Biochemical and Technological Aspects of Wheat Flour
Associated with Cookie Making Quality

By

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The Controller of Examinations,
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THIS HUMBLE EFFORT IS

Dedicated To

Holy Prophet Hazrat Muhammad (SAW)
(A father gives his child nothing better than a good education)

&

Quaid-e-Azam Muhammad Ali Jinnah
(There are two powers in the world; one is the sword and the other is the pen. There is a great competition and rivalry between the two. There is a third power stronger than both, that of the women.)
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ABSTRACT

Wheat (*Triticum aestivum* L.) is the basic ingredient of cookies formulation and its specific or consistent quality (soft wheat) is required for cookie making. In Pakistan, wheat flour is produced from a mixture of wheat varieties called mixed wheat flour (MWF) leads to unavailability of specific wheat variety suitable for specific product. However, this flour has inconsistent quality and subsequently affects product quality significantly. Present study has been aimed to evaluate the impact of various treatments on MWF. Results showed a wide range of TW and TKW as 67.00-81.00 kg hl⁻¹ and 32.82-48.70g, respectively. Wheat varieties exhibited moisture (8.50-10.78%), ash (0.33-0.83%), fat (1.36-1.49%), fiber (0.38-0.52%), protein (9.85-13.75%) and NFE (72.82-79.58%) content. Mineral composition varied as 2.20-2.64, 20.25-28.45, 2.12-4.71, 1.24-2.31, 0.54-0.85 and 0.45-0.57 mg/100g for Na, Ca, Fe, Zn, Mn and Cu content, respectively. Moreover, wheat varieties indicated wet gluten, dry gluten, starch and DS as 24.68-36.40, 7.12-11.84, 68.88-72.73 and 6.28-10.97%, respectively. SDS-sed. and AWRC were reckoned as 18.20-30.50 ml and 43.91-66.85%, respectively. Moreover, WSRC, SOCSRC, SUCSRC and LASRC values varied widely as 50.83-69.64%, 56.66-82.03%, 75.79-106.97% and 79.43-121.20%, respectively. Rheological properties were found i.e. WA (51.00-56.00%), DDT (1.20-4.20 min), dough stability (2.90-18.20 min), DoS (85.00-174.00 BU) and FQN (48.00-200.00). Pasting properties indicated variability from 65.25 to 67.80°C (PaT), 3.22 to 6.00 min (PT), 1330 to 1784 cP (PV), 645 to 1002 cP (TV), 1799 to 2565 cP (FV) 685 to 782 cP (BV) and 1154 to 1563 (SV). Physical analyses of cookies made from wheat varieties revealed 39.38-45.86 spread factor and 2.17-3.76 kg hardness. Sensory evaluation indicated that cookies made with AARI-11 were highly acceptable and also, it was selected as best variety for cookie making. In addition, different treatments were also applied and cookies made with different treatments portrayed spread factor (39.68-60.96) and hardness (0.86-3.55 kg). Cookies color determined in terms of L*, a* and b* values as 64.65-74.84, 1.22-3.75 and 31.10-35.44, respectively. Cookies revealed moisture (1.50-3.68%), ash (0.333-0.547%), fat (17.70-23.66%), fiber (0.083-0.167%), protein (5.41-6.56%) and NFE content (66.76-72.40%). Across treatments, addition of CMS (15%) produced largest spread, protease (400 mg) had highest overall acceptability. Correlation studies underline the importance of SRC values and SDS-sed. volume as major predictors of cookie making quality in addition to other physicochemical characteristics of flour.
CHAPTER 1

INTRODUCTION

Common wheat belonging to grass family (Poaceae), tribe (hordeae), genus (*Triticum*), specie (*aestivum*) is the principle cereal cultivated worldwide and its 67% is mainly utilized as food, 20% as feed and 7% for seed purpose (El-Porai *et al.*, 2013). It is main food source for mankind and almost, all the cultivated wheat is transformed to vast array of foods (Morris, 2016). It ranks 1st as world crop being a national food staple of 43 countries (Umrani and Pahoja, 2013). Likewise, it is largest grain crop and staple food of Pakistan with an area and production recorded as 9,180 thousand hectares and 25,478 thousand tonnes, respectively (GOP, 2014-15). It occupies central position in agriculture and economy with 2.1% to GDP (gross domestic product) and 10% to the value added in agriculture (Amjad *et al.*, 2010).

Wheat grain has wide utility and variety of products (Umrani and Pahoja, 2013) and mainly used for chapatti. The contribution of wheat for chapatti accounts for 70 to 80% of total production. While, the rest of wheat is utilized for production of other baked products like bread, biscuits and cakes etc. There is an eminent interest in the wheat-based foods and it is the basic ingredient in most of the baked products (David *et al.*, 2015).

Cookies are small, sweetened baked products usually based on cereals (Miller, 2016). These are very popular, relatively economical and preferred by almost all profiles of consumers (Popov-Raljic and Stojsin, 2007), not only for nutritional value but also affecting emotional status of consumers with positive mood enhancement (Turner *et al.*, 2010). Cookies are prepared in a wide range of style, size and forms (Chizoba, 2014). These are characterized by high fat and sugar level but, lower final moisture content (1-5%) (Mancebo *et al.*, 2015). Due to later, these are microbiologically stable for relatively long time (Cauvain, 2016).

Cookies quality is mainly associated with low protein content, reduced starch damage and similarly, lower values of alkaline water retention capacity (Fustier *et al.*, 2008). Most importantly, it depends on chemical components of flour that absorb water as well as quality and quantity of gluten. In general, large diameter cookies with a tender bite are characteristic of soft wheat containing low protein content and weak gluten strength (Kara *et al.*, 2005; Pasha *et al.*, 2009b).

The term “wheat quality” is a complex of various parameters, which is difficult to describe in a single term rather based on various milling, chemical, rheological and baking characteristics (Pasha *et al.*, 2006). Indeed, it reflects its suitability for a certain purpose, intended use and
particular end product (Amjad et al., 2010). It usually means consistency in the production of end product (Arif et al., 2007). Wheat flour is the basic ingredient in cookies therefore, it is imperative to evaluate the wheat flour quality. It is of great importance for baking industry as it demands desirable functionality for different products (Fustier et al., 2009a).

Different varieties of wheat and processing are required to produce wheat flour for specific use (El-Porai et al., 2013). Soft wheat varieties, which produce cookies with great spread and fine texture are thought to be good quality flour. Nevertheless, it must be realized that an ideal flour for a certain type of cookie might be imperfect for another type of product (Fustier et al., 2009b). Flour required for biscuit making usually have 7-10% protein, moisture below than 14%, unbleached and unchlorinated (Hazelton et al., 2004).

Physicochemical characteristics of flour are important to govern dough rheology and predict flour quality to develop a range of bakery products therefore, should be selected cautiously (Ahmed et al., 2015). Various predictive methods closely associated with quality of wheat flour are commonly employed in wheat breeding programs for the assessment of flour quality. These methods are easy to conduct utilizing only few grams sample (Colombo et al., 2008). Solvent retention capacity (SRC) ascribed as flour ability to hold four different solutions i.e. 5% sodium carbonate, 50% sucrose, 5% lactic acid and deionized water (Geng et al., 2012; Kang et al., 2014). In general, sodium carbonate SRC (SOCSR) is related to damaged starch, sucrose SRC (SUCSRC) linked to pentosans and gliadin, lactic acid SRC (LASRC) to glutenin characteristics and water SRC (WSRC) associated with all the four components as given earlier by Guttieri et al. (2001) and Geng et al. (2012). It is a rapid, small scale test developed to evaluate the role of all functional components in end product quality for applications of soft wheat initially by Slade and Levine (1994) and afterwards executed by AACC International Approved test (AACC, 2000) as method 56-11 (Geng et al., 2012; Kang et al., 2014).

Cookie manufacturers observe disparity in the functionality of soft wheat flour for different cookie formulation and processes. The technological problems encountered in cookie making are sticky dough, uncontrolled cookie dimensions and density, disparity in texture and surface appearance of cookie (Fustier et al., 2009b). Variations in quality are usually derived from physical and compositional characteristics of grain. These functional variations are measured through methods designed for the quality evaluation of wheat grain (Morris, 2016). Attempts have been made in the past to confront the challenges related to quality improvement of
Native starch has limiting applications in food processing because these are sensitive to pH, heat and shear. Also, the development of shelf stable refrigerated, frozen and aseptically processed food products need use of modified starches. In most of the cases, these are used to have improvement in viscosity, texture, shelf stability, emulsification and processing parameters. Starches vary in their characterization and find their way in food industry for nutritional, organoleptic and aesthetic purposes (Falola et al., 2014). Taking into account that flour made from hard wheat has less extensible gluten and great water requirement providing hard texture biscuits. Thus, these flour were improved by diluting with starch @ 10% like cornstarch, potato starch and arrowroot (Manley, 2011).

Ascorbic acid may exert oxidizing effect on dough characteristics right after its oxidation by atmospheric O₂ (Hruskova and Novotna, 2003). It exhibit a renowned strengthening impact on dough as stated previously by Faccio et al. (2012). Redox prospective of a dough is presented by components which show oxidizing and reducing ability. A certain optimal oxidation level is present for every flour for best baking performance. Though, this optimum potential is occasionally encountered naturally, specific oxidizing agents are usually employed in mill and baking sections to increase baking performance of flour (Hruskova and Novotna, 2003). Addition of some dough modifiers like potassium iodate, N-ethylmaleimide and L-cysteine into cookie formulations significantly influenced values of cookie spread (Gaines, 1990; Kara et al., 2005). Effect of oxidants such as potassium iodate and potassium bromate was investigated by Pareyt et al. (2010). Redox agents were added during cookie baking since it modified the reactivities and rheological properties of gluten without changing other flour components.

Most of the additives are neutral to health and their vigilant use make these important processing aids which provide better, economical, more attractive and safer products (Manley, 2011). Among chemical additives, not only sodium metabisulfite (Na₂S₂O₅) but, other chemical derivatives of SO₂ may also applied to relax and condition dough to aid processing operation (Zydenbos and Humphrey-Taylor, 2003). Sodium metabisulfite (SMS) reveal a dramatic impact on dough quality and develop an extensible gluten from a varied assortment of flour. This alteration in gluten quality considerably improved rheological properties of dough and significantly helped sheeting. From practical point of view, gluten quality can be
tempered though SMS or enzymes (Manley, 2011).

Even though, use of SMS in biscuit dough has been a subject of debate due to associated health concern regarding the effects of SO\textsubscript{2} in food. Nonetheless, levels of SMS used in preserved fruit are far greater as compared to biscuit dough. Thewlis and Wade (1974) explored the residual SO\textsubscript{2} in biscuits when SMS was used into dough. They established only 0.2% remained as sulphite, 10% was evaporated as volatiles, 30% oxidized to sulphate whereas, 60% come into amalgamation with several organic components of flour. Though, it is actually problematic to identify amount of SMS used in dough, probably challenging to decide whether it was added at all, because sulphur atom is a vital component of protein.

It is worthwhile to mention that treatment of flour with enzymes offer an interesting alternative to create changes in dough structure and eventually to improve functional properties of flour. Since, enzyme is protein in nature which is denatured on heating during baking process; so there is no health concern as claimed by some for addition of SMS (Manley, 2011) and has no toxicity problems. Additionally, it does not react with heat stable vitamins. These are generally recognized as safe (GRAS) (Hassan et al., 2014) so, there is no need to mention on label that is an additional market advantage (Coballero et al., 2007). The application of enzymes (proteinase) is becoming popular to modify gluten quality of dough particularly, when high protein content of flour is commonly experienced.

Proteases are added to modify the gluten network and may occasionally be employed in cookie preparation. These create less elastic dough therefore, shrinkage does not take place during sheeting and cutting. Due to the fact that, a dough having too strong gluten decreased spread hence, it becomes necessary to use proteases which improve spread ratio of biscuit, cookie and pastries (Hassan et al., 2014). Basically, these act on wheat flour protein, reduce elasticity of gluten, lowering the shrinkage of dough after moulding and sheeting (Kara et al., 2005; Cauvain and Young, 2006); for instance, gluten protein which causes dough elasticity, their degradation has remarkable effect on spread ratio of cookies (Hassan et al., 2014). It has been reported that papain is one of most efficient enzymes that were initially used to modify quality of wheat gluten (Jian-sheng et al., 2012).

Baking industry requires specific quality of flour to produce various products. Different wheat varieties are cultivated and supplied to flour mills and ultimately baking industry without taking their effect on quality into consideration (Hussain et al., 2000). Production of flour from
such varieties with unknown baking properties poses problems for both miller and bakers (Ahmed et al., 2014). Uncertain and inconsistent quality of wheat flour is also undesirable for processors.

In Pakistan, Provincial Food Departments and government procurement agencies including Pakistan Agricultural Services and Storage Corporation (PASSCO) generally implement support price and purchase wheat from farmers. Nonetheless, the primary objective of government procurements is to ensure food security and supply food to urban population instead of farmer’s protection. However, wheat support price has been main incentive for farmer community to increase more area under wheat cultivation. Food Departments usually store about 65-75% of total wheat production at the farm and the stored grains are offered to flour milling industries and small scale grinders on quota basis.

Flour millers receive a mixture of commercially available wheat varieties either from public and/or private sector for the production of straight grade flour (SGF), which subsequently used for the preparation of different baked products. Since, the production of cookies require a special grade of flour. But, flour is produced from a mixture of wheat varieties (MWF), which appeared to have inconsistent quality and consequently affected product (cookie) quality. Due to inaccessibility of a particular variety suitable for cookie making, the baking industry mainly use mixed wheat flour, which tend to have variation in cookie quality.

Moreover, high levels of fat and leavening agent are added in order to adjust the standard formulation for dough softening but, this led to economy loss for the industry and inflation. Since, wheat, sugar and shortening are the cookie ingredients, which are used in high proportion therefore, increase in the price of these raw materials always have an effect on this industry. The present research would be helpful in reducing the additional amount of fat used in cookie formula for dough softening and subsequently, cost of production.

Against this background, current study attempts to address the issue of baking industry in Pakistan and to modify the quality of mixed wheat flour by using different chemical or dough modifying agents. It is hypothesized that if, different treatments are applied to cookie dough then, baking quality of cookies will be improved. The present study was designed with the following objectives:.
1. Characterization of wheat for numerous physical, chemical and rheological parameters
2. Identification of suitable varieties, treatments and parameters in relation to cookie making quality
3. Correlation of different physicochemical and rheological parameters with cookie quality
Cereals have 60-70% starch and are energy rich source for human. These are recommended as the first food to be included into infant diet by doctors. Also, the healthy diet for adults must obtain most of its calories in the form of complex carbohydrates like cereal grain starch (Chandra and Samsher, 2013). In Pakistan, dietary pattern greatly vary in different regions but largely inclined in favor of cereals (wheat, rice etc.), legumes and meat (Farooq et al., 2001). In this section, some of more relevant literature is reviewed under the following headings:

2.1. Significance of wheat grain
2.2. Wheat flour quality
   2.2.1. Variations in wheat flour quality
2.3. Physicochemical characteristics
2.4. Rheological characteristics
   2.4.1. Farinographic parameters
   2.4.2. Rapid Visco-Analyzer (RVA) parameters
2.5. Scenario of baking industry
2.6. Cookie dough
2.7. Cookie making quality
2.8. Strategies for quality enhancement of cookies
   2.8.1. Modified starch
   2.8.2. Ascorbic acid
   2.8.3. Protease
   2.8.4. Oxidizing agent
   2.8.5. Reducing agent
2.9. Correlation of different parameters
2.1. Significance of wheat grain

Wheat is most popular, staple cereal in Pakistan and consumed in various forms (Panhwar et al., 2014). It is one of grain crops that are directly consumed by human (Wahab et al., 2007) and ranks first in area and production. It provide more than 60% of total protein and calorie requirements (Saeed et al., 2012). Since, it is generally adapted to a range of environment, cultivated in temperate, tropical and subtropical areas (Umrani and Pahoja, 2013). Likewise,
wheat varieties in Pakistan are grown in diverse climatic conditions and thus, expected to have
difference in yield and quality (Gulzar et al., 2010).

Being storehouse of essential nutrients, wheat is important part of human diet (Kumar et al., 2011). Nutrient composition of wheat showed it contain carbohydrate (78.10%), protein (14.70%), fat (2.10%), minerals (2.10%) and substantial amount of B-vitamin and sugars. Moreover, it is a worthy source of minerals (iron, zinc) and trace elements such as selenium and magnesium, which are essential nutrients for good health (Topping, 2007; Kumar et al., 2011). It is superlative among cereals generally due to its grain containing protein with exclusive physical and chemical properties (Hussain et al., 2010).

Parts of wheat grain i.e. endosperm, bran and germ are different in terms of toughness and friability thus, break in different ways on roller mill (El-Porai et al., 2013). These variations are elevated on water addition to wheat before milling in a process termed as tempering or conditioning (Sugden, 2001). Tempering is performed to achieve five objectives: (i) to toughen bran (ii) to soften endosperm means to increase its mill ability (lessening the power consumption by reduction rolls) (iii) to help bran segregation from endosperm, leading to reduction in the power consumed by break roll and eventually minimizing evaporation loss (iv) to make sure easy and appropriate sifting of stocks and lastly; (v) to guarantee moisture level of endosperm is enough to provide a final moisture level of flour in range of 14-15%.

Mostly, soft wheat is tempered to 15.0-15.5% and hard wheat to 16.0-16.5% level (El-Porai et al., 2013).

2.2. Wheat flour quality

Flour refer to fine powder obtained from cereal or other starch based materials particularly from wheat (Adeleke and Odedeji, 2010). It is final product of wheat milling and produced by a mixture of flour streams coming from a range of size reduction and sieving processes termed as streams. Flour streams obtained from a certain cultivar commonly vary in composition (Fustier et al., 2009b). Most of the wheat is processed by roller flour mill to produce refined wheat flour. Particle size reduction in roller mill is carried out by passing cleaned and tempered wheat through a sequential series of break and reduction roll. Flour resulting from each roll is blended to get straight run flour (SRF). Quality of SRF mainly depends on wheat quality and processing conditions (Prabhasankar et al., 2000).

Main factors which influence wheat quality are cultivar, cropping year, harvesting, climatic
and storage conditions (Amjad et al., 2010). Quail (1996) reported that variety and growing environment are two important factors which govern wheat quality while, Nemeth et al. (1994) stated that flour functionality is affected by various factors like protein, growing conditions, crop season and grain hardness. Quality of wheat and flour can be expressed by physical and chemical characteristic of dough but none of individual test can reflect wheat quality completely (Hruskova and Famera, 2003). Technological quality of wheat mainly depends on grain composition and end use (Igrejas et al., 2002).

Physical, chemical and nutritional aspects of wheat grain are more reliable in assessment of wheat quality. Protein content, gluten strength and hardness are basic quality parameters. Protein content predicts the appropriateness of wheat for a certain product as it influences various factors like water absorption capacity and loaf volume (Panhwar et al., 2014). Gluten roughly accounts from 78 to 85% of total protein in endosperm of wheat. Main determining factor for end product quality of wheat is endosperm texture i.e. whether the grain is soft or hard (Pasha et al., 2009a).

The term hard and soft wheat are applied to describe texture of wheat kernel. Greater force is required to disintegrate a hard wheat than soft wheat; similarly, milling of strong wheat into flour produce a coarse particle size of flour than soft wheat (Igrejas et al., 2002). Higher levels of damaged starch and pentosans tend to increase water absorption of flour and result in small diameter cookies (Moiraghi et al., 2013). Use of flour with higher water absorption in cookie making involve increased baking time and energy expenses (Guttieri et al., 2001; Barrera et al., 2007). Strong or hard wheat flour with higher protein has excellent bread making quality (Kaur et al., 2013).

Soft wheat milled into flour having low protein quantity, create a less resistant but more extensible dough. This type of “weak flour” is usually tend to be more desirable in cookie making (Hazelton et al., 2004) and exhibited a large diameter, faster spread rate with a fine grain texture than cookie prepared with strong wheat flour (Miller et al., 1997). Gaines and Finney (1989) revealed that soft wheat flour have largest spread while, hard winter wheat made smaller cookies and hard spring wheat usually produce still smaller cookies.

