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# Formulation and characterisation of sustainable nutritious gluten-reduced cookies with indigenous grains from Northeast India†

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This research article explores the formulation and characterisation of sustainable, gluten-free and gluten-reduced cookies through the utilization of a diverse range of grain-based flours. Gluten-free cookies were formulated with a diverse selection of indigenous and regionally available grains from Northeast India. While gluten-free cookie formulations incorporated rice, soy, green gram, finger millet, oats, Bengal gram, and roasted chickpea flours, gluten-reduced cookies incorporated wheat, Bengal gram, and roasted chickpea flours. The conducted study was targeted to assess the impact of alternative grain flour compositions on the sensory, nutritional and functional attributes of the cookies. The investigations contributed to the development of sustainable food products and aligned with the contemporary dietary needs, such as gluten intolerance, increased demand for plant-based protein, dietary fiber enrichment, and the utilization of locally sourced, minimally processed ingredients. Bengal gram and roasted chickpea flours were retained as fixed ingredients across formulations and served as nutrient-rich and functional base components. Comprehensive characterisation included texture, colour, nutritional profiling, and sensory analysis. The findings conveyed significant differences in the sensory appeal and nutritional content, especially in protein and fibre levels, and their dependence upon the grain combinations used. The article provided valuable insights for sustainable bakery alternatives and promotes the utility of locally sourced grains to support health-conscious and environmentally friendly food systems.

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## Sustainability spotlight

This study highlights a sustainability-driven approach to food innovation by formulating gluten-free and gluten-reduced cookies using regionally available and underutilised indigenous grain flours. These alternative ingredients not only reduce dependence on conventional wheat but also valorise the outlook of agricultural products to achieve the goals of sustainability by developing innovative cookie formulations utilising regionally available grain flours. The developed functional cookies address the dietary needs of gluten-intolerant populations, thereby supporting good health and well-being while also fostering local value chains and rural employment opportunities. Furthermore, the use of locally sourced ingredients and minimal processing contributes to lower carbon footprints, aligned with broader climate action goals. This investigative approach reinforces the role of sustainable food innovation at the intersection of nutrition, environmental responsibility and community empowerment.

## 1 Introduction

The notable differences between various cookie types are dependent upon their composition, the dough-making process and the adopted baking parameters. Among several cookies, the sugar-snap cookies are well known. Due to their higher levels of fat and sugar and lower water content, sugar-snap cookies exhibit limited gluten network development. This results in their distinctive texture and indulgent sweetness parameter of the cookies.<sup>1,2</sup> Sugar snap cookies, renowned for their indulgent sweetness and texture, hold a significant place in the global

snack market and have a steady growth trajectory that reflects the consumer demand for the classic treats. The cookies market industry is projected to grow from USD 27.56 billion in 2024 to USD 45.4 billion by 2030, and exhibits a compound annual growth rate (CAGR) of 6.43% during the forecast period (2024–2030) (Source: <https://www.marketresearchfuture.com/reports/cookies-market-1924>). Flour is the main ingredient in cookie dough formulation. It primarily consists of starch, water and protein. The most important components of the flour seem to be those that bind water. These entities, such as starch, protein and arabinoxylan, limit the spreading of the cookie.<sup>1</sup> Researchers<sup>3</sup> studied the starch–water relationship in wheat-based sugar-snap cookie dough systems and concluded that the higher levels of damaged starch are responsible for the smaller final cookie diameters. These observations have also

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been reported in several other investigations<sup>3,4</sup> and were also confirmed by the authors.<sup>5</sup> Due to the minimal gluten development of sugar-snap cookies, it is possible to produce gluten-free cookies through the utilization of gluten-free flours that do not contain gluten and with other raw materials that substitute gluten in the formulation.<sup>6</sup> Researchers<sup>3</sup> investigated the starch–water interactions in wheat-based sugar-snap cookie dough. The authors found that higher levels of damaged starch led to smaller cookie diameters. This finding is relevant to the findings of this article, as the article highlights how flour composition and processing methodology affect the cookie spread and texture. Incidentally, these will be the evaluated key parameters for the prepared gluten-free and gluten-reduced formulations with the alternate grains mentioned in this article. Also, gluten-free flours produce cookies with variant physicochemical characteristics in comparison with cookies made from wheat flour.<sup>7</sup> These properties are dependent upon the cereal origin and the milling process.<sup>8</sup> Most studies that investigated gluten-free cookies have used alternate and varied gluten-free flours such as amaranth,<sup>9–12</sup> buckwheat,<sup>2,8,11</sup> and/or rice flour<sup>13,14</sup> or legume flours.<sup>15,16</sup> Other research articles involved the utilization of oat flour<sup>17</sup> or a mixture of gluten-free flours (brown rice flour, soya flour, buckwheat flour and millet flakes) and starches (corn starch and potato starch).<sup>18</sup> Thus, this section summarizes recent international investigations related to the development of gluten-free formulations with legumes and whole grains. Notable literature being discussed and compared includes the studies of the authors in ref. 18–20 and 21.

Despite such a brief discussion, it is apparent that none of those studies analysed the effect of gluten-free flour, which combines cereals such as rice, legumes such as roasted chickpea, Bengal gram, green gram, and soy and millets such as finger millet and oats in optimized yet variegated combinations which are to be validated with the best sensory score. In this regard, the phrase “best sensory score” refers to the results obtained from the sensory evaluation being conducted for the cookies prepared in the conducted research. Thus, no literature-based sensory data were used for the reported data of the conducted research work.

The aim of the present study was to compare the suitability of different gluten-free flours with a wide range of properties. These refer to nutritional, morphological, thermal, crystallinity, and particle size distribution and are for the optimal preparation of the sugar-snap cookies. Accordingly, it is desired to establish relationships between various flour properties—nutritional composition (protein, fat, carbohydrate, and fiber content), morphological characteristics (particle size and surface structure), thermal behavior (gelatinization temperature and heat capacity), crystallinity, and functional attributes (rheological properties and antioxidant activity)—and the final cookie quality, which is to be assessed in terms of both physical observation and sensory analysis.

## 2 Materials and methods

### 2.1 Raw material and sample preparation

Rice powder (Rajdhani), refined wheat flour (Rajdhani, Select), Bengal gram flour (Besan, Rajdhani-Gram flour Grade-1),

roasted chickpea flour (Sattu, Rajdhani), soy flour (Urban Platter Soya Bean Flour), green gram (Tata Sampann, Unpolished Green Moong Dal (Whole)), finger millet flour (Pure & Sure Organic Ragi Flour), oats (Kellogg's), soybean oil (Fortune Soyabean Oil), coconut oil (Pure & Sure Organic Coconut Oil Cold Pressed), butter (AMUL Butter), ghee (Gowardhan Cow Ghee), peanut butter (Sundrop Peanut Butter), cookie shortening agent (CCDS Bakers Shortening), groundnut oil (Pure & Sure Organic Groundnut Oil), Sugar (Uttam Sugar Sulphurless Sugar), honey (Zandu Pure Honey, 100% Purity, No Added Sugar), artificial sweetener (Sugar Free Natura), and jaggery powder (Organic India) were purchased from the market complex at IIT Guwahati and from the online web portal of Amazon, India. The flours were thereafter packed in an airtight container and were stored in a refrigerator. For the developed cookie samples, proximate characteristics such as moisture content, ash content, soluble protein content, total carbohydrate content, fat content, functional properties, thermal behavior, morphology, particle size distribution *etc.* were assessed. Various chemicals and reagents (Sigma-Aldrich, India: L-ascorbic acid and Bradford reagent; Merck India: absolute methanol, petroleum ether, perchloric acid, sodium hydroxide pellets, dextrose, and oxalic acid dehydrate; Rankem: sodium bicarbonate; SRL Pvt. Ltd, India: bovine serum albumin, 2,6-dichlorophenol indophenol sodium salt (DCPIP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) extra-pure) were deployed in the conducted investigations and were procured as per the needs of the conducted experiments and analyses.

**2.1.1 Cookie formulation.** The detailed procedural descriptions are summarised in the article, and the extended formulation details are presented in the ESI.† In the cookie formulation experiment, the focus was on the optimization of the cookie recipe and the careful balancing of the ingredients for the desired combination of the cookie properties, such as the desirable flavor, texture, and nutritional value. The formulation base consisted of wheat flour (W) and was accompanied by Bengal gram flour and roasted chickpea flour. This combination imparted a harmonious blend of flavors and textures. Each batch of cookies consisted of four cookies and was achieved with standardised 30 g dough balls. The total dry mix weight for four cookies was fixed at 122 g. The selection of fats/oils, ghee,<sup>1</sup> butter,<sup>2</sup> soybean oil, groundnut oil,<sup>3</sup> cookie shortening,<sup>4</sup> or coconut oil<sup>22</sup> was tailored according to specific recipe requirements, and henceforth contributed profoundly to the depth and moisture of the cookies. In the preliminary investigations, a wheat-based cookie formulation was developed through careful consideration of the constitution of each ingredient. For every four-cookie batch, wheat flour (45 g), Bengal gram flour (15 g), roasted chickpea flour (15 g), and baking powder (2 g) were included. The choice of fat – whether ghee, butter, or cookie shortening – was set at 45 g. Such quantification was determined based on the preliminary trials and was meticulously followed to enhance the cookie's texture and flavor profile. To sweeten the cookies, various options were explored. These include sugar (S), jaggery (J), honey (H), and artificial sweeteners (A). The quantity of sweetener varied across formulations, and accordingly, a fixed choice of these (sugar (45



Table 1 Summary of cookie ingredients

Flours	Oils/fats	Sweeteners	Other additives
Rice powder (Rajdhani)	Soybean oil (Fortune)	Sugar (Uttam Sulphurless Sugar)	Baking powder
Refined wheat flour (Rajdhani)	Coconut oil (Pure & Sure)	Honey (Zandu Pure Honey)	Artificial sweetener (Sugar Free Natura)
Bengal gram flour (Besan)	Butter (AMUL)	Jaggery powder (Organic India)	Milk (double-toned, room temperature)
Roasted chickpea flour (Sattu)	Ghee (Gowardhan Cow Ghee)		Vanilla essence
Soy flour (Urban Platter)	Peanut butter (Sundrop)		
Green gram (Tata Sampann)	Cookie shortening (CCDS Bakers)		
Finger millet flour (Pure & Sure)	Groundnut oil (Pure & Sure)		
Oats (Kellogg's)			

g), jaggery (33.75 g), honey (21.95 mL), and artificial sweeteners (4.16 g) was utilized.

Each formulation was assigned a unique sample code to correlate and track with its composition. Thus, the sample codes were WS1 for wheat-sugar-ghee, WJ3 for wheat-jaggery-soybean oil, and WH5 for wheat-honey-shortening. To ensure the dough's softness and effective kneading, room temperature milk (30 °C) was incorporated. This improved the cookie's texture through the combination of the contributed moisture, aided gluten development, uniform mixing promotion and consistency across the batches. Through the addition of milk, moisture is infused into the cookie dough, thereby softening the dough and improving the mouthfeel. The constituent proteins and sugars of milk also enhance the flavour and browning properties of the cookies. Additionally, milk assists in binding ingredients and ensures a more cohesive dough structure and consistent texture in the final product. The conducted formulation experiments underscored the artistry of baking and witnessed the convergence of precision and creativity for the production of delectable treats that delight the senses and nourish the soul. To formulate gluten-free cookies, wheat flour (W) was substituted individually with rice (R), green gram (M), and soy (So) flour. All other ingredients in the dry mix were kept constant. Alternate fats and oils, along with various sweetening components, were used for the sensory variation in the cookie formulations. For the green gram-based gluten-free cookie formulation with diverse taste and dietary preference (MS2), the composition mirrored that of the wheat-based cookies, but with green gram flour (45 g) replacing wheat flour, and with the utilisation of butter (45 g). However, for all cases, the total formulation weight remained at 122 g. Similarly, for the rice-sugar and butter-based cookie formulation, only wheat was replaced with rice flour, and the composition of all other remaining constituents was retained.

