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Original article

Effect of polyphenols enriched from green coffee bean on antioxidant activity and sensory evaluation of bread

Muhammad Zuhair Mohd Zain [a], Ahmad Salihin Baba [a], Amal Bakr Shori [b,*]

[a] Biomolecular Research Group, Division of Biochemistry, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia

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ABSTRACT

The present study investigated the benefits of adding different concentrations of grounded green coffee beans (GCB) powder (3%, 5% and 7%) to wheat flour dough prior to baking at 220 °C for 30 min compared to control (0% of GCB) and commercially available bread. Each sample was analyzed for total phenolic content (TPC), antioxidant activates (DPPH radical scavenging activity (IC_{50}) and ferrous ion chelating (FIC) ability) and organoleptic properties. The highest TPC was observed in 7% GCB bread (1.61 ± 0.06 mg GAE/g) which also recorded the highest DPPH (2.80 ± 0.06 mg/ml; IC_{50}) and FIC (0.49 ± 0.01 mg EDTA/g). GCB bread (3%) showed the highest organoleptic scores among the GCB bread. In conclusion, GCB may be used in formulating functional bread which impacts high antioxidant content.

1. Introduction

The concept of functional foods was first introduced in Japan in mid-1980 (Siró et al., 2008). Functional food means food products fortified with special constituents that possess advantageous health benefits above its normal nutritional values and preference (Kwak and Jukes, 2001; Siró et al., 2008). It is a food similar form as ordinary food and can be consumed within daily dietary patterns. This distinguish functional foods from dietary or food supplement (Kwak and Jukes, 2001). The demand for such food grows steadily in recent years. One of example of functional food is bread.

Bread is one of common food source in human diet. It can act as excellent energy source and through fortification it can be used as carrier for folate, copper, thiamine, zinc, iron phytic acid and plausibly minerals (Helou et al., 2016a) and also melanoidins (Helou et al., 2016b). Bread can also be a plausibly good carrier for delivery of phenolic antioxidants and fibre polysaccharides at high concentrations (Sivam et al., 2010).

Coffee is a main dietary source of polyphenol and phenolic acid due to its high polyphenol and phenolic acid content (Ovaskainen et al., 2008; Jeszka-Skowron et al., 2016). These constituent of the coffee are correlated well with high antioxidant properties (Babova et al., 2016; Jeszka-Skowron et al., 2016), weight loss (Shimoda et al., 2006; Thom, 2007), mood enhancing and increase alertness (Smith, 2002; Williams et al., 2005), effectiveness against hypertension (Suzuki et al., 2002; Kozuma et al., 2005) and anticancer properties (Glei et al., 2006). Recently, the demand and consumption of green coffee bean has skyrocket due to health properties contain in it.

Most coffee we consumed have been roasted, a process that change the colour, flavour and odour of green coffee bean to become roasted coffee that will be ready to brew. However roasting process introduced causes 8–10% chlorogenic acid degradation and transformation per each 1% loss of dry matter (Clifford, 1999) and 11% to 45% polyphenol degradation (Budryn et al., 2015). Due to these losses, green coffee bean has the potential to be better source of these compounds than roasted coffee bean. In addition, it has the potential to be used as natural source of polyphenols and antioxidants in functional bread (Dziki et al., 2015), thus the aim of the study was to estimate the polyphenols and antioxidant properties of bread containing 3%, 5% and 7% of green coffee bean compared to control (0%) and other commercially available bread. In addition, evaluation the organoleptic properties of green coffee bean bread.

* Corresponding author.
E-mail addresses: ahmad@um.edu.my (A.S. Baba), ashouri@kau.edu.sa (A.B. Shori).
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2. Materials and methods

2.1. Preparation of green coffee bean (GCB)

Arabica green coffee bean originated from Sumatera was purchased from the Roast Things Coffee Roaster, Malaysia. The GCB was grounded to pass through a 3 mm steel strainer and kept inside an airtight container and placed in the refrigerator. The GCB then can be directly inserted into bread.

