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FULL PAPER

The effects of heat treatments on the bioactive compounds, antioxidant activity, and cosmeceutical properties (anti-pigmentation and anti-ageing) of fermented broken rice

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Abstract

Previously, broken rice was subjected to solid-state fermentation using locally isolated *Aspergillus oryzae* under optimised conditions. The fermented broken rice (FBR) has shown great promise as a bioingredient in the formulation of health foods and cosmeceutical products. However, the effects of heat treatment on the bioactive compound content and biological activities of FBR are still unknown. Thus, the goal of this study was to see how different heat treatments affected the bioactive compound content, antioxidant activity, and cosmeceutical properties of FBR, such as anti-pigmentation and anti-ageing. This study involved heating FBR at three different temperatures (autoclaving: 105°C, 5–15 mins; pasteurising: 80°C, 90°C, 10 mins; and oven heating: 60°C, 80°C, 24 hrs). The antioxidant activity was measured using ferric-reducing antioxidant power (FRAP), while the anti-pigmentation and anti-ageing activities were measured using tyrosinase inhibition and elastase inhibition activity, respectively. High-performance liquid chromatography (HPLC) was used to determine the composition of phenolic acids. Heat treatment at 60°C or 80°C for 24 hrs increases total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, anti-pigmentation, and anti-ageing activity in FBR, but oven heating at 80°C for 24 hrs produces the best results of all treatments. Additionally, oven heating at 80 °C for 24 hrs increases the content of gallic acid and syringic acid. However, some phenolic acids, such as p-catechuic, benzoic, and vanillic acids, were reduced at the same temperature. These findings suggested that heating conditions influence FBR's antioxidant activity, cosmeceutical properties, and bioactive compound content. FBR has the potential to be used as a promising bioingredient in the health food and cosmetic industries, given all of the enhanced effects from heat treatment.

1. Introduction

Rice milling generates a large volume of broken rice every year as a by-product. This agricultural by-product is rich in nutrients and beneficial to health due to the presence of a number of bioactive compounds. However, at the present time, the only practical uses for broken rice are in rice noodles and animal feed. It is important to find new, more valuable applications for broken rice.

In a previous study, broken rice was subjected to solid-state fermentation under optimised conditions using locally isolated *Aspergillus oryzae*. The fermented broken rice (FBR) has great potential as a bioingredient in the formulation of health food and cosmeceutical products due to its enhanced antioxidant activities, cosmeceutical properties (anti-pigmentation and anti-ageing), and increases in total phenolic and flavonoid content, phenolic acids, and organic acids (Abd Razak *et al.,* 2018; Abd Ghani *et al.,* 2021).

However, the potential applications of FBR as a functional ingredient in health food are mostly depending on industrial processing techniques. Different processing techniques are used by the food industries to produce food products. Therefore, it is essential to assess how the processing techniques influence and modify the bioactivities and valuable bioactive compounds in FBR. Heat treatment such as drying, pasteurization and sterilization is a common processing method in the food industry and has been widely used for microbial inactivation and to extend the shelf life of products. High heat treatment using a temperature greater than 100°C is

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required to destroy the harmful bacteria and spores and to ensure the product's safety (Inchuen *et al*., 2011). Cosmeceutical products also require preservation against microbial contamination to guarantee customer safety and increase shelf life. Even though preservatives are widely used by the cosmeceutical industry for this purpose, the use of some preservatives such as parabens is very harmful and has been linked to several health problems such as breast cancer (Darbre *et al.,* 2004) and problems related to reproductive systems (Martins *et al.,* 2017). As a result, heat sterilisation is regarded as more effective and safer for microbial contamination control.

Because the majority of phenolic compounds are relatively unstable when subjected to high heat, the content of phenolic compounds in food and the biological effects associated with these compounds are typically lost to a significant degree during high heat treatment. Recent studies, however, have shown that heat processing does not always destroy bioactivities and bioactive compounds. Heating can cause bioactivities and compound formation in some cases. It may result in complex physical and chemical reactions involving phenolic compounds and other bioactive compounds. Specifically, Călinoiu and Vodnar (2020) reported that thermal processing enhanced the antioxidant capacity, total phenolic content, and concentration of certain phenolic acids in wheat bran and oat bran. Doblado *et al.* (2005) found that heat treatment by autoclaving after fermentation of cowpeas (naturally or with *Lactobacillus plantarum*) produced functional cowpea flours with greater antioxidant capacity than the unprocessed legume. In addition, Thidarat *et al.* (2016) found that heat treatment by roasting significantly enhanced the antioxidant activities, total phenolic content, and total flavonoid content of Thai soybean cultivars.

