

Quality of Chocolate Oat Cookies Added with Whole Rice Bran Upon Storage at Room Temperature

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Rice is a staple food for over half of the global population. Rice bran, a by-product of white rice processing, is rich in bioactive components like γ -oryzanol, tocotrienols, and tocopherols, but it is usually discarded or used as animal feed. This study explored the incorporation of whole rice bran in chocolate oat cookies to enhance their nutritional profile. However, the high content of unsaturated fatty acids in rice bran makes it prone to rancidity during storage. The objective of this study was to evaluate the physicochemical properties of the cookies over time when stored at room temperature. A control group of cookies without rice bran and a batch containing 40% rice bran were prepared and analysed at three-week intervals over 15 weeks. The cookies were evaluated for their texture properties, water activity, moisture content, peroxide value, acid value, and total phenolic content. Compared to control cookies, rice bran cookies had higher initial fracturability (3601.13 g vs. 2366.03 g) and hardness (6588.57 g vs. 5937.97 g) ($p < 0.05$). Nevertheless, both cookies underwent comparable losses in fracturability and hardness across storage time. The duration of storage significantly increased the water activity and moisture content. The peroxide value and acid value of stored cookies increased during storage, whereas the highest value was observed in rice bran cookies (11.03 mEq/kg and 1.15%, respectively), indicating increased susceptibility to lipid oxidation. The addition of rice bran significantly increased the total phenolic content in chocolate oat cookies (299.44 mg GAE/100 g vs. 75.37 mg GAE/100 g), even though both formulations were decreased across duration. Although rice bran has the potential to improve the nutrient composition of cookies, careful formulation and appropriate storage conditions are essential to preserving cookie quality.

Keywords: Cookies; whole rice bran; stability; physicochemical properties; antioxidant activity

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Cookies, one type of baked good, are a popular and preferred snack for most people in the world due to several reasons, such as their ready-to-eat nature, affordable price, availability in a variety of delectable flavours, and extended shelf life. However, their traditional formulation, typically made with refined wheat flour, oil, and sugar, gives them a high-calorie content but lacks important nutrients, minerals, and fibre. This has led to a growing interest in enhancing cookies with functional ingredients that can improve their health benefits. Hence, there is a need for innovative approaches to enhance their nutritional profile. The incorporation of composite flours in cookie production has received particular interest due to their potential to enhance the physicochemical properties and nutritional value of these baked goods. Using composite flour to make baked products would help reduce reliance on imported wheat entirely. Composite flour is a novel flour that has garnered significant interest in both food product development and research. When it comes to minerals, vitamins, fibres, and proteins, composite flour is more nutritious than flour made from only a single grain [1]. Thus, it

may address nutritional deficiencies and cater to health-conscious consumers.

Recent studies have demonstrated that cookies made with composite flours, such as blends of sorghum, millet, and other alternative grains, exhibit varying physical and chemical characteristics compared to traditional wheat flour cookies. For instance, cookies formulated with a 25% sorghum and 75% millet blend showed improved moisture retention, protein content, and antioxidant activity while maintaining desirable sensory attributes such as taste and texture [2]. The use of composite flours can lead to cookies that not only provide better nutritional profiles but also satisfy consumer preferences for healthier snack options. Moreover, the addition of functional ingredients such as tempeh flour in combination with mocaf (modified cassava flour) has been shown to improve the nutritional composition of cookies. A study found that cookies made with a 75% mocaf and 25% tempeh flour blend exhibited increased protein and mineral content, alongside improved water absorption capacity, which can enhance the overall texture and shelf life of

the cookies [3]. The gelatinisation profiles of these composite flours were also comparable to that of traditional wheat flour, indicating that they can effectively replace wheat flour in cookie formulations without compromising quality. These findings underscore the potential of composite flours to create healthier, more nutritious cookies that appeal to a wide range of consumers, particularly those seeking gluten-free or nutrient-dense options.

