CHAPTER 4
RESULTS AND DISCUSSION

In present study, *Chenopodium quinoa* seeds were studied for physical properties and subjected to grinding to get optimum particle size of flour with improved functional properties. The flour thus obtained was studied for physico-chemical properties, color, functional properties, pasting properties, morphological, structural characteristics, minerals, fatty acid profile, phenolic content, flavonoid content and antinutritional factors. Since the seeds contain ample amount of starch, process for isolation of starch was also standardized and the starch isolated by standard process was studied for physico-chemical, morphological, thermal, structural and rheological properties. *Chenopodium quinoa* flour was used for development of cookies and the starch was used for development of noodles. The developed products were analyzed for various quality parameters and their stability during storage. The results obtained from this study have been discussed under the below given sections:

4.1 Physical properties of seed

4.1.1 Grain size

The length and thickness of both the varieties were significantly different (P<0.05) with V2 being wider and thicker than V1. The length and width were approximately equal, and very small differences existed as was also reported by Vilche et al. (2003). The effect of moisture content on the average values of the length and thickness of quinoa seeds is shown in Figure 4.1 (a and b). Dimensions of both the varieties increased linearly with an increase in moisture content. The length of the seeds increased from 1.81 to 2.09 mm for V1 and from 1.92 to 2.16 mm for V2, whereas thickness increased from 0.79 to 0.95 for V1 and from 0.92 to 1.07 mm for V2 with an increase in moisture content from 5 to 25%. The dimensions of the quinoa seeds were observed to fall within the range observed for quinoa from Argentina (Vilche et al., 2003) and rapeseed (Izli et al., 2009) but was higher than that
of amaranth seeds (Abalone et al., 2004).

Figure 4.1 Effect of moisture content on: a) Length and b) Thickness of quinoa seeds
The increase in moisture content showed a significant effect (p<0.05) on the length and thickness of both the varieties with a more pronounced effect on length than on thickness. Similar linear increasing trend of seed dimensions with increase in moisture content is reported in the literature (Bamgboye et al., 2009; Vilche et al., 2003; Abalone et al., 2004). Dimensions of the seeds are of paramount importance in determining the aperture size of the machine to process the seed. Apart from that, the dimensions could be useful in determining the shape of the seed.

4.1.2 Geometric and Arithmetic mean diameter

The arithmetic mean diameter and the geometric mean diameter of V2 were greater than those of V1 (Table 4.1). These diameters increased with increase in moisture content for both the varieties. The arithmetic mean diameter showed an increase of 13.92% for V2 and 16.33% for V1. In case of the geometric mean diameter an increase of 16.79% was observed for V1 and 14% for V2. There was a significant effect of a moisture content increase on the arithmetic mean diameter and the geometric mean diameter (p<0.05). The increase in the arithmetic mean diameter and geometric mean diameter with increasing moisture content might be attributed to the increase in principle dimensions of the seed. The geometric mean diameter obtained can be used to determine the volume and sphericity of the seed theoretically. Results are in agreement with the increasing trend shown by Izli et al. (2009) for rapeseed seeds and by Abalone et al. (2004) for amaranth seeds.

4.1.3 Surface area and Sphericity

The variation in the grain surface area with moisture content is shown in Table 4.1. Surface area of V1 varied from 5.9 to 8.08 mm$^2$ and for V2 varied from 7.06 to 9.17 mm$^3$ and was found to increase by 36.95% for V1 and 29.89% for V2. The surface area of both the varieties was significantly influenced by the increased moisture content (p<0.05). Similar trend for surface area was observed by Izli et al. (2009) for rapeseed. The sphericity of quinoa
seeds varied from 0.760 to 0.770 for V1 increasing by 1.32% and from 0.782 to 0.790 for V2 showing an increase of 1.02%. This shows that seeds can slide on flat surfaces easily. A similar linear increasing trend in sphericity has been reported for roselle and sunflower seeds (Bamgboye et al., 2009; Malik and Saini, 2016).

Table 4.1 Effect of moisture content on geometric properties of quinoa seeds

<table>
<thead>
<tr>
<th>Variety</th>
<th>Moisture Content (%)</th>
<th>Geometric mean diameter</th>
<th>Arithmetic mean diameter</th>
<th>Sphericity</th>
<th>Surface area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>5</td>
<td>1.37±0.03aB</td>
<td>1.47±0.03aB</td>
<td>0.760±0.01aB</td>
<td>5.9±0.26aB</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.43±0.02aB</td>
<td>1.53±0.02aB</td>
<td>0.764±0.01aB</td>
<td>6.46±0.18aB</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.50±0.03aB</td>
<td>1.60±0.03aB</td>
<td>0.766±0.01aB</td>
<td>7.10±0.25aB</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.56±0.01aA</td>
<td>1.66±0.01aB</td>
<td>0.769±0.01aB</td>
<td>7.68±0.14aB</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.60±0.02aB</td>
<td>1.71±0.02aB</td>
<td>0.770±0.01aB</td>
<td>8.08±0.21aB</td>
</tr>
<tr>
<td>V2</td>
<td>5</td>
<td>1.50±0.01cA</td>
<td>1.58±0.01cA</td>
<td>0.782±0.01aA</td>
<td>7.06±0.09cA</td>
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<tr>
<td></td>
<td>10</td>
<td>1.54±0.01cA</td>
<td>1.62±0.01cA</td>
<td>0.786±0.01aA</td>
<td>7.42±0.14cA</td>
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<tr>
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<td>15</td>
<td>1.58±0.01cA</td>
<td>1.67±0.01cA</td>
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<td>7.87±0.12cA</td>
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<td>20</td>
<td>1.65±0.01BA</td>
<td>1.73±0.02BA</td>
<td>0.790±0.01aA</td>
<td>8.51±0.14BA</td>
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<tr>
<td></td>
<td>25</td>
<td>1.71±0.02bA</td>
<td>1.80±0.02bA</td>
<td>0.790±0.01aA</td>
<td>9.17±0.23bA</td>
</tr>
</tbody>
</table>

Values followed by different lower-case letters in each column are significantly different (P<0.05) among varying moisture contents of same variety. Values followed by different upper-case letters in each column are significantly (P<0.05) different among the varieties. Lower case letters shows the effect of moisture content only within the variety and the upper case letters show the varietal effect only.

4.1.4 Thousand seed weight

The variation of the thousand seed weight (TSW) with increase in moisture content is shown in Table 4.2. Thousand seed weight increased linearly from 2.54 to 3.03 g for V1 and 2.61 to 3.13 g for V2. Thus, an increase of 19.29% and 19.92% was observed for V1 and V2 respectively with an increase in moisture content from 5 to 25%. The influence of increasing moisture content on thousand seed weight was significant (p<0.05). Similar increasing trend in thousand seed weight was found by some other researchers like Izli et al. (2009) in case of
rapeseeds and Malik and Saini (2016) for sunflower seeds.

4.1.5 Bulk density

With an increase in moisture content the values for bulk density were observed to decrease from 721 to 670 kg/m$^3$ for V2 and from 701 to 645 kg/m$^3$ for V1 as shown in Table 4.2. This decrease could be attributed to the volumetric expansion of the seed and pore spaces which became proportionally greater on moisture absorption. Bulk density showed the percentage variation of 7.07 % for V2 and 7.99 % for V1 in the given moisture range (5 to 25%). Quinoa variety V2 exhibited higher bulk density in comparison to V1 which may be due to larger size of V2 resulting in higher mass than V1. Bulk density was significantly (p<0.05) influenced by the variety and increasing moisture content. The negative linear relationship of bulk density with increasing moisture content has also been observed by various other researchers like Izli et al. (2009) for rapeseeds and Altuntaş et al. (2005) for fenugreek seeds.

4.1.6 True density

True density increased from 984 to 1097 Kg/m$^3$ for V1 and from 993 to 1166 kg/m$^3$ for V2 with increase in moisture content from 5 to 25%. The percentage increase of 11.48 % and 17.42 % was observed for V1 and V2 respectively with increase in moisture content as shown in Table 4.2. V1 exhibited lower true density than V2 which may be due to its dimensions resulting in smaller increase in true volume. True density was significantly (p<0.05) influenced by the variety and increased moisture content. The increase in true density with increase in moisture content might be attributed to the relatively lower true volume as compared to the corresponding mass of the kernel attained due to adsorption of water. Similar trend was observed by Kingsly et al. (2006) for pomegranate seeds and Vilche et al. (2003) for quinoa seeds from Argentina.
**4.1.7 Porosity**

The Porosity of the seed increased linearly from for 28.58 to 43.20% V₁ and 27.47 to 42.68% for V₂ with an increase in the moisture content from 5 to 25% (Table 4.2). This variation may be attributed to its dependence on the bulk and true densities of the seed. The effect of variety and increased moisture content on porosity was significant (p<0.05). At any moisture level, the porosity values for both quinoa varieties were lower than those for nigella seeds (Singh et al., 2015). The results for porosity are in accordance with those presented for amaranth and quinoa seeds (Abalone et al., 2004 and Vilche et al., 2003).

**4.1.8 Angle of repose**

The angle of repose increased from 19.07 to 26.57° for V₁ and 15.05 to 23.86° for V₂ as shown in Table 4.2. The lower angle of repose of V₂ seeds represents a smoother outer surface, hence the easiness to slide on each other in comparison to V₁. There was a significant influence of increasing moisture content on the angle of repose (p<0.05). The angle of repose indicates the cohesion among the individual units of the material. The higher the cohesion, higher is the angle of repose. This increasing trend of the angle of repose depending on moisture content occurs because the surface layer of moisture surrounding the particle hold the aggregate of seeds together by the surface tension. Similar results were found by Singh et al. (2015) for nigella seeds. A low angle of repose makes the seeds spread out wider on a plane surface compared to high angle of repose. Low angle of repose is often advisable during belt conveying while high angle of repose is more desirable when unloading onto a horizontal surface.
### Table 4.2 Effect of moisture content on gravimetric properties and angle of repose of quinoa seeds

<table>
<thead>
<tr>
<th>Variety</th>
<th>Moisture content</th>
<th>Bulk density (kg/m(^3))</th>
<th>True density (kg/m(^3))</th>
<th>Porosity</th>
<th>Thousand seed weight (g)</th>
<th>Angle of Repose (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>5</td>
<td>701 ±1.01(^{aB})</td>
<td>984±4.11(^{dB})</td>
<td>28.85</td>
<td>2.54±0.01(^{eB})</td>
<td>19.07±0.14(^{eA})</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>686 ±1.89(^{bB})</td>
<td>1002±4.53(^{cB})</td>
<td>31.51</td>
<td>2.65±0.01(^{dB})</td>
<td>20.99±0.18(^{dA})</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>665 ±1.10(^{dB})</td>
<td>1030±4.93(^{dB})</td>
<td>35.43</td>
<td>2.79±0.02(^{eB})</td>
<td>23.84±0.15(^{cA})</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>652 ±1.28(^{eB})</td>
<td>1058±4.99(^{eB})</td>
<td>38.37</td>
<td>2.91±0.03(^{fB})</td>
<td>24.78±0.19(^{bA})</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>645 ±1.01(^{fB})</td>
<td>1097±4.67(^{fB})</td>
<td>43.20</td>
<td>3.03±0.01(^{fB})</td>
<td>26.57±0.28(^{aA})</td>
</tr>
<tr>
<td>V2</td>
<td>5</td>
<td>721 ±1.02(^{aA})</td>
<td>993±4.18(^{dA})</td>
<td>27.47</td>
<td>2.61±0.02(^{eA})</td>
<td>15.05±0.14(^{eB})</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>708 ±1.15(^{bA})</td>
<td>1023±4.70(^{cA})</td>
<td>30.79</td>
<td>2.72±0.01(^{dA})</td>
<td>16.08±0.18(^{bB})</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>688 ±1.82(^{CA})</td>
<td>1048±5.80(^{BA})</td>
<td>34.36</td>
<td>2.88±0.02(^{cA})</td>
<td>18.09±0.15(^{eH})</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>679 ±1.02(^{dA})</td>
<td>1096±5.36(^{aA})</td>
<td>38.09</td>
<td>2.99±0.01(^{bA})</td>
<td>20.99±0.19(^{bB})</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>670 ±1.08(^{eA})</td>
<td>1166±5.83(^{aA})</td>
<td>42.68</td>
<td>3.13±0.01(^{aA})</td>
<td>23.86±0.28(^{aB})</td>
</tr>
</tbody>
</table>

Values followed by different lower-case letters in each column are significantly different (P<0.05) among varying moisture contents of same variety. Values followed by different upper-case letters in each column are significantly (P<0.05) different among the varieties. Lower case letters shows the effect of moisture content only within the variety and the upper case letters show the varietal effect only.

### 4.1.9 Coefficient of friction

The static coefficients of friction of quinoa seeds on three surfaces like plywood, glass and galvanised iron against increasing moisture content in the range of 5–25% are presented in Figure 4.2.
Figure 4.2 Effect of moisture content on static coefficient of friction of quinoa seeds against various contact surfaces

The static coefficient of friction of both quinoa varieties (V1 and V2) showed the significant difference (P<0.05) with V1 showing higher static coefficient of friction than V2. The higher coefficient of friction of V1 is in agreement with its higher angle of repose. The
Coefficient of friction increased linearly with increase in moisture content for both the varieties against all contact surfaces. An increase of 16.29, 16.00, and 18.93% was observed against glass, galvanised iron and wood respectively for V1. However, for V2 an increase of 19.12, 22.73, and 23.21% was observed against glass, galvanised iron and wood respectively with increase in moisture content from 5 to 25%. The increased friction coefficient at higher moisture content may be due to moisture present in grains offering increased cohesive force on contact surface. Among various contact surfaces plywood offered higher coefficient of static friction followed by galvanised iron and glass which may be due to smoother surfaces of glass and galvanized iron in comparison to plywood. Similar trend was reported by Izli et al. (2009) for rapeseed.