2.2. Preparation of bread

Materials used for making bread was purchased from a local hypermarket. Commercially prepared bread (white bread, whole meal bread and bran & wheat germ bread) were produced from Gardenia Bakeries (KL) Sdn Bhd (GBKL), whereas roti canai was purchased from local restaurant. The control bread was prepared by mixing 500 g flour, 6 g salts, 5.5 g yeast and 500 ml water. For green coffee bean bread, GCB was added at 3% (15 g/500 g), 5% (25 g/500 g), and 7% (35 g/500 g) in the flour. The yeast was mixed with warm water (35–45 °C) and added into the flour and salt mixture to make the dough. The dough was allowed to rise by leaving it at room temperature for 1 h. The dough then was folded and pressed repeatedly for second time to allow second fermentation to occur. The dough was leaved at room temperature for 25 min. The dough then baked at 220 °C for 30 min after which it was cooled to room temperature.

2.3. Preparation of bread extract

2.3.1. Preparation of powdered bread

Bread was prepared for analysis according to Michalska et al. (2007) with slight modification. The bread was first sliced to 1 cm thick. Then it dried in oven at 40 °C for 24 h. The bread then grinded to obtain dried and powdered bread.

2.3.2. Preparation of bread crude extract

Bread crude extract was prepared according to Michalska et al. (2007). Powdered bread was extracted with 80% aqueous methanol at a ratio of 1 g per 10 ml. The extract shaken (100 rpm) for 2 h at 37 °C followed by centrifugation (12,000g for 15 min). The extract was then used for total phenolic content (TPC) and DPPH scavenging activity. For Ferrous Ion Chelating (FIC) assay, water was used as solvent instead of 80% aqueous methanol.

2.4. Determination of total phenolic content (TPC)

The content of total phenolic was determined according to Michalska et al. (2007) with slight modification. Bread extract (0.25 ml) was mixed with 0.25 ml of diluted Folin-Ciocalteu's reagent (water 1:1 v/v), 0.5 ml of sodium carbonate (Na_2CO_3) solution (0.2 g/ml), and 4 ml of water. The mixture was allowed to stand at room temperature for 25 min followed by centrifugation (2000g for 10 min). The supernatant absorbance was measured at 725 nm (Genesis 10UV) and read against gallic acid standards (mg/ml) and expressed as gallic acid equivalents. The gallic acid was dissolved in 80% methanol (0–350 mg/ml) and used as the standard

2.5. Determination of DPPH scavenging activity

DPPH antioxidant activity was measured according to Culetu et al. (2016) by placing 20 µl sample of 80% aqueous methanol extract into microplate's well and adding 300 µl DPPH solution (1.01 × 10–4 mol/L in methanol). The sample was then incubated for 30 min in the dark at ambient temperature prior to absorbance reading (517 nm) by using microplate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, Winooski, VT). Ascorbic acid was used as positive control. Free radical scavenging activity of the sample extracts was determined by using the following formula:

$$\text{Percentage of DPPH quenched}(\%) = \frac{Absorbance(blank) - Absorbance(sample)}{Absorbance(blank)} \times 100$$

The IC_{50} (the effective concentration of extract required to scavenge 50% of DPPH free radical) was calculated.

2.6. Determination of ferrous ion chelating (FIC) ability

The chelating ability of ferrous ion was estimated according to Rajauria et al. (2013) with minor modifications. Extract of bread samples (100 µl) were mixed with 100 µl of distilled water and 25 µl of $FeO_4S{\cdot}xH_2O$, iron (II) sulphate hydrate (0.5 mM) in a microtiter plate, prior to the addition of 25 µl of ferrozine (2.5 mM). The reaction mixture was shaken vigorously. The absorbance was recorded at 562 nm with a microtiter plate reader, after 10 min of incubation at ambient temperature. Ethylenediaminetetraacetic acid (EDTA) was used as a standard compound. The percentage of inhibition of ferrozine-Fe^{2+} complex formation of the bread extracts was calculated by using the following formula and the data was expressed as mg EDTA/g bread

$$\text{Ferrous ion scavenging activity}(\%) = \frac{Absorbance(blank) - Absorbance(sample)}{Absorbance(blank)} \times 100$$

2.7. Sensory evaluation

Sensory evaluation was conducted by using the hedonic scale of 9 to 1 scale where 1 is 'dislike extremely', 5 is 'neither like nor dislike' and 9 is 'like extremely'. Participants were randomly selected (n = 30) from University of Malaya students (20–25 years old). Each of respondent was given plain water for mouth rinsing before and after each sample testing. The attribute of bread included form and shape, texture, colour, chewability, odour and taste.