Ideally, good biscuit making quality has been associated with soft kernel texture, low protein quantity (Finney et al., 1987), low AWRC values and lower percentage of vitreous kernel (Gaines, 1991). Bearing in mind that, flour and sugar are major hydrophilic constituents of a
cookie formulation. In that way, low water absorption of flour aggravates greater water absorption by sugar that increases syrup hence, viscosity of dough is decreased during baking process; in a consequence, dough can spread farther making cookies with large diameter (Slade and Levine, 1994). As good quality cookie flour have low protein content, it is usually agreed that protein composition is of no value in soft wheat. Soft wheat flour (SWF) quality mainly depends on composition as well as properties of starch, protein, lipid and non-starch polysaccharides (NSP) (Moiraghi et al., 2013).

2.2.1. Variations in wheat flour quality

From viewpoint of biscuit manufacturer, consistency in flour quality is a vital aspect and he does not like varying flour quality and, for this purpose, it is muscularly recommended that baker should be in a close liaison with miller/supplier therefore, each realizes other’s problem. It is good practice to have one grade from one mill for each biscuit formulation and not to change even for economic reasons. A great number of biscuits can be prepared with flour which has a low protein with weak and extensible gluten. Thus, flour with a protein level of <9.0% is fine while levels greater than 9.5% often causes processing issues. Major problems can be anticipated where flour is normally delivered from diverse sources (Manley, 2011). Overcoming variations in flour supplies is hence essential and these variations in biscuit flour characteristics can be experienced when flour is kept even for a few weeks. This consequently affect flour protein and mostly the effect can result in less extensible gluten. No approach is yet identified to overcome this, so flour should store for minimum or constant intervals. Flour suspended in unsighted spots of silos can also be a source of variation for this reason. Mixture of wheats is called as “grist”. Miller can produce a flour of desired moisture, ash or color, damaged starch and extraction rate using his expertise and setting the mill correctly (Manley, 2011).

Baking industry requires specific quality of flour to produce various products. Uneven distribution of constituents contained by wheat grain leads to differences in functional and compositional characteristics of various flour streams i.e. break, sizing and reduction achieved through roller milling (Prabhasankar et al., 2000; Berton et al., 2002). Compositional variations in flour produced from various wheat varieties and even flour streams resulting from same variety differ in rheological and baking characteristics (Fustier et al., 2007).
2.3. Physicochemical characteristics

2.3.1. Thousand kernel weight

Thousand kernel weight (TKW) is a valuable criteria for potential milling yield (Ghaffar et al., 2013). It is extensively applicable physical test and simple criteria to evaluate wheat quality related to its density and soundness (Mueen-ud-Din et al., 2007). Arif et al. (2002) determined highest grain weight could be achieved at 50 kg/ha seed rate. Also, Khan et al. (2002) and Mehrvar and Asadi (2006) recognized that 1000 grain weight is reduced by increasing seed rate. Similar results were also established by Laghari et al. (2011) who studied the influence of various seed rates on thousand kernel weight and reported values of grain weight as 41.4 (125kg/ha), 35.9 (150 kg/ha), 30.6 (175 kg/ha) and 24.8 g (200 kg/ha). He adduced that 1000 seed weight and yield was reduced at high seed rate due to increased lodging due to tall plants. Rafique et al. (1997) reported an increase in kernel weight at wider row spaces. Several studies have reckoned thousand kernel weight in Pakistani wheat varieties ranged 36.60-41.20 g (Amjad et al., 2010) and 35.93-45.73 g (Naseem et al., 2011). Sheikh et al. (2014) reported thousand kernel weight ranged from 35.66 to 40.2 g (dry land) and 44.16 to 47.00 g (wet land) wheat varieties grown in Sindh. Anjum et al. (2008) documented thousand kernel weight in wheat varieties of different eras ranged 28.81-49.01 g. Thousand kernel weight in wheat varieties cultivated during two consecutive crop years ranged from 26.02 to 49.03 g (Pasha et al., 2009a). TKW of Indian wheat cultivars ranged 34.3-45.7 g (Kaur et al., 2013) and 40.7-42.0 g (Kumar et al., 2013).

2.3.2. Test weight

Test weight (TW) refers to weight of a predetermined grain volume and deliberates a rough estimation of grain size and shape (Iqbal et al., 2015b). Jafri (2010) stated that wheat varieties having higher test weight will also have more flour yield. In Pakistan, test weight of wheat varieties ranged from 66.47 to 80.50 kg/hl as reported in earlier studies (Mueen-ud-Din et al., 2007; Khan et al., 2009; Safdar et al., 2009). Test weight in wheat varieties cultivated during two consecutive crop years ranged from 64.0 to 79.5 kg/ hl in a previous study (Pasha et al., 2009a). Martin et al. (2001) measured test weight in 130 hard red spring wheat recombinant inbred lines cultivated in various locations of USA ranged between 66.20 and 80.20 kg/ hl. Anjum et al. (2003) find out that wheat grains demonstrated test weight in the range of 74.80-
76.27 kg/hl. TW of two Indian wheat varieties varied between 79.5 and 82.3 kg/hl as stated earlier by Kumar et al. (2013). Test weight is deliberated as one of essential criteria in all wheat grading systems and associated with flour yield (Ahmed et al., 2014). As mentioned above, TW is a rough index of kernel density and regularity of size (Kumar et al., 2013). Variation in test weight observed in wheat cultivars might due to incongruity in genetics and environmental conditions, which prevailed during growth (Randhawa et al., 2002).

2.3.3. Moisture content

Moisture is enormously vital in any measurement related to texture of wheat kernel (Pasha et al., 2006). It predicts storability and profit margins in milling (Iqbal et al., 2015b). Moisture level in some Pakistani wheat varieties ranged between 11.78 and 13.42% as described previously (Farooq et al., 2001; Mueen-ud-Din et al., 2007; Khan et al., 2009). High moisture augments proteolytic and lipolytic activities causing nutrient loss (Jafri, 2010) and decrease storage time and flour yield as well (Anjum et al., 2003). Moisture content below 14% is considered safe for cereal storage (Manay and Shadaksharaswamy, 1987). Amjad et al. (2010) recorded moisture content in wheat varieties acquired from different areas of Punjab was found to be 12.92-13.32%.

Panhwar et al. (2014) reckoned moisture content ranged from 11.50 to 13.06% (wet land) and 10.9 to 12.66% (dry land) wheat varieties grown in Sindh. At low moisture level, there are chances that grains may be brittle and break during commercial handling operations, but this is generally the case for wheat grain, which is artificially dried to lower the moisture content (Arif et al., 2007). Moisture content in wheat flour samples ranged as 10.04-10.63% (Feyzipour et al., 2006), 12.8-14.0% (HadiNezhad and Butler, 2009) and 13.91-14.61% (Kumar et al., 2013). Moisture content is determined by genetic makeup of wheat cultivars, agronomic and climatic conditions (Mahmood, 2004). Moisture content in different wheat flour samples ranged 9.52-10.23% (Iqbal et al., 2015b).

2.3.4. Ash content

Ash content is an indication of mineral level in food sample (Olaoye et al., 2007). It is measurement of thoroughness by separating bran coat from endosperm. It also predicts flour yield and specifies milling efficiency by indirectly indicating bran contamination in flour (Amjad et al., 2010). Ash content in Pakistani wheat varieties varied from 0.50 to 0.60% (Farooq et al., 2001), 0.37-0.47% (Sameen et al., 2002) and 0.41-0.55% (Khan et al., 2009). It
influences flour color since, high level of ash imparts a dark color to end product; also it has an inverse relation to quality primarily milling process (Iqbal et al., 2015b). Anjum et al. (2008) figured ash content in wheat varieties of different eras ranged 1.34-1.85%. Ash in wheat flour was found to be in range of 0.50-0.60% by past authors (Hussain et al., 2006; Butt et al., 2007; Alam et al., 2009; Masih et al., 2014). Wheat cultivars with low ash level contain larger endosperm and eventually result more flour yield or flour extraction (Keran et al., 2009). High ash level refers to inferior wheat quality with high percentage of small or shriveled kernels (Safdar et al., 2009). Fustier et al. (2007) explained that high (0.99%) ash content of clear fraction possibly due to aleurone layer extraction which is attached to bran and contain high level of mineral. This further ratify that clear flour is made basically from kernel periphery at the end of grinding, essentially rich in ash, protein and pentosan as described by Prablasankar et al. (2000).

2.3.5. Fat content
Lipids play a vital part in determining wheat flour quality and its suitability for different baked products, which may change due to seed storage condition (Buriro et al., 2012). It is also important due to the fact that it relates to shelf life of foods since it can promote rancidity, causing development of unpleasant and odorous compounds (Okpala and Ekwe, 2013). Fat content of wheat varieties/lines varied 1.25-1.40% (Faroq et al., 2001) and 0.82-0.92% (Sameen et al., 2002). It is indispensable for appropriate gluten development of flour also because wheat oil is less stable and rapidly tends to rancid (Iqbal et al., 2015b). Previous studies found fat content in wheat flour varying from 1.11 to 1.80% (Hussain et al., 2006; Butt et al., 2007; Alam et al., 2009).

2.3.6. Fiber content
Fiber is one of essential nutrients which are required for health improvement and to combat numerous diseases. Fiber content is thought to be significantly associated with mineral and ash; levels of all the components tend to increase with an increment in yield and extraction rate of flour (Lodi and Vodovotaz, 2008). Wheat varieties portrayed fiber content ranging from 0.38 to 0.40% (Faroq et al., 2001) and 0.12 to 0.14% (Sameen et al., 2002). Wheat flour had fiber varying from 0.28 to 0.82% as reported by earlier studies (Hussain et al., 2006; Butt et al., 2007; Mebpa et al., 2007; Alam et al., 2009) whereas, much high levels of fiber content (1.03-2.36%) were reported by Ahmedani et al. (2009) and Mahmood et al. (2013).
2.3.7. Protein content

Protein is vital component of wheat enormously affecting functional, nutritional and technological characteristics of flour (Iqbal et al., 2015b). It is an essential criteria in assessment of wheat quality (Channa et al., 2015). It play a major role in wheat flour rheology (Gulzar et al., 2010) and cookie quality (Finney et al., 1987). Kim et al. (2004) reckoned protein content 8.7-10.6% in soft wheat and 10.8-12.0% in hard wheat flour. Hard wheat having high protein create a fully developed gluten matrix in dough mixing and consequently, rise bread loaf volume. High protein content is not suitable for majority of products made with soft wheat because these do not require an extensive gluten development (Gaines, 2004). Ahmed et al. (2014) investigated protein content ranged from 9.18 to 10.53% at different temperature and moisture levels. Previous studies indicated levels of protein among Pakistani wheat varieties varied between 9.20 and 13.45% (Anjum et al., 2005; Mueen-ud-Din et al., 2007; Ahmedani et al., 2009).

Panhwar et al. (2014) exhibited that protein content ranged from 13.70 to 14.83% (wet land) and 10.80 to 11.90% (dry land) wheat varieties of Sindh. Protein content in different wheat varieties was affected by seed rate as 10.15-11.55% (50 kg/ha), 9.22-11.02% (100 kg/ha), 9.45-11.52% (150 kg/ha) and 9.60-11.50% (200 kg/ha) with highest protein content of 11.55% recorded at 50 kg/ha (Hussain et al., 2010). Barrera et al. (2007) referred that protein content in wheat cultivars varied with disc mill time as 11.6-14.3 (0 min), 11.9-14.1 (2 min) and 11.7-13.9% (5 min). El-Porai et al. (2013) evaluated the effect of milling and observed that protein level in Egyptian wheat ranged 9.81-12.40 (normal milling) and 10.13-13.54% (hard milling) at different conditioning time (h).

Fustier et al. (2007) argued that protein content increased from 7.41 to 9.65 and 12.7% in patent, middle-cut and clear fractions, respectively, this might due to difference in cytoplasmic source of products resulting from distinct SWF. This elevated protein may be credited to rising level of peripheral endosperm portion which is known to have high ash, lipid and protein (Prablasankar et al., 2000). Igrejas et al. (2002) corroborated protein content in range of 8.8-18.5%. In previous work, protein content in wheat flour ranged from 7.4 to 15.8% (Hrušková and Faměra, 2003; Xiao et al., 2006; HadiNezhad and Butler, 2009).

2.3.8. Wet and dry gluten

Gluten referred to a “cohesive, viscoelastic and proteinaceous mass obtained by way of starch
isolation from wheat flour (Pasha et al., 2007) by washing to remove starch granules and water soluble components (Kaur et al., 2013). Basically, it is composite of two protein fractions namely gliadin and glutenin that coexist with starch in endosperm of some grass-related grains like wheat, barley and rye (Choudhary et al., 2013). Taken together, gliadin and glutenin consists of 80% of total protein present in wheat (Rosin, 2009) and are collectively known as gluten on mixing with water. On the basis of physical properties of grain, the miller describe wheat as hard, medium and soft. Hard wheat exhibit higher protein quantity and quality as well as a vitreous endosperm along with starch granules firmly packed in protein network (Hazelton et al., 2004).

Gluten strength is main parameter influencing the mixing properties, extensibility and resilience of dough. Usually, it is a function of both composition and concentration of protein. Greater dough resilience of hard wheat flour may pose some difficulties in cookie making process (Kara et al., 2005). It is responsible for dough strength and give texture to bakery products. Greater level of gluten is required in bread production whereas, low gluten level is desirable in biscuit and cookie making (Kumar et al., 2013). It is of technological importance for biscuit making since, it develops protein matrix surrounding starch granules, mainly add to this internal structure; which is responsible for textural quality (Fustier et al., 2007). Wet gluten in wheat varieties ranged 29.45-33.56% (Mueen-ud-Din et al., 2007) and 28.7-33.6% (Barrera et al., 2007).

Gluten content determines flour quality and has significant effect on bread making quality. Wheat proteins have great contribution to rheological properties of wheat dough. Any alteration in concentration or chemical configuration of protein in wheat considerably changes dough rheology and bread making characteristics of flour (Gulzar et al., 2010). Wet gluten in wheat flour ranged from 19.47 to 30.37% (Ahmed et al., 2014).

Since, dry gluten (DG) holds no water, it may have direct association with protein, which is an indication of flour strength and bread making quality as stated by Anjum and Walker (2000). The levels of dry gluten in wheat varieties observed in the range of 8.72-10.69% (Mueen-ud-Din et al., 2007) and 10.49-13.60% (Khan et al., 2009). Both wet and dry gluten are associated with ability of protein within wheat varieties (Gulzar et al., 2010). Panhwar et al. (2014) investigated that dry gluten ranged from 7.93 to 9.03% in wet land and from 7.53 to 8.20% in dry land wheat varieties of Sindh. Curic et al. (2001) probed dry gluten in wheat flour of
different varieties varied between 8.44 and 11.77%.

2.3.9. Starch and damaged starch content

Starch is most imperative carbohydrate in human diet (Kumar and Prabhasankar, 2013) and contributes 67-68% of whole wheat grains (El-Porai et al., 2013). Cereal grains store energy in form of starch (Belderok et al., 2000). Grain endosperm being rich in starch is used as energy food (Anderson, 2004). Panhwar et al. (2014) demonstrated starch content ranged from 69.90 to 75.83% in wet land and 61.03 to 68.66% in dry land varieties of Sindh. During milling process, starch granules intact to gluten proteins are invisibly damaged which in turn increase surface area leading to higher water absorption (Hazelton et al., 2004). It is well recognized that mechanic activities alter physicochemical properties, digestibility and configuration of starch granules (Becker et al., 2001; Huang et al., 2007). Soft type possess less compact starch-protein matrix resulting in low damaged starch (DS) and consequently, low water absorption (Hazelton et al., 2004). It seems difficult to decrease particle size of hard wheat; hence, such flour show greater particle size as compared to soft wheat (Barrera et al., 2007). DS content in wheat varieties ranged 4.0-10.0% (Farooq et al., 2001; Meintjés, 2004) and 4.00-6.25% in whole wheat flour (Iqbal et al., 2015b).

Damaged starch readily absorb water and more susceptible to α-amylase hydrolysis. Published data evidenced that DS aggravates water absorption up to 200-430% (Zivancev et al., 2012). Hard wheat always yield more DS (Barrera et al., 2007) since, high force and pressure is required to grind thus, causing damage to starch granules (Kweon et al., 2009). Tsilo et al. (2011) and Duyvejonck et al. (2012) revealed that hard wheat has starch granules which are usually imperiled to more intense damage comparing these to soft wheat granules under similar milling specifications. Therefore, it also need to exert high energy to disintegrate or reduce to yield fine flour while, lesser energy is required to reduce soft wheat resulting lower percentage of DS in soft wheat (Yu et al., 2015). El-Porai et al. (2013) explained the influence of milling on DS in Egyptian wheat observed from 3.41 to 7.10 (normal milling) and 3.62 to 7.59% (hard milling). In another study, Barrera et al. (2007) re-milled two wheat cultivars using a disc mill at different time periods and DS levels ranged as 8.4-9.3% (0 min), 12.8-14.7 (2 min) and 17.2-17.7% (5 min).

2.3.10. Alkaline water retention capacity (AWRC)

AWRC is usually considered as an indirect measure of protein quality and its low values are
thought as a predictor for soft wheat quality since, it is inversely related to cookie spread (Fustier et al., 2009a). In work of Ram and Singh (2004), AWRC values in wheat varieties were investigated in the range of 57-80%. AWRC in 6 wheat flour samples collected from flour mills in different cities of Punjab ranged 25.00-47.00% (Anjum et al., 2002) whereas, AWRC in various wheat varieties cultivated during two consecutive crop years ranged from 59.06 to 96.86% (Pasha et al., 2009a). AWRC ranged from 52.2 to 73.2% in flour and from 74.8 to 80.7% in wheat samples (Bettege et al., 2002). AWRC was found positively correlated to DS and water-soluble pentosans and subsequently, to SOCSRC and SUCSRC as well. These associations evidenced the significance of these flour components in AWRC. Likewise, a high correlation was observed between AWRC and WSRC due to the fact that both factors are influenced by similar flour constituents (Colombo et al., 2008).

2.3.11. Solvent retention capacity (SRC)

SRC test is similar to AWRC however, it gives additional knowledge (Ram and Singh, 2004). Characteristics of wheat flour depending on SRC highlight chemical, rheological and baking characteristics (Pasha et al., 2009b). Practically, it embodies flour quality and functionality that is valuable for expecting baking quality as reported by AACC (2000) and Walker et al. (2008). The suitability of this method to predict soft wheat quality has been comprehensively corroborated by past authors (Guttieri and Souza, 2003; Guttieri et al., 2004). Different tests are available to measure or evaluate gluten quality (Colombo et al., 2008). Selection of a particular method depends on numerous factors for instance country or wheat class (Gaines et al., 2006).

As discussed earlier, WSRC is dependent on all the four components (Guttieri et al., 2001). WSRC in different wheat varieties ranged 78.0-98.0% (Pasha et al., 2009b), 53.4-70.6% (Ram et al., 2005) and 55.0-77.0% (Ram and Singh, 2004). Barrera et al. (2007) examined WSRC in wheat cultivars varied from 65.0-83.9%. WSRC values ranged 49.0-60.8% in wheat flour and 69.2-81.4% in wheat varieties (Bettege et al., 2002).

SOCSRC is a measure of damaged starch or it is associated with damaged starch implying an indirect measure of hardness (Gaines, 2004) and positively related to both damaged starch and water soluble pentosans (Colombo et al., 2008). Several articles reported SOCSRC values in wheat varieties ranged 95.0-127.5% (Pasha et al., 2009b), 90.7-110.8% (Guttieri et al., 2004).
and 65.0-114.0% (Ram and Singh, 2004). In another study, Barrera et al. (2007) found SOCSRC values in wheat varieties ranged 78.1-109.6%.

SUCSRC is mainly contributed by pentosan and gliadin (Guttieri et al., 2001). Gaines (2004) recommended that SUCSRC values may reliably predict sugar-snap cookie diameter. Several publications reported the values of SUCSRC in wheat varieties ranged 125.0-163.0% (Pasha et al., 2009b) and 77.0-109.0% (Ram and Singh, 2004). Souza et al. (2012) proclaimed that low SUCSRC response perhaps due to low pentosans, could also indirectly assist in the choice of high flour yield varieties. Since, sucrose retention capacity relates to pentosan, present in seed coating, their level being typically greater in whole meal groats as compared to flour (Kweon et al., 2011). SUCSRC in wheat cultivars ranged between 86.3 and 90.6% as reported earlier (Barrera et al., 2007).

