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Ethanollic extract of rice bran: a thermally stable preservative for edible oils and cake

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Abstract

The purpose of this study was to evaluate the thermal stability of the rice bran extract (RBE) and analyze the effect of RBE on the shelf-life of sunflower oil and the quality characteristics and shelf-life of baked cake. The thermal stability of RBE was evaluated by a Rancimat test using sunflower oil. Properties such as moisture content, porosity, crumb density and pore area of cakes baked with RBE and the synthetic antioxidant butylated hydroxytoluene (BHT) were compared. Sensory properties such as taste, aroma, texture, color and overall acceptability of the cake samples were compared using a sensory panel test. The shelf-life of the cakes was evaluated by microbial counts and chemical methods. Thermally treated RBE and BHT for 2 h at 180 °C retained 75% of their initial capacity in protecting sunflower oil while RBE had a significantly higher protection factor ($p < 0.05$). Cakes baked with RBE received higher scores for taste, color and overall acceptability compared to control or BHT-added cake. BHT-added cake and RBE-added cake exceeded the aerobic plate count (APC) and yeast and mold count (YMC) on days 11 and 13 respectively, while the control cakes without added antioxidants exceeded the APC and YMC on day 7. Both BHT- and RBE-added cakes maintained hexanal levels below 5 mg/kg over 28 days while the control cake exceeded this level on day 21. The results suggest that RBE can be used as a natural food additive to improve the quality and shelf-life of baked foods and edible oils.

Keywords: Phenolic antioxidants, Food preservatives, Sensory properties, Shelf-life, Rice bran, Baked cake, Sunflower oil

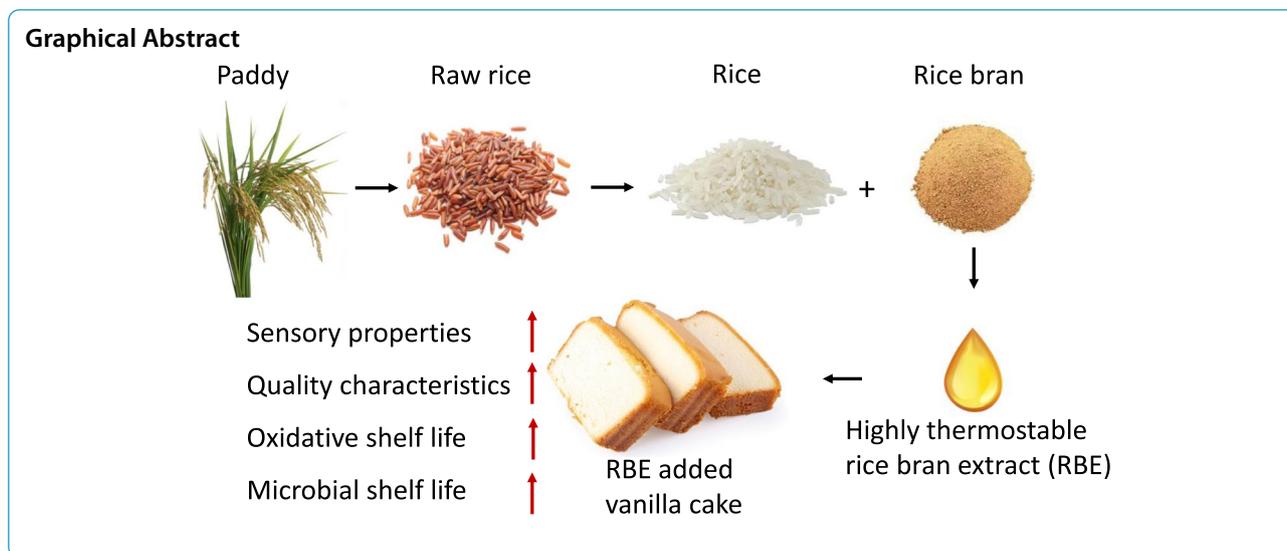
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Background

Unsaturated fatty acids found in common vegetable oils such as sunflower oil, corn oil and soybean oil form oxidation products upon exposure to heat, light, oxygen and moisture. These oxidation products affect the sensory quality of edible oil making the shelf-life of the oils shorter. The shelf-life of baked cake is also shortened by lipid oxidation as well as by microbial activity. Synthetic antioxidants such as butylated hydroxyanisole (BHA), BHT, propyl gallate and tert-butyl hydroquinone (TBHQ) are commonly used to extend the shelf-life of food products. However, there are health concerns associated with the use of synthetic antioxidants due to the cytotoxicity in cell and animal models (Eskandani et al. 2014; Mizobuchi et al. 2021). Essential oils from spices and herbs such as oregano, thyme, dittany, marjoram, lavender and rosemary have been demonstrated to be excellent sources of natural antioxidants. However, these natural antioxidants have limited applications in food industries due to their strong flavor characteristics (Lourenço et al. 2019). Vitamin A, vitamin E and vitamin C found in plant substances also protect food against lipid oxidation and enhance the nutritional quality of food. However, the activity of these vitamins in solid foods is considerably lost during high-temperature processing and storage due to limited thermal stability (Wyatt et al. 1998; Uckiah et al. 2006). The use of ascorbic acid in preserving bread is also not successful due to the quick loss of ascorbic acid during baking and storage (Nanditha & Prabhasankar 2009). Vitamin A and vitamin E can protect edible oils from oxidation. However, the role of vitamin A and vitamin E in extending the shelf-life is limited due to the sensitivity of these antioxidants to light, temperature, storage

conditions and the presence of impurities in edible oils (Mishra et al., 2021). The phenolic compounds present in edible oils, oil meal and seed hulls on the other hand display high thermal stabilities (Seneviratne & Kotuwegedara 2009; Seneviratne et al. 2009; Seneviratne et al. 2016; Senanayake et al., 2019). Similarly, the ability of ethanolic extract of *Psidium guineense* Sw. leaves constituting phenolic substances to protect sunflower oil against oxidation was not significantly affected by heat treatment compared to BHT (Senanayake et al. 2018).

