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Antioxidant efficacy of rice bran extract on stabilisation of ghee under accelerated oxidation condition

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Abstract

The antioxidant efficacy of rice bran extract on the oxidative degradation of ghee under storage conditions of 63°C was studied by adding different concentrations of methanolic extracts to ghee. The antioxidant-treated and control samples were analysed at regular intervals to assess the extent of oxidative changes and quality markers by measuring oxidative indices (free acidity, peroxide value, *p*-anisidine value, TOTOX value, thiobarbituric acid and conjugated diene), fatty acid composition, FTIR spectra and sensory attributes. During storage, the oxidative indices of samples increased. The increment was faster in untreated samples as compared to the treated samples, thus confirming the slower release of oxidation products in treated samples. Ghee incorporated with bran extract displayed a stronger ability to quench DPPH radicals; this can be attributed to the antioxidant potency of bran extract. Moreover, the solvent extract showed dose-dependent efficacy and increasing the concentration of the extract led to increased effectiveness in inhibiting lipid oxidation. In FTIR, the peak intensities in the control ghee were markedly changed in comparison with the treated ghee during storage. The effects of extract addition on ghee on fatty acid composition were unremarkable during storage. The colour and flavour scores of the samples decreased up to the end of storage and the bran extract fortified ghee samples were more acceptable to the panellists than the control ghee. It can be concluded that the bran extract was found to be quite effective towards suppressing the formation of oxidation products in the tested ghee.

Introduction

Ghee is the most famous traditional dairy product in Bangladesh and many other countries. It is usually manufactured by direct heating of cream or butter prepared from cow or buffalo milk. It has a pleasing and appetising aroma, and contributes colour and flavour richness to numerous foods used for various purposes. It is considered to be one of the best cooking and frying mediums. Ghee undergoes oxidative degradation during storage which negatively affects major quality parameters such as colour, flavour, aroma and nutritive value. This ultimately influences consumer interest and limits its storage life (Pawar *et al*., 2014; Shende *et al*., 2014). The degradation is mainly due to the lower quantity of inherent antioxidants present in ghee as compared to vegetable oils. In addition, the oxidative deterioration of ghee generates potentially toxic substances (Mehta *et al*., 2015), and consumption of these substances can lead to diarrhoea, poor growth rates, tumour growth, and carcinogenic properties (Sanders, 1989). Therefore, it is important to enhance the shelf-life of ghee by various means, and the addition of antioxidants is one of the most significant methods used to retard lipid oxidation reactions in fatty foods. In order to retard oxidative degradation of fatty foods, a number of synthetic antioxidants are frequently used. However, the use of synthetic antioxidants has been questioned because of possible toxic effects on human health and the environment (Ito *et al*., 1986; Stich, 1991). Hence, the development and utilisation of alternative antioxidants from natural sources are required. Crude extracts of plant sources rich in phenolics are of increasing interest in the food industry because of their abilities to retard the oxidative process of lipids,

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thereby improving the quality and nutritional value of food. However, the addition of herb extracts such as betel leaves, curry leaves, lemon grass (Kapadiya and Aparnathi, 2018), vidarikand, rosemary, green tea (Gandhi *et al*., 2013), shatavari, ashwagandha (Pawar *et al*., 2014) and clove extracts (Shende *et al*., 2014) to ghee is a newly emerging area. When used as main crops, some of these commodities are costly and not easily available everywhere at all time. Hence, it is essential to search for an alternative natural source that is more economical, safe and rich in antioxidants.

Rice bran, a by-product of the rice milling industry, is gaining global commercial importance as it contains many beneficial bioactive phytochemicals which possess biological effects. Rice bran contains a significant amount of oryzanols, tocopherols and tocotrienols, all of which have strong anti-oxidative activities (Perez-Ternero *et al*., 2017; Das *et al*., 2018). By taking advantage of the powerful antioxidants present in rice bran, its crude extracts can be considered as valuable additives that can enhance the oxidative stability of ghee. Therefore, the core objective of the present work was to investigate the antioxidant efficiency of crude extracts prepared from rice bran as an economic agro-food waste in improving the oxidative stability and quality of ghee during storage under accelerated oxidative conditions.

Materials and methods

Freshly milled rice bran was collected from a direct milling system in an airtight polyethylene bag. The sample was placed in a polyethylene microwave-safe bag, the moisture level was adjusted to 21% (Lakkakula *et al*., 2004) and endogenous lipase was inactivated by microwave heating (2450 MHz, output power 850 W) for 3 min. To prepare the ghee, fresh cow milk was purchased from the local market. DPPH (2,2-Diphenyl-1-picrylhydrazyl) and methyl ester standards were purchased from Sigma (Sigma Aldrich, St. Louis, MO, USA). Thiobarbituric acid was purchased from HiMedia Laboratories (Mumbai, India). The solvents and other reagents used for the analysis were of analytical- or HPLC-grade (Merck GmbH, Darmstadt, Germany).

