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Research Article

Preparation of Healthy Cookies from Germinated Flour Blends of Finger Millet and Pearl Millet Sweetened with Jaggery

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Abstract

Millets are physiologically and therapeutically healthy with high nutritious value and are in rising demand in emerging markets like India, China, Africa, and other developing countries including the Western world. Germinated Millets have high digestibility and are used as healthy food for children's growth and development. Climate change resilience technology, high nutritional value, and the announcement of the year 2023 as "International Millet Year" have made it very popular. Bakery items based on Millet, particularly cookies, are becoming more popular in both urban and rural areas. Jaggery is raw sugar prepared from sugarcane juice and is considered superior to white sugar. It offers numerous nutritional and therapeutic benefits, including anti-carcinogenic with antitoxic actions. Hence, this study aimed to prepare healthy food items with germinated finger and pearl Millets for better nutritional quality that are attracting the attention of health-conscious people on a worldwide scale. Cookies made from blends of germinated wheat flour (GWF), germinated finger millet flour (GFMF), and germinated pearl millet flour (GPMF) were examined for their physicochemical qualities, *in vitro* digestibility, antioxidant activity, and overall acceptability by consumers. *In vitro* protein digestibility (62.24-82.34%), starch digestibility (47.48-62.41%), total phenolic content (11.45–49.12 mg GAE/100 g), and antioxidant activities significantly increased as the proportion of GFMF and GPMF flour increased in the cookie samples, whereas total starch, dietary fiber, carbohydrate, and phytic acid decreased. The physical qualities of the cookies were also improved by the addition of GFMF and GPMF flours. Cookies with acceptable sensory properties, including taste, aroma, appearance, mouthfeel, crispiness, and overall acceptability, were produced by blending 60% GWF, 20% GFMF, and 20% GPMF (T_2). This study demonstrated that GFMF and GPMF flour blends may be used as functional ingredients to create superior goods.

More Information

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Introduction

Jaggery, a natural, ancient sweetener, is made by concentrating juice from sugarcane, an abundantly growing crop in India, Africa, Latin America, and Japan. India's climate makes it easier to grow sugarcane, providing the raw material for jaggery production [1]. Jaggery is a staple in Indian cuisine, used in sweet meals like sweets, candy, toffees, cakes, and various nutritious food items. It is also used in syrups and sweets. Jaggery is a healthy, unprocessed sugar, that promotes regular consumption in the diet. According to the Food Safety and Standards Authority of India (FSSAI), cane jaggery is a product of boiling or processing sugarcane juice. It is basically unrefined sugar, contains carbohydrates, proteins, choline, betaine, vitamin B complex, vitamins A, C, D, E, and PP; folate, calcium, iron, phosphorus, selenium, manganese, chromium, copper, potassium, cobalt, zinc and is referred as a powerhouse of essential nutrients with no fat of any kinds [2,3].

Post-COVID-19, innovative plant-based ingredients are being used in food production due to increasing customer interest in immunogenic, functional foods and reduced gluten products [4]. Biscuits are inexpensive, practical, and widely accepted snacks that deliver essential nutrients and health-promoting components [5]. They are easily digestible and consumable, making them essential ready-to-eat items. Children like biscuits in their meals as these are easy to handle, transport and carry with by parents. These are small, easy to make with reduced gluten, and have high sensory qualities, making them appealing to consumers. They are made by combining flour, sugar, water, and fat in dough [6]. The demand for reduced gluten (RG) foods is increasing due to the increasing prevalence of celiac disease, an immune-mediated enteropathy affecting 40-60 million people worldwide [7]. RG food items can reduce Celiac disease, but their lack of protein, dietary fibers, and micronutrients in foods, including minerals and vitamins, raises concerns about their nutritional inadequacies [8].

Eleusine coracana (L.) Gaern, known as finger millet (Hindi name- ragi), is a significant climate-resilient millet cultivated in 25 countries of Asia and Africa. Introduced to India, and it was once indigenous to Ethiopia's mountains. Finger millet has a higher calcium content than wheat or paddy rice, which is very important for proper human function. Calcium and iron are essential for bone health, and anemia can result from iron deficiency [9]. Finger millet's rich micronutrients make it a potential ingredient for creating high-quality RG products to address nutritional issues associated with gluten-free diets [10].

Pennisetum glaucum (L.) R.Br., commonly known as pearl millet (Hindi name Bajra), another climate-resilient potential millet grain crop grown in India and Africa, is the second-most-cultivated and consumed millet. It has a high mineral and protein content, attracting interest due to its high levels of dietary fibers, phytochemicals, and nutritional value. Bajra is rich in B group vitamins and minerals, with a protein content of around 6% - 12%, ether extractives of 5% (including fats), and 40% - 67% carbohydrates with sodium, folate, iron, magnesium, thiamine, niacin, zinc, riboflavin, etc. [6,11]. It is gluten-free, retains alkalinity, and has one of the greatest protein qualities. The amino acid composition significantly affects protein nutritional quality. The two amino acids that are found in pearl millet are lysine and methionine. Pearl millet has an amino acid profile that is equivalent to that of wheat, barley, and rice but superior to that of sorghum and maize, high in calories, low in starch, and has a low (55) glycemic index (GI) [12]. Both flavonoids and phenolic acid are present in amounts of 0.9% and 4.08 mg/g, respectively, which are very potent antioxidants. The total antioxidant activity of pearl millet is noted to be 1.33 ± 0.003 mg/ml in terms of ascorbic acid [13].

Germination is a low-cost bioprocessing method that can enhance sensory qualities, and consumer acceptance, and modify nutrient and health-promoting constituents while reducing anti-nutritional elements [14]. It improves the composition of underutilized grains, such as germinated finger millet (GFM) and pearl millet (GPM) flours, and enhances their potential for better food preparation. The study aimed to evaluate the physicochemical properties, *in vitro* digestibility, antioxidant activity, and consumer acceptability of cookies made from these flour blends.