Rice bran, comprising no more than 10% of whole rice, is the brown outer layer of the rice kernel that consists primarily of the pericarp, aleurone, sub-aleurone layer, and germ. Despite its relatively small proportion, it is an inexpensive, resource-rich by-product that contains noticeable amounts of fat, protein, and dietary fibre, among other nutrients [4]. Moreover, rice bran is abundant in phytonutrients, including polymeric carbohydrates, essential minerals, vitamin E, tocopherol, tocotrienols and γ -oryzanol. It also contains anthocyanins, flavonoids and various phenolic components [5]. These nutrients and phytochemicals contribute to the numerous health benefits associated with rice bran, including antioxidant, anti-inflammatory, antidiabetic, lipid-lowering, and hypotensive effects [6]. The comprehensive nutritional profile of rice bran not only underscores its potential as a functional food ingredient, but also highlights its importance in advancing public health and nutrition.

Previous studies have shown the potential of stabilised rice bran as an ingredient in composite flour for a range of baked goods, such as cookies [7], bread [8] and muffins [9]. Moreover, the application of rice bran in the food industry extends beyond baked goods. It has been incorporated into a variety of products, including sausages, noodles, and snacks, due to its ability to enhance dietary fibre content and provide functional benefits such as low glycaemic and antioxidant properties [10-12]. The versatility of rice bran makes it an attractive ingredient for food manufacturers looking to create "clean label" products that resonate with health-conscious consumers. As the food industry continues to explore innovative ways to incorporate functional ingredients, rice bran stands out as a promising option that adds nutritional value and supports sustainability by reducing food waste associated with rice processing.

However, the incorporation of rice bran into cookies does present challenges, particularly regarding stability. Due to its hygroscopic nature, which can impact texture and shelf life, and its unsaturated lipid content, rice bran is prone to rancidity. Despite these difficulties, little is known about how whole rice bran impacts the texture changes, lipid degradation, and antioxidant retention of cookies over the long term in ambient conditions. This study aims to fill this knowledge gap by examining the physicochemical changes in chocolate oat cookies enhanced with 40% rice bran during a 15-

week storage period at room temperature. The findings make a significant contribution to the field and offer valuable information for future product development, including functional additives like rice bran, by demonstrating the trade-off between shelf-life stability and nutritional enhancement.

MATERIALS AND METHODS

Materials

Stabilised whole rice bran flour was purchased from Spektrum Technology (Malaysia). Other ingredients for cookie preparation, such as wheat flour, brown sugar, butter, vanilla essence, egg, salt, corn starch, cocoa powder, baking powder, rolled oats, and chocolate chips, were purchased from a bakery shop in Shah Alam, Selangor. Kraft paper window zip-lock bags that were used to store prepared cookies were purchased from Shopee. The chemicals and solvents used were of analytical grades.

Preparation of Cookies and Storage Conditions

The preparation of control cookies was achieved by mixing brown sugar and pure unsalted butter using a hand mixer for 10 minutes until they became fluffy. Vanilla essence, eggs, and salt were added until creamy, which took five minutes. Wheat flour, cocoa powder and baking powder were added to the batter and mixed for two minutes. Rolled oats was added until it was thoroughly mixed. The dough was covered with cling wrap and kept in the chiller for 60 minutes. Ten grams of the dough were weighed and shaped into a ball, and then chocolate chips were added to each one. The balls were gently pressed and flattened with a fork to about 3 cm in diameter. The cookies were baked for 30 minutes until golden brown and fully cooked at 160°C. Then, the cookies were cooled down for 15 minutes prior to storage. Similar procedures were followed to prepare cookies added with stabilised whole rice bran, except the amount of wheat flour was substituted with rice bran flour by 40%. The cooled cookies were stored in kraft paper window zip lock bags and left at room temperature (26-27°C) for 15 weeks. Each of the bags contained approximately 60 g of cookies. All the analysis of the cookies was conducted at every three-week interval [13].

Physicochemical Analysis

Texture Analysis

The cookies' fracturability and hardness were determined by using a texture analyser (TA.XT. T2i) with a 3-point bending figure. The cookies were placed on the texture analyser's platform. The operating parameters were set to where the type of force will be compression; the approach speed was 1.0 mm/s, the test speed was 0.1 mm/s, the post-test speed was 2 mm/s, the distance was 10 mm (would be adjusted according to the size of cookies), and the rig

distance was 40.0 mm apart [14]. The force (g) required for fracturability and hardness of the cookies was recorded from the curve obtained.

