CHAPTER 5

NUTRITIONAL, SENSORY AND IN-VITRO ANTIOXIDANT CHARACTERISTICS OF GLUTEN FREE COOKIES PREPARED FROM FLOUR BLENDS OF MINOR MILLETS

5.1 Introduction

Millets also known as ragi and mandia in the Bastar region of Chhattisgarh (India) have high nutritional value as compared to major cereals such as wheat and rice and offers livelihood, security for human beings and diverse livestock populations in dry land regions of rural India (Pradhan et al., 2010). Millets are significantly rich in resistant starch, soluble, insoluble dietary fibers, minerals, antioxidants (phenolic acids, glycated flavonoids) and nutraceuticals. It contains about 92.5% dry matter which include, 2.1% ash, 2.8% crude fiber, 7.8% crude fat, 13.6% crude protein, and 63.2% starch (Ali et al., 2003). Barnyard (Echinochloa frumentacea), Kodo (Paspalum scrobiculatum) and foxtail (Setaria italic) are the important minor millets having fair amounts of protein (12%) that is highly digestible (81.13%), coupled with low carbohydrate content (58.56%) of slow digestibility (25.88%) and fat with higher polyunsaturated fatty acids (Veena, 2003). Geervani and Eggum (1989) have reported that the dietary fibre about 13% is an important phytochemical component of minor millets made up of, 35.66%, soluble and 64.34%, insoluble fractions. These are considered essential in the management of disorders like diabetes mellitus, obesity, hyperlipidemia etc. Similarly, kodo millet grain possesses a high in vitro antioxidant capacity.

Germination or malting of cereal grains may results in some biochemical modifications and produces malt with improved nutritional and sensory quality that can be used in various
traditional recipes. Apart from changing the level of nutrients, the biochemical activities, which
occur during germination, can also generate bioactive components, such as ascorbic acid,
tocopherols, tocotrienols and phenolics. Some of these compounds possess antioxidants
properties, thus resulting in an increase in the seeds antioxidant activities (Fernandez et al.,
2008). Eyzaguirre et al. (2006) have reported that the in vitro extractability and bio-accessibility
of minerals such as calcium, iron and zinc increases in finger and pearl millets due to
germination. Malting helps to improve the availability of nutrients, sensory attributes and
extends the shelf life. Millet malts also find applications in cereal based low dietary bulk and
calorie-dense weaning food, supplementary foods, health foods and in amylase-rich foods.

Cookies represent the largest category of snack foods and are commonly consumed in
most parts of the world. Dotsey (2009) has reported that urbanization has increased the
consumption of processed food and bakery products leading to high cost of production as well as
increase in the demand for importation of wheat. The main ingredients in the formulation of
cookies include flour, sugar and fat along with desired flavouring substances. The commercially
available cookies prepared from white wheat flour are considered nutritionally poor as compared
to cookies prepared from whole wheat flour (Pareyt and Delcour, 2008). Incorporating soy
protein, fibres etc can improve the nutritive value of bakery products. Gupta et al. (2011) have
reported that by incorporating navy bean, chickpea, barley can improve the nutritive values of
cookies. The present investigation includes the formulations of gluten free cookies from the flour
blends of raw and germinated foxtail, barnyard and kodo millets. This provides an insight of
ingredient delivery system and methods of producing gluten-free cookies that could be sensory
comparable to cookies prepared from wheat used as a control. At present, the literature regarding
composite flour of raw and germinated minor millets (foxtail, barnyard and kodo millets) flour
for cookies production is not available and therefore present study was conducted to explore the possibility of using germinated flour blends for the preparation of nutritional and sensory acceptable cookies. Accordingly, the developed gluten-free cookies were examined for nutritional composition, physicochemical and sensory properties that are considered important parameters in the formulation of related food products.

5.2. Materials and Methods

5.2.1. Procurement of raw materials

The procurement of raw sample of the foxtail (*Setaria italica*), barnyard (PRJ-1) (*Echinochloa frumentaceae*), and kodo (*Paspalum scrobiculatum*) millets are discussed in Section 3.2.1 of Chapter-3.

5.2.2. Germination of minor millet grains

The optimization of germination processes for the minor millets (foxtail, barnyard and kodo millet) is discussed in Section 3.2.5 of Chapter 3.

5.2.3. Preparation of minor millets flour (raw and germinated grains)

Dried minor millets of all the selected varieties were milled in a lab scale disc mill (Agrosa Pvt Ltd. India) to obtain minor millet flours. To get flour of uniform particle size, they were passed through a 60 mesh sieve. The resulting flours were packed in air tight containers and kept at 4°C for further analysis.

5.2.4. Preparation of flour blends

Blends for the preparation of cookies were prepared by mixing foxtail, barnyard and kodo millets flour in the ratios totalling to 100% as shown in Table 5.1
Table 5.1 Preparation of flour blends

<table>
<thead>
<tr>
<th>Sample</th>
<th>Foxtail millet (%)</th>
<th>Barnyard millet (%)</th>
<th>Kodo millet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>80</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>A2</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>A3</td>
<td>60</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>A4</td>
<td>50</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>A5</td>
<td>40</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>A6</td>
<td>35</td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

5.2.5. Chemical analysis of flour blends

5.2.5.1 Moisture content

Moisture content of the flours blend of cookies was determined by the method of AOAC (2000) described in Section 3.4.1.1. of Chapter 3.

5.2.5.2 Protein content

Protein content of the flours blend of cookies was determined by the method of AOAC (2000) as described in Section 3.4.1.2. of Chapter 3.

5.2.5.3 Fat content

Fat content of the flours blend of cookies was determined by the method of AOAC (2000) as described in Section 3.4.1.3. of Chapter 3.

5.2.5.4 Crude fibre content

Crude fibre content of the flours blend of cookies was determined by the method of AOAC (2000) as described in Section 3.4.1.4. of Chapter 3.

5.2.5.5 Ash content
Ash content of the flours blend of cookies was determined by the method of AOAC (2000) described in Section 3.4.1.5 of Chapter 3.

5.2.5.6 Carbohydrate content

Carbohydrate content of the flours blend of cookies was determined by the method was described in Section 3.4.1.6 of Chapter 3.

5.2.6 Dietary fibres

Dietary fibres of the flours blend of cookies was determined by IS-11062 (1984) method described in Section 3.4.3 of Chapter 3.

