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Enriched nutraceuticals in gluten-free whole grain rice cookies with alternative sweeteners

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ABSTRACT

Cookies are a popular snack worldwide, but the presence of gluten in most wheat-based cookies poses problems for people with gluten intolerance. Furthermore, gluten-free products are often deficient in nutraceuticals. This study investigated the potential of two traditional Indian rice landraces, Kalanamak and Chak-hao, as alternative cereals for producing whole grain gluten-free cookies with enriched bioactive compounds. The study also evaluated the influence of whole grain rice flours (WGRFs) and different sweeteners on the physical and biochemical properties of the cookies. The substitution of refined wheat flour with WGRFs significantly affected the physical and chemical properties of the cookies. WGRF cookies were generally crispier and had a lower spread ratio resulting in higher sensory evaluation scores. The added health benefits of WGRF derived cookies are likely due to the inherently higher levels of bioactive compounds such as quercetin equivalents with higher hydrogen peroxide scavenging (HPS) capacity and antioxidant activity derived from 2,2-diphenyl-1-picrylhydrazyl (DPPH) in Chak-hao rice and jaggery. This work shows that WGRFs from Kalanamak and Chak-hao could be viable alternatives to refined wheat flour for producing gluten-free cookies with enhanced nutraceutical benefits.

1. Introduction

Rice (*Oryza sativa* L.) has been a fundamental part of the human diet for thousands of years and has played a significant role in the green revolution to achieve food security in Asia. However, the milling process removes the outer bran and embryo, which results in white rice grain with a high glycemic index and reduced nutrient content (Anacleto et al., 2019). In contrast, brown rice is an unpolished whole grain that contains more dietary fiber, amino acids, phytosterols, phenolics, and bioactive compounds compared to white rice (Brotman et al., 2021; Tiozon et al., 2021). Additionally, pigmented rice varieties, such as red rice, black rice, and purple rice, have been found to be even more nutrient-dense than brown rice due to their enriched antioxidant properties (Itagi et al., 2023; Mbanjo et al., 2020). Kalanamak, which gets its name from the black husk (kala) and salt (namak), is a prominent landrace from Uttar Pradesh. Chak-hao, a black rice accession, is a fragrant variety of sticky rice, which derives its name from its delicious taste (Kowsalya et al., 2022). Both of these landraces are widely cultivated in geographical indicator regions. Our previous research has shown that popped rice made from these landraces retain high levels of phytochemicals and antioxidants, making them not just flavorful, but also nutritious (Itagi et al., 2023). Due to changes in lifestyle and socioeconomic conditions and increased awareness of their nutritional benefits, pigmented rice, as a stand-alone food product or as an ingredient in food products, has attracted increased attention in recent years (Itagi et al., 2023; Kasote et al., 2021). Therefore, the deployment of geographical indicator (GI)-tagged rice landraces can help in the development of additional rice food products with unique and desirable traits to diversify the consumer demands.

A considerable proportion of the world population exhibits intolerance and sensitivity to gluten, which is an integral component of grains belonging to the Triticeae tribe such as wheat, rye, barley, and oats. The development of a gluten-free (GF) diet to cater for people with gluten intolerance and celiac disease has been undertaken by studying

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alternative starch sources from grain outside of the tribe Triticeae (Vici et al., 2016). Due to the increased prevalence of gluten intolerance and celiac disease, the market for GF foods are projected to reach almost $24 billion by 2027 (Aguiar et al., 2023). It is well known that rice is extensively used to make GF foods, but most of these prior studies used refined white sugar and commercial rice flour normally made from polished rice, which is rich in starch and lacks nutrients (Paz et al., 2020). Because of this, GF diets usually lack adequate levels of essential nutrients, including micronutrients and bioactives, which can have a number of negative health implications. To ensure that people receive the best dietary intervention, it is crucial to develop GF food products derived from whole grain of nutritious rice varieties or landraces and assess the nutritional value of foods that fall within this diet group (Vici et al., 2016).

Functional foods and nutraceuticals, that offer benefits beyond meeting caloric requirements, are becoming more and more familiar to consumers. Cookies are a widely consumed snack that is crunchy and sweet, which are either baked or fried. In general, cookies are handy and have a long shelf life and these are created from a blend of ingredients including flour, sugar, eggs, and fats (Giuberti et al., 2017). Jaggery is a natural sweetener that is popular in India due to its perceived health advantages, wherein the majority of the vitamins and minerals present in sugarcane are retained. Hence substituting white sugar with sweeteners like brown sugar and jaggery in cookies offer extra functionality and health advantages (Iqbal et al., 2017).

Despite vast research on the role of rice in a GF diet, traditional whole-grain Indian rice landraces have not been fully investigated to test its potential to develop GF-free functional foods and cookies. Additionally, little information is known about the physicochemical, functional, and nutritional qualities of landraces when they are added to food matrices. The goal of the current work was to create formulations of novel, functional, nutraceutical-rich, gluten-free whole grain rice flour for making cookies using two well-known, GI-tagged, aromatic Indian rice landraces (Kalanamak from Uttar Pradesh and Chak-hao from Manipur) and raw sugarcane products (jaggery and brown sugar). Cookies made as a result were examined for their physical and chemical properties, nutritional content, nutraceuticals, and sensory qualities. The findings of this study will offer useful knowledge for manufacturing GF high-quality convenience snacks from Indian rice landraces that are nutrient-dense and palatable.

2. Material and methods

2.1. Raw materials

Popular GI-tagged landraces Chak-hao (black aromatic waxy rice) and Kalanamak aromatic rice (non-pigmented) were cultivated under well-maintained irrigated conditions during the monsoon season of 2021 at the International Rice Research Institute South Asia Regional Centre (ISARC) experimental farm in Varanasi, Uttar Pradesh. Paddies were dehusked, milled and prepared rice flour according to Itagi et al. (2023). The refined wheat flour (RWF), salted butter, white sugar, brown sugar, and jaggery available in local standard brands were purchased from the local market (Varanasi, India).

2.2. Shelling/dehulling of paddy for preparation of whole grain rice

The Paddy De-Husker (Model No. 67004, Osaw Industrial Products, Pvt. Ltd., Indosaw, Ambala, Haryana, India) was used to de-hull around 1 kg of paddy. Rubber rollers had to be adjusted in order to produce dehulled whole grain rice.

2.3. Preparation of whole grain rice flour (WGRF)

Grain that had been dehulled was washed and dried. The dehulled rice grains from the Kalanamak and Chak-hao varieties were processed in a laboratory mill (UDY Corporation, Cyclone Sample Mill, Screen-0.25 mm, Fort Collins, Colorado, USA) to make rice flour and sieved to a particle size of about 1 mm. The rice flour samples were stored in airtight containers in a refrigerator (4 °C), before being subjected to product formulations.

2.4. Reagents, solvents, and standards

Analytical grade reagents were utilized to measure nutritional components. The National Institute of Standards and Technology (NIST®) (Gaithersburg, MD, USA) provided the standard reference material (rice flour, 1568 b). Methanol and hexane of HPLC grade were procured from Fisher Scientific Co. in India. Takadiastase, DPPH (1,1-diphenyl-2-picrylhydrazyl radical), and Folin-Ciocalteu (FC) reagent were provided by Parke, Davis and Co., Ltd. in the United States and SRL (Sisco Research Laboratories Pvt. Ltd., India), respectively. Merck, India, provided the sodium hydroxide, other reagents, solvents, and standards for fatty acids, vitamins, and minerals. All aqueous solutions were prepared using ultrapure water (Milli-Q water purification system, LAB Q Ultra, India).

2.5. Cookie formulation and preparation

Cookies were prepared following the Approved Method 10-50D (AACC, 2000) with slight modifications on ingredients: RWF (control) and WGRF (from Kalanamak or Black rice) (100 g), salted butter (65 g), water (12–14 mL), sweetener (white sugar, brown sugar or jaggery) (50 g/100 g) and chopped cashew nuts as toppings (2 g/100 g). The creaming process of the salted butter and powdered sweetener was carried out in a planetary mixer (Berjaya laboratory in Kuala Lumpur, Malaysia). Subsequently, flour and water were added to the mixture. The cookie batter was portioned using a standard tablespoon and manually shaped into circular forms. The baking process was conducted in a commercial baking oven (Arise Equipments, New Delhi, India) that had been preheated to a temperature of 180 °C, for 18–20 min. After cooling for 30 min at ambient temperature, the cookies were subsequently transferred to an airtight container made of clear polyethylene terephthalate (PET). A comparative analysis was conducted to assess the physical, nutritional, nutraceutical, and sensory attributes of cookies prepared using Kalanamak and Chak-hao WGRF in contrast to those made with RWF.

2.6. Water activity (a_w) and moisture content (MC)

The MC of flour and cookie samples (2 g) was determined according to the AOAC (1990) approved method. The a_w of samples was measured using a Model Series 4 TE water activity analyzer (Aqua Lab, Meter Group Inc., Pullman, WA, USA). The experiments were conducted in triplicates (Itagi et al., 2023).

2.7. Physical characteristics and texture of cookies

Using an analytical balance (GR-202, A&D Co., Japan), cookie weight was calculated. A digital Vernier caliper with 0.001mm accuracy was used to measure the cookies diameter and thickness. The diameter/thickness formula was used to calculate the spread ratio (SR) of cookies after baking (Naseer et al., 2021). A colorimeter (Chroma Meter CR-410, Konica Minolta, Inc., Osaka, Japan) was used to assess the color of the cookies. The measuring head was placed in the center of each sample after the instrument's calibration was completed using a reference standard that was white in color. Color values using the CIE L^* a^* b^* scales were recorded using five samples for each cookie formulation. Following that, the mean values were documented as L* = lightness (100 = white, 0 = black), a* (-a* = greenness, +a* = redness), and b* (-b* = -blueness, +b* = yellowness) (Selvakumaran et al., 2019). The browning index was calculated following (Klunklin & Savage, 2018).