Water Activity (a_w)

A water activity meter was used to measure the cookies' water activity at room temperature. The sample holder was filled to the brim with an adequate amount of sample, and care was taken to prevent sample contact with the sensor. Water activity was measured up until the values coincided [15].

Moisture Analysis

Ten grams of ground sample were subjected to a hot air oven at 135°C for 3 hours [16]. The sample was weighed again after it was cooled in a desiccator until it reached a constant weight. The moisture content was estimated based on the resulting weight loss.

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W2 - W} \times 100$$

Where,

W = weight of empty petri dish

W1 = weight of petri dish with sample before drying

W2 = weight of petri dish with sample after drying to constant weight

Peroxide Value Analysis

Peroxide value (PV) was analysed using the AOAC technique [17]. Fifty mL of chloroform was aliquoted to a volumetric flask containing 5 g of sample. For three hours, the volumetric flask was shaken to extract fat. About 20 mL of the filtered extract was placed in a flask, and 2 mL of saturated potassium iodide solution and 30 mL of glacial acetic acid were added. The flask was then left for half an hour. The flask was filled with 50 mL of distilled water and 2 mL of 1% starch solution after 30 minutes. The solution turned blue-black. The solution was titrated up to a colourless solution using 0.01 N sodium thiosulphate. PV was computed using the following formula:

$$\text{PV (mEq/kg)} = \frac{S \times N \times 1000}{\text{Weight of sample}}$$

Where,

S = mL of 0.01 N $\text{Na}_2\text{S}_2\text{O}_3$

N = normality of $\text{Na}_2\text{S}_2\text{O}_3$

Acid Value Analysis

The fat extracted from the ground cookies was titrated against a 0.1 M NaOH solution in a neutralised alcohol medium using phenolphthalein as an indicator. The solution was shaken constantly until pink-colour persistence lasting 15 seconds was obtained. The free fatty acid value was calculated as follows:

$$\text{Acid value (\%)} = \frac{\text{Titre (mL)} \times 0.1 \times 5.61}{\text{Weight of sample}}$$

Total phenolic content analysis: The total phenolic content of the cookies was determined by using the Folin–Ciocalteu method [18]. Ground cookies (1 g) were extracted using 10 mL of absolute methanol that had been acidified with 1% HCl for 24 hours at 4 °C. For 10 minutes, the methanolic extracts were centrifuged at 6,953×g. Supernatants in a pool (extracted 2 times) were maintained at 4°C. 800 µL of Folin–Ciocalteu reagent, 2 mL of Na_2CO_3 , and 200 µL of extract (7.5 g/100 mL) were added. After adding distilled water to raise the level to 7 mL, it was left in the dark for 30 minutes. Absorbance was measured at 725 nm. TPC was reported as mg gallic acid equivalents (GAE)/100 g of sample.

Statistical Analysis

The measurement of each analysis was conducted in three replicates. The data were displayed as the means ± standard deviation of three samples each. An independent T-test was used to compare the physicochemical properties of the control and cookies added with rice bran. Meanwhile, for storage analysis, ANOVA and Tukey's HSD tests were used to analyse differences at $p < 0.05$ [19].

RESULTS AND DISCUSSION

The present investigation was conducted to evaluate the changes that occurred in cookies made with 100% wheat flour compared to cookies containing a 40% addition of rice bran during 15 weeks of storage at ambient temperature. Textural properties, water activity, moisture content, peroxide value, free fatty acid content, and total phenolic content were measured. During 15 weeks of storage, at every three-week interval, samples of cookies were analysed for their respective response parameters, and the results are discussed as follows.

Textural Properties of Chocolate Oat Cookies Added with Whole Rice Bran

For many years, efforts have been made to develop cookies that maintain their textural properties when they are distributed and placed on the shelves of consumers. Cookies got crumbly and tasted arid even when they were wrapped to keep moisture from evaporating [14]. A cookie's fracturability and hardness are two key textural characteristics that affect the overall quality of cookies. The ease with which a material breaks or fractures is referred to as its fracturability. It refers to how quickly a cookie cracks or snaps under pressure in the context of cookies. Figure 1 shows the textural properties of cookies throughout the period of 15 weeks of storage. Although rice bran cookies started with higher fracturability (3601.13 ± 51.47 g vs 2366.03 ± 69.46 g), both cookies showed decline overtime. No

significant difference from week 6 until week 15 implies that rice bran substitution helps maintain fracturability and matching control after long-term storage. This trait is important for bite and chewability, as cookies should not become overly crumbly or fragile after storage. Hardness, on the other hand, is the amount of force needed to cause a material to shatter or deform. Both cookies showed comparable hardness from the initial time until the end of the storage period. Similar to fracturability, the hardness of both cookies also decreased over storage time, but rice bran cookies still retained a similar texture to the control.