5.2.7. Functional properties of flour blends

5.2.7.1. Water absorption index (WAI) and water solubility index (WSI)

WAI and WSI were determined according to the method developed for cereals (Stojceska et al., 2008; Yagci and Gogus, 2008). The ground flours blends were suspended in water at room temperature for 30 min, gently stirred during this period, and then centrifuged at 3000 g for 15 min. The supernatants were decanted off into an evaporating dish of known weight. The WAI was the weight of gel obtained after removal of the supernatant per unit weight of original dry solids. The WSI was measured as the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample.

\[
WAI \left( \frac{g}{g} \right) = \frac{\text{wt. of water uptake in hydrated residue}}{\text{wt. of sample}}
\]

\[
WSI \left( \frac{g}{100g} \right) = \frac{\text{wt. of weight of dissolved solids in supernatant} \times 100}{\text{wt. of sample}}
\]

5.2.7.2. Bulk density (BD)

Bulk density was determined by the method of Wang and Kinsella (1976). Ten gram of the tested flour blends were placed in a 25 ml graduated cylinders and packed by gentle tapping
of the cylinders on a bench top, ten times, from a height of 58 cm. The final volume of the test flour blends were measured and expressed in (g/ml).

\[
\text{Bulk density } \left( \frac{g}{cm^3} \right) = \frac{\text{wt. of the flour (g)}}{\text{volume after tapping (cm}^3)}
\]

5.2.7.3 Pasting properties of flour blends

Pasting properties of the flour blends were determined by using Rapid Visco Analyzer (RVA) (model 3D, RVA; Newport Scientific Pvt. Ltd., Warriewood, Australia). Sample (3 g, 14% moisture base) was taken in an RVA aluminium canister and 25 ml of distilled water was added to it. It was then subjected to a programmed heating and cooling cycle where sample was held at 50°C for 1 min, heated to 95°C at 12°C/min and held at 95°C for 2.5 min. It was then cooled from 95 to 50°C at 12°C/min and held at 50°C for 2 min. The stirrer’s rotating speed was maintained at 160 rpm. Parameters including pasting temperature, peak viscosity, breakdown, trough, setback and final viscosity were recorded. Tests were performed in triplicates.

5.2.8 Cookies formulation and preparation

The flow sheet for the preparation of cookies given in (Figure-5.1). The formulation ingredients included the blended flour samples (100 g) (Table-5.1), sodium bicarbonate (1.0 g), salt (1.0 g), skim milk powder (20 g) shortening (50 g), sugar (40 g) and distilled water (20 ml). First of all, shortening and sugar were creamed using an electric mixer at medium speed for 5 min. Eggs and milk were added while mixing and the resultant mix was mixed for a period of 3 min. Blended flour, sodium bicarbonate, and salt were mixed thoroughly and added to the cream mixture where they were all mixed together to form a dough. The dough was kneaded to a uniform thickness of 0.25 cm using hand driven sheeter and cut into circular shapes of 5 cm diameter with the help of die cutter. Baking was carried out at 185°C for 20 ± 5 min in baking
oven. Cookie samples were cooled and stored in airtight containers until needed. Cookies made from wheat served as a control.

![Flow chart for the preparation of cookies]

Figure 5.1 Flow chart for the preparation of cookies

5.2.8.1 Physical characteristics of cookies prepared from minor millets flour blends of foxtail, barnyard and kodo millets

Diameter and thickness of cookies were measured with a Vanier calliper (CAPRI TOOLS) at two different places for each cookie and the average was calculated (one value was considered for each cookie). The average of 6 cookies was recorded for each batch, while cookies weight was determined using an electronic weighing balance. The spread ratio was calculated using the formula: diameter of cookies divided by height of cookies (Zoulias et al., 2000).
The spread ratio was calculated using the following formula:

\[
\text{Spread ratio} = \frac{\text{Diameter of cookies}}{\text{Height of cookies}}
\]

### 5.2.8.2 Textural characteristics of cookies prepared from minor millets flour blends of foxtail, barnyard and kodo millets

Hardness of the cookies was measured using a texture analyzer (TA, Stable Micro Systems, UK) in a compression mode with a sharp blade-cutting probe. The cookies were placed on a bridge with a 44 mm gap between the ridges. A blunt edge knife probe having a thickness of 4 mm attached to a 50 kg load cell and traveling at a pre, post and test speed of 1.5, 10 and 1 mms\(^{-1}\), respectively was used. Hardness (a maximum peak force) was measured for more than six cookies for each sample. The peak force to snap the cookies was reported as fracture force in ‘N’.

### 5.2.8.3 Sensory evaluation of cookies prepared from minor millets flour blends of foxtail, barnyard and kodo millets

Cookies made from wheat (control) and minor millets flour blends of foxtail, barnyard and kodo millets were subjected to sensory evaluation using thirty (30) semi-trained panellists drawn from the Department of Food Engineering and Technology which falls within the numbers as proposed by Meilgaard et al. (1999). The panellist was selected and trained following (ISO, 1985) procedure. Cookies were evaluated for colour, appearance, aroma, taste, texture and overall acceptability. The ratings were carried on a 9-point Hedonic scale ranging from 9- (like extremely) to 1- (dislike extremely). All cookie samples were coded with three-
digit random numbers and presented to individuals in a tray in individual booths. Orders of
serving were completely randomized.

The blended flour cookies of raw and germinated minor millet were optimized on the
basis of physical, textural and sensory properties. The criteria used for the optimized cookies
were weight, thickness, diameter, and spread ratio for physical properties, hardness for textural
properties and for the sensory evaluation; color, appearance, flavor, taste and overall
acceptability parameters were selected.

5.2.9 Characteristics of optimized cookies

5.2.9.1 Moisture content of optimized cookies

Moisture content of the optimized cookies was determined by the method of AOAC
(2000) as described in Section 3.4.1.1 of Chapter 3.

5.2.9.2 Protein content of optimized cookies

Protein content of optimized cookies was determined by the method of AOAC (2000)
described in Section 3.4.1.2 of Chapter 3.

5.2.9.3 Fat content of optimized cookies

Fat content of optimized cookies was determined by the method of AOAC (2000)
described in Section 3.4.1.3 of Chapter 3.

5.2.9.4 Ash content of optimized cookies

Ash content of the optimized cookies was determined by the method of AOAC (2000)
described in Section 3.4.1.5 of Chapter 3.

5.2.9.5 Carbohydrate content of optimized cookies

Carbohydrate content of optimized cookies was determined by the method of was
described in Section 3.4.1.6 of Chapter 3.
5.2.9.6 Dietary fibre of optimized cookies

Dietary fibres of optimized cookies were determined by using IS-11062 (1984) method described in Section 3.4.3 of Chapter 3.

5.2.9.7 *In vitro* antioxidant activity of optimized cookies

*In vitro* antioxidant activity assay of cookies was carried out as per the method discussed in Section 3.3.4 of Chapter 3.

5.2.9.8 Total phenolic contents of optimized cookies

Total phenolic contents of flour blends and the highly accepted cookie sample (A2) were determined by the Folin-Ciocalteu spectrophotometric method (Prior *et al.* (2005) as described in Section 3.3.1 of Chapter 3.

