

1 **Effect of partial substitution of wheat flour with millet-based gluten free**  
2 **composite flour on the quality of cookies**

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5 **Abstract**

6 Effects of partial replacement (20, 40, 60, 80 and 100%) of whole wheat flour by composite flour  
7 (based on foxtail millet, proso millet and buckwheat flours in equal proportions) on physical,  
8 nutritional, sensory and antioxidant properties of multi-millet cookies were investigated. An  
9 increase in the ratio of composite flour in the blend and formulated cookies resulted in an increase  
10 in ash, fiber, fat and protein and a decrease in moisture and carbohydrate contents. Increase in  
11 supplementation levels of composite flour added to the TPC (Total Phenolic Content), DPPH (2,2-  
12 diphenyl-1-picrylhydrazyl), reducing power and FRAP (Ferric Reducing Antioxidant Power) and  
13 decrease in metal chelating activity of cookies. Maximum gain in thickness and weight and loss in  
14 diameter, spread ratio and bake loss were observed with the highest composite flour substitution.  
15 Color values such as a\* was found to be enhanced whereas L\* and b\* were found to be weakened,  
16 simultaneously NEB (Non-Enzymatic Browning) values got increased upon substitution. A 60%  
17 blend of composite flour with whole wheat flour was selected best used in the cookie's formulation  
18 based on of sensory evaluation. Overall, this study demonstrates that millet based composite flour  
19 can effectively improve the functional values of formulated cookies, in addition to an option for  
20 the developing of gluten free products for celiac patients.

21 **Keywords:** Foxtail millet, proso millet, buckwheat, multi-millet cookies, gluten free.

22  
23 **Introduction**

24 It is evident from various researches that coarse cereals, pseudo-cereals and by-products are  
25 nutritionally superior to staple cereals such as rice and wheat which make them suitable for  
26 industrial-scale utilization for various food preparations like weaning food, snack food, composite  
27 dairy mix, and other dietary products. Possession of phenolic compounds ensures their potential  
28 health benefits. Foxtail millet (*Setaria italica*) is one of them that work to enrich prepared food

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29 products with its dietary fiber, divalent cation minerals ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Zn}^{2+}$ ) with low glycemic  
30 index attributes (Kim *et al.* 2011). It is high in polyphenolics which prevents cardiovascular  
31 disease, and elevates our immune system (Marak *et al.* 2019). Proso millet nutritive profile is also  
32 comparable to common staples even exceeding in regard to protein, crude fiber and phenolics  
33 (Amadou *et al.* 2013). It has attracted attention for its considerable health benefits, particularly  
34 anti-cancer, anti-diabetic, antiproliferative effects and anti-liver injury. Studies have shown that  
35 the extracts of proso millet possess antioxidant properties (Chandrasekara and Shahidi, 2012).  
36 Similarly, pseudo-millet are the key targets of researchers especially buckwheat (*Fagopyrum*  
37 *esculentum*) having prime importance owing to its high protein uniqueness with favorable amino  
38 acids, high dietary fiber and almost all vitamins, essential minerals, and trace elements. Buckwheat  
39 possesses health benefits like reduction of high blood pressure, blood sugar control, lower blood  
40 cholesterol, prevention of fat accumulation, constipation, colon carcinogenesis and mammary  
41 carcinogenesis, strengthen capillary blood vessels and suppresses plasma cholesterol and gallstone  
42 formation (Sedej *et al.* 2011). Another functional property of buckwheat is its gluten-free  
43 characteristics which make it a promising diet for patients suffering from celiac disease (Chopra *et*  
44 *al.* 2014).

45 Development of bakery products such as biscuits and cookies based upon the fortification of  
46 composite flour is the recent trend in the bakery industry (Hussain and Kaul, 2018: Hussain *et al.*  
47 (2018a). Research studies incorporating millets for the formulation of bakery products have been  
48 reported earlier. The foxtail millet (20, 30, and 40%) substituted wheat flours were studied and  
49 compared to the control (100% wheat flour) (Marak *et al.* 2019). The increase in incorporation  
50 levels enhanced the functional characteristics, phenolic contents and antioxidant capacities (DPPH  
51 and ABTS scavenging potentials) of the composite flour as well as the cookies developed. Sensory  
52 evaluation disclosed that cookies developed from 30% foxtail millet had comparable scores with  
53 that of control. Rice flour based gluten-free cookies prepared by blending malted foxtail millet,  
54 proso millet and buckwheat flours at 15 % and 30 % levels was investigated by Kumari *et al.*  
55 (2023). The protein, fat, ash, total dietary fiber and in vitro protein digestibility of developed cookies  
56 increased whereas glycemic and starch hydrolysis indices decreased upon increase in substitution  
57 levels of malted flours of all three grains. But till date studies having wheat based composite flour  
58 comprised of a blend of foxtail millet, proso millet, and buckwheat flours for cookies formulation  
59 are not reported. Since the selected crops are gluten-free, thus replacing wheat flour with composite

60 flour-based on these crops will dilute the wheat gluten proteins, and thereby help in preventing  
61 celiac disorders. In addition to that, the developed product will impart enhanced nutritional and  
62 health benefits due to the presence of ample quantity of essential nutrients and health promoting  
63 constituents in the composite flour based on the selected raw materials. Various chronic diseases  
64 which are prevalent these days can be managed by the consumption of such products. Since the  
65 selected crops at this stage are under under-utilization, this study will help in their revival. The  
66 objective of the present study is to compare the functional properties of the millet-based composite  
67 flour with wheat flour, to utilize it at different levels for cookie making, and to assess the nutritional  
68 characteristics and organoleptic attributes of the formulated cookies.

69  
70 **Materials and methods**  
71 Foxtail millet, proso millet and buckwheat flour was procured from farmers of Garkhon and  
72 Skurbuchan villages of Ladakh, India. Whole wheat flour was procured from farmers of Matho  
73 village. The flours so obtained were sieved through a 40 mm sieve. Ghee, baking powder, refined  
74 sugar, and corn flour were procured from the local market (Leh, India). The experiments were  
75 conducted in the Food Processing Laboratory of HMAARI, SKUAST-K, Leh, India and part of the  
76 quality analysis was done in Division of Food Science and Technology, SKUAST-K, Kashmir,  
77 India.

78  
79 **Preparation of flour blends**  
80 Blends based upon the composite flour were made by taking whole wheat flour as control. The  
81 composite flour was obtained by mixing buckwheat, foxtail millet and proso millet flours in equal  
82 proportions. Blends were prepared by mixing composite flour with wheat flour in the proportions  
83 of 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100, which correspond to 0, 20, 40,60, 80, and 100 %  
84 blends.