For the millet-based gluten-free formulations, namely RaOaJ5 (finger millet-oats-jaggery-shortening formulation) and RaRJ5 (finger millet-rice-jaggery-shortening formulation), the composition resembled the previously mentioned cookie formulation. However, jaggery (33.75 g) was utilised in both formulations, along with the cookie shortening mix (45 g) and finger millet flour (22.5 g). Oats flour (22.5 g) was used in the RaOaJ5 formulation, and rice flour (22.5 g) in the RaRJ5 formulation. These formulations exemplify the addressing of diverse dietary needs and taste preferences and offer a diverse selection of gluten-free cookie options. Thus, in total, six

optimized formulations were tested. These include MS2 (Green Moong-Sugar-Butter), RS2 (Rice-Sugar-Butter), RaRJ5 (Ragi-Rice-Jaggery-Shortening), and RaOaJ5 (Ragi-Oats-Jaggery-Shortening). Table 1 summarizes all the ingredients used for cookie formulation.

**2.1.2 Cookie preparation.** The cookie preparation process begins with the storage of raw materials in containers kept at either room temperature (26 °C) or under refrigeration (4 °C) conditions. These conditions met the specific ingredient requirements and storage recommendations. To start with, in a bowl, all the necessary ingredients are combined. This begins with the powdered sugar or alternative sweetening agents such as jaggery, honey, or artificial sweeteners, and their first phase blending with the fat or oil to eventually generate a smooth and airy mixture. A hand-held mixer was used to conduct such mixing for a duration of 5 to 10 minutes. To ensure uniformity, different flours and powders are meticulously sieved prior to their second phase addition to the fat-sweetening mixture. This prevented the formation of lumps and promoted effective mixing. The second phase mixing facilitated severe mixing for the proper entrapping of the air. Thereby, baking powder was added, and the mixture was matured to form the dough for another 5 minutes. duration of the kneading process. Thereafter, the final touches of vanilla essence and liquid double-toned milk enhanced flavor and moisture content. The flattened dough was kept in the refrigerator for 10 min for better hydration of the cookie. The dough is then portioned into 30 g balls, which are shaped to a specific height of 1 cm and a diameter of 6 cm for individual cookies. The cookies are shaped with a cookie cutter. Thus, uniform thickness and diameters of the cookies were achieved. These shaped cookies are carefully arranged on greased baking paper atop an aluminium foil-covered aluminium tray and are ready for baking. Subsequently, the tray is placed in an oven at a pre-determined temperature and time. This ensured thorough baking of the cookies and the realisation of the desired texture and colour. Once baked, the cookies are allowed to cool before being served or packaged for consumption. This meticulous process guaranteed the proper mixing of ingredients and uniform shaping of cookies. This resulted in the delectable treats for enjoyment.

The cookies were baked in an electrical oven Philips (HD6975/00 25 L Digital Oven Toaster Grill). The baking parameters were a temperature of 150 °C and a baking duration of 20 min. These conditions of the temperature and baking





Fig. 1 Flowchart depicting the preparation procedure for alternate cookie formulations.

duration were selected as per the preliminary trials that targeted the optimization of the cookie texture and color, but with an ensured thorough baking feature. Once baked, the cookies were allowed to cool for 30 min at room temperature. The cookies were individually packed in double-layered pouch bags and were then placed inside a larger pouch bag. This ensured a sealed environment for cookie samples. Silica gel packets were added to the stored system for the absorption of excess moisture and to maintain optimal freshness. The sealed plastic container was stored at room temperature. This effectively preserved the cookies' quality (Fig. 1 and 2).

## 2.2 Methods

### 2.2.1 Sensory score evaluation of the formulated cookies.

Hedonic sensory evaluation of the cookies was conducted with 28 male and female participants (age range of 10 to 70 years).

The subjects were from various socioeconomic backgrounds and were habitual cookie consumers. The considered panel size was suitable for preliminary analysis and evaluation. Hence, with this striking limitation of the conducted sensory analysis, broader consumer acceptability demands larger and diverse panels for extensive sensory analysis.

All cookie samples were analysed one day after the completion of the baking operation. For sensory evaluation, samples were presented as whole pieces on white plastic dishes coded with four-digit random numbers and were served in random order. The cookies were evaluated on the basis of acceptability of their appearance, odour, texture, taste and overall appreciation on a nine-point hedonic scale. The adopted method utilized linguistic data from both subjective and objective evaluations.<sup>23,24</sup> The scale of values ranged from "like extremely" to "dislike extremely", and correspond to the highest and lowest scores of "9" and "1" respectively. Panellists were asked to provide ratings in the form of NS (Not satisfactory), F (Fair), M (Medium), G (Good) and E (Excellent) for samples, and NI (Not Important), SI (Somewhat Important), I (Important), HI (Highly Important), and EI (Extremely Important) for the entire product for fuzzy logic analysis. These were recorded with a fuzzy linguistic score sheet. Excel 2016 (Microsoft Inc., 2016, United States) was applied in this study to analyse the sensory assessment data of the cookies with the fuzzy logic technique.

**2.2.2 Dough rheology properties.** The rheological behaviour of the cookie dough was determined immediately after the

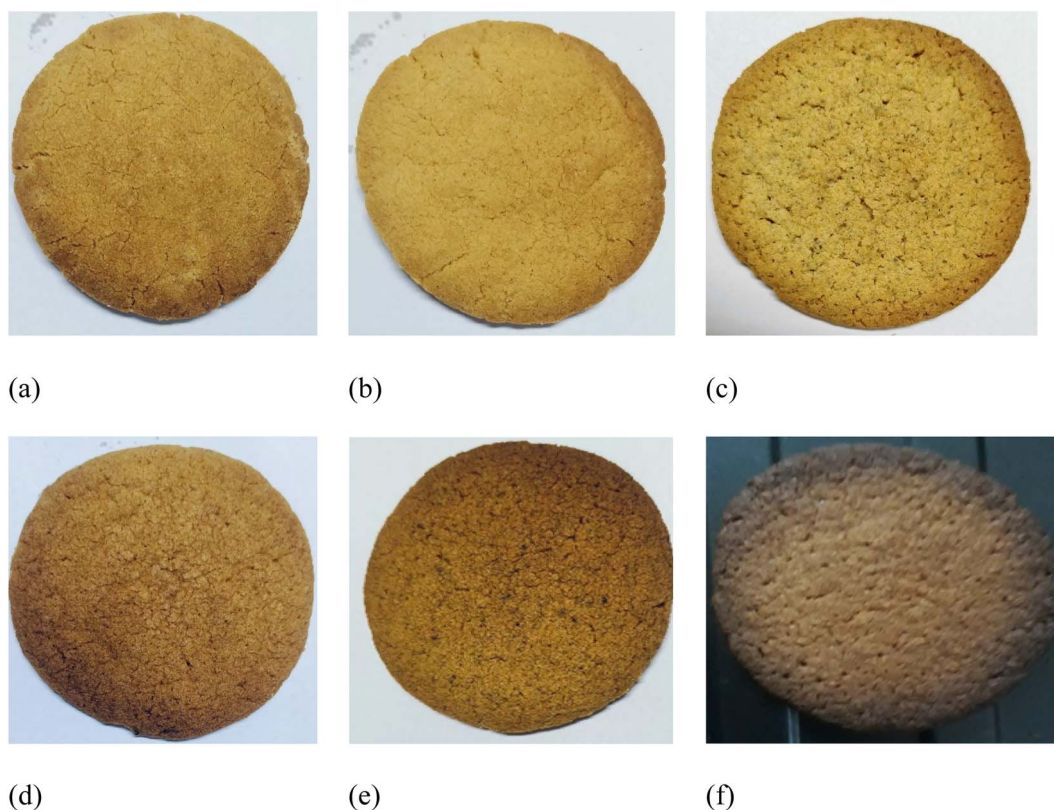


Fig. 2 Photographs depicting grain flour-based alternate cookie formulations, namely (a) WS1, (b) WS2, (c) MS2, (d) RS2, (e) RaRJ5, and (f) RaOaJ5 samples.



sheeting process with a Physica MCR 301 (Anton Paar) by adopting CP50 (cone and plate geometry) at 25 °C.<sup>25</sup> After adjusting the 3 mm gap, vaseline oil (Panreac, Panreac Química SA, Castellar del Vallés, Spain) was applied to the exposed surfaces of the samples. This prevented the drying of the sample during testing. In oscillatory tests, prior to the measurement, the dough was rested for 10 min. First, a strain sweep test was performed at 25 °C with a strain range (%) 0.1–100 at a constant frequency of 1 Hz. This enabled the identification of the linear viscoelastic region. On the basis of the obtained results, a stress value included in the linear viscoelastic region was used in the conducted frequency sweep test at 25 °C with an applied frequency range of 0.1–100 Hz. All samples were analysed in duplicate mode, and the average values were determined and reported.

**2.2.3 Measurements of thickness, spread factor, weight, color and texture.** The thickness of a cookie was determined as the average of the maximum and minimum values of the measured thickness. The spread factor was calculated by dividing the diameter by the thickness of a baked round cookie. Expansion ratio was calculated as the ratio of the cookie volume after baking to that before baking, both of which were determined by multiplying the area of the top of the cookie by its thickness. The area of the top of the cookie was measured using a planimeter. Roughness was obtained by dividing the maximum thickness by the minimum thickness of a cookie. Weight reduction was measured as a percentage of the cookie weight after baking relative to that before baking. Forty-eight replications were considered for all such assessments.

The color of cookie samples was measured with a colorimeter (Datacolor 550, USA).<sup>26</sup> The data were recorded in the L\*, a\*, b\* color system. The colourimeter was calibrated with a standard white plate. Thereafter, the samples were placed in the sample holder for measurement. Color values were recorded as “L\*” (lightness), “a\*” (redness), and “b\*” (yellowness). From a\* and b\* values, the hue angle and chroma were determined.

The texture of the cookies was analysed with a Texture Analyzer (TA.XT. plusC, Stable Microsystems, UK). The

instrument measured the maximum force required to break them in Newtons (N). The measurements were performed using a P/5 cylindrical probe with a 5 mm diameter and a test speed of 10 mm min<sup>-1</sup>. Each cookie sample was cross-checked thrice at different positions, and the average maximum force is reported in this article.

**2.2.4 Nutritional analysis of composite cookies.** The moisture, total carbohydrate, total protein, fat, soluble protein, crude fibre and mineral content of the best-rated composite flour are determined with the standard AOAC (2000) methods. These characterization studies were performed as per the previous research expertise of our research group.<sup>27</sup>

**2.2.5 Differential scanning calorimetry (DSC).** The thermal properties of all powdered cookie samples were analysed using a DSC system (Make: DSC Sirious). The heating rate of the samples was 10 °C min<sup>-1</sup> and was in the 30 to 400 °C temperature range. The onset temperature ( $T_o$ ), final temperature ( $T_f$ ), peak temperature ( $T_p$ ), and delta  $C_p$  values were obtained from the thermographs. The obtained peak temperature was considered as the gelatinization temperature ( $T_g$ ).

**2.2.6 Fourier transform infrared (FTIR) spectroscopy.** The functional groups of all dried flour samples were analysed with ATR-FTIR (FTIR: Shimadzu, Japan, IR Affinity 1). The absorbance of the powdered cookie samples was directly recorded by placing them on the ATR ZnSe crystal and in the wave number range of 4000–400 cm<sup>-1</sup> (spectral resolution of 2 cm<sup>-1</sup> through the co-addition of 64 interferograms). The measurements were carried out under the ambient conditions.

**2.2.7 X-ray diffraction (XRD) analysis.** The XRD analysis of the samples was conducted using an X-ray diffractometer instrument (Rigaku Miniflex 600, USA). The XRD patterns were recorded at a scan rate of 2° min<sup>-1</sup> in the 2 $\theta$  range of 10° to 35° diffraction angle.