5.2.9.9 Color characteristics of optimized cookies

Colour measurement of cookies was carried out by using a Hunter colorimeter fitted with optical sensor (Hunter Associates Laboratory Inc., Reston, VA, USA) on the basis of CIE L*, a*, b* colour system. Where the L* value indicates the lightness, its value range from 0 to 100, a* value gives the degree of the red and green color, with a higher positive a* value indicating more red. The b* value indicates the degree of the yellow and blue color, with a higher positive b* value indicating more yellow color.

\[
\Delta E_{ab} = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}
\]

\[
\Delta E_{ab} = \text{Total colour differences}
\]

5.2.10 Statistical analyses

The data presented in the tables represent average of three observations, (±SD) and subjected to two way analysis of variance (ANOVA) followed by Duncan's multiple range test.
(Duncan's, 1955) using statistical 7- (statistical soft, TUSA, USA) statistical software packages at $p \leq 0.05$ to determine, which means were significantly different.

5.3 Results and Discussion

5.3.1 Physiochemical and functional properties of flour blends

5.3.1.1 Chemical analyses of flour blends

The chemical composition of raw and germinated minor millets flour blends is presented in Table-5.2. Results indicated that moisture; protein and crude fiber contents of the flour blends of germinated minor millet were significantly ($p \leq 0.05$) higher than the raw minor millets flour blends.

Moisture content of blend flour of germinated minor millets varied from 8.21 % to 10.57 %. The blend flour ($A_3$) of germinated minor millets had the highest moisture content (10.57%) whereas the flour blend ($A_1$) of raw minor millet had the lowest (7.14%) moisture content. Moisture content increased with increase in germination time in foxtail, barnyard and kodo millets. The lower moisture content of raw minor millet flour blend samples as compared to germinated flour counterpart might be due to low dry matter content of later.

The germinated foxtail millet (sample ‘A’) had the highest protein content of 12.2%. This was followed by the germinated barnyard and kodo millet flour having protein contents of 9.14% and 6.7%, respectively. The flour blend ($A_2$) of germinated minor millets had the highest protein content (10.85%) and the flour blend ($A_6$) of raw minor millet had the lowest (9.02 %) protein content. The protein content decreased with decreasing levels of raw and germinated foxtail and barnyard millets in raw and germinated flour blends while the carbohydrate content increased with increasing levels of raw and germinated kodo millet in flour blends of raw and germinated minor millet because of higher carbohydrate in the later (Uwaegbute et al., 2000). The flour
blends made from germinated minor millet had significant higher protein and crude fiber than the flour blends made from raw minor millets. The increase in protein content with germination time could be attributed to net synthesis of enzyme protein by germinating seeds which might have resulted in the production of some amino acids during protein synthesis (Uwaegbute et al., 2000).

Fat content of flour blended samples (A₁-A₆) of raw and germinated minor millets varied from 3.86% to 4.61% and 3.01 to 4.22%, respectively. There were significant differences ($p \leq 0.05$) in fat content among samples. Fat content was found to be considerably high in flour blended samples of raw minor millets than flour blend samples of germinated minor millets. Fat content also decreased due to the increased activities of lipolytic enzymes during germination which hydrolyzed fat component into fatty acids and glycerol. This might be of advantage during storage of flour samples. Similar observation was made by Inyang and Idoko (2006).

Ash contents ranged from 1.02% to 4.63% in flour blend samples of raw minor millets. The A₅ flour blend sample of raw minor millet had the lowest ash value (1.45%) while; A₂ blend flour sample of germinated minor millets had the highest ash value (4.43%). There was gradual decrease in ash content after germination. However, there were significant differences ($p \leq 0.05$) in ash content of flour blend samples of raw and germinated minor millets.