85  
86 **Preparation of cookies**  
87 The multi-millet cookies formula based on flour weight was: 100 g (32.05%) flour, 20 g (6.41%)  
88 icing sugar, 40 g (12.82%) ghee, 1 g (0.32%) baking powder, 1 g (0.32%) corn flour and 150 ml  
89 (48.07%) water. The dough was sheeted to a thickness of 0.5 cm with a rolling pin. The cookies  
90 were cut round in shape with a cookie die of diameter 5 cm and transferred to a tray lined with

91 aluminum foil and were baked at 180 °C for 15 min in an electric oven. The cookies were cooled  
92 to room temperature and packed in airtight containers.

93

#### 94 **Functional properties**

##### 95 ***Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC)***

96 The WAC and OAC of flour samples were determined as per the method described by Jan *et al.*  
97 (2015) with slight modifications. The flour samples (1 g each) were mixed with 10 ml distilled  
98 water or refined mustard oil in test tubes and were kept at ambient temperature for 30 min and then  
99 centrifuged for 10 min at 2000 rpm. After centrifugation, the aqueous supernatant or clear oil  
100 obtained was decanted. The test tubes were allowed to drain for 5 min on a paper towel and  
101 followed by weighing of the residue. The WAC/OAC are expressed in g of water/oil per g of dry  
102 weight of the sample.

103

##### 104 ***Foaming Capacity (FC) and Foam Stability (FS)***

105 The determination of FC and FS was done by the method given by Jan *et al.* (2015). Exactly 2 g of  
106 flour samples were mixed with 50 ml of distilled water at 30±2 °C in a 100 ml graduated measuring  
107 cylinder. The suspension was shaken properly by whipping for 3 min in an electrical blender to  
108 form foam. The content was then transferred into the measuring cylinder and the volume of the  
109 foam was recorded after 30 seconds.

110  $FC (\%) = (\text{Volume after whipping} - \text{Volume before whipping}) \times 100 / \text{Volume before whipping}$

111 The foam volume was recorded 1 h after whipping to determine the FS as a percentage of the initial  
112 foam volume.

113  $FS (\%) = (\text{Foam volume after standing time} / \text{Initial foam volume}) \times 100$

114

##### 115 ***Swelling Power (SP)***

116 The method described by Gani *et al.* (2013) with slight modifications was followed to determine  
117 the SP. Briefly, 3 g of each sample was mixed with 30 ml distilled water in a centrifuge tube and  
118 heated at 70°C in a water bath for 30 minutes. The suspension after heating was centrifuged at 1000  
119 rpm for 15 min. The supernatant was decanted and the residue was weighed.

120  $SP = \text{Weight of the residue} - \text{Weight of dry flour}$

121

122

123 **Bulk Density (BD)**

124 The flour samples (100 g each) after tare were gently filled in a 500 ml graduated cylinder. The  
125 bottom of the cylinder was tapped gently for several times until there was no further diminution of  
126 the sample level after filling to 500 ml mark.

127  $BD \text{ (g/ml)} = \text{Weight of flour (g)}/\text{Volume taken by flour (ml)}$

128

129 **Pasting properties**

130 Pasting properties of flour were determined using a rapid visco analyser and calculated through the  
131 pasting curve using Thermocline version 2.4 software (RVA-4, Newport Scientific Pty Ltd.,  
132 Warriewood, Australia). 3.5 g of flour sample (moisture = 14%) was transferred into a canister and  
133 approximately  $25 \pm 0.1$  ml distilled water was added (corrected to compensate for 14% moisture  
134 basis). After heating the slurry at  $50^{\circ}\text{C}$ , it was stirred at 160 rpm for 10 seconds for thorough  
135 dispersion. The slurry was held at  $50^{\circ}\text{C}$  for up to 1 min, and then heated to  $95^{\circ}\text{C}$  over 7.3 min and  
136 held at  $95^{\circ}\text{C}$  for 5 min, and finally cooled to  $50^{\circ}\text{C}$  over 7.7 min. The parameters determined were  
137 peak viscosity, trough viscosity, break down viscosity, final viscosity, set back viscosity, pasting  
138 time and pasting temperature.

139

140 **Proximate composition**

141 The proximate composition of flours and blends were determined according to the methods of  
142 AOAC (2016). Crude protein was estimated according to Kjeldhal method (AOAC 992.23). The  
143 nitrogen conversion factor taken for crude protein was 5.83. Moisture was determined by hot air  
144 oven method (AOAC 952.08), ash by gravimetric method (AOAC 985.29), total fat by acid  
145 hydrolysis method (AOAC 948.15) and dietary fiber by enzymatic gravimetric method (AOAC  
146 930.30). The carbohydrate (non-fiber) content was calculated using difference method by  
147 subtracting the contents of moisture, ash, fat, fiber and protein from 100 % of dry matter.

148

149 **Antioxidant properties**

150 **Preparation of extracts**

151 Each sample (0.3 g) was dissolved in 20 ml of 70% methanol, stirred for 2 hours on a magnetic  
152 stirrer followed by a centrifugation for 10 minutes at  $3500 \text{ g}$ . The supernatant was evaporated at  
153  $40^{\circ}\text{C}$  and the supernatant was stored at  $-18^{\circ}\text{C}$ .

154

155 **Total Phenolic Content (TPC)**

156 The Folin-Ciocateu spectrophotometric method given by Jan *et al.* (2015) with some modifications  
157 was followed for the determination of TPC. The results were expressed as Gallic acid equivalents  
158 (nmolGAE) per  $\mu\text{g}$  of dried weight of sample.

159  
160 **DPPH radical scavenging activity**

161 DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extract solutions  
162 (Methanolic) of each sample was determined according to the method described by Shah *et al.*  
163 (2015). After incubation in dark for 30 minutes, the absorbance at 517 nm was measured after each  
164 sample solution.

165 
$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

166 Where  $A_{\text{control}}$  indicates the absorbance of the control and  $A_{\text{sample}}$  indicates the absorbance of the  
167 extract.

168  
169 **Reducing power**

170 The reducing power of methanol extracts were determined by the method described by Gani *et al.*  
171 (2013) with some modifications. Different volumes of extracts of six samples were mixed with 0.2  
172 M sodium phosphate buffer pH 6.6 (2.5 ml) and 1 % (w/v) of aqueous potassium ferricyanide (2.5  
173 ml). The mixture so obtained was incubated at 50 °C for 20 min. 10 % (w/v) trichloroacetic acid  
174 (2.5 ml) was added to the mixture, which was then centrifuged at 3000 g for 10 minutes. After  
175 centrifugation, 2.5 ml supernatant was collected and diluted with 2.5 ml of deionized water and 0.1  
176 % (w/v) ferric chloride 0.5 ml was added. The absorbance was recorded at 700 nm against methanol  
177 (control) and compared with ascorbic acid which was used as a standard. A higher absorbance  
178 shows a higher reducing power.