**2.2.8 Particle size distribution and morphology.** The average particle size and polydispersity index (PDI) of the cookies were evaluated using a Delsa Nano C particle analyzer (dynamic light scattering method) (DLS, Litesizer™ 500, Anton Paar, USA). Thereafter, 0.1 g of the sample was mixed with

Table 2 Characterization of cookies with their standard references

Section	Method	Standard reference
2.2.1 – Moisture, protein, fat, ash, carbohydrate, fiber	AOAC official methods	AOAC International, <i>Official methods of Analysis</i> , 22nd edn, 2023
2.2.2 – Soluble protein	AOAC 960.52 (Bradford method)	AOAC International, <i>Official methods of Analysis</i> , 22nd edn, 2023
2.2.3 – Antioxidant activity (DPPH)	Based on Blois (1958) or AOAC 2012.04	AOAC International, <i>Official methods of Analysis</i> , 22nd edn, 2023
2.2.4 – <i>In vitro</i> digestion	Adapted from standardized <i>in vitro</i> digestion protocols (INFOGEST)	FAO/WHO codex alimentarius CXS 234-1999
2.2.5 – Differential scanning calorimetry (DSC)	Thermal analysis standard protocols	Refer to ASTM E1356 or AOAC 996.11
2.2.6 – Fourier transform infrared spectroscopy (FTIR)	Standard IR spectroscopy protocols	Refer to AOAC 990.03 or ASTM E1252
2.2.7 – X-ray diffraction (XRD)	Crystallinity analysis of food powders	Refer to ISO 8124 or ASTM D5002
2.2.8 – Particle size distribution	Laser diffraction method	ISO 13320:2020 – Particle size analysis by laser diffraction



20 mL of water and was sonicated for 10 minutes. Thereafter transferred into a cuvette for analysis at 25 °C and a 170° scattering angle. For the assessment, the deployed diluent water possessed a refractive index and viscosity of 1.3328 and 0.8818 cP, respectively. Table 2 summarizes various characterization studies addressed for the prepared cookies.

The morphology of the cookies was assessed using a Gemini 300 FESEM instrument (make – Zeiss, model – Gemini).<sup>28</sup> The powders were first drop-casted onto a piece of glass and aluminium foil. Thereafter, 0.1 g of the sample was mixed with 10 mL of water, and the mixture was subjected to sonication for 10 min. Subsequently, the mixture was subjected to casting on a glass piece covered with aluminum foil. Eventually, the drop-casted sample was attached to the stub with the double-sided carbon tape. Finally, the samples were gold-coated under vacuum to achieve the pre-treated sample for analysis with the Gemini FESEM instrument.

### 2.3 *In vitro* digestion, storage and acrylamide study of sensory-based optimized cookie formulations

**2.3.1 *In vitro* digestion study.** The *in vitro* digestion studies for the sensory optimization of grain gluten-free cookies were conducted as per the procedure reported in a relevant prior art<sup>29</sup> and with a few modifications. Accordingly, the bioaccessibility of the grain-based sensory-optimized cookies. Adopting digestive enzymes, pH adjustments, digestion times, and salt concentrations, simulated environments have been achieved for oral, gastric and intestinal phases of digestion. A brief account of these phases is as follows:

(a) Gastric phase: after the oral phase, the solution was mixed with 30 mL of a 140 mM NaCl and 5 mM KCl solution. Subsequently, 0.5 mL of 11 000 U per mL Himedia pepsin (EC 232-629-3) solution, prepared in SGF electrolyte stock solution, was added to the sample mixture. After thorough mixing, the pH of the mixture was reduced to a value of 5 with 6 M HCl. Thereafter, the vessel containing the final mixture was agitated in a water bath at 100 rpm for two hours at a temperature of 37 °C.

(b) Intestinal phase: after the gastric phase, the solution was well mixed with 2.5 mL of pancreatin (Pancreatin from Porcine Pancreas, P7545-25G, Sigma-Aldrich) solution, prepared in the SIF electrolyte stock solution. To this system, 40 µL of 0.3 M CaCl<sub>2</sub> was added, and the pH of the mixture was adjusted to 7 through the addition of 0.5 cc of 1 M NaOH. Finally, the agitation procedure, similar to that mentioned for the gastric phase, was conducted.

After completing the enzymatic reaction process, the sample solution vessel was submerged in a cold bath for 10 minutes. Eventually, the sample solution was then centrifuged for 40 minutes at 5000 rpm. The resultant supernatant was then tested for total phenolic content and radical antioxidant activity (DPPH scavenging method).

**2.3.2 Storage study.** The storage study of the cookies was conducted as follows. First, the cookies were packed in biaxially oriented polypropylene pouches and were kept under ambient conditions. Thereafter, the cookies were evaluated for shelf life

and through the assessment of moisture content, water activity, free fatty acids, microbiological quality and sensory evaluation at the regular time durations of 0, 30 and 60 days. The moisture content was determined with the oven drying method. The water activity ( $a_w$ ) of freshly powdered cookie samples was measured at 25 ± 0.5 °C using a water activity meter (BIOBASE WA-60 A). The  $a_w$  data was collected from the probe itself.

The fat extracted from the cookie (0.2 g) was diluted with 0.8 mL of CDCl<sub>3</sub>. The mixture was agitated thoroughly and was thereafter transferred to 5 mm NMR tubes. Thereby, <sup>1</sup>H-NMR spectra were recorded with a Bruker Avance III 400 spectrometer (ASCEND 600, Bruker, Germany). The instrument was operated at 9.4 Tesla, which corresponds to a resonance frequency of 400.13 MHz for the <sup>1</sup>H nucleus. Also, the system was equipped with a direct detection probe head with four nuclei and field gradients on the z-axis. The typical parameters for <sup>1</sup>H-NMR spectra were as follows: 45° pulse, 2.05 s acquisition time, 6.4 kHz spectral window, 16 scans, and 26 K data points. The average acquisition time of the <sup>1</sup>H-NMR spectra was approximately 2 min. The spectral acquisition and processing were facilitated with the TopSpin 3.2 software (Bruker BioSpin).

The total plate count (TPC) and yeast and mould count (YMC) for the existence of *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* in the aqueous extracts of the stored cookie samples were analyzed. Plate count agar should be utilized under aerobic or microaerophilic conditions at a temperature of 35 °C to achieve the best results for total plate count. Potato dextrose agar is the chosen medium for the YMC. The plates should be incubated at temperatures of 25 ± 1 °C for 3 to 5 days to ensure sufficient growth of yeasts and molds. To do so, the standard methods described in the manual of the Food and Agriculture Organization, United Nations<sup>30</sup> were adopted.

**2.3.3 Acrylamide study.** Acrylamide is a suspected carcinogen required to be listed on food labels in California and products commercially traded within the European Union. Acrylamide may exist in trace levels in food products such as potato products, coffee, crackers, *etc.* Acrylamide is produced due to the high temperature-based reaction of a few amino acids, such as Asn, Arg and Lys, with the reducing sugars. Current methods for the quantitative detection of acrylamide in food samples are costly, time-consuming, and dependent on expensive scientific instrumentation. Liquid Chromatography-Mass Spectrometry (LC-MS) is a classic example of the same.

In this work, the qualitative assessment of acrylamide in the cookie sample was followed. To do so, Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy was followed, which is inexpensive, fast, and easy to use.

The Fourier transform infrared (FTIR) analysis is a powerful tool that is often used for the identification of alternative types of chemical bonds in a molecule. The infrared absorption spectrum serves as a ‘molecular fingerprint’ and thereby generates a spectrum that facilitates the identification of functional groups in the analysed organic sample. For the detection of acrylamide, an ATR-FTIR spectrometer (Perkin Elmer-Spectrum 1) was used to obtain the processed infrared spectra. The instrument is equipped with a three-reflection



diamond ATR accessory, along with a deuterated triglycine sulfate (DGTS) detector, and uses a Michelson interferometer to disperse the light. The sampling solids facility in the instrument was used to apply uniform pressure to the sample.

**2.3.4 Statistical analysis.** The obtained results were analysed with the Statistical Package for Social Sciences (SPSS), version 17 computer software. The obtained data were presented as mean  $\pm$  standard deviation. The analysis of variance was used for the data analysis related to sensory evaluation. Also, Duncan's new multiple range tests were used to separate and compare the group means. The significance was accepted for the case of  $p < 0.05$ .

## 3 Results and discussion

### 3.1 Sensory score evaluation

For various cookie samples, the assessed sensory evaluation scores conveyed variations across all the attributes. The gluten-reduced sample WS2 (Wheat-Sugar-Butter) exhibited high scores across most attributes, and thereby confirmed its favourable sensory profile. For the sample, excellent scores were obtained for the flavor, color, texture, breakability, taste, after-taste, crispiness, and overall acceptability. Similarly, sample WS1 also scored well. Similarly, the RS2 sample also obtained a good overall acceptability score (8.24). Samples MS2 (GM-Sugar-Butter), RaRJ5 (Ragi-Rice-Jaggery-Shortening) and RaOaJ5 (Ragi-Oats-Jaggery-Shortening) received commendable ratings across all attributes, and excelled particularly in taste, aftertaste, and overall acceptability. This conveyed their potential as preferred options among all evaluated samples. These findings underscore the significance of ingredient combinations and formulations in influencing and ascertaining the sensory characteristics of cookies. Such findings are also effective guides for product development and optimisation efforts. Soybean oil was also analyzed to confirm the promising attributes of a few compositions. Groundnut oil and coconut oil were analyzed for their influence on the typical aroma. This feature has been very much liked by a few sensory panellists.

Based on the fuzzy logic scale, sample MS2 (GM-Sugar-Butter) achieved the highest score of 0.82, and was placed in the "excellent" category. Following closely, sample RS2 (Rice-Sugar-Butter) attained a score of 0.78, and also fell into the "excellent" category. Subsequently, samples WS2 (Wheat-Sugar-

Butter) and WS1 (Wheat-Sugar-Ghee) received scores of 0.75 and 0.72, respectively, placing them within the same "excellent" category. These findings highlight the favourable sensory attributes of these samples, especially in terms of the fuzzy logic methodology-based assessed parameters such as flavor, color, texture, breakability, taste, aftertaste, crispiness, and overall acceptability. According to the fuzzy logic scale, sample RaOaJ5 (Ragi-Oats-Jaggery-Shortening) received the highest score of 0.86, and the sample is henceforth placed in the "very good" range. Subsequently, sample RaRJ5 (Rice-Ragi-Jaggery-Shortening) obtained a score of 0.74, and is positioned in the same "very good" category. In such an evaluation, it shall be noted that the taste and overall acceptability are deemed to be of extreme importance. This is followed by flavor, color, and breakability, which hold high importance. Aftertaste and texture are considered to be important, but to a lesser extent. Similar to the reported findings of the article,<sup>21,23</sup> reported superior sensory scores for millet-based cookies that were enriched with buckwheat and quinoa flours. Accordingly, sensory scores between 8.1 and 8.4 were achieved by the authors. These findings align with the high acceptability sensory scores obtained in the MS2 and RS2 formulations of these articles. Thus, in summary, the findings emphasize the significance of taste and overall acceptability in determining the perceived quality of the cookie samples.<sup>31</sup> They also highlight the importance of flavor, color, and breakability in enhancing the consumer scores (Table 3).