4.2 Grinding of quinoa seeds and preparation of flour

Physical structure of the grains demands breaking action of equipments in order to produce flour, convert it into various attractive products and also to make the material easy to handle. Information regarding the grinding and its influence on flour particle size and functional properties of C. quinoa flour is not available in the literature. Grinding greatly affects the functional properties and these functional properties have been found to be critical for the production of associated foods. The properties include color, water absorption, oil absorption and solubility. These properties affect the texture, processing and appearance of the final product (Kerr et al., 2000). To obtain flour of uniform particle size with consistent composition and improved functional properties quinoa seed was subjected to grinding at a moisture content of 11%. Also the effect of mills (stone and cyclotec) on average particle size and functional properties of flour was studied. Sieve analysis was used for particle size distribution as it has been considered as a cheap and uncomplicated measurement for determination of the particle size distribution.
4.2.1 Particle size distribution

The particle size distribution, percent material retained and throughs as obtained by sieve analysis of quinoa flour milled by stone and cyclotec mill is shown in Table 4.3 and 4.4. Wide range of particle size distribution was obtained depending on the variety and type of mill. The average particle size calculated was 0.274 mm for V1 and 0.254 mm for V2 in case of stone mill. However, in case of cyclotec mill average particle size was found to be 0.243 mm for V1 and 0.221 mm for V2. Data analysis showed that the average particle size of both the varieties was lower in case of cyclotec mill than stone mill. Average particle size of V2 flour was smaller than V1 for both the mills despite the same milling and screening conditions which may be attributed to the differences in the structure and breakage characteristics of the two varieties. More uniform grinding was observed by using cyclotec mill than stone mill. On the basis of particle size distribution data it can be concluded that the average particle size was lower for V2 than V1 and cyclotec mill was more effective for size reduction of both quinoa varieties in comparison to stone mill. All the particle size parameters like D (10), D (50) and D (90) obtained from the percent throughs are shown in Table 4.3 and 4.4. More pronounced effect on the particle size parameters was due to the type of mill rather than the variety.
Table 4.3 Sieve analysis of *C. quinoa* flour (V1) using stone mill (S.M) and cyclotec mill (C.M)

<table>
<thead>
<tr>
<th>Mesh No.</th>
<th>Sieve opening (mm)</th>
<th>Wt. of flour retained</th>
<th>% retained</th>
<th>Cumulative wt. retained</th>
<th>Cumulative % wt retained</th>
<th>% Throughs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S.M</td>
<td>C.M</td>
<td>S.M</td>
<td>C.M</td>
<td>S.M</td>
</tr>
<tr>
<td>30</td>
<td>0.500</td>
<td>5.08</td>
<td>3.93</td>
<td>5.11</td>
<td>3.96</td>
<td>5.08</td>
</tr>
<tr>
<td>44</td>
<td>0.355</td>
<td>23.14</td>
<td>19.00</td>
<td>23.27</td>
<td>19.14</td>
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</tr>
<tr>
<td>60</td>
<td>0.250</td>
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<td>56.11</td>
</tr>
<tr>
<td>85</td>
<td>0.180</td>
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<td>13.10</td>
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<td>75.22</td>
</tr>
<tr>
<td>100</td>
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</tr>
<tr>
<td>120</td>
<td>0.120</td>
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<td>17.23</td>
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<td>98.78</td>
</tr>
<tr>
<td>200</td>
<td>0.075</td>
<td>0.66</td>
<td>0.46</td>
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<td>99.44</td>
</tr>
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<td>Pan</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>d10</td>
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<td>0.454</td>
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</tr>
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</table>

Where; SM= Stone mill and CM= cyclotec mill.
Table 4.4 Sieve analysis of *C. quinoa* flour (V2) using stone mill (S.M) and cyclotec mill (C.M)

<table>
<thead>
<tr>
<th>Mesh No.</th>
<th>Sieve opening (mm)</th>
<th>Wt. of flour retained (S.M)</th>
<th>% retained</th>
<th>Wt. of flour retained (C.M)</th>
<th>% retained</th>
<th>Cumulative wt. retained (S.M)</th>
<th>Cumulative % wt retained</th>
<th>Cumulative wt. retained (C.M)</th>
<th>Cumulative % wt retained</th>
<th>% Throughs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S.M</td>
<td>C.M</td>
<td>S.M</td>
<td>C.M</td>
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<td>95.09</td>
<td>95.95</td>
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<td>4.91</td>
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<td>99.18</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>d90</td>
<td></td>
<td>0.461</td>
<td>0.440</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Where; SM= Stone mill and CM= cyclotec mill.
4.2.2 Effect of grinding on color and functional properties of *C. quinoa* flour

Functional properties reflect the complex interactions between structure, composition and molecular conformation of food along with the nature of environment in which they are associated and measured (Chandra et al., 2015). These are the properties which reflect the behavior of flour in food system as judged by the quality attributes of final product. The effect of milling and variety on color and various functional properties like oil binding capacity and water binding capacity was assessed as discussed below.

4.2.2.1 Color

Effect of grinding on color profile of quinoa is shown in Table 4.5. Color is an important attribute of the product and relates to its consumer acceptability. Grinding showed the significant (P<0.05) effect on color properties of quinoa flour from two varieties with V1 being lighter in color than V2. Reduction in particle size improved the lightness value of both the varieties. The increase in lightness value may be due to increased surface area allowing more reflection of light. The a and b values of the flours decreased with decrease in particle size. Increased L-value and decreased a and b values of finer particles has been reported by various researchers (Savlak et al., 2016; Ahmed et al., 2015). Values of the color analysis obtained in present study were lower than that reported by Savlak et al. (2016) and Ahmed et al. (2015) for rice flour. Color analysis result revealed that flour from both the varieties was lighter in color when milled by cyclotec mill rather than stone mill. Higher lightness of the samples obtained from cyclotec mill was also confirmed by higher whiteness index and lower yellowness index of flour.

4.2.2.2 Water binding capacity (WBC)

Water binding capacity of the flour is an important parameter as it affects the functional and sensory characteristics of the final product. WBC was significantly (P<0.05) affected by the
type of mill and quinoa variety (Table 4.6). V1 showed higher WBC than V2. Higher water binding capacity of V1 may be due to presence of more hydrophilic components like polysaccharides. Water binding capacity of the sample depends on the protein, starch and fiber with starch showing the superior contribution (Farooq and Boye, 2011). Water binding capacity was higher for samples obtained from cyclotec mill than stone mill which may be due to decrease in particle size of the quinoa flour. Higher water binding capacity due to decrease in particle size of flour may be because of larger surface area of smaller particle. Water binding capacity of the samples varied from 1.91 to 2.05 g/g for V2 and 1.96 to 2.11 % for V1.

4.2.2.3 Oil binding capacity (OBC)

Oil binding capacity of the samples varied from 1.65 to 1.70 (g/g) for V2 and 1.57 to 1.61 (g/g) for V1 (Table 4.6). Decrease in particle size increased the OBC of both the flour samples but the effect was of lesser magnitude in comparison to change in WBC. According to Kinsella (1976) OBC of a sample is mainly due to the entrapment of oil via capillary attraction by starch and presence of non polar sites in protein. Electrostatic, hydrophobic and hydrogen bonds are involved in fat-protein interactions and contribute to the oil binding capacity of the sample (Lawal, 2004). Variation in the OBC of the two varieties may be due to variation in the proportion of non polar and polar side chains of amino acids on the surfaces of their protein molecules. Higher OBC is desirable for the appropriate flavor and the mouthfeel of final product.

Based on the particle size and functional properties of the flour, cyclotec mill was used for preparation of flour from *C.quinoa* seed.
Table 4.5 Effect of grinding on color of *C. quinoa* flour

<table>
<thead>
<tr>
<th>Samples</th>
<th>Type of mill</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>whiteness Index</th>
<th>Yellowness index</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>Stone mill</td>
<td>63.40±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.99±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.97±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.52±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.24±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cyclotec mill</td>
<td>65.79±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.70±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.96±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.11±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.55±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>V2</td>
<td>Stone mill</td>
<td>62.10±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.29±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.46±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.13±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.06±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cyclotec mill</td>
<td>64.04±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.09±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.25±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.42±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.04±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Mean values in the same column followed by the different letters are significantly different (*p* < 0.05) among the mills.

Table 4.6 Effect of grinding on functional properties of *C. quinoa* flour

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of mill</th>
<th>WBC (g/g)</th>
<th>OBC (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>Stone mill</td>
<td>1.96±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.57±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cyclotec mill</td>
<td>2.11±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.61±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>V2</td>
<td>Stone mill</td>
<td>1.91±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.65±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cyclotec mill</td>
<td>2.05±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Mean values in the same column followed by the different letters are significantly different (*p* < 0.05) among the mills.
4.3 Characterization of *C. quinoa* flour

4.3.1 Proximate composition of *C. quinoa* flour

Quinoa flour showed the moisture, protein, fat, crude fibre, ash and carbohydrate content in the range of 9.52 to 9.59, 13.06 to 14.96, 5.83 to 6.32, 3.88 to 4.02, 2.94 to 3.08 and 62.59 to 64.21% respectively. V2 showed higher Protein, fibre and ash content whereas, V1 showed higher moisture, fat and carbohydrate content. Significant (P<0.05) differences were observed in the proximate composition of quinoa varieties (Table 4.7). This difference in the proximate composition of the varieties may be attributed to their genetic makeup. The protein content, ash, fat and fibre content of both the samples were higher than that of corn reported by Sandhu et al. (2007) and wheat reported by Himeda et al. (2014).

**Table 4.7 Proximate composition of *C. quinoa* flour**

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>9.59±0.10^a</td>
<td>9.52±0.08^a</td>
</tr>
<tr>
<td>Protein</td>
<td>13.06±0.14^b</td>
<td>14.96±0.17^a</td>
</tr>
<tr>
<td>Fat</td>
<td>6.32±0.11^a</td>
<td>5.83±0.10^b</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>3.88±0.08^b</td>
<td>4.02±0.07^a</td>
</tr>
<tr>
<td>Ash</td>
<td>2.94±0.06^b</td>
<td>3.08±0.07^a</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>64.21±0.21^a</td>
<td>62.59±0.15^b</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ±standard deviation. Values in a same row with different superscripts are significantly different (p≤0.05)

4.3.2 Functional properties of *C. quinoa* flour

Functional properties of the flour usually depend on its protein, complex carbohydrates, other components and the interaction between these components. These properties govern the behavior of ingredients in a complicated food processes like during processing and storage. Functional properties of flour could enhance its utilization in food products and also play an important role in novel product development and the successful performance of flour as a food ingredient.
4.3.2.1 Foaming capacity and foam stability

Foaming capacity and stability can be used to evaluate the ability of flour to act as whipping agent. The foaming capacity and stability of quinoa flour is shown in Figure 4.3.

![Foaming capacity (a) and Foam stability (b) of C. quinoa flour](image)

Foaming capacity was higher for V2 (12.36 %) than V1 (10.86 %). Foaming property of the samples on whipping is mainly due to the surface active properties of proteins. Higher
foaming capacity of V2 may be due to its higher protein content. Foaming capacity of the sample is mainly due to the ability of protein to adsorb at air water interface during bubbling, rapid conformational change and rearrangement at the interface. While as, the foam stability of the sample is mainly due to the ability of its protein to form a cohesive film around the bubbles in foam through intermolecular interactions (Tsaliki et al., 2002). With increase in time duration foaming stability varied from 68.38 to 23.72% for V1 and 70.48 to 25.08 for V2. The foaming capacity and stability observed in present study was higher than that reported by Ogungbenle (2003) for quinoa from Canada. This may be due to the variations in the protein content of the seeds as well as due to the concentration of flour used for analysis. The foaming capacity and foam stability varies with the concentration of protein; these properties also depend on the configuration of protein molecules with flexible proteins having the higher foaming capacity (Elsohaimy et al., 2015). Also the presence of saponin interferes with the foaming capacity of the sample. Higher foaming capacity of flour is considered as a desirable trait for bakery products.

4.3.2.2 Emulsifying activity (EA) and emulsion stability (ES)

Emulsifying activity and emulsion stability of C.quinoa flour are shown in Figure 4.4. V2 showed an emulsifying activity and stability of 49.61 and 47.25% in comparison to 47.19 and 44.44% shown by V1. Significant (P<0.05) differences were observed in the emulsifying activity and emulsion stability of flour varieties. EA and ES of the flour samples is usually attributed to the surface active agents (proteins) which have the ability of formation and stabilization of emulsion. Proteins act as amphiphiles by reducing the interfacial tension between two phases thereby stabilizing the dispersed droplets via steric or electrostatic effect. Emulsion activity of quinoa flour reported in current study was lower than that reported by Ogungbenle (2003) for quinoa from Canada. However, emulsion stability was almost of same range with V2 showing higher emulsion stability than that reported by Ogungbenle (2003).
Emulsion activity and stability reported in present study is higher than that reported by Suresh and Samsher (2013) for wheat and rice flour. This suggests the high fat emulsifying property of quinoa flour and its possible use as food additive and for stabilization of colloidal foods. Kaushal et al. (2012) reported that EA and ES of the sample may be related to the solubility and conformational stability of its protein with globular protein molecules providing more stable emulsions.

![Emulsifying activity and Emulsion stability of *C. quinoa* flour](image)

**Figure 4.4 Emulsifying activity and Emulsion stability of *C. quinoa* flour**

### 4.3.3 Pasting properties

Pasting characteristics of quinoa flour as analyzed by rapid visco analyzer are shown in Table 4.8. Both the varieties showed significant (P<0.05) differences in the pasting characteristics. V1 showed higher viscosity than V2 with peak, trough, breakdown, final and setback viscosity of about 845, 738, 107, 977, 239 cp. However, the pasting temperature which indicates the temperature required to cook flour was higher for V2 (89.80 °C) than V1 (86.15 °C). Lower break down (49 and 107 cP) and setback viscosity (148 and 239 cP) of
both the varieties indicate their stability and lesser tendency to retrograde. Both the flour varieties showed c-type viscosity pattern (restricted swelling). Lower viscosity of V2 can be due to its lower starch and higher protein content. Mohammed et al. (2014) reported that lower the starch content of flour lower will be its peak viscosity and proteins in the flour restrict its swelling thereby decreasing the overall viscosity. Pasting temperature of the V1 was within the pasting temperature range (74.9 to 88.8 °C) observed by Sandhu et al. (2007) for corn varieties. However, V2 showed slightly higher pasting temperature than this range. Higher pasting temperature of V2 can be due to its higher protein and fibre content that obstruct the swelling of granules and increase the amount of heat required as observed by Falade and Okafor (2015) for cocoyam flours.

**Table 4.8 Pasting properties of C. quinoa flour**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak viscosity (cP)</td>
<td>845±10.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>512±9.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Trough viscosity (cP)</td>
<td>738±10.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>463±10.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breakdown viscosity (cP)</td>
<td>107±11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49±4.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final viscosity (cP)</td>
<td>977±11.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>611±10.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Setback viscosity (cP)</td>
<td>239±12.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148±17.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pasting temperature (°C)</td>
<td>86.15±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.80±0.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ±standard deviation. Values in a same row which are followed by the different superscripts are significantly different (p≤0.05)

**4.3.4 Morphological Characteristics**

Scanning electron micrographs of the quinoa flours are shown in Figure 4.5. Both the varieties revealed the presence of many irregular chunks. From the micrographs clear distinction between the two varieties can be made. Most of the starch granules in V1 were scattered distinct and few were embedded within a dense matrix while as, in V2 granules were agglomerated and attached in a continuous structure of either fibre, protein or other constituents. Ahmed et al. (2016) also observed the presence of granules in distinct and
agglomerated form for lentil flours. V2 showed the compact structure where as the V1 showed the porous structure. According to Becker et al. (2014) porous particles reduce the milling efficiency by the damping effect resulting in coarser flour. However, denser structure facilitates the rupture of endosperm and produces the particles of small size.

![Scanning electron micrographs of C. quinoa flour](image)

**Figure 4.5 Scanning electron micrographs of C. quinoa flour**

**4.3.5 X-ray diffraction pattern and relative crystallinity**

XRD is used to determine the crystalline structure of the sample. The X-ray diffractograms of *C.quinoa* flours are shown in Fig. 4.6. Both the samples exhibited the typical A-type diffraction pattern with strong reflection peaks at 2θ of 15, 17, 18 and 23°. The relative crystallinity was 19.35 % for V1 and 20.18 % for V2. As a general trend higher the amylose content lower will be the crystallinity of sample. Hence, higher crystallinity of V2 can be due to its lower amylose content. Crystallinity values observed in present study were lower than the range (16.97-23.81%) observed by Oliveira et al. (2017) for corn flour. Crystallinity is also affected by the amount of crystalline region, type of amylopectin chain, orientation of helices and the extent of interaction between double helices (Shujun et al.,
Crystallinity in case of flour also depends on the composition of flour like fat and proteins in addition to starch (Sankhon et al., 2014).