The effects of heat treatment on the bioactivities and bioactive compounds of FBR must therefore be clarified. Despite the fact that FBR is a promising bioingredient for healthy food and cosmeceutical products, proper heating methods should be considered not only to control microbial contamination but also to preserve the activities and qualities of FBR's naturally occurring health-promoting constituents or even to boost its potential beneficial effects. The biological activities and bioactive compound content of fermented broken rice after heat treatment have not been previously reported, as far as we are aware. Therefore, the objective of this study was to determine the effects of various heat treatments, including oven heating, pasteurisation, and autoclaving, on the total phenolic content, total flavonoid content, antioxidant activity, cosmeceutical activities (anti-pigmentation and anti-ageing), and the content of phenolic acids in FBR.

2. Materials and methods

2.1 Preparation of inoculum and substrate

The fungus *A. oryzae* F0017 was obtained from MARDI's Collection of Functional Food Cultures (CFFC), Selangor, Malaysia while broken rice was obtained from Padiberas Nasional Berhad (Bernas, Selangor, Malaysia). The culture was grown on potato dextrose agar (PDA) and maintained at 30°C. The inoculum was prepared from 5 days old slant by suspending the fungal spores in Tween 80 (0.01%) and adjusted to a concentration of 1×10^6 spores/mL. Prior use, broken rice was washed thoroughly and dried at 40° C for 24 hrs.

2.2 Solid state fermentation

Approximately 90 g of broken rice was added to Erlenmeyer flasks and autoclaved at 121°C for 15 mins. Then, the moisture content was adjusted to 37% with sterilized distilled water. Next, 1% (v/w) of fungal spores was inoculated into the substrate, mixed properly and incubated at 30°C for 12 days. The samples were then harvested and heated at 50°C for 24 hrs in the oven before they were extracted with distilled water and filtered through Whatman No. 1 filter paper. These samples were used as control and the processing was done in triplicates.

2.3 Thermal processing of fermented broken rice

2.3.1 Oven heating

Following incubation in solid state fermentation (SSF), FBR samples were subjected to a pre-heated laboratory oven at 60°C and 80°C for 24 hrs prior to extraction with distilled water and filtered through Whatman No. 1 filter paper. The processing was done in triplicates.

2.3.2 Pasteurization

Following incubation in SSF, FBR samples were pasteurized in a laboratory pasteurizer prior to extraction with distilled water and filtered through Whatman No. 1 filter paper. The thermal process was carried out at 80°C and 90°C for 10 mins. The processing was done in triplicates.

2.3.3 Autoclaving

Following incubation in SSF, FBR samples were subjected to a laboratory autoclave and heated at a temperature of 105°C for 5, 10, and 15 mins prior to extraction with distilled water and filtered through Whatman No. 1 filter paper. The processing was done in triplicates.

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2.4 Total phenolic content

Folin-Ciocalteu reagent (5 mL) and 7.5% Na_2CO_3 (4 mL) was mixed to 1 mL aliquot of each sample. The mixture was allowed to react at room temperature for 2 hrs in the dark. The absorbance was read at 765 nm using a spectrophotometer. The calibration curve was plotted by using gallic acid as a standard. Results are expressed as mg/g gallic acid equivalent (GAE).

2.5 Total flavonoid content

The total flavonoid content of samples was determined according to a method as described by Chang *et al.* (2002), with some modifications. One mL of each sample was added to 0.3 mL of 5% $NaNO_2$ solution. After 5 mins of incubation, 0.3 mL of 10% AlCl3 solution was added to the mixture, followed by incubation for 6 mins. Then, 1 M NaOH solution (2 mL) was added. The final volume of the mixture was brought to 10 mL by using distilled water and the final mixture was allowed to react for 15 mins. Absorbance was measured at 430 nm. The total flavonoid content was calculated from a quercetin calibration curve, and the result was expressed as mg/g quercetin equivalent (QE).