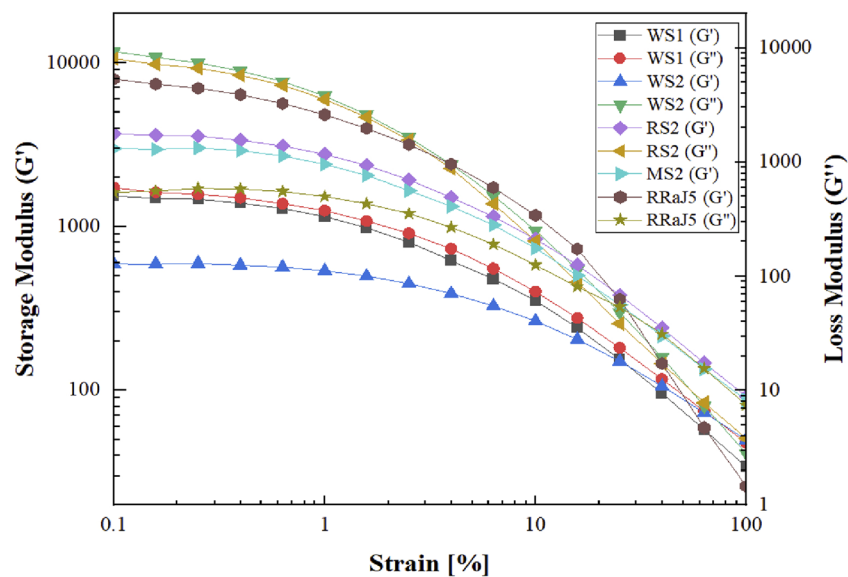
### 3.2 Dough rheology

The measured dough rheological analysis in terms of the amplitude sweep data provided a detailed insight into the rheological characteristics of various dough samples and for the range of compositions for alternate formulations such as the wheat-sugar-ghee (WS1), wheat-sugar-butter (WS2), rice-sugar-butter (RS2), green moong-sugar-butter (MS2), rice-finger millet-jaggery-shortening (RaRJ5), and finger millet-oats-jaggery-shortening (RaOaJ5). For each sample, its behaviour is evaluated in terms of its strain, storage modulus, and loss modulus. The strain, represented as a percentage, serves as the independent variable (plotted on the *x*-axis), and the storage and loss moduli, both measured as dependent variables in Pascals, are depicted on the *y*-axis. The data examination

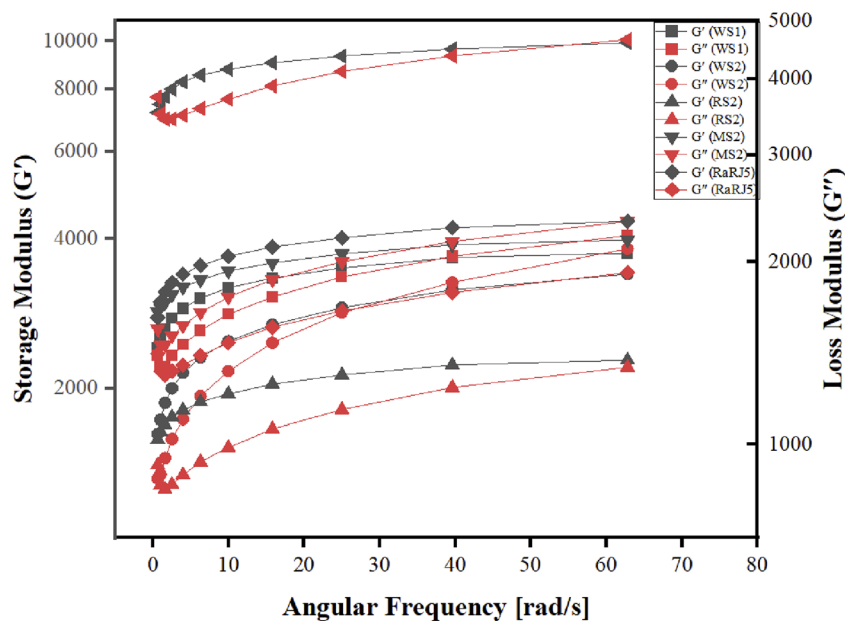
Table 3 Sensory score of gluten-free and gluten-reduced grain-based cookies

S. no.	Sample description	Sample ID	Flavor	Color	Texture	Breakability	Taste	After taste	Crispiness	Overall acceptability
1	Wheat-sugar-ghee	WS1	8.29 $\pm$ 0.8	8.14 $\pm$ 0.69	8.5 $\pm$ 0.76	8.35 $\pm$ 1.1	8.5 $\pm$ 1.25	8.42 $\pm$ 1.2	8.33 $\pm$ 1.09	8.44 $\pm$ 0.94
2	Wheat-sugar-butter	WS2	8.29 $\pm$ 0.5	8.4 $\pm$ 0.81	8.64 $\pm$ 0.89	8.28 $\pm$ 1.11	8.07 $\pm$ 0.83	8.07 $\pm$ 0.83	8.21 $\pm$ 0.91	8.56 $\pm$ 1.11
3	GM-sugar-butter	MS2	8.21 $\pm$ 0.9	8.35 $\pm$ 1.07	8.47 $\pm$ 1.34	8.16 $\pm$ 0.89	8.71 $\pm$ 1.11	8.57 $\pm$ 0.97	8.36 $\pm$ 0.95	8.78 $\pm$ 1.04
4	Rice-sugar-butter	RS2	8.14 $\pm$ 1.1	8.14 $\pm$ 1.1	8.48 $\pm$ 1.62	8.59 $\pm$ 1.28	8.45 $\pm$ 1.43	8.27 $\pm$ 1.4	8.13 $\pm$ 1.28	8.24 $\pm$ 1.31
5	Ragi-rice-jaggery-shortening	RaRJ5	8.07 $\pm$ 0.92	8.05 $\pm$ 1.43	7.91 $\pm$ 1.34	7.89 $\pm$ 1.27	7.99 $\pm$ 1.11	8.29 $\pm$ 1.15	7.98 $\pm$ 1.26	8.09 $\pm$ 1.02
6	Ragi-oats-jaggery-shortening	RaOaJ5	8.28 $\pm$ 1.25	8.08 $\pm$ 1.11	8.17 $\pm$ 1.27	7.85 $\pm$ 1.07	8.64 $\pm$ 1.11	7.85 $\pm$ 1.10	8.00 $\pm$ 1.41	8.21 $\pm$ 0.99





(a)



(b)

Fig. 3 Dough rheology of optimal cookies: (a) amplitude sweep and (b) frequency sweep.

revealed distinct trends in the mechanical response of the dough samples to independently varying strain levels. Generally, an increase in strain correlates with elevated storage and loss moduli across most samples and is indicative of heightened stiffness and viscosity under greater deformation. An increase in dough stiffness (storage modulus) and viscosity (loss modulus) conveys a more solid-like and less deformable dough structure. Such a property influences baking performance in terms of the reduced cookie spread during baking, enhanced shape retention, and firmer texture in the final product. Also, increased viscosity conveys better absorption during mixing

and baking, and this affects the texture and mouthfeel characteristics of the cookies. However, the specific nuances in the behaviour of each dough composition underscore the importance of understanding the unique rheological properties of different formulations. Such insights are invaluable for the optimization of the processing parameters and for the development of tailored food products with desired textural attributes.

The rheological behavior of various dough samples, including wheat-sugar-ghree (WS1), wheat-sugar-butter (WS2), rice-sugar-butter (RS2), green moong-sugar-butter (MS2), ragi-



rice-jaggery-shortening (RaRj5), and ragi-oats-jaggery-shortening (RaOaJ5), was investigated at different angular frequencies. For all samples, the storage modulus, representing the dough's stiffness, generally increased with increasing angular frequency. This conveyed its greater ability to resist deformation. Additionally, the loss modulus, reflecting the dough's viscous behavior and energy dissipation, exhibited varying trends across samples. While some samples demonstrated an increase in loss modulus with angular frequency and suggested higher viscosity, others displayed relatively constant or reduced loss modulus values. These findings provide valuable insights into the rheological properties of dough under different processing conditions. Such insights aid in the baking parametric optimization tasks and in ascertaining the desired product characteristics (Fig. 3).

The rheological analysis of rice sugar cookie formulations, incorporating different fats and oils such as cookie shortening (CS), peanut butter (PB), soybean oil (SB), and coconut oil (CN), elucidates distinct characteristics in storage and loss modulus for each variant. Ghee-based doughs demonstrate notably higher storage modulus values in comparison to butter, PB, SB, and CN oil-based doughs. The trends convey a more solid-like behaviour. This is due to the ghee's higher saturated fat content and crystalline structure. In contrast, butter doughs exhibit relatively lower storage modulus values and signify a softer consistency. PB and SB doughs show intermediate storage modulus values and reflect their semi-solid nature. Additionally, CN oil-based doughs display the lowest storage modulus and convey a more fluid-like behaviour. Regarding loss modulus, ghee doughs have a higher parametric value in comparison to the other formulations. This suggests greater energy dissipation during deformation. The rheological behavior of the reported dough formulations of the article is consistent with the trends reported by the author.<sup>32</sup> In this article, the authors reported that the soy-enriched doughs exhibited higher consistency coefficients.<sup>32</sup> Thus, the comparative analysis underscores the significant influence of fat and oil composition on the rheological properties of rice sugar cookie dough, and provides useful insights into formulation optimisation for the desired product attributes.<sup>33</sup> Such rheological insights are crucial for the optimization of cookie formulations, as they assist in the prediction of dough behavior during mixing, shaping, and baking operations. Thus, a greater understanding of the viscoelastic properties allows formulators to adjust ingredient ratios—such as flour type, fat content, and moisture level—to achieve desired texture, spreadability, and structural integrity in the final baked product. Thus, through the analysis of the storage and loss moduli, formulations can be fine-tuned to balance softness and crispiness, prevent excessive spreading, and ensure consistent quality across batches (Fig. 4).

### 3.3 Nutritional characteristics

**3.3.1 Mineral content.** The assessed data offers useful insight into the mineral content of various cookie samples, and in terms of the assessed concentrations of elements such as sodium (Na), magnesium (Mg), aluminum (Al), potassium (K),

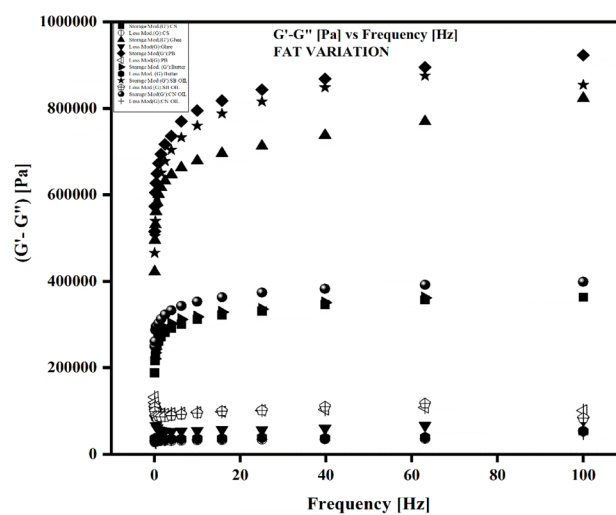


Fig. 4 Dough rheology for RS2 with different fats and oils.

calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) (measured in parts per million (ppm)). Conversely, the rice-based cookie (RS2) displays lower concentrations of most elements (potassium at 17.97 ppm) and suggests a comparatively lower mineral content. MS2 contains Na content of 46.67 ppm and a good amount of Mg (7.7 ppm) and Al (6.21 ppm). This may impact the nutritional value of the cookies by providing fewer essential micronutrients such as calcium, magnesium, and potassium. These minerals do have a critical influence on bone health, muscle function, and electrolyte balance. Therefore, formulations with lower mineral concentrations are likely to be less beneficial for individuals seeking nutrient-dense snack options and for scenarios in which populations are at a greater risk of mineral deficiencies. The findings are also in agreement with our previous findings on the characterisation of green gram, roasted chickpea and Bengal gram mineral content. Similarly, WS2, another variant consisting of wheat-sugar-ghee, displayed substantial concentrations of potassium (22.22 ppm), calcium (0.56 ppm), and manganese (0.042 ppm). However, its levels of magnesium (2.85 ppm) and copper (0.02 ppm) are marginally lower in comparison to the MS2 sample. Comparatively, RaRj5 and RaOaJ5, millet-based gluten-free formulations, display notable concentrations of calcium (3.36 ppm and 3.49 ppm, respectively), along with substantial levels of magnesium (8.85 ppm and 9.94 ppm, respectively) and manganese (0.56 ppm and 0.68 ppm, respectively). These findings underscore the significance of ingredient choice in determining the nutritional quality of cookies and have certain desired implications for dietary diversity and health (Table 4).