The seed of the grass species *Oryza sativa* (Asian rice) is a staple food in more than 100 countries. Rice is recorded as the second highest consumed cereal grain worldwide after maize. Once the hull is removed, rice contains three edible components: the pericarp, germ and endosperm. Rice without the hull in its intact form is known as whole grain rice such as brown, purple, red and black rice. Milling and polishing the rice removes the pigmented pericarp and produces white rice and rice bran as a byproduct. The fibrous bran layer of rice has been reported to be rich in phenolic compounds that have antioxidant properties (Arab et al. 2011; Jun et al. 2012). Numerous studies have reported antioxidant and antimicrobial activities of rice bran extracts suggesting possible applications of these compounds as natural food preservatives to extend the oxidative and microbial shelf-life of food products (Martillanes et al. 2020).

Recently reported findings show that ethanol-water extract from rice bran decreased the oxidation and mold and yeast proliferation in preserving a mayonnaise-type emulsion (Martillanes et al. 2020). Further, hexane soluble fraction of methanolic extracts from purple rice bran limited the microbial growth in raw

channel catfish (*Ictalurus punctatus*) belly flap meat. However, the study implied that the color change of the food products may limit the applications (Min et al. 2009). A process for improving the water retention in meat and seafood products by treating a meat or seafood product with a composition comprising a rice bran extract having 3 to 9% (w/w) total phosphorous content has also been reported (Soma & VanAlstyne 2020). The use of rice bran in foods that are processed at high temperatures is less frequent. Rice bran has been touted as a natural antioxidant component in breadmaking (Irakli & Katsantonis 2017). However, the incorporation of more than 10% of rice bran as it is had a negative effect on the sensory properties of bread in terms of color, crumb texture and texture during storage (Lima et al. 2002).

Even though rice bran has been added to baked food in order to cause favorable changes, phenolic extracts of rice bran have not been used in baked food. Alcoholic extracts of rice bran are rich in phenolic compounds (Arab et al. 2011). The potential of the ethanolic extracts of rice bran for improving the quality characteristics and extending the shelf-life of edible oils and baked food and the thermal stability of the extracts during thermal processing such as cooking or baking has not been tested. Due to the high thermal stability of natural phenolic substances, we hypothesized that ethanolic extracts of rice bran may be effective in extending the shelf-life of baked foods without negatively affecting cake properties or sensory properties.

Materials and methods

Sample collection, preparation and evaluation of antioxidant activity

Phenolic compounds of rice bran were extracted without defatting rice bran. Rice bran of BG-409 White Nadu variety rice (*Oryza sativa* L.) collected within 12 h of milling was air-dried at 40 °C in a hot air oven (YC0-010(200L), Gemmy, Taiwan) until a constant weight was obtained. The dried rice bran was ground using a kitchen grinder, sieved through a 180 µm mesh and kept at -20 °C until further analysis. Phenolic compounds from rice bran were extracted at 27 °C using ethanol:water (70:30 v/v) solvent system within 24 hrs. The solvent system (1.0 mL) was mixed with the prepared rice bran powder (0.2 g). Phenolic antioxidants were extracted using a vortex at 40 Hz for 2 min (twice). The resultant extract was centrifuged at 400 g for 10 min using a centrifuge (75,007,214 Heraeus Megafuge 8R, Thermofisher Scientific, Germany). Four successive extractions were performed for each sample. The

resultant ethanolic extracts of rice bran (RBE) were collected, diluted to 5.0 mL with ethanol:water (70:30 v/v) solvent system and stored in dark brown vials for further analysis.

Soluble phenolic content and antioxidant activity assay in chemical systems

RBE (100 µL) was mixed with diluted Folin-Ciocalteu reagent (2.5 mL). After 5 min, 7.5% Na₂CO₃ solution (2.0 mL) was added and the mixture was diluted to 5 mL with distilled water. After 1 h, the absorbance of the reaction mixture was measured at 765 nm using a UV-visible spectrophotometer with respect to a blank with ethanol:water (70:30 v/v) solvent system without phenolic extract. The soluble phenolic content was expressed as gallic acid equivalents (GAE).

DPPH assay, ferric reducing antioxidant power assay and deoxyribose degradation assay were conducted as reported (Seneviratne & Kotuwagedara 2009).

Potential of RBE to improve the shelf- life of sunflower oil and the thermal stability of RBE based on Rancimat test

The thermal stability of RBE was assessed based on the potential of heat-treated RBE to protect sunflower oil under accelerated oxidation in a Rancimat apparatus. Naturally present phenolic substances and free fatty acids were removed from sunflower oil by passing through a column of alumina as reported (Senanayake et al. 2018). Volumes of RBE or BHT stock solutions (1000 µg /mL) needed to achieve a final concentration of 100 µg/g of sunflower oil were transferred into the reaction tubes of the Rancimat apparatus (892 Professional Rancimat, Metrohm Switzerland). Then, the solvent was removed under a stream of nitrogen. Phenol-stripped sunflower oil (3.2 g) was added to each reaction tube and vortexed (40 Hz, 2 min). A control was prepared by adding striped sunflower oil (3.2 g) in the absence of the RBE or BHT.