**Figure 4.6 X-ray diffraction pattern of C.quinoa flour**

**4.3.6 Mineral analysis of C.quinoa flour**

Mineral composition of *Chenopodium quinoa* flour is outlined in Table 4.9. Quinoa is considered as a good source of minerals. The data revealed that magnesium, calcium, potassium, iron are present in abundant amounts in both the varieties while as zinc and sodium are present in least abundant amounts. Significant (p<0.05) differences were observed between the mineral content of two varieties. V1 was found to be higher in potassium, iron and zinc while as, V2 was higher in calcium, magnesium and sodium. These differences may be attributed to the genetic makeup of the crop. The values of minerals reported in current study were almost within the range reported in literature for quinoa and higher than that of some cereals like wheat, rice and barley (Koziol, 1992). Variations in the mineral
composition in comparison to Koziol (1992) may be due to the differences in the geographic and climatic conditions.

### Table 4.9 Mineral composition of *C. quinoa* flour (mg/kg)

<table>
<thead>
<tr>
<th>Minerals</th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>412±11.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>595±11.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1358±32.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1409±26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium</td>
<td>9870±25.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9400±24.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron</td>
<td>69.50±2.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.30±2.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc</td>
<td>35.91±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.54±1.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sodium</td>
<td>43.57±1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.01±2.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Values in a same row which are followed by the different superscripts are significantly different (p≤0.05)

### 4.3.7 Fatty acid profile

The fatty acid composition of *C. quinoa* (V1 and V2) lipids as obtained by gas chromatographic analysis is given in Table 4.10. Significant (P<0.05) differences existed in the fatty acid profile of two varieties. Linoleic acid was found to be most abundant fatty acid in both varieties V1 and V2 contributing about 58.23 % and 47 % of lipid fraction respectively. Palmitic acid was the main saturated fatty acid and accounted for about 10.13 % lipid fraction in case of V1 and 9.43 % in case of V2. Saturated fatty acids like stearic acid and arachidic acid were below limit of quantification in case of V1 whereas, contributed 2.06 % and 2.40 % of lipid fraction respectively in case of V2. Over all V2 was higher in saturated fatty acids and monounsaturated fatty acids while as V1 was higher in polyunsaturated fatty acids. Results for fatty acid profile were in agreement with the previous studies which observed almost similar composition (Hager et al., 2012; Ando et al., 2002). Unsaturated fatty acids are considered to have a beneficial effect on serum lipoprotein profile, blood pressure, parameters of glycemic control and cardiovascular risk factors (Schwingshackl and Hoffmann 2012; Joris and Mensink 2016).
Table 4.10 Fatty acid profile of *C. quinoa* flour samples [% (w/w) of total lipids]

<table>
<thead>
<tr>
<th>Fatty acids (%)</th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>10.13±0.22(^a)</td>
<td>9.43±0.24(^b)</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>BLQ</td>
<td>2.06±0.08(^a)</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>BLQ</td>
<td>2.40±0.07(^a)</td>
</tr>
<tr>
<td>Oleic acid (C18:1)</td>
<td>21.36±0.18(^b)</td>
<td>31.90±0.23(^a)</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>58.23±0.25(^a)</td>
<td>47.00±0.22(^b)</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>8.54±0.20(^a)</td>
<td>5.66±0.16(^b)</td>
</tr>
<tr>
<td>Others</td>
<td>1.74±0.19(^a)</td>
<td>1.55±0.09(^b)</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>66.77±0.22(^a)</td>
<td>52.66±0.19(^b)</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Values in a same row which are followed by the different superscripts are significantly different (p ≤ 0.05); BLQ = Below the Limit of Quantification.

### 4.3.8 Dietary fibre

Dietary fibre content of *C. quinoa* flour is shown in Table 4.11. Significant (p<0.05) differences were observed between the dietary fibre content of two varieties with V2 showing higher dietary fibre content than V1. Insoluble dietary fibre comprised about 86.39 % and 86.93 % of the total dietary fibre of V1 and V2 respectively. Total, soluble and insoluble dietary fibre content of V1 and V2 was within the range reported in literature for various pseudocereals (Alvarez-Jubete et al., 2009). Dietary fibre content values reported in current study for the two varieties was higher than that reported for most of the cereals (Dhingra et al., 2012). High intake of dietary fiber has been associated with decreased hyperinsulinemia, lower plasma lipid concentrations and improved glycemic control (Chandalia et al., 2000).
### Table 4.11 Dietary fibre content of *C. quinoa* flour

<table>
<thead>
<tr>
<th>Parameter</th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble dietary fibre (%)</td>
<td>1.83±0.07</td>
<td>1.99±0.09a</td>
</tr>
<tr>
<td>Insoluble dietary fibre (%)</td>
<td>11.62±0.22b</td>
<td>13.24±0.26a</td>
</tr>
<tr>
<td>Total dietary fibre (%)</td>
<td>13.45±0.15b</td>
<td>15.23±0.17a</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Values in a same row which are followed by the different superscripts are significantly different ($p \leq 0.05$).

#### 4.3.9 Antioxidant Properties

##### 4.3.9.1 Radical scavenging activity (% DPPH inhibition)

Radical scavenging activity of methanolic extracts of quinoa flour was examined and compared as given in Table 4.12. Significant ($P < 0.05$) differences existed in the radical scavenging activity of extracts from both the flours. V2 (19.24 %) showed higher radical scavenging ability than V1 (17.46 %) which means V2 has higher total antioxidant activity than V1. As, the higher DPPH radical scavenging activity has been correlated with higher antioxidant activity (Sultana et al., 2009). Higher antioxidant activity of V2 may be due to presence of more pigments, which can also be verified from its slightly darker color in comparison to V1. Ladjal and chibane (2015) also found the weak antioxidant activity for chickpea with light colored seed coat in comparison to the darker one. The radical scavenging activity of flours observed in current study was found to be within the range (11.8 to 28.9 %) observed by Carciochi et al. (2015) during the optimization of phenolic compound extraction from quinoa. The radical scavenging activity has been found to vary according to the composition, genetic diversity and quantity of secondary metabolites (Reddy et al., 2016).

##### 4.3.9.2 Total phenolic content (TPC)

Phenolic compounds present in food are thought to be responsible for its antioxidant activity. Total phenolic content of the samples is shown in Table 4.12. Both the varieties showed significant ($P < 0.05$) differences in the total phenolic content with V1 (196.59 mg
GAE/100g) showing lower value than V2 (218.37 mg GAE/100 g). According to Marathe et al. (2011) differences in the phenolic content of the varieties exist due to degree of maturity, genetic factors and environmental conditions. Since, in our case degree of maturity and environmental conditions were same the differences may be due to genetic makeup of the variety. Marathe et al. (2011) also found the color of seed coat to be responsible for total phenolic content with samples possessing darker color seed coat exhibiting higher total phenolic content. Quinoa contains higher phenolic compounds in comparison to cereals and some pseudocereals as reported by Asao and Watanabe (2010). Total phenolic content reported in present study was higher than the range (0.16 to 1.35 mg GAE/g) reported by Asao and Watanabe (2010). But, lower than that reported by Jan et al. (2016b) for pseudocereals like raw Chenopodium album (241 mg/100 g). These differences may be due to environmental conditions genetic factors and degree of maturity of the crops (Liu et al., 2016b).

4.3.9.3 Total flavonoid content

Total flavonoid content of the quinoa flours differed significantly (P<0.05) as shown in Table 4.12. V2 (94.67 mg CE/100g) showed higher total flavonoid content than V1 (80.44 mg CE/100g). The difference exists due to genotypic variation in the two varieties. Flavonoids are known to have antioxidant activity and also show synergistic effect with other antioxidant compounds. Recently attention has been focused on these compounds because of their health benefits. Flavonoid content observed in present study was much higher than that reported by Park et al. (2017) for quinoa seeds cultivated in Korea. However, they reported flavonoid values as quercetin equivalent. Flavonoid content values reported in present study were comparable to that reported for sweet quinoa seeds (8.1 CE/10 g) but lower than that reported for bitter quinoa seeds (13.9 mg CE/10 g) (Dini et al., 2010). Flavonoid content of
the sample can vary according to environmental conditions and genetic background (Liu et al., 2016).

Table 4.12 Anti-oxidant activity (RSA), total phenolics, total flavonoid and ascorbic acid content of C.quinoa flour

<table>
<thead>
<tr>
<th>Parameters</th>
<th>V1</th>
<th>V2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant activity (%)</td>
<td>17.46±0.43b</td>
<td>19.24±0.72a</td>
</tr>
<tr>
<td>Total phenolic content (mg GAE/100g)</td>
<td>196.59±1.36b</td>
<td>218.37±1.56a</td>
</tr>
<tr>
<td>Total flavonoid content (mg CE/100 g)</td>
<td>80.44±2.04b</td>
<td>94.67±1.33a</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100 g)</td>
<td>4.34±0.71a</td>
<td>3.43±0.62a</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ±standard deviation. Values in a same row which are followed by the different superscripts are significantly different (p≤0.05)

4.3.9.4 Ascorbic acid

Ascorbic acid can act as radical scavenger, acceptor/donor in electron transport system and hence has antioxidant properties. Total ascorbic acid content of the quinoa flour samples is shown in Table 4.12. No significant (P>0.05) differences were observed among the two quinoa varieties in the total ascorbic acid content with V2 (3.34 mg/100 g) showing lower value than V1 (4.43 mg/100 g). Slight variations in the ascorbic acid content between the two varieties can be due to their genetic makeup. The ascorbic acid content values reported in present study were lower than that reported by Dini et al. (2010) for sweet (1.3 mg/10 g) and bitter (1.2 mg/10 g) quinoa seeds from Ecuador and Peru. These differences in ascorbic acid content of quinoa from the literature may be attributed to difference among crop varieties. different environmental conditions and method used for determination of ascorbic acid. Ihara et al. (2004) in a clinical study found that the recommended daily allowance value of ascorbic acid content ranged from 66 to 79 mg/day with an optimal daily dosage of 75 mg/day.
4.3.10 Effect of treatments on antinutritional constituents of quinoa

Tannins were either found to be present in very low (safe) levels or in some case have not been detected in quinoa. While as, protease inhibitors were not detected in quinoa (Ruales and Nair, 1993b; Pachauri et al., 2017). Further, tannins have been considered as a double edged sword because of being both beneficial (chemopreventive against mutagenesis and carcinogenesis) and undesirable (Chung et al., 1998). Hence, in this study phytates and saponins were studied as antinutritional components.

4.3.10.1 Saponin content

The results for saponin content of quinoa varieties are shown in Table 4.13. Significant (P<0.05) differences existed between the two varieties on the basis of saponin content. Variety V2 (3.22 g/100g) showed higher saponin content than V1 (2.95 g/100g). Saponins are the major objection in consumption of quinoa. These are water soluble glycosides bitter in taste and cause the disruption of red blood cells membranes. Saponin content found in present study is higher than that reported for quinoa seeds (0.21 %) from Argentina (Gonzalez et al., 1989). However, the results from present study are within the range (0.4-5.6 %) reported by Koziol (1992) for different quinoa varieties. These differences in the saponin content exist due to the growing conditions of the crop and also due to the method used for estimation. In present study gravimetric method was used for estimation of saponins. Soaking (3 and 6 h) reduced the saponin content from 2.95 to 1.61 % for V1 and from 3.22 to 1.94 % for V2. Whereas, soaking along with rubbing reduced the saponin content to 0.31 % for V1 and 0.52 % for V2. Loss of saponins during soaking may be due to leaching. Saponins being water soluble result in foaming during washing along with rubbing and hence effective removal. Quispe-fuentes et al. (2013) also observed the leaching behavior of saponin while studying the kinetic approach to saponin extraction during washing of
quinoa. Oral toxicity of saponins in case of warm-blooded animals has been found to be relatively low with lethal dose in the range of 1.9-6000 mg/Kg (Ruales and Nair, 1993b).

4.3.10.2 Phytate content

The results for phytate content of quinoa varieties are shown in Table 4.13. Significant (P<0.05) differences existed between the phytate content of two varieties with V2 (652 mg /100g) showing higher phytate content than V1 (510 mg /100g). Phytic acid at higher concentrations has been found to lower the bioavailability of nutritional components and has also been involved in hard-to cook phenomenon in case of legumes. However, in lower levels phytates can show positive effects like radical scavenging (antioxidant activity) (Sreerama et al., 2012). Ruales and Nair (1993b) found the phytic acid content of about 1.04 g/100 g in raw quinoa seeds which is much higher than the values reported in present study. Phytic acid content of both the quinoa varieties observed in present study was much lower than that observed by Sreerama et al. (2012) for chick pea, horse gram and cowpea. Soaking (3 and 6 h) alone showed a reduction from 510 mg/100g to 489 mg/100 g for V1 and 652 mg/100g to 617 mg/100g for V2. Whereas, soaking (6 h) along with rubbing reduced the phytate content to 417mg/100g for V1 and 544 mg/100g for V2. Reduction in phytic acid content by soaking was also observed by Ologhobo and Fetuga (1984) for some nigerian legumes and Alonso et al. (2000) for faba and kidney beans. Reduction by soaking has been attributed to the leaching of phytate ions in soaking water due to difference in chemical potential (concentration gradient) (Bishnoi et al., 1994; Alonso et al., 2000). Valencia et al. (1999) also reported the reduction in phytate content (61 to 76 %) due to soaking of quinoa.

Soaking along with rubbing in comparison to soaking alone was found to be effective technique for reduction of antinutritional components and hence the process was used for reduction of antinutritional content of quinoa. For both the processes saponin content showed higher reduction than phytate content.
Table 4.13  Effect of pre-treatments on antinutritional content of *C. quinoa* flour

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatments</th>
<th>Saponin (%)</th>
<th>Phytate (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>2.95±0.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>510±0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Soaking (3 h)</td>
<td>2.30±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>496±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>Soaking (6 h)</td>
<td>1.61±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>489±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Soaking (6 h) + rubbing</td>
<td>0.31±0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>417±0.94&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Native</td>
<td>3.22±0.15&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>652±0.41&lt;sup&gt;aA&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Soaking (3 h)</td>
<td>2.71±0.12&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>641±0.17&lt;sup&gt;bA&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>V2</td>
<td>Soaking (6 h)</td>
<td>1.94±0.10&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>617±0.55&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Soaking (6 h) + rubbing</td>
<td>0.52±0.08&lt;sup&gt;dA&lt;/sup&gt;</td>
<td>544±0.65&lt;sup&gt;dA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ±standard deviation. Values in a same column which are followed by the different superscripts are significantly different (p≤0.05). Lower case letters within the column shows the effect of treatments within the variety and the upper case letters within the column show the varietal effect only.

4.4 Extraction and characterization of starch

There is a growing demand of starch, as the new food processing industries are increasingly dependent on both native and modified starches for the manufacture of various fabricated foods. This demand has created interest in finding the new sources of this polysaccharide. Starch is a renewable raw material and can be used as energy source after conversion to ethanol (Pascoal et al., 2013). Most of the native starches have limited number of uses, which demands the exploration of non-conventional starch sources with better end use. Starch has been widely used for improving moisture retention and maintenance of the quality of stored food products (Abegunde et al., 2013). Selection of an appropriate extraction method is one of the critical steps in the starch extraction process. Appropriate starch extraction method is followed with a target to obtain pure product with maximum recovery and lowest cost. Process was standardized for isolation of starch from *Chenopodium quinoa*
varieties in order to obtain maximum starch yield with better aesthetic appeal and minimum impurities. Starch extracted by standard process from both the varieties was then compared for various physico-chemical, functional, thermal, structural and rheological properties.