Carbohydrate contents of flour blends of raw and germinated minor millets varied from 68.02 to 72.71% and 66.67 to 71.92%, respectively. There was a decrease in carbohydrate contents of foxtail, barnyard and kodo millets during germination. The flour blended sample (A₃) of raw minor millet had the highest (72.71%), while the flour blend sample (A₂) of germinated minor millet had the lowest carbohydrate value (66.67%). The decrease in carbohydrate after the germination might be attributed to increase in alpha-amylase activity which had broken down
complex carbohydrates into simpler and more absorbable sugars. Chinma et al., (2009) reported similar results of decrease in carbohydrate from 60.50–53.60% in brown and yellow varieties of tiger nut (Cyperus esculentus) as a result of germination.
Table 5.2 Analysis of raw and germinated minor millets flour blends (dry weight basis)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Protein (g/100 g)</th>
<th>Fat (g/100 g)</th>
<th>Ash (g/100 g)</th>
<th>Moisture (g/100 g)</th>
<th>Crude fiber (g/100 g)</th>
<th>CHO (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.2 ± 0.32&lt;sup&gt;[ap]&lt;/sup&gt;</td>
<td>14.32 ± 0.01&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>3.6 ± 0.01&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>3.31±0.02&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>7.54±0.02&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>8.21±0.02&lt;sup&gt;[aq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>9.14±70.036&lt;sup&gt;[bp]&lt;/sup&gt;</td>
<td>11.22±0.03&lt;sup&gt;[cq]&lt;/sup&gt;</td>
<td>3.47±0.01&lt;sup&gt;[cq]&lt;/sup&gt;</td>
<td>4.52±0.01&lt;sup&gt;[cq]&lt;/sup&gt;</td>
<td>9.21±0.02&lt;sup&gt;[cq]&lt;/sup&gt;</td>
<td>9.87±0.04&lt;sup&gt;[cq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>6.7±0.012&lt;sup&gt;[bp]&lt;/sup&gt;</td>
<td>7.9±0.041&lt;sup&gt;[dq]&lt;/sup&gt;</td>
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<td>10.21±0.01&lt;sup&gt;[dq]&lt;/sup&gt;</td>
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<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>4.21±0.07&lt;sup&gt;[eq]&lt;/sup&gt;</td>
<td>8.78±0.04&lt;sup&gt;[eq]&lt;/sup&gt;</td>
<td>9.75±0.04&lt;sup&gt;[eq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10.05±0.02&lt;sup&gt;[cp]&lt;/sup&gt;</td>
<td>10.85±0.03&lt;sup&gt;[fq]&lt;/sup&gt;</td>
<td>3.01±0.05&lt;sup&gt;[fq]&lt;/sup&gt;</td>
<td>4.35±0.04&lt;sup&gt;[fq]&lt;/sup&gt;</td>
<td>9.51±0.07&lt;sup&gt;[fq]&lt;/sup&gt;</td>
<td>9.86±0.06&lt;sup&gt;[fq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;3&lt;/sub&gt;</td>
<td>9.80±0.05&lt;sup&gt;[cp]&lt;/sup&gt;</td>
<td>10.49±0.01&lt;sup&gt;[gq]&lt;/sup&gt;</td>
<td>3.87±0.08&lt;sup&gt;[gq]&lt;/sup&gt;</td>
<td>2.21±0.07&lt;sup&gt;[gq]&lt;/sup&gt;</td>
<td>2.63±0.04&lt;sup&gt;[gq]&lt;/sup&gt;</td>
<td>9.54±0.10&lt;sup&gt;[gq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;4&lt;/sub&gt;</td>
<td>9.56±0.04&lt;sup&gt;[cp]&lt;/sup&gt;</td>
<td>9.88±0.05&lt;sup&gt;[ip]&lt;/sup&gt;</td>
<td>4.02±0.04&lt;sup&gt;[ip]&lt;/sup&gt;</td>
<td>2.35±0.04&lt;sup&gt;[ip]&lt;/sup&gt;</td>
<td>2.31±0.03&lt;sup&gt;[ip]&lt;/sup&gt;</td>
<td>9.57±0.02&lt;sup&gt;[ip]&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;5&lt;/sub&gt;</td>
<td>9.26±0.02&lt;sup&gt;[cp]&lt;/sup&gt;</td>
<td>9.79±0.05&lt;sup&gt;[jq]&lt;/sup&gt;</td>
<td>3.74±0.07&lt;sup&gt;[jq]&lt;/sup&gt;</td>
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<td>1.02±0.09&lt;sup&gt;[jq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;6&lt;/sub&gt;</td>
<td>9.02±0.03&lt;sup&gt;[cp]&lt;/sup&gt;</td>
<td>9.29±0.03&lt;sup&gt;[kq]&lt;/sup&gt;</td>
<td>4.61±0.08&lt;sup&gt;[kq]&lt;/sup&gt;</td>
<td>3.72±0.07&lt;sup&gt;[kq]&lt;/sup&gt;</td>
<td>3.23±0.07&lt;sup&gt;[kq]&lt;/sup&gt;</td>
<td>8.98±0.05&lt;sup&gt;[kq]&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (Raw)</td>
<td>9.39±0.02&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>9.39±0.02&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>2.3±0.03&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>1.97±0.04&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>1.97±0.04&lt;sup&gt;[aq]&lt;/sup&gt;</td>
<td>10.45±0.08&lt;sup&gt;[aq]&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*n = 3, Mean± standard deviation values in the same column as well as row<sup>[p,q]</sup> (for individual properties) which are not followed by the same letter are significantly different (p < 0.05). Foxtail millet (A), Barnyard millet (B) and Kodo millet flour (C) in the proportions of 80:15:5, 70:20:10, 60:25:15, 50:30:20, 40:35:25, 35:35:30, which refer to A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, and A<sub>6</sub> flour blends respectively and wheat flour as control. Germ=Germinated.
5.3.1.2 *In vitro* antioxidant properties of flour blends

A significant difference was observed in the phenolic contents and DPPH radical scavenging activity in flour blends of raw and germinated minor millets. The *in vitro* antioxidant activity of flour blends of germinated minor millets varied from 58.23 % to 89.23%. The flour blend (A_6) of germinated minor millets had the highest *in vitro* antioxidant activity (89.23%) whereas, the flour blend (A_1) of raw minor millet having the lowest (55.23 %) *in vitro* antioxidant activity. The phenolic content and *in vitro* antioxidant activity increased with increase in kodo millet flour level in flour blends of minor millet (Table-5.3). Highest phenolic content and DPPH radical scavenging activity was found in the flour blend made from germinated minor millet in comparison with raw millets due to increased availability of some bound polyphenolic compounds from polysaccharides and proteins during germination. The bound phenolic compounds get liberated by the action of cell wall-degrading enzymes (mainly esterases) on these bonds (Janitzio *et al*., 2014). Similar results of were found by Yang *et al*., (2001), who reported that during the germination process, antioxidant activity and total phenolics increased in wheat grain. Gorinstein *et al.* (2007); Zielinski and Kozłowska (2000) reported a linear correlation between antioxidant activity and total phenolic contents and stated that when total polyphenols content increased, antioxidant activity also increased in grains. Adom and Liu (2002) have postulated that the insoluble phenolics as the major contributors in enhancing the antioxidant capacity of grains

5.3.1.3 Functional properties of flour blends

The functional properties of flour play an important role in the product development. Table- 5.3 shows the various functional properties of flour blends. Housson and Ayenor (2002) studied that water absorption capacity is an important variable in the development of ready to eat
foods, and high absorption capacity may assure product cohesiveness. Several factors such as number of hydration positions, physical environment, pH, solvent, presence of lipids and carbohydrates, greatly affect the water absorption capacity of the millets (Kinsella, 1982). Water absorption capacity (WAC) of blended flour samples (A1-A6) of raw foxtail, barnyard and kodo millet varied from 1.46 g/g to 2.57g/g and water absorption capacity of blend flour sample of germinated foxtail, barnyard and kodo millet varied from 2.01 to 4.68g/g when compared to control (1.39g/g). This shows that the WAC of flour blends was significantly improved as a result of germination due to more exposure to hydrophilic molecules.

Water solubility index (WSI) is related to the presence of soluble molecule. For blended flour samples of germinated minor millet flours WSI of raw foxtail, barnyard and kodo millet varied from 2.25 g/g to 7.23/g. The solubility could imply to the amount of amylose leaching out from starch granule when swelling, therefore the higher the solubility the higher will be the amylose leaching (Reungmaneepaitoon, 2009). The water absorption index (WAI) and water solubility index (WSI) decreased in flour blends with increased kodo millet proportion, due to decreased protein content. The WAI and WSI of flour blends of germinated minor millet were found significantly (p≤ 0.05) higher than the flour blends of raw minor millets. The WAI increased after germination was due to increase in protein content, since the protein hold maximum water by binding water molecules in its structure. In the same way, an increase was noticed in WSI of flour blends of germinated minor millet due to the formation of lower molecular weight compounds due to the activity of amylases and proteases. Gamel et al. (2006) reported that the water absorption capacities of cowpea, green gram, lentil and Bengal gram were significantly improved by germination while the water solubility index of corn flour improved after germination.
The bulk density of the flour blends of raw and germinated minor millet flours is presented in Table-5.3. Bulk density is an important parameter that determines the packaging requirement of a product (Parde et al., 2003). In comparison to germinated blends of millet flours, the bulk density of the raw blends of millet flour samples were significantly high (p<0.05). Bulk densities of flour blends of germinated minor millet were lower as compared to flour blends of raw minor millet flour. Nazni and Devi (2016) also attributed lowered bulk density in germinated foxtail and barnyard millet flour. Merill and Walt (1973) have reported that the decreased bulk density of the germinated millet flour indicates low porosity or air spacing in the flour, therefore less auto oxidation. This is an advantage in respect to spoilage, packing and transportation as goods in relation to weight.