179 
$$\text{Reduction (\%)} = (1 - A_C - A_S) \times 100$$

180 Where,  $A_C$  absorbance of standard

181  $A_S$  absorbance of the sample

182  
183 **FRAP (Ferric Reducing Antioxidant Power) value**

184 The method described by Jan *et al.* (2015) was followed for its estimation. 3 ml of the FRAP  
185 reagent [10 ml of acetate buffer (300 mM, PH 3.6), 1 ml TPTZ (10 mM) in hydrochloric acid  
186 solution (40 mM) and 1 ml of  $\text{FeCl}_3$  solution (20 mM)] was added to 0.1 ml of each sample extract.

187 After incubation for 4 minutes at room temperature, absorbance was taken at 593 nm immediately  
188 and the results were expressed as  $\mu\text{M Fe}^{2+} /\text{L}$

189  $\text{FRAP} = \text{Change in absorbance of sample from 0-4 minutes} / \text{Change in absorbance of standard}$   
190  $(\text{ascorbic acid}) \text{ from 0-4 minutes} \times 2$

191 Where 2 indicated the FRAP value of ascorbic acid.

192  
193 ***Metal chelating activity***  
194 The metal chelating activity of samples was determined as described by Jan *et al.* (2015) which  
195 was calculated as follows:

196  $\text{Iron (Fe}^{2+}\text{) chelating activity (\%)} = 1 - (A_{S562}/A_{C562}) \times 100$

197 Where,  $A_{S562}$  Absorbance of the sample at 562 nm

198  $A_{C562}$  Absorbance of the control at 562 nm

199  
200 **Physical characteristics**

201 The thickness of the cookies was measured by a Vernier Caliper and the diameter was determined  
202 using a measuring scale placing them edge-to-edge. From the measurements taken, the spread ratio  
203 (W/T) was calculated, where W is the diameter and T is the thickness of the cookies.

204  
205 **Color analysis**  
206 The color was measured using a Hunter Lab Colorimeter, (Color Flex Reston, Virginia, USA).  
207 Color values were measured as  $L^*$ ,  $a^*$ , and  $b^*$ . Each value represents the average of four  
208 measurements at different points of the cookies. An  $L^*$  value is the lightness, while as  $a^*$  and  $b^*$   
209 values are the chromaticity values that indicate redness and yellowness, respectively.

210  
211 **Non-enzymatic browning index (NEB)**  
212 The NEB of the samples was determined as previously described by Sharma and Gujral (2014).  
213 The browning index ( $\Delta A$ ) was calculated as:

214  $\Delta A = \text{Absorbance at 420 nm} - \text{Absorbance at 550 nm}$

215  
216 **Sensory evaluation**  
217 A panel of 20 judges (semi-trained panelists) from the scientific staff members of HMAARI,  
218 SKUAST-K, Leh, India and students of the FoA, SKUAST-K, Kashmir, India was selected after

219 explaining different terminologies, characteristics of good quality cookies, score-sheet, and  
220 methods of scoring used in the sensory evaluation. The criteria for selection of judges include  
221 availability, capability and reliability. The samples were evaluated on the basis of color, texture,  
222 taste, flavour and overall acceptability using 9-point Hedonic Scale assigning scores 9-like  
223 extremely to 1-dislike extremely. A score of 5.5 and above was considered acceptable.

224  
225 **Texture analysis**  
226 The textural properties of the products were investigated using a Texture analyzer (TA XP plus  
227 Exponent Stable MicroSystem, Brookfield, USA). A 3-point Bending Rig and 5 kg load cell were  
228 used to measure the fracture strength. The distance between the two beams was 7 mm. Another  
229 identical beam was brought down from above at a pre-test speed of 1.0 mm/sec, test speed of 3.0  
230 mm/sec, and post-test speed of 10.0 mm/sec which was continued until the cookie broke. The peak  
231 force was considered as the fracture strength.

232  
233 **Statistical analysis**  
234 The data obtained from experiments performed in triplicates were subjected to one-way ANOVA  
235 and Duncan test by SPSS (version 27.0.1).

236  
237 **Results and discussion**  
238 Although it was difficult to sheet the dough, cookies were developed using 100% composite flour  
239 with extra effort. However, this flour resulted in relatively fragile and darkest cookies among the  
240 other cookie samples studied (Fig. 1).

241  
242 **Functional properties of flour**  
243 The WAC of composite flour was significantly ( $p \leq 0.05$ ) higher than that of wheat flour (Table-1).  
244 Variations in the WAC between both the flour might be responsible due to the difference in protein  
245 structure and the presence of different hydrophilic carbohydrates (Kaur *et al.* 2014). The OAC of  
246 composite flour was significantly lower than that of wheat flour and these variations may be  
247 explained by the variation in the presence of hydrophobic amino acids having an affinity for the  
248 hydrocarbon side chain of the oil. Similar results were observed by Mohamed *et al.* (2009). The  
249 SC of composite flour was lower than that of wheat flour which corroborated with the results of  
250 Poongodi Vijayakumar *et al.* (2010). The SC of the flour depends upon particle size, types of



251 variety, and processing methods used to obtain flour. The difference may also be attributed to the  
252 high level of gluten content in wheat flour which helps it in the retention of water, and increase in  
253 volume by absorption of water. The FC and FS of 100% composite flour were found significantly  
254 higher than that of 100% wheat flour. The results of FC are in line with those found by Kaur *et al.*  
255 (2014). FC is considered to be dependent upon the configuration of protein molecules. Flexible  
256 proteins have a good FC but the reverse is true for highly ordered globular molecules (Baljeet *et*  
257 *al.* 2010). Composite flour having 100% mixed millet flour had the maximum whereas, the control  
258 reported minimum BD's. Present results are in line with the findings of Poongodi Vijayakumar *et*  
259 *al.* (2010).

260  
261 **Pasting properties of flour**

262 Pasting properties are important factors in determining the applications of flours for baking  
263 purposes. The results obtained (Table-2) revealed significant differences in the pasting profiles of  
264 flour blends of millets. As the proportion of composite flour increased, the peak, trough, breakdown  
265 final viscosity, pasting time and pasting temperature decreased while as setback viscosity increased  
266 in flour blends of composite flour from F<sub>1</sub> (0% composite flour) to F<sub>6</sub> (60% composite flour). The  
267 pasting properties depend upon the amount of starch content, amylase activity,  
268 amylose/amylopectin ratio, protein and lipid content present in the flours. Anberbir *et al.* (2024)  
269 reported significant differences between the different types of viscosities in teff, pearl millet and  
270 buckwheat composite flour.