Preparation of sample

The rice bran was defatted using n-hexane before being added to methanol at a solid to solvent ratio of 1:10 (w/v). Next, it was stirred for 3 h at room temperature followed by filtering through a Whatman No. 4 filter paper. The residue was re-extracted twice with the same solvent. The filtrates were pooled and dried under reduced pressure at 45°C. The ghee was prepared by the direct creamery method as described by Antony *et al*. (2018). Methanolic extracts of rice bran and synthetic antioxidant (butylated hydroxytoluene; BHT) were added directly to the freshly prepared ghee. The treatments were ghee containing 0.025% methanolic extract (GBM_1), ghee containing 0.05% methanolic extract (GBM_2), ghee containing 0.10% methanolic extract (GBM_3), ghee containing 0.02% BHT (GBH) and ghee without any additives (G). The ghee samples were stored in an incubator at 63°C and then were analysed at regular intervals of 0, 7, 14, and 21 d.

Radical scavenging activity of ghee sample by DPPH assay

The capacity of antioxidants to quench DPPH radicals in ghee was measured before and after the accelerated oxidation tests (Pawar *et al*., 2014). The ghee sample (0.2 mL) was mixed with 3.8 mL of ethyl acetate to obtain 4 mL of the mixture followed by the addition of 1 mL of DPPH ($6.09 \times 10{-}5$ mol/L) solution in ethyl acetate. After 10 min, the absorbance was recorded at 520 nm wavelength. A mixture of 1 mL of DPPH solution and 4 mL ethyl acetate was used as a reference sample.

Oxidative indices

The official methods of American Oil Chemists' Society (AOCS, 1987) were utilised to determine free acidity (method Ca 5a-40), peroxide values (method Cd 8-53) and conjugated diene (method Ti 1a-64). The *p*-anisidine value (method p2.4) was determined by PORIM test methods (PORIM, 1995). The oxidation value was estimated using Holm's equation of TOTOX = 2PV + *p*-AV (Wai *et al*., 2009). Thiobarbituric acid value was determined by the method of Patton and Kurtz (1951).

Fatty acid composition

Fatty acids were reduced to their corresponding derivative methyl esters based on method p3.4 (PORIM, 1995), and were analysed by gas chromatography (Clarus 590 GC PerkinElmer, USA) joined with a flame ionisation detector. Methyl ester samples were injected and GC separations were performed on a capillary column (Elite-FFAP, 0.25 mm i.d. × 30 m × 0.25 µm). The flow of carrier gas (He) was set to 2 mL/min. The initial oven temperature was set to 120°C, and increased to 240°C at a rate of 4°C/min. The temperatures of the injection port and detector were maintained at 120°C and 250°C, respectively. Fatty acid methyl esters (FAMEs) were

identified by comparing their retention times with those of pure FAME standards (Supelco, Bellefonte, USA) under the same operating conditions. The peak areas of target compounds were obtained from the computer, and the percentage of the fatty acids were calculated as the ratio of partial area to total area.

FTIR spectroscopy

The FTIR spectra of ghee samples were measured using the Fourier Transform Infrared (FTIR) Spectroscopy (IRAffinity- 1S, Shimadzu Corporation, Kyoto, Japan) joined with a high sensitivity pyroelectric detector (deuterated L-alanine doped triglycine sulphate). The spectra were obtained with a spectral resolution of 2 cm^{-1} and the frequency ranged from 4,000 to 850 cm^{-1}.

Sensory evaluation

The samples of ghee fortified with rice bran and the control sample were evaluated for their sensory attributes (colour, flavour and overall acceptability) on a nine-point hedonic scale by a panel of 10 experienced judges. First, necessary training was imparted to the panellists to avoid any bias during the evaluation of the sample. Sensory panel members were then requested to score the samples from 1 to 9 as described by Asha *et al*. (2015).

Statistical analysis

All analyses were performed in triplicate, except for the GC data. The data were expressed in terms of mean and standard deviation (SD), and were subjected to a one-way analysis of variance (ANOVA). The mean values were compared at $p < 0.05$ significant level by Duncan's multiple range test using IBM SPSS 22 statistics.