The texture analysis of cookies was conducted to determine their breaking strength, utilizing a TA. XT plus texture analyzer (Stable Micro Systems Ltd, Surrey, UK) equipped with a 50 kg load cell. The peak force required to break a single whole cookie was recorded and the average value of ten replicates was reported (Pal et al., 2019).

2.8. Nutritional characteristics and bioactive potential of flours and cookies

2.8.1. Nutrient composition

The Kjeldahl method was employed to determine the protein content (Kjeltec™ 8200, Kjeltec, Foss, Sweden). The fat was extracted with petroleum ether (40–60 °C) through a Foss ST243 Soxtec Extraction Unit and quantified through gravimetric analysis (Itagi et al., 2023). The determination of ash content was carried out through the incineration of the samples at a temperature of 550 °C (AOAC, 2000). The estimation of total carbohydrate in the samples was carried out following FAO (2003, p. 77) and the Gross Energy (Calories/100 g dry matter) was calculated based on the methods outlined by Ganogpichayagrai and Suksaard (2020).

2.8.2. Micronutrient analysis

All the trace mineral compounds and metals were quantified using an ICP-MS (Agilent 7800 ICP-MS) by following the prior protocol outlined in Itagi et al. (2023). For the vitamin analyses, finely ground samples (5 g) were weighed in a 100 mL volumetric flask, added with HCl (30 mL, 0.1 mol/L), and incubated at 120 °C for 30 min. Then, the pH was adjusted to 7 with NaOH (0.1 N). Takadiastase (5 mL, 1 mol/L) was added, followed by overnight incubation at 35 °C. The sample was diluted to 100 mL with distilled deionized water and filtered (0.22 μm, PVDF Whatman filter paper). A 10 μL of the filtrate and standards were analyzed using LC-MS/MS that had an autosampler and MS detector (Agilent Technologies, 6470 Triple Quad LC-MS/MS). This system was equipped with a 1.8-μm Agilent ZORBAX RRHD Eclipse Plus C-18 stationary phase in 3.0 mm × 100 mm formats. The mobile phase of gradient delivery composed of a mixture of solvent A (water: formic acid, 100:0.3, v/v) and B (methanol: formic acid, 100:0.3, v/v) and had a flow rate of 0.50 mL/min (0–0.4 min, 1% B; 0.4–6 min, 1%–45% B; 6–7.5 min, 45–90% B; 7.5–9min, 90%–1% B). The standard vitamins B1, B2, B5, and B6 appear at 0.96, 7.2, 4.8, and 2.2 min retention times (Rezaei et al., 2022).

2.8.3. Dietary fiber (DF) content

The Megazyme K-TDFR kit (Megazyme Wicklow, Ireland) was utilized to evaluate the levels of total dietary fiber (TDF), insoluble (IDF), and soluble (SDF). The computation of IDF and SDF was performed utilizing the Megazyme Mega-Calculation method (Itagi et al., 2023).

2.8.4. Total sugar, total starch, and amylose content

The total starch was conducted utilizing a Megazyme assay kit specifically designed for total starch (Megazyme K-TSTA, Wicklow, Ireland) (Itagi et al., 2023). The determination of amylose content was conducted in accordance with the methodology outlined by Cuevas et al. (2018), and the categorization of samples was based on the contents as described by Graham (2002). The quantification of the overall quantity of soluble sugar present in the cookies was carried out using the anthrone method as described in Roy et al. (2021).

2.8.5. Extraction and estimation of oryzanol

Oryzanol extraction and estimation were done following the method described in Itagi et al. (2023). The findings were reported in mg per 100 g, and all subsequent measurements were taken with the same spectrophotometer.

2.8.6. Estimation of total phenolic content and antioxidant potential

2.8.6.1. Extraction of phenolic content. A 1g sample was subjected to extraction using 10 mL of petroleum ether in an ultrasonicator (PCI Analytics, India) for 15 min. After centrifugation (5 min at 2520×*g*), the supernatant was decanted and collected. The polyphenols were extracted from defatted samples following Itagi et al. (2023).

2.8.6.2. Total phenolic content (TPC). FC reagent (800 μl) and Na_2CO_3 (7.5 g/100 mL) (2 mL) were added to 200 μl of sample. Then, the sample volume was increased to 7 mL with deionized water and then left to stand in a lightprotected environment for 30 min. The absorbance was taken at 725 nm. TPC was reported according to Itagi et al. (2023).

2.8.6.3. Total flavonoid content (TFC). The extract (1 mL) was diluted to 5 mL with ultrapure water, followed by the addition of $NaNO_2$ (5 g/100 mL) (300 μl). The mixture was incubated for 5 min. Next, $AlCl_3$ (10 g/100 mL) (600 μl) was added, and the mixture was incubated for an additional 6 min. A 2 mL NaOH (1 mol/L) was added, and the volume was adjusted to 10 mL. The quantification of TFC was performed by measuring the absorbance at a wavelength of 510 nm, and the resulting values were expressed as milligrams of catechin equivalents (CE) per 100 g of the sample, as previously reported by Itagi et al. (2023).

2.8.6.4. Total anthocyanin content (TAC). A mixture comprising of 2 mL of potassium chloride buffer (0.03 mol/L, pH 1.0) and 2 mL of sodium acetate buffer (0.4 mol/L, pH 4.5) was introduced to 20 μl of extract. Following a 15min incubation period, the absorbance was quantified at 550 nm and 700 nm relative to a blank sample consisting of ultrapure water. TAC was recorded in terms of milligrams of cyanidin-3-glucoside (C-3-G) equivalents per 100 g of the sample (Itagi et al., 2023).

2.8.6.5. Total antioxidant capacity. The extract (500 μL) was combined with phosphomolybdenum reagent (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate, and 4 mmol/L ammonium molybdate) (1.23 mL) and incubated at 90 °C for 90 min. The total antioxidant capacity was reported as quercetin equivalents (QE) per 100 g of the sample at an absorbance of 695 nm (Itagi et al., 2023).

2.8.6.6. Hydrogen peroxide scavenging capacity (HAS). The extract (0.4 mL) was combined with 40 mmol/L H_2O_2 (0.6 mL). The mixture was diluted to 2 mL with 50 mmol/L sodium phosphate buffer (pH 7.4) and then incubated for 40 min at 30 °C. The findings were reported as mg quercetin equivalents (QE) per 100 g of sample (Itagi et al., 2023).

2.8.6.7. DPPH radical scavenging activity. The DPPH assay was conducted on 500 μL of extract according to Itagi et al. (2023). The results were represented in terms of % DPPH radical scavenging activity.

2.8.6.8. Ferric reducing antioxidant power (FRAP). A 200 μL of volume of freshly made FRAP reagent (300 mmol/L acetate buffer (pH 3.6):10 mmol/L 2,4,6-tri (2- pyridyl)-1,3,5-triazine solution:20 mmol/L $FeCl_3$ solution, (10:1:1, v:v:v)) was added to 20 μL of extract. At 620 nm, the mixture's absorbance was measured after 5 min at 37 °C incubation. Results were expressed as mmol/L Trolox Equivalents per gram of material (Tomasina et al., 2012).

2.8.6.9. Targeted bioactive profiling. The defatted samples were used to extract the phenolic compounds. One milliliter of 80% aqueous methanol (acidified with 1% HCl) was added to a 50 mg sample. After sonication for 10 min at 120 W, 800 μL of the supernatant was collected and centrifuged (28000×*g*, 10 min). Extraction was done twice, and the pooled supernatant was filtered through a 0.22 PVDF membrane filter, then subjected to liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-MS/MS) to profile and identify phenolic compounds. The HPLC system used in this study was an Agilent 6470 Triple quad LC/MS System (Agilent Technologies, Santa Clara, CA, USA) equipped with column C18, 2.1 × 100 mm, 1.8 μm which was used for phenolic compound separation. Mobile phases A and B were composed of solvent A (water: formic acid, 100:0.3, v/v) and B (methanol: formic acid, 100:0.3, v/v), respectively. The flow rate was set at 0.40 mL/min (0–0.5 min, 10% B; 0.5–5 min, 10%–40% B; 5–12 min, 40–80% B; 12–15 min, 80%–2% B) and the column was maintained at 40 °C. An aliquot of each sample solution (10 μL) was injected into the system equipped with an ESI source and a triple-quadrupole mass spectrometer (MS/MS). The ESI source and the MS/MS were operated in the negative ion and positive ion multiple reaction monitoring (MRM) modes, respectively. All measurements were conducted in duplicate. Calibrations with $R^2 = 0.99$ were used.

2.8.7. Fatty acid (FA) profiling

FA content in samples was analyzed following a method by Jarukas et al. (2020), with slight modifications. Powdered samples were extracted with hexane (1:7, w/v) in a shaking water bath (65 °C, 30 min), then centrifuged at 7000×*g* for 15 min. Total lipid fraction was recovered after solvent removal in a stream of nitrogen. The samples were then derivatized using 2 mL of 7% (v/v) BF_3 in methanol and 1 mL of toluene and placed in a warm bath at 80 °C for 45 min. After the addition of 5 mL of distilled water, the *trans*-methylated FAs were extracted with 1 mL hexane. The aliquot of the hexane phase was analyzed by gas chromatography. An Agilent 7890B gas chromatograph (Agilent Technologies, USA) with a Flame-Ionization Detector was used to separate and quantify FAs. One microliter aliquot of the hexane phase was injected in split-mode onto a DB-Wax column (30 m × 0.25 mm ID, 0.25 μm DB-Wax (J&W 122–7032)). The injector temperature was set at 250 °C, detector at 280 °C, oven at 50 °C initially, then 50 °C, 1 min, 25 °C/min to 200 °C, 3 °C/min to 230 °C, 18 min. The carrier gas was Hydrogen. Detector gasses were Hydrogen: 40 mL/min; Air: 450 mL/min; Helium make-up gas: 30 mL/min. An electronic pressure control in the constant flow mode was used. The FAME calibration standards were used for the quantification of FAs in the various lipid extracts.