Materials and methods

Materials

The variety of finger millet (FM), pearl millet (PM), and wheat (W) grains, as well as additional baking ingredients (sunflower oil, baking powder, vanilla essence, and jaggery) for cookie production, were procured from the local market in Meerut (UP). The FM, PM, and W grains were sorted, cleaned, and stored in airtight containers at ambient conditions till subsequent use. All analytical grade reagents and standards (boric acid, quercetin standard, Folin-Ciocalteu reagent, 2,2-diphenyl-lpicrylhydrazyl (DPPH), gallic acid standard, trichloroacetic acid) used were procured from Merk Chemicals (PTY) Ltd., Delhi, India.

Germination of finger millet

Finger millet (FM), Pearl millet (PM), and Wheat (W) grains were washed, drained, and dried in an oven dryer (Model No. DHG-9101 ISA) at 40 °C for 24 h. The dried materials were milled and sieved (mesh size 100 μm diameter) to produce raw finger millet flour (RFMF), raw pearl millet (RPMF), and raw wheat flour (RWF) which served as control. For the germination process, cleaned grains were washed and soaked in water at room temperature for 24 hours. Water was drained from grains after soaking, spread individually on clean jute bags covered with a damp cotton cloth, and left for 48 h at 35 °C to germinate. Grains were sprinkled with water at 4 h intervals to stimulate the germination process. The optimum germination temperature of finger millet, pearl millet, and wheat grains has been reported at 28-35 °C [15] and the germination rate increased until the time reached 42-72 h. After germination, these were washed thoroughly under tap water and finally with distilled water, drained, and dried in an oven dryer at 40 °C for 24 h and were milled, sieved (mesh size100 μm) to obtain germinated finger millet flour (GFMF), germinated pearl millet flour (GPMF) and germinated wheat flour (GWF). Those flours were packed in airtight containers and stored at room temperature for further use and analysis.

Cookies preparation

Cookies were made using the modified Sharma, et al. [16] technique, where 100% GWF served as a control and GWF-GFMF-GPMF composite flour samples as shown in Figure 1. In order to achieve homogeneous mixing, several ratios of GWF, GFMF, and GPMF flours were combined (spiral dough mixture), including 100:0 (T_0), 80:10:10 (T_1), 60:20:20(T_2), 40:30:30(T_3), and 20:40:40 (T_4) respectively. The ingredients for the cookies were 100 g of flour, 60 g of jaggery, 3 mL of vanilla flavoring (Ossoro French Vanilla Flavour Essence, India), 40 g of vegetable shortening (Confect Vegetable Shortening, India), 1 g of baking powder (Weikfield Baking Powder, India), 3 g of skim milk powder and 30 mL of water. With the aid of a cookie cutter, the dough was rolled out to a thickness of 5.80 mm. The cookies were baked in a preheated

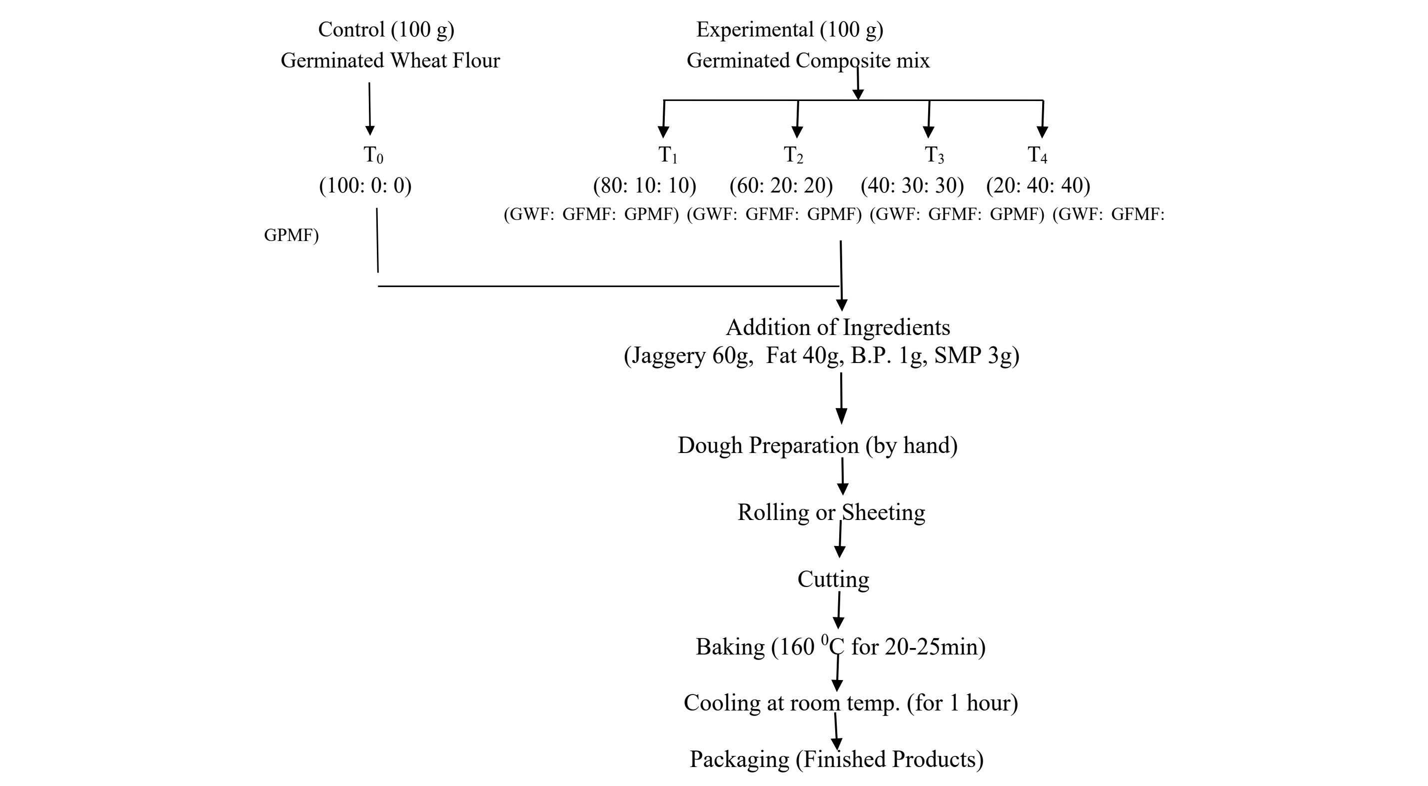

Figure 1: Flow diagram for preparation of cookies

oven (Electric Operated Single Deck Baking Oven, India) at 160 °C for 20-25 min. Triplicate cookie samples were prepared and subsequently analyzed.