Results and discussion

Radical scavenging activity of ghee samples

On day 0, the ghee that had been incorporated with bran extracts displayed higher scavenging activity as compared to the control ghee, possibly due to lack of antioxidants in the control sample (Figure 1). The scavenging activity was reduced in all the samples as antioxidant compounds were utilised in the oxidation process to quench free radicals as the storage period progressed. During storage, a slow decrease in the scavenging activity of the treated samples as compared to that of the control was noted. This clearly showed the antioxidant potency of the methanolic extract of rice bran. Asha *et al*. (2015) stated that the ghee that had been incorporated with orange peel extract showed maximum potential to quench the DPPH radicals as compared to the control ghee throughout the storage period. At the end of day 21, the sample containing 0.1% extract (GBM_3) exhibited the highest activity (39.42%) as compared to the rest of samples containing extracts (26.81% for GBM_1 and 32.17% for GBM_2). Furthermore, the scavenging activity of the ghee containing extracts exhibited a significantly lower value than that of BHT at all corresponding storage periods.

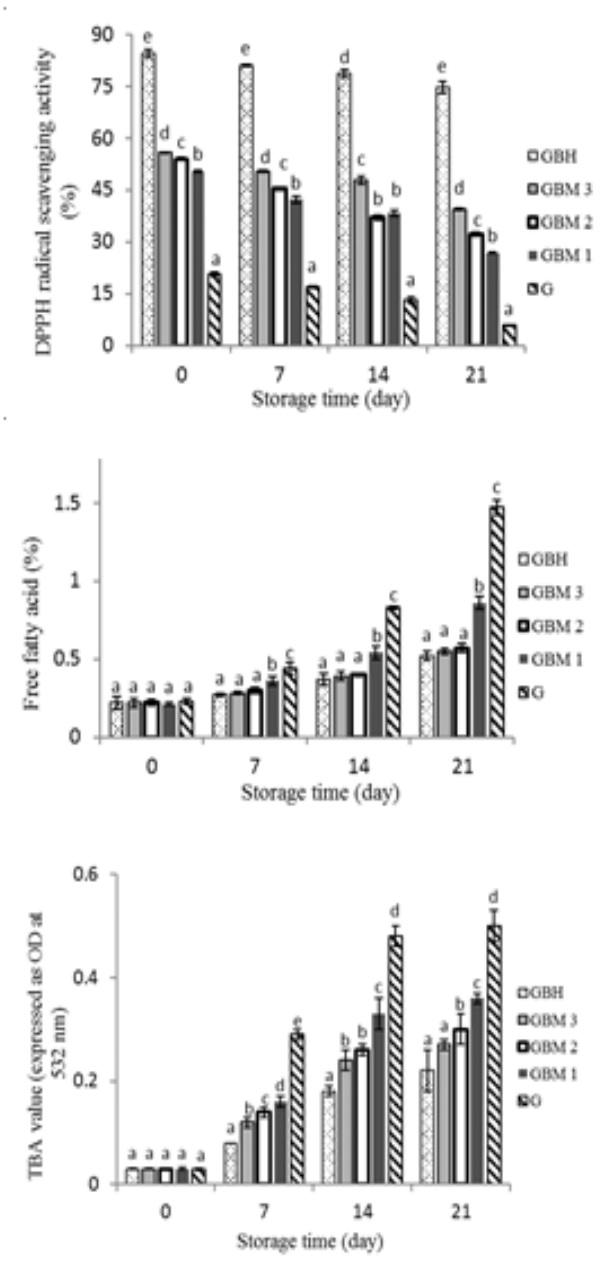

Figure 1. Changes in DPPH radical-scavenging activity, free fatty acid value and TBA value of ghee samples during storage at 63°C. G: ghee without any additive; GBH: ghee containing 0.02% BHT; GBM_1: ghee containing 0.025% methanolic extract; GBM_2: ghee containing 0.05% methanolic extract; GBM_3: ghee containing 0.1% methanolic extract. Data are means of three replicates (n = 3) ± standard deviation. Data in each storage grouping with different letters on bar are significantly different ($p < 0.05$).