2.6 Ferric reducing antioxidant power

The FRAP assay was performed as previously described (Benzie and Strain, 1999). A freshly prepared FRAP working solution (2850 µL) was mixed with an aliquot of each sample (150 µL) and was allowed to react at room temperature in the dark. Absorbance was measured at 593nm. Antioxidant activity was determined from a ferrous sulphate calibration curve. The result was expressed as mM/g ferrous equivalent (FE).

2.7 Tyrosinase inhibition activity

Tyrosinase inhibition activity was performed using the dopachrome method using l-3,4-dihydroxyphenylalanine (L- DOPA) as the substrate according to the method by Alam *et al*. (2012) with minor modifications. About 40 µL of the test sample solution were mixed with 40 µL of mushroom tyrosinase (31 U/mL) and 80 µL of 0.1 M phosphate buffer (pH 6.8) in a 96-well plate. Then, the mixture was incubated at 25°C for 5 mins. Then, 40 µL of 10 mM L-DOPA solution was added into the mixture and incubated again for 10 mins. Next, the absorbance was measured at 475 nm using the microplate reader (Versamax). Each sample was accompanied by a blank containing all components except L-DOPA. Kojic acid was used as positive control.

2.8 Elastase inhibition activity

The anti-wrinkle or anti-ageing potential of samples was evaluated by measuring their elastase inhibition activity using Elastase Assay Kit (EnzChek, USA). An aliquot of the sample (50 µL) was added to 100 µL of 0.5 U/mL porcine pancreatic elastase and incubated in the dark at room temperature for 15 min. Then, 50 µL of DQ™ elastin working solution (25 µg/mL) was added into the mixture followed by incubation in the dark for 30 mins. Absorbance at 505/515 nm (Ex/Em) was measured using a fluorescent microplate reader. N-methoxy (N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone) was used as a reference inhibitor.

Tyrosinase and elastase inhibition activities were calculated using the following equation:

$$\% \text{ inhibition} = \{[(A - B) - (C - D)] / (A - B)\} \times 100$$

Where A = absorbance of blank solution with enzyme, B = absorbance of blank solution without enzyme, C = absorbance of sample solution with enzyme, and D = absorbance of sample solution without enzyme

2.9 Phenolic acid composition

The determination of phenolic acids composition was carried out by using High-Performance Liquid System (HPLC) Alliance (Waters 2695) equipped with a photo-diode array detector (Waters 2996). The phenolic acids in the sample were separated on a reversed-phase analytical column (150 mm × 4.6 mm × Bridge C18, 3.5 µm, Waters). The separation carried out in the mobile phase consisted of 0.1% formic acid (A) and methanol (B) with the flow rate set at 0.7 mL/min. The detector was set at 270 nm and 325 nm and peak identification were performed by comparing the retention times to the standard phenolic acids. Quantification of phenolic acids was performed using the calibration curves obtained by injecting known amounts of standard compounds.

2.10 Statistical analysis

Mean values and standard deviations were calculated from the data obtained from triplicate experiments. In determining the significance of the data, a one-way analysis of variance (ANOVA) was conducted using Minitab Statistical Software (Version 18). Differences between means with a p-value of <0.05 were considered statistically significant.

3. Results and discussion

3.1 Effect of thermal processing on total phenolic content of fermented broken rice

Figure 1 displays the TPC of FBR following various heat treatments. The TPC value of samples heated in an oven at 60°C and 80°C was significantly higher than that of the control samples. Oven heating at 60°C increased

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the TPC value of FBR from 4.76 mg GAE/g sample to 7.44 mg GAE/g sample. With the increase of oven heating temperature from 60°C to 80°C, the content of total phenolic increased more significantly to 14.5 mg GAE/g sample. The increase in TPC may be attributable to the release of phenolic substances from their bound state into their free state due to the disruption of cell walls caused by oven heating. In contrast, neither pasteurisation nor autoclaving affected the TPC of FBR significantly. This result is consistent with the findings of Rahman *et al.* (2021), who observed a positive effect of oven heating on the TPC of spent grain from breweries. Similarly, Xu *et al.* (2007) demonstrated that heat treatment in an oven increases the TPC of citrus peel extract. In addition, Escudero-López *et al.* (2016) found that pasteurisation had no significant impact on the concentration of TPC in fermented orange juice.