2.9. Sensory evaluation

A hedonic sensory evaluation was conducted on cookies made from whole grain rice flour. The evaluators were 27 volunteer staff members from ISARC (Varanasi) (untrained), ranging in age from 23 to 58 years and including both male and female participants. Each of the panelists has provided their informed consent to partake in the study. The sensory evaluation was conducted within the confines of the sensory and product development laboratory at CERVA. The cookies were prepared in advance of the sensory evaluation and were subsequently stored at ambient temperature. In the context of sensory evaluation, the samples were presented in their entirety on white plastic dishes that were labeled with codes. The panelists were then served the samples in a randomized sequence. The panelists were furnished with distilled water and unsalted crackers to rinse their palates in between tastings. The cookies underwent an evaluation process that assessed their surface color, surface cracking pattern, crumb color, texture, mouth feel, flavor, aroma, and overall acceptability. The evaluation was conducted using a nine-point hedonic scale which ranged from "like extremely" to "dislike extremely," corresponding to the highest and lowest scores of "9" and "1" respectively (Naseer et al., 2021).

2.10. Statistical analysis

The experiments were performed in triplicate (n = 3), unless stated otherwise, and the results were reported as the mean values along with their standard deviations. Data from the results were analyzed statistically using one-way ANOVA, followed by a Tukey's post hoc test ($P < 0.05$) using R statistical package, version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). To reveal a distinct clustering between data sets, the principal component analysis (PCA) scores plot was used. The correlation between physical properties and texture profile, nutritional composition, bioactives, and antioxidant activity for each rice genotype was analyzed by Pearson's correlation. All data visualizations were performed using the R statistical package.

3. Results and discussion

Kalanamak from Uttar Pradesh and Chak-hao from Manipur are popular GI-tagged Indian rice landraces that are renowned for their aroma and superior grain quality (Kowsalya et al., 2022). The bran and broken rice (byproducts of the rice milling process) of these two landraces are likewise known to possess higher nutraceutical properties. Rather than being used as a low-value commodity for fodder, these byproducts could be instead leveraged as raw material in food applications with enriched functional properties for different value-added product development purposes. In this study, whole grain rice flour derived from the well-known aromatic rices, Kalanamak (greenish brown) and Chak-hao (black rice), and raw sugarcane products (jaggery and brown sugar) were used to explore novel formulations of nutraceutical-rich gluten-free cookies. The physicochemical, nutritional, and sensory properties of these cookies were compared with that of refined wheat-based cookies and discussed.

3.1. Physicochemical properties of flour and cookie samples

Along with texture and flavor, the surface color of a baked product is a crucial factor in the initial acceptability of baked products among consumers. The flour and sweetener had remarkable and complex effects on the color formulation of the resulting cookies, as shown in Fig. 1. A higher L^* value, which indicates lightness, is observed in refined wheat flour (RWF) and Kalanamak whole grain rice flour (KWGRF)based cookies than in black whole grain rice flour (BWGRF)based cookies. RWF cookies made with white sugar were noted with the highest L^* values, which could be attributed to the typical light color of well-milled wheat kernels. The lower L* values of all BWGRF cookies, on the other hand, can be attributed to the presence of pigmented bran in the present study. These findings align with previous studies by Joo and Choi (2012) and Jang et al. (2010) that observed a decreasing L^* value with increasing rice bran substitution in a cookie formulation. Moreover, the L^* value of Kalanamak whole grain rice flour white sugar (KWGRFWS) cookie was not substantially different from refined wheat flour white sugar (RWFWS) cookie and was similar to that reported by Joo and Choi (2012) for cookies made from commercially available rice flour. Differences in rice flour color were attributed to their polyphenols, which relate to the purple color of the rice grain (Buenafe et al., 2022; Klunklin & Savage, 2018). The L* values generally decreased with the addition of jaggery as it contains reducing sugars that could participate in the Maillard reaction. Chand et al. (2011) reported that reducing sugars in jaggery typically increases with storage regardless of the storage conditions. The Maillard reaction, which occurs during the baking process, is another factor that affects the final color of baked food items (Giuberti et al., 2017). This reaction could result in reddish-brown hues from the interaction of reducing sugars with proteins and explain the lower L^* value of 60.49 for refined wheat flour brown sugar (RWFBS) cookie compared to a higher value of 67.00 for Kalanamak whole grain rice flour brown sugar (KWGRFBS) cookie (Ryan & Brewer, 2006). All WGRF cookies had significantly lower redness (a^*) and yellowness (b^*) compared to RWF cookies, with BWGRF showing the lowest values for both parameters. The differences in a^* and b^* values were presumably correlated with the differences in the amino acid profile and content of reducing sugars responsible for the intensity of the Maillard reaction (Torbica et al., 2012; Zucco et al., 2011).

The physical and chemical characteristics of RWF and WGRF from

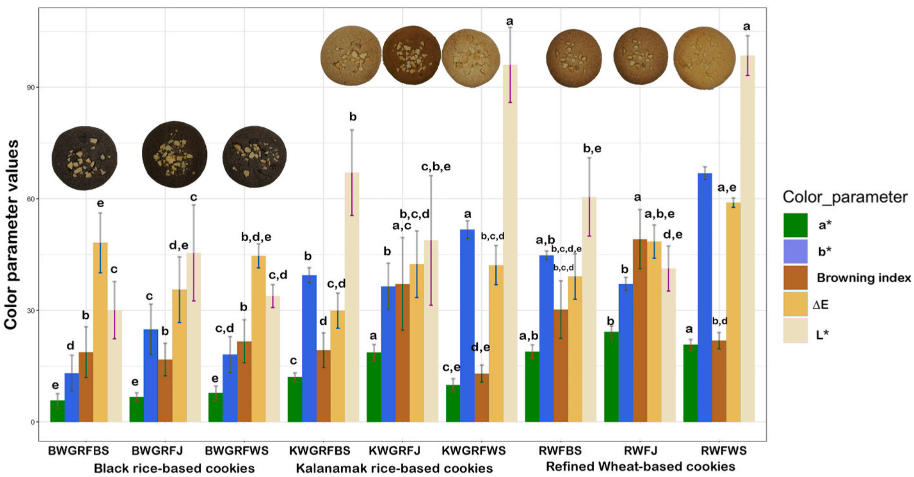

Fig. 1. Color measurements of cookies from RWF and WGRFs from Kalanamak and Chak-hao rice with different sweeteners.
Abbreviations: RWF–Refined wheat flour; WGRF–Whole grain rice flour. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Kalanamak and Chak-hao rice, and their corresponding cookies were shown in Table 1. The WGRF samples exhibited lower moisture content (9.93% and 10.13% for KWGRF and BWGRF, respectively) compared to RWF (13.71%). These findings are consistent with previous studies that have also reported lower moisture content in rice flour relative to wheat flour (Islam et al., 2012; Rai et al., 2014; Torbica et al., 2012). Moisture content is a crucial consideration for flour storage since flour with a moisture content exceeding 13% is more prone to microbial deterioration (Oppong et al., 2021). Accordingly, the moisture content of WGRFs in the present study was below the threshold level, indicating that both flour samples (BWGRF and KWGRF) have a satisfactory shelf life. Due to water evaporation during baking, which results in the distinctive crusty features of cookies, all cookie formulations exhibited considerably lower moisture content in PC1 than flour samples (Fig. 2b). Regardless of flour type, the moisture content values of cookies prepared with jaggery were up to 51% higher than those prepared with refined white sugar. This distinction may be ascribed to the hygroscopic character of inverted sugar and the presence of mineral ions in jaggery (Lamdande et al., 2018; Rao & Singh, 2022). Apart from moisture content, the water activity (a_w) also exhibited lowest in cookies, in comparison to flour (PC1,

Table 1
Physicochemical properties of refined wheat flour and whole grain rice flour samples and resulting cookies with different sweeteners.