4.4.1 Process standardization for isolation of starch from C. quinoa

For standardization of starch isolation process alkali concentration was varied with seed being used in two forms whole and ground form. For alkaline steeping, samples were treated with varying alkali concentration (0.20, 0.25, and 0.30%). The performance of extraction methods was evaluated on the basis of yield, residual protein fraction and color (L* value) of starch. Effect of process conditions on the yield, color (L-value) and protein content of quinoa starch is shown in Table 4.14 and 4.15. Increasing the alkali concentration up to 0.25% increased the starch yield for seeds and flour of both the varieties. For alkali steeping flour of V1 and V2 showed the higher starch yield of 48.52% and 41.28% in comparison to the yield of 43.77% and 39.05% respectively obtained from quinoa seeds under same conditions. However, further increase in alkali concentration (0.30%) decreased the yield and color value with increase in residual protein of starch. Yield of starch obtained from current process was almost comparable to the starch yield results (45.3-53.3%) obtained for various pseudocereals (Wright et al., 2002; Jan et al., 2016a) for lab scale extraction of quinoa starch. The decrease in starch yield at alkali concentration greater than 0.25% (w/v) might be due to swelling or gelatinization of the starch granule which resulted in the increased viscosity of slurry. Increase in alkali concentration to 0.30% increased the residual protein content which might be due to salting out effect (Nnadozie et al., 2015). Higher alkali concentration also resulted in the formation of mucilaginous starch layer on the surface after centrifugation posing the difficulty in starch separation thereby leading to reduced starch yield. The lower residual protein for flour in comparison to seed may be due to effective contact between the protein and alkali due to prior grinding which favored the maximum
release of protein into alkaline solution. Therefore, on the basis of high color (L-value), higher yield and lower residual protein, steeping of flour rather than seed in 0.25% alkali concentration was selected as the standard process for isolation of starch.

Table 4.14 Yield, color and protein fraction of quinoa starch (V1) as obtained from different extraction conditions

<table>
<thead>
<tr>
<th>Alkali (NaOH)</th>
<th>Sample</th>
<th>Starch Yield (%)</th>
<th>Protein Fraction (%)</th>
<th>Color (L*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20 (%)</td>
<td>Seeds</td>
<td>37.78±0.28f</td>
<td>1.10±0.02abc</td>
<td>94.41±0.20d</td>
</tr>
<tr>
<td></td>
<td>Flour</td>
<td>39.15±0.35e</td>
<td>1.07±0.01bd</td>
<td>95.01±0.14c</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>43.77±0.37b</td>
<td>1.05±0.01be</td>
<td>96.13±0.16b</td>
</tr>
<tr>
<td>0.25 (%)</td>
<td>Flour</td>
<td>48.52±0.48a</td>
<td>0.95±0.09f</td>
<td>97.27±0.15a</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>40.89±0.31d</td>
<td>1.14±0.03a</td>
<td>93.84±0.17e</td>
</tr>
<tr>
<td>0.30 (%)</td>
<td>Flour</td>
<td>43.53±0.38c</td>
<td>1.06±0.05cde</td>
<td>95.08±0.10f</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Values in a same column which are followed by the different superscripts are significantly different (p≤0.05)

Table 4.15 Yield, color and protein fraction of quinoa starch (V2) as obtained from different extraction conditions

<table>
<thead>
<tr>
<th>Alkali (NaOH)</th>
<th>Sample</th>
<th>Starch Yield (%)</th>
<th>Protein Fraction (%)</th>
<th>Color (L*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20 (%)</td>
<td>Seeds</td>
<td>34.12±0.62f</td>
<td>1.08±0.02ab</td>
<td>94.20±0.22d</td>
</tr>
<tr>
<td></td>
<td>Flour</td>
<td>36.01±0.54e</td>
<td>1.04±0.01b</td>
<td>94.68±0.20e</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>39.05±0.64c</td>
<td>1.02±0.03b</td>
<td>95.82±0.26b</td>
</tr>
<tr>
<td>0.25 (%)</td>
<td>Flour</td>
<td>41.28±0.31a</td>
<td>0.89±0.09c</td>
<td>96.80±0.12a</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>37.51±0.61d</td>
<td>1.12±0.02a</td>
<td>94.08±0.33c</td>
</tr>
<tr>
<td>0.30 (%)</td>
<td>Flour</td>
<td>40.18±0.70b</td>
<td>1.03±0.03b</td>
<td>95.15±0.23c</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± standard deviation. Values in a same column which are followed by the different superscripts are significantly different (p≤0.05)
4.4.2 Characterization of *C. quinoa* starch

4.4.2.1 Physicochemical properties

4.4.2.1.1 Proximate composition and purity

Proximate composition (except fat) of isolated starches did not show any significant difference (Table 4.16) which may be due to extraction, drying and storage conditions being same for both. However, a significant difference was observed in the fat content of the starches with V1 showing higher values than V2, which may be due to the genetic makeup of the seeds or due to high lipid content of V1. Lower value of protein for both starches shows the effectiveness of extraction process for removal of residual protein. Low lipid, fibre and protein content of starch extracted by alkaline steeping has been reported by other researchers as well (Belhadi et al., 2013). The purity level of starch in present study was higher than that reported for other quinoa starches (Steffolani et al., 2013). The purity level of starch was higher for V2 than V1.

4.4.2.1.2 Color

The color parameters (L*, a*, b*, hue, and chroma) of quinoa starches are shown in Table 4.16. The L*, a*, b* values of the starches showed significant differences. Isolated starches were pure white in color because of higher hue angle and L* values being greater than 90, with V1 being slightly much whiter than V2 (Boudries et al., 2009). The slight difference in color parameters can be due to the presence of pigments like carotene and phenolic compounds present in quinoa seed (Mir et al., 2016). Starches from present study are suitable from consumer point of view because of low chroma and high lightness values.

4.4.2.1.3 Amylose and amylopectin content

Table 4.16 shows the amylose content of quinoa starch varieties. Amylose content varied significantly from 9.46 % for V2 and 12.10 % for V1 and is consistent with the values of 0.3-12.1 % reported in literature for quinoa (Lindeboom et al., 2005; Tang et al., 2002).
Similar amylose content range (8.22-9.30 %) was observed by Steffolani et al. (2013) for Q-J.Grano and other quinoa starches. Amylopectin content was higher for V2 (90.54 %) than V1 (87.9 %). The ratio of Amylose/amylopectin for V1 and V2 was 1:7.26 and 1:9.5 respectively. The results were also in agreement with the amylose content range (1.87-19.11 %) for different pseudocereals like amaranth and Chenopodium album (Jan et al., 2016a). Variations in amylose contents among different starches can be because of different botanical sources (Srichuwong et al., 2005), varying harvesting periods (noda et al., 2003) and climatic conditions (Asaoka et al., 1985). Studied starches contained low amylose content and hence could form gels with a lower retrogradation tendency (BeMiller, 1993).

4.4.2.1.4 Water binding capacity (WBC) and Oil binding capacity (OBC)

Water binding capacity of the Chenopodium quinoa starches varied significantly and was higher for V1 (92.15%) and lower for V2 (88.27%) as shown in Table 4.16. These values were within the range of values reported by Lindeboom et al. (2005) for some other quinoa varieties. Water binding capacity has been reported to be affected by loose association of amylose and amylopectin molecules, with low water binding capacity attributing to the close association of starch polymers (Soni et al., 1987; Lorenz et al., 1990). Oil binding capacity of the starches also showed the significant difference (p<0.05) with V1 (159%) showing higher oil binding capacity than V2 (156.28%). As the starch usually is devoid of proteins its oil binding capacity has been found mainly due to physical entrapment of oil within the starch structure due to capillary attraction (Kinsella 1976; Wani et al., 2015). However, some studies have also attributed this higher oil absorption capacity to the non-polar protein residues (Majeed et al., 2017).
Table 4.1 Physico-chemical properties of native starches from Indian quinoa varieties

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
</tr>
<tr>
<td>Starch yield (g 100g(^{-1}))</td>
<td>48.52±0.48(^a)</td>
</tr>
<tr>
<td>Purity (%)</td>
<td>98.30±0.10(^a)</td>
</tr>
<tr>
<td>Moisture</td>
<td>8.49±0.29(^a)</td>
</tr>
<tr>
<td>Fat</td>
<td>0.40±0.05(^a)</td>
</tr>
<tr>
<td>Ash</td>
<td>0.22±0.02(^a)</td>
</tr>
<tr>
<td>Protein</td>
<td>0.95±0.09(^a)</td>
</tr>
<tr>
<td>Fibre</td>
<td>0.13±0.03(^a)</td>
</tr>
<tr>
<td>Amylose (%)</td>
<td>12.10±0.13(^a)</td>
</tr>
<tr>
<td>Amylopectin (%)</td>
<td>87.9±0.13(^b)</td>
</tr>
<tr>
<td>Amylose/amylopectin ratio 1:2</td>
<td>1:7.3±0.13(^b)</td>
</tr>
<tr>
<td>L-value</td>
<td>97.27±0.15(^a)</td>
</tr>
<tr>
<td>Hue angle</td>
<td>126.36±0.15(^a)</td>
</tr>
<tr>
<td>Chroma</td>
<td>6.12±0.15(^a)</td>
</tr>
<tr>
<td>Water binding capacity (%)</td>
<td>92.15±0.65(^a)</td>
</tr>
<tr>
<td>Oil binding capacity (%)</td>
<td>159.00±0.79(^a)</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SD. Means in a row with different superscripts are significantly different (p < 0.05).
### 4.4.2.1.5 Swelling power & solubility

Swelling power (SP) and solubility of native quinoa starches at different temperatures (55-95 °C) are shown in Figure 4.7 (a) and (b).

**Figure 4.7 Effect of temperature on a) Swelling power and b) solubility of *C.quinoa* starches**
Both the starches showed increased SP and solubility with increase in temperature. The maximum SP observed for V1 and V2 varieties was 12.53 g/g and 13.89 g/g respectively and can be considered as highly restricted swelling behavior as in both cases swelling power was below 16 g/g. V2 starch had the higher swelling power and solubility in comparison to V1 starch. High SP and low water binding capacity of V2 can be linked to its low amylose content as amylose reinforces internal network within granules thus restricting swelling. These types of starches are stable against shearing action during cooking in water (Galvez and Resurreccion, 1992). The solubility and swelling power gives the evidence of magnitude of interaction between starch chains within amorphous and crystalline domains. Solubility of starches differs due to different chain length distributions (Liu et al., 1999; Bello-Perez et al., 2000). Restricted swelling behavior shown by both starches is desired for manufacture of products like noodles. The restricted swelling power and solubility of starches can be due to high linear content and natural cross-linkages of native starches (Schoch and Maywald, 1968).

4.4.2.1.6 Turbidity

The turbidity values of quinoa starch gels are shown in Figure 4.8. Turbidity values of both starch gels increased progressively with increasing storage time at 4 °C which may be due to leaching of amylose and amylopectin chains from functional zones, which scattered a significant amount of light (Perera and Hoover, 1999). V2 starch showed lower turbidity values (1.382-1.441) than V1 (1.428-1.474). The lower turbidity values of V2 starch may be due to its lower amylose content. Turbidity development in starch pastes during storage may be affected by factors like amylose and amylopectin chain lengths, leached amylose and amylopectin granule swelling and granule remnants (Jacobson et al., 1997). Lower turbidity of V2 can also be due to its smaller particle size in comparison to V1.
4.4.2.2 Pasting properties

Pasting properties of quinoa starches are summarized in Table 4.17. There was a significant difference in pasting properties of quinoa starches. The Peak viscosity (PV), Breakdown viscosity (BD), Setback viscosity (SB) and Final viscosity (FV) were higher for V1 starch compared to V2 starch. PV gives the maximum viscosity attained by starch granules during heating and also indicates the water binding capacity of starch (Gupta et al., 2009). High PV (4637 Cp) of V1 starch correlates well with its higher water binding capacity. Higher blue value (amylose content) has been associated with higher peak viscosity (Noda et al., 2003; Osundahunsi et al., 2003). This high viscosity and lower breakdown of both the starches is desirable because of the non-cohesive nature of their paste being suitable for many industrial and food applications. The pasting temperature (PT) which is the minimum temperature required to cook the starch was lower for V1 (69.45 °C) starch than V2 (72.85 °C).
°C) starch. Higher PT of V2 starch regardless of its lower amylose than V1 showed that PT can be probably affected by factors like degree of branching of amylopectin and higher degree of crystallinity (Kim et al., 1996; Wang et al., 2011). The PV, BD, FV and SB of the present starches were almost similar to that observed for quinoa. But the PT (62.7 °C) observed was lower than the values (69.45-72.85 °C) observed in present study (Steffolani et al., 2013).

Table 4.17 Pasting properties of *C. quinoa* starches

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Varieties</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
<td></td>
</tr>
<tr>
<td>PT (°C)</td>
<td>69.45±1.83b</td>
<td>72.85±1.11a</td>
<td></td>
</tr>
<tr>
<td>Peak viscosity (cP)</td>
<td>4637±11.92a</td>
<td>2488±29.82b</td>
<td></td>
</tr>
<tr>
<td>Trough viscosity (cP)</td>
<td>3694±6.91a</td>
<td>1790±30.51b</td>
<td></td>
</tr>
<tr>
<td>Breakdown viscosity (cP)</td>
<td>943±33.40a</td>
<td>698±22.68b</td>
<td></td>
</tr>
<tr>
<td>Final viscosity (cP)</td>
<td>4869±37.52a</td>
<td>2684±34.60b</td>
<td></td>
</tr>
<tr>
<td>Setback viscosity (cP)</td>
<td>1175±33.59a</td>
<td>894±21.47b</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SD. Means in a row with different superscripts are significantly different (*p* < 0.05). PT = Pasting temperature; Breakdown = (Peak- Trough); and Setback = (Final- Trough)

4.4.2.3 Thermal properties

The starch gelatinization transition temperatures (To, Tp and Tc) and associated enthalpies of native quinoa starches are shown in Table 4.18. V2 starch showed slightly higher To, Tp, Tc (66.61°, 71.56° and 76.98°C) than V1 (64.32°, 69.36° and 75.78°C). Higher gelatinization temperature (GT) of V2 can be an indication of higher crystallite size or stability of starch crystallites in starch of particular variety which makes granule more resistant towards gelatinization. This high GT of V2 is also justified by its lower breakdown viscosity and higher PT. The results are in agreement with the findings of Boudries et al.
(2009) for sorghum starch, where higher GT (To, Tp and Tc) values were recorded for starch with higher crystallinity. Starches from various botanical sources vary in composition and reveal different transition temperatures and gelatinization enthalpies (Singh et al., 2003). Gelatinization temperature range (R) was higher for V1 (10.08) than V2 (9.9). Extended temperature range reflects a wide range of crystals stability (Fredriksson et al., 1998).