Proximate analysis of flour and cookies

AOAC techniques of 925.09, 992.23, 923.03, and 920.39 [17], respectively for moisture, protein, ash, and fat were used. AACC International's methodologies of 32-45.01 and 76-13.01, respectively, were used to determine the total amounts of dietary fiber and starch content [18]. Carbohydrate content (calculated by difference) and energy value (calculated based on 4.0 kcal/g for protein and carbohydrate, and 9.0 kcal/g for fat) were determined following a standard method [17].

Mineral analysis of flour and cookies

The Association of Official Analytical Chemists (AOAC) procedures were used to estimate the mineral content (calcium, magnesium, potassium, zinc, and iron) of previously analyzed ash samples (section 2.4) of flours and cookies [17].

Anti-nutritional factors in flour and cookies

The concentration of phytic acid (PA) was profiled based on the AOAC [17] method, using a UV-spectrophotometer at 640 nm and values expressed on a dry weight basis as mg/100 g. Trypsin inhibitor activity was assayed using 0.04% (w/v) of BAPA (Nα-benzoyl-L-arginine 4- nitroanilide hydrochloride) as trypsin substrate [19]. Measurements were made using a UV-spectrophotometer at 410 nm and values expressed as trypsin inhibitor (TI) unit per mg (dw).

Polyphenols and antioxidant activity of flour and cookies

By mixing 50 g of each type of flour with 500 mL of methanol for 24 h, we measured the extraction of polyphenols, DPPH, and iron reduction power. The mixture was then centrifuged for 10 min at 3000 rpm using a Rotina 380 R centrifuge (Labotech Ecotherm, Midrand, South Africa), filtered using Whatman paper into beakers and transferred into several centrifuge tubes that were maintained in the freezer for future analysis [20].

Total Phenolic Content (TPC)

A technique used by Mudau, et al. [5] was used to evaluate the TPC of flour and millet cookie extract. Test tubes were filled with an extract (0.2 mL), 2.5 mL of five-fold diluted Folin-Ciocalteu, and 5 mL of distilled water. After five minutes, 15% sodium carbonate (7.5) was added to the tubes, the mixture was vortexed, and it was left in the dark for thirty minutes. The absorbance at 760 nm values was measured using a Spectrophotometer. The findings were represented in milligrams of gallic acid per gram of flour and cookie sample, and the standard curve was made with gallic acid.

Total Flavonoid Content (TFC)

The study examined the TFC of flour and millet cookie extract using a modified method by Mahloko, et al. [21]. The extract was combined with 5% NaNO2 and reacted for 5 minutes before adding 10% AlCl3. After 6 minutes, distilled water and 1 M NaOH were added and vortexed. A

Spectrophotometer was used to record absorbance at 510 nm, and the quercetin standard (R2 = 0.9992) was used for a standard curve. Results were measured in milligrams of Quercetin per gram of samples.

DPPH Radical Scavenging

The DPPH assay of flour and millet cookies was analyzed as described by De Ancos, et al. [22], wherein 3.9 mL 0.1 mM DPPH was added to the mixture of flour and millet cookies extract (10 µL) and distilled water (90 µL). The combination was thoroughly mixed before being left in the dark to react for 30 min, after which the absorbance of the mixture was measured at 517 nm using a Spectrophotometer.

Ferric Reducing Antioxidant Power (FRAP)

Lou, et al. [23] used a method to measure the FRAP assay of flour and millet cookie samples. They mixed sample extract and methanol in a test tube, blended with 0.2 M phosphate buffer and 1% K3[Fe (C N)6], and centrifuged for 20 minutes. The supernatant was combined with 0.1 mM FeCL3 and distilled water, and the absorbance was measured using a Spectrophotometer at 700 nm. A higher absorbance combination indicated higher reducing power.

In vitro protein digestibility analysis of flour and cookies

The *in vitro* protein digestibility (IVPD) was assessed by weighing 200 mg of the sample into a 100 mL Erlenmeyer flask containing 1.5 g of pepsin and 35 mL of sodium citrate tribasic dehydrate [24]. The mixture was incubated for two hours at 37 °C, then centrifuged at 10,000 g for 15 minutes. Following the AOAC [17] technique, the supernatant was collected, cleaned, dried, and the nitrogen content was examined. The IVPD was computed as the percentage of protein in the supernatant compared to the total protein content of the sample.

In vitro digestibility of starch in flour and cookies

Using the methods described by Goni, et al. [25], the parameters of starch hydrolysis of each flour and millet cookie sample were identified. In order to determine the proportion of hydrolyzed starch, Eq. (1) was used.

$$C = C_{\infty} - (1- e^{-kt}) \qquad (1)$$

Where C is the percentage of hydrolyzed starch at time t, C∞ is the equilibrium hydrolyzed starch after 180 min and k is the kinetic constant).

Thereafter, the hydrolysis index (HI) of the products was obtained (by dividing the areas under the hydrolysis curve of each sample). From the HI value, the estimated glycemic index (eGI) of the samples was obtained using Eq. (2) [25]

$$eGI = 39:7 + 0:548HI \qquad (2)$$

Where eGI ¼ estimated glycemic index (%); HI ¼ hydrolysis index (%).