Samples/ Parameter	Refined Wheat Flour	Refined Wheat Flour Cashew Cookies			Kalanamak Whole grain Rice Flour	Kalanamak Whole Grain Rice Cashew Cookies			Black Whole grain Rice Flour	Black Whole Grain Rice Cashew Cookies		
		White Sugar	Brown Sugar	Jaggery		White Sugar	Brown Sugar	Jaggery		White Sugar	Brown Sugar	Jaggery
Moisture Content (%)	13.71 ± 0.04a	2.44 ± 0.09g	4.10 ± 0.04c	3.74 ± 0.03d,e	9.93 ± 0.12b	2.02 ± 0.15h	2.70 ± 0.16g	3.42 ± 0.10e,f	10.13 ± 0.04b	2.53 ± 0.09g	3.18 ± 0.05f	3.78 ± 0.12c,d
Water Activity (a_w)	0.71 ± 0.01a	0.28 ± 0.02e,f	0.35 ± 0.02c	0.31 ± 0.01d,e	0.40 ± 0.00b	0.22 ± 0.01g	0.26 ± 0.01f,g	0.29 ± 0.01d,e,f	0.43 ± 0.01b	0.29 ± 0.002d,e,f	0.33 ± 0.01c,d	0.33 ± 0.01c,d
Total starch (g/100g)	75.13 ± 0.04b	56.29 ± 0.12d	56.87 ± 0.65d	54.86 ± 1.66d	80.0 ± 0.32a	55.10 ± 0.07d	56.48 ± 0.72d	61.62 ± 0.20c	72.85 ± 0.72b	56.14 ± 0.96d	57.36 ± 0.53c,d	57.57 ± 1.60c,d
Total amylose (g/100g)	21.80 ± 0.00a	16.41 ± 0.01b,c	16.26 ± 0.1c	17.03 ± 0.1b	16.11 ± 0.4c	13.87 ± 0.00d	14.29 ± 0.2d	13.96 ± 0.00d	11.17 ± 0.04e	5.16 ± 0.1f	4.75 ± 0.01f	3.79 ± 0.1g
Total sugar (g/100g)	–	23.97 ± 1.40b,c,d	23.27 ± 3.71c,d	20.33 ± 1.85d	–	30.77 ± 2.04a	26.97 ± 1.24a,b,c	20.77 ± 2.07d	–	30.40 ± 1.99a	27.80 ± 2.54a,b	22.20 ± 1.33d
Diameter (D, mm)	–	40.44 ± 0.87a	39.00 ± 0.72a,b	39.44 ± 0.42a	–	37.33 ± 0.27b,c	37.00 ± 0.27c	36.00 ± 0.27c	–	39.89 ± 0.16a	39.67 ± 0.54a	39.00 ± 0.27a,b
Thickness (T, mm)	–	12.00 ± 0.27b	12.78 ± 0.16a,b	12.78 ± 0.16a,b	–	12.67 ± 0.58a,b	12.78 ± 0.16a,b	12.67 ± 0.27a,b	–	12.67 ± 0.27a,b	13.22 ± 0.16a	13.33 ± 0.27a
Spread ratio (D/T)	–	3.37 ± 0.15a	3.05 ± 0.06b,c,d	3.09 ± 0.05b,c	–	2.95 ± 0.04b,c,d	2.90 ± 0.04c,d	2.84 ± 0.05d	–	3.15 ± 0.06a,b	3.00 ± 0.02b,c,d	2.93 ± 0.07b,c,d
Weight (g)	–	8.11 ± 0.17b,c,d	7.78 ± 0.11d	8.40 ± 0.05a,b,c	–	8.11 ± 0.03b,c,d	7.96 ± 0.19d	8.02 ± 0.06c,d	–	8.69 ± 0.06a	8.76 ± 0.11a	8.44 ± 0.13a,b
Hardness (N)	–	3.97 ± 0.54c	5.01 ± 0.62a,b	4.31 ± 0.44b,c	–	2.33 ± 0.37d	2.49 ± 0.20d	2.25 ± 0.23 d	–	3.98 ± 0.40c	4.60 ± 0.64b,c	5.40 ± 0.69a

Values are expressed as the mean of three (3) replicates for moisture content, a_w, total amylose, and total sugar, and ten (10) for thickness, diameter, spread ratio, weight and, hardness parameters ± standard deviation; "- "–Not applicable parameters; Different lowercase and uppercase letters denote a significant difference ($P < 0.05$).

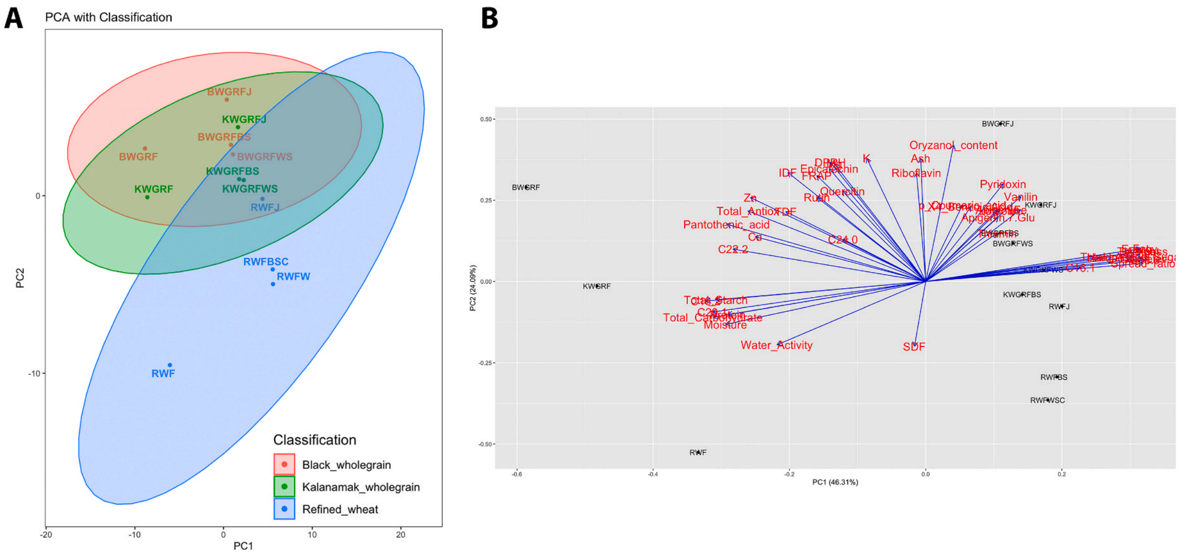

Fig. 2. Principal component analysis of RWF and WGRF and resulting cookies with different sweeteners.
Abbreviations: RWF–Refined wheat flour; WGRF–Whole grain rice flour; RWFWS (Refined wheat flour white sugar); RWFBS (Refined wheat flour brown sugar); RWFJ (Refined wheat flour jaggery); KWGRF (Kalanamak whole grain rice flour); KWGRFWS (Kalanamak whole grain rice flour white sugar); KWGRFBS (Kalanamak whole grain rice flour brown sugar); KWGRFJ (Kalanamak whole grain rice flour jaggery); BWGRF (Black whole grain rice flour); BWGRFWS (Black whole grain rice flour white sugar); BWGRFBS (Black whole grain rice flour brown sugar); BWGRFJ (Black whole grain rice flour jaggery). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 2b). All cookie samples had values in the range of 0.28–0.35, while the wheat flour exhibits less than 0.85. Interestingly, the KWGRFWS cookies have the lowest a_w value (0.22) (Table 1). Lower MC and a_w values are crucial for extending the shelf life of the final product and reducing the risk of foodborne illnesses. In addition, appropriate packaging materials are also necessary for quality preservation and shelf-life extension (Itagi et al., 2023; Yildiz & Gocmen, 2021).

The term "hardness", which describes the amount of force used to distort a sample, is measured among various cookies. In general, the hardness values of RWF (3.97–5.01N) and BWGRF (3.97–5.40N) cookies were comparable. Interestingly, all KWGRF cookies had a low breaking point (2.25–2.49 N) (Table 1). The contribution to PC1 (Fig. 2b) was significantly influenced by the hardness value and spread ratio, which accounts for the characteristic features of the cookie samples. These results are consistent with those of Yildiz and Gocmen (2021) and Paz et al. (2020), who reported a reduction in hardness values when substituting rice flour for wheat flour in a cookie formulation. In addition, these studies attribute the high hardness values to the higher protein content of RWF compared to WGRF. Proteins on the surface of starch granules function as adhesives, which strengthen the starch–-protein bond in cookie dough and increase the overall hardness of the cookie (Ryan & Brewer, 2006). In addition, a higher DF content may also enhance the texture of cookies. This was the case with GF cookies made with almond flour (Yildiz & Gocmen, 2021). Likewise, BWGRF had a DF that was up to 49% greater than the other two flour types, which may explain the higher hardness values of BWGRF cookies. Yildiz and Gocmen (2021) propose that DF can reduce the amount of free water in the dough, thereby decreasing the resulting spread ratio of cookies.

Differences in starch, DF, and protein proportions in the flour could account for the dimensional property variations (Mudgil et al., 2017). Diameter, thickness, and spread ratio are typical parameters used to determine cookie quality. The cookies in the study varied in diameter (37–40.44 mm), with significant differences observed primarily among KWGRF cookies (Table 1). In addition, the WGRF substitution led to an increase in the overall thickness of the cookies. These changes are reflected in spread ratio values. In general, cookies with a higher spread ratio are regarded as the most commercially appealing (Giuberti et al., 2017; Mudgil et al., 2017). Table 2 suggests that replacing RWF with WGRF resulted in a slight reduction in the spread ratio of cookies. However, the spread ratio values of BWGRF cookies were almost equivalent to those of the control, indicating that they are commercially viable. Additionally, BWGRF cookies were heavier than RWF and KWGRF cookies. Ryan and Brewer (2006) observed that cookies made with low-protein flour tended to be smaller in diameter and have lower spread ratios, indicating denser properties.

3.2. *Nutritional properties of flour and cookie samples*

3.2.1. *Macronutrient content*

Although RWF had the highest protein levels (13.24 g/100g), followed by BWGRF (12.41 g/100g), and KWGRF (10.55 g/100g), the cookies derived from the flour has substantially lowered the protein content (Table 2). RWF cookies contained protein ranging between 7.90 and 8.50%. Compared to KWGRF cookies (7.09–7.80 g/100g), BWGRF cookies retained higher protein levels (7.17–8.50 g/100g). Prior studies reported cookies made from rice and wheat flour of regular varieties (Islam et al., 2012; Klunklin & Savage, 2018; Torbica et al., 2012). Also observed was a significant difference in the total carbohydrates of the flour samples. KWGRF was found to have the highest carbohydrate level (74.15 g/100g), which was 3% and 4% higher than BWGRF and RWF, respectively. Variations in flour carbohydrate content could be attributed to differences in protein and lipid content (Oppong et al., 2021). However, in this study, only protein showed a strong positive correlation with total carbohydrates (Fig. 4b). All cookie samples exhibited a significant drop in carbohydrates, which may be ascribed to amylose and amylopectin leaching from the starch granules when it swells during the thermal process (Itagi et al., 2023).

The fat content of flour samples ranged from 0.87 in RWF to 3.34 g/100g in KWGRF (Table 2). These differences in lipid content are primarily attributed to the presence of bran components in both WGRFs, which are virtually absent from RWF due to the nature of processing (Ciccoritti et al., 2017; Oppong et al., 2021). The addition of extra fat resulted in an increase of total fat in the range 25.83–27.76 g/100g (Table 2). The fat content of resulting cookies remains higher, regardless of the sweetener used (Klunklin & Savage, 2018).