The findings of the current study were supported by Yang et al. [20], who stated that the hardness and fracturability of the cookies were substantially decreased. Using germinated brown rice in cookie processing yielded similar results [13]. Hui [21] and Kaszuba et al. [22] stated that high-fibre ingredients like bran and whole grain flour added bulk, making the dough denser. Fibres could absorb more water, resulting in a thicker dough. Regular cookies had fats that coated flour particles evenly, resulting in a delicate texture. In high-fibre cookies, fats did not spread as evenly; thus, it contributed to a harder texture in high-fibre cookies [23]. Apart from product composition, the texture of dry foods is also affected by the moisture content and water activity. Hough et

al. [19] reported that the loss of crispness of biscuits during storage is evident when water activity values range between 0.43 and 0.60.

Water Activity (aw) and Moisture Content of Chocolate Oat Cookies Added with Whole Rice Bran

To forecast the cookie's stability and safety with respect to microbiological growth and lipid oxidation rates, its water activity was crucial. Water activity for the cookies was analysed for 15 weeks at room temperature. Figure 2 clearly demonstrates an increase in water activity for both cookie formulations over a 15-week storage period. The control cookies reported significantly higher water activity during weeks 3 and 6 than rice bran-containing cookies. Furthermore, it is evident that moisture content values in both types of cookie samples increased significantly across the storage time. Moisture content also plays a critical role in determining the shelf stability of food products. The result shows that moisture content in control cookies progressively increased from 3.56% to 7.28% throughout storage time. Meanwhile, cookies containing rice bran increased significantly by week 3 from 3.63% to 5.51% and then gradually increased to 6.98% in week 15. The control formulation gains more moisture content as compared to rice bran-containing cookies at the end of the storage period ($p < 0.05$).

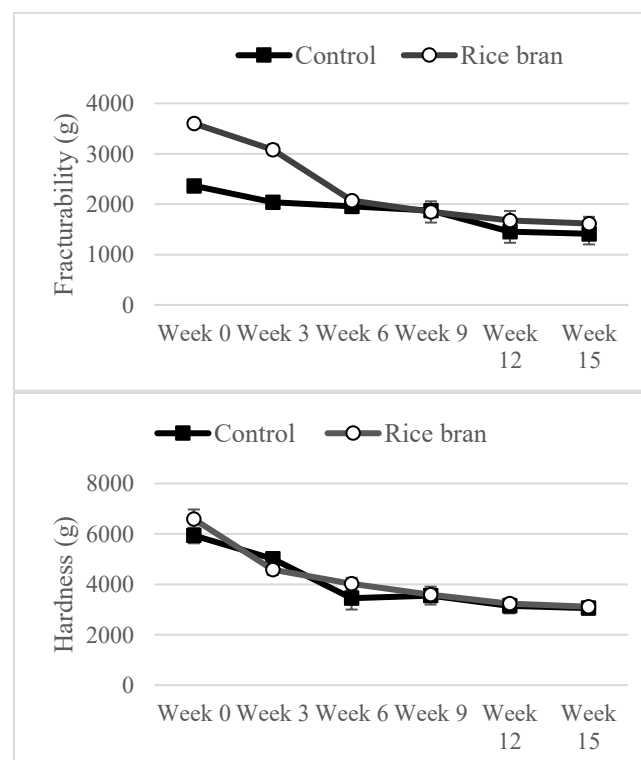


Figure 1. Textural properties of chocolate oat cookies added with whole rice bran over 15 weeks of storage
a) Fracturability b) Hardness.