Use of LASRC in order to consider bread quality of hard winter wheat is proposed by Xiao et al. (2006), but there is limited data regarding application of this method as an analytical tool in bread wheat products (Colombo et al., 2008). LASRC in wheat varieties ranged 101.5-139.0% (Pasha et al., 2009b), 72.0-122.8% (Ram et al., 2005) and 81.6-108.0% (Guttieri and Souza, 2003). In an earlier study, Zhang et al. (2007) reported that diameter of cookies from Chinese wheat can be projected by SUCSRC and particle size up to 83%. LASRC values indicated positive correlation with damaged starch as both values increase in coordination. It has been reported earlier that greater values of glutenins made biscuit of smaller diameter due high dough elasticity (Wieser et al., 2003). LASRC values in wheat varieties ranged between 102.5 and 132.6% (Barrera et al., 2007).

2.3.12. Sodium dodecyl sulphate (SDS) sedimentation

SDS sedimentation test is an assessment of wheat flour strength and indirect gluten quality. This test is influenced by hydration capacity of flour proteins and their sedimentation through SDS acting as detergent to eliminate oil, which adsorbed to protein molecule and to assist their hydration ability (Ahmed et al., 2014). SDS sedimentation values in whole wheat flour of different varieties varied from 18.17 to 28.33 ml (Randhawa et al., 2002), 17.0 to 32.0 ml (Pedersen et al., 2004) and 27.33 to 30.32 ml (Iqbal et al., 2015b). SDS-sedimentation in wheat varieties ranged 19.67-36 mL (Mueen-ud-Din et al., 2007; Pasha et al., 2007). Sedimentation volume of flour is mainly related to protein and kernel hardness; wheat cultivars having low SDS-sedimentation could be most desirable for cake and cookies (Pasha et al., 2009a). While,
wheat cultivars presenting greater values of sedimentation values were categorized as strong wheat and can be used for yeast leavened products (Randhawa et al., 2002).

2.4. Rheological characteristics

For cereal technologists, rheology is recognized as a remarkable instrument for flour quality evaluation (Hadnadev et al., 2011). Cereal scientists employ rheological tests throughout processing chain to observe mechanical characteristics, molecular structure and composition, and behavior of materials during processing and to assess end product quality (Dobraszczyk and Morgenstern, 2003). Rheometry estimates important characteristics of flour viscosity, elasticity and plasticity pertaining to dough behavior and end product quality (Bloksma and Bushuk, 1988). Parameters selected as major rheological characteristics include water absorption, dough development time, dough stability and softening of dough (Mueen-ud-Din et al., 2007).

2.4.1. Farinographic parameters

Brabender Farinograph is a great dynamic dough analysis equipment and most commonly recognized for determining physical properties of dough (Hadnadev et al., 2011). It is normally used for determination of water absorption in flour, particularly in industrial settings (Mondal and Datta, 2012). It provides knowledge about mixing behavior of flour by recording the mechanical resistance of dough during process of mixing and kneading. It is also used to compare different rheological parameters of dough (Liu et al., 2005). Different baked products need wheat flour with varying quality (Diosi et al., 2015). It is able to maintain the effect of additives and therefore, optimization of flour processing to standardize flour quality made with varied quality of raw materials (Hadnadev et al., 2011). Rheological properties of dough like farinograph properties are related to predict mixing properties, sheeting and baking quality of food product (Nikolic et al., 2013).

From practical point of view, water absorption is a farinographic parameter which is of immense importance and represents quantity of water that could be absorbed by a specific amount of flour (Channa et al., 2015). Farinographic water absorption in wheat varieties ranged 54.40-58.00% (Farooq et al., 2001) and 53.33-55.83% (Mueen-ud-Din et al., 2007). It has a direct relation with yield of end product and tend to be one of most significant factors in evaluating “flour strength” (Hadnadev et al., 2011). Earlier studies found water absorption in wheat flour varied from 51.5 to 63.0% (Konopka, 2004; Pedersen et al., 2004; HadiNezhad
Dough development time (DDT) refers to elapsed time from start of kneading until maximum consistency is reached (Hadhadev et al., 2011). DDT in wheat varieties ranged 2.00-3.75 minutes (Farooq et al., 2014) and 3.53-4.63 min (Mueen-ud-Din et al., 2007). Values of DDT in wheat flour ranged from 1.5 to 2.0 min (HadiNezhad and Butler, 2009; Paraskevopoulou et al., 2010; Mohammed et al., 2012; Voicu et al., 2012).

Dough stability denotes time through which maximum dough consistency undergoes no change or varies slightly. It is a gluten quality indicator describing viscoelastic behavior (Hadhadev et al., 2011). Dough stability in wheat varieties varied 1.50-17.25 min (Farooq et al., 2001) and 6.27-8.13 min (Mueen-ud-Din et al., 2007). Mahmood (2004) reported dough stability in wheat varieties ranged 1.5-19.0 min. Dough stability in flour samples ranged from 1.40 to 6.10 min (HadiNezhad and Butler, 2009; Paraskevopoulou et al., 2010; Mohammed et al., 2012).

Degree of dough softening is presented on farinograph curve for a kneading period of 10 minutes (using the ICC) (Voicu et al., 2012). Softening of dough in wheat varieties fall within the range of 70-100 BU (Anjum et al., 2002) and 59.00-80.33 BU (Mueen-ud-Din et al., 2007). Similar to dough stability, DoS is also a gluten quality indicator which defines the viscoelastic behavior of gluten network formed (Hadhadev et al., 2011). DoS of flour varied from 40 to 80 BU (HadiNezhad and Butler, 2009).

2.4.2. Rapid Visco-Analyser (RVA) parameters

RVA is a versatile and valuable instrument for evaluating pasting properties of starch. Main advantages of RVA as compared to Brabender Amylograph/Viscograph include: only a small sample of 3-4 g (starch, wholemeal, or flour) is required, the analysis is rapid and data is recorded directly by computer (Bhattacharya and Corke, 1996). Pasting curve gained from RVA is an extent of viscosity of starch or cereal suspension in heating cycle revealing molecular events present in starch granules (Majzoobi et al., 2011). Pasting parameters of starch are usually thought to be measured through relation of starch structural properties, involving starch morphology and rigidity (Yu et al., 2015).

Peak viscosity of untreated flour was observed to be 2137.7 cP (Jian-sheng et al., 2012) and 2621cP (Gomez et al., 2008). Peak viscosity of wheat flour was recorded as 1125BU (Ojinnaka et al., 2009) and 195.01 RVU (Falola et al., 2014). Peak viscosity of starch resulting from
various flour millstreams varied from 1635 to 1743 cP for hard wheat and from 1678 to 1921 cP for soft wheat (Yu et al., 2015).

Trough viscosity which demonstrates minimum hot paste value and is a measure of holding strength of dough at that temperature is strongly related to end quality (Ragae and Abdel-Aal, 2006). Trough viscosity of starch resulting from various flour millstreams varied 1310-1641 cP for hard wheat and 1375-1668 cP for soft wheat (Yu et al., 2015). Furthermore, trough viscosity of untreated wheat flour was observed as 1385.7 cP (Jian-sheng et al., 2012).

Final viscosity of wheat flour was recorded as 2524.3 cP (Jian-sheng et al., 2012) and 3213 cP (Gomez et al., 2008). Final viscosity of starch obtained from various flour millstreams varied from 1814 to 2022 cP for hard wheat and from 1873 to 1975 cP for soft wheat as reported by Yu et al. (2015). In another study, final viscosity of wheat flour was noted as 30.55 RVU (Falola et al., 2014).

Breakdown viscosity of wheat flour during heating indicates the difference between peak viscosity and viscosity at the start of first holding phase (Alam et al., 2009). Breakdown viscosity, the difference between peak viscosity and minimum viscosity, was further used to study response of starch to shear thinning during 93°C hold (El-Porai et al., 2013). Breakdown viscosity of wheat flour was established as 751.3 cP (Jian-sheng et al., 2012) and 893.0 cP (Gomez et al., 2008). Breakdown of starch in various flour millstreams varied from 177 to 326 cP for hard wheat and from 252 to 369 cP for soft wheat (Yu et al., 2015).

Setback is reclamation of viscosity during cooling of heated suspension of starch and also an indication of short term retrogradation (Kaur et al., 2013). Setback viscosity of wheat flour was observed as 1485.0 cP (Gomez et al., 2008). Setback correlates with anti-staling effects, represents shelf life of end products (El-Porai et al., 2013). Setback of starch in various flour millstreams varied from 381 to 526 cP for hard wheat and 307 to 446 cP for soft wheat (Yu et al., 2015). The earlier studies have reported pasting temperature of wheat flour varying from 64.45°C (Jian-sheng et al., 2012) to 78.44°C (Falola et al., 2014). Gelatinization temperature of wheat flour was reported as 72°C (Ojinnaka et al., 2009). Pasting temperature in flour resulting from different break and reduction flour streams ranged from 84.4 to 90.9 cP for hard wheat and 87.2 to 90.1°C for soft wheat (Yu et al., 2015).

2.5. Scenario of cookies in baking industry

Baked products based on wheat flour are popular foodstuff worldwide and are widely
consumed in subcontinent particularly, in Pakistan (Butt et al., 2007). Among baked products, cookies are essentially used as snacks by children and adults as well (Hussain et al., 2006). Cookies are given to infants as weaning foods furthermore, school going children consume biscuits as snacks while at school. Cookies offer a valuable vehicle for nutritional enhancement and are recommended as a better use as compared to bread for their wide consumption, ready-to-eat (RTE) form and extended shelf life (Arshad et al., 2008). Biscuits are concentrated source of energy and provides two time more energy than bread (Masih et al., 2014). In Pakistan, there has been remarkable growth in cookie manufacturing accredited to introduce innovations, improved distribution and increased product varieties. In addition to the regular distribution all over the country, cookies have also been distributed as one of basic foods during natural catastrophes.

The term “cookie” refers to a small, sweet, flat bakery product, prepared normally from cereal flour whereas equivalent term “biscuit” has specific meanings of twice baked representing lower moisture of this family of baked products. Cookie is originated from Dutch word, “koekje” that meaning small cake, according to geographical location, the term cookies is used in USA while biscuit in UK, Australia and NewZealand (Hazelton et al., 2004). Basic property which discriminate biscuit, cookie and crackers from other bakery products like bread or cake, is their distinct moisture (<5%) (Zydenbos and Humphrey-Taylor, 2003). Hazelton et al. (2003) reported moisture content of bread (35-40%), cakes (15-30%) and cookie/biscuits (1-5%). Due to low moisture content, there is less risk of spoilage by microorganisms. Weak flour having <9.5% protein is used for cookies but, there is no rule for proportion of sugar and shortening (Zydenbos and Humphrey-Taylor, 2003).

Three major ingredients commonly used of cookie making include flour, shortening and sugar hence, these are used in large proportion. Wheat flour is the main ingredient (HadiNezhad and Butler, 2009) however, the amount of fat and sugar solution found in a system produce a plastic and cohesive dough with minimum development of gluten matrix and therefore lacking elasticity. In that way, there is no continuous protein network, due to low moisture (Manley, 2000) and higher sugar and shortening levels (Sciarini et al., 2013). Biscuit, cookies, cracker contain a cereal base (at least 60%) and in most of the cases, it is commonly wheat however often rice, oat, rye and barley may also be used. Other constituents which can be added in cookie dough are salt, syrup, emulsifier and chemical agents although, these are added
relatively at low level (Pareyt and Delcour, 2008).

2.6. Cookies dough

Cookies are made with dough resulting from a mixture of flour with other ingredients, which underwent mixing and resting for a period and passed through rollers to make sheet (Chizoba, 2014). Consistency of dough have direct relation to its water absorption which is influenced by several factors. Of total absorption of flour, about 46% is related to starch, 31% to protein and 23% to pentosan content (Hazelton et al., 2004). In cookie making, mixing is carried out to evenly distribute ingredients and improve water absorption, instead of evolving a true dough network. Owing to their high amount of fat and sugar, the gluten development is restricted (Pareyt et al., 2008). Nonetheless, protein of soft wheat flour is considered not to be functionally inert in cookie formulation, particularly in baking. Cookie dough components greatly affect dough handling, cookie baking and quality of end baked product (Pareyt and Delcour, 2008).

Hazelton et al. (2003) characterized biscuit dough as hard and soft dough whereas, Wade (1988) categorized into three groups including hard, short and soft dough as used in England and also cutting machine, rotary and wire cut dough in US. Hard dough have tight and much stiff consistency which require extensive mixing or work resulting an increase in dough temperature. Viscoelastic characteristics of hard dough indicate occurrence of three dimensional well developed gluten structure during mixing and sheeting (Hazelton et al., 2003). These resemble to bread dough i.e. both elastic and extensible (Zydenbos and Humphrey-Taylor, 2003).

Short dough are like cake dough due to high fat content. Increase fat content decrease dough extensibility so it break easily as there is little mixing of flour so, gluten network is minimized. Their consistency is like wet sand which hold together under pressure however, crumble with great ease. A variety of biscuit and cookies made worldwide are from short dough and subsequently, offer a huge range of size, shape, flavor and ingredients. Short dough is mixed in two stages firstly, letting the creaming of fat and sugar while in modern “all-in” mixing process. These dough have consistency of being cohesive and plastic however, lack elasticity and extensibility e.g. rotary molding and wire cutting.

Contrasting to hard dough that undergo shrinkage during baking process, short dough spread possibly due to their high sugar and fat levels. Soft dough is a subgroup of short dough i.e.
high sugar and fat. These have pourable consistency and require fat (65-76%), sugar (35-40%), weak flour and mixing in two stages. Flour and other ingredients are introduced at the end with minimum mixing action to avert dough being tough. These result in soft, delicious, melt in mouth feel but are fragile and tend to breakage thus, offer difficulties in packaging (Zydenbos and Humphrey-Taylor, 2003).

2.7. Cookie making quality
A standard AACC International sugar-snap cookie baking test is executed in order to evaluate quality of SWF by calculating diameter and spread factor of cookies reported previously by Nemeth et al. (1994) and Method 10-52 AACC (2000). Flour components, which absorb water and quality and content of gluten affect the cookie quality most. Large diameter and high spread ratio are characteristics of soft wheat having low protein with weak gluten strength (Pasha et al., 2009b). Cookie diameter is a tremendously important parameter of baking quality in relation to soft wheat (Hoseney et al., 1988). It is main parameter measured during cookie baking and may be defined by rate at which dough spreads and by rate at which dough sets up (Gaines and Finney, 1989).

Width is suggested as most reliable evaluation of flour quality (HadiNezhad and Butler, 2009). Jacob and Leelavathi (2007) stated that cookies from sunflower oil had higher width (8.8cm) as compared to non-emulsified shortening ‘dalda’ (8.1cm). HadiNezhad and Butler (2009) reported width of six type of flour varied from 7.36 to 7.80 cm whereas, Butt et al. (2007) presented width of wheat flour cookies (WFC) as 2.43 cm. Thickness of wheat flour cookies was observed as 43.33 mm (Hussain et al., 2006) and 11.0 mm (Siddiqui et al., 2003). Spread factor of wheat flour cookies was ranged from 36.80 to 58.33 (Pasha et al., 2002; Siddiqui et al., 2003; Hussain et al., 2006; Butt et al., 2007; Masih et al., 2014).

2.8. Strategies for quality enhancement of cookies

2.8.1. Modified Starch
Modification of starch alters its properties such as water absorption, pasting, gelling, digestibility, and many other characteristics according to requirement. There are different methods to modify starch properties i.e. through physical, chemical and enzymatic or combination of these. Starch modification can be achieved to increase their stability against diverse conditions such as extreme heat, shear, acidic condition, cooling or freezing to alter texture, to decrease or increase viscosity. These are usually modified to have improved
performance in various applications (Kumar and Prabhasankar, 2013). Some investigations were focused on use of various chemical treatments to alter starch properties (Singh et al., 2007). In work of Ojinnaka et al. (2009), cocoyam starch was modified by acid and enzyme treatments. These starch samples were substituted with wheat flour at different levels for the production of cookies. After two month storage in polythene bags, cookies produced with 5 and 10% substitution levels were reported to be most acceptable.

2.8.2. Ascorbic acid
Treatment of flour with small level of oxidizing compounds is a legitimate practice. Impact of oxidizing agents is twofold i.e. flour improvement and bleaching. Flour improvement tends to alter rheological characteristics of dough while, flour bleaching is responsible to destroy yellow pigments, eventually resulting in white flour and bread. Ascorbic acid, though being itself a reducing agent, may apply an oxidizing impact on characteristics of dough after its oxidation by atmospheric oxygen. Occurrence of thiol groups in dough suggests that ascorbic acid is active only with the presence of oxygen and its impact in resting fermenting dough is largely decreased reducing effect. After resting period, ascorbic acid showed a noticeable strengthening impact on gluten matrix triggered by an increase of its protein cross-linking (Hrušková and Novotná, 2003).

2.8.3. Protease
Commercially, protease has long history of usage applications in production of baked products, bread, crackers. These enzymes may be added to reduce dough consistency, ensure dough uniformity, reduce mixing time, maintain gluten strength in bread and to improve texture and flavor (Goesaert et al., 2005). Moreover, protease has become a replacement of bisulfite previously used to regulate dough consistency by reducing disulfide bonds in gluten protein whereas, proteolysis degrades peptide bonds (Hassan et al., 2014). In each case, final impact is similar weakening of gluten network (Ganzle et al., 2008).
Proteases are also used to make biscuit, cookie and pastries. These act on wheat flour protein, reduce elasticity of gluten and hence, lowering dough shrinkage after moulding and sheeting (Cauvain and Young, 2006); for example degradation of gluten protein which causes dough elasticity, has remarkable effect on spread ratio of cookies (Kara et al., 2005). Above all, papain was evaluated as one of first and more effective enzymes used to modify quality of gluten. Dynamic rheology also indicated that use of enzymes like amylases, proteases and bug
damaged wheat flour (up to 10%) caused less elastic dough (Koksel et al., 2001).

### 2.8.4. Oxidizing agent (potassium iodate)

Gluten-modifiers like oxidizing and reducing (redox) agents are employed to govern rheological characteristics of wheat flour (Sandhu et al., 2011). Cookie dimension and break strength excluding their weight, were observed significantly affected by adding redox agents. Adding protein modifying agents at low levels, though, affected neither macroscopic dough properties (hardness, elasticity) nor cookie (weight, dimension) parameters significantly. This is due to the fact that in a cookie formula, whether gluten is undeveloped or inaccurately plasticized likely due to lower water and higher sugar and shortening levels (Pareyt et al., 2010).

However, similar concentration of these oxidizing and reducing compounds had a marked influence on developed bread and cookie formulations (Pedersen et al., 2005). Moreover, comparatively high cookie pH than bread or cakes may possibly decreased reactivity i.e. oxidizing capacity of oxidizing agents added (Wieser, 2003). To compensate low reactivity of oxidizing and reducing agents, different additives were added at considerably greater levels. During baking process, set time and collapse of cookie dough were considerably influenced by high concentration of these redox compounds. Adding potassium iodate at much high levels improved cookie width while, reduced height and break strength of cookies (Pareyt et al., 2010).

### 2.8.5. Reducing agent (Sodium metabisulfite)

Sodium metabisulfite (SMS) has a dramatic impact on dough quality leading to extensible gluten even from a varied range of flour. This alteration to gluten quality considerably changed rheological behavior of dough and greatly helped sheeting. The amount of SMS usage is a pragmatic control parameter for variation in quality and quantity of flour protein and acts promptly. It acts as a reducing agent breaking a number of disulphide (S=S) linkages which, tightly join protein chains to one another, to S=H linkages. Various studies used L-cysteine to replace SMS but, it is economical to use SMS than L-cysteine. It seems that any restriction for use of SMS is unreasonable since, it offers a technical issue for biscuit manufacturers to search for an appropriate alternate processing aid. Another advantage of SMS is that it acts instantaneously and can be introduced at the end of dough mixing with satisfactory result.
Reducing agents split intermolecular and intra-molecular S-S bridges in gluten protein (Kumar et al., 2013). Consequently, this cleavage cause a reduction in molecular weight of protein whereas, dough extensibility is augmented (Stauffer, 1994).

2.9. Correlation of different parameters

Several articles have evinced negative correlations between flour protein and cookie width (Miller and Hoseney, 1997). It is ascribed to gluten protein, as established previously (Chung et al., 2014), that spread factor tend to increase with a rise of non-wheat protein level. Likewise, a negative relationship was delved between spread factor and AWRC (Abboud et al., 1985), both were influenced by damaged starch. A high and negative association of cookie width was found with WSRC ($r = -0.63$) as established by Ram and Singh (2004).