The fatty acid profile of BWGRF and KWGRF cookies generally

Table 2
Nutritional composition of refined wheat flour and whole grain rice flour samples and resulting cookies with different sweeteners.

Samples/ Parameter	Refined Wheat Flour	Refined Wheat Flour Cashew Cookies			Kalanamak Whole grain Rice Flour	Kalanamak Whole Grain Rice Cashew Cookies			Black Whole grain Rice Flour	Black Whole Grain Rice Cashew Cookies		
		White Sugar	Brown Sugar	Jaggery		White Sugar	Brown Sugar	Jaggery		White Sugar	Brown Sugar	Jaggery
Total Carbohydrate (g/100g)	71.43 ± 0.13b	62.27 ± 0.38c	60.77 ± 0.18d,e	59.94 ± 0.30e	74.15 ± 0.44a	62.27 ± 0.49c	61.60 ± 0.23c,d	58.62 ± 0.62f	72.29 ± 0.14b	62.01 ± 0.29c	61.08 ± 0.30c,d,e	58.70 ± 0.18f
Protein (g/100g)	13.24 ± 0.11a	7.90 ± 0.22c,d	7.90 ± 0.20c,d	8.50 ± 0.30c	10.55 ± 0.39b	7.09 ± 0.09d	7.80 ± 0.21c,d	7.27 ± 0.45d	12.41 ± 0.16a	7.17 ± 0.23d	7.27 ± 0.14d	8.50 ± 0.07c
Fat (g/100g)	0.87 ± 0.13h	25.87 ± 0.20f	25.83 ± 0.09f	26.01 ± 0.28e,f	3.34 ± 0.02g	27.76 ± 0.19a	26.67 ± 0.18c,d	27.67 ± 0.44a	3.02 ± 0.26g	26.35 ± 0.15d,e	27.15 ± 0.15b	26.98 ± 0.16b,c
Ash (g/100g)	0.64 ± 0.07g	1.19 ± 0.06f	1.19 ± 0.06e,f	1.80 ± 0.07c	1.85 ± 0.10c	1.41 ± 0.04d,e	1.46 ± 0.05d	2.20 ± 0.04a	1.90 ± 0.05b,c	1.40 ± 0.04d,e,f	1.44 ± 0.08d	2.07 ± 0.01a,b
Energy (Calories/ 100g)	347.5 ± 0.18e	516.6 ± 1.80a,b	509.0 ± 1.24c	508.1 ± 0.90c	370.6 ± 0.62d	522.3 ± 2.60a	518.3 ± 2.94a	517.5 ± 0.61a	368.3 ± 0.24d	518.8 ± 2.41a	516.7 ± 1.57a,b	511.4 ± 0.89b,c
Insoluble Dietary Fiber (g/100g)	1.88 ± 0.21c,d	1.31 ± 0.00d	1.33 ± 0.40d	1.67 ± 0.09c,d	3.10 ± 0.02a,b	2.51 ± 0.12b,c	2.18 ± 0.18b,c,d	2.23 ± 0.06b,c,d	4.12 ± 0.32a	2.62 ± 0.09b,c	2.72 ± 0.17b,c	3.24 ± 0.26a,b
Soluble Dietary Fiber (g/100g)	1.28 ± 0.05a	1.34 ± 0.13a	0.71 ± 0.16a,b	0.75 ± 0.04a,b	0.77 ± 0.03a,b	0.67 ± 0.41a,b	0.44 ± 0.04a,b	0.13 ± 0.03b	1.03 ± 0.27a,b	0.17 ± 0.01b	0.56 ± 0.20a,b	0.89 ± 0.12a,b
Total Dietary Fiber (g/100g)	3.16 ± 0.26b,c,d	2.65 ± 0.14c,d	2.05 ± 0.24d	2.42 ± 0.14c,d	3.13 ± 0.01b,c,d	3.19 ± 0.30b,c	2.62 ± 0.22c,d	2.37 ± 0.10c,d	5.16 ± 0.05a	2.79 ± 0.08c,d	3.28 ± 0.38b,c	4.13 ± 0.14a,b
Minerals												
Magnesium (mg/ kg)	428.1 ± 4.11g	286.7 ± 2.74h	323.0 ± 11.09h	520.5 ± 3.26f	1692 ± 5.82a	1010 ± 15.99c,d	1069 ± 0.46c	1497 ± 20.92b	1491 ± 20.11b	899.4 ± 2.27e	966.6 ± 1.81d	1466 ± 16.55b
Potassium (mg/ kg)	1806 ± 20.31g	1082 ± 13.79i	1406 ± 48.97h	2287 ± 16.98e,f	3056 ± 23.42d	1840 ± 30.72g	1997 ± 24.90g	4124 ± 62.56b	3770 ± 28.97c	2234 ± 5.82f	2458 ± 6.75e	4759 ± 70.18a
Manganese (mg/ kg)	5.89 ± 0.00g	3.90 ± 0.05h	4.41 ± 0.15h	5.84 ± 0.02g	24.24 ± 0.07a	17.43 ± 0.36d	17.48 ± 0.01d	19.33 ± 0.22c	21.71 ± 0.12b	9.69 ± 0.05f	10.29 ± 0.08f	13.25 ± 0.14e
Iron (mg/kg)	15.25 ± 0.40c	15.70 ± 0.66c	29.21 ± 5.32a,b	25.12 ± 0.14a,b,c	18.33 ± 0.50c	19.76 ± 0.73b,c	22.27 ± 1.69b,c	34.25 ± 2.61a	20.60 ± 0.63b,c	21.99 ± 0.78b,c	19.65 ± 1.33b,c	25.65 ± 0.32a,b,c
Copper (mg/kg)	1.80 ± 0.01d,e	1.31 ± 0.07f	1.33 ± 0.11f	1.36 ± 0.02f	4.96 ± 0.10a	1.67 ± 0.01e,f	1.90 ± 0.17c,d,e	1.82 ± 0.00d,e	3.19 ± 0.00b	2.23 ± 0.00c	2.01 ± 0.05c,d,e	2.18 ± 0.03c,d
Zinc (mg/kg)	7.42 ± 0.14f	4.68 ± 0.13h	4.93 ± 0.22g,h	5.39 ± 0.05g	17.45 ± 0.05b	8.72 ± 0.13e	9.24 ± 0.18d,e	9.88 ± 0.09d	18.95 ± 0.09a	9.42 ± 0.13d	9.64 ± 0.01d	11.23 ± 0.01c
Selenium (mg/ kg)	0.09 ± 0.01c	0.07 ± 0.02c	0.06 ± 0.00c	0.06 ± 0.01c	0.20 ± 0.01a	0.11 ± 0.01b,c	0.10 ± 0.00b,c	0.14 ± 0.00a,b	0.08 ± 0.00c	0.11 ± 0.01b,c	0.09 ± 0.02b,c	0.11 ± 0.01b,c
Vitamins												
Thiamine (Vitamin B1) (ug/g)	0.40 ± 0.06b,c	0.23 ± 0.03c	0.17 ± 0.06c	0.35 ± 0.04b,c	0.32 ± 0.02b,c	0.71 ± 0.05a	0.56 ± 0.10a,b	0.41 ± 0.02b,c	0.29 ± 0.02b,c	0.71 ± 0.05a	0.56 ± 0.1a,b	0.41 ± 0.02b,c
Riboflavin (Vitamin B2) (ug/g)	0.25 ± 0.02a,b	0.20 ± 0.00b	0.26 ± 0.01a,b	0.31 ± 0.02a,b	0.22 ± 0.07a,b	0.25 ± 0.02a,b	0.25 ± 0.02a,b	0.29 ± 0.02a,b	0.41 ± 0.11a,b	0.41 ± 0.01a,b	0.39 ± 0.02a,b	0.43 ± 0.04a
Pantothenic acid (Vitamin B5) (ug/g)	2.05 ± 0.08c	0.61 ± 0.00h	0.54 ± 0.04h	1.38 ± 0.06d,e,f,g	2.98 ± 0.04b	1.15 ± 0.01f,g	1.12 ± 0.02g	1.45 ± 0.07f,g	4.40 ± 0.12a	1.49 ± 0.01d,e	1.28 ± 0.01e,f,g	1.66 ± 0.08d
Pyridoxine (Vitamin B6) (ug/g)	0.95 ± 0.04d,e	0.70 ± 0.01e	0.82 ± 0.00e	1.22 ± 0.03c,d	0.81 ± 0.02e	0.96 ± 0.02d,e	0.95 ± 0.02d,e	1.29 ± 0.04b,c	1.01 ± 0.08c,d,e	1.55 ± 0.04a,b	1.60 ± 0.09a,b	1.73 ± 0.15a

Values are expressed as the means of (3) replicates for total carbohydrate, protein, ash, energy, fat, and minerals two (2) replicates for fiber and vitamins ± standard deviation; Different lowercase letters between rows denote a significant difference ($P < 0.05$).

contain higher levels of unsaturated and polyunsaturated FAs such as oleic, myristic, linoleic, linolenic, and gadoleic acid (shown in Fig. 5a), compared to RWF and RWFbased cookies (Supplementary Table 1). Unsaturated and polyunsaturated FAs are crucial in reducing cholesterol levels which provides various health benefits (Ciccoritti et al., 2017; Joo & Choi, 2012; Kasote et al., 2021; Ruan et al., 2015). Among the fatty acids profiled across samples, oleic acid is particularly abundant in WGRF and its cookies (Fig. 5d). This is particularly the case in KWGRF. Several studies suggest that oleic acid is the most prevalent fatty acid in rice bran (Joo & Choi, 2012; Ruan et al., 2015).