Table 4.18 Thermal properties of *C. quinoa* starches

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Varieties</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
</tr>
<tr>
<td>T&lt;sub&gt;o&lt;/sub&gt; (°C)</td>
<td>64.32±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;p&lt;/sub&gt; (°C)</td>
<td>69.36±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;c&lt;/sub&gt; (°C)</td>
<td>75.78±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔH&lt;sub&gt;gel&lt;/sub&gt; (J/g)</td>
<td>5.10±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>R (°C)</td>
<td>10.08±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SD. Means in a row with different superscripts are significantly different (*p* < 0.05).

4.4.2.4 Morphological properties

The scanning electron micrograph of quinoa starches is shown in Figure 4.9. Micrographs showed that the starch granules from both quinoa varieties were irregular, angular and polygonal in shape with almost smooth surfaces and no obvious signs of damage or fissures due to isolation conditions. According to the micrograph average granule size was 1.23 µm and 1.19 µm for V1 and V2 respectively. Quinoa starches from different varieties have shown irregular, polygonal shapes (Lindeboom, et al., 2005; Steffolani et al., 2013). Being small in size in comparison to corn starch, quinoa starches can act as carriers for colorants and flavors (Zhao and Whistler, 1994).
4.4.2.5 X-ray diffraction pattern and relative crystallinity

The X-ray diffractograms of quinoa starches are presented in Figure 4.10. Quinoa starches from varieties V1 and V2 exhibited a typical ‘A’ type pattern, characterized by strong intensity peaks at around 2θ of 15.41, 17.64, 18.20 and 23.31° for V2 and 15.23, 17.19, 18.17, 23.32° for V1. The XRD intensities of both starch varieties were almost identical. The degree of crystallinity ranged from 21.46 % for V1 and 22.19 % for V2. Crystallinity is affected by short and long-side chain amylopectin and amyllose content. In general lower the amyllose content, higher will be the degree of crystallinity of starch as observed by Cheetham et al. (1998) in case of maize starch. Crystallinity values were lower than that observed for horse chestnut and some pseudocereal starches like Chenopodium album and Chenopodium quinoa (Rafiq et al., 2015; Jan et al., 2016a; Steffolani et al., 2013).
4.4.2.6 Particle size distribution

The Particle size of the starches as determined by Laser diffraction analyzer is shown in Figure 4.11. Both starches showed unimodal granule size distribution profile. The average particle size of V1 (1.30 µm) was larger than V2 (1.15 µm). The granule diameter varied from 0.766 to 4.050 µm for V1 and from 0.668 to 2.784 µm for V2. The variation in starch granule morphology may be due to variations in amylose, amylopectin and its structure all these play an important role in controlling the size and shape of starch granules (Kaur et al., 2007). Due to small granule size quinoa starch can be used as an economic and safe fat replacer in comparison to corn starch which requires acid catalyzed hydrolysis and high pressure shearing for reduction of the granule size to act as fat replacer (Jane et al., 1992). Starch granule size has been found to significantly affect the processing ability and quality of end product (edible films, noodles etc) (Chen et al., 2003).
Figure 4.11 Particle size distribution pattern of starches (a) *C. quinoa* V1 (b) *C. quinoa* V2

4.4.2.7 Fourier transform infrared (FTIR) spectroscopy

The major functional groups present in extracted quinoa starch were identified using
FTIR spectra as shown in Figure 4.12. The dominant functional groups present in carbohydrates are hydroxyl groups responsible for the intra and inter molecular bonding with other hydroxyl groups (Joshi et al., 2013).

Figure 4.12 FTIR Spectra of *C. quinoa* starches

The starch is mainly characterized by the strong absorption bands at 3500-3200 cm\(^{-1}\) (due to O-H stretching) and at 1200-1000 cm\(^{-1}\) (due to C-O-C stretching). The broader bands were observed for both the starches at 3300-3500 cm\(^{-1}\) attributed to the O-H stretching. Small peaks at 2928 cm\(^{-1}\) and 2924 cm\(^{-1}\) for V1 and V2 respectively were attributed to the C-H stretches. The bands at 1456 cm\(^{-1}\) and 1416 cm\(^{-1}\) for V1 and 1454 cm\(^{-1}\) and 1418 cm\(^{-1}\) for V2 were believed to be due to angular deformation of C-H. The bands in the region 800 to 400 cm\(^{-1}\) are due to skeletal mode of pyranose ring (Ogunmolasuyi et al., 2016). FTIR spectra of both the starches were almost similar. However, the comparison showed that the intensity and
shape of the -OH absorption bands was higher for V2 than V1 which depicts the higher crystallinity of the V2. The reason for this change has been attributed to the presence of higher amyllopectin that shifts the band to higher wave numbers (Kizil et al., 2002).

4.5 Development of product using C. quinoa flour

Bakery products are widely consumed and have been considered as an important part of balanced diet for many years with variety of products being available on supermarket shelves (Smith et al., 2004). Cookies represent the largest category of snacks in bakery industry and can serve as effective vehicle of nutrient supply to consumer. Development of cookies can be a better choice than any other product due to their relatively longer shelf life, wide consumption, ready to eat form and better palatability (Tsen et al., 1973). Rising demand for gluten free products has been created due to sharp increase in celiac disease and other intolerances to gluten (Gallagher et al., 2004). Quinoa can be a better choice due to its protein quality, higher dietary fibre, unsaturated fat and gluten-free nature. Some studies (Wang et al., 2015; Harra et al., 2011; Wang and Zhu 2016) have evaluated the potential of quinoa for development of cookies. However, in these cases quinoa was used in combination with wheat flour or some other flour which decreases the nutritional value of quinoa and also in some cases invalidates the concept of the prepared cookies being gluten-free. Available gluten-free cookies are of low quality with poor flavour and mouthfeel (Gallagher et al., 2004; Pestorić et al., 2017). Also optimization of formulations and processing parameters of cookies from quinoa has not been explored. Cookies prepared by optimized process were then evaluated for various physical, functional, textural and sensory characteristics as given below:

4.5.1 Estimation of responses

The experimental variables in actual form along with values of responses are given in Table 4.19.
Table 4.19: The central composite rotatable design with process variables and experimental results of responses.

<table>
<thead>
<tr>
<th>Runs</th>
<th>Variables</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
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<tr>
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</tr>
</tbody>
</table>

Where $Y_1$ = color (L-value); $Y_2$ = spread factor; $Y_3$ = hardness (N); $Y_4$ = antioxidant activity (% DPPH inhibition); $Y_5$ = overall acceptability.
ANOVA data for response variables along with correlation coefficient is shown in Table 4.20. Lack-of-fit, model analysis and $R^2$ were used to determine the adequacy of models. The lack-of-fit measures the ability of a model to represent the data in experimental domain and cannot be accounted for random error. If lack-of-fit is insignificant then model is considered as adequate in describing the response. The aptness of the model to signify real relationship among selected parameters is given by $R^2$. The $R^2$ values of models for this study were $> 0.70$ representing a good fit between the model and experimental data. The difference between the experimental and predicted values was less indicating the suitability of the model used.

Table 4.20 Estimated regression coefficients of the fitted second order polynomial and their significance

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DF</th>
<th>$Y_1$</th>
<th>$Y_2$</th>
<th>$Y_3$</th>
<th>$Y_4$</th>
<th>$Y_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>7.22</td>
<td>46.53</td>
<td>20.32</td>
<td>7.76</td>
</tr>
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<td>0.151</td>
<td>-2.96</td>
<td>0.014</td>
<td>0.125</td>
</tr>
<tr>
<td>$X_2$</td>
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<td>0.065</td>
<td>0.124</td>
<td>0.185</td>
<td>0.163</td>
</tr>
<tr>
<td>$X_3$</td>
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<td>-0.142</td>
<td>5.57</td>
<td>0.63</td>
<td>-0.098</td>
</tr>
<tr>
<td>$X_4$</td>
<td>1</td>
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<td>-0.050</td>
<td>2.45</td>
<td>0.233</td>
<td>0.050</td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>1</td>
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<td>-0.073</td>
<td>-1.34</td>
<td>-0.136</td>
<td>-0.198</td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>1</td>
<td>-0.554</td>
<td>-0.023</td>
<td>-0.223</td>
<td>-0.064</td>
<td>-0.170</td>
</tr>
<tr>
<td>$X_3^2$</td>
<td>1</td>
<td>-1.10</td>
<td>-0.253</td>
<td>1.34</td>
<td>-0.206</td>
<td>-0.521</td>
</tr>
<tr>
<td>$X_4^2$</td>
<td>1</td>
<td>-0.198</td>
<td>-0.142</td>
<td>-1.17</td>
<td>0.018</td>
<td>-0.317</td>
</tr>
<tr>
<td>$X_1 X_2$</td>
<td>1</td>
<td>-0.008</td>
<td>0.021</td>
<td>0.009</td>
<td>-0.003</td>
<td>0.042</td>
</tr>
<tr>
<td>$X_1 X_3$</td>
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<td>-0.004</td>
<td>-0.014</td>
<td>-0.441</td>
<td>-0.001</td>
<td>-0.014</td>
</tr>
<tr>
<td>$X_1 X_4$</td>
<td>1</td>
<td>0.001</td>
<td>0.003</td>
<td>-0.566</td>
<td>-0.004</td>
<td>-0.012</td>
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<tr>
<td>$X_2 X_3$</td>
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<td>-0.059</td>
<td>-0.020</td>
<td>-0.003</td>
<td>-0.068</td>
<td>-0.023</td>
</tr>
<tr>
<td>$X_2 X_4$</td>
<td>1</td>
<td>0.226</td>
<td>-0.011</td>
<td>-0.015</td>
<td>-0.068</td>
<td>-0.018</td>
</tr>
<tr>
<td>$X_3 X_4$</td>
<td>1</td>
<td>0.351</td>
<td>0.004</td>
<td>1.37</td>
<td>-0.076</td>
<td>-0.142</td>
</tr>
</tbody>
</table>

$R^2$ = 0.991 0.997 0.994 0.987 0.993

| Lack of fit | 10  | NS     | NS     | NS     | NS     | NS     |

*Significant at p ≤0.05. **Significant at p ≤0.01. ***Significant at p ≤0.001. NS- Not-significant; DF- Degree of freedom

$Y_1$ = color (L-value); $Y_2$ = spread factor; $Y_3$ = hardness (N); $Y_4$ = antioxidant activity (% DPPH inhibition); $Y_5$ = overall acceptability.
Effect of optimization on the responses and quality of product is given below as follows;

4.5.1.1 Color

Surface color has been considered as an important indicator of the degree of baking of cookies and contributes to consumer preference hence needs to be controlled strictly. The lightness value of the cookies ranged from 46.08 to 56.90 (Table 4.19). The p value (Table 4.20) indicates that linear (except fat) and quadratic effect of all the variables was significant. In contrast among interactions only “sugar and time” and “temperature and time” showed a significant effect on color of cookies. The magnitude of regression coefficients (Table 4.20) showed that the linear term of temperature had a maximum negative effect (B= -1.81) followed by time (B= -1.15) and sugar content (B= -1.04). Figure 4.13 (a and b) shows the decrease in color value with increase in baking temperature, time and sugar content. It can be observed that the major effect was of baking temperature as with rise in temperature lightness decreases remarkably. The decrease in lightness value with increase in temperature, time and sugar content may be due to caramelization of sugar and Maillard browning reactions causing malanoidin formation during heating and thus, resulting in the darkening of product (Manzocco et al., 2000). The results are in conformity with the Farris and Piergiovanni (2008) who reported the decrease in color (L-value) of cookies with increase in temperature and sugar content. Gan et al. (2007) also reported the similar decreasing trend of colour value for cake.

The model F-value of 126 implies that the model is significant. The pred R-Squared of 0.963 is in reasonable agreement with the Adj R-Squared of 0.983.
Figure 4.13 Response plots showing the effect of process parameters on color (L-value) of cookies
4.5.1.2 Spread factor

Spread factor has been considered as an important quality parameter for cookies. Higher spread factor means the higher product yield. The spread factor of cookies varied from 5.89 to 7.26. The effect of process variables on the spread factor of cookies is shown in Table 4.19.

The magnitude of p value indicates that linear and quadratic effect of all variables was significant. However, among interactions “fat and sugar”, “fat and temperature” and “sugar and temperature” showed a significant effect on spread factor of cookies. The analysis of regression coefficients (Table 4.20) showed that the linear term of temperature and time had a negative effect with temperature showing higher effect (B = -0.14) than time (B = -0.050). While as fat and sugar had a positive effect on spread factor with magnitude being higher for fat content (B = +0.15) followed by sugar content (B = +0.065). The response plots for effect of temperature, fat content and time on spread factor are shown in Figure 4.14 (a and b). Increase in sugar content increased the spread factor of cookies. Similar increased mobility of dough and higher spread of cookies by increase in sugar content was observed by Doescher et al. (1987). Kulthe et al. (2014) also observed that the spread factor is decreased by decrease in sugar content. Spread factor increased gradually with increase in fat content. Singh et al. (2002) also observed the increase in spread factor by increase in sugar level and attributed it to the increase in fluidity of dough allowing two dimensional extensible film formation rather than three-dimensional elastic network formation. The spread factor increased from 6.66 to 7.26 with increase in temperature from 170 to 180 °C. However, further increase in temperature decreased the spread factor to 5.89. Cookie spread rate is controlled by the viscosity of dough. Sugar contributes to dough viscosity and is related to the dough expansion during baking (Abboud and Hoseney 1984).
Figure 4.14 Response plots showing the effect of process parameters on spread factor of cookies
During low temperature and time combinations sugar gets dissolved in available water content of the dough which lowers the initial viscosity of dough and the cookie spreads at a faster rate and vice-versa occurs during high temperature and time combinations due to lower availability of water content. Another reason for decrease in spread factor of cookies at higher temperature may be that the cookies set up before getting a chance to spread.

The model F-value of 378 implies that the model is significant. The predicted R-Squared of 0.986 is in reasonable agreement with the Adjusted R-Squared of 0.994.

### 4.5.1.3 Hardness

Hardness has been considered as an important characteristic of cookie quality as it affects consumer acceptance and repeat sales (Gaines et al., 1992). Hardness refers to the ease with which the product will break. Hardness of the quinoa cookies varied from 34.05 N to 58.09 N. The effect of the process variables on the hardness of quinoa cookies is shown in Table 4.19.

The magnitude of p value indicates that linear as well as quadratic terms of all variables had a significant effect on hardness of cookies (Table 4.20). Among interactions “fat and temperature”, fat and time and “time and temperature” showed significant effect on hardness of cookies. The regression coefficients revealed that linear term of fat showed negative effect on the hardness of cookies (B= -2.96). The response plots for effect of temperature, fat and time on hardness of cookies are shown in Figure 4.15 (a and b). The decrease in hardness with increase in fat content may be attributed to the tenderizing effect exerted by fat. The decreased hardness may also be due to the encapsulation of flour particles by fat, thereby isolating the flour particles from each other and making them more easily detachable. The linear terms of temperature, sugar and time showed positive effect the hardness of cookies with the higher magnitude observed for temperature (B= +5.57) than time (B= +2.45) as shown in Table 4.20.
Figure 4.15 Response plots showing the effect of process parameters on hardness of cookies
Increased hardness with increase in baking temperature and time may be due to the higher water loss from the dough which may lead to a more rigid fiber frame after baking. Further formation of fiber-protein complexes during high temperature time combinations can also promote the hardening of product (Farris and Piergiovanni 2009). Sugar content showed the non-significant positive effect of lower magnitude (B= +0.12) on hardness. Increase in hardness with increase in sugar content may be due to its conversion from solution to harder glass-like state after cooling. Singh et al. (2002) also observed the increase in hardness of cookies by sugar and attributed it to its conversion to hard glassy state.