The induction time (IT) for the appearance of oxidized products of sunflower oil was measured at 120 °C and at an airflow rate of 20 L/h by Rancimat apparatus using software StabNet (1.0 version). To evaluate the effectiveness of thermally treated antioxidants in protecting sunflower oil, the solvent of the RBE or BHT solutions in another set of Rancimat tubes was removed under a stream of nitrogen and the remaining contents of the tubes were heated at 180 °C for 2 h. Then, the Rancimat experiment was conducted as above with sunflower oil (3.2 g). IT was evaluated by Rancimat curves and protection factor (PF) was calculated as, $PF = \frac{IT_{\text{antioxidant added oil}}}{IT_{\text{control}}}$. Retained stability was calculated as, $\text{Retained stability} = \frac{PF_{(180\text{ }^\circ\text{C})}}{PF_{(RT)}} \times 100$.

Effect of RBE on the quality characteristics, sensory properties and shelf-life of baked cake

Preparation of vanilla cake

Vanilla cake with a formula containing whole purpose wheat flour (100 g), sugar (85 g), margarine (Astra, palm oil-based, 100 g), baking powder (2 g), beaten eggs (25 g), milk (20 mL), and vanilla (2 g) was prepared with RBE or BHT at 20 mg/100 g of margarine and baked at 160 °C for 30 min in a preheated electric oven (TR02000/TR2000R/TR050/TR055/ TR060, Black & Decker, USA) as reported (Senanayake et al. 2018). Control cake was prepared without adding any antioxidant extract.

Cake properties

The baked cake samples were cooled to room temperature (RT). Moisture content, porosity, crumb density and pore area of cakes were determined as reported (Senanayake et al. 2019). Sensory evaluation of cake samples was performed as reported using 30 trained panelists (Seneviratne et al. 2011). Cake samples (about 3 × 3 × 1.5 cm) were served to each panelist in a randomized order within 10 h of baking. Panelists were asked to wash their mouths with water to remove any traces of residual food. Each panelist rated the quality attributes of cakes according to appearance, color, odor, taste, texture (hardness and softness) and overall acceptability based on a 5-point hedonic scale (1: dislike very much to 5: like very much).

Microbial shelf life of cake

Aerobic plate count (APC) and yeast and mold count (YMC) of cakes were determined according to the standard protocols (Wang et al. 2016). Cake samples were stored at RT in stomacher bags under sterile conditions. Each cake sample (1.0 g) was homogenized in 9.0 mL of 0.1%-buffered peptone water (CM1049). Homogenized serial dilutions (1 mL) were transferred to Plate Count Agar (CM0325) plates in triplicate by pour plate technique and incubated at 37 °C for 48 hours according to ISO 7218 (International Organization for Standards 2014) to determine APC. 1.0 mL of each dilution was transferred to sterile Potato Dextrose Agar (M096) plates in triplicate by pour plate technique and incubated at 25 °C for 4 days according to ISO 7218 (International Organization for Standards 2014) to determine the YMC.

Measurement of oxidation products of cake

Each cake sample (50 g) was ground well, and chloroform-methanol (2:1 v/v, 150 mL) was added and shaken in an orbital shaker for 20 min. The lipid extract was separated by decantation, and the extraction was continued with two fresh portions of the solvent system. All the

extracts were combined, dried over anhydrous sodium sulfate, filtered and the solvents were evaporated in a rotary evaporator. The traces of the solvents in the lipid were removed by flushing with a stream of nitrogen and stored at −20 °C.

The oxidative stability of the cake samples was determined based on the peroxide value (PV) of oil extracted from the cake samples according to a previously reported method (Shantha & Decker 1994). The absorbance of the reaction mixture was recorded at 500 nm using a UV-visible spectrophotometer. A control was prepared without adding the sample. A standard curve was constructed using iron (III) chloride. PV was expressed as milliequivalents (meq) of peroxides per kg of oil. PV was determined according to the following formula.

$$PV = [(A_s - A_c) \times m] / [55.84 \times m_0 \times 2]$$

(A_s = Absorbance of the sample, A_c = Absorbance of the control, m = Slope obtained from the calibration curve, m_0 = mass in grams of the sample)

Hexanal content in cakes was measured as previously reported (Senanayake et al. 2018) by headspace solid-phase microextraction (SPME) gas chromatography (GC). Each baked cake sample (4.0 g) cooled to RT was placed in a headspace vial and sealed with PTFE/silicone septum within 1 h and stored at RT. The first batch of stored cake samples was processed on the same day of baking by heating the contents in the vials at 55 °C while shaking for 13 min in an autosampler heating block of the gas chromatograph. A solid-phase microextraction fiber needle (100 μm PDMS, Supelco, St. Louis, MO) was injected into the vial and the needle was left in the vial for 3 min to absorb volatiles and the needle was then transferred to the injector port (250 °C, split ratio 1:5) of a gas chromatograph 2010 Plus equipped with an AOC-5000 Plus autosampler (Shimadzu, Japan) and a capillary column RtxR- WAX (cross-bond with PEG, 30 m × 0.32 mm i.d. 0.25 μm) for 1 min. The column temperature was 65 °C and the running time was 10 min. The carrier gas (helium) was passed through the column at a flow rate of 2.0 mL min⁻¹ and hexanal was detected by a flame ionization detector set at 250 °C. Hexanal contents in the samples were quantified using a standard curve prepared with authentic hexanal. The analysis was repeated at different time intervals for stored samples.