5.3.1.4 Pasting properties of flour blends

Pasting properties are the important factors in determining the applications of flours. The results obtained of pasting profile (Table-5.4) revealed that the significant differences were observed in pasting profiles of flour blends of minor millets. Pasting temperature of the raw and germinated minor millet blend flour sample ranged from 78.00°C to 86.85°C and 77.68 to 85.20°C respectively, being the highest for blended flour sample (A_2) of germinated minor millet (85.20°C) and lowest for blended flour sample (A_6) of germinated minor millet (77.68°C).

Kaur and Singh (2005) studied that peak viscosities attained during the heating portion of tests indicates the water binding capacity of starch mixture. This often correlates with the final product qualities. High peak viscosity indicates the high swelling capacity of the starch granules. High and lower peak viscosities were observed for blended flour sample (A_1) of raw minor millet (610cP) and blended flour sample (A_6) of raw minor millet (155cP), respectively.
Table 5.3 Antioxidant and functional properties of raw and germinated minor millets flour blends*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antioxidant properties</th>
<th>Functional properties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPC (mg/100g)</td>
<td>DPPH activity (%)</td>
</tr>
<tr>
<td>Sample</td>
<td>Raw</td>
<td>Germ.</td>
</tr>
<tr>
<td>A₁</td>
<td>23.46±0.51&lt;sup&gt;fp&lt;/sup&gt;</td>
<td>34.23±0.42&lt;sup&gt;fq&lt;/sup&gt;</td>
</tr>
<tr>
<td>A₂</td>
<td>27.84±0.57&lt;sup&gt;ep&lt;/sup&gt;</td>
<td>42.12±0.63&lt;sup:eq&lt;/sup&gt;</td>
</tr>
<tr>
<td>A₃</td>
<td>35.32±0.46&lt;sup&gt;dp&lt;/sup&gt;</td>
<td>49.53±0.32&lt;sup&gt;dq&lt;/sup&gt;</td>
</tr>
<tr>
<td>A₄</td>
<td>46.56±0.62&lt;sup&gt;ep&lt;/sup&gt;</td>
<td>51.03±0.26&lt;sup&gt;cq&lt;/sup&gt;</td>
</tr>
<tr>
<td>A₅</td>
<td>52.01±0.53&lt;sup&gt;bp&lt;/sup&gt;</td>
<td>62.23±0.76&lt;sup&gt;bq&lt;/sup&gt;</td>
</tr>
<tr>
<td>A₆</td>
<td>58.39±0.66&lt;sup&gt;ap&lt;/sup&gt;</td>
<td>68.24±0.45&lt;sup&gt;aq&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>18.34±0.23&lt;sup&gt;gp&lt;/sup&gt;</td>
<td>18.52±0.19&lt;sup&gt;gpql&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*n = 3, Mean± standard deviation values in the same column as well as row <sup>(p,q)</sup> (for individual properties) which are not followed by the same letter are significantly different (p < 0.05). Foxtail millet, Barnyard millet and Kodo millet flour in the proportions of 80:15:5, 70:20:10, 60:25:15, 50:30:20, 40:35:25, 35:35:30, which refer to A₁, A₂, A₃, A₄, A₅, and A₆ flour blends respectively and wheat flour as control. Germ=Germinated
The blended flour sample (A₁) of raw minor millet indicated its high water binding capacity resulting in more swelling of the starch granules. The ranges observed in trough viscosity of blended flour samples the raw and germinated minor millet were 87 to 305cP and 35 to 76cP, respectively. Zhang and Hamauzu (2004) observed that the breakdown is caused by the disintegration of gelatinized starch granules structure during continued stirring and heating, thus, indicating the shear thinning property of starch. The breakdown viscosity of blended flour sample of raw and germinated minor millet ranged from form 68 to 30cP and 13 to 57.03cP, respectively. Final viscosity indicates the ability of the starch to form a viscous paste. The high final viscosity of blended flour sample of raw minor millet flour as compared to blend flour sample of germinated minor millet indicates its high resistance to shear.

The lower setback value of blended flour samples (A₁-A₆) of germinated minor millet (16 to 79cP) as compared to blended flour samples of (A₁-A₆) of raw minor millet (93 to 326cP) indicated their lowest rate of retrogradation and hence the product made of low set back viscosity flour will have prolonged shelf life period. Karim et al. (2007) studied that the increase in setback viscosity results from the rearrangement of amylose molecules that have leached out from the swollen starch granules during cooling and is generally used as a measure of gelling ability or retrogradation tendency of the starch. Therefore, setback viscosity is an index of retrogradation. As the proportion of kodo millet increased the peak viscosity, breakdown, setback, final viscosity and pasting temperature decreased in flour blends of minor millet from A₁ to A₆ samples. Nazni and Devi (2016) indicated that the germination significantly affects the pasting profiles of flour blends of minor millet as it decreases the peak viscosity, breakdown, setback, final viscosity and pasting temperature, since germination alters the native confrontation of starch, proteins, lipids and fiber components in flour that would interfere with the pasting
process. The decrease in viscosity after the germination may be due to degradation of starch during germination. Nazni and Devi (2016) also found similar results for peak viscosity, breakdown, setback, final viscosity and pasting temperature value in processed barnyard and foxtail millets flour. Similar trend was observed in wheat and brown rice flour (Xu et al., 2011).
Table 5.4 Pasting properties of raw and germinated minor millets flour blend