Oxidative indices

Free fatty acids (FFA) are often used as an indicator for lipid hydrolysis by lipase, and hydrolysis may be promoted by the reaction of oil with moisture. After 21 days of storage, the amounts of FFA in G, GBH, GBM_1, GBM_2, and GBM_3 significantly ($p < 0.05$) increased from 0.23, 0.22, 0.21, 0.22, and 0.22% to 1.47, 0.52, 0.86, 0.57, and 0.55%, respectively (Figure 1). But no significant difference was found between the FFA contents in GBM_2 and GBM_3. The resistance against the accumulation of FFA was more in the ghee treated with bran extract or BHT than in the control ghee which could be due to the antioxidant potency of extract or BHT. Asha *et al*. (2015) reported that the formation of FFA was significantly higher in the control than in the ghee incorporated with the orange peel extract or BHA during storage at 60°C. The thiobarbituric acid (TBA) value is used to measure secondary lipid peroxidation products. As can be seen in Figure 1, the TBA content showed a significant increasing trend during storage, but no regular pattern of increase followed that. A sharp increase in TBA values was noticed up to 14 days of storage, followed by a decrease. At the end of 21 days of storage, control sample G exhibited the highest TBA (0.50) while the BHT incorporated sample exhibited the least (0.22). The higher level of TBA value in the control sample as compared to rice bran treated samples indicated a higher extent of degradation products in the control. A similar trend was also confirmed by Asha *et al*. (2015) for ghee incorporated with orange peel extract during storage. A slow rise in the TBA value of the treated samples as compared to that of the control clearly showed the antioxidant efficacy of the bran extract. In addition, with the increasing extract concentration in ghee, malonaldehyde developed accordingly which resulted in significant ($p < 0.05$) decrease of the absorbance at 532 nm. The bran extract significantly ($p < 0.05$) reduced the peroxide value (PV) throughout the storage period as compared to the control, thus indicating that ghee containing extracts were effective in retarding hydroperoxides development (Figure 2). However, the development of peroxides in GBM_3 was lowest as compared to GBM_2, GBM_1 or G. This could be due to the presence of antioxidants and its stability at lower temperature. Our findings are in agreement with Pawar *et al*. (2014) who observed that the addition of herb extracts to ghee greatly inhibited the rise in peroxides under storage conditions at