Identification and quantification of phenolic compounds

The ethanolic extract (5 mL) was concentrated under vacuum at 45 °C until it reached a viscous oily consistency. This viscous liquid was dissolved in acetonitrile (1.0 mL) and the mixture was washed three times with hexane (2.0 mL). Then, the acetonitrile layer was separated and the solvent was evaporated. The resultant

residue was dissolved in methanol (50 μ L) and filtered through a 0.45 μ m filter unit. HPLC experiments were performed using an Agilent 1100-Infinity liquid chromatographic system (Agilent Technologies, Waldbronn, Germany) equipped with an Agilent 1200 diode array detector following the exact conditions and procedure reported (Weerakoon et al. 2021). The injection volume was 20 μ L. Phenolic compounds were quantified as described by Seneviratne and Dissanayake (2008) using authentic standards.

Statistical analysis

All analyses were run in triplicate unless otherwise indicated. Two sample t-test and ANOVA were carried out for the determination of significant differences ($p < 0.05$) between the means. Data were analyzed using Minitab (Version 17 for Windows) and presented as the mean \pm SD).

Results and discussion

Soluble phenolic content and antioxidant activity

The soluble phenolic content of RBE as determined by Folin Ciocalteu assay is 4141 ± 463 mg GAE/kg (dry weight). The yield of soluble phenolic compounds in rice bran varies among the different varieties of rice as well as the solvent system used for extraction. Non-defatted red rice bran (10g) extracted with 60–80% ethanol extract (400 mL) yields 11,510 mg GAE/kg (Jun et al. 2012). Non-defatted rice bran from Iranian rice yield 3310 mg GAE/kg (Arab et al. 2011). The amount of total phenol content in RBE reported in the present study is within the reported limits and the use of ethanol:water for extraction ensures consumer acceptance as a food-grade preservative. Further, the use of ethanol: water solvent system facilitates the use of rice bran as animal feed after extracting the soluble fraction of the phenolic compounds.

As the ethanol-water system has been successfully used to extract phenolic compounds from plant extracts, RBE can be considered as a phenolic extract of rice bran. The DPPH radical scavenging activity, reducing power and the ability to inhibit deoxyribose degradation of RBE is significantly ($p < 0.05$) higher or comparable to that of synthetic antioxidants at 30 μ g mL⁻¹ concentration (Additional file 1). Many plant extracts also display DPPH radical scavenging activity, ferric reducing power and trolox equivalent antioxidant capacity. However, reports indicate that in vitro antioxidant activity may not always strongly correlate with the antioxidant activities in food systems due to several limitations of in vitro assays (Sadeer et al. 2020). The antioxidant activity of synthetic

or natural antioxidants may also change due to the processing conditions of foods. Plant extracts may also affect sensory properties negatively (Dzikia et al. 2014). Therefore, the suitability of RBE as a food additive or preservative was tested in different food systems.

Thermal stability of RBE by Rancimat test

Limited studies have reported the potential uses of extracts from rice bran as a food preservative (Martilanes et al. 2020; Min et al. 2009). However, the thermal stability of rice bran extracts during food processing which is critical for the application of rice bran extracts as a food preservative has not been reported. The shelf-life of polyunsaturated oils can be improved by the addition of phenolic extracts from natural sources (Taghvaei et al. 2014). Therefore, the effectiveness of thermally treated BHT and RBE as food preservatives was evaluated by monitoring the formation of oxidation products in polyunsaturated oils under accelerated oxidation conditions using the Rancimat apparatus. Rancimat curves for the formation of oxidation products in sunflower oil are given in Fig. 1. The PF values based on the IT determined from the Rancimat curves are given in Table 1. Naturally present phenolic compounds in sunflower oil also extend the shelf-life of sunflower oil (Mishra et al. 2021). Therefore, such natural antioxidants present in sunflower oil were removed from sunflower oil prior to the enrichment of sunflower oil with RBE or BHT. The comparison of curve 3 with curve 5 and the relevant PF values indicates that RBE is more effective in extending the shelf-life of sunflower oil than BHT. The comparison of curve 2 with curve 4 and the corresponding PF indicates that heated RBE is more effective in extending the shelf-life of sunflower oil than heated BHT (Fig. 1, Table 1). The thermal stability of the synthetic antioxidants used has been reported to be in the order of BHT > BHA > TBHQ where TBHQ lost more than 60% of the antioxidant activity within 1 h at 185 °C (Hamama & Nawar 1991). Similarly, natural antioxidants present in rosemary such as rosmarinic acid, carnosol and carnosic acid have been shown to destroy during thermal processing of food products (Zhang et al. 2012). Both RBE and BHT retained about 75% activity (calculated based on PF) after heat treatment at 180 °C for 2 h (Table 1). However, higher PF of heated RBE compared to heated BHT suggests that RBE may serve as a better preservative of thermally processed foods than common synthetic or rosemary-based antioxidants.