Water activity, the most important determinant of the storage life of dehydrated products, is a reliable assessment of the microbial growth and chemical stability of foods following manufacture. A higher water activity value of >0.8 may facilitate diverse microbial growth. The physicochemical and microbiological stability of food also depends significantly on the water content and its interaction with food ingredients. All measured cookies had water activity between 0.32 and 0.74. As a point of reference, the range (0.4–0.6) of water activity values denotes the potential for shelf stability of the products [24]. Most bacteria, yeasts, and moulds cannot thrive at water activity 0.3. Therefore, there is very little chance of microbial spoiling. Meanwhile, certain

yeast strains and moulds may begin to grow at water activity level 0.6. Lipids are most stable to oxidation when water activity is within the range of 0.05–0.7, and all water activities under 0.70 values are considered acceptable for preventing microbial growth [15]. The cookies are still thought to have a moderate shelf life, although they are more prone to microbial growth than at lower water activity levels. The increase of a_w and moisture content of packed cookies over time of storage may be due to the hygroscopic nature of the dried product, the nature of the packaging materials, and the storage environment (temperature and relative humidity) that causes moisture migration from the surrounding air or the food itself to seep into the product [25].

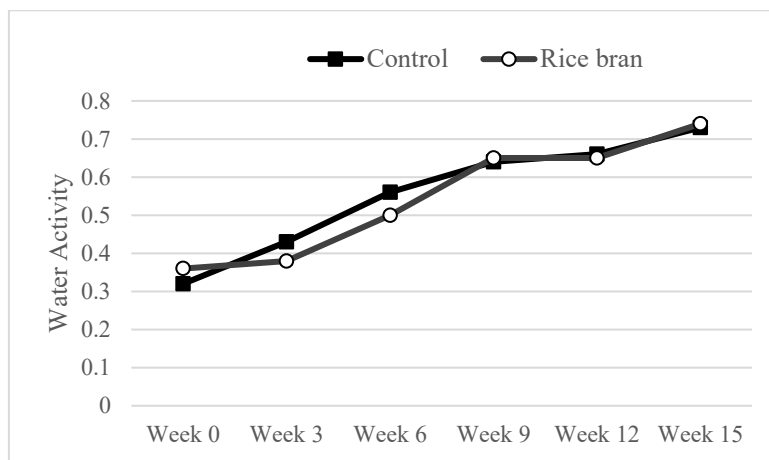


Figure 2. Water activity of chocolate oat cookies added with whole rice bran over 15 weeks of storage.

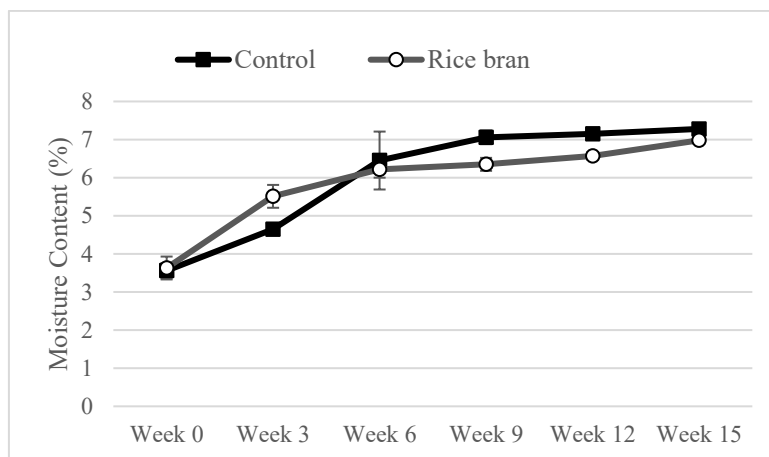


Figure 3. Moisture content of chocolate oat cookies added with whole rice bran over 15 weeks of storage.

Peroxide Value (PV) and Acid Value of Chocolate Oat Cookies Added with Whole Rice Bran

Table 1 shows the effect of storage on the peroxide value and acid value of chocolate oat cookies. Peroxide value is an indicator of rancidity development during storage. A significant increase in PV was recorded across the storage period from 5.12 to 10.73 mEq/kg in control cookies, while it was 6.01 to 11.03 mEq/kg in rice bran cookies. Among the cookies, PV was found to be significantly highest in cookies added with 40% rice bran at the initial, week 6 and week 9 of storage compared to control cookies. Storage of cookies for 15 weeks at ambient temperature significantly increased the free fatty acid content from 0.41 to 1.02% in control cookies and 0.45 to 1.15% in rice bran cookies at 15 weeks of the storage period. Rice bran cookies show higher acid values than control cookies during weeks 3, 6 and 12 ($p < 0.05$).