271  
272 **Proximate composition of flour and cookies**

273 The proximate composition as given in Table-3. The moisture content is partially affected by the  
274 fiber content in a sample. However, in our study blended flour had lower moisture content as  
275 compared to wheat flour despite having higher fiber content with a similar trend in its products.  
276 This might be due to the higher fat content which reduces moisture in the blended flour (Sreerama  
277 *et al.* 2010). Hussain *et al.* (2018b) also found similar results during multigrain biscuit formulation.  
278 Ash, fiber, fat and fiber of flour increased while carbohydrate decreased upon blending. Cookies  
279 with composite flour showed higher ash as compared to that with wheat flour. The higher ash  
280 content may be due to the high mineral content in the former. The analyzed formulated cookies  
281 had dietary fiber ranging from and 3.26 to 7.84% with cookies formulated from composite flour

282 had higher fiber content. Chhavi and Savita (2012) observed similar trend in finger millet  
283 incorporated bread. All the cookies formulated contained high fat content ranging from 19.81% to  
284 25.27% which is mainly due to the use of ghee in the formulation of these cookies. Increased total  
285 fat content in flour decreases its shelf life but improves binding ability, flavor retention capacity  
286 and reduces moisture in the final product (Sreerama *et al.* 2010). The formulated cookies had crude  
287 protein content ranging from 8.19 to 9.79% which may be explained by the fact that the composite  
288 flour contain comparatively higher protein. The carbohydrate content of cookies ranged between  
289 51.34 and 63.59%. The carbohydrate levels decreased upon the supplementation which could be  
290 attributed to the higher fraction of other constituents (moisture, protein, ash, fiber and fat content)  
291 present in the composite flour. This trend is also probably because wheat flour contains endosperm  
292 as major portion which is a good source of carbohydrates.

293  
294 **Antioxidant properties of flour and cookies**  
295 The blended flour as well as the cookies formulated by replacing whole wheat flour with composite  
296 flour exhibited significantly ( $p < 0.05$ ) high antioxidant activities for most of the investigated  
297 properties when compared with the control samples (Table-3).

298  
299 **Total phenolic content**  
300 An increasing trend in the formulated cookies was observed upon increase in the ratio of composite  
301 flour. Higher TPC was observed in 100% composite flour (12.07nmolGAE/ $\mu$ g) and cookies  
302 prepared with 100% composite flour (7.65 nmol GAE/ $\mu$ g) as compared to their controls. Other  
303 researchers (Chen *et al.* 2021) revealed increasing trend in TPC upon the substitution of finger  
304 millet flour in rice and wheat-based food products. Sharma and Gujral (2014) suggested that a  
305 continuous baking process could modify the chemical structure of a phenolic compound,  
306 specifically due to polymerization, and consequently reduce extractable polyphenols in baked  
307 products.

308  
309 **DPPH scavenging potential**  
310 DPPH radical scavenging activity was higher in composite blends than in wheat flour and their  
311 formulated products as consequence of higher polyphenolic content in composite flour. The  
312 examined extracts of composite blends possess radical inhibitors or scavengers with the possibility  
313 to act as primary antioxidants. Similar results were found by Sedej *et al.* (2011) for milling fractions

314 of wheat and buckwheat. The extracts might react with free radicals (peroxy radicals) which are  
315 the major contributors of the auto-oxidation chain of fat, thereby culminating the chain reaction  
316 (Sharma and Gujral, 2014). Baking led to an increase in the scavenging activity of radicals in  
317 cookies which has earlier been reported by Sharma and Gujral (2014). The possible reason behind  
318 this might be the formation of brown pigments melanoidins, the products of maillard reaction,  
319 which takes place during the process of baking.

320  
321 **Reducing power**  
322 Better reducing power was found in composite flour as well as cookies with 100% composite flour  
323 than their respective controls thus showing increasing trend on increase in blending ratios.  
324 Incorporation of buckwheat and rye flour to wheat flour for bread preparation led to the increase  
325 in reducing power on baking (Filipev *et al.* 2011). The reducing power of an antioxidant  
326 compound is because of the presence of reductones and their antioxidant capacity is due to the  
327 breaking of the free radical chain reaction by donating a hydrogen atom and preventing peroxide  
328 formation. Baking of cookies led to an increase in reducing power. The products which were  
329 generated during the baking of cookies due to maillard reaction, might resulted in increasing the  
330 reducing power (Sharma and Gujral, 2014).

331  
332 **FRAP value**  
333 FRAP value of flour blends increased with the incorporation of composite flour. FRAP was found  
334 to be lowest for wheat flour-based cookies (control) and it increased as the proportion of composite  
335 flour increased in the blends. These results are in accordance with that of Jayawardana *et al.* (2022)  
336 in finger millet blended biscuits. The polyphenolic content of composite flour as well as  
337 supplemented cookies is responsible for their ferric ions reducing ability.

338  
339 **Metal chelating activity**  
340 The metal chelating activity was significantly higher for wheat flour extracts than for composite  
341 flour extracts as well as their respective cookies. Although the methanolic extracts of wheat flour  
342 exhibited more potent chelating activity on  $Fe^{2+}$  than the millet extracts despite rutin content of  
343 millets possesses more structural features than ferulic acid for complexing metal ions. Lower  
344 antioxidative capacity of rutin as metal chelator in comparison to ferulic acid (and other  
345 hydroxycinnamic acids from wheat flour) might be due to steric hindrance of the sugar moiety of

346 rutin (Sedej *et al.* 2011). The presence of ferulic acid, a high potent chelating component in wheat  
347 flour extract might be responsible for its higher chelating capacity than composite flour extract.