We also studied the quickly digested starch (RDS, hydrolyzed at 20 min), slowly digested starch (SDS, hydrolyzed between 20 and 120 min), and resistant starch (RS, undigested after 120 min).

Physical properties of millet cookies

The Vernier Caliper was used to measure jaggery-based millet cookies' thickness and diameter in two perpendicular directions. The spread ratio was calculated by dividing biscuit diameter by thickness [5]. Chroma-Metre, the L* (lightness), a* (redness), and b* (yellowness) values were used to assess color characteristics, while an Instron universal testing machine was used to assess texture at 28 °C. The study aimed to improve the quality of jaggery-based millet cookies [26].

Consumer acceptability of value-added cookies

Consumer acceptance of the most acceptable cookies was carried out among 25 untrained panelists. The consumers were served with one serving of the cookies and opinions were solicited as to whether the product was acceptable. The opinions were tabulated and expressed on a percent basis.

Statistical analysis

Obtained experimental values were analyzed by analysis of variance (ANOVA) and student's t - test for comparisons. SPSS software (version 16.0) was used to analyze the data Figure 2.

Results and discussions

Physico-chemical and anti-nutritional composition of germinated flours and cookies

The proximate composition, antioxidant activity, and anti-

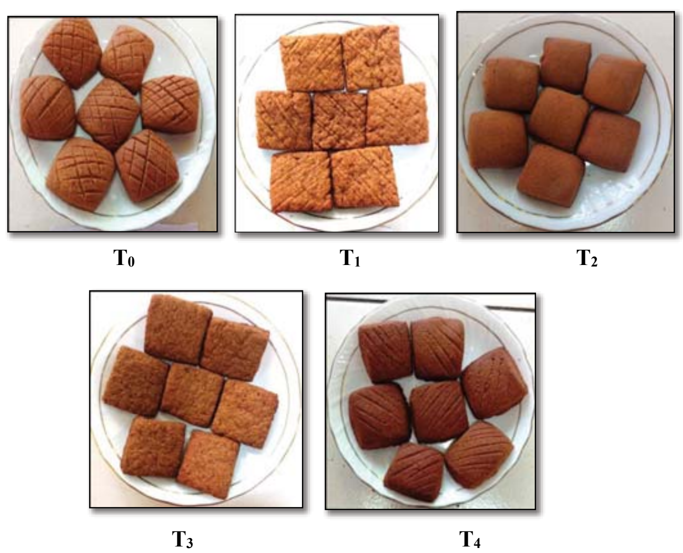

Figure 2: Photographs of millet cookies prepared. [T_0 = 100 % germinated wheat flour (GWF); T_1 = 80% germinated wheat flour + 10% germinated finger millet flour (GFMF) + 10% germinated pearl millet flour(GPMF); T_2 = 60% germinated wheat flour + 20% germinated finger millet flour (GFMF) + 20% germinated pearl millet flour(GPMF); T_3 = 40% germinated wheat flour + 30% germinated finger millet flour (GFMF) + 30% germinated pearl millet flour(GPMF); T_4 = 20% germinated wheat flour + 40% germinated finger millet flour (GFMF) + 40% germinated pearl millet flour(GPMF).]

nutritional content (dry basis) of the flour and cookies made from GWF, GFMF, and GPMF blends are shown in Tables 1,2. Comparing the GPMF to the GFMF and GWF, significantly higher amounts of protein (12.24 g/100g), ash (5.15 g/100g), and fat (5.64 g/100g) were found (Table 1). Cookies made from GWF, GFMF, and GPMF and their blends revealed moisture, ash, protein, fat, total dietary fiber, carbohydrate, starch, and energy value of 6.01–6.66 g/100 g, 1.01–2.25 g/100 g, 11.25–16.62 g/100 g, 12.88–14.42 g/100 g, 12.63–17.22 g/100 g, 60.23 68.64 g/100 g, 34.36–40.20 g/100 g and 362.24–372.61 kcal/100 g, respectively (Table 2). The increase in T2 in the blend was followed by an increase in the moisture content of the cookies. The increased water absorption capacity of GPMF's proteins may be responsible for this increase. Arepally, et al. [27] also found high water absorption ability of pearl millet's proteins was responsible for the rise in the moisture content of multi-millet biscuits. All of the cookie samples had usually low moisture contents, which could indicate good shelf stability since low moisture contents would prevent microbes from proliferating as much.

As GFMF and GPMF proportions increased, cookies' carbohydrates and starch decreased, while ash, fat, protein, and energy increased. This may be due to high levels of ash, protein, and fat in GFMF and GPMF, as well as the substitution effect, which may decrease dietary fiber, carbohydrates, and starch (Table 1). Our findings confirm previous reports [5,28-30] which found increased protein, fat, and ash content in finger millet flour and pearl millet flour.

This study revealed that biscuits/cookies made from wheat flour had lower protein, ash, dietary fiber, and energy values when compared to germinated finger and pearl millets [31]. This suggests that germinated finger and pearl millets

Table 1: Physicochemical composition, antioxidant properties, and Anti-nutritional factors of germinated wheat flour (GWF), germinated finger millet flour (GFMF), and germinated pearl millet flour (GPMF).