Quality of vanilla cakes baked with the antioxidants

The physical characteristics and the sensory qualities of vanilla cake baked with and without antioxidants were

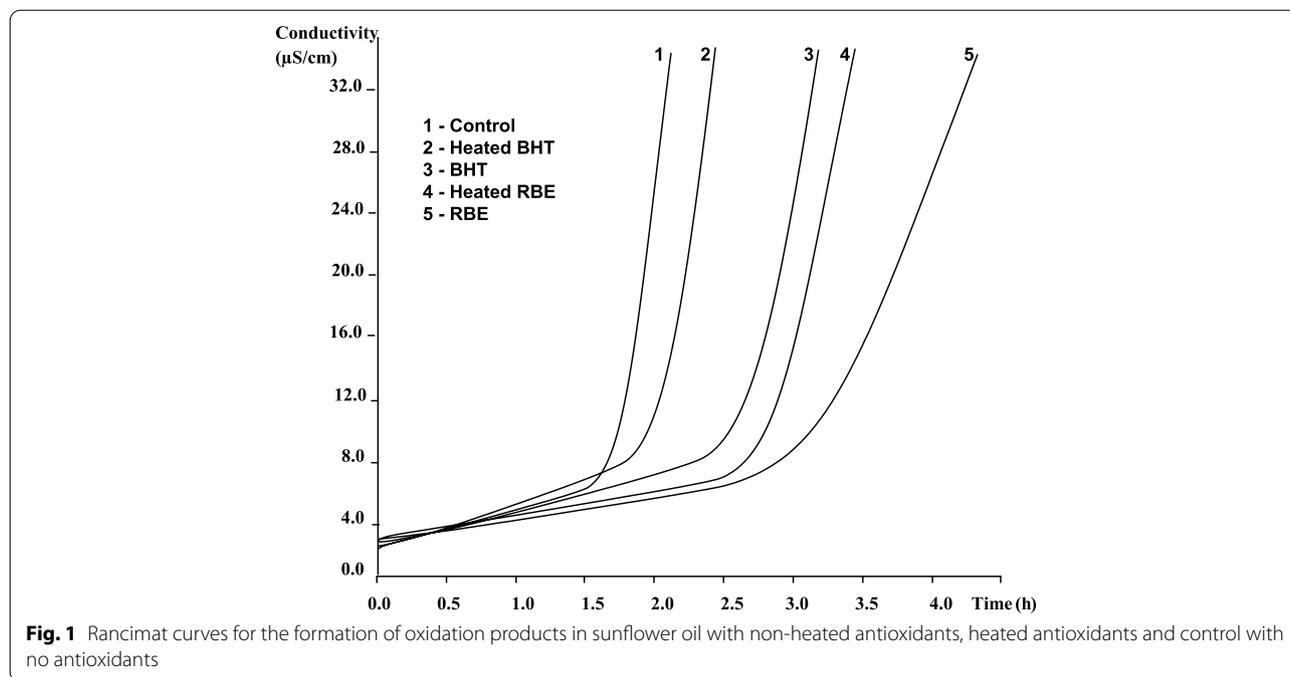


Table 1 IT and PF of BHT- and RBE-added sunflower oil by Rancimat

	IT (h)		PF		Retained stability (%)
	RT	180°C (2 h)	RT	180°C (2 h)	
RBE	3.15 ± 0.12 ^{ax}	2.62 ± 0.04 ^{bx}	2.37 ± 0.12 ^{ax}	1.87 ± 0.04 ^{bx}	79 ± 3 ^x
BHT	2.51 ± 0.05 ^{ay}	1.84 ± 0.06 ^{ay}	1.69 ± 0.05 ^{ay}	1.24 ± 0.06 ^{by}	75 ± 3 ^x

IT of the control = 1.48 ± 0.03 h. Data presented as mean ± SD (n = 3). The letters a and b indicate a significant difference in a row between the induction times (IT) or protection factors (PF). Letters x and y indicate significant difference in a column (p ≤ 0.05)

evaluated to assess the potential of RBE as a food additive in baked foods processed at high temperatures. There is no statistically significant difference between the percentage moisture content, porosity and pore area between the cakes baked with and without antioxidants (Table 2). The crumb density of the cakes is significantly (P < 0.05) reduced due to the addition of the antioxidants. Reported data indicate that cakes prepared with added clove essential oil at 400 ppm showed comparable levels of peroxides and reduced microbial counts during storage as cakes with BHT added at 200 ppm. However, when high concentrations are used to match the effectiveness of synthetic antioxidants, clove oil affects the sensory properties of cake (Ibrahim et al. 2013). High percentages (10–30%) of green tea powder in sponge cake did not affect the sensory properties but negatively affected the crust color and cohesiveness (Lu et al. 2010). These negative effects were probably due to the presence of

volatile aroma compounds present in clove oil and the tannins present in green tea powder. The present study indicates that the RBE-added cakes received significantly higher (P < 0.05) scores in the panel tests for appearance, taste, color and overall acceptability compared to the BHT-added cakes and control cakes without any added antioxidants. In addition, the aroma of RBE-added cake is comparable to that of BHT-added cake and the control cake indicating that RBE does not unfavorably affect the aroma of baked vanilla cakes. Therefore, RBE incorporates positive sensory qualities to baked cake.