Significant and positive associations of WSRC were found with AWRC, SOCSRC, SUCSRC and spread ratio whereas, negative relationships were observed in WSRC, wet gluten, dry gluten, water absorption, SDS-sedimentation and cookie thickness as reported earlier (Pasha et al., 2009b). A linear relationship of SUCSRC was found with AWRC, cookie thickness, SOCSRC, WSRC and LASRC however, SUCSRC was negatively correlated to SDS-sedimentation volume and spread ratio of cookies (Guttieri et al., 2001; Ram et al., 2005).

The relationships of spread factor with damaged starch, SOCSRC, SUCSRC and WSRC suggest that pentosans, damaged starch and their capacity to absorb aqueous solution demonstrate a strong impact on cookie quality although, protein had no significant role (Moiraghi et al., 2013). Similarly, SOCSRC was observed to be positively associated with AWRC, SUCSRC and WSRC while, negatively correlated to spread ratio of cookies (Ram et al., 2005) however, no relationship was found with protein ($r = 0.03$) and LASRC ($r = 0.18$) as stated previously (Guttieri et al., 2004).

Cookie width exhibited negative associations with protein, damaged starch, SRC values and cookie thickness (Kang et al., 2010). Spread factor was found to be negatively correlated with damaged starch ($r = -0.47$) (Mancebo et al., 2015). A negative relationship was also observed between biscuit width and damaged starch ($r = -0.90$) as reported by Pauly et al. (2013).

Cookie diameter was found to decrease with more starch damage for both wheat and triticale flour as damaged starch absorbs more water as compared to undamaged starch (Gaines et al., 1988). Negative associations of cookie width with solvent retention capacities were found in Pakistani, Argentinean, Chinese and Indian wheat as well as European commercial flour (Ram et al., 2011).
and Singh, 2004; Zhang et al., 2007; Moiraghi et al., 2011; Duyvejonck et al., 2012). It is documented that higher levels of damaged starch reduce baking quality although, wet gluten was found independent of damaged starch (Barrera et al., 2007).

In Pakistan, mixed wheat is delivered to miller that is main source of varying flour properties like protein and gluten content. These variations in flour characteristics considerably influence product quality. Since, baking industry do not have access to particular varieties which are suitable for cookies development so, mixed wheat flour resulted in hard texture cookies; also, high levels of fat and leavening agent are added as compared to regular formulation to make dough soft leading to economical loss. Problem in achieving the desired consistency in cookie quality is the most important challenge for baking industry which needs to be addressed. So, a practical approach is necessary, which can overcome variations in flour supplies from varied source.
CHAPTER 3 MATERIALS AND METHODS

3.1. Procurement of raw materials

Commercia"y available varieties of wheat (*Triticum aestivum* L.) in Punjab were procured from Wheat Research Institute, Ayub Agricultural Research Institute, Faisalabad., while wheat varieties of Sindh were purchased from Wheat Research Institute Sakrand, Nawabshah, Sindh, Pakistan. Commercially available mixed wheat flour was obtained from commercial flour mill. All chemicals and reagents (Analytical grade) were purchased from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan), Merck (Merck KGaA, Darmstadt, Germany) and local market. Present study was conducted in different laboratories of National Institute of Food Science of Technology (NIFSAT).

3.2. Physical analyses of wheat grains

3.2.1. Test weight

Test weight of five wheat varieties was determined through Schopper Chondrometer (OHAVS; Chicago) and values were expressed in kilogram per hec"oliter (kg/hl) following the method No. 55-10 given in AACC (2000).

3.2.2. Thousand Kernel weight

A representative specimen (100 g) of each variety was taken and 1000 grain weight was noted by counting clean, sound and unbroken kernels by adopting the method No. 84-10 as described in AACC (2000). Kernel weight was expressed as grams per 1000 kernels.

3.3. Wheat milling

Wheat grains from all the varieties were manually cleaned in order to remove dirt, straw and stones. The tempering of wheat grains was carried out at 15.5% moisture level for 24 hours at room temperature to equilibrate moisture within wheat grains. The amount of water required for tempering was calculated as per method No. 26-95 (AACC, 2000).

\[
\text{Amount of water (mL) required} = \left[ \frac{100 - \text{Original moisture} (\%) - 1}{100 - \text{Desired moisture} (\%)} \right] \times \text{Weight of sample}
\]

Tempered wheat samples were milled through Brabender Quadrumate Senior Mill (C.W. Brabender Instruments, Inc.) to acquire four milling fractions *i.e.* break flour, reduction flour, bran and shorts according to method No. 26-21A (AACC, 2000). Straight grade flour was prepared by blending the break and reduction flour.
3.4. Chemical analyses of wheat flour

Chemical analyses of straight grade flour (SGF) of each variety were carried out including moisture, ash, fat, fiber and protein content according to their respective methods as given below. Nitrogen free extract (NFE) was calculated by difference method.

3.4.1. Moisture content

Moisture content of wheat flour was determined using an air forced draft oven (Memmet Germany) at a temperature of 105± 5°C following the method No. 44-15A described in AACC (2000). The following formula was used to calculate the percentage of moisture.

\[
\text{Moisture (\%) = } \frac{\text{Wt. of original sample (g) - Wt. of dried sample (g)}}{\text{Wt. of original sample (g)}} \times 100
\]

3.4.2. Ash content

Flour samples were evaluated for ash content according to method No. 08-01 (AACC, 2000). The samples were placed in pre-weighed crucibles and their charring was performed on bunson burner before incinerating in muffle furnace while, maintaining the temperature at 550ºC, until the flour sample converted to grayish white residue.

\[
\% \text{ Ash} = \frac{\text{Weight of residue}}{\text{Sample weight}} \times 100
\]

3.4.3. Fat content

Crude fat of flour sample was determined by using Soxhlet apparatus following the method No. 30-25 outlined in AACC (2000). Flour sample (5.0 g) was extracted using petroleum ether at a condensation rate of 2-3 drops/sec. for 8 hours. After distilling excess ether the residue of extraction flask was dried at 100ºC for 30 min. until a constant weight. Fat content was calculated by the formula given below.

\[
\% \text{ Fat} = \frac{\text{Wt. of ether extract}}{\text{Wt. of flour sample}} \times 100
\]

3.4.4. Fiber content

Flour samples were evaluated for fiber content by adopting the method No. 32-10 (AACC, 2000). Fat and moisture free flour samples (2 g) were used for the determination of fiber content and boiled for 30 min. in the presence of 1.25% H₂SO₄, and then filtered and washed. Then, these samples were again boiled in 1.25% NaOH for 30 min. and then filtered and washed. Drying of resultant residue was conducted at 130ºC for 2 hours and weighed. The dried residue was ignited at 600ºC±15ºC, cooled and reweighed. Fiber content was calculated
according to following expression.

\[
\% \text{ Fiber} = \frac{\text{Loss in weight on ignition - blank}}{\text{Weight of sample}} \times 100
\]

3.4.5. Protein content

Nitrogen content in flour samples was determined by Kjeldahl’s method as described in AACC (2000) method No 46-10. Nitrogen percentage was determined by the following expression:

\[
\% \text{ N} = \frac{\text{Titer of 0.1 N } \text{H}_2\text{SO}_4 \text{ used} \times 0.0014 \times 250}{\text{Weight of sample} \times \text{Vol. of aliquot sample}} \times 100
\]

Protein %age was calculated by multiplying % nitrogen with a factor 5.7.

3.4.6. Nitrogen free extract (NFE)

NFE content was calculated according to the following expression.

\[
\text{NFE} = 100 - (\text{Moisture} \% + \text{Protein} \% + \text{Fat} \% + \text{Fiber} \% + \text{Ash} \%)
\]

3.5. Solvent retention capacity (SRC)

SRC values of wheat flour were reckoned by using four solvents i.e. deionized water, 5% sodium carbonate, 50% sucrose and 5% lactic acid according to AACC method No. 56-11 (AACC, 2000) with minor modifications as suggested by Ram and Singh (2004) and Pasha et al. (2009b). SRC tests were conducted using 1 g flour sample instead of 5 g (AACC 56-11) in 15 mL tubes with conical bottoms. The material was dispersed in 5 mL solvent and kept for 20 min with intermittent agitation on a vortex mixer at 5, 10, 15, and 20 min, followed by 15 min centrifugation at 1000×g at room temperature.

3.6. Alkaline water retention capacity (AWRC)

Flour samples were tested for AWRC according to method No. 56-10 (AACC, 2000). Flour (1 g) was suspended in 5 mL of 0.1N NaHCO₃, hydrated for 20 min. and centrifuged at 1000×g for 15 min at room temperature. The precipitate obtained was weighed and AWRC was calculated.

3.7. Wet and dry gluten

Wet and dry gluten content in wheat flour samples were measured by using Glutomatic (Pertin GM-2200; Sweden) according to method No. 38-12A given in AACC (2000) and centrifuged on an especially constructed sieve under standardized conditions. Wet gluten forced through the sieve and the total weight of wet gluten (passed through and remaining on the sieve) was
observed. Total wet gluten was then dried under standardized conditions and weighed.

\[
\text{Wet Gluten (14\% moisture basis)} = \frac{\text{Total wet gluten (g)} \times 860}{100 - \%\text{Sample moisture}}
\]

\[
\text{Dry Gluten (14\% moisture basis)} = \frac{\text{Total dry gluten (g)} \times 860}{100 - \%\text{Sample moisture}}
\]

3.8. Sodium Dodecyl Sulfate (SDS)-sedimentation test

SDS- sedimentation test for wheat flour were carried out according to AACC method No. 56-70 (AACC, 2000). 3.15 ± 0.02 g flour (on 14.0\% moisture basis) of five samples was weighed into weighing papers or boats. 50 ml distilled water was added by automatic pipet to five 100-ml graduated cylinders. Mixing and hydration schedule was proceeded by hand procedures described in method No. 56-70 AACC (2000) with slight modifications. After mixing, 25 ml SDS-lactic acid reagent was added to each cylinder using pipet. Then, cylinders were positioned upright and sedimentation volumes were noted to nearest 0.5 ml after 20 min. The temperature of sedimentation liquid was recorded and corrected sedimentation volumes to 25°C.

3.9. Mineral profile

Mineral profile of wheat flour was determined using Atomic Absorption Spectrophotometer (AA240, Varian, Australia) and Flame Photometer (Sherwood, UK) according to AOAC method No. 3.014-016 (AOAC, 2006). Wet digestion of flour sample of each variety was performed in di-acid mixture (3:1) of HNO\textsubscript{3}:HClO\textsubscript{4} at hot plate for 2 hours. Mineral content \textit{i.e.} Fe, Cu, Zn and Mn in the digested samples were determined using Atomic Absorption Spectrophotometer on an acetylene air flame while Ca, Na and K were determined by Flame Photometer.

3.10. Starch content
Starch content in wheat flour was determined following AACC method No. 76-13 (AACC, 2000) with slight modification. 0.1 g accurately weighed milled sample of each variety was added to a glass test tube. Then, 0.2 mL of aqueous ethanol was added to wet the samples and aid dispersion. Tubes were stirred on a vortex mixer. Immediately, 3 mL of thermostable α-amylase (contents of bottle 1) was added and tubes were incubated in a boiling water bath for 6 min with vigorous stirring (after 2, 4 and 6 min). The tubes were placed in a water bath at 50°C; and 0.1 mL of the contents of bottle 2 were added. The tube were stirred on a vortex mixer and incubated at 50°C for 30 min. The volume of the contents of test tubes was made up to 100 ml and centrifuged at 2,000 rcf for 10 min. The clear, undiluted filtrate was used for the assay. 3.0 mL of GOPOD reagent was added to each tube (including the D-glucose controls and reagent blanks), and tubes were incubated at 50°C for 20 min. Here, D-Glucose control consist of 0.1 mL of D-glucose standard solution (1 mg/mL) and 3.0 mL of GOPOD reagent while, Reagent Blank solutions comprise of 0.1 mL of water and 3.0 mL of GOPOD Reagent. The absorbance for each sample, and the D-glucose control was recorded at 510 nm against the reagent blank.

3.11. Damaged starch

Damaged starch content in wheat flour was determined according to AACC method No. 76-30A (AACC, 2000) with slight modification. 1.00 g (14% moisture basis) of flour sample was weighed into 125-ml Erlenmeyer flask. 0.05 g fungal α-amylase enzyme was accurately weighed and introduced into Erlenmeyer flask. 45 ml acetate buffer (reagent 1) was added and a uniform suspension was obtained by using glass rod. Then, it was incubated at 30°C in a thermostatically controlled water bath for exactly 15 min from time of adding acetate buffer (reagent 1). At end of 15 min, 3.0 ml H$_2$SO$_4$ solution (reagent 2) and 2.0 ml sodium tungstate solution (reagent 3) were added and mixed thoroughly. It was allowed to stand for 2 min, and filtered through (Whatman No. 1) filter paper, and first 8-10 drops of filtrate were discarded. Immediately, 5 ml extract was pipet into test tube (approximately 50-ml capacity; length 20 cm, diameter 2 cm) as described in method No 80-60. 10 ml alkaline ferricyanide reagent was added, mixed, and test tubes were immersed in vigorously boiling water bath. Care was taken that surface of liquid in test tube be 3-4 cm below surface of boiling water. Test tubes were left in boiling water bath for exactly 20 min. Then, test tube and contents were cooled under running water and poured at once into 100- or 125-ml Erlenmeyer flask. These test tube were rinsed out with 25 ml acetic acid-salt solution, adding rinsings to solution in Erlenmeyer flask. 1 ml of soluble starch-KI solution was added, mixed thoroughly, and titrated with 0.1N thiosulfate to complete disappearance of blue color. (A 10-ml microburet is recommended for this titration.). The ml ferricyanide reduced was calculated by subtracting ml thiosulfate required for the determination from the thiosulfate used for enzyme-reagent blank. It was
converted to maltose by means of Ferricyanide-Maltose (Damaged Starch) Conversion Table given in AACC method No. 80-60 and percent maltose produced from damaged starch or percent damaged starch was calculated.

3.12. Rheological properties
Rheological properties of wheat flour were determined using farinograph (method No. 54-21) and rapid visco analyzer (method No. 76-21) as described in AACC (2000).

3.12.1. Farinographic studies
Physical dough properties of five wheat varieties were determined by using Farinograph (Brabender D-4100, Germany) according to the procedure described in AACC (2000). Farinograms were interpreted for different characteristics like water absorption, dough development time, dough stability, mixing tolerance index and softening of the dough as described below.

3.12.1.2. Water Absorption
Water absorption of the flour is the percentage of water required to reach at the center of the curve on the 500 Brabender Units (BU) line with maximum consistency of the dough (peak). For each sample water absorption was recorded directly from the burette attached to the farinograph.

3.12.1.3. Dough development time
It was the time interval in minutes from first addition of water to the point of maximum consistency just before the indication of weakening.

3.12.1.4. Dough stability
Dough stability was recorded as the time difference between the point where top of curve first intersected 500 BU line (Arrival time) and point where top of curve left 500 BU line (Departure Time).

3.12.1.5. Softening of dough
Dough softening was measured as the difference in BU from centre of curve at peak and centre of curve 12 min. after peak.

3.12.2. Rapid visco analyzer (RVA) studies
Pasting properties of wheat varieties were determined using Rapid Visco Analyzer (Super 4 Newport Scientific Ltd. Australia) by following method No. 76-21 (AACC, 2000). 3.50 g of
flour sample (14% moisture basis) was weighed into the weighing vessel before transfer into the test canister. 25.0 ± 0.1 ml water was dispensed into the new test canister. Sample was transferred onto the water surface in the canister. The stirrer was placed into the canister and vigorously jogged the blade through the sample up and down 10 times. Jogging action was repeated to ensure that the samples remaining on the water surface or on the paddle were dissolved. The paddle and canister assembly were inserted firmly into the paddle coupling so that the paddle is properly centered. The measurement cycle was initiated by depressing after initiation and terminated automatically.

3.13. Chemical modification of starch

For cookies preparation, maize starch was employed for the preparation of chemically modified starch (CMS) following the procedure as stated previously (Yousif et al., 2012) with minor changes. For this, maize starch (750 g) was taken in a beaker following the addition of 375 ml dist. water. Afterwards, 375 mL of 0.1 N HCL solution was also added in the beaker holding the mixture of starch and dist. water. Then, pH (7.0) was maintained using an alkali (1 N NaOH). Further, washing of starch was carried out three times with dist. water and filtration through Whatman Filter Paper No. 1. Also, neutralized starch was dried at ambient temperature for 24 hours.

3.14. Preparation of cookies

Cookies were prepared using straight grade flour (SGF) obtained from different wheat varieties according to AACC method No. 10-50D (AACC, 2000). Moreover, cookies were also prepared from selected wheat variety (SWV) and mixed wheat flour (MWF) by applying various treatments as described in Table 1. For treatments, wheat flour was substituted with chemically (acid) modified starch (CMS) whereas, other chemical agents *i.e.* ascorbic acid (AsA), potassium iodate (KIO₃) and sodium metabisulfite (SMS) were added to dough formulation. However, for protease a resting time of half an hour was given to dough for the reason of enzyme action on dough. The ingredients required for the preparation of cookies were weighed accurately. Then, creaming of shortening and sugar was achieved until fluffy, and then, eggs were added. Flour and baking powder were added to the creamy mass and mixed to a homogenous mass. Batter was then rolled out on a sheeting board to a uniform thickness and was cut into circular shapes with the help of a cookie cutter. Cookies were placed on baking trays with their respective labelling at a proper distance and were baked at 180°C in the baking
oven for 10-12 min. until these were fully baked. After baking, the cookies were cooled at room temperature for 20 min. and packed in polythene bags/zip bags and evaluated for storage stability fortnightly during 30 days storage at room temperature.

3.15. Analyses of cookies

3.15.1. Chemical analyses
Cookies were analyzed for proximate composition using respective methods as stated earlier and described in AACC (2000).

3.15.2. Physical analyses
Weight, diameter and thickness of cookies were measured and their average were noted. Width of cookies was measured by placing six cookies horizontally (edge to edge) and rotated at 90° angles for replication. Similarly, thickness of cookies was measured by placing six cookies on one another and replicate reading was recorded. Spread factor was calculated according to method No. 10-50D (AACC, 2000), using following formula:

\[
SF = \frac{W \times CF \times 10}{T}
\]

Where, CF = Correction factor at constant atmospheric pressure (1.0 in this case).

3.15.3. Color
Color values of cookies were recorded by color meter (Color Test II, Nehaus Neotech) following the method of Kara et al. (2005). The instrument was calibrated before test with standards (54Ctn for dark and 151CTn for light). Cookie samples were placed under photocell of color meter. Sample values were taken in triplicates and compared with standards.
Table 1: Treatment plan for preparation of cookies

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Modifying/chemical agents</th>
<th>Description</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Control (MWF)</td>
<td>T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>-</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>SWV + Chemically (acid) modified starch (CMS)</td>
<td>T&lt;sub&gt;1&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;1&lt;/sub&gt;C&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>15 %</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>SWV + Ascorbic acid (AsA)</td>
<td>T&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>10 ppm</td>
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<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>15 ppm</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>SWV + Enzyme (protease)</td>
<td>T&lt;sub&gt;3&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>200 mg/100 g</td>
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<td>T&lt;sub&gt;3&lt;/sub&gt;C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>600 mg/100 g</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>SWV + Oxidizing agent (potassium iodate/KIO&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>T&lt;sub&gt;4&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>500 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>1500 ppm</td>
</tr>
<tr>
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<td>SWV + Reducing agent (sodium metabisulfite/SMS)</td>
<td>T&lt;sub&gt;5&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1000 ppm</td>
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<td></td>
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<td>3000 ppm</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>MWF + Chemically (acid) modified starch (CMS)</td>
<td>T&lt;sub&gt;6&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5 %</td>
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<tr>
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<td>15 %</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>MWF + Ascorbic acid (AsA)</td>
<td>T&lt;sub&gt;7&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5 ppm</td>
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<td></td>
<td></td>
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<tr>
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<td></td>
<td>T&lt;sub&gt;7&lt;/sub&gt;C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>15 ppm</td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt;</td>
<td>MWF + Enzyme (protease)</td>
<td>T&lt;sub&gt;8&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>200 mg/100 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;8&lt;/sub&gt;C&lt;sub&gt;2&lt;/sub&gt;</td>
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<td></td>
<td>T&lt;sub&gt;8&lt;/sub&gt;C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>600 mg/100 g</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt;</td>
<td>MWF + Oxidizing agent (potassium iodate/KIO&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>T&lt;sub&gt;9&lt;/sub&gt;C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>500 ppm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;9&lt;/sub&gt;C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1000 ppm</td>
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<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;9&lt;/sub&gt;C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1500 ppm</td>
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<td>T&lt;sub&gt;10&lt;/sub&gt;</td>
<td>MWF + Reducing agent (sodium metabisulfite/SMS)</td>
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<td>1000 ppm</td>
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<td>2000 ppm</td>
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<tr>
<td></td>
<td></td>
<td>T&lt;sub&gt;10&lt;/sub&gt;C&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3000 ppm</td>
</tr>
</tbody>
</table>

SGF: straight grade flour; SWV: selected wheat variety; MWF: mixed wheat flour; C: concentration
3.15.4. Hardness value

Hardness value of cookies was determined according to the method of Piga et al. (2005) using Texture Analyzer (TA-XT2, Plus, Stable Microsystems, Surrey, UK) interfaced with a computer. For the data analysis, the Texture Expert program version 4.0.9.0 was used. Three repeated observations were taken for every formulation.