2.8. Statistical analysis

Calculation of data (n = 9 for TPC & FIC ability; n = 3 for DPPH inhibition activity) for all the parameter's mean and standard error (ER) was carried out by SPSS 16 software, using one-way analysis of variance (ANOVA). The statistical significance was tested at $p < .05$ using post hoc Tukey's analysis at 95% least significant difference (LSD).

3. Results and discussions

3.1. Total phenolic content of GCB bread

Fig. 1 shows the TPC in bread added with different concentrations of grounded GCB (3%, 5% and 7%) compared to control bread (0% of GCB) and commercially available bread. The addition of 7%, 5% and 3% GCB increased ($p < .05$) TPC in bread (1.61 ± 0.06, 1.14 ± 0.07 and 0.93 ± 0.04 mg GAE/g; respectively) compared to control (0.26 ± 0.02 mg GAE/g). Even at 3% GCB addition, the TPC was nearly twice compared to the whole meal bread (0.50 ± 0.04 mg GAE/g). Moreover, all the three types of GCB bread showed

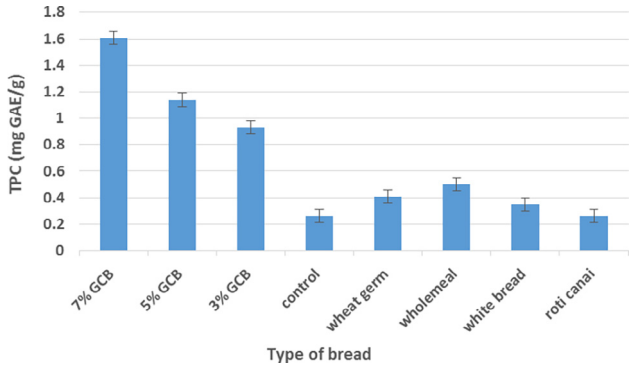

Fig. 1. Total phenolic content (TPC; mg GAE/g) of green coffee bean (GCB; 3%, 5% & 7%) bread versus control bread (0% GCB) and other commercially available bread. Value are presented as mean value ± SE (n = 9). The level of significant was present at $p < .05$.

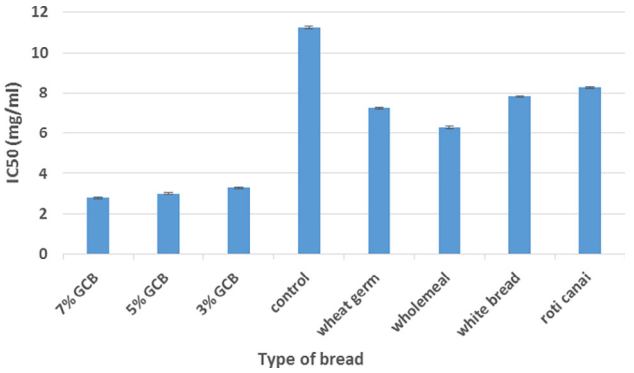

Fig. 2. DPPH radical scavenging activity (IC_{50}; mg/ml) of green coffee bean (GCB; 3%, 5% & 7%) bread versus control bread (0% GCB) and other commercially available bread. Value are presented as mean value ± SE (n = 3). The level of significant was present at $p < .05$.

significantly ($p < .05$) higher TPC compared to other commercially available bread (Fig. 1).

The increase of TPC observed in GCB bread, may due to presence of polyphenols and phenolic acid in GCB mainly coffee chlorogenic acid (Clifford, 1999). In the present study, GCB extract showed total phenolic content of 36.41 ± 0.77 mg GAE/g (data not shown). In addition, increasing amount of GCB addition resulted in increased TPC in bread (Fig. 1). Similarly, the addition of broccoli sprouts (1–5%) influenced TPC of bread ranged from 1.90–2 mg GAE/g (Gawlik-Dziki et al., 2014). The lower TPC of 0.2264 mg GAE/g has been shown in steamed bread made from whole wheat flour (Li et al., 2015). Likewise, TPC of bread made from whole wheat and refined flour showed the average of 1.58 and 0.87 mg FAE/g, respectively (Yu et al., 2013). Li and Beta, (2011) reported low TPCs of 1.01 and 0.52 mg FAE/g for purple wheat bread from the whole and refined flour respectively. These values were significantly lower than the present results. TPC in control bread was associated with phenolic compound (ferulic acid) found naturally in wheat flour after the milling process (Jackson and Hoseney, 1986) and amino acid as well as smaller peptides formed in proteolysis of protein wheat flour during fermentation of bread (Di Cagno et al., 2002).