348  
349 **Physical properties of cookies**

350 Since appearance is used to evaluate a food product before the taste, physical characteristics are  
351 equally important determinants of consumer acceptability and marketability. The physical  
352 properties of cookies prepared from blended flour and wheat flour are shown in Table-4. The  
353 diameters of cookies made from composite flour were found significantly lower than 100% wheat  
354 flour. The thickness of cookies ranged between 0.75 to 0.81 cm, which increased with increase in  
355 the level of composite flour incorporations. Increase in thickness might be due to the decrease in  
356 diameter on addition of composite flour content in cookie dough. The changes in diameter and  
357 thickness were reflected in spread ratio. Spread ratio decreased with the addition of composite  
358 flour. Other workers, Sharma and Gujral (2014) and Kaur *et al.* (2014) obtained similar results in  
359 barley and buckwheat blended cookies, respectively. Reduced spread ratios of composite flour  
360 fortified cookies were attributed to the fact that composite flours form aggregates with increased  
361 numbers of hydrophilic sites available that compete for the limited free water in biscuit dough. The  
362 thicknesses and spread ratios of the cookies were inversely proportional to the level of millet based  
363 composite flour. The weight of cookies increased as the concentration of composite flour increased  
364 in the blends. The range of cookie weight was 10.30 g to 11.35 g with maximum value in 100%  
365 composite flour cookies. The results are in agreement with the that of Chauhan *et al.* (2016) which  
366 they have attributed to the high water-holding capacity of amaranth flour when compared to wheat  
367 flour. The decrease in bake loss of cookies with the increase in the level of composite flour can be  
368 attributed to the ability of their dietary fiber to retain more water.

369  
370 **Color analysis of cookies**

371 Composite flour incorporation significantly ( $p < 0.05$ ) affected the colors of the cookies. The surface  
372 color of the cookie decreased for  $L^*$  and  $b^*$  as the composite content increased in the blends (Table-  
373 4). While as, increase in  $a^*$  was observed with an increase in composite flour content in the cookies.  
374 The decrease of  $L^*$  and  $b^*$  values as well as the increase of  $a^*$  values indicated that the darkness  
375 of incorporated biscuits was proportional to the level of composite flour. The crumb color of  
376 barnyard millet-wheat composite flour bread (Singh *et al.* 2012) and finger millet supplemented

377 wheat biscuits Jayawardana *et al.* (2022) was reported to be lower as compared to their controls.  
378 Maillard browning and caramelization of sugar in addition to oxidation of phenolic compounds  
379 considered to produce brown pigments during baking (Sharma and Gujral, 2014).

380  
381 **Non-enzymatic browning index of cookies**  
382 The NEB index of pure wheat and pure blended cookies was observed to be 0.008 and 0.024,  
383 respectively (Table-4). Incorporation led to a significant increase in non-enzymatic browning index  
384 which might be due to possible dilution of the sugars and proteins of the wheat flour upon  
385 incorporation of composite flour. Sharma and Gujral (2014) and Jan *et al.* (2015) reported a  
386 significant increase in browning upon baking of *chapati* and cookies, respectively, which  
387 corroborate with our results. Increase in browning is associated with Maillard browning,  
388 caramelization of sugar occurring during baking of cookies. (Sharma and Gujral, 2014).

389  
390 **Texture profile analysis of cookies**  
391 The hardness of the cookies is determined from curve shown in Fig. 2(a). Once the trigger force is  
392 attained, the force is seen to increase until the cookie fractures and falls into two pieces. This is  
393 observed as the maximum force and can be referred to as the ‘hardness’ of the sample. The distance  
394 at the point of break is the resistance of the sample to bend and so relates to the ‘fracturability’ of  
395 the sample i.e., a sample that breaks at a very short distance has a high fracturability. The cookies  
396 made from 100% composite flour were found to be the least hard and most fragile thus hardness  
397 of cookies decreased with the incorporation of composite flour. The hardness of wheat flour  
398 cookies (100%) and composite flour cookies (100%) were 6952.48 g and 2987.25 g, respectively.  
399 The order of the hardness was found to be as follows;  $C_1 > C_2 > C_3 > C_4 = C_5 < C_6$ . The values of  
400 hardness and fracturability are given in Table 4. Baljeet *et al.* (2010) found similar results for  
401 buckwheat biscuits. The addition of gluten free component (composite flour) to flour blends may  
402 be responsible for the softer cookies.

403  
404 **Sensory attributes of cookies**  
405 From the results, Fig. 2(b), it was observed that most of the sensory score of the blended flour  
406 cookies displayed the decreasing trend from  $C_1$  to  $C_6$  samples because of increasing substitution  
407 levels of composite flour. The sensory scores for color, texture and appearance saw a decline,  
408 simultaneously a rise in scores for taste and flavor in cookies formulated using composite flour was

409 observed. Our results in terms of appearance and texture are also in accordance with that of  
410 Poongodi Vijayakumar *et al.* (2010). The composite flour had a dark colour, due to its high  
411 phenolics, ash content and sandiness, which might have contributed to the reduction in panel  
412 sensory perceptions and hence decreased the scores for sensory attributes like colour, texture and  
413 appearance. Other studies of millet have also found that, as the amount of millet addition increases,  
414 sensory scores decrease (Shimray *et al.* 2012). Most of the sensory attributes were significantly  
415 affected however the decline in the scores was not very high due to supplementation. The sensory  
416 results indicated that the inclusion of 60% millet based composite flour seems to be the most  
417 preferred amount to add to a cookie.

418  
419 **Conclusions**  
420 From our study, it can be concluded that addition of blended flour (foxtail millet, proso millet and  
421 buckwheat) to whole wheat flour enhances the protein, fiber, fat, ash, total phenolic content and  
422 antioxidant properties of the multi-millet cookies. The Overall acceptability of cookies was highest  
423 at the 60% level of blending. This study may provide ample scope for utilization of these under-  
424 utilized gluten free crops in other food products. This will also be contributing step to the  
425 celebration of International Year of Millets recognized by FAO and UNO for the year 2023.

426  
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**Table-1:** Functional properties of flour.

Sample	WAC (g/g)	OAC (g/g)	SC (%)	FC (%)	FS (%)	BD (g/mL)
F <sub>1</sub>	1.20 <sup>a</sup> ±1.76	1.88 <sup>b</sup> ±1.43	5.78 <sup>f</sup> ±0.09	09.42 <sup>a</sup> ±0.29	52.12 <sup>a</sup> ±0.94	0.72 <sup>a</sup> ±0.05
F <sub>2</sub>	1.34 <sup>a</sup> ±2.19	1.67 <sup>b</sup> ±1.45	5.31 <sup>e</sup> ±1.07	09.39 <sup>a</sup> ±0.11	54.61 <sup>b</sup> ±0.63	0.75 <sup>b</sup> ±0.02
F <sub>3</sub>	1.53 <sup>a</sup> ±1.08	1.34 <sup>a</sup> ±2.76	5.13 <sup>d</sup> ±0.31	09.16 <sup>a</sup> ±0.09	60.51 <sup>c</sup> ±1.43	0.80 <sup>c</sup> ±0.11
F <sub>4</sub>	1.61 <sup>a</sup> ±0.76	1.19 <sup>a</sup> ±2.04	4.93 <sup>c</sup> ±0.04	13.39 <sup>b</sup> ±0.45	62.13 <sup>d</sup> ±0.32	0.87 <sup>d</sup> ±0.07
F <sub>5</sub>	1.67 <sup>a</sup> ±1.45	1.14 <sup>a</sup> ±0.09	4.38 <sup>b</sup> ±2.06	21.64 <sup>c</sup> ±0.37	64.41 <sup>e</sup> ±0.41	0.94 <sup>d</sup> ±0.09
F <sub>6</sub>	1.70 <sup>a</sup> ±0.37	1.09 <sup>a</sup> ±0.98	3.89 <sup>a</sup> ±0.09	30.38 <sup>d</sup> ±0.34	70.62 <sup>f</sup> ±0.14	1.02 <sup>d</sup> ±0.05