**3.3.2 Other proximate contents.** Table 5 presents the nutritional composition of different samples labelled WS1 to RaOaJ5. Sample WS1 contained 23.58 g per 100 g of total carbohydrate, 2.56 g per 100 g of total protein, 1.7 g per 100 g of soluble protein, 11.14 g per 100 g of total fat, and 0.98 g per 100 g of ash. Among the formulations, MS2, a green gram-based gluten-free cookie, stands out with the highest protein content



Table 4 Mineral content analysis of optimized grain flour cookies

S. no.	Sample code	Mg (ppm)	K (ppm)	Ca (ppm)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
1	WS1	2.39 ± 0.25	19.60 ± 0.42	0.37 ± 0.08	0.04 ± 0.01	0.21 ± 0.04	0.03 ± 0.01	0.07 ± 0.01
2	WS2	2.85 ± 0.55	22.22 ± 1.8	0.56 ± 0.28	0.04 ± 0.05	0.34 ± 0.62	0.02 ± 0.08	0.076 ± 0.04
3	MS2	7.70 ± 0.57	60.98 ± 3.9	1.03 ± 0.16	0.06 ± 0.04	0.34 ± 0.15	0.38 ± 0.27	0.13 ± 0.04
4	RS2	2.55 ± 0.95	17.97 ± 2.33	0.38 ± 0.27	0.03 ± 0.07	0.18 ± 0.67	0.015 ± 0.02	0.089 ± 0.09
5	RaRJ5	8.85 ± 1.06	54.13 ± 0.53	3.36 ± 0.49	0.56 ± 0.38	0.29 ± 0.07	0.03 ± 0.20	0.08 ± 0.05
6	RaOaJ5	9.94 ± 2.04	57.01 ± 0.85	3.49 ± 1.56	0.68 ± 0.08	0.33 ± 0.13	0.03 ± 0.01	0.13 ± 0.05

Table 5 Nutritional composition of best-rated composite cookies

S. no.	Sample	Total carbohydrate (g per 100 g)	Total protein (g per 100 g)	Soluble protein (g per 100 g)	Total fat (g per 100 g)	Ash (g per 100 g)
1	WS1	23.58 ± 0.21	2.56 ± 0.16	1.7 ± 0.67	11.14 ± 0.5	0.98 ± 0.3
2	WS2	23.58 ± 0.36	2.56 ± 0.54	1.05 ± 0.34	9.11 ± 0.82	1.44 ± 0.6
3	MS2	22.87 ± 1.05	14.31 ± 0.43	5.66 ± 0.48	11.43 ± 0.97	2.08 ± 0.5
4	RS2	30.5 ± 1.1	2.9 ± 0.66	1.22 ± 0.07	11.5 ± 0.8	3.01 ± 0.84
5	RaRJ5	17.77 ± 2.05	2.57 ± 0.88	0.91 ± 0.27	11.78	1.26 ± 0.41
6	RaOaJ5	19.86 ± 1.07	3.49 ± 0.75	1.21 ± 0.68	12.07	2.36 ± 1.01

(14.31 g per 100 g) and soluble protein (5.66 g per 100 g). While RS2, a rice-sugar-butter-based formulation, exhibits the highest total carbohydrate content (30.5 g per 100 g), RaRJ5, a millet-rice-jaggery formulation, ascertains relatively lower total carbohydrate content (17.77 g per 100 g). In terms of fat content, RaOaJ5, another millet-based gluten-free formulation, demonstrates a notable level of total fat (12.07 g per 100 g). Additionally, the ash content, indicating the mineral content of the cookies, varies across formulations, and RS2 has the highest ash content (3.01 g per 100 g). Similarly, other samples exhibited variations in their nutritional content. The protein and fiber enhancement being confirmed for the MS2 formulation aligns with those reported by the authors.<sup>32</sup> The authors inferred that the soy and chickpea flour together significantly improved nutritional density in gluten-free cookies.<sup>32</sup> These nutritional values were evaluated in the context of recommended daily intake levels.<sup>34</sup> For example, the protein content in sample MS2 (14.31 g per 100 g) contributes significantly toward the Recommended Dietary Allowance (RDA) of 46 g per day for adult females and 56 g per day for adult males (USDA, 2019). Similarly, the fiber content across all samples (approximately 2 g per 100 g) is modest in comparison to the Adequate Intake (AI) of 25–30 g per day. This conveys that there is a good scope for the enhancement of dietary fiber. The fat and carbohydrate contents fall within the Acceptable Macronutrient Distribution Ranges (AMDRs) of 20–35% and 45–65% of daily energy intake (<https://ods.od.nih.gov/HealthInformation/nutrientrecommendations.aspx>). Such variations in the nutritional composition provide insights into the dietary profile and potential health implications of the samples. Additionally, they can inform dietary choices and formulations in food product development and can cater to specific nutritional needs and preferences.

**3.3.3 In vitro study.** The DPPH radical scavenging activity of baked samples WS1 and WS2 was measured to be 26.78% and 24.93%, respectively. RaRJ5 and RaOaJ5 displayed elevated levels of antioxidant activity at 34.42% and 37.81%, respectively. Similarly, RS2 and MS2 showed antioxidant activities of 32.22% and 33.51% respectively. Following *in vitro* digestion, a loss of antioxidant activity was observed across all samples.<sup>35</sup> While WS1 and WS2 exhibited the lowest retention of antioxidant activity at 4.06% and 4.47% respectively, RS2 and MS2 retained higher levels at 9.13% and 10.38% respectively. Notably, RaRJ5 and RaOaJ5 exhibited the highest retention of antioxidant activity post *in vitro* digestion, and had values of about 17.25% and 19.82%, respectively. The reported DPPH findings for RaOaJ5 and RaRJ5 in this article are comparable to the antioxidant activity values reported for soy and corn flour-based cookies in ref. 32. Thus, the findings are in agreement with the general trend of the antioxidant potential of legume and millet-based formulations.<sup>36</sup>

### 3.4 Physical properties

Table 6 presents the physical characteristics and hardness measurements of different samples labelled WS1 to RaOaJ5. Sample WS1 exhibited a weight of 27.59 g, a diameter of 79.34 mm, and a thickness of 8.03 mm, and had a spread ratio of 9.88. The color parameters indicated a hue angle of 68.17°, a chroma value of 39.51, and color coordinates ( $b^*$ ,  $a^*$ ) of (14.69, 36.68). Its hardness was measured at 11.81 N. Similarly, other samples showed variations in weight, diameter, thickness, spread ratio, color parameters (hue angle, chroma, and color coordinates), and hardness. The analysis of the cookie samples revealed significant variations in their physical characteristics and hardness. Conversely, cookies with ragi and oats (RaRJ5 and RaOaJ5) tend to have lower hardness values despite having



Table 6 Physical properties of best-rated cookies

S. no.	Sample code	Wt. (g)	Dia (mm)	Thickness (mm)	Spread ratio	Color				Hardness
						$b^*$	$a^*$	Hue Angle	Chroma	(N)
1	WS1	27.59	79.34	8.03	9.88	36.68	14.69	68.17	39.51	11.81 ± 0.51
2	WS2	26.96	76.27	8.2	9.3	39.15	14.31	69.92	41.68	15.27 ± 22.08
3	MS2	27.93	81.27	8.1	10.03	38.05	14.58	69.03	40.75	5.07 ± 2.8
4	RS2	26.55	83.26	7.48	11.13	39.65	15.88	68.17	42.71	2.62 ± 1.6
5	RaRJ5	28.27	76.3	8.9	8.57	19.47	10.89	60.78	22.31	13.36 ± 2.55
6	RaOaJ5	26.71	69.93	5.1	13.71	24.14	13.25	61.24	27.54	16.05 ± 5.32

similar spread ratios. This is mostly due to the fibrous nature of these ingredients, which may contribute to a softer texture. These findings underscore the influence of ingredient composition on cookie texture and hardness, and highlight the potential for tailored formulations to achieve desired sensory attributes and quality. Higher  $\Delta C_p$  values convey that the cookie matrix requires more energy to undergo phase transitions (e.g., gelatinization or melting). Such an aspect typically correlates with stronger molecular interactions and a more cohesive texture. Such a value can also imply better shelf stability due to reduced susceptibility to moisture or temperature fluctuations.

Thus, the higher energy absorption observed in the DSC analysis, inferring elevated  $\Delta C_p$  values, reflects greater thermal transitions within the cookie matrix. This suggests a more complex and stable internal structure, which contributes to improved texture—such as enhanced crispiness or firmness—and better resistance to structural breakdown during baking and storage.

### 3.5 DSC curves

Differential scanning calorimetry (DSC) analysis revealed distinct thermal characteristics among the various cookie formulations. Conversely, MS2 exhibited the lowest melting point at 190 °C, suggesting a comparatively weaker structure, and is supported by its lower  $\Delta C_p$  value of 1.014 J (g K)<sup>-1</sup>. Remarkably, RaOaJ5 displayed the highest  $\Delta C_p$  value of 2.316, which is indicative of significant energy absorption during the melting process. WS1, with a melting peak at 220 °C and  $\Delta C_p$  of 0.762, exhibited relatively lower thermal stability. WS2 showed a slightly lower melting peak at 217.9 °C but a higher  $\Delta C_p$  of 1.422, suggesting enhanced thermal stability in comparison to the WS1 sample. Fig. 5 shows the DSC curves for all the optimal cookie formulations.

### 3.6 FTIR curves

Fig. 6 depicts the FTIR spectra of the alternate best-performing grain flour-based gluten-free cookie formulations. Broadband absorption observed between 3600 and 3000 cm<sup>-1</sup> and across all cookie formulations conveys significant water absorption, and is consistent with previous studies.<sup>37</sup> Peaks at 3360 and 3180 cm<sup>-1</sup> correspond to N–H valence stretching vibrations from cross-linking bridges and hydroxyl [OH] groups, and suggest the presence of proteinaceous and polysaccharide components.<sup>37</sup> The FTIR peaks corresponding to N–H stretching and carbohydrate-related vibrations suggest the presence of proteinaceous and polysaccharide components in the cookie formulations. These biomolecules contribute to the nutritional value by supplying essential amino acids and dietary fiber. These support muscle repair, satiety, and digestive health. The presence of the C=O functional group, typically associated with lipids, is evident in the spectral region between 1746 and 1720 cm<sup>-1</sup> across all formulations. Bands corresponding to peptide linkages, such as amide I (1690–1600 cm<sup>-1</sup>), amide II (1575–1480 cm<sup>-1</sup>), and amide III (1301–1229 cm<sup>-1</sup>), further

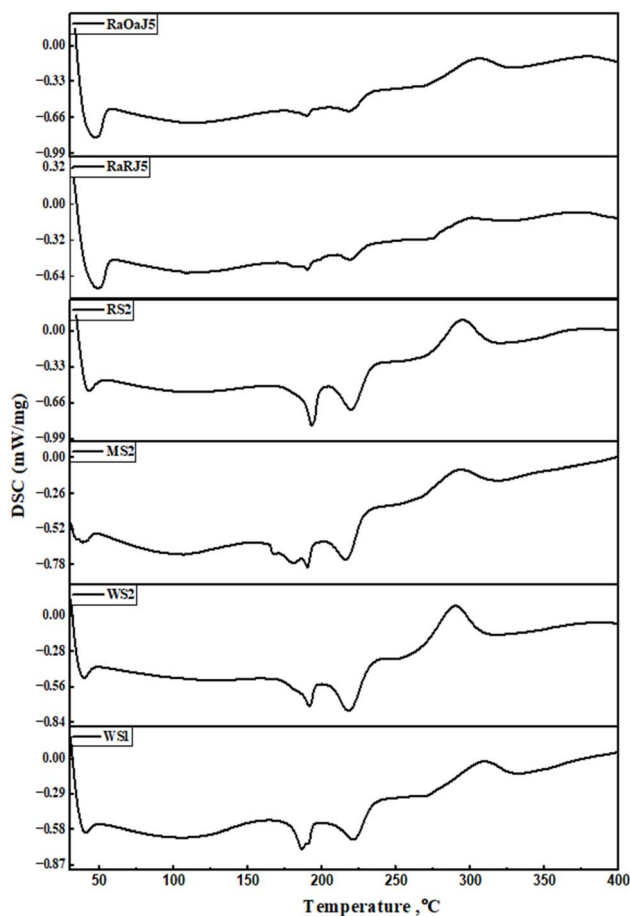


Fig. 5 DSC curves of the best-rated optimal formulation of the cookies.



support the presence of proteins within the cookies.<sup>38</sup> Additionally, bands located at  $1200\text{--}900\text{ cm}^{-1}$  are attributed to C–O and C–C stretching vibrations, as well as C–O–H and C–O–C deformations of carbohydrates. These indicate the carbohydrate content within the cookie formulations.<sup>39</sup> These spectral features collectively provide insights into the chemical composition and structural characteristics of the cookies, and highlight the presence of proteins, lipids, and carbohydrates, all of which contribute to their overall texture and flavour.