Whole meal, wheat germ, white bread and roti canai is among local bread tested due to their availability and high consumption rate. The white bread, whole meal and wheat germ bread contained higher amount of TPC compared to control bread and roti canai. These might due to addition proteins, vitamins, fibrous whole meal, wheat germ and bran to the bread formulation. Roti canai used high amount palm oils in the food preparation which seem does not cause any significant amount of difference in TPC compared to control bread since their main ingredient consist of wheat flour.

3.2. Antioxidant potential of GCB bread

Fig. 2 shows IC_{50} DPPH inhibition in bread added with different concentrations of grounded GCB (3%, 5% and 7%) compared to control and commercially available bread. The IC_{50} DPPH inhibition of bread with 3% GCB addition (3.28 ± 0.07 mg/ml) was approximately 3 times lower than control (11.25 ± 1.13 mg/ml) and two times lower than Whole meal bread (6.30 ± 0.35 mg/ml). The IC_{50} DPPH inhibition decreased significantly ($p < .05$) upon increased the constrictions of GCB to bread when compared to control bread (Fig. 2). However, there were no significant differences ($p > .05$) in IC_{50} DPPH inhibition of bread among the three concentrations of GCB. The range of IC_{50} DPPH inhibition of other local bread was between 8.27–6.30 mg/ml (Fig. 2).

Fig. 3 shows FIC ability in GCB bread compared to control and commercially available bread. A great increased ($p < .05$) in FIC ability was observed in bread added with GCB in comparison with control and local bread. However, there were no significant differences in FIC ability between the three concentrations of GCB bread ranged from 0.44 to 0.49 mg EDTA/g. Furthermore, white bread showed lower FIC ability (0.02 ± 0.01 mg EDTA/g; $p < .05$) among other local bread (Fig. 3).

Antioxidant properties of plant can be classified on the ability to chelate metal ions or directly scavenge the free radical ions (Valko et al., 2006). The primary antioxidant activity is the ability of compound donating hydrogen to the stable radicals and ability to reduce ferric ions by donating electron to inhibit chain initiation and break chain propagation while secondary antioxidant is ability of compound to bind strongly to metal ions to suppress the formation of radicals via Fenton reaction and protect against oxidative damage (Lim et al., 2007). Therefore, DPPH assay is used to measure primary antioxidant properties and FIC assay for secondary antioxidant properties measurement (Lim et al., 2007; Deetae et al., 2012).

DPPH radical scavenging activity and FIC ability were observed in GCB bread in comparison to control bread and other local bread (Figs. 2 and 3). This may occur due to presence of phytochemical in bread mainly coffee chlorogenic acid carried upon addition of GCB (Dziki et al., 2015). Similarly, the antioxidant activity of broccoli sprouts breads (1–5%) using free radicals scavenging ability were ranged from 65.64 to 83.93 EC50 mg DW/mL which related to active compounds derived from broccoli sprouts (Gawlik-Dziki

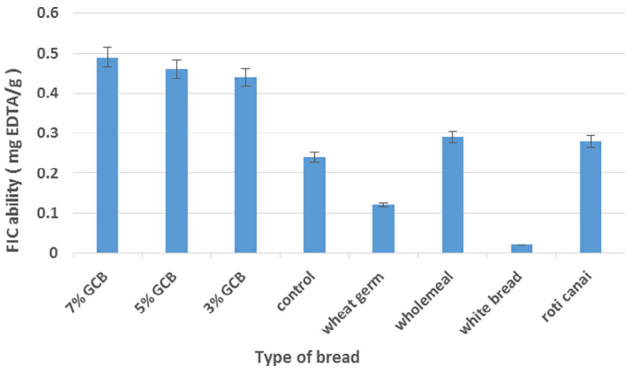

Fig. 3. Ferrous ion chelating (FIC) ability (mg EDTA/g) of green coffee bean (GCB; 3%, 5% & 7%) bread versus control bread (0% GCB) and other commercially available bread. Value are presented as mean value ± SE (n = 9). The level of significant was present at $p < .05$.

et al., 2014). DPPH radical scavenging activity values of bread made from refined flour (2.83–3.90 μmol TE/g; Yu et al., 2013) was almost similar to the present results. However, Liu et al. (2010) reported higher DPPH values ranged between 6.48 ± 1.46 μmol TE/g and 8.57 ± 1.46 μmol TE/g for six different bread wheat grains. The difference of DPPH scavenging abilities may be due to the different raw flour materials with varying phenolic compounds, the processing conditions along with the extraction procedures (Mpofu et al., 2006, 2007).