3.15.5. Sensory evaluation

Sensory quality of cookies was evaluated following the method of Meilgaard et al. (2007). Sensory evaluation was carried out using a panel consisting of eight members. Cookie samples prepared from each treatment were presented in coded white plastic plate, using a 9 point hedonic scale with a scale ranging from 1 to 9 with 1 representing the least score (dislike extremely) and 9 the highest score (like extremely). Order of presentation of samples was randomized. Water was provided to rinse the mouth between evaluations. Panelists were instructed to evaluate the coded samples for crispiness, color, taste, flavor, texture and overall acceptability.

3.16. Statistical analysis

All analysis were carried out in triplicate and data were reported as mean± standard error. Significant differences among treatments were evaluated through analysis of variance (ANOVA) under completely randomized design (CRD) and beyond ANOVA contrast analysis was applied according to method described by Montgomery (2008) using different statistical software like SPSS version (version 13, 2004) and Minitab (version 11.2, 1996). Mean± standard deviation and graphs computed through excel 2013. Analysis of variance was carried out on data for five wheat varieties and different treatments applied to cookies as well. Three factor factorial analysis was performed for storage data. Relationship of different physicochemical parameters with cookie making characteristics was determined by Pearson’s correlation coefficient.
CHAPTER 4

RESULTS AND DISCUSSION

4.1. Physical properties of wheat varieties

4.1.1. Test weight

Mean squares pertaining to test weight of wheat varieties have been shown in Table 2. Statistical results depicted that differences among wheat varieties were found to be highly significant for test weight. Results evidence that test weight varied greatly from 67.00 to 81.00 kg hl$^{-1}$ (Table 3). In present work, highest value for test weight was reckoned in TD-1 (81.00 kg hl$^{-1}$) followed by Benazir-13 (79.00 kg hl$^{-1}$), Lasani-08 (71.00 kg hl$^{-1}$) and AARI-11 (70.00 kg hl$^{-1}$) whilst, lowest (67.00 kg hl$^{-1}$) was recorded in FSD-08. Test weight is a rough indication of flour yield and have become a main parameter in wheat grading (Iqbal et al., 2015b) possibly due to the fact that, most of grains are sold at a certain test weight (Amjad et al., 2010). In general, hectoliter mass is one of parameters used for quality evaluation of durum wheat (Torbica et al., 2016).

Present results support outcome of several publications related to test weight of Pakistani wheat varieties ranged from 76.22 to 81.50 kg/hl (Amjad et al., 2010; Naseem et al., 2011; Ahmed et al., 2014; Iqbal et al., 2015b). Similar values of test weight (73.3 to 82.5 kg/hl) in wheat varieties reported formerly by Kumar et al. (2013) and Uhlen et al. (2015) further supports interpretation of present results. Significant differences observed in test weight might due to varying genetic makeup of wheat varieties, diverse environmental conditions prevailing in each region as reported earlier by Safdar et al. (2009) and Ahmed et al. (2014).

4.1.2. Thousand kernel weight

Statistical results for thousand kernel weight (TKW) of different wheat varieties presented in Table 2 indicate that variations in TKW were highly significant among wheat varieties. Mean values for TKW have been described in Table 3. Results clearly indicated that a wide range of TKW was observed from 32.82 to 48.70g. Across varieties, TD-1 yielded highest thousand kernel weight (48.70g) followed by FSD-08 (38.94g), Lasani-08 (38.27g), Benazir-13 (33.20g) while, lowest (32.82g) was noted in AARI-11. Present research come up with highest thousand kernel weight in TD-1 which was statistically different from other wheat varieties. Present observations are in conformity with several investigators who published thousand kernel weight in Pakistani wheat varieties in the range of 33.49-46.9 g (Ahmad et al., 2012; Ghaffar et al., 2013; Iqbal et al., 2015b).
Table 2: Mean squares for physical properties of wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TW</td>
</tr>
<tr>
<td>Variety</td>
<td>4</td>
<td>110.3700**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>12.2700</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Table 3: Mean values for physical properties of wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mean± SE</th>
<th>TW (kg/hl)</th>
<th>TKW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSD-08</td>
<td>67.00±1.74c</td>
<td>38.94±1.01b</td>
<td></td>
</tr>
<tr>
<td>Lasani-08</td>
<td>71.00±1.89bc</td>
<td>38.27±1.02b</td>
<td></td>
</tr>
<tr>
<td>AARI-11</td>
<td>70.00±1.90bc</td>
<td>32.82±0.89c</td>
<td></td>
</tr>
<tr>
<td>T.D-1</td>
<td>81.00±2.29a</td>
<td>48.70±1.38a</td>
<td></td>
</tr>
<tr>
<td>Benazir-13</td>
<td>79.00±2.23ab</td>
<td>33.20±0.94c</td>
<td></td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).

TW: Test weight; TKW: Thousand kernel weight

Similar values of thousand kernel weight have been reported in wheat varieties of Sindh ranged
Variations in 1000 kernel weight may attributed to genetic make-up of wheat varieties/lines, environment and growing conditions (Amjad et al., 2010; Naseem et al., 2011). Moreover, it is also controlled by soil fertility particularly, planting density as stated earlier (Ghaffar et al., 2013).

4.2. Chemical properties of wheat varieties

4.2.1. Moisture content

Statistical data for moisture content in wheat varieties conferred in Table 4 revealed highly significant variations within wheat varieties. Mean values related to moisture content have been shown in Table 5. Present results show that moisture content ranged between 8.50 and 10.78%. Moisture content in wheat varieties was corroborated as FSD-08 (9.54%), Lasani-08 (10.78%), AARI-11 (8.50%), TD-1 (10.58%) and Benazir-13 (9.76%). According to this, highest moisture content was recorded in Lasani-08 (10.78%) whereas, the lowest was appraised in AARI-11 (8.50%). It is proposed that wheat varieties investigated in present study for moisture content presented a large genetic diversity.

Present observations are in agreement with the results reported by various authors (Naseem et al., 2011; Ahmed et al., 2014; Iqbal et al., 2015b) but, antithesis to findings of few researchers (Nikolić et al., 2013; Masih et al., 2014). Results are also in line with Arif et al. (2007) and Umrani and Pahoja (2013), who reported moisture in wheat varieties of Sindh ranged between 8.8 and 10.86%. Wheat flour containing low moisture has more storage stability since, the values above 14.5% entices insect, mold and bacteria (Keran et al., 2009). Moisture level of wheat is mainly influenced by genetic and non-genetic factors including environmental and storage conditions due to hygroscopic nature of wheat grains (Ahmed et al., 2014). According to Pasha et al. (2006), moisture level was significantly affected by genetic variations of wheat cultivars and crop years however, other researchers agreed upon the effect of climatic and agronomic conditions (Channa et al., 2015).

4.2.2. Ash content

Mean squares (Table 4) for ash content of wheat flour apprised a highly significant influence of wheat varieties on ash content. Mean values regarding ash content are depicted in Table 5. From these results, it can be observed that ash content was estimated in range of 0.33 to 0.83%. In this piece of work, ash content in wheat varieties was figured as 0.63% (FSD-08), 0.49% (Lasani-08), 0.33% (AARI-11), 0.83% (TD-1) and 0.66% (Benazir-13). Accordingly, TD-1
possessed highest ash content (0.83%) than did others. In present investigations, ash content was found to be influenced by varietal differences. It is referred that Lasani-08, AARI-11 and Benazir-13 were statistically different from FSD-08 and TD-1. Since, high amount of ash represents the contamination of bran in wheat flour therefore, present study established that high ash level may adversely affect cookie quality. Present results are in congruence with past authors (Amjad et al., 2010; Mushtaq et al., 2010; Saeed et al., 2012; Masih et al., 2014) who delved 0.52-0.68% ash in wheat varieties of Pakistan. However, contrary to present results, high ash content was stated earlier by Naseem et al. (2011) and Choudhary et al. (2013).

Ash refers to the inorganic residue remained after burning of organic material. It is a significant factor in milling and clarity of flour is evaluated by flour ash (Wei, 2002). Ash content was affected by wheat varieties, growth and environmental conditions as stated earlier (Buriro et al., 2012; Iqbal et al., 2015b). Sameen et al. (2002) stated that ash level was significantly enhanced with fertilizer doses while, another study conducted by El-Porai et al. (2013) showed that an increment in ash was observed with hard milling as compared to normal milling. Other studies supported a strong genotype by environment interaction on ash level (Buriro et al., 2012).

4.2.3. Fat content

Mean squares regarding fat content of wheat varieties (Table 4) put out that fat content varied non-significantly due to differences in wheat varieties. Mean values regarding fat content are portrayed in Table 5. According to results, a narrow range of fat content (1.36 and 1.49%) was pointed out in wheat varieties. Fat content is recorded as 1.34%, 1.39%, 1.36%, 1.49% and 1.46% in wheat varieties i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. As in present case, TD-1 (1.49%) possessed highest fat content whereas, FSD-08 had lowest (1.34%). However, wheat varieties studied in present investigation were all the same with respect to fat content. Present observations are assent with data obtained by Gulzar et al. (2010) and Choudhary et al. (2013) who noticed fat content varied from 1.18 to 1.43% in wheat varieties/lines. But, in contradictory to findings reported earlier by Mushtaq et al. (2010) and El-Sharnouby et al. (2012).
4.2.4. Fiber content

Mean squares (Table 4) pertaining to fiber content in SGF portrayed a highly significant influence of wheat varieties on fiber content. Mean values for fiber content have been depicted in Table 5. Present findings showed that fiber content in wheat varieties varied between 0.38 and 0.52%. Maximum value was ascertained in TD-1 (0.52%) while, minimum (0.38%) was found out in AARI-11. A significant reduction in fiber content was observed in Benazir-13 (0.51%) followed by FSD-08 (0.47%) and Lasani-08 (0.45%). It can be realized that in present study, AARI-11 had low fiber content which was statistically different from other varieties. This work confirms that variations in fiber content are primarily dictated by wheat genetics and may also relate to grain size and bran. Present results are in parity with other authors (Choudhary et al., 2013; David et al., 2015) who determined fiber content in wheat flour in range of 0.42-0.51%. However, contrasting with results of earlier workers (Gulzar et al., 2010; Mushtaq et al., 2010; Masih et al., 2014). They rather reported fiber content in wheat varieties ranged from 0.10 to 0.31%.

4.2.5. Protein content

Table 4 divulged mean squares regarding protein content of wheat varieties. It is obvious from statistical results that all experimental measures had significantly more variability due to wheat varieties in respect to protein content. As shown in Table 5, mean values for protein content of wheat varieties varied from 9.85 to 13.75%. Amongst, protein content in wheat varieties was corroborated as 12.87%, 10.94%, 9.85%, 13.75% and 13.13% in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. In this case, maximum protein was discerned in TD-1 (13.75%) whereas, minimum was observed in AARI-11 (9.85%). Wheat varieties examined in present research showed that protein content was influenced by varietal differences. Present study divulge that Lasani-08, AARI-11 and TD-1 were statistically different from FSD-08 and Benazir-13 with respect to protein content. From nutrition viewpoint, TD-1 and Benazir-13 had highest protein content and may find their potential for bread making. Above all the others, AARI-11 having lower protein content which acquired eminent interest and it is supported by the fact that, cookie quality is related to low protein content (soft wheat). Based on these results, present work is pragmatically important and may be useful for miller and processors to make selection of an appropriate variety depending on their intended use. Present results commensurate with findings of several researchers who reported protein content.
of Pakistani wheat varieties ranged between 8.35 and 13.68% (Gulzar et al., 2010; Naseem et al., 2011; Iqbal et al., 2015a; Nisar et al., 2015). Some published data reported protein content ranged as 10.32-10.67%, 7.61-13.32%, 9.4-11.2%, 10.57-13.95% and 7.86-13.45% in Iranian, Argentinean, Chinease, Indian and Korean wheat cultivars, respectively (Feyzipour et al., 2006; Colombo et al., 2008; Geng et al., 2012; Kaur et al., 2013; Kang et al., 2014). Protein level in numerous wheat varieties of Sindh varied from 8.28 to 16.0% as stated earlier (Arif et al., 2007; Buriro et al., 2012; Khan et al., 2013).

Protein content is controlled by a combination of agronomy and genetics, indicating a general trend for wheat varieties to develop kernel of high, medium, or low protein, which may be altered by factors entailing fertilizer application, planting density and sowing time (Hussain et al., 2010). In another study, Khan et al. (2013) stated numerous factors, which affect protein content include genetic and non-genetic (environment, soil, fertilizer and storage conditions).

In view of Ahmad et al. (2001), protein content was invariably affected by different seed densities and fertilizer rates. Alam et al. (2009) suggested that high temperature in Pakistan is responsible for high protein (12.0 %) level in wheat and marks it strong wheat. Panhwar et al. (2014) found protein content of dry land wheat varieties was low than wet land wheat varieties.

4.2.6. Nitrogen free extract (NFE) content

Statistical data for nitrogen free extract (NFE) content of wheat varieties has been demonstrated in Table 4. It is apparent from results that NFE content did not differ significantly within wheat varieties. NFE content in wheat varieties varied between 72.82 and 79.58% (Table 5). Nitrogen free extract in wheat varieties i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13 was find out as 75.15%, 75.94%, 79.58%, 72.82% and 74.48%, respectively. Here, highest nitrogen free extract was found in AARI-11 (79.58%) however, lowest content was exhibited in TD-1 (72.82%). Data suggests that wheat varieties analyzed in present investigation were all the same statistically with respect to NFE content. Present study comply with Yadav et al. (2012) who speculated as carbohydrate of flour was calculated by difference, the variations may due to differences in other component. Values reported in this work are in good agreement with findings established by other researchers (Alam et al., 2009; Saeed et al., 2012; Masih et al., 2014; Omah and Okafor, 2015) who calculated nitrogen free extract in wheat flour ranged from 72.50 to 78.60%.
### Table 4: Mean squares for chemical properties of wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Moisture</th>
<th>Ash</th>
<th>Fat</th>
<th>Fiber</th>
<th>Protein</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>4</td>
<td>2.4922**</td>
<td>0.1040**</td>
<td>0.0132 NS</td>
<td>0.0094**</td>
<td>15.9330**</td>
<td>27.540 NS</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>0.2191</td>
<td>0.0009</td>
<td>0.0044</td>
<td>0.0005</td>
<td>0.3360</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

### Table 5: Mean values for chemical properties (%) of wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Moisture</th>
<th>Ash</th>
<th>Fat</th>
<th>Fiber</th>
<th>Protein</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSD-08</td>
<td>9.54 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.63 ± 0.017&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.34 ± 0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.012&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.87 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.15 ± 1.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>10.78 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.015&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.39 ± 0.038&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45 ± 0.012&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.94 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.94 ± 2.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AARI-11</td>
<td>8.50 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33 ± 0.009&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.36 ± 0.035&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.012&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.85 ± 0.44&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.58 ± 3.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T.D-1</td>
<td>10.58 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83 ± 0.017&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.49 ± 0.043&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52 ± 0.012&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>13.75 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.82 ± 3.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>9.76 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66 ± 0.023&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46 ± 0.040&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 ± 0.017&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.13 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.48 ± 2.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).
However, values observed in present study incommensurate with findings of past authors (Falola et al., 2014; Jagannadham et al., 2014) who calculated comparatively low values of nitrogen free extract ranged as 69.16-69.47%. Moreover, NFE content was found to be varied 75.15-76.53% in wheat varieties/lines and 63.29-76.78% in different milling fractions (Gulzar et al., 2010; Iqbal et al., 2015a).

4.2.7. Mineral content

4.2.7.1. Sodium (Na)
Statistical results (Table 6) for sodium content in wheat varieties indicated that it was found to be statistically different among wheat varieties. In Table 7, mean values related to sodium content varied from 2.20 to 2.64 mg/100g. Across all wheat varieties, highest sodium content was determined in TD-1 (2.64 mg/100g) followed by Benazir-13 (2.51 mg/100g), FSD-08 (2.34 mg/100g) and Lasani-08 (2.22 mg/100g) but, lowest was observed in AARI-11 (2.20 mg/100g). Present investigation bring forward that variations in sodium content are likely to cause by wheat varieties.

Determination of mineral composition in food has attracted more scientific attention. In Pakistan, wheat being staple food for major population, so does main source of trace and essential elements in addition to major nutrients (Shar et al., 2002). These observations are in conformity with previous investigations who figure out sodium content in wheat ranged from 2.0 to 2.3 mg/100g (Gulzar et al., 2010; El-Sharnouby et al., 2012). In past, the impact of genetics and environmental conditions affecting variations in mineral content of wheat has been evaluated (Peterson et al., 1983).

4.2.7.2. Calcium (Ca)
Mean squares (Table 6) pertaining to calcium content in wheat varieties referred that calcium content differed significantly among wheat varieties. In Table 7, mean values for calcium content can be observed to be ranged from 20.25 to 28.45 mg/100g. As can be seen, calcium content in wheat varieties was probed as FSD-08 (22.41 mg/100g), Lasani-08 (20.36 mg/100g), AARI-11 (20.25 mg/100g), TD-1 (28.45 mg/100g) and Benazir-13 (24.79 mg/100g). Maximum calcium content was delved in TD-1 (28.45 mg/100g) though, minimum was observed in AARI-11 (20.25 mg/100g). It is understood that variations in calcium content are probably caused by wheat varieties.
Values observed in present research are somewhat higher than those obtained by Ragaee et al. (2006) who figured calcium in hard wheat (159.50 mg/kg) and soft wheat (202.2 mg/kg). Calcium content in wheat was ranged from 15.0 to 53.5 mg/100g in some previous investigations (Khan et al., 2009; Gulzar et al., 2010; Akhter et al., 2012; El-Sharnouby et al., 2012). In Pakistan, reported prevalence of hypocalcaemia was observed as 58.9% in pregnant and 52.1% in non-pregnant women (NNS, 2011).

4.2.7.3. Iron (Fe)

Statistical results (Table 6) showed that iron content was significantly affected by the wheat varieties. Table 7 represents mean values of iron content varying from 2.12 to 4.71 mg/100g. It could be seen that, iron content in wheat varieties was ascertained as FSD-08 (2.69 mg/100g), Lasani-08 (2.28 mg/100g), AARI-11 (2.12 mg/100g), TD-1 (4.71 mg/100g) and Benazir-13 (4.09 mg/100g). Highest iron content was found out in TD-1 (4.71 mg/100g) but, lowest was determined in AARI-11 (2.12 mg/100g). Variations in mineral content found in present study may due to soil composition, varietal differences and genetic variations.