Microbial shelf life and chemical shelf life of cakes

Cakes or other baked foods expire when the microbial counts exceed permissible limits and due to the formation of products, such as free fatty acids, lipid peroxides and volatile lipid degradation products above the tolerable limits. The microbial shelf-life of baked vanilla cakes

Table 2 Cake properties and sensory evaluation

	Control cake	RBE added cake	BHT added cake
Cake properties (n = 3)			
Moisture (%)	21.8 ± 1.1 ^a	20.1 ± 0.5 ^a	20.8 ± 1.6 ^a
Porosity (%)	44.6 ± 2.4 ^a	41.4 ± 0.4 ^a	39.7 ± 4.0 ^a
Crumb density (g/cm ³)	0.50 ± 0.02 ^a	0.45 ± 0.00 ^b	0.42 ± 0.00 ^c
Pore area (cm ²)	0.006 ± 0.002 ^a	0.007 ± 0.002 ^a	0.006 ± 0.002 ^a
Sensory properties by panel test (n = 30)			
Taste	2.75 ± 1.16 ^b	4.05 ± 0.83 ^a	2.55 ± 1.23 ^b
Aroma	3.10 ± 0.91 ^a	3.50 ± 1.00 ^a	3.05 ± 0.89 ^a
Texture	3.70 ± 0.73 ^a	3.75 ± 0.79 ^a	2.60 ± 1.14 ^b
Color	3.75 ± 0.79 ^b	4.15 ± 0.59 ^a	3.05 ± 0.89 ^b
Overall acceptability	3.45 ± 0.51 ^b	4.10 ± 0.45 ^a	3.35 ± 0.51 ^b

Data represent mean ± SD. Letters a, b and c indicate significant difference in a row ($p \leq 0.05$)

was evaluated based on the APC and YMC. Time taken to exceed APC (1×10^5 CFU/g) and YMC (1×10^3 CFU/g) was considered as the expiration of shelf-life of cake as reported (Fig. 2) (Senanayake et al. 2019). Vanilla cake baked without any antioxidant exceeded the maximum allowable APC and YMC within 7 days of storage at RT in sterile Stomacher bags. The cakes baked with BHT exceeded those levels on day 11. Cakes baked with RBE on the other hand took 13 days to exceed the maximum allowable APC and YMC levels suggesting that RBE extends the microbial shelf-life of vanilla cakes better than BHT.

Peroxide value acts as a preliminary indicator of chemical shelf-life although peroxide value itself does not affect the sensory quality as much as hexanal levels do. Hexanal is a better indicator of sensory perception

of bakery products (Purcaro et al. 2008). Most of the sensory properties are directly related to the off-flavors in baked products due to the generation of secondary oxidation products such as hexanal. Hexanal contents above 5 mg/kg create easily observable rancid odors (Fritsch & Gale 1977). The effect of RBE on the formation of peroxides and hexanal during storage of the cake samples was measured as indicators of oxidative deterioration of cake (Fig. 3a and b). The addition of both BHT and RBE into cake samples resulted in a significant ($p \leq 0.05$) decrease in peroxide value and hexanal formation compared to the control cake samples during the storage period. Both RBE and BHT maintain hexanal levels below 5 mg/kg over a period of 28 days. Rancid odors from food deterioration can be observed at hexanal concentrations above 5 ppm. Figure 3b shows

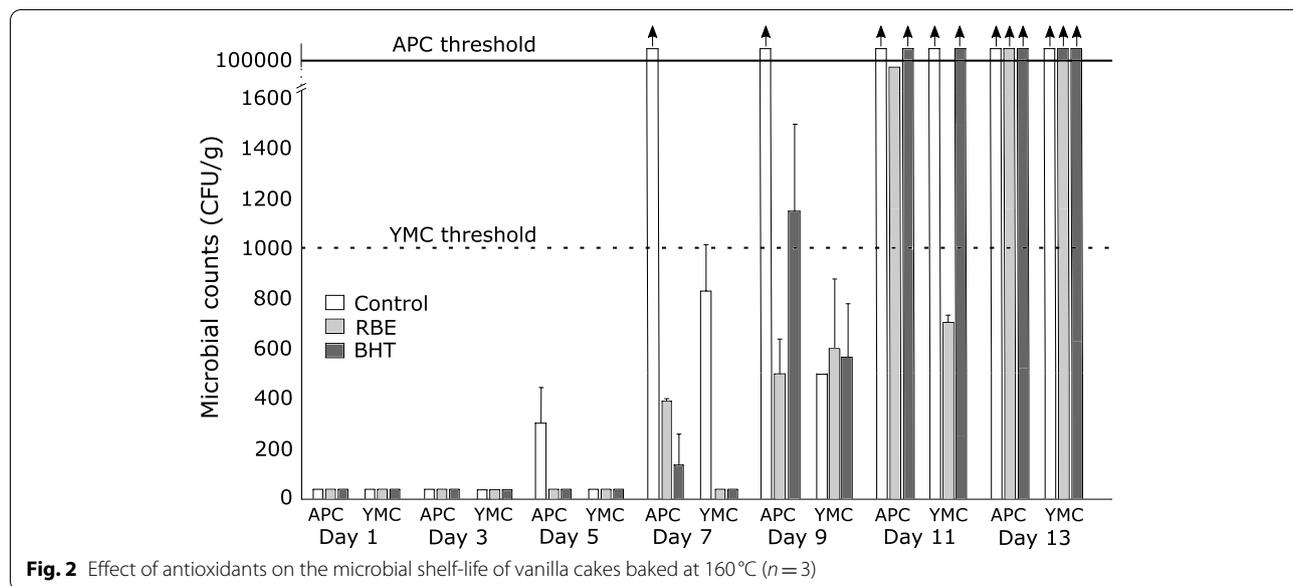


Fig. 2 Effect of antioxidants on the microbial shelf-life of vanilla cakes baked at 160°C (n = 3)