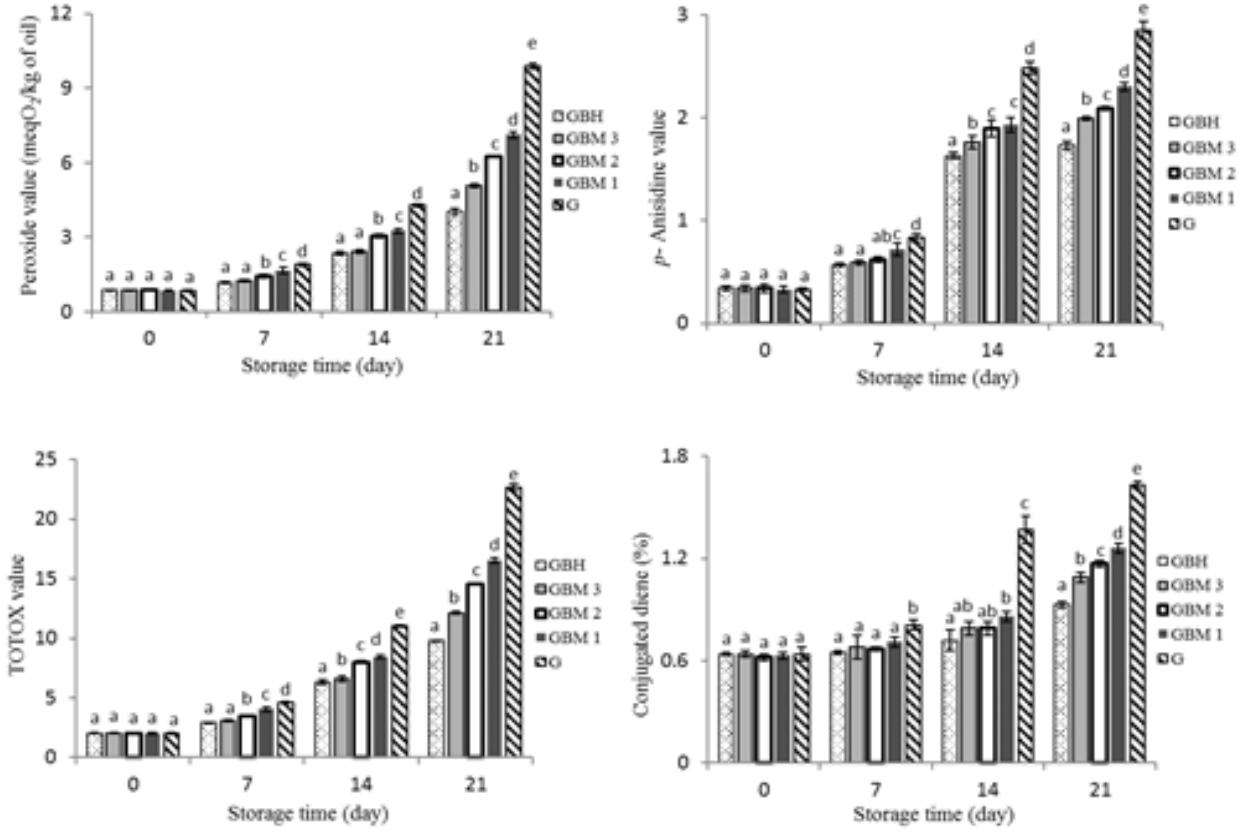

Figure 2. Changes in peroxide value, p-anisidine value, TOTOX value and conjugated diene of ghee samples during storage at 63°C. G: ghee without any additive; GBH: ghee containing 0.02% BHT; GBM_1: ghee containing 0.025% methanolic extract; GBM_2: ghee containing 0.05% methanolic extract; GBM_3: ghee containing 0.1% methanolic extract. Data are means of three replicates ($n = 3$) ± standard deviation. Data in each storage grouping with different letters on bar are significantly different ($p < 0.05$).

80°C. In the present work, the ghee incorporated with synthetic BHT displayed a lower peroxide value (4.03 meq O2/kg of oil) as compared to the rest of the samples (5.08-9.89 meq O2/kg of oil) after 21 days of storage. The peroxide value and *p*-anisidine value (*p*-AV) are commonly used to estimate the degree of oxidative deterioration in heated oils. The former is the measure of primary and the latter is of secondary oxidation products (Anjum *et al*., 2006). At the end of storage period, the *p*-AV reached 2.85, 1.73, 2.30, 2.09 and 1.99 for the samples G, GBH, GBM_1, GBM_2, and GBM_3, respectively (Figure 2). The addition of bran extract to ghee slowed down the rate of formation of secondary oxidation products as reflected by the *p*-AV in ghee during storage. Mehta *et al*. (2015) confirmed that the *p*-AV of ghee gradually increased and reached its maximum on the 4th day of storage, then reduced on the 10th day of storage at 80°C. In the present work, the increment in *p*-AV was high at the earlier phase and low at the later phase of storage. This might be due to the volatilisation of secondary oxidation products or their further breakdown. Significant differences ($p < 0.05$) in the TOTOX values of ghee samples were noted after 21 days of the oxidation test (Figure 2). The TOTOX values, from lowest to highest, were found in the samples GBH, GBM_3, GBM_2, GBM_1, and G. The lower TOTOX values of the treated samples implied their higher stability to oxidative rancidity than the control. The methanolic extract of rice bran significantly ($p < 0.05$) reduced the generation of conjugated diene (CD) in ghee during storage (Figure 2). After 21 days of storage, the CD contents for the samples G, GBH, GBM_1, GBM_2, and GBM_3 increased from 0.64, 0.64, 0.63, 0.62, and 0.64 to 1.63, 0.93, 1.26, 1.17, and 1.09, respectively. The inhibition of the formation of CDs by natural antioxidants were also reported by Pawar *et al*. (2014) who indicated that the herb extracts retarded the formation of CD in ghee during storage at 80°C.

Fatty acid composition

The prominent fatty acids in fresh ghee were oleic (32.96%), palmitic (32.78%), stearic (15.92%) and myristic (9.27%), while octanoic, decanoic, lauric, palmitoleic, linoleic and linolenic acids were present in minor quantities (< 2%). As shown in Table 1, fresh ghee contained 62.65% saturated fatty acids (SFA) and 34.69% monounsaturated fatty acids (MUFA). Only 2.66% of polyunsaturated fatty acids (PUFA) were detected in the fresh ghee. The present results indicated that the addition of rice bran extract or storage conditions did not greatly affect the change of fatty acid composition in ghee; this might be due to the naturally high percentage of SFA in fresh ghee. However, the reduction in PUFA was more than in MUFA. This reduction could be due to the greater number of double bonds in the structure of PUFA. At the end of storage, the relative contents of PUFA in G, GBH, GBM_1, GBM_2, and GBM_3 decreased to 80.82, 98.47, 82.03, 90.62, and 97.00%, respectively. An appreciable retention of PUFA was observed in the stabilised ghee when compared with the control sample. These results are consistent with previous results reported by Bhanger *et al*. (2008) when stabilising cookies with rice bran extracts under ambient storage.

Table 1. Changes in SFA, MUFA and PUFA in ghee samples during storage at 63°C.