Present results are well justified by various authors who reported iron content in SGF of different wheat varieties in range of 1.30-5.55 mg/100g (Ihsanullah et al., 2002; Khan et al., 2009; Akhter et al., 2012; El-Sharnouby et al., 2012). Mineral composition of wheat varieties divulges iron content ranged between 14.80 and 21.37 mg/kg (Amjad et al., 2010) while, Ragaee et al. (2006) figured iron content in hard wheat (13.20 ppm) and soft wheat (13.9 ppm). Iron is an important component of hemoglobin which carry oxygen from respiratory organ to other parts of body through blood (Attaullah et al., 2017). Iron is also a constituent of myoglobin which help muscle cell. It is required in all living organisms for normal bioactivities such as oxygen transport, DNA synthesis, cell division and electron transport (Hentze et al., 2010). Level of hemoglobin below 11 g/dL is considered abnormal during pregnancy and may cause anemia due to iron deficiency (Shill et al., 2014). Iron deficiency is major nutritional problem worldwide and have reached at epidemic level in various developing countries (Sirdah et al., 2014). Iron deficiency anemia (IDA) can be characterized by reduced production of red blood cells owing to less storage of iron in body. In Pakistan, 61.9% children and 50.4% of adult women were reported to be anaemic (NNS, 2011). Also, IDA in children has been related to growth retardation, less physical activity, impaired cognition and proposed to contribute in high infant mortality rate (Akhtar et al., 2013; NIPS, 2013).
Wheat flour containing higher Fe is valuable for people suffering from anemia and may also help to alleviate iron deficiency anemia (IDA) (Khan et al., 2009). Standard Fe content in wheat flour is established as 3.3 mg/100 g and daily intake of Fe is recommended as 14-28 mg/day and 18 mg/day recommended by RDA is equivalent. Marketed mill flour have lower Fe content indicating deficiency of element. It can be explained that minerals are mainly present in germ and outer layer of grain. As a matter of fact, milling is aimed to remove germ and most of outer grain layer to yield white flour which contain a high level of starch and caloric value than whole wheat flour but, during this process most of vitamin and minerals of grain lost (Ihsanullah et al., 2002).

4.2.7.4. Zinc (Zn)

Variance analysis for zinc content in wheat varieties shown in Table 6 pointed out that it was found to be statistically different among wheat varieties. Mean values (Table 7) showed that zinc content varied between 1.24 and 2.31 mg/100g. Zinc content in wheat varieties was reckoned as FSD-08 (1.83 mg/100g), Lasani-08 (1.48 mg/100g), AARI-11 (1.24 mg/100g), TD-1 (2.31 mg/100g) and Benazir-13 (2.07 mg/100g). This could infer that, highest zinc content was investigated in TD-1 (2.31 mg/100g) however, lowest was demonstrated in AARI-11 (1.24 mg/100g). It is known that varietal differences are the main reason to cause variation in zinc content of wheat varieties. Zinc play a significant role in plant nutrition as it is a functional, structural or regulatory co-factor of large number of enzymes. More than 70 metallo enzymes having Zn are reported in all six classes of enzymes (Khan et al., 2006).

Similar observations have been described by various investigators who revealed 0.60 to 3.00 mg/100g zinc in SGF of different wheat varieties (Khan et al., 2009; Akhter et al., 2012; El-Sharnouby et al., 2012). Several authors have found zinc content in Pakistani wheat varieties ranged between 9.0 and 81.0 mg/kg (Anjum et al., 2002; Wahab, 2003; Khan et al., 2006; Amjad et al., 2010). Soil is an important factor which can reduce mineral content in wheat grain and flour. Variations in mineral content might due to varying genetic makeup of wheat varieties and agro climatic factors experienced by varieties (Khan et al., 2009).

Zinc supports the cellular growth and serves as a key for immune system being a cofactor of more than 300 enzymes. Micronutrient deficiency is more prevalent in developing countries due to large consumption of cereals for protein and energy. Zinc requirement for malnourished children is assessed as 2-4 mg/kg body weight (Müller et al., 2001), which is much greater as
compared to healthy children aged 1-3 years (0.17 mg/kg). In Pakistan, 39.2% children and 41.3% adult women were found zinc deficient (NNS, 2011).

4.2.7.5. Manganese (Mn)

Statistical results for manganese content in wheat varieties expressed in Table 6 apprised that it was significantly influenced by wheat varieties. Mean values (Table 7) represent that manganese content varied from 0.54 to 0.85 mg/100g. Manganese content in wheat varieties was discerned as 0.68 mg/100g (FSD-08), 0.62 mg/100g (Lasani-08), 0.54 mg/100g (AARI-11), 0.76 mg/100g (TD-1) and 0.85 mg/100g (Benazir-13). It is obvious that, highest manganese content was appraised in Benazir-13 (0.85 mg/100g) while, lowest was revealed in AARI-11 (0.54 mg/100g). Variations in manganese content determined in the present research may be a result of different factors including soil, genetics, varietal differences and environmental conditions. Present findings fall within range reported by Amjad *et al.* (2010) and El-Sharnouby *et al.* (2012). Nonetheless, these values were divergent to findings by Shar *et al.* (2002) and Khan *et al.* (2006) who established Mn content in wheat varieties of Sindh ranged from 23.14 to 60.37 mg/kg.

4.2.7.6. Copper (Cu)

Statistical results (Table 6) for copper content in wheat varieties construe highly significantly variance for copper content within wheat varieties. Mean values divulge copper content in wheat varieties ranged between 0.45 and 0.57 mg/100g (Table 7). Copper content was figured as 0.51, 0.48, 0.45, 0.57 and 0.52 mg/100g in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. Highest copper content was reckoned in TD-1 (0.57 mg/100g) while, lowest was shown in AARI-11 (0.45 mg/100g). It is established that variations in copper content are likely due to differences in wheat varieties. Present observations are in agreement with various investigations, who found copper in wheat varieties ranged as 0.10-1.32 mg/100g (Shar *et al.*, 2002; Khan *et al.*, 2009; Amjad *et al.*, 2010; Akhter *et al.*, 2012). Copper is indispensable for the maintenance of normal hemoglobin level and being a part of several enzymes (Khan *et al.*, 2009). According to findings of Wahab (2003) and Khan *et al.* (2006), copper content of wheat grains varied between 10.40 and 41.82 mg/kg.
**Table 6: Mean squares for mineral content of wheat varieties**

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Variety</td>
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<td>10.728*</td>
</tr>
<tr>
<td>Error</td>
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<td>2.364</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

**Table 7: Mean values for mineral content (mg/100g) of wheat varieties**

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>FSD-08</td>
<td>2.34±0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>2.22±0.83&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>AARI-11</td>
<td>2.20±0.86&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>T.D-1</td>
<td>2.64±0.97&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>2.51±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).


4.2.8. Wet gluten

Statistical analysis for wet gluten content given in Table 8 revealed a highly significant influence of wheat varieties on wet gluten content. Table 9 displays that a wide range for wet gluten content was found between 24.68 and 36.40%. Highest wet gluten content was ascertained in TD-1 (36.40%) followed by Benazir-13 (33.92%), FSD-08 (30.24%), Lasani-08 (29.16%) however, lowest was observed in AARI-11 (24.68%). Wheat varieties investigated in present study showed that wet gluten was influenced by varietal differences. Amongst, Lasani-08 was statistically different from other varieties in terms of wet gluten. Highest wet gluten was found in TD-1 probably due to fact that it also has high protein. Results regarding wet gluten content in present investigation are akin to earlier outcomes of different researchers, who reported wet gluten ranged 24.17-38.83% in wheat varieties (Khan et al., 2009; Amjad et al., 2010; Gulzar et al., 2010; Naseem et al., 2011). These observations are further justified by results of Arif et al. (2007) and Buriro et al. (2012) who found wet gluten in wheat varieties of Sindh in range of 23.36-39.50%. According to studies of Barrera et al. (2007) as well as of Kumar et al. (2013), wet gluten ranged between 24.10 and 33.60% in wheat cultivars.

Several articles reported wet gluten content in the range of 24.7-30.3, 25.4-30.1, 21.68-33.31, 29.1-29.2 and 14.48-28.97% in Chinese, Egyptian, Indian, Iranian and Korean wheat varieties, respectively (Feyzipour et al., 2006; Geng et al., 2012; El-Porai et al., 2013; Kaur et al., 2013; Kang et al., 2014). Gluten quality is determined by different factors such as genetics, growing conditions and milling process (Hazelton et al., 2004) as well as genetic potentiality of wheat to accumulate protein in seed as reported previously (Buriro et al., 2012). Quality and concentration of gluten is affected by genotype (variety), cultural practices, growing conditions like soil, climate and use of fertilizer (Kumar et al., 2013).

4.2.9. Dry gluten

Mean squares for dry gluten content presented in Table 8 point out that dry gluten content was significantly different within wheat varieties. Mean values (Table 9) for dry gluten content ranged between 7.12 and 11.84% in wheat varieties. Of all wheat varieties, TD-1 possessed highest dry gluten (11.84%) followed by Benazir-13 (9.80%), FSD-08 (9.08%) and Lasani-08 (7.20%) whilst, lowest was evaluated in AARI-11 (7.12%). Within wheat varieties, Benazir-13 was statistically different whereas, FSD-08 was statistically similar to AARI-11; likewise,
Lasani-08 was statistically same to TD-1. This work is consistent with earlier workers who found dry gluten in wheat varieties ranged 7.27-10.84% (Gulzar et al., 2010; Naseem et al., 2011; Ahmed et al., 2014). The level of dry gluten in Chinese, Indian and Korean wheat cultivars was ranged 8.7-10.8, 7.48-11.56 and 4.48-10.24%, respectively (Geng et al., 2012; Kaur et al., 2013; Kang et al., 2014). The level of dry gluten of wheat varieties in Sindh was observed from 7.53 to 15.80% (Arif et al., 2007; Panhwar et al., 2014; Soomro et al., 2014).

4.2.10. Starch content

Statistical analysis for starch content given in Table 8 promulgate that starch content did not differ significantly within wheat varieties. Table 9 display mean values for starch content ranged from 68.88 to 72.73%. Starch content in wheat varieties was find out as FSD-08 (70.27%), AARI-11 (72.73%), Lasani-08 (71.91%), TD-1 (68.88%) and Benazir-13 (69.92%). Highest starch content was pointed out in AARI-11 (72.73%) however, lowest was observed in TD-1 (68.88%). Regardless of wheat variety, it is known that all the wheat varieties tested in present study were statistically similar with respect to starch content. In parallel, starch content of numerous wheat varieties of Sindh was found to be 61.03-75.83% (Burito et al., 2012; Panhwar et al., 2014; Channa et al., 2015). In contrast to these results, low values (66.17-66.43%) of starch were found previously by Bashir et al. (2013) and Mahmood et al. (2013). Starch content was ranged 60-70% of total dry weight of wheat grain (Belderok et al., 2000).

4.2.11. Damaged starch (DS)

Statistical data for damaged starch presented in Table 8 construe that all the experimental measures had significantly more variability due to wheat varieties. Mean values for damaged starch content are given in Table 9. A broad range of variations was observed for damaged starch in wheat varieties ranged from 6.28 to 10.97%. Damaged starch in wheat varieties was ascertained as 8.43%, 7.33%, 6.28%, 10.97% and 10.38% in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. The lowest value of damaged starch was found in AARI-11 (6.28%) while, highest was observed in TD-1 (10.97%).

In present investigation, wheat varieties showed great genetic variations in damaged starch. It is also noteworthy that all wheat varieties were statistically different. TD-1 has highest damaged starch level followed by Benazir-13 implying that their high protein content might be one explanation in this regard. This would help establishing that wheat varieties having high protein may also have high damaged starch. Present findings commensurate to results of
numerous investigations which reported damaged starch content in wheat flour varied as 6.07-12.81% (Farooq et al., 2001; Colombo et al., 2008; Mancebo et al., 2015). However, in contrast, different authors observed higher damaged starch levels in wheat varieties ranging from 26 to 30% (Mulla et al., 2010).

Zivancev et al. (2012) determined 4.35 to 7.12% damaged starch in 18 wheat varieties. Likewise, Kang et al. (2014) found damaged starch varied from 2.08 to 7.90% in 30 Korean wheat cultivars. Yu et al. (2015) reported DS increased over entire successive break and reduction streams i.e. 3.4-15.7% in hard wheat and 1.8-6.0% for soft wheat. It has been reported that damaged starch increase water absorption up to 200-430% level (Barrera et al., 2007). Degree of DS is affected by origin of starch, wheat hardness, type and action of force exerted (Becker et al., 2001), amylase and lipid content as stated earlier (Zivancev et al., 2012). Tempering has great impact on milling and may possibly reduce the level of damaged starch (Kweon et al., 2009). Damaged starch is directly affected by grain hardness, which in turn influenced by genotype and environment (Kang et al., 2014). Many researchers reported that flour from reduction millstreams displayed greater starch damage as compared to those from break millstreams (Obuchowski et al., 2010; El-Porai et al., 2013; Sakhare et al., 2013).

4.2.12. SDS-sedimentation test

Statistical results (Table 10) represented that SDS-sedimentation values of wheat varieties were highly significantly affected by wheat varieties. Table 11 lists mean values for SDS-sedimentation with varying levels from 18.20 to 30.50ml. Highest SDS-sedimentation values was noticed in TD-1 (30.50ml) followed by Benazir-13 (29.20ml), FSD-08 (25.40ml), Lasani-08 (22.80ml) while, lowest was measured in AARI-11 (18.20 ml). It is reported that wheat varieties showed genetic variations with respect to SDS-sedimentation volume. Also, AARI-11 had lowest SDS-sedimentation value which was also statistically different from other wheat varieties. Low protein content of AARI-11 could explain this fact of low SDS-sed. volume in AARI-11. This study accede with outcome of Naseem et al. (2011) as well as of Kumar et al. (2013) who measured SDS-sedimentation volume in wheat varieties in range of 23.83-33.02 ml. However, present observations were found to be incongruent with findings of past authors who reported SDS-sedimentation ranged between 36 and 89 ml (Kaur et al., 2013; Uhlen et al., 2015).
Table 8: Mean squares for wet gluten, dry gluten, starch and damaged starch content of wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Wet gluten</th>
<th>Dry gluten</th>
<th>Starch</th>
<th>Damaged starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>4</td>
<td>107.94**</td>
<td>11.615**</td>
<td>5.920NS</td>
<td>34.676**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>2.00</td>
<td>0.178</td>
<td>10.760</td>
<td>0.362</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Table 9: Mean values for wet gluten, dry gluten, starch and damaged starch content (%) of wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Wet gluten</th>
<th>Dry gluten</th>
<th>Starch</th>
<th>Damaged starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSD-08</td>
<td>30.24±0.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.08±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.27±1.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.43±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>29.16±0.83&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.20±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.91±1.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.33±0.28&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>AARI-11</td>
<td>24.68±0.62&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.12±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.23±1.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.28±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T.D-1</td>
<td>36.40±0.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.84±0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.88±1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.97±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>33.92±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.80±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.92±1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.38±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).
In work of Xiao et al. (2006) and in agreement with Kang et al. (2014), a wide range of SDS sedimentation values in wheat cultivars was found as 8.40-45.75 ml. SDS-sedimentation test is an estimate of gluten strength and influenced by degree of hydration of wheat proteins and on their degree of oxidation (Pasha et al., 2009b). It was significantly influenced by wheat cultivar (Shin et al., 2012; Kang et al., 2014). Another study (Mueen-ud-Din et al., 2007) pointed out that the differences in SDS-sedimentation of wheat varieties are revealed by variations in the levels of moisture and protein.

4.2.13. Alkaline water retention capacity (AWRC)
Statistical results for AWRC values in wheat varieties presented in Table 10 indicated that all experimental measures had significantly more variability due to wheat varieties. Mean values regarding AWRC can be observed in Table 11 which showed that AWRC values varied between 43.91 and 66.85%. AWRC values in wheat varieties were discerned as FSD-08 (54.96%), Lasani-08 (47.12%), AARI-11 (43.91%), TD-1 (66.85%) and Benazir-13 (55.80%). Highest AWRC value was determined in TD-1 (66.85%) although, lowest was found in AARI-11 (43.91%). In present investigation, varietal differences were observed with respect to AWRC. This study put forward that Lasani-08 was statistically different from other wheat varieties.

Outcome of this work endorse the findings of past authors who found AWRC in wheat varieties ranged from 53.7 to 80.0% as recorded by previous studies (Ram and Singh, 2004; Morris et al., 2004). However, values found in this study were incompatible with Colombo et al. (2008) who reported higher AWRC values in 18 Argentinean wheat varieties ranged between 71.98 and 93.63%. AWRC values ranged from 62.6, 64.2 and 71.1% in patent, middle-cut and clear fraction, respectively, deducing more hydrophilic nature of peripheral layers of endosperm (Fustier et al., 2009a).

4.2.14. Solvent retention capacity (SRC)
4.2.14.1. Water solvent retention capacity (WSRC)
Statistical results pertaining to WSRC values in wheat varieties (Table 10) divulge that WSRC values appeared to be significantly affected by wheat varieties. Mean values for WSRC values presented in Table 11 varied from 50.83 to 69.64%. WSRC values were find out in wheat varieties i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13 as 58.77%, 63.67%, 50.83%, 66.38% and 69.64%, respectively. Highest WSRC values were observed in Benazir-13
(69.64%) whilst, lowest was exhibited in AARI-11 (50.83%). It is reported that wheat varieties showed genetic variations with respect to WSRC. Also, AARI-11 was found to be statistically different from other wheat varieties.

Present research is alike with various investigations which report WSRC in two wheat cultivars ranged from 47.92 to 83.9% (Xiao et al., 2006; Barrera et al., 2007; Zhang et al., 2007; Švec et al., 2012). Švec et al. (2012) also gave idea that values of WSRC were higher in wheat i.e. groat than flour, likely due to occurrence of non-starch polysaccharides. Several publications have reported that WSRC varied 66.26-80.99%, 52.4-71.3% and 57.42-83.76% in Argentinean, Chinese and Korean wheat varieties, respectively (Colombo et al., 2008; Geng et al., 2012; Kang et al., 2014).

4.2.14.2. Sodium carbonate solvent retention capacity (SOCSRC)

Mean squares for SOCSRC values in wheat varieties demonstrate highly significant variance for SOCSRC values among wheat varieties (Table 10). Mean values showed SOCSRC values vary widely between 56.66 and 82.03% (Table 11). SOCSRC values in wheat varieties were examined as 68.27%, 68.20%, 56.66%, 73.12% and 82.03% in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. Highest SOCSRC value was recorded in Benazir-13 (82.03%) whilst, lowest was exhibited in AARI-11 (56.66%). In present case, SOCSRC was influenced by wheat varieties. Though, there were statistical similarities among FSD-08, Lasani-08 and TD-1.

Results found in present work are assent to findings of few scientists who established SOCSRC values in different wheat lines/varieties ranged from 62.23 to 98.68% (Xiao et al., 2006; Zhang et al., 2007; Švec et al., 2012). Barrera et al. (2007) found SOCSRC values in wheat varieties ranged between 78.1 and 109.6%. Lower values for SOCSRC were observed in soft wheat by many researchers (Gaines et al., 2006; Colombo et al., 2008). Several articles have reported SOCSRC varied as 62.5-95.7, 77.28-116.71 and 78.84-113.93% in Chinese, Korean and Argentinean wheat cultivars, respectively (Colombo et al., 2008; Geng et al., 2012; Kang et al., 2014).

4.2.14.3. Sucrose solvent retention capacity (SUCSRC)

Statistical results (Table 10) for SUCSRC values in wheat varieties point out that SUCSRC values were found to be significantly different among wheat varieties. SUCSRC values varied over wide ranges from 75.79 to 106.97% (Table 11). SUCSRC values in wheat varieties were
noticed as 85.38% (FSD-08), 81.99% (Lasani-08), 75.79% (AARI-11), 106.97% (TD-1) and 95.75% (Benazir-13). Highest SUCSRC value was demonstrated in TD-1 (106.97%) while, lowest was observed in AARI-11 (75.79%). Present study suggests that SUCSRC differ due to genetic variations in wheat varieties. These observations were found to be in parity with previous studies which reported SUCSRC in wheat cultivars ranged from 82.67 to 142.10% (Xiao et al., 2006; Barrera et al., 2007; Zhang et al., 2007; Švec et al., 2012). Several authors have reported SUCSRC values varied over wide ranges 99.7-133.0%, 97.10-132.82% and 95.11-120.06% in Chinese, Korean and Argentinean wheat cultivars, respectively (Colombo et al., 2008; Geng et al., 2012; Kang et al., 2014). Lower values for SUCSRC were observed in soft wheat by many researchers (Guttieri et al., 2004; Gaines et al., 2006).

