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Research article

Fruiting-body-base flour from an oyster mushroom—a waste source of antioxidative flour for developing potential functional cookies and steamed-bun

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Abstract: In a commercial oyster mushroom farm, fruiting body base (FBB) was not harvested compared to the common cap and stem of the fruiting body parts, and thus remained as waste. In the present study, unused FBB was powdered and subjected to proximate analysis as floured FBB (FFBB). FFBB was found to contain 71.2% carbohydrate, 8.93% moisture, 7.18% fibre, 5.72% ash, 5.57% protein, and 1.4% fat, while raw-FBB (RFBB) contained 7.57% carbohydrate, 84.4% moisture, 5.17% fibre, 5.72% ash, 1.54% protein, and 0.85% fat. The high carbohydrate content of FFBB was subjected to hot-water extraction and yielded 7.40 g of FFBB polysaccharide (FFBBP). Total phenolic content (TPC) of FFBBP contained 1.80 mg gallic acid equivalents (GAE)/g, exhibiting the reducing activity of 1.74 mM Fe(II)/g by ferric reducing antioxidant power assay, and reduced the stable 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate radical forms at IC₅₀ of 25.08 mg/mL, which was comparable with other parts of oyster mushroom species. Different percentages of FFBB mixtures were utilized in the production of cookies (10% and 20%) and steamed buns (10%, 20% and 30%). Thirty-seven panellists were selected for sensory testing, which showed that 10% of

FFBB in a steamed bun was more acceptable than 30% of FFBB, while the overall acceptance of cookies with 10% FBB was insignificant (p > 0.05) but 20% FFBB in cookies was significantly different from control (p < 0.05). This study indicates that potential value of FFBB as an economical antioxidative flour in the development of functional foods.

Keywords: oyster mushroom; fruiting body waste; antioxidant properties; functional cookies; functional steamed buns

1. Introduction

Cookies and steamed buns are delicacy snacks that contain wheat flour, yeast, and sugar and are typically found in Chinese and Western cuisine, respectively [1]. Cookies are typically sold in bakeries and consumed at any time of the day, while steamed buns are commonly sold in dining outlets, where they are served on hot trays and consumed with sweet fillings, savoury fillings, or anchovies with chili paste [2]. Improving the nutritional quality of these foods may increase their value, as consumers are becoming more health conscious and increasing the demand for foods with added value [3].

Flour is the main ingredient in both snacks; therefore, as a healthy substitute, mushroom flour can be utilized. Mushroom flour is a gluten-free flour [4,5], which may benefit people with celiac disease, a condition in which the consumption of gluten can trigger the immune system in the small intestine and damage the intestinal lining, leading to malabsorption (prevention of nutrient absorption) [6]. In a study conducted by Quinones et al. [7], rice flour, potato flour, cassava starch, millet flour, and corn flour were used at different percentages to prepare four different treatments, but no significant difference from the control (gluten-free composite) was observed in terms of preference and acceptability.

There are collective demands to utilize agricultural food by-products for economic, environmental, and sustainability reasons [8]. Edible mushroom plays a major role in the agricultural food industry. Of the few species of macrofungi (fleshy fruit bodies) that are edible [9], *Pleurotus sp.* in particular, which is also known as "delicious oyster" or "purple spore oyster" and originated from Japan in the 1990s [10], is popular due to its important "Umami taste" [11] and pleasant savoury flavour attributed to its monosodium glutamate (MSG) and free amino acid content [12]. Previous studies have mostly included only the fruiting body of the mushroom, with the bases or stools considered as waste products and used in animal feed mixtures or as organic compost, known as "mushroom spent" [13].

Oyster mushroom is also known for its high antioxidative activity in every part of the mushroom. Oxidative damage is caused by free radicals naturally produced in the body, and the most common form of oxidative damage in the human body is aging [14]. Neutralizing free radical molecules using an antioxidant compound may protect cells against damage and reduce the prevalence of cancer, cardiovascular disease, cataract, diabetes mellitus, and stress [15]. Therefore, antioxidants have been of particular interest to researchers due to their anti-inflammation and anti-radicalization effects, and their ability to lower blood lipids, mimic anticoagulants, and modify biological responses [16]. According to Chirinang et al. [17], the major antioxidant compounds found in mushrooms are phenolic compounds.