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The values with different superscript in a column differ significantly ( $p \leq 0.05$ ). F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub> and F<sub>6</sub> refer to 0, 20, 40, 60, 80, 100% composite flour, respectively.

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WAC: Water Absorption Capacity, OAC: Oil Absorption Capacity, SC: Swelling Capacity, FC: Foaming Capacity, FS: Foaming Stability,

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BD: Bulk Density.

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**Table 2:** Pasting properties of flour.

Sample	Peak Viscosity (cp.)	Trough Viscosity (cp.)	Breakdown Viscosity (cp.)	Final Viscosity (cp.)	Setback Viscosity (cp.)	Pasting time (min)	Pasting Temperature (°C)
F <sub>1</sub>	2341 <sup>c</sup> ±35.14	1636 <sup>d</sup> ±41.88	971 <sup>f</sup> ±0.09	2982 <sup>c</sup> ±24.63	1352 <sup>a</sup> ±0.94	6.702 <sup>d</sup> ±0.08	85.72 <sup>c</sup> ±0.61
F <sub>2</sub>	2218 <sup>d</sup> ±28.38	1670 <sup>d</sup> ±9.64	834 <sup>e</sup> ±1.07	2911 <sup>e</sup> ±41.39	1461 <sup>c</sup> ±2.63	5.175 <sup>b</sup> ±0.07	85.56 <sup>c</sup> ±0.88
F <sub>3</sub>	1975 <sup>c</sup> ±10.50	1541 <sup>c</sup> ±1.15	619 <sup>d</sup> ±49.31	2816 <sup>d</sup> ±31.09	1405 <sup>b</sup> ±7.43	5.380 <sup>c</sup> ±0.11	84.28 <sup>b</sup> ±1.91
F <sub>4</sub>	1580 <sup>b</sup> ±31.95	1216 <sup>b</sup> ±26.02	493 <sup>c</sup> ±10.04	2539 <sup>c</sup> ±0.45	1413 <sup>b</sup> ±0.32	5.287 <sup>c</sup> ±0.07	84.62 <sup>b</sup> ±5.73
F <sub>5</sub>	1406 <sup>b</sup> ±16.18	1288 <sup>b</sup> ±33.19	339 <sup>b</sup> ±2.06	2464 <sup>b</sup> ±22.17	1644 <sup>d</sup> ±82.41	5.094 <sup>a</sup> ±0.09	83.42 <sup>a</sup> ±1.19
F <sub>6</sub>	1153 <sup>a</sup> ±12.27	1024 <sup>a</sup> ±19.16	173 <sup>a</sup> ±19.89	2309 <sup>a</sup> ±1.34	1770 <sup>e</sup> ±11.14	5.028 <sup>a</sup> ±0.05	84.19 <sup>b</sup> ±0.48

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The values with different superscript in a column differ significantly ( $p \leq 0.05$ ). F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub> and F<sub>6</sub> refer to 0, 20, 40, 60, 80, 100% composite flour, respectively.

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**Table 3:** Proximate composition and antioxidant properties of flour and cookies.