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Peak viscosity (cP)</th>
<th>Trough viscosity (cP)</th>
<th>Breakdown (cP)</th>
<th>Final viscosity (cP)</th>
<th>Setback (cP)</th>
<th>Pasting temperature (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>610±25.09&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>133±16.4&lt;sup&gt;ap&lt;/sup&gt;</td>
<td>305±12.5&lt;sup&gt;bq&lt;/sup&gt;</td>
<td>76±1.7&lt;sup&gt;ip&lt;/sup&gt;</td>
<td>305±4.5&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>57±0.32&lt;sup&gt;ip&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt;</td>
<td>437±21.95&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>128±14.2&lt;sup&gt;bp&lt;/sup&gt;</td>
<td>402±16.4&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>76±1.6&lt;sup&gt;ip&lt;/sup&gt;</td>
<td>35±6.5&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>52±0.76&lt;sup&gt;ip&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;3&lt;/sub&gt;</td>
<td>244±15.23&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>115±14.8&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>215±4.3&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>69±1.6&lt;sup&gt;ip&lt;/sup&gt;</td>
<td>290±12.5&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>46±0.21&lt;sup&gt;ip&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;4&lt;/sub&gt;</td>
<td>287±22.34&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>115±25.3&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>182±3.2&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>115±2.1&lt;sup&gt;ip&lt;/sup&gt;</td>
<td>105±9.5&lt;sup&gt;mo&lt;/sup&gt;</td>
<td>54±0.65&lt;sup&gt;ip&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;5&lt;/sub&gt;</td>
<td>313±21.23&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>108±15.2&lt;sup&gt;dp&lt;/sup&gt;</td>
<td>217±27.5&lt;sup&gt;eq&lt;/sup&gt;</td>
<td>95±1.2&lt;sup&gt;ip&lt;/sup&gt;</td>
<td>96±1.0&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>13±0.45&lt;sup&gt;ip&lt;/sup&gt;</td>
</tr>
<tr>
<td>A&lt;sub&gt;6&lt;/sub&gt;</td>
<td>155±17.23&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>68±1.3&lt;sup&gt;eq&lt;/sup&gt;</td>
<td>87±1.8&lt;sup&gt;eq&lt;/sup&gt;</td>
<td>35±1.0&lt;sup&gt;aqi&lt;/sup&gt;</td>
<td>68±1.3&lt;sup&gt;eq&lt;/sup&gt;</td>
<td>33±1.99&lt;sup&gt;ip&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*n = 3, Mean± standard deviation values in the same column as well as row (p<sub>j</sub>) (for individual properties) which are not followed by the same letter are significantly different (p < 0.05). Foxtail millet, Barnyard millet and Kodo millet flour in the proportions of 80:15:5, 70:20:10, 60:25:15, 50:30:20, 40:35:25, 35:35:30, which refer to A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub>, and A<sub>6</sub> flour blends respectively and wheat flour as control. Germ=Germinated
5.3.2 Physical, textural and sensory properties of cookies prepared from blend flour of raw and germinated minor millet

5.3.2.1 Physical properties of cookies

The results of various physical properties of cookies are shown in Table-5.5. The significant differences (p<0.05) were observed in the weight, thickness, diameter, and spread ratio of the control and the cookies made with the flour blends of raw and germinated minor millets. The maximum diameter of 53.76 mm was noticed in cookies made from A2 flour blend formulation of germinated minor millet followed by cookies made from A2 flour blend formulation of raw minor millet (52.18 mm) and minimum diameter value was found in wheat cookies (52.49 mm). Similarly, cookie samples A1-A2 made from flour blend formulation of germinated minor millet showed significantly higher thickness from 8.68 and 10.23 mm, respectively, than the cookie samples A1-A2 made from flour blend formulation of raw minor millet.

The spread ratio is an important character in determining the quality of cookies. The cookies made from A2 flour blend formulation of raw minor millet had higher spread ratio of 6.21 followed by cookies made from A2 flour blend formulation of germinated minor millet (6.01) and least was observed in wheat flour cookies (5.99). Cookies made from the flour blends of raw minor millets had higher spread ratio than germinated minor millet flour blends and wheat flour cookies. Cookies having higher spread ratios are considered the most desirable. The decrease in spread ratio of germinated blend flour cookies may be due to the enzymatic degradation of the starch and protein into the smaller sugars and peptides resulting in the increase of hydrophilic nature within cookies. Hoojjat and Zabik (1984) indicated that spread factor decreases with increase in the amount of hydrophilic activities in cookie dough. Similar
results were observed in raw and germinated rice flour cookies (Chung et al., 2014). Singh et al. (2003) reported that the spread ratio of cookies increased as non-wheat protein content increased. The diameters of cookies made from raw and germinated minor millets from all the samples (A1-A6) were found significantly lower than control (whole wheat flour) cookies. The thickness of cookies made from flour blends of raw and germinated minor millets was however higher than the control cookies.

5.3.2.2 Textural properties of cookies

The significant differences (p<0.05) were observed in the textural properties of the cookies made with the flour blends of raw and germinated minor millets and control cookies (Table 5.5). The peak force required to puncture the cookies prepared from flour blend (A2) of raw and germinated flour, 44.71 and 39.95 N, respectively, was found significantly (p≤0.05) lower than the cookies prepared from wheat (control). The wheat flour cookies required significantly higher force to break than cookies of flour blends of raw and germinated minor millet flour. This is also indicative of lower bulk density of flour blends of millet flours. The order of the hardness of cookies of flour blends of raw minor millets was found to be as follows; A2<A1<A6<A5<A4<A3<C. Also a similar order of hardness was found in cookies made from flour blends of germinated minor millets. The hardness of cookies of flour blends of raw minor millets was higher than cookies of flour blends of germinated minor millet flour. Hardness of cookies decreased during germination due to the structural degradation of starch and protein induced by germination.
Table 5.5 Physical and textural properties of cookies prepared from flour blend of raw and germinated minor millets*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Weight (g)</th>
<th>Thickness (mm)</th>
<th>Diameter (mm)</th>
<th>Spread ratio</th>
<th>Hardness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Raw</td>
<td>Germ</td>
<td>Raw</td>
<td>Germ</td>
<td>Raw</td>
</tr>
<tr>
<td>A₁</td>
<td>12.45±0.02</td>
<td>12.98±0.03</td>
<td>8.68±0.1</td>
<td>9.07±0.03</td>
<td>51.93±0.07</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>eq</td>
<td>fp</td>
<td>eq</td>
<td>b</td>
</tr>
<tr>
<td>A₂</td>
<td>12.58±0.01</td>
<td>13.44±0.02</td>
<td>8.68±0.02</td>
<td>8.68±0.03</td>
<td>53.08±0.05</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>dq</td>
<td>eq</td>
<td>fp</td>
<td>a</td>
</tr>
<tr>
<td>A₃</td>
<td>13.44±0.04</td>
<td>14.31±0.03</td>
<td>9.13±0.01</td>
<td>9.31±0.04</td>
<td>51.87±0.09</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>bq</td>
<td>eq</td>
<td>fp</td>
<td>e</td>
</tr>
<tr>
<td>A₄</td>
<td>13.37±0.01</td>
<td>13.98±0.01</td>
<td>9.91±0.02</td>
<td>10.19±0.03</td>
<td>50.79±0.07</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>aq</td>
<td>dp</td>
<td>cq</td>
<td>a</td>
</tr>
<tr>
<td>A₅</td>
<td>14.23±0.03</td>
<td>14.95±0.05</td>
<td>9.46±0.03</td>
<td>9.94±0.05</td>
<td>51.23±0.09</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>eq</td>
<td>bq</td>
<td>pq</td>
<td>p</td>
</tr>
<tr>
<td>A₆</td>
<td>14.55±0.05</td>
<td>14.91±0.04</td>
<td>9.63±0.02</td>
<td>10.23±0.04</td>
<td>51.66±0.08</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>aq</td>
<td>pq</td>
<td>eq</td>
<td>d</td>
</tr>
<tr>
<td>Control (Raw)</td>
<td>12.02±0.02</td>
<td>12.02±0.02</td>
<td>9.23±0.01</td>
<td>9.23±0.01</td>
<td>52.49±0.06</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>dp</td>
<td>dp</td>
<td>dp</td>
<td>c</td>
</tr>
</tbody>
</table>