In the present study, the fruiting body waste from commercial oyster mushroom production was utilised as gluten-free flour. In a preliminary study, the proximate composition of raw fruiting body base (RFBB) and floured fruiting body base (FFBB) was analysed, including the moisture, ash, lipid, protein, fibre, and carbohydrate content. Next, the total carbohydrate content and the antioxidative properties of the polysaccharide extracted from FFBB were screened. Finally, functional steamed buns and cookies were prepared using FFBB and sensory analysis was conducted.

2. Materials and Method

2.1. Mushroom sample

Fresh mushrooms (*Pleurotus sapidus*) were harvested from the Mushrooms Unit, Agrotechnology Section, University Park, Serdang, Malaysia. The lower part of the stem, also known as the fruiting body base (FBB), were taken as samples (Figure 1D). Samples were then cleaned from soil and insects, dried in a food dehydrator at low temperature of 40 °C (Creative Marketplace, Malaysia), and ground to a fine powder before being sieved to remove clumps. This procedure was necessary to prevent lignin artefacts in the sample [18]. The sample preparation flow is shown in Figure 1 from picture A to F.

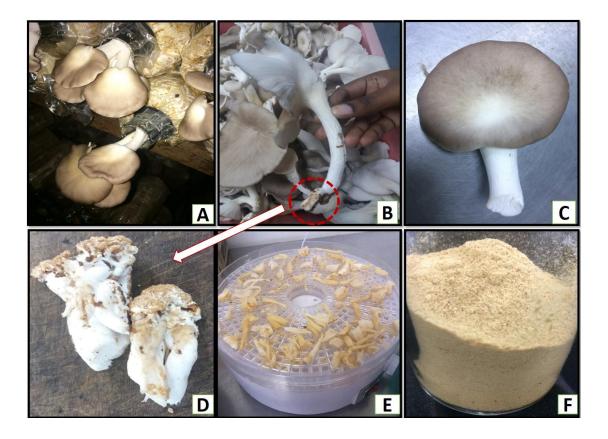


Figure 1. Flow chart of the harvesting of fruiting body base (FBB) from commercial oyster mushroom *Pleurotus sapidus*. A: First week of harvesting time, B: whole fruiting body (cap, gills, stem, and base), C: fruiting body (cap and stem), D: raw FBB, E: dried FBB, F: floured FBB (FFBB).

2.2. Proximate composition of raw and floured mushroom

Proximate analysis of *P. sapidus* was performed to determine the proportion of moisture, ash, lipids, protein, and carbohydrates [18]. The fruiting body base (RFBB and FFBB) was subjected to proximate analysis (AOAC 1984). Moisture content was determined by drying in an oven. To determine the ash content, the sample was ignited at 550 °C in a muffle furnace. Next, using the Kjeldahl method, the total nitrogen content (crude protein) of mushrooms was determined by multiplying by a factor of 6.25. Lastly, the crude fibre content was determined according to AOAC (1984) extraction method 962.09.

2.3. Hot water extraction of crude polysaccharide from FFBB

Extraction of polysaccharide was performed using FFBB (100 g) as in Figure 1F according to improvised method of Klaus et al. [19]. FFBB was washed using 96% of ethanol (v/v) for 24 h under continuous stirring at room temperature and subsequently filtered. The filtered cake of FFBB was dried in a food dehydrator for 60 min at 40 °C before being added to 2 L of distilled water and autoclaved for 45 min at 121 °C. Next, the temperature was reduced to room temperature and the sample was centrifuged for 10 min at 6000 rpm. Using a rotatory evaporator (low temperature), the supernatant concentration was reduced to 10% of the initial concentration. The concentrated supernatant was mixed with cold ethanol 99.8% (1:3) and stored overnight at 4 °C. Finally, the precipitated crude polysaccharide component from FFBB was centrifuged at 4000 rpm for 10 min and freeze-dried.