Parameters	GWF	GFMF	GPMF
Proximate analysis			
Moisture (g/100 g)	10.78 ± 0.21	10.23 ± 0.45	9.85 ± 0.09
Ash (g/100 g)	2.12 ± 0.75	4.22 ± 0.24	5.15 ± 0.15
Fat (g/100 g)	2.15 ± 0.05	3.12 ± 0.60	5.64 ± 0.01
Protein (g/100 g)	9.85 ± 0.18	10.85 ± 0.11	12.24 ± 0.89
Starch (g/100 g)	73.11 ± 0.68	52.26 ± 0.31	56.65 ± 0.90
Crude fiber (g/100 g)	2.04 ± 0.21	4.55 ± 0.18	2.11 ± 0.09
carbohydrate (g/100 g)	70.06 ± 0.25	66.65 ± 0.13	67.12 ± 0.21
Energy value (kcal/100 g)	298.42 ± 0.17	310.57 ± 0.35	326 ± 0.55
Minerals content			
Calcium (mg/100 g)	2.58 ± 0.11	210.4 ± 1.25	71.21 ± 0.36
Iron (mg/100 g)	3.62 ± 0.31	40.12 ± 0.14	10.23 ± 0.78
Magnesium (mg/100 g)	30.18 ± 0.22	1589.2 ± 0.125	97.43 ± 0.11
Potassium (mg/100 g)	7.53 ± 0.15	565.09 ± 0.5	523.02 ± 0.21
Zinc (mg/100 g)	4.26 ± 0.3	9.45 ± 0.14	7.21 ± 0.11
Anti-nutritional factors			
TIA (TIU/mg)	0.15 ± 0.01	ND	ND
Phytic acid (mg/100 g)	222.0 ± 0.90	125.65 ± 4.28	168.50 ± 2.15
In vitro digestibility			
In vitro protein digestibility (%)	69.34 ± 0.75	78.33 ± 1.21	82.43 ± 1.12
Polyphenols and Antioxidant Activity			
TPC (mg GAE/100 g)	69.37 ± 3.13	150.1 ± 0.25	112.4 ± 1.0
TFC (mg RE/g)	700.85 ± 15.5	982.65 ± 26.3	865.44 ± 28.32
FRAP (µmol TE/100 g)	158.17 ± 12.41	398.55 ± 7.35	410.46 ± 8.25
DPPH (%)	62.46 ± 1.75	80.0 ± 2.5	82.42 ± 1.25

Mean and standard deviation of triplicates. Mean values with different superscripts in a row are significantly ($p \leq 0.05$) different from each other. TIA: Trypsin Inhibitor Activity; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; FRAP: Ferric Reducing Antioxidant Power; DPPH: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay; ND: Not detected.

Table 2: Proximate composition of jaggery-based cookies prepared from GWF, GFMF and GPMF.

Parameters	T_0	T_1	T_2	T_3	T_4
Moisture (g/100 g)	6.21 ± 0.21	6.01 ± 0.21	6.66 ± 0.21	6.35 ± 0.21	6.48 ± 0.21
Ash (g/100 g)	1.02 ± 0.21	1.37 ± 0.21	1.85 ± 0.21	2.07 ± 0.21	2.25 ± 0.21
Fat (g/100 g)	12.88 ± 0.21	13.15 ± 0.21	13.61 ± 0.21	14.05 ± 0.21	14.42 ± 0.21
Protein (g/100 g)	11.25 ± 0.21	13.47 ± 0.21	14.32 ± 0.21	15.46 ± 0.21	16.62 ± 0.21
Total Starch (g/100 g)	40.20 ± 0.21	39.12 ± 0.21	37.8 ± 0.21	36.29 ± 0.21	34.36 ± 0.21
Total dietary fiber (g/100 g)	17.22 ± 0.21	16.48 ± 0.21	14.61 ± 0.21	13.21 ± 0.21	12.63 ± 0.21
carbohydrate (g/100 g)	68.64 ± 0.21	66.01 ± 0.21	63.56 ± 0.21	62.07 ± 0.21	60.23 ± 0.21
Energy value (kcal/100 g)	362.24 ± 0.21	365.39 ± 0.21	369. ± 0.21	371.57 ± 0.21	372.61 ± 0.21

Mean and standard deviation of triplicates. Mean values with different superscripts in a row are significantly ($p \leq 0.05$) different from each other.

have a better nutritional profile and could be suitable raw materials for food items with added value. High-fiber biscuits, such as the control (GWF-T0) and composite cookies (T_1, T_2, T_3, & T_4), contain between 12.63-17.22 g/100 g dietary fiber (Table 2). Consuming foods high in dietary fiber has numerous health advantages, including lowering the risk of chronic illnesses like diabetes, cardiovascular disease, obesity, and certain types of cancer, and promoting the growth of the gut microbiota, which positively impacts various physiological processes [32].

Additionally, given the physiological effects of high-starch diets on humans, the reduction in starch content (from 40.20 to 34.36 g/100g) of the cookies with increasing GFMF and GPMF levels might imply an improved nutritional advantage. The composite biscuits' starch level is equivalent to that of gluten-free biscuits made with a gluten-free flour mix and sorghum starch (55:45), which had a starch content of 55.80–64.70 g/100 g [33], equivalent to the values (444.55–451.38 kcal/100 g) reported for multi-millet biscuits [27] for which the energy values of the composite biscuits were 362.24–372.61 kcal/100 g.

It was found that adding GFMF and GPMF to cookies had a significant impact on their phytic acid and TIA content, the two anti-nutritional elements. Table 3 shows the anti-nutrients found in cookies as the proportion of GFMF and GPMF in blends of germinated wheat and millet flour increased, the amount of phytic acid (PA) in the cookies decreased from 239.21 mg/100 g to 121.59 mg/100 g. Malting of Millets is known to reduce the phytic acid content as demonstrated by Boncompagni, et al. [34]. Trypsin-inhibitor activity (TIA) was not found in the cookies made from the mixture of GFPM and GPMF, but a low level of TIA (0.09 TIU/mg) was found in the cookies made from 100% GWF. In the cookies, this value was significantly decreased. For wheat, millet, and cowpea flours, previous researchers have noted a similar pattern in trypsin inhibitory activity [15]. Cookie samples with low levels of polyphenol A (PA) and high levels of GFMF and GPMF may indicate better macro-mineral bioavailability, especially for calcium. These samples are typically low in PA and TIA compared to flours (Table 1). Anti-nutrients' low content may be due to thermal breakdown during baking. Consumption improves digestibility and bioavailability of vital nutrients. The PA content in the product is safe for human consumption, considering the reference daily intake (RDI) value of phytate (631-746 mg RDI/day) for the USA and UK [4].