*n = 3, Mean± standard deviation values in the same column as well as row(p,q) (for individual properties) which are not followed by the same letter are significantly different (p < 0.05). Foxtail millet, Barnyard millet and Kodo millet flour in the proportions of 80:15:5, 70:20:10, 60:30:20, 40:35:25, 35:35:30, which refer to A₁, A₂, A₃, A₄, A₅, and A₆ flour blends respectively and Wheat flour as control. Germ=Germinated.
The degradation of macromolecules contributed to the formation of weaker matrix in cookies, resulting in the softer texture. Chauhan et al. (2015) have reported the similar results for cookies prepared from raw and germinated amaranth flour. Hadnadev et al. (2013) reported that the replacement of rice with buckwheat flour led to a decrease in cookie hardness.

5.3.2.3 Sensory characteristics

The average values of panel perceptions of cookies prepared from flour blends of raw and germinated minor millets and that of control flour were evaluated for their color, appearance, flavor, taste and overall acceptability using 9-point Hedonic scale (Fig.5.2a-b). From the results, it was observed that the sensory score of the blended flour cookies displayed the decreasing trend from A₁ to A₆ samples as a result of increasing substitution levels of kodo millet flour. The kodo millet flour had the dark colour, due to its high phenolics, ash content and sandiness/gritty, which might have contributed to reduction in panel sensory perceptions and hence decrease in scores for sensory attributes like colour, appearance and mouthfeel.

The highest surface color score was achieved by cookies prepared from blended flour samples from A₁-A₆, of germinated minor millet. The score for surface appearance also decreased when kodo millet flour was incorporated up to 20 % level. The scores for texture decreased as the level of kodo millet flour increased. The highest score for all sensory attributes were observed for cookies produced by incorporating germinated foxtail, barnyard and kodo millet in the proportion of 70:20:10 (A₂) than control cookies as well as cookies produced by incorporating raw foxtail, barnyard and kodo millets in the proportion of 70:20:10 (A₂ sample cookies produced by incorporating germinated foxtail, barnyard and kodo millet in the proportion of 70:20:10 (A₂) with overall mean score of 7.85 were more acceptable than the cookies produced by incorporating raw foxtail, barnyard and kodo millet in the proportion of
70:20:10 (A₂) having sensory score of 7.23 as well as wheat flour cookies with score of 6.75 in all respects. This may be attributed to the reduction in bitterness and enhanced flavor properties as a result of malting in germinated minor millet cookies in comparison to raw minor millet flour cookies (Sinha and Kawatra, 2003). Jan et al. (2016) obtained similar results for cookies prepared from raw and germinated Chenopodium flour.

![Figure - (a)](image)

Superscripts a, b, c, d, e and f show significant (p ≤ 0.05) difference among the cookies of blend; Error bars represent standard deviation of three replicate. Foxtail millet, Barnyard millet and Kodo millet flour in the proportions of 80:15:5, 70:20:10, 60:25:15, 50:30:20, 40:35:25, 35:35:30, which refer to A₁, A₂, A₃, A₄, A₅, and A₆ flour blends respectively and wheat flour as control. Germ=Germinated.
Superscripts a, b, c, d, e and f show significant ($p \leq 0.05$) difference among the cookies of blend; Error bars represent standard deviation of three replicate. Foxtail millet, Barnyard millet and Kodo millet flour in the proportions of 80:15:5, 70:20:10, 60:25:15, 50:30:20, 40:35:25, 35:35:30, which refer to $A_1$, $A_2$, $A_3$, $A_4$, $A_5$, and $A_6$ flour blends respectively and wheat flour as control. Germ=Germinated

![Figure-(b)](image)

**Figure-5.2 Sensory analysis of cookies made from flour blends of (a) raw minor millets (foxtail, barnyard and kodo), (b) germinated minor millets (foxtail, barnyard and kodo)**

### 5.3.3 Optimization of cookies

The blended flour cookies of raw and germinated minor millet were optimized on the basis of physical, textural and sensory properties. Cookies prepared with incorporation of raw and germinated foxtail, barnyard and kodo millets flour in the proportion of 70:20:10 ($A_2$) were found to be acceptable in terms of sensory score. The peak force required to puncture the cookies prepared from flour blend ($A_2$) from raw and germinated flour, 44.71 and 39.95 N, respectively was found to decrease significantly ($p \leq 0.05$) than the cookies prepared from wheat (control). The cookies made from $A_2$ flour blend formulation of raw minor millet had higher spread ratio of
6.21 followed by cookies made from A₂ flour blend formulation of germinated minor millet 6.01 and least was observed in wheat flour cookies 5.99. On the basis of these results the A₂ formulation cookies were selected for further characterization

5.3.3.1 Characteristics of optimized cookies (A₂ sample)

5.3.3.1.1 Chemical composition

The nutritional analysis of cookies prepared from flour blends of germinated minor millets optimized sample (A₂) had higher amounts of moisture, ash, protein and dietary fiber followed by that of raw minor millet flour blend (A₂) of same formulations and control cookies (Table 5.6).

The protein content of cookies prepared from flour blends of germinated minor millet was higher, attributed either to losses in dry weight, particularly in carbohydrates through respiration during germination and synthesis of new peptides/amino acids as a result of biochemical process. The losses in dry weight can be accounted mainly by loss in sugars during respiration and due to the production of carbon dioxide and water, which escaped from the seeds (Mbithi-Mwikya et al., 2000). The increase in dietary fibers during baking and germination may occur due to the formation of fiber-protein complexes that are resistant to heating and are quantified as dietary fibers (Caprez et al., 1986). The fat (6.69 g/100 g) and carbohydrate (37.74 g/100 g) contents of cookies prepared from flour blends of germinated minor millets optimized sample were significantly lower than the raw minor millet flour blend and control cookies. The highest moisture content of germinated flour cookies may be due to de-polymerization of organic molecules which might have increased water holding capacity during baking and also due to higher dietary fiber contents.
Significantly higher total dietary fiber content of 34.34 % was perceived in optimized cookie prepared from flour blends of germinated minor millets followed optimized cookie prepared from flour blends of raw minor millets 29.7 % and minimum in wheat flour cookies (Table 5.6). After the germination and baking has been reported to further improve the dietary fiber content of cookies. The total dietary fiber of cookies was increased, not only due to new synthesis, but rather to the formation of fiber-protein complexes that were resistant to heating and were quantified as dietary fiber (Caprez et al., 1986). Lattimer and Haub (2010) have demonstrated many health benefits of dietary fiber content: cancer prevention, protection against cardiovascular diseases, lowering glycemic index of diabetic people and prevents or relieves constipation in human due to the absorption of water from the digestive track.