2.4. Determination of total carbohydrate analysis using phenol-sulphuric acid assay

One mL of sample solution (0.1 g/100 mL) was added to 80% phenol (0.05 mL) and concentrated sulphuric acid (5 mL). The solution was cooled to room temperature and the absorbance was measured using a spectrophotometer at 490 nm. To obtain a standard curve, a stock solution of 0.1% glucose and distilled water was prepared. The glucose was diluted in distilled water in the range of 0–100 µg glucose/2 mL and the absorbance was measured at 490 nm.

2.5. Antioxidant assay

2.5.1. Total phenolic content (TPC)

The F-C (Folin-Ciocalteu) reagent method with minor modifications was used to determine the TPC of the floured fruiting body base polysaccharide (FFBBP) extract [20]. The FFBBP extract (10 μ L) was diluted in distilled water (1 g/mL). The diluted extract was then added to 25 μ L of fresh F-C reagent in the well of a 96-well plate. The mixture was allowed to rest for 5 min, and was then added to 20% of Na₂CO₃ solution (25 μ L) and 150 μ L of distilled water. The reaction was next incubated for 30 min at room temperature in the dark. Using a microplate reader, the absorbance was read at 760 nm. The TPC of FFBBP extract was calculated by comparison with the absorbance of the standard curve [gallic acid calibration curve (0–1000 μ g/mL) and ascorbic acid (0–1000 μ g/mL)]. TPC was expressed as mg GAE/g (mg of gallic acid equivalent per gram of dry weight of the sample).

2.5.2. Ferric reducing antioxidant power (FRAP)

A method modified from Sulaiman et al. [20] and Benzie et al. [21] was used for the FRAP assay. First, the FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl, and 20 mM FeCl₃ 6H₂O at a ratio of 10:1:1 (v/v). To determine the activity of FRAP, the FFBBP extract (20 μL) was diluted in distilled water (0–250 mg/mL), mixed with FRAP reagent (180 μL) in 96-well plate, and incubated at room temperature for 30 min in the dark. Using a plate reader, the absorbance was measured at 593 nm. FRAP activity was determined by comparison with the standard [ferrous sulfate (FeSO₄) solution (0.1, 0.2, 0.4, and 0.6 mM)] and expressed as ferrous equivalent (mM Fe (II)). Ascorbic acid (0–1000 μg/mL) was used as a positive control.

2.5.3. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)

The ABTS radical scavenging activity of the extract was measured as described by Kim et al. [22], with slight modifications. Briefly, a solution of 2.45 mM $K_2S_2O_8$ and 7 mM ABTS was prepared and incubated in the dark at room temperature for 16 h prior to use. The absorbance of the resulting blue-green ABTS radical solution was adjusted to 0.7 \pm 0.2 before use. Next, 20 μ L of the FFBBP extract was mixed with 180 μ L of the diluted ABTS solution and incubated for 20 min at room temperature. Using a microplate reader, the absorbance was read at 734 nm. Ascorbic acid (0–1000 μ g/mL) was used as a positive control. The scavenging ability of the extract was expressed as an IC₅₀ value.

2.6. Preparation of steamed bun

First, 250.0 g wheat flour and 3.0 g baking powder were sieved and mixed in a bowl to prepare a control steamed bun. FFBB was then used to replace the wheat flour at different percentages (10%, 20%, and 30%). Next, 11.0 g instant yeast, 40.0 g of sugar and 60.0 mL of water was added to form a crumb paste, and 30.0 g of vegetable shortening and 100.0 mL of milk were added before the mixture was kneaded. The dough was proved for one hour at room temperature until it had doubled in size, and it was then kneaded again to release trapped air. Next, the dough was divided into smaller portions, each of which was rolled and proved again for 15 min. The buns (with red bean fillings) were placed in a steaming basket, steamed for 15 min, and cooled at room temperature. Steamed buns were prepared the night prior to sensory testing.

2.7. Sensory evaluation of steamed bun

For the sensory assessment, sample steamed buns served were sliced into four quadrants and evaluated by blinded sensory evaluators (students and staff of the Faculty Food Science and Technology, University Putra Malaysia). The sensory attributes of the steamed bun, including appearance, colour, flavour, texture, aroma, aftertaste, and overall appearance, were measured using a nine-point hedonic scale. In the hedonic scale, one point indicates 'extreme dislike' and nine points indicating 'extreme like'.