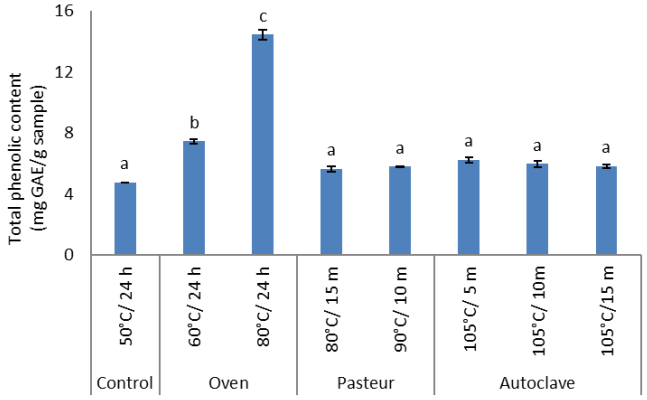

Figure 1. Total phenolic content of fermented broken rice at different thermal processing treatments. Bars with different notations are significantly different ($p < 0.05$).

3.2 Effect of thermal processing on total flavonoid content of fermented broken rice

Figure 2 depicts the effect of thermal processing on the TFC of FBR. The TFC of FBR extract obviously increased with oven heating treatment and temperature. TFC increased slightly from 0.1 mg QE/g sample to 0.2 mg QE/g sample after being heated in an oven at 60°C for 24 hours. When FBR samples were heated at 80°C for 24 hrs, the TFC concentration increased and reached its peak (1.1 mg QE/g sample). This could be due to the fact that higher temperatures promote the release of phenolic compounds from the food matrix. Higher heat treatment temperatures, as described by Rahman *et al.* (2021), were effective in facilitating the release of bound phenolic compounds from their esterified and insoluble bound forms in cell vacuoles and walls. The lower temperature, on the other hand, was unable to disrupt the cell vacuoles and thus release phenolic compounds or cleave the covalent bonds between phenolic compounds and other plant molecules. The results of this study, however, contradict previous findings by Zhang *et al.* (2010) and Yadav *et al.* (2018), who found that TFC levels in tartary buckwheat extracts and cowpea seed extracts were significantly reduced after oven heating treatment, respectively. According to Bunea *et al.* (2008), flavonoids are a diverse group of phenolic compounds, differing in both chemical structure (number and distribution of hydroxyl groups) and thermal stability. It is also important to note that pasteurisation and autoclaving had no discernible effect on TFC levels in FBR extract. Extremely high TFC levels in comparison to other treatments may be due to the extended heating exposure of oven heating (24 hrs). Ajatta *et al.* (2019) reported a similar finding, demonstrating that prolonged durations of heat treatment by hot air oven (roasting) favoured increased amounts of TFC in varieties of marble vine (*Dioclea reflexa*).

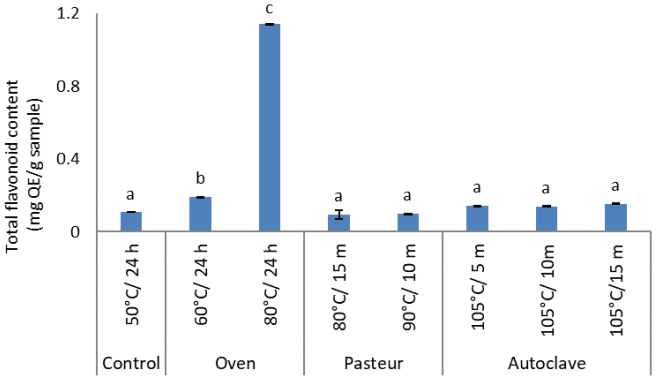

Figure 2. Total flavonoid content of fermented broken rice at different thermal processing treatments. Bars with different notations are significantly different ($p < 0.05$).

3.3 Effect of thermal processing on antioxidant capacities of fermented broken rice

The antioxidant potential of FBR was assessed using a widely used method called the FRAP assay. Figure 3 depicts the results. In comparison to the control (14 mM Fe/g sample), the antioxidant capacity of FBR extracts increased significantly up to 1.5-fold and 5-fold after being heated in an oven at 60°C (20.2 mM Fe/g sample) and 80°C (68.6 mM Fe/g sample) for 24 hrs. This finding was consistent with the findings of Lee *et al.* (2019) and Rahman *et al.* (2021), who discovered that the antioxidant activities of oven heated *Lonicera japonica* and Brewer's spent grain were higher than the corresponding untreated extracts.