Table 3 reveals the mineral profiles of the cookie samples, the addition of GFMF and GPMF significantly raised the levels of Ca, Fe, Mg, K, and Zn. Jaggery greatly enhanced the estimated mineral content when it was added. This could be a result of the greater mineral profile of GFMF and GPMF in comparison to GWF (Table 1). Cookies' levels of calcium, iron, magnesium, potassium, and zinc increased from 80.76 to 312.32 mg/100 g, 1.92 to 2.89 mg/100 g, 125.2 to 152.28 mg/100 g, 280.43 to 401.43 mg/100 g, and 3.3 to 4.19 mg/100 g, respectively. In T_4 cookies, potassium and calcium were found to be the minerals in the highest concentrations, followed by magnesium, zinc, and iron (i.e., P > Ca > Mg > Zn > Fe). In germinated finger and pearl Millet flour, calcium concentrations increased from 210.4 mg/100 g to 2.58 mg/100 g and 71.21 mg/100 g to 2.58 mg/100 g respectively (Table 1) in comparison to germinated wheat flour. As a result, cookies made with GFMF and GPMF are a rich source of calcium for the growth of bones and teeth [20]. Potassium is a crucial mineral component necessary for the body's utilization of iron and the control of high blood pressure [35]. Iron was the least abundant in the cookies, with values ranging from 1.92 mg/100 g in T_0 to 2.89 mg/100 g in T_4 cookies, but it rose with rising GFMF and GPMF, which may be related to millet's high iron content and the customers' increased packed cell volume [36]. Kulthe, et al. [37] found that replacing wheat flour with pearl millet flour significantly increased iron, calcium, and phosphorus content in cookies from 2.48%, 18.26%, and 86.7% to 6.71%, 29.36%, and 208.1%, respectively. This resulted in a greater concentration of minerals in the biscuit samples compared to GFMF, GPMF, and GWF flours (Table 1). The release of bound mineral elements during baking may contribute to this, indicating a higher micronutrient density in cookies. Minerals play crucial roles in human physiology, including regulating the immune system, heartbeat, hormone production, bone tissue, and nerve impulse transmission [28].

Polyphenols and antioxidant properties of germinated flours and cookies

As GFMF and GPMF increased (T_1 to T_4), their polyphenols and antioxidant activity increased, as measured by TPC, TFC, flavonoid, FRAP, and DPPH values of GWF (T_0)-based cookies (Table 3). This may be due to their greater antioxidant capacities (Table 1). Plant-based meals containing phenolic compounds have the ability to serve as antioxidant agents against various degenerative illnesses [38]. Phenolic chemicals can chelate metals, activate antioxidant enzymes, and protect the body from free radicals [29]. The cookies' total phenolic and flavonoid contents ranged from 11.45 mg GAE/g in T_0 to 49.12 mg GAE/g in T_4, as well as from 2.99 mg QE/g in T_0 to 4.10 mg QE/g in T_4. According to Chiemela, et al. [14] and Mudau, et al. [5], the levels of GFMF and GPMF may be responsible for the greatest total phenol and total flavonoid contents of T_4. The DPPH radical scavenging capacity and FRAP of the cookies improved with the rise in GFMF and GPMF, with T_0 having the lowest values and T_4 having the greatest values. The ranges were 62.11% to 73.49% and 0.864 to 1.008 mg GAE/100 g, respectively. The scavenging activity of the sample increases with the DPPH value of the cookies. Adebo, et al. [39] suggest that the release of soluble bioactive chemicals, like oligosaccharides and peptides, may be the cause of the rise in DPPH seen following the addition of GFMF and GPMF cookies. This indicates that the cookie samples'

Table 3: Mineral composition, Anti-nutritional, Polyphenols, and Antioxidant Activity of cookies.

Parameters	T_0	T_1	T_2	T_3	T_4
Mineral composition					
Calcium (mg/100 g)	80.76 ± 0.81	182.32 ± 0.47	213.67 ± 1.01	274.22 ± 1.09	312.32 ± 1.12
Iron (mg/100 g)	1.92 ± 0.21	2.03 ± 0.21	2.27 ± 0.21	2.58 ± 0.21	2.89 ± 0.21
Magnesium (mg/100 g)	125.2 ± 0.21	138.85 ± 0.21	143.38 ± 0.21	149.22 ± 0.21	152.28 ± 0.21
Potassium (mg/100 g)	280.43 ± 0.21	313.11 ± 0.21	365.71 ± 0.21	386.38 ± 0.21	401.43 ± 0.21
Zinc (mg/100 g)	3.3 ± 0.21	3.8 ± 0.21	4.01 ± 0.21	4.14 ± 0.21	4.19 ± 0.21
Anti-nutritional factors					
TIA (TIU/mg)	0.09 ± 0.01	ND	ND	ND	ND
Phytic acid (mg/100 g)	239.21 ± 0.21	207.36 ± 0.21	162.51 ± 0.21	144.22 ± 0.21	121.59 ± 0.21
Polyphenols and Antioxidant Activity					
TPC (mg GAE/100 g)	11.45 ± 0.21	20.82 ± 0.21	35.39 ± 0.21	41.66 ± 0.21	49.12 ± 0.21
TFC (mg QE/g)	2.99 ± 0.21	3.27 ± 0.21	3.61 ± 0.21	3.93 ± 0.21	4.10 ± 0.21
FRAP (mg GAE/100 g)	0.864 ± 0.21	0.903 ± 0.21	0.978 ± 0.21	0.992 ± 0.21	1.008 ± 0.21
DPPH (%)	62.11 ± 0.21	64.55 ± 0.21	68.13 ± 0.21	71.80 ± 0.21	73.49 ± 0.21

Mean and standard deviation of triplicates. Mean values with different superscripts in a row are significantly ($p \leq 0.05$) different from each other. TIA: Trypsin Inhibitor Activity; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; FRAP: Ferric Reducing Antioxidant Power; DPPH: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay; ND: Not Detected.

functional composition has improved and an increase in antioxidant activity can reduce free radicals and peroxides while strengthening the body's own anti-oxidative enzymes [40].