2.8. Preparation of cookies

Cookies were prepared using three different compositions with varying percentages of FFBB (0%, 10%, and 20%). The other ingredients used were butter, caster sugar, wheat flour, corn flour, custard flour, and almond. The cookies were baked at 140 $\,^{\circ}$ C for 30 min.

2.9. Sensory evaluation of cookies

Sensory analysis of cookies with 0%, 10% and 20% FFBB were conducted with 35 untrained panellists. Panellists were given a questionnaire with a five-point scale, where five represented extreme like and one represented extreme dislike. The attributes evaluated were appearance, aroma, texture, colour, taste, aftertaste, and overall acceptance. Samples were prepared the night before the analysis and stored in an airtight container.

2.10. Statistical analysis

Minitab 18 software was used for statistical analysis using analysis of variance (ANOVA) (Minitab Inc. USA), and values were expressed as mean \pm standard deviation from triplicates, unless stated otherwise, to determine the significant differences among means for all testing at a p-value of 1 at $\alpha = 0.05$.

3. Results and Discussion

3.1. Proximate composition of raw fruiting body base (RFBB) and floured fruiting body base (FFBB)

Table 1 shows the proximate composition of RFBB (raw fruiting body base) and FFBB (floured fruiting body base) of P. sapidus strain QDR. First, the moisture content was analysed. The moisture content of RFBB (84.4 \pm 3.08) was significantly higher than that of FFBB (8.93 \pm 0.15). FFBB was stored at low-humidity conditions to prevent weight loss, veil opening, and browning [23]. According to Guo et al. [10], P. sapidus has high moisture levels and should not be stored in humid conditions, as fungal infection and insect infestation may occur. Next, the ash content was analysed. Ash content represents the minerals remaining after incineration. FFBB showed a higher ash content of 5.72 \pm 0.04 compared with RFBB (0.76 \pm 0.06). Therefore, FFBB contains more minerals than RFBB. According to Mallikarjuna et al. [24], Pleurotus florida and Pleurotus djamor flour are good sources of potassium and phosphorus and are low in sodium, and are therefore suitable for consumption by hypertension patients. The protein content in FFBB (5.57 \pm 0.2) was also higher than in RFBB (1.54 \pm 0.17). When FFBB is mixed with wheat flour, it adds protein, which also adds value to the food. According to the World Health Organization (WHO), an average of 0.66 g of protein per kg of body weight should be consumed per day [25]. We next analysed the fat content of FFBB and RFBB, and found no significant difference (1.40 \pm 0.06 versus 0.85 \pm 0.23, respectively) as mushroom in general has a low fat content. In a study conducted by Chen et al. [26] on Pleurotus eryngii, polysaccharide was found to reduce the plasma serum triglyceride cholesterol level, indicating that the use of mushroom powder in food may benefit individuals with cardiovascular disease. Next, the crude fibre content was determined and FFBB (7.18 ± 1.3) was found to contain more fibre than RFBB (5.17 \pm 0.17). Fibre is known to be crucial for good bowel and digestive health. In a study conducted by James et al. [27], fibre was found to play a major role in obesity, type 2 diabetes, cancer, and cardiovascular disease. Fibre such as β -glucan is abundantly available in mushroom, and has been extensively studied for its anti-cancer properties [28], indicating its potential to have a therapeutic effect in chronic disease. The most common source of energy in the food pyramid is carbohydrate. The carbohydrate content of RFBB and FFBB was determined using a phenol-sulphuric acid assay. As shown in Table 1, FFBB has a higher carbohydrate content (71.2 \pm 1.38), 9.4-fold higher than that of RFBB (7.57 \pm 3.51).

Table 1. Proximate composition (g/100 g sample) of raw and floured fruiting body base of oyster mushroom *Pleurotus sapidus* strain QDR

Sample	Moisture	Ash	Protein	Fat	Fibre	Carbohydrate
RFBB	84.40 ± 3.18^{a}	0.76 ± 0.06^{a}	1.54 ± 0.17^{a}	0.85 ± 0.23^{a}	5.17 ± 0.17^{a}	7.57 ± 3.51^{a}
FFBB	8.93 ± 0.15^{b}	5.72 ± 0.04^{b}	5.57 ± 0.20^{b}	1.40 ± 0.06^{b}	7.18 ± 1.30^{a}	71.20 ± 1.38^{b}

^{*}Each value represents the mean of three replicates \pm standard deviation. Means denoted with different superscript letter within the same column indicate a significant difference (p < 0.05) between the two types of sample. Note: **RFBB: raw fruiting body base; FFBB: floured fruiting body base.