**3.6.1 Qualitative detection of acrylamide.** Acrylamide, an unsaturated amide, is found in various thermally processed foods. It is generated in food products containing a high content of reducing sugars, such as glucose, and proteins, especially rich in asparagine, an amino acid. It is often achieved after heating the constituent to a high temperature ( $>170\text{ }^{\circ}\text{C}$ ).

Fourier transform infrared (FT-IR) is a powerful tool for the identification of various types of chemical bands in a molecule. It is produced through an infrared absorption spectrum and is referred to as the molecular “fingerprint”. FT-IR is also the most useful method for the identification of constituent chemicals in organic samples. The presence of all the mentioned peaks associated with acrylamide in a food sample would strongly suggest the presence of acrylamide. However, the absence of one or more peaks does not necessarily mean that acrylamide is not present.

The formation of acrylamide in food is a complex chemical process.<sup>40</sup> It is influenced by various factors such as temperature, moisture content, pH, and the composition of the food matrix. Therefore, the presence and intensity of peaks in the FTIR spectrum may vary the acrylamide content, and these are dependent on these factors.

Thus, in some cases, acrylamide may form in trace amounts. This leads to weak or less prominent peaks in the FTIR spectrum. Additionally, the detection limit of the FTIR instrument and the sensitivity of the method may influence the ability to detect low levels of acrylamide.

While the presence of characteristic peaks associated with acrylamide is a strong indicator of its presence, it's essential to consider other analytical methods and techniques for confirmation, especially when dealing with lower concentrations or complex food matrices. Thus, the FTIR provides preliminary qualitative insights, and the article acknowledges its limitations in terms of specificity and sensitivity. A paragraph in the discussion section explicitly conveys that the inferences are qualitative in nature, and quantitative inferences shall be explored in the future through the conduct of LC-MS or GC-MS for the affirmed presence of acrylamide in the cookie samples. Furthermore, even enzyme-linked immunosorbent assays (ELISA) can be followed for quantitative analysis and confirmation of acrylamide presence.

Based on the provided peaks, the presence of peaks at  $1644\text{ cm}^{-1}$  and  $3330\text{ cm}^{-1}$  suggests that acrylamide may have existed in samples WS1 and WS2; the presence of peaks at  $1630\text{ cm}^{-1}$ ,  $3322\text{ cm}^{-1}$ , and  $3325\text{ cm}^{-1}$  suggests that acrylamide may indeed be present in sample MS2; the presence of peaks at  $1625\text{ cm}^{-1}$  and  $3290\text{ cm}^{-1}$  suggests that acrylamide may be

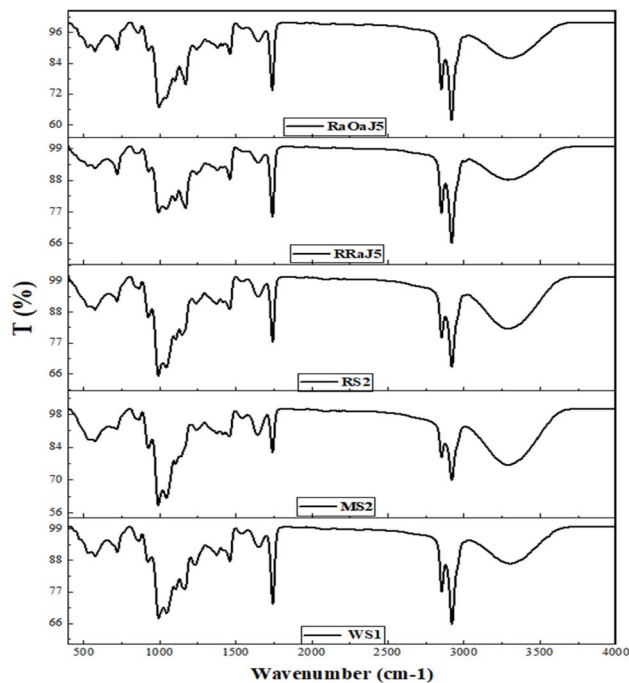


Fig. 6 FTIR curves of the best-rated optimal formulation of the cookies.

present in sample RS2; and the presence of a peak at  $1632\text{ cm}^{-1}$  suggests that acrylamide may be present in sample RaRJ5.

### 3.7 X-ray diffraction

The relative crystallinity percentages for various dough samples reveal notable differences in their structural organization. Wheat-Sugar-Ghee (WS1) and Ragi-Oats-Jaggery-Shortening (RaOaJ5) exhibit the highest crystallinity percentages of 41.8% and 33.03%, respectively. This conveyed a relatively ordered structure and its association with the firmer texture and improved shelf stability. Higher crystallinity in baked products often results in a denser, more cohesive matrix that resists moisture absorption and structural degradation over time. All these enhance the crispness and extend shelf life. Crystalline regions in starches and fats contribute to a more stable structure that is less prone to staling or softening. This is particularly beneficial to maintain the desired crunchiness and for the prevention of spoilage during storage. First, the presence of ghee in WS1 and shortening in RaOaJ5 may contribute to the formation of crystalline structures. Ghee and shortening contain saturated fats, which tend to solidify and form crystalline arrangements under appropriate conditions, such as cooling. Additionally, the combination of wheat flour and sugar provides a framework for crystallisation to occur, as both ingredients have molecular structures that are conducive to forming ordered arrangements. Conversely, Rice-Sugar-Butter (RS2) displays the lowest crystallinity at 7.2% and suggests a more disordered or amorphous composition. The lower relative crystallinity observed in the Rice-Sugar-Butter (RS2) dough sample in comparison to others could be attributed to several



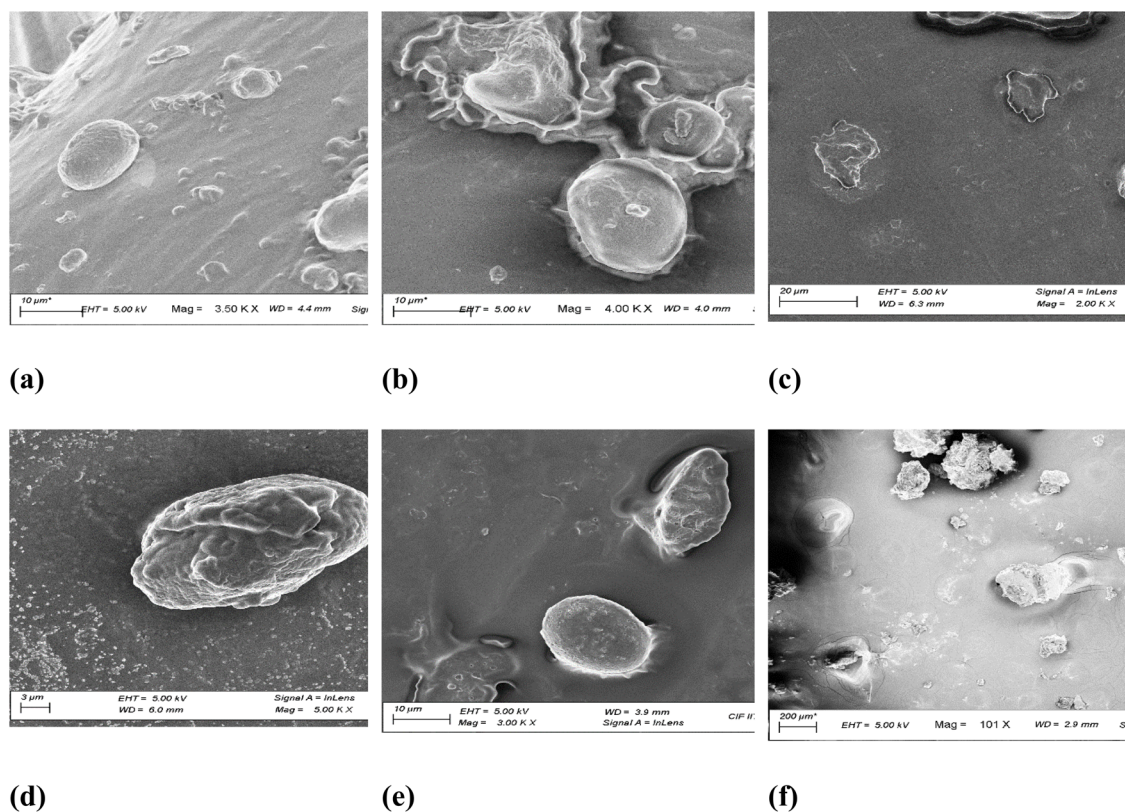


Fig. 7 FESEM images of best-rated gluten-free and reduced cookies: (a) WS1, (b) WS2, (c) MS2, (d) RS2, (e) RaOaJ5 and (f) RaRJ5.

factors. First, the ingredients themselves could contribute to the lower crystallinity. Rice contains starches, which tend to form amorphous structures upon being mixed with other ingredients and water. Additionally, the presence of sugar and butter in these dough formulations could inhibit the formation of crystalline structures. This is due to their interference with molecular arrangements during dough formation and baking. Samples such as Wheat-Sugar-Butter (WS2) and Rice-Ragi-Jaggery-Shortening (RaRJ5), exhibit intermediate levels of crystallinity ranging from 25.66% to 32.86%. These variations are possibly arising due to the differences in ingredient composition, processing methods, and formulation techniques. All these highlight the diverse structural characteristics that are inherent in each dough sample.

### 3.8 FESEM and particle size distribution

**3.8.1 FESEM images.** The FESEM (Field Emission Scanning Electron Microscopy) morphology analysis reveals distinct features among different cookie samples and provides insights into their structural characteristics. The regular circular structures observed in WS1 and WS2 suggest a homogenous distribution of ingredients within the cookie matrix and contribute to their uniform appearance. In contrast, the triangular-like structure of MS2 resembles the original morphology of green gram flour and indicates the presence of intact particulates within the cookie. RS2 displays an elliptical regular structure and conveys a different composition or processing method in

comparison to other samples. Finally, the comparable structures observed in RaRJ5 and RaOaJ5 indicate similarities in ingredient composition or processing techniques. These findings underscore the relationship between the cookie structure and formulation and highlight the potential impact on sensory attributes and consumer acceptance (Fig. 7).

**3.8.2 Particle size distribution.** The results from the dynamic light scattering (DLS) analysis reveal variations in the particle size distribution and average diameter among the various cookie formulations mentioned. Among the samples, RaOaJ5 exhibits the largest average diameter of 2557 nm, followed by RS2 with an average diameter of 2270 nm. Conversely, RaRJ5 has the smallest average diameter of 1097 nm. These differences in particle size can be attributed to variations in ingredient composition and processing methods. Formulations with larger average diameters may indicate the presence of larger aggregates or particles. These potentially result from the interaction between ingredients during mixing or baking. Conversely, smaller average diameters suggest finer particle sizes or more uniform dispersion of ingredients. The polydispersity index (PdI) provides insight into the uniformity of particle sizes within each sample, with values closer to zero indicating a narrower size distribution. Overall, these findings highlight the importance of particle size characterization in understanding the physical properties and potential sensory attributes of food products such as cookies (Table 7).



Table 7 Particle size distribution of best-rated cookies

S. no.	Sample name	PdI	Average diameter (nm)
1	WS1	0.594	1822 ± 19.36
2	WS2	0.572	1743 ± 31.98
3	RaOaJ5	0.485	2557 ± 10.50
4	RaRJ5	0.933	1097 ± 7.83
5	RS2	0.322	2270 ± 23.49
6	MS2	0.682	1626 ± 18.02

### 3.9 Storage study

Moisture and water activity play an important role in the storage of cookies. There was a minor increase in the moisture content of cookies from the 0th day to the 60th day (Table 8). Such a marginal increase in moisture could be related to the general hygroscopic nature of the packed cookies.<sup>41</sup> The nature of the packaging material and its porosity play an important role in the moisture uptake. The authors in ref. 42 reported that stored biscuits packed in metalised polyester or biaxially oriented polypropylene exhibit higher moisture content than those packed in paper-aluminium foil polyethylene laminate pouches. Cookies packed in laminate pouches absorbed less moisture during storage. This could be due to the impermeable nature of aluminium foil in the laminate to air and water vapour.<sup>43–45</sup> Similar findings with respect to the increased moisture content of cereal bran incorporated biscuits at the end of 60 storage days were reported by the authors in ref. 27 and 28. A corresponding

enhancement in the Aw value in both cookie samples was also observed.