The influence of phenolic content to antioxidant activity of foods samples was studied (Marathe et al., 2011; Budryn et al., 2015). In the present study, good correlation was observed between TPC and DPPH radical scavenging activity ($R^2 = 0.7753$) or ferrous ion chelating ability ($R^2 = 0.6359$).

3.3. Organoleptic properties of GCB bread

The organoleptic properties of different concentrations of GCB added to bread were shown in Table 1. The addition of GCB in bread resulted in reduced scores for odour and taste (3.90–4.30 and 4.17–4.27 respectively) compared to control bread (5.20 and 5.30 respectively). The highest scores of GCB bread were observed for chewability (5.77–6.10) and texture (5.47–6.03) which was not significantly different ($p > .05$) from control bread (6.50 and 5.90 respectively). A green colour of bread crust was observed in GCB bread compared to control bread (Fig. 4) imposed slightly negative effect on the colour score (Table 1). The other attributes such as attractiveness and shape were being negatively affected by the addition of GCB (5.17–4.57 and 5.47–4.93; respectively). Furthermore, 3% GCB bread had highest scores among other GCB bread for all the six attributes. Meanwhile, the average score of roti canai, white bread, whole meal and wheat germ bread were higher than GCB bread (Table 1).

Bread is one of staple food in many country where its formulation depend on the cultural resignation or designed upon requirement of the local area. Therefore, making of enriched bread containing natural products packed with bioactive ingredient is a sound approach. Such product however must be palatable to consumer judgment. Dziki et al. (2015) reported that the addition of GCB introduce slightly green colour of bread crumb and crust. Attributes such as chewability and texture seemed to be fairly affected by the addition of grounded GCB. The GCB powder may disrupt the continuity of the gluten matrix microstructure thus, affecting the volume and texture of bread (Indrani et al., 2010).

Table 1
Organoleptic properties of green coffee bean (GCB; 3%, 5% & 7%) bread versus control bread (0% GCB) and other commercially available bread.

Evaluations	Score							
	Roti canai	White bread	Whole meal	Wheat germ	Control	3% GCB	5%GCB	7%GCB
Shape	6.90 ± 1.18Afgh	7.53 ± 1.25Befgh	6.57 ± 1.41Cgh	6.70 ± 1.26Dfgh	5.76 ± 1.48Egh	5.47 ± 1.61abdF	5.07 ± 1.78abcdG	4.93 ± 1.93abcdeH
Texture	6.97 ± 1.03Aef	7.37 ± 1.56Bdef	6.50 ± 1.31	6.77 ± 1.38Ce	5.90 ± 1.60bD	6.03 ± 1.50b	5.47 ± 1.59abcE	5.70 ± 1.58abF
Attractiveness	6.67 ± 1.09Aefgh	7.37 ± 1.25Befgh	6.30 ± 1.80Cgh	6.27 ± 1.60Dgh	5.33 ± 1.75abE	5.17 ± 1.86abF	4.57 ± 1.83abcdG	4.67 ± 1.73abcdH
Colour	6.90 ± 1.30Afgh	7.27 ± 1.28Bdfgh	6.17 ± 1.82	6.53 ± 1.43Cgh	5.73 ± 1.78D	5.27 ± 1.51abF	5.00 ± 2.07abcG	5.03 ± 1.93abcH
Chewability	7.43 ± 0.77Acdf	7.47 ± 1.33Bcdf	6.60 ± 1.30	6.90 ± 1.58	6.50 ± 1.48	6.10 ± 1.75abC	5.77 ± 1.65abD	5.77 ± 1.85abF
Odour	7.43 ± 0.97Ae	7.23 ± 1.33Be	6.57 ± 1.36Ce	6.50 ± 1.50D	5.20 ± 1.88abE	4.30 ± 2.20abcd	3.90 ± 1.84abcd	3.90 ± 1.86abcd
Taste	7.63 ± 0.89Acefg	7.27 ± 1.44Befgh	6.07 ± 1.85aCfgh	6.53 ± 1.78Dfgh	5.30 ± 1.84abE	4.27 ± 1.95abcdF	4.20 ± 2.01abcdG	4.17 ± 2.12abcdH

Value are presented as mean value ± SE (n = 30). Values with the same alphabet (per row) are considered significantly difference in reference to subject in capital letter ($p < .05$). 1 = Dislike Extremely, 2 = Dislike Very Much. 3 = Dislike Moderately, 4 = Dislike Slightly, 5 = Neither Like nor Dislike, 6 = Like Slightly, 7 = Like Moderately, 8 = Like Very Much, 9 = Like Extremely.