4.2.14.4. Lactic acid solvent retention capacity (LASRC)

Mean squares (Table 10) pertaining to LASRC values in wheat varieties show that LASRC values differed highly significantly among wheat varieties. From Table 11, mean values for LASRC values varied greatly from 79.43 to 121.20%. TD-1 possessed maximum values of LASRC (121.20%) followed by Benazir-13 (116.91%), Lasani-08 (95.48%) and FSD-08 (80.44%) however, lowest was found in AARI-11 (79.43%). It is considered that variations in LASRC likely due to genetic variations in wheat varieties. Though, there were statistical similarities between FSD-08 and AARI-11 and likewise, in TD-1 and Benazir-13 but, basic differences of Lasani-08 were pointed out. Present findings are in line with Barrera et al. (2007) and Zhang et al. (2007), who found LASRC values of wheat varieties in the range of 65.85-132.60%. Švec et al. (2012) observed that LASRC in wheat flour from four localities and two harvest years ranged from 124.2 to 154.7%. LASRC values reckoned in Argentinean (104.92-133.07%), Chinese (77.5-135.0%) and Korean wheat cultivars (81.21-145.06%) as reported previously (Colombo et al., 2008; Geng et al., 2012; Kang et al., 2014). LASRC may differ likely due to genotypic variations in wheat varieties (Pasha et al., 2009b). In this context, Kang et al. (2014) described that differences in SOCSRC, LASRC and WSRC values were generally accounted by wheat cultivar (> 81%). They inferred that SRC values revealed larger variations in genotypes rather than crop years.
4.3. Rheological properties of wheat varieties

4.3.1. Water absorption (WA)

Statistical results (Table 12) pertaining to water absorption in wheat varieties demonstrate that water absorption varied significantly among wheat varieties. Mean values (Table 13) for water absorption showed that it varied from 51.00 to 56.00%. Water absorption in wheat varieties was reported as FSD-08 (55.00%), Lasani-08 (53.00%), AARI-11 (51.00%), TD-1 (56.00%) and Benazir-13 (54.20%). Maximum water absorption was observed in TD-1 (56.00%) whereas, minimum was found in AARI-11 (51.00%). It is believed that wheat varieties characterized in this study for water absorption showed a large genetic diversity. Here, wheat varieties having lower rate of water absorption tended to be more suitable for cookie making and vice versa. Present study establish that wheat flour with high protein and DS also has higher water absorption. Present data is comparable with various researchers who noted water absorption in wheat varieties ranged as 51.41-63.35% (Amjad et al., 2010; Naseem et al., 2011; Ahmed et al., 2014). But, inconsonant with findings of Kaur et al. (2013), who reported high water absorption of wheat flour ranging from 58.1 to 68.4%. The values of water absorption were observed in Iranian (56.6-60.3%) and Chinese wheat flour (54.0-61.2%) in earlier studies (Feyzipour et al., 2006; Geng et al., 2012).

To comply with Farooq et al. (2014), present study also confirms that rheological properties of flour differ within varieties. Water absorption of wheat flour is determined by different factors such as particle size, protein quality, damaged starch, starch properties and pentosan content (Vizitiu and Danciu, 2011). Starch absorbs about 0.3% of water based on its mass but, in case of damaged starch, it might be increased up to 10 times (Rasper and Walker, 2000). Taken together, an increase in the levels of protein and damaged starch levels with reduced flour particle size tend to increase water absorption of flour (Fustier et al., 2009a).

4.3.2. Dough development time (DDT)

Mean squares (Table 12) related to dough development time in wheat varieties indicated that it differed highly significantly among wheat varieties. Mean values for DDT (Table 13) presented that it varied widely from 1.20 to 4.20 min. Dough development time in wheat varieties was noted as 3.20, 2.50, 1.20, 4.20 and 3.50 min in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively.
Table 10: Mean squares for SDS sedimentation volume, AWRC and SRC values of wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>SDS-sed. Volume</th>
<th>AWRC</th>
<th>WSRC</th>
<th>SOCSRC</th>
<th>SUCSRC</th>
<th>LASRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>4</td>
<td>74.166**</td>
<td>238.540**</td>
<td>161.580**</td>
<td>253.540**</td>
<td>453.810**</td>
<td>1164.70**</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Table 11: Mean values for SDS- sedimentation volume, AWRC and SRC values of wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>SDS-sed. volume (ml)</th>
<th>AWRC (%)</th>
<th>WSRC (%)</th>
<th>SOCSRC (%)</th>
<th>SUCSRC (%)</th>
<th>LASRC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSD-08</td>
<td>25.40±0.92&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>54.96±1.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.77±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.27±1.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85.38±2.22&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>80.44±2.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>22.80±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.12±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.67±1.69&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>68.20±1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.99±2.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>95.48±2.53&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AARI-11</td>
<td>18.20±0.77&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.91±1.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.83±1.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.66±1.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75.79±2.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>79.43±2.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T.D-1</td>
<td>30.50±1.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.85±1.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66.38±1.88&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>73.12±2.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>106.97±3.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>121.20±3.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>29.20±0.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>55.80±1.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>69.64±1.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.03±2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.75±2.49&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>116.91±3.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SDS-sed. volume: SDS-sedimentation volume; AWRC: Alkaline water retention capacity; WSRC: Water solvent retention capacity; SOCSRC: Sodium carbonate solvent retention capacity; SUCSRC: Sucrose solvent retention capacity; LASRC: Lactic acid solvent retention capacity
Maximum dough development time was computed in TD-1 (4.20 min) while, minimum was found in AARI-11 (1.20 min). It is regarded that variations in dough development time may possibly be a consequence of differences among wheat varieties. Though, there were statistical similarities between FSD-08 and Lasani-08 but, basic differences in AARI-11, TD-1 and Benazir-13 were also pointed out. Similar observations have made by Amjad et al. (2010) and Naseem et al. (2011) who recorded dough development time in wheat varieties ranged from 1.3 to 5.5 minutes. A wide range of dough development time in wheat varieties of Sindh was obtained as 1.00-11.46 min (Ahmed et al., 2014; Channa et al., 2015).

High values of DDT indicates strong flour while, lower DDT values are associated with weak flour (Naseem et al., 2011). A positive association has been observed between DDT and water absorption, which may be attributed to starch swelling producing changes in rheological properties (Farooq et al., 2014). Hadnadev et al. (2011) had agreed that it is influenced by gluten quality, starch granules and damaged starch content. Moreover, DDT increase with an ascent in proteolytic dilapidation of protein. It also tend to increase with a reduction in starch granule size. Dough development time is related to farinographic stability and DoS; for instance DDT and dough stability increases, softening of dough declines (Mueen-ud-Din et al., 2007).

4.3.3. Dough stability

Mean squares (Table 12) pertaining to dough stability in wheat varieties interpret that it differed highly significantly within wheat varieties. It can be observed from mean values regarding dough stability (Table 13) varied from 2.90 to 18.20 min. Results showed that dough stability in wheat varieties was computed i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13 as 10.90 min, 4.20 min, 2.90 min, 18.20 min and 13.70 min, respectively. Across varieties, TD-1 (18.20 min) gave highest dough stability while, lowest was found in AARI-11 (2.90 min). This study confirms that variations in dough stability are possibly a result of genetic differences in wheat varieties. Although, there were statistical similarities between AARI-11 and TD-1 but, FSD-08, Lasani-08 and Benazir-13 were statistically different for dough stability.

Similar values of dough stability in wheat varieties ranged 1.50-18.80 min were noticed by earlier workers (Amjad et al., 2010; Naseem et al., 2011; Farooq et al., 2014). From literature, dough stability was found to be ranged 1.0-6.4 min., 3.33-4.36 min. and 1.7-13.8 min. in
Chinese, Iranian and Indian wheat flour, respectively (Feyzipour et al., 2006; Geng et al., 2012; Kaur et al., 2013). Higher dough stability is an indication of high quality protein and deliberated as stronger wheat (Ahmed et al., 2014). A decrease in dough stability indicates lower sedimentation (Kaur et al., 2013) and weak dough strength (El-Porai et al., 2013).

4.3.4. Degree of softening (DoS)

Mean squares (Table 12) regarding degree of softening in wheat varieties indicated that it varied highly significantly due to wheat varieties. Mean values for degree of softening are given in Table 13. Broadest variations from 85.00 to 174.00 BU were observed for degree of softening in wheat varieties. Degree of softening in wheat varieties was recorded as 122.00 BU (FSD-08), 135.00 BU (Lasani-08), 174.00 BU (AARI-11), 85.00 BU (TD-1) and 117.00 BU (Benazir-13). A high degree of softening was observed in AARI-11 (174.00 BU) while, low degree of softening was found in TD-1 (85.00 BU). Building on present data, it is reported that a large genetic diversity was observed for degree of softening in wheat varieties. As shown by these results, AARI-11 and TD-1 were statistically different from other wheat varieties with respect to degree of softening.

Present findings were somewhat alike to Amjad et al. (2010) and Bashir et al. (2013) who observed degree of softening in wheat varieties ranged from 46.67 to 141.67 BU. However, a discrepancy was observed between present results and findings of other authors who found degree of softening ranged from 44 to 70BU (Mohammed et al., 2012; Nikolić et al., 2013; Iqbal et al., 2015b). Flour with lower degree of softening are thought to be stronger flour whereas, the flour having higher values are weak (Farooq et al., 2014). Practically, high stability and low degree of softening is the representation of dough capability to withstand long processing treatment. Increase DoS is mainly important parameter of proteolytic breakdown of gluten (Hadnadev et al., 2011).

4.3.5. Farinograph quality number (FQN)

Mean squares (Table 12) pertaining to farinograph quality number in wheat varieties construe that it differed highly significantly due to wheat varieties. Table 13 shows that farinograph quality number varied in a wide range from 48.00 to 200.00. Based on present observations, highest farinograph quality number was recorded in AARI-11 (200.00) followed by Lasani-08 (184.00), FSD-08 (181.00) and Benazir-13 (109.00) and while, lowest was recorded in TD-1 (48.00).
Present outcome suggests that variations in farinograph quality number may be related to varietal differences. In this way, statistical similarities were observed for FSD-08, Lasani-08 and TD-1 however, AARI-11 and Benazir were statistically different. Data here on, thus appears consonant with Kaur et al. (2013) who reported farinograph quality number ranged from 35 to 150. They further reported that flour having high levels of protein, wet gluten and dry gluten exhibited low dough stability and FQN. As for power of flour, valued by FQN i.e. how soft it becomes at battering that is a flour quality index (Voicu et al., 2012). The differences in rheological parameters of flour determined by farinographic depends upon wheat varieties (Farooq et al., 2014). Moreover, properties of farinograph curve for a particular variety differ with respect to location (Ahmed et al., 2014).

4.4. Pasting properties of wheat varieties

4.4.1. Pasting temperature (PaT)

Mean squares (Table 14) pertaining to pasting temperature in wheat varieties interpret that it had significantly more variability due to wheat varieties. Table 15 represents mean values of pasting temperature varied in a quite narrow range from 65.25 to 67.80°C. Pasting temperature was computed as 66.90°C, 66.95°C, 67.80°C, 65.25°C and 66.73°C in wheat varieties i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. Maximum pasting temperature was recorded for AARI-11 (67.80°C) while, lowest was found in TD-1 (65.25°C). Regardless of variety, all the wheat varieties tested in present study were statistically similar with respect to pasting temperature.

Present observations accede to data obtained by previous studies which revealed pasting temperature of Indian wheat flour in range of 64.45-68.75°C (Jian-sheng et al., 2012; Kaur et al., 2013). On contrary, few scientists reported higher values (70.18-96.7°C) of gelatinization temperature for wheat (Falola et al., 2014; Jagannadham et al., 2014; Channa et al., 2015). Temperature required to start gelatinization and FV is associated with starch retrogradation, which tend to decrease with more starch damage. Gelatinization behavior of starch may be governed by α-amylase activity and useful in the prediction of flour baking quality (Channa et al., 2015). During first holding stage, flour-gum paste underwent heating to 95°C for 30 min. and inverse of viscosity change (before and after this phase) provides heating stability of mixture and high values are consideration of increased heating stability.
Table 12: Mean squares for rheological properties of wheat varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WA</td>
</tr>
<tr>
<td>Variety</td>
<td>4</td>
<td>11.184*</td>
<td>30.999**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>6.258</td>
<td>0.086</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Table 13: Mean values for rheological properties of wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>WA (%)</th>
<th>DDT (min)</th>
<th>DST (min)</th>
<th>DoS (BU)</th>
<th>FQN</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSD-08</td>
<td>55.00±1.44b</td>
<td>3.20±0.15a</td>
<td>10.90±0.29a</td>
<td>122±3.17b</td>
<td>181±4.79a</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>53.00±1.50ab</td>
<td>2.50±0.06a</td>
<td>4.20±0.12b</td>
<td>135±3.76c</td>
<td>184±5.21a</td>
</tr>
<tr>
<td>AARI-11</td>
<td>51.00±1.39a</td>
<td>1.20±0.06c</td>
<td>2.90±0.06d</td>
<td>174±4.91d</td>
<td>200±5.20c</td>
</tr>
<tr>
<td>T.D-1</td>
<td>56.00±1.50bc</td>
<td>4.20±0.19d</td>
<td>18.20±0.46d</td>
<td>85±2.31a</td>
<td>48±1.33a</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>54.20±1.39ab</td>
<td>3.50±0.16b</td>
<td>13.70±0.35c</td>
<td>117±3.18bc</td>
<td>109±2.83b</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).

WA: Water absorption; DDT: Dough development time; DST: Dough stability time; DoS: Degree of Softening; FQN: Farinograph quality number
However, inverse of viscosity change (before and after second holding phase) is related to cooling stability of flour, where paste was allowed to set at 50°C for 10 min. (Alam et al., 2009). In view of El-Porai et al. (2013), highest pasting temperature of Egyptian wheat was obtained by hard milling than normal milling. Likewise, Barrera et al. (2013) evaluated the impact of DS on pasting properties of starch suspensions and deduced that pasting temperature increased with increasing the level of DS. Increased pasting temperature may be credited to lower starch swelling, higher water absorption capacity (WAC) and gelatinization of starch granules (Kaur et al., 2013).

4.4.2. Peak time (PT)

Statistical results (Table 14) pertaining to peak time in wheat varieties infer that PT differed highly significantly due to wheat varieties. Table 15 showed that mean values for peak time varied from 3.22 to 6.00 min. Peak time in wheat varieties was noted as FSD-08 (4.33 min), Lasani-08 (3.47 min), AARI-11 (3.22 min), TD-1 (6.00 min) and Benazir-13 (5.73 min). This shows that maximum PT was observed in TD-1 (6.00 min) while, lowest was found in AARI-11 (3.22 min). It is believed that wheat varieties investigated in present study are more likely to cause variations in peak time. Amongst, AARI-11 was found to be statistically different from other wheat varieties. Nasir (2015) reported that peak time was non-significantly affected by quinoa lines and ranged from 6.97 to 6.99 min. Pasting parameters of starch are usually thought to be measured through relation of starch structural properties, involving starch morphology and rigidity (Yu et al., 2015) and are largely affected by milling process (Dhital et al., 2010).

4.4.3. Peak viscosity (PV)

Mean squares (Table 14) related to peak viscosity in wheat varieties showed that peak viscosity differed highly significantly among wheat varieties. From Table 15, mean values for peak viscosity can be found to vary from 1330 to 1784 cP. PV in wheat varieties was reported i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13 as 1496cP, 1624cP, 1784cP, 1330cP and 1435cP, respectively. Maximum peak viscosity was observed in AARI-11 (1784cP) while, lowest was found in TD-1 (1330cP). It can be reasoned here that variations in peak viscosity are possibly a result of genetic variations in wheat varieties. Of all, TD-1 and Benazir-13 were statistically different as compared to others.

These findings are compatible to Kaur et al. (2013) and Jagannadham et al. (2014) who found
peak viscosity ranged from 1278 to 2311 cP for Indian wheat flour. It has been established that peak viscosity of whole wheat flour is determined by wheat variety and environment and variations in flour peaks of different wheat lines may due to differences in amylase activity (Farooq et al., 2001), which was further confirmed by El-Porai et al. (2013), who described the differences in RVA viscosity of wheat flour can be related to variations in damaged starch and α-amylase activity. This could be explained by the fact that peak viscosity decreased with an increase in DS and α-amylase activity.

Peak viscosity designates equilibrium point between swelling and rupturing of starch granules (Kaur et al., 2013). Peak viscosity, which reflects swelling properties of starch is generally associated with amyllopectin however, amyllose or amyllose-lipid network, might inhibit granule swelling, which led to a decline in peak viscosity (Wang and Copeland, 2012; Yu et al., 2015). Protein strengthens associations between continuous and dispersed phase, which provides greater strength towards shearing (Fitzgerald et al., 2003). This implies that higher values of peak viscosity are likely due to higher glutenin levels and glutenin: gliadin ratio (Kaur et al., 2013).

4.4.4. Trough viscosity (TV)

Mean squares (Table 14) pertaining to trough viscosity in wheat varieties point out that TV differed highly significantly among wheat varieties. Mean values for trough viscosity can be observed from Table 15 and ranged between 645 and 1002 cP. Trough viscosity in wheat varieties was established as 795cP, 905cP, 1002cP, 645cP and 740cP in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. Maximum trough viscosity was observed in AARI-11 (1002cP) while, minimum was found in TD-1 (645cP). Variations in trough viscosity might be a result of differences in wheat varieties. Present study suggests that AARI-11, TD-1 and Benazir-13 were statistically different with respect to trough viscosity. These results were somewhat analogous to findings of previous studies who computed trough, viscosity of flour from wheat varieties ranged from 930 to 1471 cP (Jian-sheng et al., 2012; Kaur et al., 2013; Jagannadham et al., 2014). However, in contrast to present findings, El-Porai et al. (2013) found trough viscosity of Egyptian wheat flour ranged from 1235 to 2772 cP.
In fact, granules rupturing owing to shearing contributes to trough viscosity. It is affected by extent of granule swelling, amylose exudation, amylose-lipid network development (Kaur et al., 2013).

4.4.5. Final viscosity (FV)

Mean squares (Table 14) pertaining to final viscosity in wheat varieties designated that FV differed highly significantly among wheat varieties. Results evidence that mean values for final viscosity have been shown in Table 15. A wide variation in final viscosity was observed from 1799 to 2565 cP. Final viscosity in wheat varieties was recorded as 2002cP (FSD-08), 2135cP (Lasani-08), 2565cP (AARI-11), 1799cP (TD-1) and 1952cP (Benazir-13). Maximum final viscosity was observed in AARI-11 (2565cP) while, minimum was found in TD-1 (1799cP). As anticipated, varietal differences might be the main reason for variations in final viscosity. In that manner, TD-1 and Benazir-13 were statistically differed in terms of final viscosity. Variations in final viscosity are likely due to genetic variations in wheat varieties. Present results regarding final viscosity are endorsed by Kaur et al. (2013) who reported final viscosity in the range of 1774-2365 cP for Indian wheat flour. For final viscosity, the last part of RVA pasting curve determines the increase in viscosity related to gelation and retrogradation during cooling. When paste is cooled to 50°C, starch granules reached a final viscosity, due to re-association or retrogradation of amylose (Ragaee and Abdel-Aal, 2006; Kaur et al., 2013).

4.4.6. Breakdown viscosity (BV)

Mean squares (Table 14) pertaining to breakdown viscosity in wheat varieties point out that BV differed highly significantly among wheat varieties. Table 15 show mean values for breakdown viscosity and wide ranges can be observed for BV indicating variability from 685 to 782 cP. Breakdown viscosity in wheat varieties was calculated as 701cP, 719cP, 782cP, 685cP and 695cP in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. Maximum breakdown viscosity was observed in AARI-11 (782cP) whereas, minimum was found in TD-1 (685cP). It is observed that wheat varieties analyzed in present study for breakdown viscosity showed a large genetic diversity. Of all wheat varieties, Lasani-08 was statistically different regarding breakdown viscosity. Values found in current study are akin to results, as shown by Kaur et al. (2013) and Jagannadham et al. (2014) who found breakdown viscosity of Indian wheat flour ranged from
277 to 901 cP. El-Porai et al. (2013) found breakdown viscosity of Egyptian wheat ranging between 533 and 1450 cP. Breakdown was related to starch stability under the influence of high shear (Ragaee and Abdel-Aal, 2006). Breakdown tend to decrease by protein, may due to maintenance of continuous network or association between continuous and dispersed phases (Fitzgerald et al., 2003).