Sample	Storage time (day)	Fatty acid composition (%)		
		SFA	MUFA	PUFA
G	0	62.65 (100.00)	34.69 (100.00)	2.66 (100.00)
	21	63.92 (102.03)	33.94 (97.83)	2.15 (80.82)
GBH	0	63.15 (100.00)	34.25 (100.00)	2.61 (100.00)
	21	63.47 (100.50)	33.95 (99.12)	2.57 (98.47)
GBM_1	0	62.52 (100.00)	34.90 (100.00)	2.56 (100.00)
	21	63.15 (101.01)	34.74 (99.54)	2.10 (82.03)
GBM_2	0	62.23 (100.00)	35.53 (100.00)	2.24 (100.00)
	21	62.45 (100.35)	35.51 (99.94)	2.03 (90.62)
GBM_3	0	62.67 (100.00)	34.66 (100.00)	2.67 (100.00)
	21	63.03 (100.57)	34.38 (99.19)	2.59 (97.00)

Number in parenthesis is relative % of SFA, MUFA and PUFA based on the initial SFA, MUFA and PUFA content before storage. G: ghee without any additive; GBH: ghee containing 0.02% BHT; GBM_1: ghee containing 0.025% methanolic extract; GBM_2: ghee containing 0.05% methanolic extract; GBM_3: ghee containing 0.1% methanolic extract.

FTIR spectroscopy

Figure 3 exhibits the important spectral changes produced in ghee samples under the oxidative conditions at 63°C. An increase or decrease in the intensity of some of the wave number regions was observed. However, only the regions that were known to be due to certain oxidation products were evaluated. The most significant wave numbers (Srivastava and Semwal, 2015) are 3,003 cm^{-1} [stretching = C-H (cis)], 2,955 cm^{-1} [stretching -C-H(CH_3)], 2,926 cm^{-1} [asymmetric stretching -C-H(-CH2)], 2,854

cm^{-1} [symmetric stretching -C-H(-CH_2)], 1,745 cm^{-1} [stretching -C=O], 1,710 cm^{-1} [stretching -C=O acid], 1,465 cm^{-1} [bending -C-H(CH_2)], 1,238, 1,163 and 1,114 cm^{-1} [stretching -C-O].

The intensity (absorbance) of the cis-double bond near 3,003 cm^{-1} (shoulder) suffered a slow shift towards higher values during oxidative stress. This rise might be due to the generation of free radicals by heating which initiated the primary oxidation reaction of unsaturated fatty acids. This reaction resulted in primary oxidation products that contain cis and conjugated double bonds as previously happened in the case of the auto-oxidation of oleic and linoleic acids (Belitz and Grosch, 1999; Moharam and Abbas, 2010). The intensities of the bands at 2,926, 2,854 and 2,955 cm^{-1} increased as the oxidation progressed. This might be related to the surrounding chemical changes as a consequence of the oxidation process (Liang *et al*., 2013; Vlachos *et al*., 2006). Vlachos *et al*. (2006) stated that the bands at 2,854 and 2,962 cm^{-1} increased their intensities but the band at 2,925 cm^{-1} reduced its absorbance and increased its width during heating of corn oil. In the present work, the band at 2,955 cm^{-1} was absent whereas the band at 2,926 cm^{-1} was present at the earlier phase of oxidation (0 and 7 d). This is likely to depend on the availability of -CH3 or -CH2 functional groups in the sample that corresponds to oxidative changes in the sample (Vlachos *et al*., 2006; Tiencheu *et al*., 2013). In terms of the change occurring at the C=O region, the increase in peak intensity at 1,745 cm^{-1} could be found during storage. This change is related to the degradation of hydroperoxides and the formation of saturated aldehydes or other secondary oxidation products (Raba *et al*., 2015). A higher intensity at 1,745 cm^{-1} resulted in the presence of more carbonylic compounds (Rohman, 2017). Valdés *et al*. (2015) also reported increments in absorbance intensity at the bands near 2,959, 2,856, and 1,745 cm^{-1} during the accelerated oxidation of