3.2. Antioxidant assay (TPC, FRAP, and ABTS) of FFBBP and comparison with similar studies

Table 2 shows the antioxidant properties of the FFBBP extract of P. sapidus QDR. Three antioxidant assays were conducted: total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] (ABTS) assays, thus showing a high potential for an antioxidative flour replacement in food technology. As shown in Table 2, 250 mg/mL of extract contains 1.8 ± 0.09 mg GAE/g of phenolic content. The FFBBP showed a lower TPC value compared with that reported in other studies, but was nonetheless an appreciable amount. The reducing activity was observed by FRAP assay, and for 250 mg/mL of extract, 1.74 ± 0.01 mM Fe(II)/g was reduced. The reducing power was higher than that of P. ostreatus 32783, 0.001 mM Fe(II)/g [29], but was similar to that of P. sapidus and P. sajor-caju at 1.97 mM Fe(II)/g and 1.98 mM Fe(II), respectively [30]. The ABTS assay showed the reduction of free ABTS radical into non-radical ABTS. The IC₅₀ of FFBBP extract concentration was 25.08 mg/mL, indicating superior free radical scavenging activity compared with P. ostreatus 32783 (IC₅₀ = 317.09 mg/ml). When compared with the results of a study conducted by Adebayo et al. [31] using P. levis CP-30 (IC₅₀ = 0.005 mg/mL), P. pulmonarius CP-799 (IC₅₀ = 0.0037 mg/mL) and P. tuber-regium CP-182 (IC₅₀ = 0.005 mg/mL) showed very high free radical scavenging activity, but all comparison studies have used the fruiting body (cap and stem), which is generally known to have a high polyphenol content. To the best of our knowledge, the current study is that the first to use the fruiting body base of *Pleurotus* sp. for the development of novel functional foods.

Table 2. Antioxidant properties of FFBB polysaccharide extract of *P. sapidus* strain QDR and comparison with similar studies.

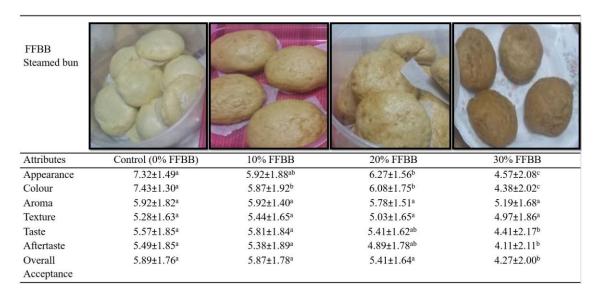
Pleurotus sp./Polysaccharide	Fruiting Body Parts	TPC	FRAP	ABTS	Reference
concentration		Gallic acid (mg GAE/g)	Ferrous sulfate (mM Fe(II)/g)	IC ₅₀ (mg/mL)	
P. sapidus strain QDR (250 mg/mL)	Base	1.80	1.74	25.08	Current work
P. ostreatus 32783 (1000 mg/mL)	Cap and stem	11.36	0.001	317.09	[32]
P. sapidus (1000 mg/mL)	Cap and stem	1.10	1.97	NA	[33]
P. sajor-caju (1000 mg/mL)	Cap and stem	1.53	1.98	NA	
P. levis CP-30 (0.02 mg/mL)	Cap and stem	0.57	NA	0.005	
P. pulmonarius CP-799 (0.02 mg/mL)	Cap and stem	0.98	NA	0.0037	[31]
P. tuber-regium CP-182 (0.02 mg/mL)	Cap and stem	0.50	NA	0.005	

^{*}NA = Not available.