In vitro protein and starch digestibility of germinated flours and cookies

The *in vitro* starch digestibility (IVSD) and *in vitro* protein digestibility (IVPD) of the flours and cookies, respectively, are shown in Table 1 and Figure 3. Protein quality and bioavailability in food items may be assessed using *in vitro* protein digestibility. The cookies' IVPD varied from 62.24% in T_0 to 82.34% in T_4. With an increase in GFMF and GPMF levels, the digestibility of cookies significantly improved ($p \leq 0.05$). Millets' germination significantly impacts protein absorption by promoting structural changes in proteins like globulin, improving chain flexibility and accessibility to proteases. Prior research by Akusu, et al. [41] found that this is due to the high concentration of soluble globular proteins and amino acids in GFMF and GPMF, which result from the change in protein structures during processing. Germination also promotes chain flexibility and accessibility to proteases, further enhancing the protein's availability [42]. This increases the protein digestibility of the offspring. From 47.48% in T_0 to 62.41% in T_4, the IVSD was present. Due to the high fat, protein, dietary fiber, and phenolic content of GFMF and GPMF, the IVSD in the GFMF and GPMF mix cookies was considerably ($p \leq .05$) higher (Table 1). Therefore, those who have diabetes and obesity will benefit from its ingestion [43].

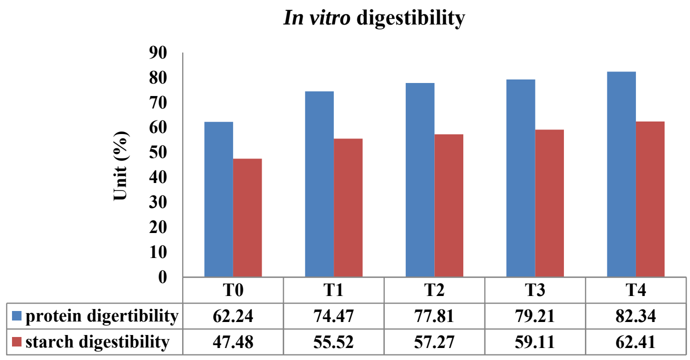

Figure 3: *In vitro* protein and starch digestibility of germinated wheat and germinated finger and pearl millet cookies.
Mean values with different superscripts in a row are significantly (p - 0.05) different from each other. T_0 = 100% GWF; T_1 = 80% GWF + 10% GFMF + 10% GPMF; T_2 = 60% GWF + 20% GFMF + 20% GPMF; T_3 = 40% GWF + 30% GFMF + 30% GPMF; T_4 = 20% GWF + 40% GFMF + 40% GPMF.

Physical properties of cookies

The GFMF and GPMF mix cookies' physical characteristics are displayed in Table 4. We found that the diameter and thickness of the cookies were unaffected by the rising levels of GFMF and GPMF. As the GFMF and GPMF mix concentration increased, the weight of T_0 to T_4 cookies dropped. Cookies baked using local GWF flours have a greater weight. High fiber content prevents more moisture from escaping during baking, increasing the weight of the cookies [5]. This is consistent with a study by Omoba, et al. [36], which found a correlation between the weight of cookies and their greater fiber content. The reduction in the fiber content of flours (Table 1) may be the cause of the weight loss in GWF (T_0) cookies. And the functional qualities of jaggery may have something to do with the weight fluctuation. The modest weight gain may be caused by variations in the amount of moisture that jaggery retains.

The spread ratio (SR) in cookies is influenced by the quality of the flour used and their expansion capacity [44]. GFMF and GPMF-based cookies (T_1 to T_4) have a low SR compared to the control (T_0), which is desirable. The high fiber content of the cookies (Table 2) may be responsible for the reduction in SR compared to the high SR reported in T_0 cookies, with a tendency that is consistent with the research of Chinma, et al. [4]. The increase in protein content in millet flour may also contribute to the observed drop in spread ratio in germinated cookies. Mahalaxmi, et al. [1] suggest that flours with high protein content yield cookies with a low spread ratio. Takawale, et al. [31] linked greater dough protease or proteinase activity with a smaller spread ratio. Additionally, Mashau, et al. [45] found that cookies made with fermented, protein-rich powdered cassava had a lower spread ratio.

As the quantity of germinated millet flour (GFMF-GPMF) was increased, the hardness of the cookies dramatically decreased from T_0 (71.19 N) to T_4 (63.84 N) (Table 4). A higher percentage of germinated millets may have diluted the gluten concentration, which might explain why the hardness has decreased. The increased carbohydrate content of GWF also contributes to the reduced hardness (Table 1). According to Mashau, et al. [45], the high starch content of tortillas made from 100% maize flour resulted in a worse water-holding capacity as well as a faster rate of starch retrogradation and shrinkage. The findings also revealed that the hardness of the biscuit decreased noticeably as the amount of multi-millet flour increased, and textural alterations suggested a softer texture for the biscuits. The samples' rising levels of protein and fat may be the cause of this. Arepally, et al. [27] speculate that the elevated protein content may have sped up the pace of water absorption and reduced stiffness. As a result, the findings of Singh, et al. [6] on the production of biscuits using wheat and pearl millet flour showed that when the proportion of pearl millet flour was increased, the hardness of the biscuits reduced from 5798 to 4323 g.

Table 4 shows that baking browning, regulated by dissolved reducing sugar and water, significantly affects cookies' color [5]. In T_0 to T_4 samples, the L* values decreased significantly from 64.24 to 51.37 ($p \leq 0.05$). Finger millet and pearl millet's dark tint, compared to wheat flour's white color, was attributed to the increased amounts of GFMF and GPMF. Millet flour's strong phenolic and mineral content also contributed to its black color. The * values, which indicate how red the cookies are, considerably increased from 3.83 in sample T_0 to 5.87 in T_4. Cookies samples T_0 through T_4 had b* values that varied from 30.61 to 15.35, which clearly indicates a color. According to Arepally, et al. [27], the overall color difference (ΔE) > 3 is often easy to see with the human eye. Table 4 shows that when the ratio of GFMF and GPMF to GWF increased, ΔE considerably increased for the samples T_0 to T_4 from 0.00 to 13.27. As a result, the samples T_0 through T_4 can clearly be distinguished by their different colors.