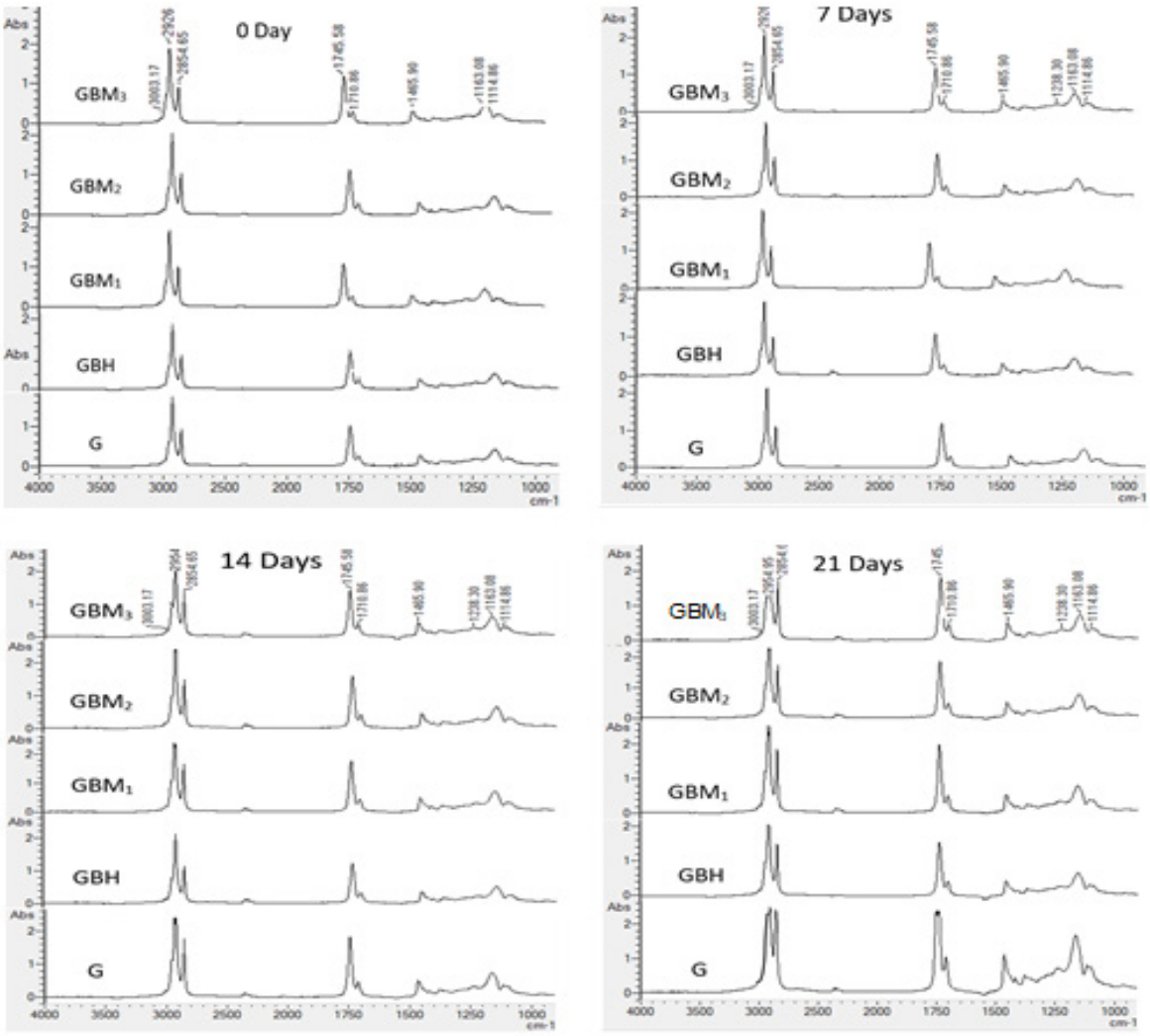

Figure 3. Changes in FT-IR spectra of ghee samples during storage at 63°C. G: ghee without any additive; GBH: ghee containing 0.02% BHT; GBM_1: ghee containing 0.025% methanolic extract; GBM_2: ghee containing 0.05% methanolic extract; GBM_3: ghee containing 0.1% methanolic extract.

blanched, roasted and fried almonds, respectively. Not only that, a small shoulder was found at 1,710 cm^{-1} that might be attributed to the formation of FFAs (Goburdhun *et al*., 2001). Its intensity increased with storage time but the rate of increment was lower in treated samples as compared to fresh ghee. The intensities of the bands near 1,238, 1,163 and 1,114 cm^{-1} showed similar changes to that observed in the band at 1,710 cm^{-1}. Liang *et al*. (2013) observed a similar trend at bands 1,238 and 1,163 cm^{-1} in walnut oils during heating. Of course, in most cases, the changes in absorbance of the different bands in the FTIR spectra were found to be lower in the sample incorporated with bran extracts as compared to fresh ghee throughout the storage period. This observation clearly shows that bran extract effectively reduced the oxidative reactions in ghee samples. The data from FTIR led to the same conclusions as per the changes in the chemical properties previously described.