The model F-value of 202 implies that the model is significant. The pred R-Squared of 0.975 is in reasonable agreement with the Adj R-Squared of 0.989.

4.5.1.4 Antioxidant activity (% DPPH inhibition)

Antioxidants have gained increased interest among consumers because the epidemiological studies have revealed the lower risk of cancer and cardiovascular diseases with frequent consumption of antioxidants (Temple 2000). Antioxidant activity of optimized cookies varied from 18.12 % to 20.97 % (Table 4.19). The p value indicates that linear and quadratic effect of all variables (except fat content) was significant (Table 4.20). However, in case of interactions “sugar-time”, “sugar-temperature” and “temperature-time” showed the significant effect. The analysis of regression coefficients showed that the variables sugar, temperature and time had a positive effect on antioxidant activity with magnitude being higher for temperature (B= +0.630) followed by time (B= +0.233) and sugar content (B= +0.185). The response plots for effect of temperature, sugar content and time on antioxidant activity are shown in Figure 4.16 (a and b). It can be observed that the major effect was that of temperature as with increase in temperature antioxidant activity of cookies increased remarkably.
Figure 4.16 Response plots showing the effect of process parameters on antioxidant activity of cookies
Increase in antioxidant activity with increase in sugar, temperature and time may be due to the formation of melanoidins during baking process. These compounds have been reported to show antioxidant activity (Manzocco et al., 2000). The antioxidant activity of cookies remained almost constant after 180 °C suggesting the stability of molecules bearing radical scavenging ability. Increase in antioxidant activity can also be attributed to the possible breakdown of phenolics or their degradation products which could react with the Folin ciocalteu reagent (Sun et al., 2014). Lindenmeier and Hofmann (2004) determined the influence of baking conditions on antioxidant activity of bread and the antioxidant activity was found to be higher in crust in comparison to the crumb and untreated flour. They found a 3-5 fold increase in antioxidant activity with increase in baking temperature and time and attributed it to the formation of antioxidant compound pronyl-L-lysine. The increase in antioxidant activity due to baking was also observed by Sharma and Gujral (2014).

The model F-value of 84 implies that the model is significant. The pred R-Squared of 0.937 is in reasonable agreement with the Adj R-Squared of 0.975.

4.5.1.5 Overall acceptability

Overall acceptances of cookies as the sum of various quality characteristics (surface color, texture, mouth feel and taste) can be used to assess the quality of the cookies prepared. The overall acceptance score of cookies varied from 5.54 to 7.84 (Table 4.19). The magnitude of p value indicates that linear as well as quadratic terms of all variables had a significant effect on overall acceptability of cookies (Table 4.20). Among interactions “fat and sugar” and “time and temperature” showed a significant effect on overall acceptability of cookies. The regression coefficients revealed that linear terms of fat and sugar showed positive effect on the overall acceptability of cookies with the sugar content (B= +0.16) showing effect of slightly higher magnitude than fat content (B= +0.13).
Figure 4.17 Response plots showing the effect of process parameters on overall acceptability of cookies
While as linear terms of temperature and time showed negative effect on the overall acceptability of cookies with the temperature showing effect of higher magnitude ($B = -0.10$) than time ($B = -0.05$). Figure 4.17 (a and b) shows the effect of temperature, fat, sugar content and time on overall acceptability of cookies. The cookies with higher fat and sugar content seemed to be more acceptable by the panellists. Positive effect of fat and sugar can be due to the reason that sugar acts as flavour enhancer and fat provides the better mouth feel. The negative effect of temperature on overall acceptability of cookies may be due to higher temperature resulting in decreased color ($L$-value) and increased hardness of cookies.

The model F-value of 153 implies that the model is significant. The pred R-Squared of 0.969 is in reasonable agreement with the Adj R-Squared of 0.986.

### 4.5.2 Optimization

The simultaneous optimization of the responses was done numerically using statistical software Design-expert 11. The criterion for optimization of cookies was to obtain maximum values for spread factor, antioxidant activity and overall acceptability. However, color and hardness were kept in range (Table 4.21). Numerical analysis report showed that fat content, sugar content, baking temperature and baking time of 41.83 %, 33.95 %, 181 °C and 18 min, respectively gave an optimized product of desirability 0.93. High desirability value indicated the suitability of process conditions for achieving favourable results in terms of responses. Predicted and experimentally determined values for responses were color 52.72 and 53.05 spread factor 7.26 and 7.16, hardness 46.40 and 47.05, antioxidant activity 20.54 and 20.67 ($\%$ DPPH inhibition) and overall acceptability 7.76 and 7.61, respectively. Optimized solution provided the optimum range of variables for production of best quality cookies in terms of color, spread factor, hardness, antioxidant activity and overall acceptability. The optimum conditions obtained may be recommended for preparation of quinoa cookies.
Table 4.21 Criteria and outputs of the numerical optimization of the responses for cookies

<table>
<thead>
<tr>
<th>Variables</th>
<th>Goal</th>
<th>Experimental range</th>
<th>Importance</th>
<th>Optimum values</th>
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<td>X₂</td>
<td>In range</td>
<td>25</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>X₃</td>
<td>In range</td>
<td>170</td>
<td>190</td>
<td>3</td>
</tr>
<tr>
<td>X₄</td>
<td>In range</td>
<td>15</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

Responses

<table>
<thead>
<tr>
<th>Responses</th>
<th></th>
<th>Predicted values</th>
<th>Actual Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y₁</td>
<td>In range</td>
<td>46.08</td>
<td>56.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>52.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53.05</td>
</tr>
<tr>
<td>Y₂</td>
<td>Maximum</td>
<td>5.89</td>
<td>7.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>7.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.16</td>
</tr>
<tr>
<td>Y₃</td>
<td>In range</td>
<td>34.05</td>
<td>58.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>46.40</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>47.05</td>
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<tr>
<td>Y₄</td>
<td>Maximum</td>
<td>18.12</td>
<td>20.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>20.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.67</td>
</tr>
<tr>
<td>Y₅</td>
<td>Maximum</td>
<td>5.54</td>
<td>7.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>7.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.61</td>
</tr>
</tbody>
</table>

Where Y₁ = color (L-value); Y₂ = spread factor; Y₃ = hardness (N); Y₄ = antioxidant activity (% DPPH inhibition); Y₅ = overall acceptability.

4.6 Development of product using C. quinoa starch

Noodles made from starch are popular throughout oriental countries as well in oriental dishes served in western countries. Good quality noodles should have high tensile strength and low cooking loss and can be obtained from starches having high amylose content and restricted swelling power (Kim et al., 1996). Due to the absence of gluten, physico-chemical, functional, rheological and thermal properties of the starches would decide the quality of noodles prepared from them. Demand for starch noodles is increasing worldwide and the limited output from commercial sources is unable to meet the needs. Quality of the noodles is mainly assessed on the basis of its appearance, cooking quality and texture. Different treatments like heat moisture treatment and addition of hydrocolloid into starch formulations are practiced in order to impart the improved properties to the final product. Selection of the gum however depends on its characteristics and suitability for the process (Charles et al.,
Despite the known numerous advantages of quinoa starch its potential for noodle preparation remains untapped. Further, the utilization of hydrocolloids and hydrothermal treatment for improvement of quinoa starch characteristics and noodle preparation and quality has not been exploited yet. Hence, the objective of the present study was addition of hydrocolloid and hydrothermal treatment of quinoa starch to observe their effect on starch properties and noodle quality.

4.6.1 Characteristics of native and modified C.quinoa starch

4.6.1.1 Color

Lightness values of native quinoa starch (NQS), gum modified quinoa starch (GM-QS) and heat moisture treated quinoa starch (HMT-QS) are shown in Table 4.2. Both the modification methods decreased the lightness value of starch but the decrease in lightness for gum modified starch was not significant (P>0.05). Among the studied starches HMT-QS showed lower lightness value followed by GM-QS and NQS. The lightness value of 97.27, 96.97 and 90.21 was observed for NQS, GM-QS and HMT-QS respectively. Decreased lightness value of HMT-QS may be due to separation of some heterogeneous materials like sugars, salts, proteins and pigment among others as reported by Falade and Ayetigbo (2015). Slight variation in lightness value of GM-QS may be due addition of gum which is darker in color in comparison to starch as shown by Bolanle otegbayo (2017). Lightness values reported in present study were higher than those reported for native and HMT amaranth starch by Chandla et al. (2017).

4.6.1.2 Amylose content

The amylose content of the native and modified starches is shown in Table 4.2. Both gum addition and HMT treatment increased the amylose content of starch. The difference in amylose content from HMT treatment was statistically significant (P<0.05). Order of amylose content in native and modified starches was HMT-QS (12.94%) > GM-QS (12.12%) > NQS
Increase in amylose content of starch due to HMT treatment has been attributed to the interaction of starch chains within the amorphous area of starch granule (Li et al., 2011). Gunaratne and Hoover (2002) have reported that additional interactions between amylose-amylopectin and/or amylose-amylose can be the cause of increase in amylose content. Positive impact of HMT treatment on amylose content of starch was also shown by Li et al. (2011) and Chandla et al. (2017). Addition of gum also increased the amylose content of the starch but the effect was not significant (P>0.05). Slight increase in amylose content due to gum addition may be due to minor amount of amylose present in the gum itself (Bolanle Otegbayo, 2017).

4.6.1.3 Water and oil binding capacity (WBC and OBC)

The water and oil binding capacity of Native and modified starches is shown in Table 4.2. Highest water and oil binding capacity was observed in starch modified by HMT. The water and oil absorption capacities of the samples were 92.15 and 159.00 % for native, 115.49 and 163.69 % for HMT and 108.29 and 157.18 % for GM-QS respectively. Water absorption capacity measures the strength of association between amylose and amylopectin chains with higher absorption being associated with loose association of molecules (Lutfi et al., 2017). Increase in water and oil absorption capacity after HMT treatment may be attributed to the fact that HMT increases both hydrophilic and lipophilic nature of starch. Similar pattern of increase in water and oil absorption capacity by HMT was reported by Singh et al. (2009) for water chestnut starch and Chandla et al. (2017) for amaranth starch. Addition of gum increased the water absorption capacity but the oil absorption capacity decreased. The decrease in oil absorption capacity on addition of xanthan gum can be attributed to its higher hydrophilic nature. Decreased oil absorption capacity can be justified by the fact that gums are used to reduce oil uptake in various snacks. Sakhale et al. (2011) also observed decrease in oil uptake of snacks due to addition of xanthan gum. Water
absorption capacity of starch depends on the structural properties of the type of biopolymer added. Xanthan gum being high in hydroxyl groups facilitates water molecules for more feasible interaction through hydrogen binding eventually leading to higher water absorption (Lutfi et al., 2017).

Table 4.22 Physico-chemical properties of native, heat moisture treated and gum modified starches

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Native</th>
<th>HMT-QS</th>
<th>GM-QS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color (L- value)</td>
<td>97.27±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.21±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.97±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amylose content (%)</td>
<td>12.10±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.94±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.12±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water absorption capacity (%)</td>
<td>92.15±0.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>115.49±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108.29±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oil absorption capacity (%)</td>
<td>159.00±0.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>163.69±0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157.18±0.64&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Where HMT-QS is heat moisture treated quinoa starch, GM-QS is gum modified quinoa starch. Results are expressed as mean values ± SD. Means in a row with different superscripts are significantly different (p < 0.05).

4.6.1.4 Morphological properties

Scanning electron micrographs of native and modified starches are shown in Figure 4.18. Native quinoa starch granules were polyhedral in shape with smooth surface. HMT treatment resulted in the surface degradation and aggregation of starch granules which can be possibly due to high moisture, partial gelatinization and rearrangement of molecular structure of starch. Rafiq et al. (2016) reported the similar results of loss of smoothness (Surface degradation) and aggregation of starch granules for horse chestnut starch and attributed it to the partial gelatinization of starch. Sharma et al. (2015) also reported the formation of dents or holes on surface of starch granules due to HMT. Scanning electron micrograph of gum added starch revealed the presence of some spherical and rod shaped particles of irregular sizes which are the characteristic of gum (Parvathy et al., 2007).
4.6.1.5 Pasting properties

Pasting profile of native and modified starches is shown in Table 4.23. Native quinoa starch itself also exhibited quite stable viscosity. However, the modifications improved the viscosity profile. HMT modification showed significant (p<0.05) positive effect on pasting properties (Peak, trough Final, setback viscosity and pasting temperature). Higher pasting temperature (73.65°C) indicates the strengthening of starch paste by HMT. This higher pasting temperature may be due to presence of more crosslink’s. Increase in paste viscosity due to heat treatment has also been reported by Jacobs et al. (1995) in case of rice, wheat and pea starches. Increase in final viscosity of HMT quinoa starch from 4869 to 5068 and setback
viscosity from 1175 to 1260 cP may be due to increased cross links between starch chains resulting in additional junction zones in starch gel. Increase in final and set back viscosity due to HMT was also observed by Adebowalea et al. (2005) for red sorghum starch and attributed it to the larger units of dissolved starch molecules as a result of cooling. Addition of gum also resulted in significant (P≤0.05) increase in paste viscosity.

Table 4.23 Pasting properties of native, gum modified and heat moisture treated starches

<table>
<thead>
<tr>
<th>Sample</th>
<th>PT (°C)</th>
<th>Peak viscosity (cP)</th>
<th>Trough viscosity (cP)</th>
<th>Final viscosity (cP)</th>
<th>Setback viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>69.45± 1.83b</td>
<td>4637±11.92c</td>
<td>3694±6.91c</td>
<td>4869±37.52c</td>
<td>1175±33.59a</td>
</tr>
<tr>
<td>HMT-QS</td>
<td>73.77± 0.74a</td>
<td>4749±16.65b</td>
<td>3808±7.09b</td>
<td>5068±12.77b</td>
<td>1260±18.82b</td>
</tr>
<tr>
<td>GM-QS</td>
<td>70.98± 0.98b</td>
<td>4878±18.08a</td>
<td>3915±7.77a</td>
<td>5118±15.04a</td>
<td>1203±11.59a</td>
</tr>
</tbody>
</table>

Where HMT-QS is heat moisture treated quinoa starch, GM-QS is gum modified quinoa starch. *n=3; Results are expressed as mean values ± SD. Means in a column with different superscripts are significantly different (p < 0.05). PT = Pasting temperature; and Setback = (Final - Trough)

The increase in paste viscosity on addition of gums might be due to the interaction (hydrogen bonding) between amylose and solubilised gum. It can also be due to the ability of gums to act as thickener. Alloncle et al. (1989) assumed that gum acts as the continuous phase and the starch paste remains dispersed as suspension in this phase. As longer the starch granules start swelling the concentration of the gum within continuous phase increases resulting in increased viscosity of the phase. Increase in viscosity also causes greater shear forces on swollen granules much higher than that encountered by simple starch-water suspensions resulting in the granular disruption. Alloncle and Doublier (1991) also observed the same increase and indicated that the changes resulting from addition of gum to starch are
complex and can be attributed to phase separation process in relation incompatibility phenomena between unlike polymers. Effect of both modifications was found to be more pronounced on paste viscosity than pasting temperature.

