23} during the 7-day storage period. The decrease in overall exchangeable proton mobility corresponds with retrogradation of the starch phase and redistribution of gluten and water in the amorphous regions of the bread samples (Hallberg and Chinachoti, 2010). BP at moderate levels in fortified bread could compete with other macromolecules for water and weaken the hydrogen bonds between water molecules and other substances. Thus, the water migration and redistribution were hampered, which delayed bread staling.

Sensory parameters of the breads

The sensory evaluation results are shown in Table 4. Based on the results, bread without BP obtained higher overall acceptability. The addition of BP reduced the sensory quality of the bread. The main reasons could be that the colour of the bread crumb changed from white to brown with increasing BP addition, and that when BP was used in high quantities, the breads had a slightly bitter taste. Biscuits enriched with BP had similar sensory properties (Krystyjan *et al.,* 2015). However, the enriched bread with 10% BP was superior to the other fortified breads in all attributes. During storage, the samples with 10 - 15% BP underwent smaller changes in scores than the white bread. Therefore, it can be concluded that appropriate addition of BP could help maintain the sensory qualities of bread.

Conclusion

In the present work, BP was added to white bread to examine its influence on the physical and nutritional properties of breads. The addition of BP significantly increased the dietary fibre, thiamine, and riboflavin levels in breads. The enriched wheat bread with BP contained more polyphenol components, as expected, and the fortified breads had better antioxidant activity than the white bread. However, at the 20 - 25% BP supplementation level, there were some detrimental effects such as decreased specific volume, increased hardness and chewiness, poorer water retention, and, in particular, a darker and slightly more bitter bread crumb. Substitution of 10% of the wheat flour in the recipe with BP delayed bread staling during storage. Overall, a proper balance between the nutritional and antioxidant properties and the physical and sensory properties of the bread was obtained in enhanced breads with 10 - 15% BP. The enriched breads with BP had better nutritional profiles, more polyphenols, and stronger antioxidant properties than the white bread.

Acknowledgments

The present work was financially supported by the Apicultural Industry Technology System (CARS45-KXJ8).

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