The antioxidant activity of FBR samples, on the other hand, did not change significantly when heated by pasteurisation and autoclave. Our finding was contrary to a report by Doblado *et al.* (2005), which found that heat treatment in an autoclave after fermentation produced functional cowpea flours having higher antioxidant capacity than the raw legume, while Escudero-López *et al.* (2016) reported a significant decrease of antioxidant activities in the fermented orange juice after pasteurization.

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The improvement of antioxidant properties of FBR during heating by oven could result from the increase in free phenolic content related to antioxidant properties of FBR which was resulted from the breakage of linkages between bound phenolics and cell walls by thermal hydrolysis of cell walls and subcellular components (Lee *et al.,* 2019). The increasing trend of the antioxidant activity of FBR was in accordance with the content changes of total phenolic and total flavonoid. It can be speculated that phenolics were the main antioxidant compounds in the FBR.

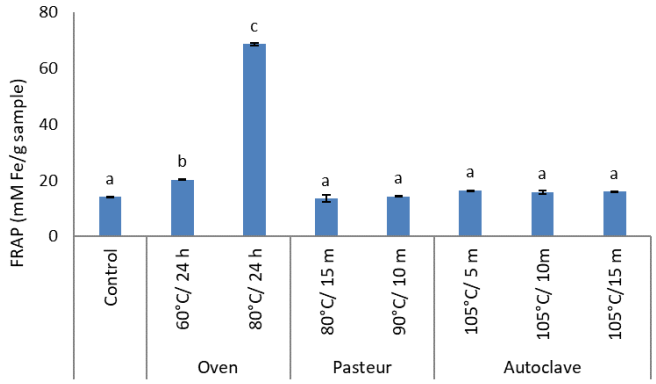

Figure 3. Ferric-reducing antioxidant potential (FRAP) of fermented broken rice at different thermal processing treatments. Bars with different notations are significantly different ($p < 0.05$).

3.4 Effect of thermal processing on anti-pigmentation and anti-ageing activity of fermented broken rice

3.4.1 Tyrosinase inhibition activity

Overexpression of tyrosinase, also known as polyphenol oxidase, causes an increase in the amount of melanin produced by the skin of humans. This increase in melanin production can cause hyperpigmentation effects such as freckles, melasma, age spots, and even melanoma (Yamaguchi *et al.,* 2017). In most cases, a greater quantity of melanin is produced as a consequence of a combination of genetic and environmental factors. Therefore, the development of nutraceutical or topical products containing bioactive ingredients with high tyrosinase inhibition activity for the purpose of whitening and depigmenting the skin is of the utmost importance. The cosmeceutical properties of FBR will be preserved and maintained if the proper method of thermal processing is selected. Figure 4 depicts the tyrosinase inhibition activity in the FBR after thermal processing at different temperatures and exposure times. In comparison to the control (71.1%), exposing FBR to oven heat at 60°C (80.1%) and 80°C (90.3%) for 24 hrs significantly increased tyrosinase inhibition activity. Pasteurisation at 80°C for 15 mins (82.4%) and 90°C for 10 mins (80%) also increased tyrosinase inhibition activity in FBR significantly.

Previous studies by Zocca *et al.* (2010) found that 15 mins of cooking *Brassica oleracea* leaves in 85°C distilled water increased the water's ability to inhibit both commercial tyrosinase and plant polyphenol oxidase. There was a high concentration of total sulphur in this water, and it also contained glucosinolates, phenols, anthocyanins, and organic acids, all of which are known tyrosinase inhibitors. Lee and Lee (2012) also reported that the tyrosinase inhibition activity of non-astringent persimmon fruit juice increased as the heating temperatures and times were increased. The juice's tyrosinase inhibition activity increased by more than 30% after three hours of heating at 120°C and 130°C.

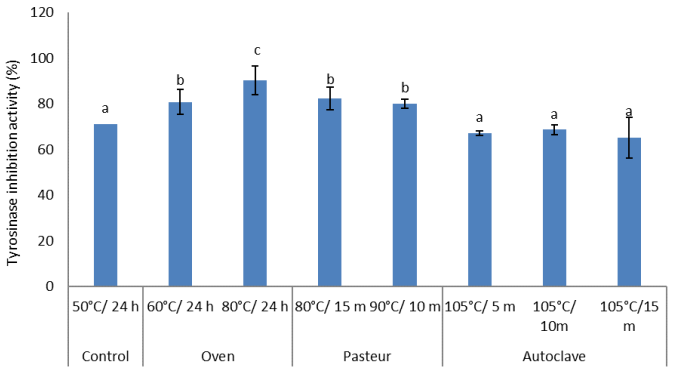

Figure 4. Tyrosinase inhibition activity of fermented broken rice at different thermal processing treatments. Bars with different notations are significantly different ($p < 0.05$).