The structure of foods is changed during processing, and as a result, lipids may become more exposed to oxygen. In addition, naturally occurring antioxidant systems are impaired during processing, making processed food more susceptible to oxidation. To provide a comprehensive view of the state of lipid oxidation, it was advised to measure the PV trend as a function of time, as peroxides tend to degrade over time. According to a study by Sharma and Riar [25], the peroxide value rose throughout the course of 120 days of storage. Arogba [26] also found in other studies that cookies' peroxide value increased while they were being stored. According to Rajiv et al. [27],

cookies' increased moisture content during storage encourages fat breakdown, which raises the peroxide value of the cookies. Peroxide levels that are much lower than 10 mEq/kg are considered fresh. Thus, a rotten taste begins to emerge in cookies when their peroxide levels are between 20 and 40 mEq/kg. The fat in butter has the potential to oxidise over time, raising the peroxide value.

Additionally, unsaturated lipids in oats could oxidise and increase PV. Moreover, because of its fat content, rice bran flour may also be prone to oxidation. Unsaturated fats found in rice bran can significantly affect the peroxide value. The peroxide value was higher in weeks 12 and 15, which may be due to the packaging used in this study, which consisted of lock bags made of kraft paper. The barrier qualities of kraft paper are generally moderate, so it would not be as successful in preventing lipid oxidation from light, moisture, and oxygen as more advanced packaging materials. The samples within the kraft paper bags may be more exposed to oxygen over time if they are poorly sealed or have a high oxygen permeability. Such an event would result in increased peroxide readings. Aside from that, oxidation may be worsened by storing the bags in a manner that exposes them to heat or light, which would result in the higher peroxide readings shown in weeks 12 and 15. Maintaining product quality and customer satisfaction required controlling the peroxide value of items through proper storage, particularly for products like cookies, where oils and fats contribute to texture and shelf life.

Table 1. Peroxide and acid value of chocolate oat cookies added with whole rice bran.

	Peroxide value (mEq/kg)		Acid value (%)	
	Control	Rice bran	Control	Rice bran
Week 0	5.12 ± 0.22 ^{Cb}	6.01 ± 0.16 ^{Da}	0.41 ± 0.03 ^{Da}	0.45 ± 0.06 ^{Ca}
Week 3	6.58 ± 0.44 ^{Ba}	6.11 ± 0.21 ^{Da}	0.56 ± 0.01 ^{Cb}	0.67 ± 0.05 ^{Ba}
Week 6	6.97 ± 0.03 ^{Bb}	8.79 ± 0.14 ^{Ca}	0.60 ± 0.03 ^{Cb}	0.78 ± 0.06 ^{Ba}
Week 9	7.26 ± 0.27 ^{Bb}	9.00 ± 0.29 ^{Ca}	0.78 ± 0.05 ^{Ba}	0.82 ± 0.03 ^{Ba}
Week 12	10.64 ± 0.24 ^{Aa}	10.31 ± 0.13 ^{Ba}	0.86 ± 0.03 ^{Bb}	1.04 ± 0.09 ^{Aa}
Week 15	10.73 ± 0.31 ^{Aa}	11.03 ± 0.15 ^{Aa}	1.02 ± 0.08 ^{Aa}	1.15 ± 0.05 ^{Aa}

Results are presented in mean ± standard deviation (n=3). Means with different uppercase letters indicate significant differences between weeks at ($p < 0.05$). Means with lowercase letters indicate significant differences between samples at ($p < 0.05$).

Table 2. Total phenolic content of chocolate oat cookies added with whole rice bran.