**4.6.2 Evaluation of starch noodles**

In order to maintain good quality, cooked noodles should have less cooking loss, relatively strong bite with smooth surface, shorter cooking time and good mouthfeel.

**4.6.2.1 Optimum cooking time**

Optimum cooking time of native, HMT-QS and GM-QS is shown in Figure 4.19. Native starch noodles showed shorter cooking time followed by GM-QS and HMT-QS noodles. HMT and addition of gum increased the cooking time of the noodles with the effect from HMT being significant (P<0.05) and more pronounced. The longer cooking time of HMT (4.07 min) and GM-QS (3.11 min) noodles in comparison to native (3.04 min) noodles may be due to their higher gelatinization temperature. Kaur et al. (2015) also reported the longer cooking time of mung bean and corn starch noodles due to addition of gum and attributed it to the limited water availability and delayed gelatinization of starch granules. Shorter cooking time of native and gum modified noodles is a desirable characteristic. Cooking time of native and modified noodles ranged from 3.04 to 4.07 min which is comparable to the cooking time of mung bean and corn starch noodles and lower than that of commercial rice noodles marketed in India (Kaur et al., 2015; Sandhu and Kaur 2010).
Figure 4.19 Optimum cooking time of native and modified starch noodles

Figure 4.20 Cooking loss of native and modified starch noodles
4.6.2.2 Cooking loss

The values for cooking loss of native and modified starch noodles are shown in Figure 4.20. Cooking loss has been considered as an important indicator of noodle quality as it affects the appearance and texture of the product. Native starch noodles (7.18 g/100g) showed higher cooking loss than HMT (6.17g/100g) followed by GM-QS (5.25 g/100g). Lower cooking loss of gum modified noodles in comparison to native starch noodles may be attributed to the complex formation between the gum and amylose thereby preventing its leaching. The reduced cooking loss due to addition of gum depicts that the process facilitates the formation of a network which is more stable during cooking process. However, higher cooking loss of HMT noodles in comparison to GM-QS noodles may be attributed to the longer cooking time of HMT noodles due to higher gelatinization temperature. Although the HMT results in more ordered crystalline starch matrix causing less amylose leaching but the higher cooking loss observed in this study may be due to prolonged cooking resulting in the partial disintegration of external structure of noodles (Cham and Suwannaporn. 2010). Reduced cooking loss on addition of gum has also been reported by Han et al. (2011) and attributed it to the formation of more stable network.

4.6.2.3 Water uptake

Figure 4.21 shows the water uptake values for native and modified quinoa starches. Both HMT and GM showed the significant (P<0.05) positive effect on the water uptake of starch noodles. Water uptake showed the following order; HMT-QS>GM-QS>NS. The porous structure of the dried noodles allows the entry of water into noodles causing the rehydration and is considered as a desirable phenomenon. Water uptake of native and modified noodles ranged from 139.47 to 148.02 (g/100g). Higher water uptake of HMT-QS (148.02 g/100g) and GM-QS (144.15 g/100g) may be correlated to their higher water absorption capacity in comparison to the native starch. Increased rehydration of noodles on
addition of gums was also observed by Yu and Ngadi (2004). Collado et al. (2001) showed the increased water uptake for HMT modified sweet potato starch noodles.

Figure 4.21 Water uptake of native, heat moisture treated and gum modified starch noodles

4.6.2.4 Textural properties

The textural characteristics of native and modified starch noodles are shown in Table 4.24. Both HMT and gum addition exerted significant (P<0.05) positive effect on textural characteristics of quinoa starch noodles. Native starch noodles were low in firmness. HMT and hydrocolloid addition increased the firmness of noodles with the effect from addition of hydrocolloid being more pronounced. The increase in hardness of noodles due to hydrocolloid and HMT has been attributed to the formation of strong network. Han et al. (2011) also reported the increase in hardness for rice starch noodles due to addition of gums and attributed it to the strong network development and interaction between starch molecules and gum.
Table 4.24 Textural profile analysis of dried cooked starch noodles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hardness (N)</th>
<th>Adhesiveness (N.sec)</th>
<th>Chewiness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>11.47±0.27c</td>
<td>0.149±0.02a</td>
<td>368±34.64c</td>
</tr>
<tr>
<td>HMT-QS</td>
<td>13.19±0.36b</td>
<td>0.105±0.01b</td>
<td>1339±23.94a</td>
</tr>
<tr>
<td>GM-QS</td>
<td>16.27±0.26a</td>
<td>0.074±0.02c</td>
<td>1084±18.77b</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SD. Means within a column with different superscripts are significantly different (p < 0.05).

Increase in noodle firmness upon addition of xanthan gum was also observed by Silva et al. (2013) for sweet potato starch noodles. Higher hardness value observed for HMT noodles may also be due to higher amylose content water binding capacity, higher final and setback viscosity of HMT starch (Chandla et al., 2016). Higher firmness in noodles means lesser susceptibility to breakage during cooking. Adhesiveness is the undesirable characteristic of noodles and is the measure of work required to overcome the attractive forces between surface of food and teeth. GM-QS and HMT starch noodles showed decrease in adhesiveness. Among all the samples gum noodles showed the lowest adhesiveness (0.074 N.sec) indicating the lesser stickiness of treated noodles. Gum addition and HMT resulted in the improved noodle quality due to parameters like increased firmness and decreased adhesiveness in comparison to native starch noodles. Padalino et al. (2014) observed the decrease in adhesiveness and stickiness of spaghetti at low gum concentrations and attributed it to the strong network that entraps starch granules. Reduction in adhesiveness due to HMT and gum addition may be attributed to the increased stability of granules thereby decreasing the association and interaction of granules within the noodles. Chewiness refers to the energy required to masticate or disintegrate the noodle strands for easy swallowing. Good quality noodles usually require firm bite and chewy texture (Hou, 2001). Chewiness values of the native and treated starch noodles ranged from 368 to 1339. With HMT starch noodles
exhibiting higher chewiness (1339) followed by Gum added starch noodles (1084) and native starch noodles (368). Galvez and Resurreccion (1992) suggested that cooked starch noodles should neither be too hard nor too soft and the results obtained in the present study are in accordance with this proclamation.

4.6.2.5 Sensory analysis

Sensory characteristics have been considered as an important indicator of consumer preferences and most reliable method for measuring the quality attributes of product. Variation in sensory analysis attributes of noodles prepared with native and modified starches is shown in Table 4.25. Gum modified noodles scored higher than native and HMT starch noodles in the aforementioned sensory quality attributes. Hydrocolloid noodles scored higher in all attributes than HMT noodles followed by native noodles. Lower score of appearance in HMT noodles may be due to the lower lightness of HMT starch as shown by the L value of 90.21 in comparison to 97.27 and 97.12 for native and gum modified starches. Firmness score was highest for gum modified noodles followed by HMT and native noodles. Chewiness indicates the time taken to masticate a strand of noodle to reduce its particle size sufficiently for swallowing. Firmness and chewiness of a sample should neither be too low nor too high. HMT noodles scored higher for chewiness followed by GM-QS noodles and native starch noodles. Non-significant difference existed between the chewiness of HMT noodles and GM-QS noodles which may be due to the large variation in sensory data as some of the panellists liked the higher chewiness of HMT noodles while others found it less attractive (too chewy) in comparison to that of GM-QS noodles. Slipperiness defines the extent up to which the product slides across the tongue. Higher slippery surface is desirable in starch noodles. Gum modified noodles scored higher for slipperiness followed by HMT and native starch noodles. Tooth packing refers to the sticking of the noodle strands to the teeth during mastication. Tooth packing score was higher for native starch noodles followed by HMT and gum
modified noodles. Over all acceptability score was highest for gum modified starch noodles followed by HMT and native noodles.

Based on the total score of aforementioned sensory quality attributes, cooking and textural properties gum modified starch noodles were judged to be best among all experimental samples followed by HMT and native starch noodles.

Table 4.25 Sensory analysis of dried cooked starch noodles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appearance</th>
<th>Slipperiness</th>
<th>Firmness</th>
<th>Tooth packing</th>
<th>Chewiness</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>8.26±0.11a</td>
<td>6.75±0.14c</td>
<td>6.40±0.14c</td>
<td>7.02±0.13c</td>
<td>6.41±0.13c</td>
<td>6.95±0.11c</td>
</tr>
<tr>
<td>HMT-QS</td>
<td>7.50±0.24b</td>
<td>7.71±0.12b</td>
<td>7.73±0.16b</td>
<td>7.71±0.12b</td>
<td>8.05±0.27a</td>
<td>7.82±0.12b</td>
</tr>
<tr>
<td>GM-QS</td>
<td>8.18±0.16a</td>
<td>8.07±0.07a</td>
<td>8.12±0.09a</td>
<td>7.89±0.08a</td>
<td>8.00±0.12a</td>
<td>8.05±0.08a</td>
</tr>
</tbody>
</table>

Results are expressed as mean values ± SD. Means within a column with different superscripts are significantly different (p < 0.05).

4.7 Storage stability of products

Storage study is important for determining the quality, shelf stability as well as profitability of the product. Storage study of the product means understanding the effect of various environmental conditions on the quality of the product and to determine the acceptability of the product under such conditions (Bajaj et al., 2012). For effective storage study products are to be packed according to the composition and need of the product. Packaging materials serve as a barrier against light, moisture, air (precisely oxygen) and also prevent the chances of contamination from undesirable microorganisms. Optimized cookies prepared from quinoa were stored in different Packaging materials (LDPE and MET-PPE) under same atmospheric conditions and analysed for various physico-chemical, functional and textural parameters.
4.7.1 Effect of storage conditions on the quality of optimized C. quinoa cookies

Storage stability in case of cookies was examined on the basis of changes in physico-chemical, functional and textural parameters like moisture content, water activity, free fatty acids, peroxide value, hardness and overall acceptability.

4.7.1.1 Moisture content

Moisture content is known to have a significant effect on the quality attributes of the snack products with low moisture content resulting in longer shelf life and crispier product. Effect of packaging material and storage period on moisture content of the C. quinoa cookies is shown in Figure 4.22. Moisture content of the freshly prepared samples prior to packaging was 2.89 to 2.94% which is within the range (1-5 %) suitable for cookies (Pareyt and Delcour, 2008). With increase in storage duration from 0 to 120 days moisture content of the cookies increased from 2.94 to 3.92 % in case of LDPE and 2.89 to 3.17% for MET-PPE. Possible reasons for increase in moisture content of the cookies may be; a) higher humidity of storage environment b) permeability of the packaging material and c) hygroscopicity of the product. Significant moisture absorbance in case of LDPE may be due to its less impermeable nature in comparison to MET-PPE. Therefore, in case of moisture content MET-PPE proved to be better packaging material than LDPE. Increase in moisture content of bakery products during storage in different packaging materials was also observed by Kumar et al., (2014), Romani et al. (2015) and Nagi et al. (2012) for baked products (biscuits). However, in case of Nagi et al. (2012) increase in moisture content observed during the storage was much higher than that observed in current study which may be due to the variation in packaging material and storage conditions.
4.7.1.2 Water activity

Moisture alone is insufficient to determine the quality of product hence water activity serves as a measure of water availability. Water activity has been considered as an important parameter to describe microbial stability, water migration and textural changes occurring during storage (Ergun 2010). Figure 4.23 shows the effect of packaging material and storage period on water activity of the *C. quinoa* cookies. Analysis of the data revealed that both packaging material and storage period showed the significant (P<0.05) increase in the water activity of the cookies in case of LDPE throughout the study. However, marginal increase in water activity was observed in case of cookies packed in MET-PPE. With increase in storage days from 0 to 120 water activity of the cookies increased from 0.24 to 0.54 in case of LDPE and 0.23 to 0.40 for MET-PPE. It has been observed that the choice of formula or package is not always simple. During storage product changes like crystallization of sugar will release...
water and cause an increase in water activity of the product even if the moisture content of product remains constant (Kerry and Butler 2008).

**Figure 4.23 Effect of storage periods and packaging materials on water activity of *C. quinoa* cookies**

### 4.7.1.3 Free fatty acid (FFA)

Determination of free fatty acids is a simple and rapid method for quality control of product. Increase in free fatty acids in a sample is an evidence of hydrolytic rancidity. Table 4.26 shows the effect of packaging material and storage period on free fatty acid content of the *C. quinoa* cookies. Analysis of the data revealed that there was a slow increase in FFA in case of MET-PPE in comparison to LDPE. Overall, LDPE packed cookies showed significantly higher free fatty acids than those packed in MET-PPE. Free fatty acid content of the cookies increased from 0.33 to 1.20 in case of LDPE and from 0.30 to 0.92 for MET-PPE with increase in storage days from 0 to 120. Generation of free fatty acids depends on the initial conditions of the fat, its processing and chemical changes occurring during storage.
Increase in free fatty acids in quinoa cookies may be due to its higher unsaturated fatty acid. As mentioned by choe and min (2007) moisture from foods is easily accessible to unsaturated fatty acids and oil for hydrolysis with increased moisture resulting in rapid hydrolysis. According to Australian Macadamia Society (AMS) FFA content of about 0.6% is considered acceptable in some foods (Wallace and walton 2011). Another recommendation mentions that the product usually develops rancid flavor or is considered unfit for consumption when FFA content is greater than 1.5% (Nagi et al., 2012). Formation of free fatty acids has been found to be independent of formation of hydro peroxides though they may run concurrently as well (Pomeranz 1982). Aggarwal (2017) also observed the significant increase in free fatty acid content of the product in LDPE and attributed it to the increase in moisture content. Walton et al. (2017) observed the significant increase in free fatty acid in vacuum packed nuts at temperatures above 25 °C and attributed it to the hydrolytic cleavage of triglycerides due to activation of enzymes by higher moisture content. However, in some cases rancidity measures like free fatty acid and peroxide value had been considered as unreliable with sensory analysis being the more reliable test (Wallace and walton 2011).

4.7.1.4 Peroxide value

Rancidity caused by oxidation of fat results in the formation of peroxides or hydroperoxides which are measured as peroxide value. For many years peroxide value has been used as an index of rancidity (Pomeranz 1982). Peroxide value of the product increased from 3.84 to 6.15 meqO₂/Kg in case of LDPE and 3.76 to 4.72 meqO₂/Kg in case of MET-PPE. Cookies packed in LDPE presented higher peroxide values in comparison to MET-PPE packed cookies (Table 4.26). Increase in peroxide value may be due to oxidation of unsaturated fatty acids present. The observed oxidation during storage could be due to residual oxygen present at the time of packaging. Apart from the inner package conditions, storage temperature and incorporation of air into the dough during kneading may also
facilitate the oxidation process (Hu and Jacobsen 2016). Ajith et al. (2015) also found the increase in peroxide value of cashew nut oil with increase in the relative humidity and temperature during storage.