Furthermore, sixty days of storage under controlled conditions only induced marginal sensory changes in the cookies. After the storage period, panellists reported reduced cookie hardness, and this is also confirmed by the instrumental analysis (Table 8). Reduced breaking strength, also measured by the three-point peak force or hardness tests, is usually reported in stored cookies being prepared with or without fat replacement.<sup>46</sup> No rancid off-flavour was reported by the panellists up to 60 days of storage in any of the cookie formulations. Sensory evaluation post-storage confirmed continued acceptability, and scores remained above 8.0 for key attributes such as taste, texture, and overall appreciation. This conveys the retention of sensory quality in the formulations over time.

Food-borne illness is caused by microorganisms. Human consumption warrants their absence from food products. For the cookie samples, the microbial count for TPC, yeast and mold, *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* was undetected. This suggests that the microbial quality of cookies during storage for 60 days remained good. All nine best-rated cookie formulations had nil plate counts (CFU mL<sup>-1</sup>) and yeast and mould counts after processing.<sup>47</sup> In the conducted study, microbial counts were assessed with standard plate count methods on nutrient agar for total plate count (TPC) and potato dextrose agar for yeast and mold count (YMC). For this case, the incubation conditions were 37 °C and a 24–48-hour duration. The minimum detection limit for these methods, as per the plated sample volume and dilution factor, was approximately 10

Table 8 Storage parameters of best-rated gluten reduced cookies

S. no.	Cookie formulation	Storage parameters								
		0th			30th			60th		
		Mc	Aw	Hardness	Mc	Aw	Hardness	Mc	Aw	Hardness
1	WS1	3.88 ± 0.12	0.44 ± 0.3	11.81 ± 0.51	4.04 ± 0.37	0.48 ± 0.5	12.5 ± 2.03	4.52 ± 0.4	0.49 ± 0.24	12.07 ± 1.33
2	WS2	4.05 ± 0.11	0.46 ± 0.14	15.27 ± 22.08	4.46 ± 0.51	0.47 ± 0.5	15.89 ± 2.76	4.79 ± 0.6	0.48 ± 0.27	15.49 ± 5.06
3	MS2	3.95 ± 0.7	0.45 ± 0.09	15.07 ± 2.8	4.00 ± 0.08	0.47 ± 0.2	17.2 ± 1.36	4.06 ± 0.43	0.48 ± 0.07	16.9 ± 1.81
4	RS2	4.13 ± 0.34	0.44 ± 0.15	12.62 ± 1.6	4.27 ± 0.59	0.45 ± 0.06	13.45 ± 2.7	4.31 ± 1.04	0.47 ± 0.54	13.04 ± 2.55
5	RaRJ5	4.59 ± 0.34	0.47 ± 0.09	13.36 ± 2.55	4.65 ± 1.06	0.49 ± 0.06	14.28 ± 5.03	4.82 ± 0.86	0.5 ± 0.08	14.09 ± 7.89
6	RaOaJ5	4.42 ± 0.51	0.46 ± 0.03	16.05 ± 5.32	4.7 ± 0.93	0.47 ± 0.02	17.6 ± 7.44	4.8 ± 0.61	0.48 ± 0.15	17.8 ± 8.04

Table 9 Microbial analysis of best-rated gluten-reduced cookies

S. no.	Cookie formulation	Microbial count					
		0th		30th		60th	
		TPC	YMC	TPC	YMC	TPC	YMC
1	WS1	ND	ND	0.4 ± 0.02	ND	1.0 ± 0.5	ND
2	WS2	ND	ND	0.1 ± 0.03	ND	1.4 ± 0.6	ND
3	MS2	ND	ND	0.1 ± 0.01	ND	0.13 ± 0.02	ND
4	RS2	ND	ND	0.12 ± 0.02	ND	0.17 ± 0.04	ND
5	RaRJ5	ND	ND	0.65 ± 0.06	ND	2.82 ± 0.47	ND
6	RaOaJ5	ND	ND	0.7 ± 0.09	ND	3.3 ± 0.45	ND



CFU mL<sup>-1</sup>. Therefore, the nil plate counts being reported in the article convey that the microbial growth was below the detection threshold of 10 CFU mL<sup>-1</sup>. Researchers<sup>48</sup> found similar results for beniseed and unripe plantain-enriched wheat biscuit. After 30 days, WS1 and WS2 had 0.4 and 0.1 CFU mL<sup>-1</sup> TPC, and both types of cookies had no yeast or mould growth. Millet-based cookies, such as RaRJ5 and RaOaJ5, had a TPC count ranging from 0.5–0.8 CFU per mL TPC, but no YMC growth was detected. RS2 and MS2 had TPC less than 0.2 CFU mL<sup>-1</sup> up to 60 days. After 60 days, wheat-based cookies (WS1 and WS2) had TPC 1.0 and 1.4 CFU mL<sup>-1</sup>, respectively. Millet-based cookies, *i.e.* RaRJ5 and RaOaJ5, had the maximum number for the total plate count (Table 9).

### 3.10 <sup>1</sup>H-NMR spectra based fatty acid profile of cookies during storage

The <sup>1</sup>H-NMR spectra of the fat extracted from the grain-based gluten-free cookies were used to determine their fatty acid profiles. Fig. 8 illustrates the <sup>1</sup>H-NMR spectra of the fat extracted from the millet grain-based optimal cookie formulation RaRJ5. The parameter was assessed during the storage study for the 0th and 60th day cases of the prepared cookies. For MS2, WS2, WS1, RS2 and RaOaJ5 cookie formulations, the stability of fatty acids in cookies during storage was also assessed with proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectroscopy. In Fig. 8, the peaks at 0.81 ppm, 1.19 ppm, 1.51 ppm, 2.20 ppm, 2.35 ppm, 2.77 ppm, 3.73 ppm, 4.04 ppm, 4.29 ppm, 5.09 ppm, 5.53 ppm, 5.74 ppm, 6.06 ppm, 6.49 ppm, 7.28 ppm, 7.59 ppm, 9.14 ppm, 9.41 ppm, and 9.97 ppm in the NMR spectra correspond to various proton environments that are typically found in fatty acids. The peaks at lower chemical shifts (0.81–3 ppm) are indicative of alkyl chain protons such as methyl and methylene groups. Peaks around 4–6 ppm suggest the presence of olefinic protons and thereby indicate unsaturated fatty acids. The higher chemical shift regions (around 7–10 ppm) are often associated with protons in more complex environments. These possibly include aromatic compounds or oxidized fatty acids. The consistent presence of these peaks suggests that the fatty acid profile includes a mix of saturated and unsaturated fatty acids, and with desired stability in the chemical structure for a period of 60 days. Similar findings can be analyzed from the graphical representations presented in ESI file† part B for all other optimally reported grain flour-based cookie formulations.

In summary, the conducted research investigations were able to identify relevant changes that would occur in the fatty acid profile with storage time. Accordingly, insights could be gained into the optimal practices that shall be followed to maintain cookie quality and extend shelf life. The <sup>1</sup>H NMR spectrum of the cookies conveys a mixture of saturated and unsaturated fatty acids. No significant changes occurred in the NMR spectra of the cookie formulation RaRJ5 in the 60-day storage period. The fatty acid profile, as indicated by the positions and intensities of the peaks in the NMR spectra, remained stable. This affirmed that the fatty acids in the cookies did not undergo substantial chemical alteration during the storage period. The consistent <sup>1</sup>H-NMR spectral profiles observed

between the 0th and 60th day of storage indicate that the lipid components in the cookies remained chemically stable and had no significant oxidation or degradation. Such good stability of the parameter is a strong indicator of good shelf life, particularly in terms of resistance to rancidity and preservation of nutritional quality. Additionally, the absence of off-flavours reported in sensory evaluations further supports the chemical integrity of the lipid fraction. These findings suggest that the selected fat sources and packaging methods effectively preserved the cookies' quality during the 60-day storage period.

### 3.11 Comparative assessment with commercially available gluten-free cookies

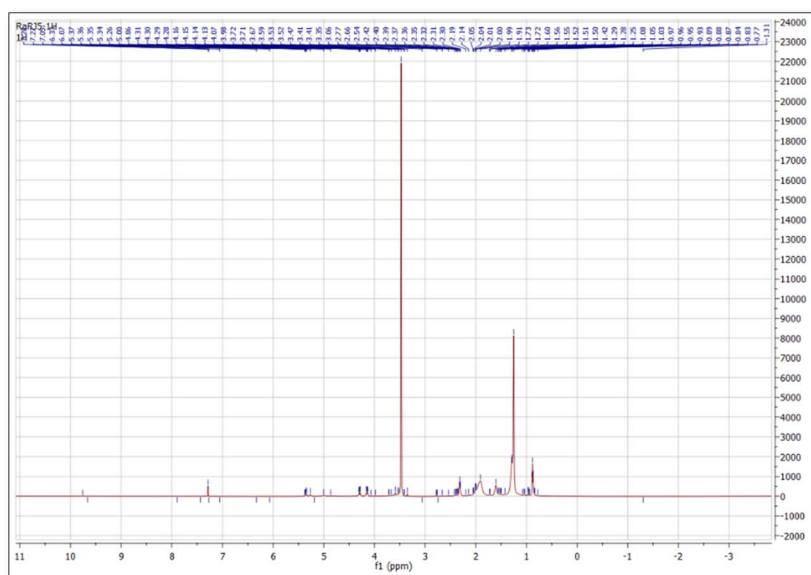
Most grain flour-based cookies have been designed to serve as a gluten-free alternative. Such formulations mimic traditional sugar cookies. The deployment of a diverse gluten-free flour blend facilitates a similar texture and flavour to that of conventional cookies. However, in such conventional cookies, the nutritional profile is dominated by simple carbohydrates and sugars. Thus, the cookies with little protein or fiber have lower nutritional density in comparison to other gluten-free snacks that are constituted with ingredients such as legumes or whole grains.<sup>49</sup> The absence of fat could be appealing for some dietary preferences. However, such cookies lack the satiety and potential health benefits of healthy fats. The commercial gluten-free sugar cookies from the brand “Gluten-Free Heaven” offer a convenient option for those needing to avoid gluten. Addressing the nutritional difference between the respective values of the RS cookie formulation and other commercial cookies (“Gluten free heaven”), the following can be summarized for the nutritional characteristics:

- RS2 cookies contain approximately 11.5 g per 100 g of protein and 3.01 g per 100 g of dietary fiber. These promising values contribute to satiety and muscle maintenance.
- In contrast, the commercial cookies contain 0 g of protein and 0 g of fibre, and with 9 g of sugar per 24 g serving, a higher carbohydrate content has been apparent in the commercial cookies.

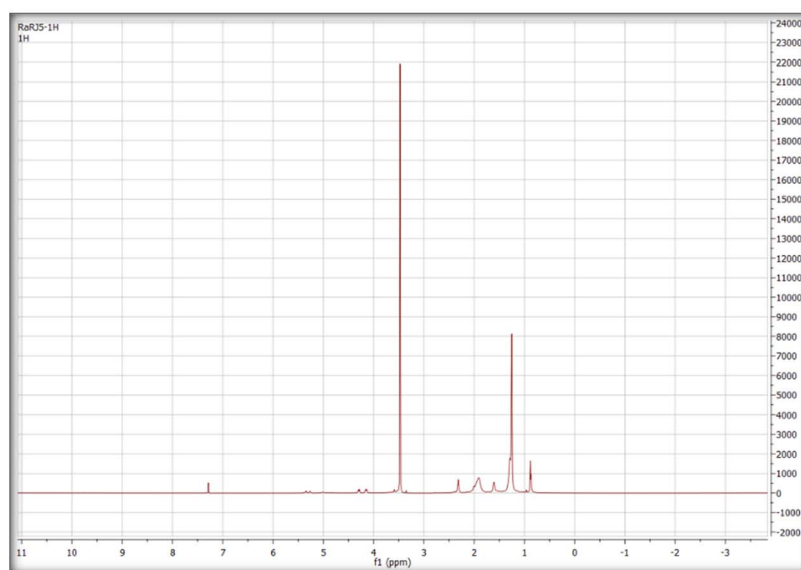
Thus, comparatively, a protein difference of over 11.5 g per 100 g and a fiber difference of 3 g per 100 g has been apparent. In other words, the significantly higher nutritional density of the RS2 cookies has been apparent with respect to the commercial cookies.