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3.3. Sensory evaluation

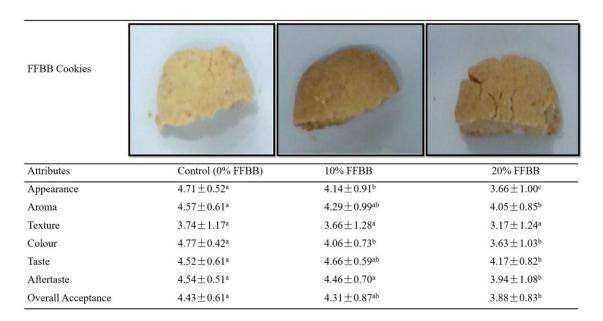
Sensory analysis is a scientific method to measure and analyse the response to a product according to sight, smell, touch, taste, and sound [34]. Figure 2 shows the sensory evaluation of steamed buns while Figure 3 shows the sensory evaluation for cookies, using a nine-point hedonic scale and a five-point scale, respectively. The attributes for sensory analysis were appearance, colour, aroma, texture, taste, aftertaste and overall acceptance. As shown in Figure 2, the steamed buns were categorised according to the percentage of FFBB introduced into the buns, which was control (0% FFBB), 10%, 20%, and 30%. The sensory analysis results indicated that all four types of buns had moderate acceptance (range 4.11–7.43). In terms of colour, the 20% FFBB score was significantly higher than that of 10% and 30% FFBB. As FFBB darkens upon steaming, the colour of buns changes, and 30% FFBB buns were likely too dark and less preferred by panellists. Aroma plays a major role in sensory analysis, and 10% FFBB buns scored the same as control buns while 20% FFBB buns were not significantly different from the control. However, 30% FFBB buns scored the lowest among the four categories as the smell of mushroom was considered too strong. Texture scoring was not significantly different among the four categories; as mushroom flour contains high levels of crude fibre. For taste, 10% FFBB buns were scored as significantly acceptable by panellists, suggesting that the 'Umami' flavour of P. sapidus may have contributed to the high score. For the aftertaste attribute, control buns scored highest, but the 10% FFBB bun score was not significantly difference than that of the control. However, 30% FFBB buns scored the lowest, possible due to the bitter taste of mushroom flour. For overall acceptance, the 10% FFBB buns had a similar acceptance to the control. The acceptability of steam buns decreased with increasing percentage of FFBB.



^{*}Each value represents the mean of 37 panellists \pm standard deviation. Means denoted with different superscript letters within the same row indicate a significant difference (p < 0.05) between the four samples. FFBB: Floured fruiting body base.

Figure 2. Sensory analysis of steamed buns prepared with different FFBB percentages with a red bean filling

As shown in Figure 3, cookies were categorised according to the percentage of FFBB content and included control (0% FFBB), 10% FFBB, and 20% FFBB samples. The appearance of cookies varied across the different percentages of FFBB. As the percentage of FFBB increased, the score decreased significantly. The sensory analysis results indicated that only the control and 10% FFBB cookies had moderate acceptance (range 3.66–4.77) in all attributes. Cookies prepared using 20% FFBB scored the least in all the attributes. As overall acceptance, the 10% FFBB cookies were not significantly different than control cookies, and 20% FFBB cookies were significantly lower than the control.



^{*} Each value represents the mean of 37 panellists \pm standard deviation. Means denoted with different superscript letters within the same row indicate a significant difference (p < 0.05) between the four samples. FFBB: Floured fruiting body base.

Figure 3. Sensory analysis of cookies prepared with different FFBB percentages

4. Conclusion

The study indicates that FFBB can be utilised as a healthy flour substitute. The results from antioxidant assays showed that FFBBP shows antioxidant activity, and may therefore represent a functional food. TPC assay showed that 250 mg/mL contained 1.8 \pm 0.09 mg GAE/g, while FRAP assay showed that 250 mg/mL can reduce 1.74 \pm 0.01 mM Fe(II)/g, and ABTS assay showed an IC₅₀ = 25.08 mg/mL. Sensory evaluation showed that steamed buns and cookies prepared with 10% FFBB were considered acceptable by the panellists. FFBB has high potential to be used as a healthy substitute for wheat flour due to its high free radical scavenging properties.

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Conflict of interest

There is no conflict of interest for this journal article.

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