5.3.3.1.2 Total phenolic contents (TPC)

The analysis indicated that the germinated minor millets optimized sample (A2g) blended cookies had higher amounts of total phenolic contents (45.43 mg/100g) as compared to raw minor millet flour blend (A2r) and control cookies. As mentioned previously in the phenolic content analysis of flour blends, that the germination increased the total phenolic contents attributed to increased availability of some bound polyphenolic compounds from polysaccharides and proteins. Similar observations have been made for baked rhubarb, whereby TPC was found increased during the baking process (McDougall et al., 2010). Lindenmeier and Hofmann, (2004) reported increase in TPC might be due to the production of Maillard reaction products in the cookies during thermal processing and the release of bound phenolic acids from cell walls during baking (Dewanto et al., 2002).
5.3.3.1.3 Antioxidant activity

Antioxidants activity determined in terms of DPPH free radical scavenging activity of the compounds are capable of preventing, delaying or retarding the development of rancidity or other flavor deterioration in foods or as protective factors against the oxidative damage in the human body. The optimized cookies prepared from blended flour of raw and germinated minor millet flour cookies exhibited higher DPPH free radical scavenging activity 60.34% and 64.23%, respectively, as compared to wheat flour cookies (42.34%) (Table 5.6). During the baking process formation of dark color pigments (brown color) due to the Maillard browning and these pigments have been reported to have antioxidant activity which further increased the antioxidant activity (Manzocco et al., 2000; Xu and Chang, 2008). Sharma and Gujral (2014) have reported that baking further increased the antioxidant activity in cookies prepared with barley flour. Increase in the activity of the endogenous hydrolytic enzymes during sprouting is one of the many metabolic changes resulting in the increase in antioxidant activity (Alvarez-Jubete et al., 2010). Baking has been further reported to increase the antioxidant activity of cookies incorporated with Amaranth flour (Chauhan et al., 2015)

5.3.3.1.4 Color characteristics

Color is a principal factor in determining the acceptability of cookies. Significant differences were found in L*, a* and b* values for optimized cookie prepared from flour blends of raw and germinated minor millets. Hunter colour values of wheat flour cookies, optimized cookies prepared from blended flour of raw and germinated minor millet is shown in Table 5.6. The $L^*$ (lightness) values for optimized cookies prepared from flour blends of raw and germinated minor millets were 59.13 and 50.01, respectively. The $a^*$ (amber colour) values for optimized cookies prepared from flour blends of raw and germinated minor millets were 9.71
and 11.45, respectively and The $b^*$ (yellowness) values for optimized cookies prepared from flour blends of raw and germinated minor millets were 31.27 and 30.86, respectively. Xu and Chang (2008) reported that the formation of dark colour pigments (brown colour) due to the Maillard browning during baking process can increase the antioxidant properties of the baked product. The cookies prepared from flour blend of germinated minor millet displayed significant lower value for lightness (L-value) and yellowness (b-value) but significantly higher value for redness (a-value) followed by raw minor millets flour blended cookies and control wheat flour cookies. The lightness (L-value), yellowness (b-value) decrease during germination might be due to increased protein contain that had negatively correlated with lightness of a cookies, indicating that the Maillard reaction played the major role in colour formation (Chevallier et al., 2000). The browning reactions are also influenced by many factors such as water activity, pH, temperature, sugars, type and ratio of amino compounds (Sharma and Gujral, 2013). Thus alteration in chemical composition of germinated minor millet flours blended cookies indicated development of cookies with enhanced nutritional value and product functional characteristics.
Table 5.6 Nutritional and functional characteristics of cookies prepared from optimized flour blend of raw and germinated minor millets

<table>
<thead>
<tr>
<th>Samples</th>
<th>Moisture (g/100 g)</th>
<th>Protein (g/100 g)</th>
<th>Fat (g/100 g)</th>
<th>Ash (g/100 g)</th>
<th>Total CHO (g/100 g)</th>
<th>Dietary fiber (g/100 g)</th>
<th>TPC (mg/100g)</th>
<th>DPPHA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.32±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.89±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.99±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.45±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.99±2.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.36±0.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.23±0.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.34±0.87&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Raw (A&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>6.74±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.72±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.72±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.54±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.65±1.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.7 ±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.23±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.34±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germ (A&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>7.34±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.06±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.69±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.78±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.74±1.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.34±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.43±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.23±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Samples</th>
<th>L-value</th>
<th>a-value</th>
<th>b-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72.04±1.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.24±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Raw (A&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>59.13±2.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.71±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.27±1.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Germ (A&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>50.01±1.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.45±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.86±1.23&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

n=3, Mean ±standard deviation values in the same column which are not followed by the same letter are significantly different (p < 0.05). A<sub>2</sub> refers to cookies made from flour blend of raw and germinated; foxtail, barnyard and kodo millets flour in the proportions of 70:20:10, respectively.

5.4 Conclusion

Six flour blends were prepared by homogenously mixing raw and germinated foxtail, barnyard and kodo millets flours which were later used to make cookies. The germination of foxtail, barnyard and kodo millets resulted in an increase in protein, fibre, total polyphenolic content, and antioxidant activities. Analyses revealed that germinated flour blends contained higher proteins, total phenolics and possessed higher in vitro antioxidant activity, less fat and carbohydrate contents than that of raw millets flour blends. The sensory evaluation revealed that cookies prepared from incorporation of germinated foxtail, barnyard and kodo millet were
superior than the cookies prepared from raw combinations. The cookies (A\textsubscript{1}-A\textsubscript{6}) prepared from flour blends of raw and germinated minor millets were optimized on the basis of physical, textural and sensory properties. The nutritional analysis of cookies prepared from flour blends of germinated minor millets optimized sample had higher amounts of moisture (7.34 g/100 g); ash (2.78 g/100 g); protein (11.06 g/100 g), dietary fiber (34.36 g/100 g), total phenolic contents (45.43 mg/100) and DPPH radical scavenging activity (64.23\%) as compared to raw minor millet flour blended and control cookies. This study may exploit the scope for utilization of raw and germinated minor millets in various nutritional food products development.