Sensory evaluation

Consumer acceptance or rejection of ghee mainly depends on important sensory attributes such as colour and flavour. These sensory attributes (colour and flavour) of ghee samples with added rice bran methanolic extract were evaluated during 21 days of storage at 63°C and compared with the control. The samples treated with bran extract were found to be acceptable for their colour and flavour during sensory evaluation by the panellists (Table 2). Kapadiya and Aparnathi (2018) concluded that the herb extract fortified ghee samples were found to have acceptable sensory attributes. At day 0, the sensory panel enjoyed the colour and flavour of the ghee samples very much as their score was above 8. But the fresh ghee samples added with extract acquired slightly lower scores than that of the control sample, indicating that the treatment with extract had slightly adverse effects on such attributes (colour and flavour). Rahila *et al*. (2018) observed similar results for ghee that had different levels of rosemary extract added. The control ghee sample acquired a golden yellow colour. Most of the fortified ghee extracts acquired a yellow to golden yellow colour, almost similar to that of the control ghee. At the end of storage period, the initial golden yellow colour of the fresh ghee changed to dark golden yellow. During storage, the colour and flavour scores of ghee samples gradually decreased towards the end of storage; this reduction was highest in the control sample and the flavour score of the control sample was below the acceptable level (4.77). Conversely, the bran extracts fortified ghee samples retained scores that were above the acceptable level. The overall acceptance rating of ghee samples was done based on colour, flavour, texture and mouth feeling. The sensory panel gave a significantly higher rating to GBM_3 as compared to all the other samples. However, the reduction in sensory scores for ghee during storage might be due to the oxidation of ghee with the formation of peroxides and other oxidative products. The food lipid oxidation degraded not only PUFA but also flavouring compounds, colouring pigments, and some of the vitamins (Rahila *et al*. 2018).

Table 2. Sensory scores of ghee samples during storage at 63°C.

Parameter	Day	Sample			
		G	GBM_1	GBM_2	GBM_3
Colour	0	8.46 ± 0.04^{aD}	8.44 ± 0.09^{aD}	8.37 ± 0.28^{aD}	8.41 ± 0.09^{aC}
	7	6.56 ± 0.12^{aC}	7.09 ± 0.10^{bC}	7.30 ± 0.09^{bC}	7.40 ± 0.29^{bB}
	14	6.00 ± 0.04^{aB}	6.60 ± 0.06^{bB}	6.68 ± 0.35^{bB}	7.18 ± 0.36^{cB}
	21	5.05 ± 0.08^{aA}	5.63 ± 0.21^{bA}	5.76 ± 0.17^{bA}	5.92 ± 0.24^{cA}
Flavour	0	8.43 ± 0.15^{aD}	8.41 ± 0.18^{aD}	8.38 ± 0.27^{aC}	8.40 ± 0.18^{aC}
	7	7.02 ± 0.20^{aC}	7.23 ± 0.21^{aC}	7.30 ± 0.14^{aB}	7.38 ± 0.34^{aB}
	14	6.13 ± 0.07^{aB}	6.22 ± 0.18^{aB}	7.01 ± 0.17^{bB}	7.11 ± 0.20^{bB}
	21	4.77 ± 0.14^{aA}	5.20 ± 0.21^{bA}	5.76 ± 0.15^{cA}	5.85 ± 0.07^{cA}
Overall acceptability	0	8.45 ± 0.11^{aD}	8.39 ± 0.12^{aD}	8.42 ± 0.23^{aC}	8.39 ± 0.11^{aD}
	7	7.00 ± 0.15^{aC}	7.19 ± 0.18^{aC}	7.37 ± 0.26^{bB}	7.40 ± 0.29^{bC}
	14	6.00 ± 0.06^{aB}	6.39 ± 0.06^{bB}	7.01 ± 0.15^{cB}	7.05 ± 0.15^{cB}
	21	5.02 ± 0.14^{aA}	5.23 ± 0.17^{aA}	5.73 ± 0.21^{bA}	5.82 ± 0.07^{bA}

G: ghee without any additive; GBM_1: ghee containing 0.025% methanolic extract; GBM_2: ghee containing 0.05% methanolic extract; GBM_3: ghee containing 0.1% methanolic extract. Data are means of three replicates ($n = 3$) ± standard deviation. Data within a row with the same lowercase letters are not significantly different at $p > 0.05$; values within a column with the same uppercase letters are not significantly different at $p > 0.05$.

Conclusion

The present work provides an insight into the efficacy of added methanolic rice bran extract as a natural antioxidant to reduce ghee oxidation. By combining the results obtained from different tests such as oxidative indices, fatty acid composition and FT-IR spectral patterns, it can be asserted that the

addition of bran extract slowed down the oxidative deterioration of ghee by inhibiting or retarding the generation of hydroperoxides and secondary oxidation products during storage at 63°C. When incorporated with bran extract, ghee showed stronger activity in quenching DPPH radicals. The sensory properties of ghee were also significantly affected by the addition of extract. However, the extract sample showed dose-dependent capability of retarding oxidation and retaining the quality of ghee. It was also less effective than synthetic BHT antioxidants. The present work also suggests that rice bran extract should be explored as a viable source of potent natural antioxidants. Not only can it be used in dairy products such as ghee to retard oxidative deterioration, its antioxidant capabilities can also be explored in other food and nutraceutical systems.

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