Each RS2 sample serving (1/24 of the package) contains 90 calories, a value recommended as a relatively low-calorie snack. The cookies have 0 g of fat, including no saturated or trans fats. This appeals to individuals desiring a low-fat diet. They contain 45 mg of sodium. In the total carbohydrate content of 15 g, 9 g corresponds to that of the sugars. In other words, a significant portion of the calories comes from sugar. The cookies do not have any dietary fibre and hence do not offer much desired satiety or digestive benefits. Lastly, without protein content, the commercial cookies serve as a simple source of carbohydrates and sugars and are not a balanced snack.<sup>50</sup> The RS2 gluten-free cookies (serving size: 30 g), containing 30.5 ± 1.1% moisture, 2.9 ± 0.66% ash, 1.22 ± 0.07% fat, 11.5 ± 0.8% protein, and 3.01





(a)



(b)

Fig. 8  $^1\text{H}$ -NMR spectra of fat extracted from the RaRJ5 cookie formulation (a) for 0th day and (b) 60th day storage period cases.

$\pm 0.84\%$  fiber, offer a more nutritionally balanced option in comparison to the commercially available “Gluten-Free Heaven” sugar cookies. The RS2 cookies are rich in protein and fiber, which contribute to better satiety, muscle maintenance, and digestive health. This makes them a healthier choice. The commercial gluten-free cookies referenced in the comparative assessment were not tested under the same laboratory conditions as the experimental formulations. Instead, their nutritional data were obtained from the manufacturer’s published nutrition label and were accepted as it is from the

publicly available product information. This approach was followed to provide a general benchmark for comparison. In contrast, the commercial cookies are fat-free and have a higher sugar content. Thus, they are sweeter but nutritionally less dense due to no protein or fibre content. Thus, the comparative analysis highlights that while “Gluten-Free Heaven” sugar cookies may appeal to those seeking a low-fat sweet treat, the RS2 cookies provide significant health benefits and a more balanced nutritional profile.



Table 10 A summary of the best data achieved in this work and in the literature for the grain-based cookie formulations

S. no.	Cookie composition	Proximate composition	Bioactive components	References
1	<ul style="list-style-type: none"> <li>• C3: 50% wheat flour</li> <li>• 10% chickpea flour</li> <li>• 10% barley</li> <li>• 10% maize flour</li> <li>• 10% barley</li> <li>• 10% pearl millet flour</li> <li>• 10% finger millet flour</li> </ul>	<ul style="list-style-type: none"> <li>• Carbohydrate content: 66.9%</li> <li>• Crude fiber range: 1.12–1.55%</li> <li>• Ash: 1.29–1.76%</li> </ul>	—	57
2	<ul style="list-style-type: none"> <li>• MS2: 60% green gram flour</li> <li>• 20% Bengal gram flour</li> <li>• 20% chickpea flour</li> </ul>	<ul style="list-style-type: none"> <li>• Total carbohydrate content: 22.87 g per 100 g</li> <li>• Total protein: 14.31 g per 100 g</li> <li>• Soluble protein content: 5.66 g per 100 g</li> <li>• Fat: 11.43 g per 100 g</li> </ul>	<ul style="list-style-type: none"> <li>• MS2-AA: 33.51% after <i>in vitro</i></li> <li>• AA: 10.38%</li> </ul>	This work

### 3.12 Literature comparison

The findings from the literature<sup>51</sup> on composite flour cookies, which included chickpea flour, pigeon pea flour, moong bean flour, cowpea flour, and wheat flour, highlight similarities with the formulation in the present study, especially in terms of protein and fat content. The multigrain cookies formulated by the authors in ref. 52, incorporating chickpea flour, wheat flour, barley, maize, pearl millet, and finger millet flour, also exhibit comparable nutritional profiles, and emphasize the potential of multigrain formulations in enhancing protein and fat content along with the maintenance of the acceptable levels of moisture, ash, and carbohydrates. Furthermore, the cookies formulated by the authors in ref. 52 containing ragi, oats, and brown rice align closely with the nutritional composition observed in the present study, and accordingly reinforce the versatility of alternative grain-based formulations in producing cookies with desirable nutritional attributes. These studies collectively support the feasibility and effectiveness of the incorporated diverse flour blends in cookie formulations to enhance nutritional value and antioxidant properties, and thereby provide valuable insights for further exploration and optimization in product development.

The study conducted by the researchers<sup>53</sup> sheds light on the chemical composition of cookies using FT-IR spectroscopy. They precisely delve into the identification of lipid oxidation products and their influence on cookie quality. The reported best research findings complement the discussion on lipid-related peaks in the provided paragraph and offer additional context to the interpretation of spectral data related to lipid components and their oxidation states in cookies. Furthermore, the work by researchers<sup>54</sup> has investigated the presence of acrylamide in baked goods utilizing FT-IR spectroscopy, and provided very useful insights into the characteristic peaks that are associated with its formation and detection.

The investigations conducted by the authors<sup>17</sup> into the structural properties of cookies, using FESEM morphology analysis, provided valuable insights into the impact of various ingredients and processing techniques on cookie texture and appearance. This research aligns with the observations made in this article and reinforces the need to understand the relationship between formulation parameters and cookie

characteristics, as well as the assessed structural features, with the microscopy technique. Additionally, studies conducted for the rheological properties of dough and cookie formulations, such as those by the authors in ref. 55, contribute further to the insights associated with the interplay between ingredient composition and dough texture. Through the exploration of the rheological behaviour of dough under variant conditions, the conducted research provided additional context for the rheological analysis discussed in the article and elucidated the underlying mechanisms that influence dough consistency and processing suitability.

Investigations into the sensory changes and quality attributes of stored cookies, as studied in ref. 56, provide valuable insights into factors influencing cookie shelf-life and consumer acceptability. They accordingly support the observations made with respect to the sensory alterations during storage. Examining sensory parameters and quality attributes with respect to time, the conducted research improved the understanding of the influence of the storage conditions and duration on the sensory properties and overall quality of cookies. This is also aligned with the findings of this article.

Similarly, studies on microbial safety and shelf-life extension techniques for baked goods, such as those by the researchers,<sup>47</sup> offer crucial insights into effective strategies for the control of microbial growth and for the preservation of the product quality during storage. Through the exploration of microbial analysis and storage stability, such studies provide valuable information on the maintenance of food safety and extending shelf life. This complements the discussion on microbial analysis and the observed changes in cookies during storage. This body of research contributes to our understanding of storage-related factors affecting cookie quality and safety, and provides practical implications for food industry professionals for the optimization of storage conditions and enhanced product stability (Table 10).

## 4 Conclusions

The sensory evaluation scores of the cookie samples reveal notable differences in their sensory attributes. WS2 shows consistently high scores across various attributes and conveys its favourable sensory profile in terms of flavour, colour, texture,



and overall acceptability. Samples MS2 and RaOaJ5 receive commendable ratings across all attributes, demonstrating superior ratings in key sensory attributes such as taste, lingering flavour (aftertaste), and overall consumer preference. All these indicate strong acceptance traits of the evaluations conducted by the sensory panel. The fuzzy logic analysis further supports these findings, with samples MS2, RS2, WS1, and WS2 being classified in the “excellent” category, and RaOaJ5 and RaRj5 being categorized as “very good.” These results underscore the significant role of ingredient combinations in affirming the good sensory characteristics of cookies and guiding effective product development efforts. These are suggested as follows for future applications:

- Commercial scaling of the MS2 and RS2 formulations for gluten-free bakery products targeting health-conscious consumers.
- Integration into school and hospital nutrition programs and through the provision of healthy protein- and fibre-rich snacks.
- Development of ready-to-eat mixes using optimized flour blends for home baking.
- Further research on the shelf-life extension and packaging innovations that support market viability.
- Exploration of low-glycemic sweeteners and fortification strategies and thereby enhance the functional food profile.

These targets convey the greater ability of the research findings to achieve practical, consumer-oriented applications.

The rheological analysis offers valuable insights into the mechanical behaviour of dough samples under varying conditions.<sup>58</sup> While an increase in strain generally correlates with elevated stiffness and viscosity across most samples, each formulation exhibits unique rheological properties. For instance, ghee-based doughs demonstrate higher stiffness compared to butter, peanut butter, soybean oil, and coconut oil-based doughs, and convey a more solid-like behavior. Conversely, while butter doughs display relatively softer consistency, peanut butter and soybean oil doughs convey intermediate stiffness, and coconut oil-based doughs exhibit the lowest stiffness. These distinctions underscore the influence of fat and oil composition on dough rheology and are crucial for the optimization of the processing parameters and for the achievement of the desired product attributes.

The analysis of mineral content highlights nutritional differences among cookie samples. In comparison to the wheat-based cookies, the cookies containing unripe papaya exhibit notably higher levels of potassium and calcium and do convey a potential nutritional advantage. Conversely, rice-based cookies display lower mineral concentrations and indicate comparatively lower nutritional content. These findings underscore the significance of ingredient choice in determining the nutritional quality of cookies, with implications for dietary diversity and health.

The Fourier transform infrared (FT-IR) analysis of cookie formulations reveals characteristic peaks associated with their chemical composition and offers insights into their structural characteristics. Broadband absorption in the range of 3600 to 3000  $\text{cm}^{-1}$  suggests significant water absorption, and these are

consistent with previous studies. Peaks corresponding to N–H valence stretching vibrations and C=O functional groups indicate the presence of proteinaceous, polysaccharide, and lipid components within the cookies. Additionally, bands attributed to peptide linkages and carbohydrate stretching vibrations further support the presence of proteins and carbohydrates. These spectral features collectively contribute to the cookies' texture and flavor. The detection of peaks associated with acrylamide formation provides further insights into the potential presence of this compound in selected cookie formulations. However, considering the complex nature of acrylamide formation and detection limitations, the absence of characteristic peaks does not rule out its presence. Moreover, the rheological analysis and FESEM morphology reveal structural differences among dough and cookie samples, and are influenced by the ingredient composition and processing methods. The dynamic light scattering (DLS) analysis further elucidates variations in particle size distribution, and the moisture content and sensory changes during storage underscore the importance of formulation and packaging processes for the maintenance of the cookie quality. Microbial analysis indicates satisfactory microbial quality during storage and highlights the safety of the evaluated cookie formulations. Overall, these comprehensive analyses provide valuable insights into the chemical, structural, and quality attributes of the cookie formulations, and convey the need for future efforts targeting optimisation for enhanced nutritional and sensory profiles of the best reported cookie formulations.

The key findings of the article have been listed as follows:

- MS2 (Green gram-based) and RS2 (Rice-based) gluten-free cookies demonstrated the highest sensory acceptability and nutritional balance.
- While MS2 exhibited the highest protein content (14.31 g per 100 g), RS2 affirmed the highest carbohydrate content (30.5 g per 100 g).
- RaOaJ5 and RaRj5 (millet-based) cookies conveyed superior antioxidant retention and mineral content.
- Rheological analysis revealed that the fat type significantly influenced dough stiffness and baking performance.
- FTIR and DSC analyses confirmed the presence of stable protein, lipid, and carbohydrate structures.
- <sup>1</sup>H-NMR spectra indicated no significant lipid degradation during a 60-day storage period and inferred greater shelf-life stability.
- Microbial analysis confirmed safety with negligible microbial growth up to 60 days of storage.

In conclusion, the comprehensive analysis of sensory attributes, rheological behaviour, and nutritional composition provides valuable insights into the quality and characteristics of various cookie formulations. These insights can inform product development efforts and guide the formulation of cookies with improved sensory profiles, nutritional content, and textural attributes. All such efforts will be able to meet consumer preferences and will effectively address the growing concern for the balanced yet holistic dietary needs of the cookie consumers in India and abroad.



## Data availability

The data of this study are available upon request.

## Author contributions

The comprehensive research pedagogy was collaboratively conceived by all the authors. The experimental research, along with the collection of data and subsequent analysis, was conducted by Paushali Mukherjee. She also composed the initial draft. The subsequent improvisations were appropriately considered by the other author.

## Conflicts of interest

No conflict of interest exists for the reported findings.

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