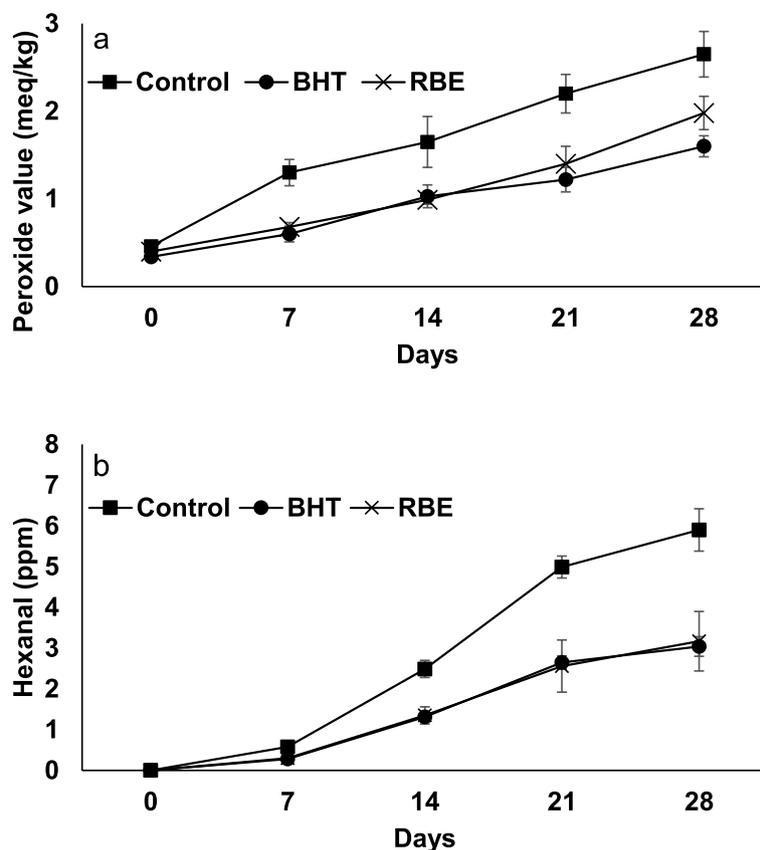


Fig. 3 Effect of RBE and BHT on the formation of peroxides and hexanal in cake samples during storage at room temperature; **a** peroxide formation of cakes and **b** hexanal formation in cakes over time. Each data point represents mean \pm SD ($n = 3$)

that both RBE and BHT maintain the hexanal levels below 5 mg/kg even at day 28 while the control cake sample exceeds this hexanal limit around 21 days. Even though the antioxidant capacity of BHT is significantly lower than that of RBE, both BHT and RBE maintained comparable peroxide and hexanal levels in cakes (Additional file 1, Fig. 3). Studies done with oil emulsions indicate that the inhibition of lipid oxidation does not always positively correlate with antioxidant activities such as DPPH radical scavenging activity and reducing power (Zhang et al. 2015). Such correlations of DPPH radical scavenging activity and oxidation of lipids in cakes are even more complicated due to the complexity of the matrix.

Identification of phenolic compounds

The present study was conducted with the aim of utilizing easily extractable phenolic compounds present in rice bran for improving the quality and the shelf-life of selected foods. Only ethanol and water were used for the extraction of phenolic compounds because the extracted phenolic compounds are meant for food

purposes. Therefore, attempts were not made to extract less soluble bound phenolic compounds using other organic solvents. Three major phenolic compounds present in RBE are ferulic acid, *p*-coumaric acid and vanillic acid. Ferulic acid is the most abundant while catechin is present in smaller quantities compared to the quantities of the three major phenolic compounds (Fig. 4) (Table 3). These results are consistent with the previous observations (Butsat & Siriamornpun 2010). In addition to the reported phenolic compounds in the present study, crude extracts of rice bran from different rice varieties prepared by different extraction methods such as extraction with methanol, alkaline digestion and ultrasound-assisted extraction have been reported to contain a few other phenolic substances such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid and bound phenolic compounds. Alcoholic extracts of rice bran are also known to contain γ -oryzanol and tocopherols (Butsat & Siriamornpun 2010; Das et al. 2017; Wang et al. 2015). However, the sample preparation for HPLC analysis in the present study involves ethanolic extraction followed by the