Sample	Proximate composition (%)						Antioxidant properties				
	Moisture content	Ash	Fiber	Fat	Protein	Non-fiber carbohydrate	TPC (nmolGAE/ μg)	DPPH Inhibition (%)	Reducing power (%)	FRAP (μM Fe <sup>2+</sup> /L)	Metal chelating activity (%)
F <sub>1</sub>	13.08 <sup>a</sup> ±0.09	1.69 <sup>e</sup> ±0.06	2.62 <sup>f</sup> ±0.06	01.75 <sup>d</sup> ±0.01	10.14 <sup>c</sup> ±0.15	70.72 <sup>a</sup> ±0.07	07.67 <sup>f</sup> ±0.01	38.19 <sup>e</sup> ±0.01	33.15 <sup>f</sup> ±1.03	2.08 <sup>d</sup> ±0.10	52.51 <sup>a</sup> ±8.5
F <sub>2</sub>	12.77 <sup>b</sup> ±0.02	1.93 <sup>d</sup> ±0.01	3.13 <sup>e</sup> ±0.01	01.96 <sup>d</sup> ±0.07	10.15 <sup>c</sup> ±0.15	70.06 <sup>a</sup> ±0.11	08.08 <sup>e</sup> ±0.01	44.26 <sup>d</sup> ±0.05	37.30 <sup>e</sup> ±1.60	2.08 <sup>d</sup> ±0.24	30.06 <sup>b</sup> ±1.63
F <sub>3</sub>	12.26 <sup>c</sup> ±0.08	2.34 <sup>c</sup> ±0.02	3.66 <sup>d</sup> ±0.01	02.37 <sup>c</sup> ±0.03	11.23 <sup>b</sup> ±0.04	68.14 <sup>b</sup> ±0.27	08.67 <sup>d</sup> ±0.02	48.31 <sup>c</sup> ±0.04	41.07 <sup>d</sup> ±1.48	2.23 <sup>c</sup> ±0.13	28.61 <sup>c</sup> ±7.20
F <sub>4</sub>	11.88 <sup>d</sup> ±0.04	2.63 <sup>b</sup> ±0.07	4.34 <sup>c</sup> ±0.01	02.78 <sup>c</sup> ±0.04	11.25 <sup>b</sup> ±0.22	67.12 <sup>c</sup> ±0.08	09.75 <sup>c</sup> ±0.01	52.16 <sup>b</sup> ±0.07	43.18 <sup>c</sup> ±1.56	2.30 <sup>c</sup> ±0.09	13.23 <sup>d</sup> ±3.00
F <sub>5</sub>	10.38 <sup>e</sup> ±0.25	3.39 <sup>a</sup> ±0.02	5.72 <sup>b</sup> ±0.05	03.79 <sup>b</sup> ±0.05	11.57 <sup>a</sup> ±0.03	64.11 <sup>d</sup> ±0.01	10.64 <sup>b</sup> ±0.04	56.87 <sup>a</sup> ±0.02	44.48 <sup>b</sup> ±3.97	2.56 <sup>b</sup> ±0.19	05.34 <sup>e</sup> ±0.45
F <sub>6</sub>	10.13 <sup>c</sup> ±0.05	3.40 <sup>a</sup> ±0.08	6.71 <sup>a</sup> ±0.01	04.81 <sup>a</sup> ±0.01	11.68 <sup>a</sup> ±0.07	62.27 <sup>e</sup> ±0.07	12.07 <sup>a</sup> ±0.14	56.91 <sup>a</sup> ±0.03	45.40 <sup>a</sup> ±1.14	2.86 <sup>a</sup> ±0.01	03.35 <sup>f</sup> ±1.92
C <sub>1</sub>	06.76 <sup>e</sup> ±1.14	1.26 <sup>a</sup> ±0.08	3.26 <sup>a</sup> ±0.07	19.81 <sup>a</sup> ±0.32	08.19 <sup>a</sup> ±0.10	63.59 <sup>c</sup> ±0.03	06.58 <sup>a</sup> ±0.09	40.61 <sup>a</sup> ±0.09	30.08 <sup>a</sup> ±0.91	2.09 <sup>a</sup> ±0.06	60.09 <sup>e</sup> ±1.51
C <sub>2</sub>	06.23 <sup>d</sup> ±0.68	1.55 <sup>b</sup> ±0.05	4.29 <sup>b</sup> ±0.03	20.27 <sup>b</sup> ±0.02	08.30 <sup>a</sup> ±0.07	61.94 <sup>d</sup> ±0.06	06.71 <sup>a</sup> ±0.10	55.53 <sup>b</sup> ±0.08	37.16 <sup>b</sup> ±0.77	2.16 <sup>b</sup> ±0.01	52.54 <sup>d</sup> ±8.52
C <sub>3</sub>	05.67 <sup>c</sup> ±0.03	1.63 <sup>c</sup> ±0.01	5.94 <sup>c</sup> ±0.04	21.86 <sup>c</sup> ±0.19	08.62 <sup>b</sup> ±0.16	58.52 <sup>c</sup> ±0.16	06.85 <sup>a</sup> ±0.01	57.18 <sup>c</sup> ±0.11	45.50 <sup>c</sup> ±0.91	2.18 <sup>b</sup> ±0.06	49.08 <sup>c</sup> ±1.45
C <sub>4</sub>	04.19 <sup>b</sup> ±0.05	1.69 <sup>d</sup> ±0.01	6.26 <sup>d</sup> ±0.04	23.17 <sup>d</sup> ±0.21	08.71 <sup>b</sup> ±0.10	57.01 <sup>c</sup> ±0.27	07.21 <sup>b</sup> ±0.33	61.65 <sup>d</sup> ±0.09	49.82 <sup>d</sup> ±4.14	2.61 <sup>d</sup> ±0.05	32.47 <sup>b</sup> ±1.87
C <sub>5</sub>	03.56 <sup>a</sup> ±0.07	1.73 <sup>c</sup> ±0.02	7.51 <sup>e</sup> ±0.02	24.98 <sup>e</sup> ±0.09	09.20 <sup>c</sup> ±0.19	53.51 <sup>b</sup> ±0.17	07.53 <sup>c</sup> ±0.19	64.79 <sup>e</sup> ±0.02	63.41 <sup>e</sup> ±2.87	2.64 <sup>d</sup> ±0.04	24.68 <sup>a</sup> ±1.19
C <sub>6</sub>	03.09 <sup>a</sup> ±0.52	2.72 <sup>i</sup> ±0.03	7.84 <sup>e</sup> ±0.08	25.27 <sup>f</sup> ±0.04	09.79 <sup>d</sup> ±0.15	51.34 <sup>a</sup> ±0.04	07.65 <sup>c</sup> ±0.06	67.81 <sup>f</sup> ±0.10	65.09 <sup>e</sup> ±0.39	3.76 <sup>f</sup> ±0.40	23.01 <sup>a</sup> ±2.00

530 The values with different superscript in a column differ significantly ( $p \leq 0.05$ ). F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub> and F<sub>6</sub> refer to 0, 20, 40, 60, 80, 100% composite flour, respectively and C<sub>1</sub>,  
531 C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub> and C<sub>6</sub> refer to cookies made from 0, 20, 40, 60, 80, 100% composite flour, respectively.

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**Table 4:** Physical properties, color values, NEB and textural properties of cookies.

Sample	Diameter (cm)	Thickness (cm)	Spread ratio	Weight (g)	Bake loss (%)	L*	a*	b*	NEB	Hardness (g) force <sup>-1</sup>	Fracturability (mm) distance <sup>-1</sup>
C <sub>1</sub>	06.32 <sup>d</sup> ±0.16	0.75 <sup>a</sup> ±1.89	08.42 <sup>d</sup> ±1.15	10.30 <sup>a</sup> ±2.10	11.39 <sup>f</sup> ±1.39	62.55 <sup>e</sup> ±0.71	03.00 <sup>a</sup> ±0.94	25.39 <sup>e</sup> ±0.75	0.008 <sup>a</sup> ±0.17	6952 <sup>e</sup> ±0.29	1.22 <sup>d</sup> ±0.06
C <sub>2</sub>	06.32 <sup>d</sup> ±0.09	0.75 <sup>a</sup> ±0.15	08.42 <sup>d</sup> ±0.63	10.68 <sup>b</sup> ±0.49	11.05 <sup>e</sup> ±1.47	51.92 <sup>d</sup> ±0.88	03.49 <sup>b</sup> ±1.83	23.19 <sup>d</sup> ±0.43	0.014 <sup>b</sup> ±0.13	5551 <sup>d</sup> ±0.19	1.22 <sup>d</sup> ±0.17
C <sub>3</sub>	06.29 <sup>c</sup> ±2.27	0.78 <sup>b</sup> ±0.67	08.06 <sup>c</sup> ±0.49	10.75 <sup>c</sup> ±1.27	10.53 <sup>d</sup> ±1.60	48.56 <sup>c</sup> ±0.61	03.83 <sup>c</sup> ±1.73	21.04 <sup>c</sup> ±0.86	0.015 <sup>b</sup> ±0.14	4700 <sup>c</sup> ±0.27	1.18 <sup>d</sup> ±0.34
C <sub>4</sub>	06.27 <sup>c</sup> ±1.15	0.79 <sup>b</sup> ±1.33	07.93 <sup>b</sup> ±1.28	10.84 <sup>d</sup> ±0.82	10.00 <sup>c</sup> ±0.68	46.94 <sup>b</sup> ±0.35	04.71 <sup>d</sup> ±1.29	18.56 <sup>b</sup> ±0.51	0.016 <sup>b</sup> ±0.18	4625 <sup>b</sup> ±0.36	1.11 <sup>c</sup> ±0.08
C <sub>5</sub>	06.23 <sup>b</sup> ±0.62	0.80 <sup>b</sup> ±3.15	07.78 <sup>b</sup> ±1.23	11.03 <sup>e</sup> ±0.21	09.24 <sup>b</sup> ±0.91	46.51 <sup>b</sup> ±0.40	04.91 <sup>e</sup> ±1.45	17.44 <sup>b</sup> ±0.37	0.019 <sup>c</sup> ±0.13	4614 <sup>b</sup> ±0.05	1.01 <sup>b</sup> ±0.43
C <sub>6</sub>	06.17 <sup>a</sup> ±1.39	0.81 <sup>b</sup> ±0.96	07.61 <sup>a</sup> ±2.31	11.35 <sup>f</sup> ±0.58	08.49 <sup>a</sup> ±1.97	38.13 <sup>a</sup> ±0.44	06.10 <sup>f</sup> ±0.51	13.54 <sup>a</sup> ±0.38	0.024 <sup>d</sup> ±0.10	2987 <sup>a</sup> ±0.64	0.92 <sup>a</sup> ±0.09