	Total phenolic content (mg GAE/100 g)	
	Control	Rice bran
Week 0	75.37 ± 2.45 ^{Ab}	299.44 ± 5.56 ^{Aa}
Week 3	47.59 ± 3.21 ^{ABb}	223.52 ± 7.86 ^{Ba}
Week 6	38.33 ± 5.56 ^{Bb}	214.26 ± 6.41 ^{Ba}
Week 9	30.93 ± 3.21 ^{Bb}	192.03 ± 8.50 ^{BCa}
Week 12	17.96 ± 8.48 ^{Cb}	169.81 ± 3.12 ^{Ca}
Week 15	12.26 ± 2.83 ^{Cb}	156.92 ± 7.10 ^{Ca}

Results are presented in mean ± standard deviation (n=3). Means with different uppercase letters indicate significant differences between weeks at ($p < 0.05$). Means with lowercase letters indicate significant differences between samples at ($p < 0.05$).

Formation of FFA is also a measure of the rancidity of foods. Triglyceride hydrolysis led to the creation of free fatty acids, which were made worse by the oil's contact with moisture [28]. Acid value (AV) was a useful metric for assessing free fatty acid content and played a significant role in defining the degree of refining and quality fluctuations of fats and oils over storage. Based on the current findings, control cookies have a better shelf life and are less likely to go rancid than rice bran cookies because they maintain a lower acid value over time. Aside from that, control cookies show superior oxidative stability than rice bran cookies, and the rice bran cookies' quicker rise in acid value indicates they would lose freshness sooner. A similar trend of subsequent increase in the FFA content of flaxseed- and modified rice starch-incorporated cookies was reported by Omran et al. [29] and Muttagi and Ravindra [30], respectively.

Total Phenolic Content (TPC) of Chocolate Oat Cookies Added with Whole Rice Bran

The total phenolic content is shown in Table 2. The addition of rice bran significantly increased the total phenolic content in chocolate oat cookies by four times higher than control cookies ($p < 0.05$). There was a significant decrease in total phenolic content across storage. Although the reduction is there, the amount present in rice bran-containing cookies is still higher than in control cookies.

It was well known that phenolic chemicals exhibited excellent antioxidant action. The antioxidant properties of phenolic substances were due to the

hydroxyl group that was present on their aromatic ring. When it came to white, red, and black rice, rice bran has the highest TPC when measured against whole grain, embryo, and endosperm. Current findings have shown that the enriched cookies' TPC remained higher than the control group throughout storage, suggesting that they had greater antioxidant potential. It is evident that the reduction of TPC in cookies added with germinated millet during the first three months of storage was less prominent compared to the last three months [25]. According to Bhat et al. [31], the breakdown of polyphenols during storage might be the cause of the decrease in total phenolic content. A significant loss could be attributed to the possible oxidation of antioxidant components under favourable conditions during storage. Higher storage temperatures had the potential to accelerate the content reduction of phenolic compounds through their decomposition. Phenolics can also be degraded by light, which results in a decrease in their quantity. Additionally, high humidity levels can have an impact on the stability of phenolic compounds, which, over time, will affect their content.

CONCLUSION

The effect of stabilised rice bran flour addition on selected quality characteristics of cookies was evaluated during storage for 15 weeks at room temperature. Remarkable differences in textural properties, moisture, water activity and the development of lipid oxidation products were found in cookies packed with kraft paper bags. The addition of 40% stabilised rice bran had a substantial impact on the physicochemical parameters of chocolate oat cookies after 15 weeks of ambient storage. The initial

hardness (6588.57 ± 382.10 g) and fracturability (3601.13 ± 51.47 g) of rice bran-enriched cookies decreased over storage time; however, at week 15, it was still on par with control cookies. Texture softening is contributed by an increase in moisture content from 3.63% to 6.98% and an increase in water activity from 0.36 to 0.74. Additionally, rice bran cookies showed greater acid (1.15%) and peroxide (11.03 mEq/kg) values, suggesting faster lipid oxidation than the control. These modifications imply that in comparison to control cookies, rice bran cookies might have a lower shelf life because of their quicker rates of lipid degradation and moisture absorption. Despite these stability issues, rice bran cookies showed improved antioxidant potential by maintaining a noticeably greater total phenolic content over storage, beginning at 299.44 mg GAE/100 g and ending at 156.92 mg GAE/100 g. Future research should concentrate on refining formulations to minimise moisture absorption and lipid oxidation, possibly by adding natural antioxidants or sophisticated barrier packaging, to optimise commercial viability. Studies on consumer acceptability and sensory evaluation are also advised for determining market readiness.

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