In this study, the increasing trend of tyrosinase inhibition activity of oven heated FBR paralleled the changes in total phenolic, total flavonoid, and antioxidant activity. Increased tyrosinase inhibition activity may be largely attributable to phenolic compounds, which have been reported to be potent tyrosinase inhibitors (Briganti *et al.,* 2003).

3.4.2 Elastase inhibition activity

Skin ageing is characterized by a progressive loss of skin tissue and by alterations in the extracellular matrix macromolecules of the dermis. These include elastin, a component of the elastic fibers of the papillary dermis that is crucial for the structural integrity and biomechanics of the skin. UV exposure is one factor that may contribute to the modification of the dermal extracellular matrix and the degradation of elastin. Elastase is known to be the enzyme capable of degrading elastin; therefore, elastase inhibition activity may be a useful method for measuring the skin's protection against UV-induced ageing. Figure 5 depicts the elastase inhibition activity of FBR extract after thermal processing. Compared to the control (25.6%), oven heating at 60°C (41.1%) and 80°C (67.6%) significantly increased the elastase inhibition activity in FBR, whereas pasteurisation and autoclave at any temperature and exposure time did not affect the elastase inhibition activity.

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The research on the effects of heat processing on elastase inhibition activity is limited, despite the abundance of studies on the topic. Nevertheless, it is commonly asserted that compounds with antioxidant activity exhibit anti-ageing, anti-inflammatory, and anti-pigmentation/whitening properties (Choi *et al.,* 2008). Antioxidant activity may be one of the mechanisms for elastase inhibition. As previously reported in the literature, tomato extract exhibited a linear correlation between antioxidant and anti-elastase activity (Henderson *et al.,* 2010). Thus, in this study, the increase in elastase inhibition activity of FBR can be related to the increase in antioxidant activity.

3.5 Effect of thermal processing on phenolic acids composition of fermented broken rice

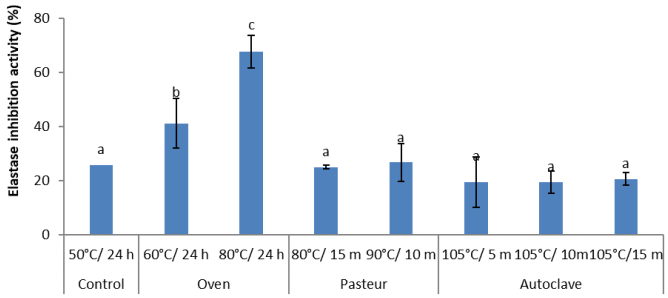

Figure 5. Elastase inhibition activity of fermented broken rice at different thermal processing treatments. Bars with different notations are significantly different ($p < 0.05$).

HPLC was used to identify and quantify the effects of thermal processing, heating temperature, and exposure time on the compositional changes of phenolic acids (gallic, p-catechuic, OH-benzoic, vanillic, and syringic) in FBR. Figure 6 shows the changes in the phenolic acid composition of heated FBR. The most common phenolic acid discovered was OH-benzoic acid. In comparison to the control (8.4 µg/g), oven heating at 60°C (5.5 µg/g) and 80°C (2.2 µg/g) significantly reduced p-catechuic acid in FBR extract. Furthermore, autoclave treatment at 105°C for 5, 10, and 15 mins reduced the p-catechuic acid content to 3.9 µg/g, 6.5 µg/g, and 5.3 µg/g, respectively. Similarly, OH-benzoic acid decreased with heating treatment in an oven at 80°C (4.7 µg/g) and an autoclave at 105°C for 5, 10, and 15 mins (2.8 µg/g, 3.8 µg/g, and 1.8 µg/g, respectively). Vanillic acid was significantly reduced when heated in an oven at 80°C for 24 hrs (5 µg/g) and autoclave at 105°C for 5 mins (5 µg/g) compared to the control (7.1 µg/g). Pasteurisation at 80°C and 90°C, as well as autoclaving at 105°C, destroyed the gallic acid in the samples. This suggests that some phenolic acids were most likely destroyed by a specific type of heat treatment at high temperatures. Bryngelsson *et al.* (2002) previously reported that autoclaving at 100-120°C increased p-coumaric acid, ferulic acid, and vanillin in oat grains while decreasing caffeic acid to undetectable levels.