534 The values with different superscript in a column differ significantly ( $p \leq 0.05$ ). C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub> and C<sub>6</sub> refer to cookies made from 0, 20, 40, 60, 80, 100%  
535 composite flour, respectively.

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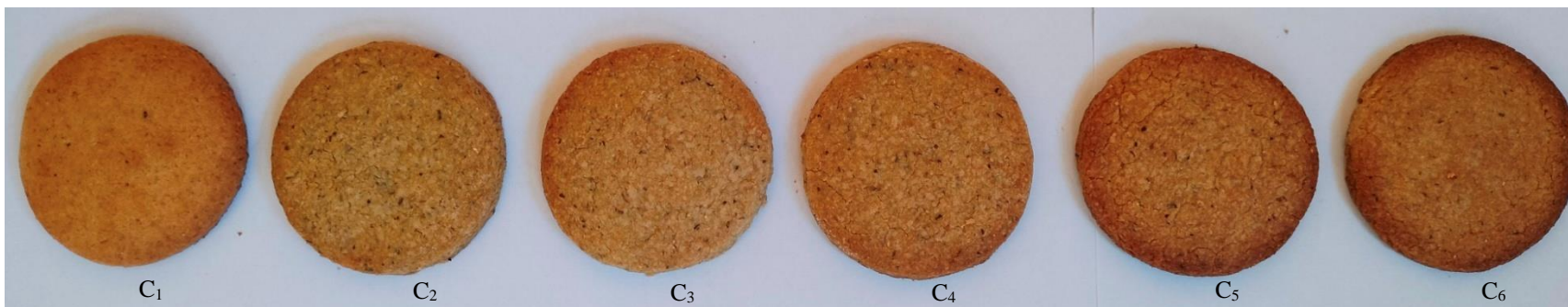


Fig. 1. Multi-millet cookies.

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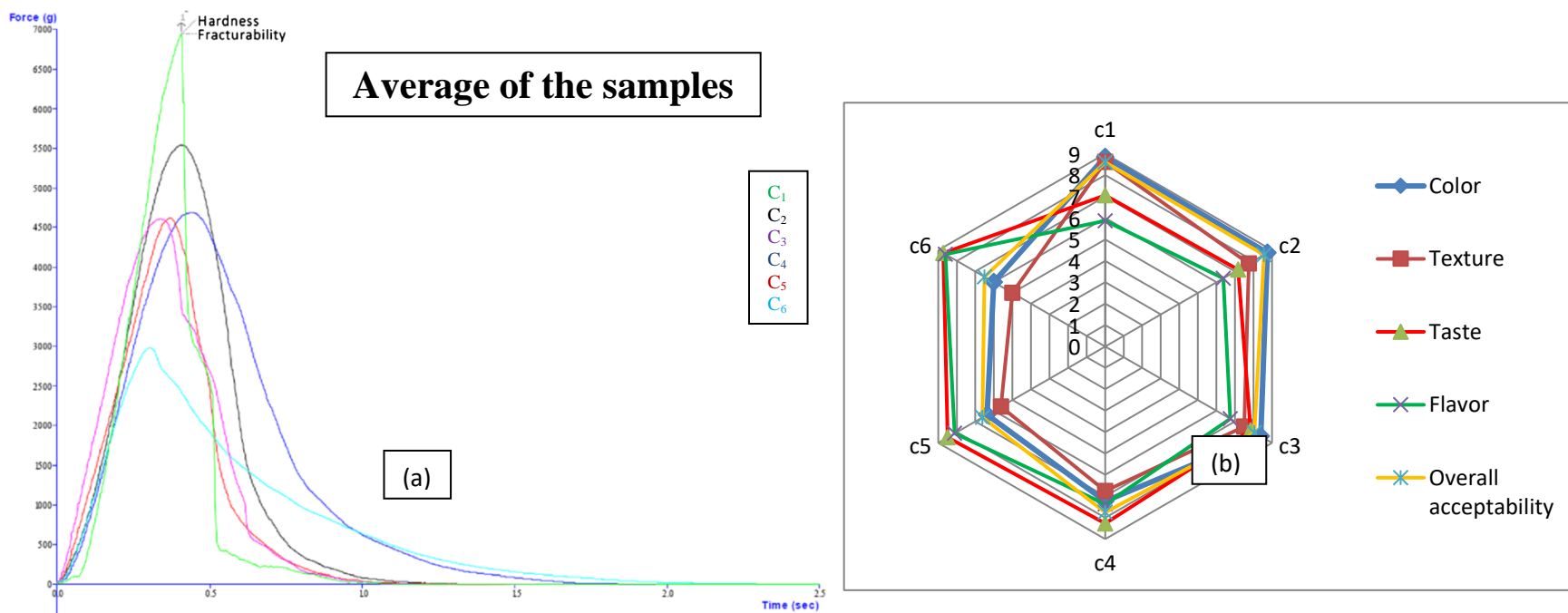


Fig. 2. (a) Hardness, and (b) Sensory scores of cookies.

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