Ash content across flour samples ranged from 0.64 to 1.90 g/100g. Similar trends were observed among the cookies. The ash content contributes to the distinction of BWGRFJ cookies from other cookie samples (PC2, Fig. 2b). Cookies made with jaggery had more ash content (1.80–2.20 g/100g) than those made with brown sugar (1.19–1.44 g/100g) and white sugar (1.19–1.44 g/100g). Similar results were observed by Lamdande et al. (2018) when substituting jaggery for sugar in a muffin formulation. Previously, it was reported that white sugar has an ash value of approximately 0.015% (McKee et al., 2015), while brown sugar and jaggery have values of approximately 0.20% and 1.56%, respectively (Lamdande et al., 2018). The ash content reflects the mineral, fiber, and inorganics remaining in the sample after it has been heated to a very high temperature, eradicating moisture, volatiles, and organics compounds (Altındağ et al., 2015; Islam et al., 2012).

3.2.2. Micronutrient content

ICP-MS data of flour samples revealed varying mineral concentrations between samples (Table 2). KWGRF flour samples had the highest Mg content (1692 mg/kg), followed by BWGRF (1491 mg/kg) and RWF (428.1 mg/kg). The K, Fe, and Zn values in BWGRF were substantially higher than those in the other two varieties of flour. Interestingly in the baked cookies of BWGRF made with white and brown sugar, the Mg and K levels were reduced, and substituting with jaggery maintained the Mg and K levels to the same basal level as in the flour. The mineral content of jaggery-sweetened cookies was found to be generally higher than that of white and brown sugar. For instance, KWGRF cookies with jaggery offer the highest % daily value for Mn per 32g serving (26.99%, KWGRF with jaggery). In PC1, Fe distinguishes KWGRF jaggery-formulated cookies from the other formulations (Fig. 2a). KWGRF jaggery-formulated cookies could provide a 6.26% daily value of Fe per 32g serving. The enhanced mineral content in KWGRF and BWGRF cookies could therefore be attributed to the application of mineral rich WGRFs and the inclusion of jaggery in the formulation. During dough preparation and baking, jaggery crystals dissolve as a result of their interaction with water molecules. This may have caused micronutrients from jaggery crystals to migrate throughout the cookie matrix resulting in mineral-rich cookies (Verma et al., 2019). The presence of vitamin and mineral-rich rice bran in pigmented varieties as compared to non-pigmented varieties also likely accounted for these results (Ciccoritti et al., 2017; Oppong et al., 2021). Lamdande et al. (2018) made comparable findings on muffins formulated with jaggery. In many regions of Asia and Africa, jaggery has a well-established reputation as a nutraceutical due to its abundance of essential amino acids, antioxidants, phenolics, minerals such as Mg, K, Fe, Zn, and Cu, and vitamins. This nutrient-dense profile made jaggery a suitable substitute for white and brown sugar (Lamdande et al., 2018; Rao & Singh, 2022).

The WGRF cookies, regardless of the sweetener used, generally retained the highest levels of B-vitamin (B1, B2, B5, and B6) than RWF-based cookies (Table 2). The vitamin B5 content of cookie samples ranged from 1.66 to 0.54 μg/g, with the BWGRFJ cookies containing the most vitamin B5 and RWFBS cookies containing the least. BWGRFJ cookies also retained the highest vitamin B2 (1.43 μg/g) and vitamin B6 (1.73 μg/g), while BWGRFWS and KWGRFWS had the highest vitamin B1 level (0.71 μg/g), among cookie samples examined in the present study. These results are predominantly attributed to the presence of bran components in both WGRFs, as these components are a known abundant source of B-vitamins, which provide a variety of health benefits (Kasote et al., 2021; Tiozon et al., 2021).

3.3. Antioxidant-based nutraceutical properties of flour and cookie samples

Table 3 presents the levels of γ-oryzanol, phenolics, and total antioxidant capacities in flour and cookie samples. In general, our findings concur with previous research indicating that whole grain rice is an abundant source of bioactive compounds. Furthermore, pigmented whole grain rice is far superior to non-pigmented rice with enriched nutraceutical properties (Goufo & Trindade, 2014; Itagi et al., 2023; Kasote et al., 2021; Tiozon et al., 2021). BWGRF (37.56mg/100 g rice flour) had 4-folds and 1.7-folds more γ-oryzanol than KWGRF and RWF, respectively. γ-oryzanol distinguishes jaggery-sweetened BWGRF cookies (48.82 mg/100 g rice flour) from the other formulations (PC2, Fig. 2b). These BWGRF cookies had 35% and 105% γ-oryzanol than KWGRFJ and RWFJ cookies. Previous reports have shown that γ-oryzanol concentrates on the rice bran (Goufo & Trindade, 2014; Kumari et al., 2015). The presence of bran in both WGRFs may account for the high oryzanol content of the resulting cookies. Recent research evidence suggests that γ-oryzanol may alleviate obesity and cognitive impairment, highlighting its importance to bioactive rice research (Mastinu et al., 2019; Masuzaki et al., 2019).

There was a general decline in the levels of total phenolic compounds (TPC) upon baking. Be that as it may, in comparison to RWC cookies with added refined white sugar, the cookies made from KWGRWS have 2-folds higher phenolics and BWGRWS cookies have 5-folds higher phenolics content (Table 3). Interestingly, the KWGR and BWGR formulated with jaggery not only retained the highest levels of phenolics, but also flavonoids, and anthocyanins (Table 3; Fig. 3b). The TPC value of BWGRFJ cookies (178.1 mg GAE/100g) was 1.5 times greater than in KWGRFJ cookies, 6 times greater than in RWFJ, and 8-times greater than that of RWFWS cookies, which is the formulation with the lowest TPC observation. The total flavonoid content (TFC) of the

Table 3
Nutraceutical properties of refined wheat flour, wholegrain rice flour samples, and resulting cookies with different sweeteners.

Samples/ Parameter	Refined Wheat Flour	Refined Wheat Flour Cashew Cookies			Kalanamak Whole grain Rice Flour	Kalanamak Whole Grain Rice Cashew Cookies			Black Whole grain Rice Flour	Black Whole Grain Rice Cashew Cookies		
		White Sugar	Brown Sugar	Jaggery		White Sugar	Brown Sugar	Jaggery		White Sugar	Brown Sugar	Jaggery
Total oryzanol (mg/100g flour)	10.14 ± 0.37k	11.65 ± 0.29j	13.26 ± 0.15i	15.27 ± 0.20h	21.49 ± 0.43g	32.80 ± 0.31e	31.62 ± 0.23f	40.81 ± 0.36b	34.31 ± 0.28d	37.56 ± 0.15c	37.78 ± 0.26c	48.82 ± 0.79a
Total phenolics (mg GAE/100g flour)	18.76 ± 1.22d	22.57 ± 0.57d	27.07 ± 0.47d	31.80 ± 0.37d	411.0 ± 8.47a	44.61 ± 0.49c	47.28 ± 0.71c	119.0 ± 1.53	632.6 ± 16.20a	103.3 ± 0.84b	166.4 ± 1.27a	178.1 ± 1.84a
Total flavonoids (mg CE/100g flour)	26.17 ± 1.66g	11.80 ± 1.92g,h	9.75 ± 1.81h	41.31 ± 1.92g	166.3 ± 2.74c	88.52 ± 1.66f	118.0 ± 1.31d	169.3 ± 5.14c	271.7 ± 10.90a	116.5 ± 2.83d	98.01 ± 4.28e	222.7 ± 3.10b
Total anthocyanin (mg C-3-GE/ 100g flour)	ND	ND	ND	33.40 ± 1.67f	239.6 ± 25.51d	116.9 ± 16.70e	139.2 ± 9.64e	144.7 ± 25.51e	634.6 ± 33.40a	356.2 ± 9.64c	339.5 ± 34.76c	434.2 ± 16.70b
Total Antioxidant Capacity (mg QE/100g flour)	18.76 ± 1.22f,g	5.80 ± 0.57g	4.96 ± 0.75g	32.11 ± 1.31e,f	164.4 ± 3.39b	44.61 ± 0.49d	47.28 ± 0.71d	54.60 ± 0.47d	289.4 ± 16.20a	34.62 ± 0.84d,e	29.13 ± 1.27f	77.40 ± 5.64c
HAS (mg QE/100g flour)	0.5 ± 0.1f	ND	ND	25.83 ± 0.73d	201.0 ± 1.43b	14.29 ± 0.25f	14.75 ± 0.19f	21.91 ± 0.19e	275.1 ± 1.64a	24.73 ± 0.42d	23.17 ± 0.42d	32.85 ± 0.62c
DPPH radical scavenging activity (%)	16.96 ± 1.16g	14.78 ± 1.55g	30.80 ± 0.45f	26.30 ± 0.35f	43.62 ± 1.61d	35.87 ± 0.47e	17.61 ± 3.12g	40.36 ± 0.51d,e	79.06 ± 0.37a	45.36 ± 0.80d	50.58 ± 1.21c	59.57 ± 1.34b
FRAP (mmol/L TE/g flour)	0.61 ± 0.01f	0.28 ± 0.02g	0.13 ± 0.01g	0.49 ± 0.07f,g	1.75 ± 0.02d	0.80 ± 0.01f	0.83 ± 0.01e,f	1.05 ± 0.05e	8.56 ± 0.23a	2.87 ± 0.04c	3.10 ± 0.02c	6.17 ± 0.11b

Abbreviations: C-3-GE–Cyanidin-3-Glucoside Equivalents; CE–Catechin Equivalents; GAE–Gallic Acid Equivalents; HAS–Hydrogen Peroxide Scavenging Activity; QE–Quercetin Equivalents TE– Trolox Equivalents; ND–Not detected; QE–Quercetin Equivalents; Values are mean ± standard deviation of three independent determinations (n = 3); Different lowercase letters between rows denote a significant difference ($P < 0.05$).