On the other hand, gallic acid levels increased significantly when FBR was heated in an oven at 60°C (2.4 µg/g) and 80°C (2.6 µg/g) compared to the control (1.6 µg/g). Similarly, syringic acid was significantly elevated when FBR was heated in an oven at 80°C (1.5 µg/g) compared to the control (0.9 g/g). Our findings are consistent with those of Xu and Chang (2008), who observed an increase in the concentration of gallic acid in heated yellow and black soybeans. The high increase in antioxidant activity, tyrosinase inhibition activity, and total phenolic content of FBR can be attributed to the increased concentration of these compounds. Kim (2007) found that gallic acid inhibited tyrosinase, decreased melanin synthesis, and suppressed oxidative stress simultaneously. Various studies with diverse processing conditions yielded varying results. Sapna *et al.* (2018) found that toasting black rice flour at 90-100°C for 30 mins increased vanillic by 101%, decreased ferulic acid by 73%, but had no effect on caffeic acid content. According to the same study, toasting red rice flour decreased caffeic acid by 64% but had no effect on ferulic acid levels. Due to their varying distribution in the grain and susceptibility to oxidation as a result of their distinct chemical structures, processing affects the selected phenolic acids differently. Whether they are

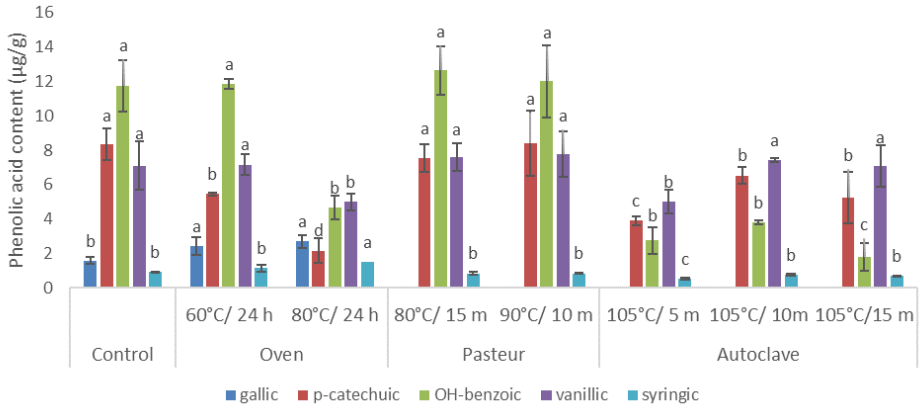

Figure 6. Phenolic acids content of fermented broken rice at different thermal processing treatments. Bars with different notations are significantly different ($p < 0.05$).

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presently bound to carbohydrates or other components or not is also significant (Bryngelsson *et al.,* 2002).

In general, oven heating at 80°C for 24 hrs produces the least amount of phenolic acid. This may suggest that the increase in antioxidant, tyrosinase, and elastase inhibition activity caused by this treatment is not due to phenolic acids alone, but rather to a synergistic effect among phenolic compounds. FBR's antioxidative and cosmeceutical properties may be enhanced by phenolic compounds and other components besides phenolic acids. According to Wang *et al.* (2014), grain nature, phenolic compound type, and location, as well as heat treatment duration and intensity, can influence phenolic content.

4. Conclusion

Proper heating may preserve or even improve FBR's potential medicinal and cosmeceutical properties. Heat treatment by oven heating at 60°C and 80°C for 24 hrs increases the total phenolic content, total flavonoid content, antioxidant activity, anti-pigmentation activity, and anti-ageing activity of FBR, with oven heating at 80° C producing the best results. Additionally, 24 hrs of oven heating at 80°C increases the concentration of gallic acid and syringic acid. However, at the same temperature, certain phenolic acids, including p-catechuic, benzoic, and vanillic acids, decreased. This study demonstrated the potential use of FBR as a bioingredient in the health, cosmetics, and functional food industries.

Conflict of interest

The authors declare no conflict of interest.

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