Table 4.26 Effect of storage period and packaging materials on free fatty acids and peroxide value of C. quinoa cookies

<table>
<thead>
<tr>
<th>Storage duration (days)</th>
<th>Low density polyethylene</th>
<th>Metalized polyester polyethylene laminate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FFA (%)</td>
<td>P.V (meqO₂/Kg)</td>
</tr>
<tr>
<td>0</td>
<td>0.33±0.02&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>3.84±0.08&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>0.38±0.02&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>3.96±0.17&lt;sup&gt;bA&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>0.44±0.02&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>4.12±0.11&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>45</td>
<td>0.51±0.03&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>4.32±0.11&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>0.60±0.04&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>4.64±0.09&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>0.75±0.04&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>4.96±0.09&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>0.88±0.06&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>5.36±0.09&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>105</td>
<td>1.01±0.02&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>5.72±0.11&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>1.20±0.04&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>6.15±0.08&lt;sup&gt;cA&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by different lower-case letters in each column are significantly different \((P<0.05)\). Values followed by different upper-case letters in each row are significantly different \((P<0.05)\). Lower case letters shows the effect of storage duration within the packed sample and the upper case letters show the effect of packaging materials on samples.

Samples packed in MET-PPE showed lower increase in peroxide value which is an indicative of the lower oxygen transmission rate of the package. However, cookies in both the packages were stable throughout the storage study as the values were less than the permissible limit of 10 meqO₂/Kg (Hemery et al., 2015; CAC, 1999).
4.7.1.5 Hardness

Hardness is commonly used as an index of quality in case of bakery products (Gaines et al., 1992). Hardness of the cookies decreased as the storage duration increased with the effect being significant (P<0.05) in case of LDPE packed cookies (Figure 4.24).

![Figure 4.24](image_url)

**Figure 4.24 Effect of storage period and packaging materials on hardness of *C. quinoa* cookies**

Hardness in case of cookies packed in LDPE decreased from 46.92 to 44.48 N in comparison to a decrease of 47.05 to 45.74 N for MET-PPE package. Prominent decrease in LDPE packed cookies may be due to less moisture barrier properties of LDPE. The change in texture during storage may be due to moisture redistribution, sucrose crystallisation and loosening of the matrix due to moisture migration. Decrease in the hardness during storage was also observed by Rajiv et al. (2012) in case of flaxseed incorporated cookies.
4.7.1.6 Microbial analysis

Yeast and mold count was found to be nil in all cookies samples throughout the storage study. Hozova et al. (1997) and Banusha and Vasanharuba (2014) also did not found any fungal colony during storage of the biscuits prepared from wheat flour and composite flour. Total plate count (TPC) of the samples is shown in Figure 4.25.

![Figure 4.25 Effect of storage period and packaging material on total plate count (cfu/g) of C. quinoa cookies](image)

With increase in storage duration total plate count of the samples in both the packages increased. Samples packed in LDPE showed higher total plate count varying from $4.67 \times 10^3$ to $7.87 \times 10^3$ cfu/g in comparison to the $5.33 \times 10^2$ to $2.87 \times 10^3$ cfu/g shown by MET-PPE samples. Increase in total plate count with increase in storage duration may be attributed to the increased moisture content. Increase in total plate count during storage has also been attributed to the presence of spores in the raw material which might have survived technological process implemented and in convenient conditions, proliferated (Hozova et al.,
1997). Though the total plate count of the samples increased with increase in duration of storage but was within the permissible limits ($10^4$ cfu/g) given by International commission on microbiological specification for foods (ICMSF, 2010). Total plate count for high protein biscuits according to ISI specifications (1974) is 50000 cfu/g (maximum).

### 4.7.1.7 Overall acceptability

Sensory analysis has been considered as a vital tool for consumer acceptability of the product. Overall acceptability of *C. quinoa* cookies as influenced by storage period and packaging materials is shown in Figure 4.26.

![Figure 4.26 Effect of storage periods and packaging materials on overall acceptability of C. quinoa cookies](image)

During the storage period (0-120 days), overall acceptability of cookies based on sensory scores decreased from 7.54 to 4.61 for LDPE and 7.61 to 5.74 for MET-PPE (Figure 4.26). Significant reduction in overall acceptability of product in LDPE may be due to its higher moisture and air permeability in comparison to MET-PPE which results in the loss of
texture, change in color and also the development of off-flavours and off-odours. Marginal decrease in overall acceptability was observed in MET-PPE cookies stored up to 75 days. However, further increase in storage duration showed the significant difference in overall acceptability. It was observed that *C. quinoa* flour cookies were in almost acceptable quality up to 120 days in MET-PPE (metalized polyester polyethylene) packages. Rajiv et al. (2012) also observed the marginal decrease in total sensory score of flaxseed fortified cookies stored for 90 days and attributed it to the increase in fat acidity, peroxide value and decrease in breaking strength.

4.7.2 Effect of storage on quality parameters of *C. quinoa* starch noodles

Storage stability in case of starch noodles was examined on the basis of changes in parameters like moisture content, water activity, cooking loss, firmness, and overall acceptability at a time interval of 0, 30, 60, 90, 120, 150 and 180 days.

4.7.2.1 Moisture content

The effect of storage duration and packaging material on moisture content of the *C. quinoa* starch noodles is shown in Figure 4.27. Increase in storage duration from 0 to 180 days increased the moisture content of the noodles from 6.05 to 6.70% in case of LDPE and 6 to 6.21% for BOPP. Migration of the moisture to the sample depends on the permeability of the packaging material used and storage environment. Higher moisture gain in the LDPE may be due to its higher permeability whereas; BOPP showed marginal increase in moisture content during storage. Increase in moisture content of noodles may be due to movement of water vapors through the packaging material from the storage environment of high relative humidity.
Figure 4.27 Effect of storage period and packaging materials on moisture content of *C. quinoa* starch noodles

### 4.7.2.2 Water activity

Water activity has been considered as an important parameter to describe microbial stability, water migration and textural changes occurring in product during storage. Figure 4.28 shows the effect of packaging material and storage duration on water activity of *C. quinoa* starch noodles packed in LDPE and BOPP. Water activity of the products stored in LDPE and BOPP varied from 0.47 to 0.55 and 0.46 to 0.49 respectively with increase in storage duration from 0 to 180 days. Product packed in BOPP showed marginal increase in water activity which may be due to the lower permeability of the packaging material in comparison to LDPE. Kaur et al. (2012) also observed the increase in water activity of pasta during storage and attributed it to the surroundings.
Cooking loss plays a crucial role in determining the cooking quality in case of snack products like noodles with lower leaching out of solids indicating good cooking quality (Chang and Wu, 2008). Effect of packaging material and storage duration on cooking loss of C. quinoa starch noodles packed in LDPE and BOPP is shown in Table 4.27. Cooking loss of the products stored in LDPE and BOPP varied from 5.32 to 6.18% and 5.25 to 5.81 % respectively with increase in storage duration from 0 to 180 days. Increase in cooking loss with increase in storage duration may be due to weakening of intermolecular interaction of starch. Product packed in BOPP showed marginal increase in cooking loss in comparison to LDPE noodles which may be attributed to greater loosening of matrix due to comparatively higher water activity and moisture gain in case of LDPE. Overall cooking loss of C. quinoa starch noodles in both the packages remained below 10% throughout the storage period, which is within the accepted range of Chinese and Thai standards for starch noodles (Kim et
Kaur et al. (2012) studied the effect of storage (up to 4 months) on cooking loss of pasta enriched with cereal bran and found no significant difference.

### 4.7.2.4 Firmness

Firmness has been considered as an important parameter for assessing the quality and integrity of cooked noodles. Firmness of the noodles decreased as the storage duration increased with the effect being significant in case of LDPE packed noodles.

<table>
<thead>
<tr>
<th>Storage duration (days)</th>
<th>LDPE</th>
<th>BOPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cooking loss</td>
<td>Firmness</td>
</tr>
<tr>
<td>0</td>
<td>5.32±0.14&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>16.12±0.21&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>5.40±0.07&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>16.03±0.06&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>5.44±0.10&lt;sup&gt;deA&lt;/sup&gt;</td>
<td>15.94±0.11&lt;sup&gt;abA&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>5.58±0.11&lt;sup&gt;daA&lt;/sup&gt;</td>
<td>15.84±0.04&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>5.69±0.06&lt;sup&gt;caA&lt;/sup&gt;</td>
<td>15.63±0.03&lt;sup&gt;cdB&lt;/sup&gt;</td>
</tr>
<tr>
<td>150</td>
<td>5.87±0.07&lt;sup&gt;baA&lt;/sup&gt;</td>
<td>15.44±0.04&lt;sup&gt;dbB&lt;/sup&gt;</td>
</tr>
<tr>
<td>180</td>
<td>6.18±0.11&lt;sup&gt;caA&lt;/sup&gt;</td>
<td>15.15±0.03&lt;sup&gt;xB&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values followed by different lower-case letters in each column are significantly different (P<0.05). Values followed by different upper-case letters in each row are significantly different (P<0.05). Lower case letters shows the effect of storage duration within the packed sample and the upper case letters show the effect of packaging materials on samples.

Firmness in case of noodles packed in LDPE decreased from 16.12 to 15.15 N in comparison to a decrease of 16.27 to 15.89 N for BOPP package. Prominent decrease in LDPE packed noodles may be due to its less moisture barrier properties of LDPE. The change in firmness of noodles during storage may be due to moisture redistribution and subsequent loosening of the matrix due to moisture migration.
4.7.2.5 Microbial analysis

Total plate count (TPC) of the noodles during storage is shown in Figure 4.29.

Figure 4.29 Effect of storage periods and packaging materials on total plate count (cfu/g) of C. quinoa starch noodles

With increase in storage duration total plate count of the samples in both the packages increased. Noodles packed in both LDPE and BOPP didn’t show any yeast and mold count throughout the storage study. Noodle samples packed in LDPE showed higher total plate count varying from $6.67 \times 10^2$ to $7.33 \times 10^3$ cfu/g in comparison to the BOPP samples ($6 \times 10^3$ to $2.73 \times 10^3$ cfu/g). Increase in total plate count with increase in storage duration may be attributed to the increase in moisture content and water activity. Increase in total plate count during storage has also been attributed to the presence of spores in the raw material which might have survived technological process implemented in convenient conditions, proliferated (Hozova et al., 1997). According to Health Protection Agency (2009) for ready to eat foods like noodles $10^4$ to $<10^6$ cfu/g is the borderline. However, if the total plate count exceeds $10^6$cfu/g, noodles are considered as spoiled. Total plate count of the samples
increased with increase in duration of storage but was within the permissible limits throughout the study.

4.7.2.6 Overall acceptability

Sensory attributes have been considered as better indicators for any adverse chemical changes during storage. The sensory score obtained for overall acceptability of quinoa starch noodles stored in LDPE and BOPP is shown in Figure 4.30.

![Figure 4.30 Effect of storage periods and packaging materials on overall acceptability of C. quinoa starch noodles](image)

During the initial storage days non significant effect was observed on overall acceptance of the samples. However, further increase in storage duration resulted in the significant decrease of overall acceptability of sample packed in LDPE. As the storage progressed overall acceptability for noodles stored in BOPP decreased from 8.05 to 7.79 while as in case of noodles stored in LDPE overall acceptability decreased from 8 to 7.12. Decline in sensory score was slightly higher for the product stored in LDPE than BOPP. The
overall acceptability of quinoa starch noodles in both the packages remained within acceptable limits during the entire storage study. Duszkiewicz et al. (1988) studied the shelf life of spaghetti and found no significant differences for external appearance and general acceptability of the product up to the six months of storage.

4.8 *In vitro* digestibility of developed products

To determine the *in vitro* digestibility of the optimized products, flour and starch from the same variety was taken for comparison purpose. Nosworthy et al. (2017) while studying the effect of processing on *in-vitro* and *in-vivo* protein quality of peas suggested that *in-vitro* digestibility can be used as a surrogate for *in vivo* analysis since the digestibility values from both the methods were well correlated.

4.8.1 *In vitro* protein digestibility (IVPD)

Digestibility of protein has been considered as an important characteristic of protein nutritional quality. Significant differences existed in the *in vitro* protein digestibility of *C. quinoa* flour (V2) and the product (cookies) developed from it. In present study *C. quinoa* flour (81.73%) showed lower *in vitro* protein digestibility than the developed product (83.89%) as shown in Figure 4.31. Increase in *in vitro* protein digestibility due to baking may be related to the unfolding of protein at high temperature due to denaturation leading to its higher accessibility to digestive enzymes. Resistance of the protein to enzymatic hydrolysis in its native form can be attributed to the compact structure of native protein. Increase in *in vitro* protein digestibility due to baking has been reported by various researchers (Sathe et al., 1982; Gahlawat & Sehgal, 1998; Abdel-Aal, 2008). Positive effect of baking on IVPD may also be due to inactivation or structural disintegration of some antinutritional components at higher temperature (baking). Vijayakumari et al. (1998) studied the effect of heat processing on antinutrients and *in vitro* protein digestibility in pulses and reported that heat processing results in higher protein digestibility. They attributed this increased protein digestibility to the
reduction in antinutritional factors thereby increasing the accessibility of the protein to enzymatic attack.

![Figure 4.31 In vitro protein digestibility of C.quinoa flour and cookies](image)

4.8.2 In vitro starch digestibility (IVSD)

The readily digestible starch (RDS) and slowly digestible starch (SDS) fractions of starch and optimized products are shown in Figures 4.32 and 4.33. Readily digestible starch is rapidly hydrolyzed in small intestine and has been associated with rapid elevation of postprandial glucose whereas the slowly digestible starch is slowly hydrolyzed in small intestine and hence is the desirable form of digestible starch fraction. Structure of the starch has been considered as an important factor for its digestion with high amylose starches being hydrolyzed less efficiently than low amylose starches (Htoon et al. 2009). IVSD of the flour sample (V2) was lower than the optimized product developed from same variety. Lower SDS (20.58 %) and higher RDS (31.51 %) fraction of cookies in comparison to its flour could be due to partial gelatinization of starch at higher temperatures. However, the difference was marginal which may be due to the baking resulting in partially intact starch granules that are
less susceptible to digestion (Englyst et al., 2003). Relevant literature for the digestible starch fractions of quinoa flour, starch, cookies and noodles is not available. However, the results found in current study were almost comparable to the digestible and resistant starch fractions of rice starches observed by Hung et al. (2016).

![Figure 4.32](image-url) **Figure 4.32 In vitro starch digestibility expressed as rapidly digestible starch (RDS) and slowly digestible starch (SDS) fractions of *C. quinoa* flour and cookies**

Noodles made from starch showed lower RDS (53.75%) but higher SDS (33.42%) in comparison to the native starch. Development of higher amount of slowly digestible starches due to temperature cycled retrogradation has been reported by many researchers (Tian et al., 2012; Zhang et al., 2011). Lower digestibility and higher resistant starch content of optimized starch noodles may be due to processing conditions like cooking, cooling, drying and re-heating (retrogradation) resulting in the formation of a complex structure (strong bonding) which is more resistant to enzymatic degradation. Kayisu and Hood (1979) also observed the decrease in enzyme susceptibility of cooked dried starch pastes from different sources and attributed it to the retrogradation occurring during drying. Reduced enzyme susceptibility by retrogradation was also observed for sago starch by Cui and Oates (1997). From the results of
*in vitro* starch digestibility it can be predicted that flour and cookies could be considered as an ideal resistant starch material due to their low slowly and readily digestible starch in comparison to the starch, whereas the noodles could be more suitable as a slowly digestible starch material. Further, retrogradation makes starch more resistant to enzymatic hydrolysis and hence retrograded starches can be used for preparation of low-calorie or low glycemic index foods.

![Starch digestibility graph](image)

**Figure 4.33** *In vitro* starch digestibility expressed as rapidly digestible starch (RDS) and slowly digestible starch (SDS) fractions of *C. quinoa* starch and noodles