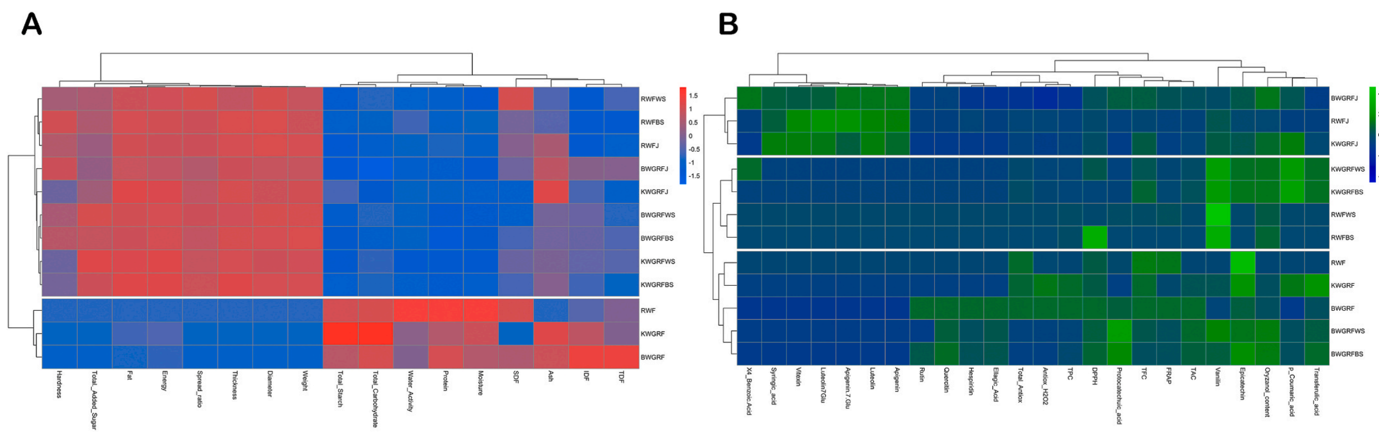

Fig. 3. Heatmaps of the physical attributes and macromolecules (a) and antioxidant components and capacity (b) of RWF and WGRF and resulting cookies with different sweeteners.
Abbreviations: RWF–Refined wheat flour; WGRF–Whole grain rice flour; RWFWS (Refined wheat flour white sugar); RWFBS (Refined wheat flour brown sugar); RWFJ (Refined wheat flour jaggery); KWGRF (Kalanamak whole grain rice flour); KWGRFWS (Kalanamak whole grain rice flour white sugar); KWGRFBS (Kalanamak whole grain rice flour brown sugar); KWGRFJ (Kalanamak whole grain rice flour jaggery); BWGRF (Black whole grain rice flour); BWGRFWS (Black whole grain rice flour white sugar); BWGRFBS (Black whole grain rice flour brown sugar); BWGRFJ (Black whole grain rice flour jaggery). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

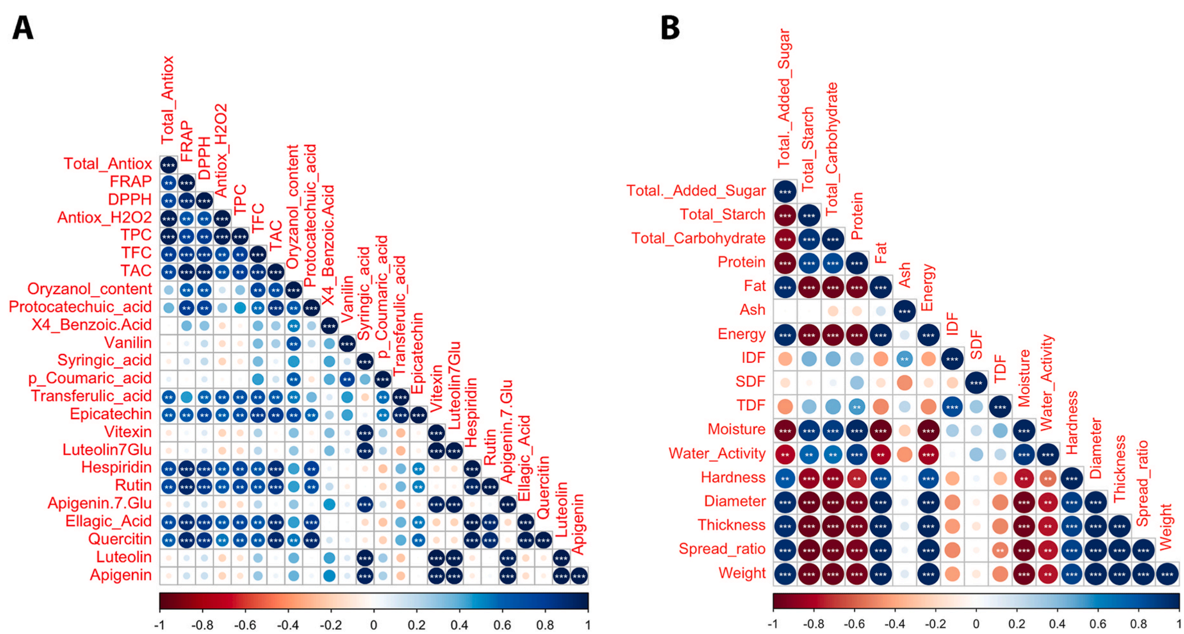

Fig. 4. Correlation plots for antioxidant components and capacity (a) and physical attributes and macromolecules (b) of RWF and WGRF and resulting cookies with different sweeteners. The p-values were signified as follows: ** = $P < 0.05$, ***$P < 0.001$.
Abbreviations: RWF–Refined wheat flour; WGRF–Whole grain rice flour; RWFWS (Refined wheat flour white sugar); RWFBS (Refined wheat flour brown sugar); RWFJ (Refined wheat flour jaggery); KWGRF (Kalanamak whole grain rice flour); KWGRFWS (Kalanamak whole grain rice flour white sugar); KWGRFBS (Kalanamak whole grain rice flour brown sugar); KWGRFJ (Kalanamak whole grain rice flour jaggery); BWGRF (Black whole grain rice flour); BWGRFWS (Black whole grain rice flour white sugar); BWGRFBS (Black whole grain rice flour brown sugar); BWGRFJ (Black whole grain rice flour jaggery). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cookie samples ranged from 9.75 to 222.7 mg CE/100g, with BWGRFJ cookies having the highest values and RWFBS cookies having the lowest. BWGRF-based cookies had a higher total anthocyanin content (TAC) than cookie samples derived from other flours. In addition, the TAC values of BWGRFJ cookies (434.2 mg C-3-GE/100g) were 13-folds greater than those of RWFJ cookies, which had the lowest TAC results.

Antioxidants derived from pigmented rice protect vital lipids, proteins, and DNA from oxidative stress. Therefore, the potential of the antioxidants to combat free radicals was assessed through *in vitro* techniques (Goufo & Trindade, 2014; Tiozon et al., 2021). Across antioxidant capacity assays (total antioxidant capacity, hydrogen peroxide scavenging activity (HAS), DPPH radical scavenging activity, and FRAP), phytochemical rich BWGRF consistently demonstrated the highest values (289.4 mg QE/100g, total antioxidant capacity; 275.1 mg QE/100g, HAS; 79.06%, DPPH; 8.56 mM TE/g, FRAP). A study conducted by Iqbal et al. (2017) on the antioxidant content and capacity of raw and processed sugars showed that jaggery had 118 and 138 times more phenolic compounds than brown and white sugar, respectively. The DPPH radical scavenging and reducing power of the samples followed a similar pattern. Retaining higher levels of TPC, TFC and TAC correlated strongly with antioxidant properties (Fig. 4a). This is further supported by the strong positive correlation between bioactive