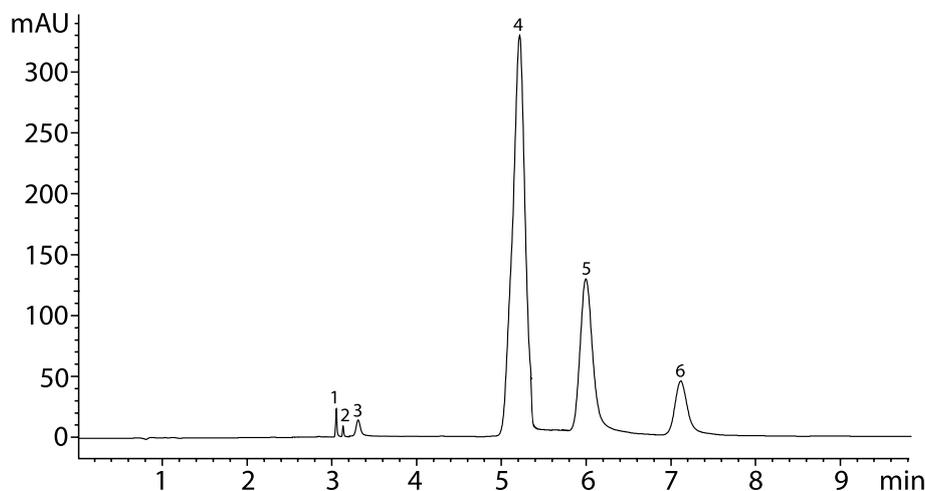


Fig. 4 HPLC chromatogram of the phenolic compounds of RBE: (1) catechin, (2) Gallocatechin gallate, (3) Ellagic acid, (4) Ferulic acid, (5) *p*-coumaric acid, (6) vanillic acid (means of three samples \pm standard deviation, mg/kg RBE powder)

Table 3 Quantities of easily extractable phenolic compounds in rice bran

Phenolic compound	Quantity $\mu\text{g g}^{-1}$
Catechin	2.7 ± 0.1
Gallocatechin gallate	2.5 ± 0.1
Ellagic acid	4.4 ± 0.1
Ferulic acid	203.2 ± 1.4
<i>p</i> -Coumaric acid	63.1 ± 0.1
Vanillic acid	25.9 ± 0.1

Data presented as mean \pm SD ($n=3$)

extraction with acetonitrile and hexane wash, which removes γ -oryzanol and tocopherols. Therefore, the observed clean phenolic profile of 6 phenolic antioxidants does not represent all the compounds in rice bran extracts that are responsible for antioxidant activity and food preservative properties.

Exposure of catechin, gallic acid and vanillin to high temperatures (100°C) revealed that phenolic compounds are relatively stable with degradation ranging from 15 to 30% after 4 h of exposure (Volf et al. 2013). Thermal decomposition of ferulic acid (major constituent in RBE) in the air at temperatures above 300°C has been shown to result in the generation of vanillin and vanillic acid (Fiddler et al. 1967). These thermal decomposition compounds also display strong antioxidant activity (Tai et al. 2011). Ferulic acid and *p*-coumaric acid have also been reported to remain intact in cookies and muffins after baking while some reports also indicate that baking increases the free phenolic acids in bread (Abdel-Aal & Rabalski 2013). Hydroxycinnamic acids such as ferulic acid and *p*-coumaric acid have been reported to

react with Maillard intermediates in a baking model stimulated with glucose and glycine at 200°C (Jiang et al. 2009). Even though phenolic acids are known to combine with carbohydrates, proteins and lipids, such interactions under present baking conditions have not been reported (Juanying et al. 2019; Limwachiranon et al. 2018).

Phenolic acids act as antioxidants mainly by hydrogen atom transfer which generates phenolic radicals that are more stable than the substrate molecules due to resonance stabilization. The antimicrobial activity of *p*-coumaric acid and vanillic acid is due to the ability of these compounds to bind to DNA and to change the permeability of cell membrane (Alves et al. 2013). Both dissociated and undissociated forms of ferulic acid and *p*-coumaric acid display antimicrobial activity (Pernin & Guillier 2019). Phenolic acids are responsible for sour and bitter flavors in foods. The taste threshold for *p*-coumaric acid and ferulic acid in corn flour products have been established to be 48 and 90 ppm respectively (Huang & Zayas 1991). The amount of *p*-coumaric acid and ferulic acid in the cakes prepared in the present study is below 10 ppm suggesting that the phenolic acids do not impart any negative flavors to the cake. However, further studies are necessary to explain the improvement of the taste of RBE-added cakes compared to BHT-added cakes because BHT is a single antioxidant compound while RBE is a mixture of antioxidant compounds.

Conclusions

RBE can be used as an alternative to synthetic food additives in the baked cake to improve the quality characteristics and sensory properties. Due to the high thermal stability, RBE present in cakes after baking extends

the microbial and chemical shelf life of cakes. Therefore, RBE can be considered as a food preservative, which provides additional functional properties to baked cake. RBE also improves the thermal stability and shelf life of sunflower oil suggesting that the oxidation of polyunsaturated oils is retarded by RBE.

Abbreviations

APC: Aerobic plate count; BHA: Butylated hydroxyanisole; BHT: Butylated hydroxytoluene; GAE: Gallic acid equivalents; PF: Protection factor; PV: Peroxide value; RBE: Phenolic extract of rice bran; RT: Room temperature; SPME: Solid-phase microextraction; TBHQ: Tert-butyl hydroquinone; YMC: Yeast and mold count.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43014-022-00094-0>.

Additional file 1. Antioxidant activities of RBE and synthetic antioxidants.

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Not applicable.

Authors' contributions

KPS: Formal analysis, Visualization, Writing- Original draft preparation. NVPA: Investigation. CMS: Investigation, Validation, Writing- Original draft preparation. NJ: Project administration, Supervision, Writing- Review and Editing. KNS: Conceptualization, Supervision, Fund acquisition, Writing- Review and Editing. The author(s) read and approved the final manuscript.

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Availability of data and materials

All the data used to support the findings of this study are included in the article and in supplementary materials.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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