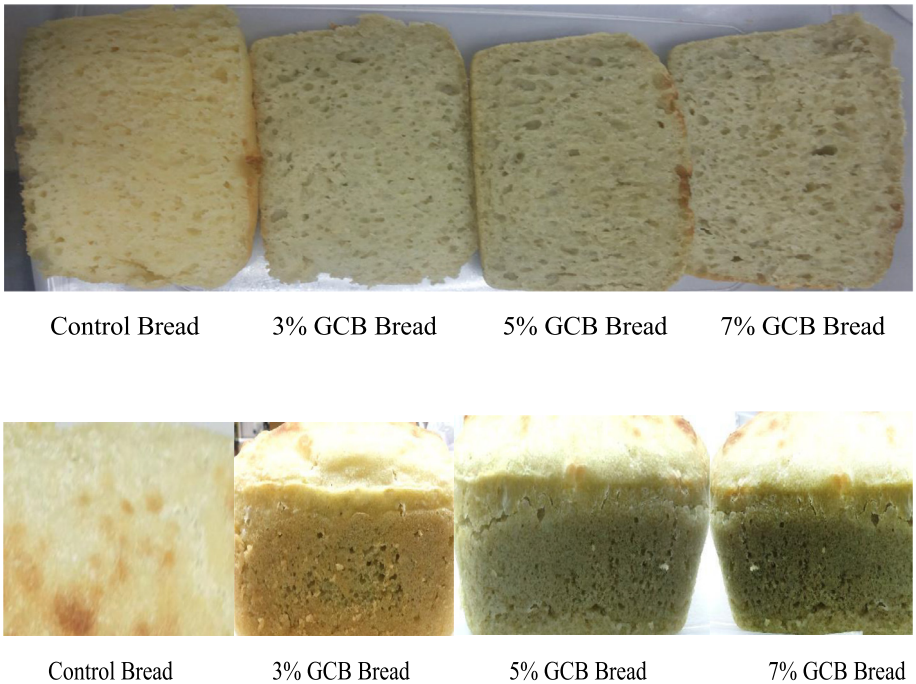

Fig. 4. Colour crumb (top) and crust (bottom) of green coffee bean (GCB; 3%, 5% & 7%) bread versus control bread (0% GCB).

Other attributes of GCB bread such as shape and attractiveness were negatively affected by GCB addition. These results may influenced by lower score of odour, colour and taste of GCB bread. The attribute for odour and taste showed lower score at higher level of GCB addition (Table 1). These two bread attributes might be influenced by certain off-flavour specifically 2,4,6-Trichloroanisole (TCA) (Spadone et al., 1990) known to be responsible for stale or musty aroma (Callejón et al., 2016). Probiotic fermentation i.e. *Bacillus subtilis* R0179 and *Lactobacillus rhamnosus* R0011 (Côté et al., 2013) could be used to enhance the negative organoleptic impacts of GCB in terms of odour, colour and flavours since some probiotics have the ability to degrades the off-odour and off-flavour compounds in the plant (Montet et al., 1999).

4. Conclusion

The addition of GCB can increase significantly the functional properties of bread by increasing the phenolic content and antioxidant properties compared to control bread and other commercially available bread. Increasing amount of GCB addition resulted in increased phenolic content and antioxidant activity in GCB bread. However, the addition of GCB resulted in reduced organoleptic properties score of overall characteristic especially for odour and taste. The increased in both DPPH radical scavenging activity and FIC ability of GCB bread showed the GCB bread was a potentially good source of radical scavenging and iron-chelating antioxidant properties. Thus, the present results can help further in the formulation of functional bread which contains high antioxidant values. This is important as bread is staple food in many country. The GCB bread is not only targeting the whole population but also to the consumers who believe in the benefit of merging 'traditional' with pro-health food. Further study is needed to estimate the proximate analysis of GCB bread.

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