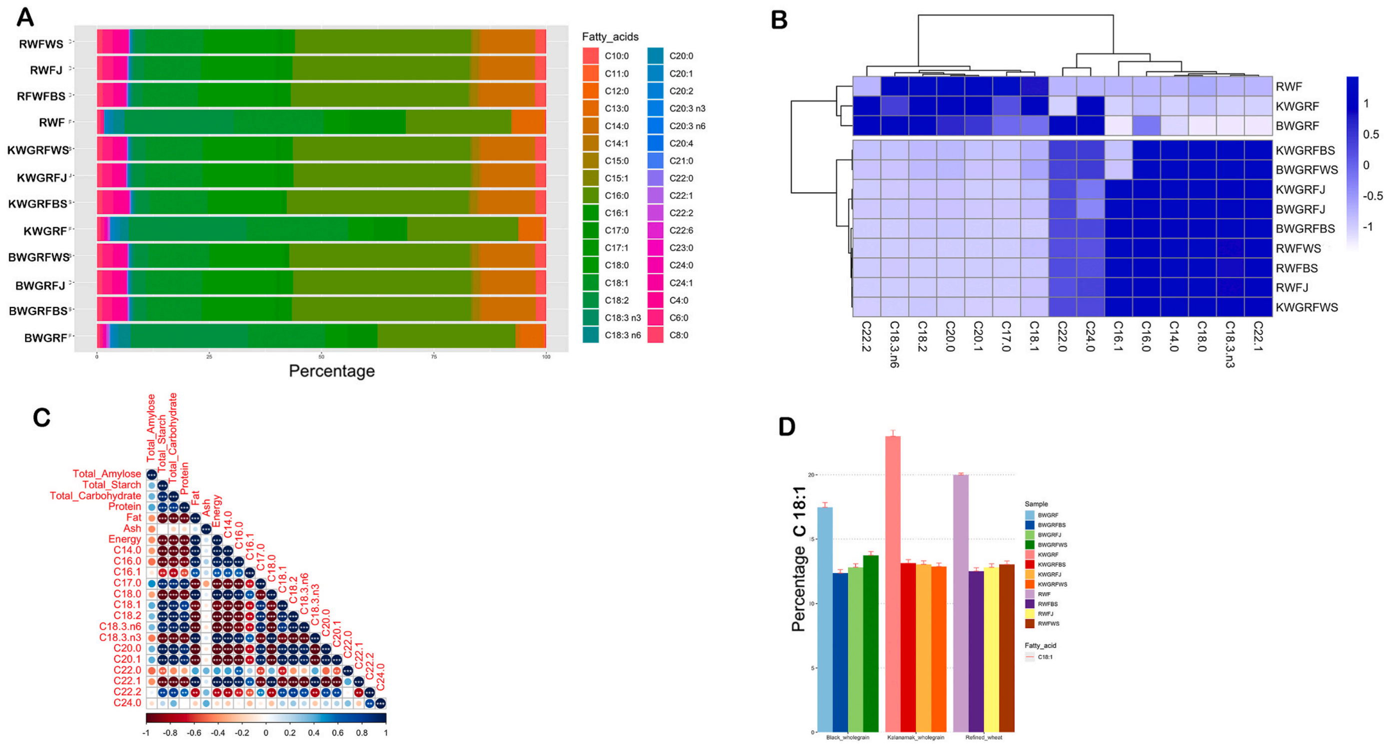

Fig. 5. Fatty acid profile (a), heatmap (b), correlation plot (c), and level of oleic acid in RWF and WGRF and resulting cookies with different sweeteners. Abbreviations: RWF–Refined wheat flour; WGRF–Whole grain rice flour; RWFWS (Refined wheat flour white sugar); RWFBS (Refined wheat flour brown sugar); RWFJ (Refined wheat flour jaggery); KWGRF (Kalanamak whole grain rice flour); KWGRFWS (Kalanamak whole grain rice flour white sugar); KWGRFBS (Kalanamak whole grain rice flour brown sugar); KWGRFJ (Kalanamak whole grain rice flour jaggery); BWGRF (Black whole grain rice flour); BWGRFWS (Black whole grain rice flour white sugar); BWGRFBS (Black whole grain rice flour brown sugar); BWGRFJ (Black whole grain rice flour jaggery). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

compounds and antioxidant capacity, especially hesperidin, rutin, ellagic acid, and quercetin with FRAP and DPPH (Fig. 4a). Heating starch in water causes the granules to rupture. Phenolic and bioactive compounds in the matrix could complex with starch molecules either through inclusion (where the compounds are captured within the starch helices), or non-inclusion (where the compounds are trapped between the helices) (Sudlapa & Suwannaporn, 2023). This complexation process occurred during baking. These results highlight the potential of formulating nutritionally superior GF-products from whole grain rice and nutrient-dense sweetener such as jaggery.

3.4. Sensory analysis of cookie samples

The sensory properties of cookies produced using WGRF and RWF with three different sweeteners (white sugar, brown sugar, and jaggery) were evaluated, and the results are presented in Fig. 6. KWGRF cookies sweetened with white sugar were rated the highest in all sensory properties (8.00) except for crumb color (Fig. 6c). The panelists preferred the crumb color of BWGRF cookies with jaggery (8.00), followed by RWF with white sugar (7.85). In terms of surface color (8.00) and cracking patterns (8.00), BWGRF cookies formulated with jaggery were a close second to KWGRF cookies with white sugar. For aroma, the panelists showed the highest preference for KWGRF cookies with jaggery (7.89). The preference for mouthfeel in KWGRF and RWF cookies (both versions of sweetened with white sugar), could be due to a clean mouthfeel without any residue formation, attributed to lower TDF compared to BWGRF cookies. Similar findings were reported by Baumgartner et al. (2018) on cookies made with dephytinized oat flour. Yildiz and Gocmen (2021) argued that gluten-free bakery products have weaker sensory properties and may not meet consumer expectations due to their harder structure, darker color, unpleasant appearance, and dry-sandy feeling in the mouth compared to conventional gluten-containing products. However, our results suggest that KWGRF and BWGRF cookies, particularly those made with white sugar and jaggery, could compete with RWF-based cookies and thus may be more preferred by consumers.

4. Conclusions

The whole grain cookies made from GI-tagged rice landraces, Kalanamak and Chak-hao, offer distinct sensory qualities and are nutritious that are rich in polyunsaturated fatty acids, minerals, fiber, and bioactive compounds. The Chak-haobased cookies retained the highest levels of phytochemicals with greater antioxidant activities and adding jaggery as sugar alternative exhibited higher levels of Fe and helped to retain higher antioxidant compounds upon baking. These cookies demonstrate good shelf-life stability with a_w levels under 0.85. Although gluten-free formulations spread less than the wheat control, sensory evaluation suggests that acceptability of KWGRF and BWGRF cookies are comparable to RWFbased cookies. The rising demand for nutritious foods provides manufacturers an opportunity to diversify their products to cater to specific markets. Future research can explore the development of more gluten-free functional foods using Kalanamak and Chak-hao rice, catering to both local and global demands. Investigating packaging and storage options is also essential for maintaining shelf stability and nutritional quality.

Informed consent declaration

All panelists granted informed consent before taking part in the study. The research protocol was explained to the panelists, detailing the cookies and their ingredients. Panelists could opt out of evaluation sessions without needing to explain their choice. Evaluations were conducted at the Center of Excellence in Rice Value Addition, Product

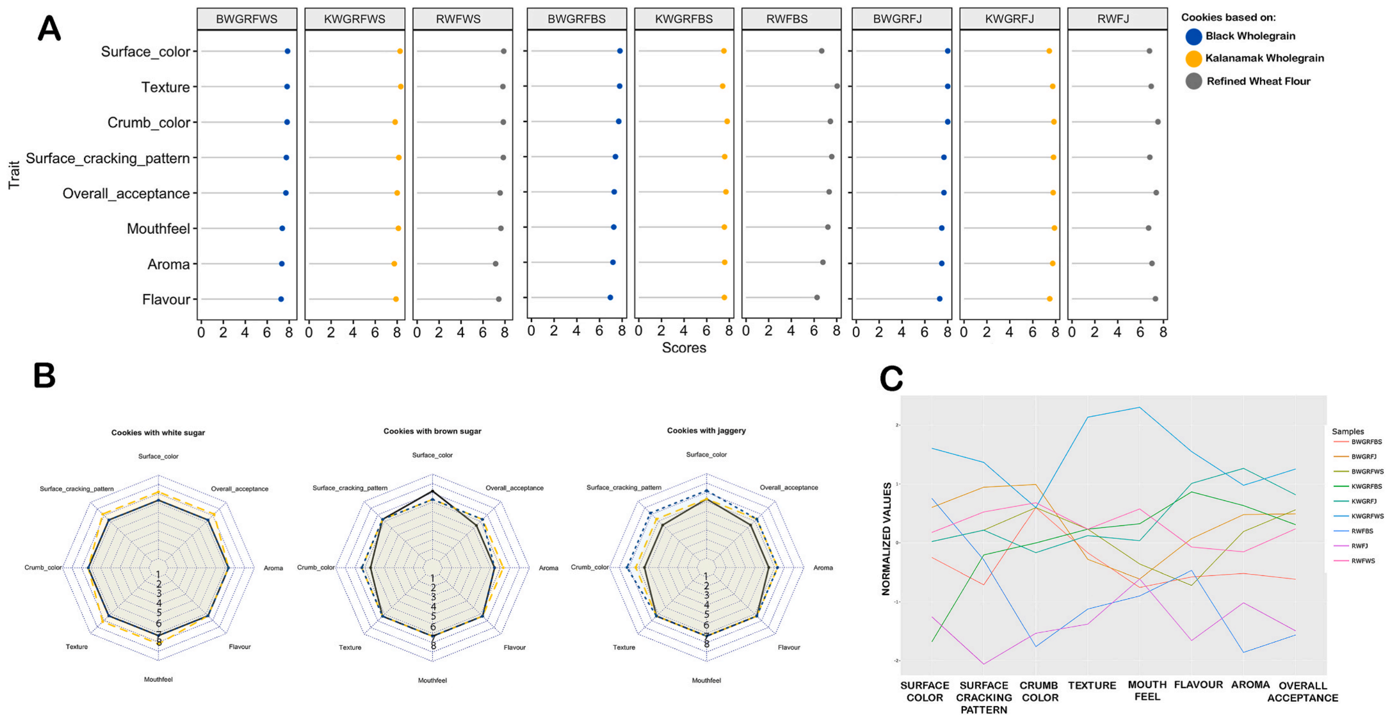

Fig. 6. Sensory profile (a), dot plots (b), spider plots (c), and normalized scores in RWF and WGRF and resulting cookies with different sweeteners. Abbreviations: RWF–Refined wheat flour; WGRF–Whole grain rice flour; RWFWS (Refined wheat flour white sugar); RWFBS (Refined wheat flour brown sugar); RWFJ (Refined wheat flour jaggery); KWGRF (Kalanamak whole grain rice flour); KWGRFWS (Kalanamak whole grain rice flour white sugar); KWGRFBS (Kalanamak whole grain rice flour brown sugar); KWGRFJ (Kalanamak whole grain rice flour jaggery); BWGRF (Black whole grain rice flour); BWGRFWS (Black whole grain rice flour white sugar); BWGRFBS (Black whole grain rice flour brown sugar); BWGRFJ (Black whole grain rice flour jaggery). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Development, and Sensory Laboratory, located at the IRRI South Asia Regional Center in Varanasi, Uttar Pradesh, India. Cookies suitable for consumption were included in the study.

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CRediT authorship contribution statement

Hameeda Banu Itagi: Conceptualization, Investigation, Resources, Supervision. **Kristel June D. Sartagoda:** Methodology, Investigation, Writing – original draft, Writing – review & editing, Data curation. **Nitesh Gupta:** Methodology, Formal analysis, Investigation. **Vipin Pratap:** Methodology, Formal analysis, Investigation. **Priyabrata Roy:** Methodology, Formal analysis, Investigation. **Rhowell N. Tiozon:** Jr, Software, Data curation, Visualization, Writing – review & editing. **Ahmed Regina:** Conceptualization, Supervision, Funding acquisition. **Nese Sreenivasulu:** Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

Authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115245.

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