Sensory properties of cookies

As per the recommendations of Sugumar & Guha [46], a panel of 25 untrained individuals participated in the sensory evaluation. To the best of our knowledge, there isn't much research on making germinated millet cookies with a combination of germinated finger and pearl millet flour. Before conducting any further investigation, the authors first wanted to determine if germinating multi-millet cookies made with these flours would indeed be edible to a consumer. This variety of cookies' customer acceptability was determined in this study. Table 4 lists the typical panel impressions of cookies produced using different GFMF and GPMF to GWF ratios for various sensory qualities. For cookie samples T_3 and T_4, it was shown that the sensory score differed statistically (p 0.05) for each characteristic. Sample T_0 received the highest score for scent, followed by samples T_1 and T_2 for mouthfeel, and sample T_3. The biscuit sample T_3, which had an overall acceptability score of 8.04, received the highest marks for appearance, color, and crispiness. The dextrinization and browning reactions of starch and protein molecules [47] may have contributed to the high consumer preference for cookies with 10% and 20% GFMF and GPMF (T_1 & T_2) in terms of appearance and crispiness (Table 4). Lower ratings for sensory characteristics indicate deteriorating sensory perceptions. Arepally, et al. [27] found that biscuits made with wheat flour had the highest overall acceptability score, followed by biscuits made with 30% multi-millet flour to wheat flour ratio. Kulkarni, et al. [47] found similar results

Table 4: Physical and sensory properties of cookies prepared from germinated finger millet and germinated pearl millet flour blends

Parameters	T_0	T_1	T_2	T_3	T_4
Physical Properties					
Thickness (cm)	0.64 ± 0.03	0.68 ± 0.06	0.68 ± 0.9	0.74 ± 0.11	0.76 ± 0.15
Diameter (cm)	4.52 ± 0.23	4.51 ± 0.42	4.52 ± 0.28	4.50 ± 0.36	4.51 ± 0.23
Spread ratio	5.09 ± 0.67	5.07 ± 0.25	5.04 ± 0.41	5.02 ± 0.26	5.01 ± 0.38
Hardness (N)	71.19 ± 0.99	70.6 ± 0.94	68.24 ± 0.95	66.77 ± 0.57	63.84 ± 0.95
Weight (g)	4.14 ± 0.12	4.12 ± 0.23	4.11 ± 0.73	4.09 ± 0.18	4.07 ± 0.17
Color					
L* (lightness)	64.24 ± 0.81	62.85 ± 0.97	56.22 ± 0.97	53.93 ± 0.96	51.37 ± 0.88
a* (redness)	3.83 ± 0.31	4.11 ± 0.22	4.52 ± 0.25	5.09 ± 0.65	5.87 ± 0.48
b* (yellowness).	30.61 ± 0.92	26.22 ± 0.57	21.58 ± 0.54	18.11 ± 0.82	15.35 ± 78
ΔE	0.00 ± 0.00	3.25 ± 0.16	5.66 ± 0.36	9.89 ± 0.46	13.27 ± 0.71
Sensory properties					
Appearance	7.90 ± 0.14	7.99 ± 0.72	8.06 ± 0.34	7.88 ± 0.33	7.05 ± 0.42
Taste	7.75 ± 0.42	7.88 ± 0.34	7.95 ± 0.17	8.00 ± 0.45	7.34 ± 0.27
Aroma	7.50 ± 0.38	7.44 ± 0.13	7.12 ± 0.25	7.10 ± 0.24	7.00 ± 0.29
Mouthfeel	7.44 ± 0.29	7.68 ± 0.43	7.92 ± 0.23	7.51 ± 0.36	7.24 ± 0.19
Crispiness	7.40 ± 0.18	7.92 ± 0.21	7.75 ± 0.31	8.11 ± 0.57	7.43 ± 0.27
Overall acceptability	7.50 ± 0.21	7.95 ± 0.59	7.55 ± 0.46	8.04 ± 0.42	7.21 ± 0.16

Mean values with different superscripts in a row are significantly (p - 0.05) different from each other. T_0 = 100 % GWF; T_1 = 80% GWF + 10% GFMF + 10% GPMF; T_2 = 60% GWF + 20% GFMF + 20% GPMF; T_3 = 40% GWF + 30% GFMF + 30% GPMF; T_4 = 20% GWF + 40% GFMF + 40% GPMF.

when cookies with different amounts of pearl millet (10% - 50%) were sensory-wise evaluated, performing better than the control cookies.

Conclusion

The study revealed that incorporating 60% - 80% GWF with ≤ 20% GFMF and GPMF in cookies yielded favorable nutritional, antioxidant, textural, and sensory qualities. The addition of GFMF and GPMF significantly enhanced color attributes, making them more appealing. The most suitable choice is T_2 cookies with 60% GWF, 20% GFMF, and 20% GPMF. Similar healthy elements, nutritive and sensory aspects, and health-promoting features may be found in these cookies. Additionally, they have high *in vitro* digestibility, which suggests that they have the potential to be useful products. While the current findings are promising, further investigations are necessary to address important aspects of these cookies. It is imperative to evaluate the storage stability of the developed cookies, ensuring they maintain their quality over time. Additionally, structural elucidation of the products will provide valuable insights into their composition, aiding in better understanding their potential benefits, along with descriptive sensory analysis. These additional investigations will provide better insights into the sensory characteristics and overall acceptance of the products. *In vitro* protein and starch digestibility suggest that the cookies made from germinated millets are easily digestible and have numerous health benefits.

Declarations

Author contribution statement: Shikha Sharma: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Wrote the paper.

Professor Dr. A.P. Garg, designed and refined the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools, or data; Finalized the paper.

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