4.4.7. Setback viscosity (SV)
Statistical results (Table 14) regarding setback viscosity in wheat varieties represented that SV differed highly significantly among wheat varieties. Table 15 displays that there is a wide range of setback viscosity in wheat varieties varying from 1154 to 1563 cP. Setback viscosity in wheat varieties was calculated i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13 as 1207cP, 1230cP, 1563cP, 1154cP and 1212cP, respectively. According to results, maximum setback viscosity was observed in AARI-11 (1563cP) while, lowest was found in TD-1 (1154cP). Present observations had agreed to data obtained by El-Porai et al. (2013). Other studies conducted by Kaur et al. (2013) and Jagannadham et al. (2014) who reported setback viscosity in Indian wheat flour varied from 844 to 1009 cP. It can be inferred that baked products made with flour with higher setback viscosity may have had greater staling rate since, greater setback is related to high retrogradation rate. Breakdown, peak and final viscosities were interrelated therefore, flour with high peak viscosity also revealed high breakdown and final viscosities. Studies have established that protein provide some protection against breakdown (Kaur et al., 2013).

4.5. Physical analyses of cookies produced from wheat varieties
4.5.1. Width
Mean squares (Table 16) regarding width of cookies prepared from wheat varieties have shown that width differed significantly among wheat varieties as compared to control. Mean values (Table 17) for width indicated that width of cookies prepared from wheat varieties varied from 25.20 to 26.60 cm and 26.50 cm in mixed wheat flour (MWF) selected as control. Width of cookies prepared from wheat varieties was measured as FSD-08 (25.90 cm), Lasani-08 (26.00 cm), AARI-11 (26.60 cm), TD-1 (25.20 cm) and Benazir-13 (25.50 cm).
### Table 14: Mean squares for pasting properties of wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PaT</td>
</tr>
<tr>
<td>Variety</td>
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<tr>
<td>Error</td>
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<tr>
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<td></td>
</tr>
</tbody>
</table>

** ** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

### Table 15: Mean values for pasting properties of wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PaT (°C)</td>
</tr>
<tr>
<td>FSD-08</td>
<td>66.90±1.78a</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>66.95±1.74a</td>
</tr>
<tr>
<td>AARI-11</td>
<td>67.80±1.76a</td>
</tr>
<tr>
<td>T.D-1</td>
<td>65.25±1.85a</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>66.73±1.81a</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).

PaT: Pasting temperature; PT: Peak time; PV: Peak viscosity; TV: Trough viscosity; FV: Final viscosity; BV: Breakdown viscosity; SV: Setback viscosity
Maximum width was reckoned in AARI-11 (26.60 cm) while, lowest was found in TD-1 (25.20 cm). It is established that width of cookies is likely to vary with wheat varieties and MWF examined in present study. Further, AARI-11 was statistically different from other wheat varieties as well as control. This study comply with Doescher et al. (1987) and Miller and Hoseney (1997) who pointed this out that biscuits made with soft wheat displayed higher width than hard wheat cultivars. Present findings are assent to previous results of Pasha et al. (2002) who determined 25.0 cm width of cookies.

In fact, higher levels of DS, arabinoxylan and protein exist in hard and durum wheat, which bind significant level of water than soft wheat and decreased biscuit spread (Pauly et al., 2013). In view of Igrejas et al. (2002), the composition of flour protein have low impact on quality as compared to its level. Cookie diameter was largely affected by cultivar for Korean wheat varieties and experimental lines (Kang et al., 2014). Souza et al. (2004) stated that cookie quality of soft wheat is determined by wheat genotypes, environment and their interaction.

**4.5.2. Thickness**

Statistical data (Table 16) pertaining to thickness of cookies prepared from wheat varieties explored that it differed significantly among wheat varieties as compared to control. Mean values for thickness can be observed from Table 17. Thickness of cookies prepared from wheat varieties varied from 5.80 to 6.40 and 6.40 cm in MWF (control). Thickness of cookies prepared from wheat varieties was measured as 6.10, 6.00, 5.80, 6.40 and 6.30 cm in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. Maximum (6.40 cm) thickness was observed in TD-1 while, lowest (6.00 cm) was found in Lasani-08. Varietal differences are most probably to cause variations in thickness of cookies within wheat varieties and MWF. Data here suggests that, AARI-11 and TD-1 were observed statistically different from other wheat varieties with respect to thickness.

To comply with Akbar et al. (2016), cookie thickness increased with increase in protein content. Findings of current study were found to be in compliance with Pasha et al. (2002) who noted 6.70 cm width of cookies. Thickness of cookies for Korean wheat cultivars was influenced by genotype and environmental conditions (Kang et al., 2014). Cookie thickness was also determined by spread ratio, which in turn affected by relative ability or competition of different ingredients for water availability as well as flour constituents for water absorption during dough development (Masih et al., 2014).
4.5.3. Spread factor
Mean squares (Table 16) related to spread factor of cookies prepared from wheat varieties show that spread factor differed significantly among wheat varieties as compared to control. Table 17 lists the mean values for spread factor of cookies. Spread factor varied from 39.38 to 45.86 for cookies prepared from wheat varieties and 41.41 in MWF (control). Across varieties, AARI-11 yielded cookies with highest (45.86) spread factor followed by Lasani-08 (43.33), FSD-08 (42.40) and Benazir-13 (40.48) while, lowest was calculated in TD-1 (39.38). Varietal differences might be the main reason to cause variations in spread factor of cookies. Though, there were statistical similarities among wheat varieties and MWF however, AARI-11 was statistically different from other varieties. Present findings commensurate to results of Pasha et al. (2002) who reported spread factor ranged from 44.26 to 64.15. This work and in agreement with Pareyt et al. (2008), spread factor of cookies was found to be decreased with increased protein and high gluten levels. Flour having lower absorption will formulate cookies with larger spread (Mancebo et al., 2015).

4.5.4. Weight
Mean squares (Table 16) regarding weight of cookies prepared from wheat varieties point out that weight did not varied significantly within wheat varieties as compared to control. Mean values for weight have been revealed in Table 17. Weight of cookies prepared from wheat varieties varied from 9.01 to 9.49 g/cookie and 9.25 g/cookie in MWF (control). As shown, maximum weight was measured in FSD-08 (9.49 g/cookie) followed by TD-1 (9.38 g/cookie), Lasani-08 (9.11 g/cookie) and AARI-11 (9.07 g/cookie) however, lowest was found in Benazir-13 (9.01 g/cookie). However, wheat varieties and MWF studied in present research were statistically similar with respect to weight of cookies. In parallel, weight of cookies made from wheat flour cookies (control) was found to be 7.55-13.834 g/cookie in previous studies (Arshad et al., 2008; Olagunju and Ifesan, 2013).

4.5.5. Hardness
Mean squares (Table 16) related to hardness of cookies prepared from wheat varieties infer that hardness differed highly significantly among wheat varieties as compared to control. Mean values for hardness have been presented in Table 17. Force required to break cookie was recorded as 3.45 kg, 2.95 kg, 2.17 kg, 3.91 kg and 3.76 kg in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively.
Based on these observations, maximum hardness was observed in TD-1 (3.91 kg) while, lowest was found in AARI-11 (2.17 kg). Peak force varied for cookies made with five wheat varieties and mixed wheat flour. Values observed in present investigation are incommensurate with those observed by Jacob and Leelavathi (2007) who reported breaking strength of cookies ranged from 4.6 to 9.7 kg. Fustier et al. (2007) found that specific tearing force (Fs) for biscuit made with parent flour increased from 1.61N, 1.90N and 2.14 N in patent, middle-cut and clear fractions, respectively. Another study conducted by Mancebo et al. (2015) described that peak force for wheat cookies was found to be 40.21 N.

Hardness is one of main property defining textural assessment of cookies and appraised as peak force to snap cookie (Mancebo et al., 2015). During baking, since fat melts gluten entanglements occur with great ease to form a continuous matrix due to low physical hindrance (Pareyt et al., 2010) and these gluten entanglements produced hard cookies (Sciarini et al., 2013). In order to retain a soft texture, it is essential to store cookies in good packaging condition under a constant relative humidity (HadiNezhad and Butler, 2009).

4.6. Sensory evaluation of cookies produced from wheat varieties

4.6.1. Color

Statistical data (Table 18) for color of cookies prepared from wheat varieties represented that color differed significantly within wheat varieties as compared to control. Experimental values (Table 19) related to color of cookies prepared from wheat varieties varied from 5.88 to 7.50 while, 7.00 in control. Results clearly indicated that maximum score of color was examined in AARI-11 (7.50) followed by Benazir-13 (7.26), TD-1 (6.88) and Lasani-08 (6.38) while, minimum was found in FSD-08 (5.88). Color is very critical since our choice of food is greatly affected by this trait and also evaluating accurately baked cookies.

Findings of present study are quite consonant with various researchers who found color scores of cookies produced from wheat ranged as 6.46-7.33 (Arshad et al., 2008; Mushtaq et al., 2010; Aziah et al., 2012; Akbar et al., 2016). While, contrary to present results, color score of wheat cookies was found to be 8.00, previously (Hussain et al., 2006). Color of cookies is not only related to flour color that is used but also linked to baking reactions for instance Maillard and caramelisation process (Ameur et al., 2007; Mancebo et al., 2015).
Table 16: Mean squares for physical properties of cookies prepared from wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Width</td>
<td>Thickness</td>
<td>Spread factor</td>
<td>Weight</td>
<td>Hardness</td>
</tr>
<tr>
<td>Variety</td>
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<td>0.1740*</td>
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<td>0.1327&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1.4694&lt;sup&gt;**&lt;/sup&gt;</td>
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<td>Total</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Table 17: Mean values for physical properties of cookies prepared from wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Width (cm)</th>
<th>Thickness (cm)</th>
<th>Spread factor</th>
<th>Weight (g)</th>
<th>Hardness (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.50±0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.40±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.41±1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.25±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.50±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FSD-08</td>
<td>25.90±0.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.10±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.40±0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.49±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.45±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lasani-08</td>
<td>26.00±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.33±1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.11±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.95±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>AARI-11</td>
<td>26.60±0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.80±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.86±1.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.07±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.17±0.13&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T.D-1</td>
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<td>6.40±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>3.91±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benazir-13</td>
<td>25.50±0.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.30±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>3.76±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).
4.6.2. Crispiness
Mean squares (Table 18) pertaining to crispiness of cookies prepared from wheat varieties delved that crispiness differed significantly due to wheat varieties as compared to control. Mean values for crispiness of cookies prepared from wheat varieties (Table 19) varied from 6.69 to 7.56 and 7.25. Score of crispiness of cookies prepared from wheat varieties was evaluated as FSD-08 (7.56), Lasani-08 (7.38), AARI-11 (7.38), TD-1 (6.69) and Benazir-13 (7.00). Maximum crispiness was observed in FSD-08 (7.56) but, minimum was found in TD-1 (6.69). Evaluation of sensory attributes of cookies was found to be in harmony with results of Arshad et al. (2008) and Aziah et al. (2012) who assessed crispiness values of cookies produced from wheat ranged 7.09-7.20. Whereas, crispiness scores were contradictory to those evaluated by Hussain et al. (2006) as well as of Akbar et al. (2016).

4.6.3. Flavor
Mean squares (Table 18) related to flavor of cookies prepared from wheat varieties conferred that flavor differed significantly among wheat varieties as compared to control. Mean values related to flavor of cookies prepared from wheat varieties as shown in Table 19 varied from 6.56 to 7.31 and 6.38 in MWF (control). Maximum flavor of cookies prepared from wheat varieties was determined as 6.56 (FSD-08), 7.31 (Lasani-08), 7.25 (AARI-11), 7.13 (TD-1) and 7.00 (Benazir-13). This further clarified that maximum flavor was evaluated in Lasani-08 (7.31) whereas, lowest was found in FSD-08 (6.56). Present results are close to the values reported by Arshad et al. (2008) and Akbar et al. (2016) who judged flavor scores of wheat cookies ranged 6.50-7.33. However, flavor scores were found to be inconsistent to those established previously (Hussain et al., 2006; Mushtaq et al., 2010; Aziah et al., 2012). Flavor is main criterion which play an important part in selection of products. Maillard reaction occurring between amino acid/peptides and reducing sugars may be liable for aroma of baked, fried and other heat processed foods (HadiNezhad and Butler, 2009).

4.6.4. Taste
Mean squares (Table 18) referring taste of cookies prepared from wheat varieties interpreted that it differed significantly among wheat varieties as compared to control. Mean values for taste of cookies prepared from wheat varieties presented in Table 19 varied from 6.56 to 7.75 and 7.25 in MWF (control). Taste of cookies prepared from wheat varieties was assessed as
6.56, 7.50, 7.75, 7.19 and 6.75 in FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13, respectively. It seems that maximum score of taste was evaluated in AARI-11 (7.75) while, minimum was noticed in TD-1 (7.19). Values observed in present investigation are similar to those observed by Akbar et al. (2016) who found taste scores of cookies produced from wheat as 7.33. However, in contrast to findings by previous studies (Mushtaq et al., 2010; Mancebo et al., 2015).

4.6.5. Texture
Mean squares (Table 18) related to texture of cookies prepared from wheat varieties demonstrate that texture differed significantly within wheat varieties as compared to control. Mean values for texture of cookies prepared from wheat varieties given in Table 19 varied from 6.38 to 7.63 and 7.00 in MWF (control). Texture of cookies prepared from wheat varieties was assessed i.e. FSD-08, Lasani-08, AARI-11, TD-1 and Benazir-13 as 6.88, 7.44, 7.63, 6.38 and 6.69, respectively. Maximum texture was observed in AARI-11 (7.63) while, lowest was found in TD-1 (6.38). Cookies prepared with AARI-11 were evaluated best regarding breakage and surface appearance.

Similar values (7.00) for texture of cookies produced from wheat were noticed by earlier workers (Arshad et al., 2008; Akbar et al., 2016). But, in contrast to findings by few scientists (Hussain et al., 2006; Mushtaq et al., 2010; Mancebo et al., 2015). Texture is one of key parameters which contribute to eating quality of cookies (Mancebo et al., 2015). Both protein and damaged starch absorb higher amount of water and escalate dough viscosity (Pauly et al., 2013). The functionality is mainly affected by various factors including wheat cultivar, variation within a cultivar, grain hardness, differences in crop season, protein level and milling process (Fustier et al., 2009b). Moreover, cookies produced with soft wheat had more tender bite and better eating quality and appearance (Delcour and Hoseney, 2010).

4.6.6. Overall acceptability
Statistical data (Table 18) pertaining to overall acceptability of cookies prepared from wheat varieties divulge that overall acceptability differed significantly among wheat varieties comparing these to control. Mean values pertaining to overall acceptability of cookies made from wheat varieties (Table 19) were found to vary from 6.75 to 7.75 and 7.06 in MWF (control). Overall acceptability of cookies prepared from wheat varieties was evaluated as FSD-08 (7.25), Lasani-08 (7.69), AARI-11 (7.75), TD-1 (6.75) and Benazir-13 (6.94). For
purpose of comparison, maximum overall acceptability was observed in AARI-11 (7.75) while, lowest was found in TD-1 (6.75). Panelists found cookies from AARI-11 had good color, surface appearance and highly acceptable as compared to other varieties. Low protein of AARI-11 may be one explanation for this attribute. Present findings regarding overall acceptability scores are inconsistent to several articles who have reported low (5.66-6.64) overall acceptability scores of wheat cookies (Arshad et al., 2008; Mushtaq et al., 2010; Aziah et al., 2012; Mancebo et al., 2015).
Table 18: Mean squares for organoleptic properties of cookies prepared from wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Color</th>
<th>Crispiness</th>
<th>Flavour</th>
<th>Taste</th>
<th>Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.5458*</td>
<td>0.9875*</td>
<td>1.5958*</td>
<td>1.7375*</td>
<td>1.2208*</td>
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<tr>
<td>Error</td>
<td>42</td>
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<td>0.5342</td>
<td>0.3289</td>
<td>0.5997</td>
<td>0.6384</td>
<td>0.4137</td>
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<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Table 19: Mean values for organoleptic properties of cookies prepared from wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mean± SE</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Overall acceptability</th>
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<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Crispiness</td>
<td>Flavour</td>
<td>Taste</td>
<td>Texture</td>
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<td></td>
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<tr>
<td>Control</td>
<td>7.00±0.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.25±0.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.38±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.25±0.25&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.00±0.27&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.06±0.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>FSD-08</td>
<td>5.88±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.56±0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.56±0.22&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.56±0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.88±0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.25±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lasani-08</td>
<td>6.38±0.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.38±0.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.31±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.50±0.27&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.44±0.37&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.50±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>AARI-11</td>
<td>7.50±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.38±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.25±0.23&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.75±0.31&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>7.63±0.30&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.75±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T.D-1</td>
<td>6.88±0.40&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.69±0.26&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.13±0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.19±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.38±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.75±0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Benazir-13</td>
<td>7.26±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00±0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.75±0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.69±0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.94±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means sharing similar letter in a column are statistically non-significant (P>0.05).
4.7. Characterization of cookies prepared with different treatments

On the basis of different physicochemical, rheological and sensory parameters, AARI-11 was selected as best wheat varieties for cookie making and it was further utilized for the preparation of cookies along with application of different treatments. These treatments were applied to cookie formulation for the reason that if AARI-11 is best variety for cookie making but, it may be commercially inaccessible due to lack of adequate wheat classification system. So, core objective was to improve quality of mixed wheat flour. For this reason, various treatments from T₁ to T₅ were applied to selected wheat variety (SWV) while T₆ to T₁₀ to mixed wheat flour (MWF) at various levels. Treatment combinations can be described as chemically (acid) modified starch (CMS) i.e. T₁ & T₆, ascorbic acid i.e. T₂ & T₇, protease i.e. T₃ & T₈, potassium iodate (KIO₃) i.e. T₄ & T₉ and sodium metabisulfite (SMS) i.e. T₅ & T₁₀. Moreover, their cookie baking quality was also evaluated to study the effect of treatments and their concentrations, which is the basis of research.

4.7.1. Physical properties of cookies

4.7.1.1. Width

Mean squares (Table 20) regarding width of cookies prepared with different treatments have shown highly significant differences among all combinations. Further, splitting statistical results revealed that treatments caused highly significant effect on cookies width while, the effect of concentration was non-significant on cookies width. Also, there were significant variations in width due to interaction of concentration and treatments. Data show that, all the treatments were statistically different as compared to control with respect to width.

Table 21 lists the mean values for width of cookies made with different treatments varying from 25.4 to 31.7 cm and 26.50 cm in mixed wheat flour which was selected as control (T₀). To have useful interpretation of results, the results regarding width are summarized as 30.8-31.7 cm (CMS), 25.4-26.0 cm (ascorbic acid), 29.7-30.5 cm (protease), 27.8-28.2 cm (KIO₃) and 28.5-29.5 cm (SMS). On the whole, cookies prepared from wheat flour substituted with CMS (85:15) produced largest width (31.7 cm) whereas, cookies treated with ascorbic acid (15 ppm) had lowest width (25.4 cm). In order to further support the interpretation, the results regarding treatments were also discussed as herein.

Width of cookies made with the replacement of chemically modified starch (CMS) at different levels (5, 10 and 15%) ranged from 30.8 to 31.7 cm. Within treatments, highest width (31.7
cm) of cookies was measured in T6C3 followed by 31.5 cm (T6C2), 31.2 cm (T1C3), 31.0 cm (T1C2), 30.9 cm (T6C1) and 30.8 cm (T1C1). In this work, width of cookies was improved by increasing the replacement of wheat flour with CMS. Largest width of cookies was observed with substitution of wheat flour by CMS at 15% level (85:15). This may probably due to dilution of protein of wheat flour with starch and alteration in flour characteristics due to acid modified starch. Pareyt et al. (2011) described that diameter ranged as 88.3-93.8 mm for cookies made with substitution of flour with 10 to 40% starch.

Treatment of flour with ascorbic acid at different (5, 10 and 15ppm) concentrations have shown width of cookies varied from 25.4 to 26.0 cm. Regarding treatments, width of cookies measured as T2C1 (26.0 cm), T2C2 (25.9 cm), T2C3 (25.5 cm), T7C1 (25.8 cm), T7C2 (25.6 cm) and T7C3 (25.4 cm). Present data reported a decreasing trend for width of cookies, as the concentration of ascorbic acid was increased. To comply with Faccio et al. (2012), this may possibly due to the fact that development of too much S-S linkages in gluten network lead to more firm dough without capability to stretch adequately.

Addition of protease (papain) at different (200, 400 and 600 mg) concentrations impacted width of cookies and it ranged between 29.7 and 30.5 cm. For treatments, width of cookies was determined as 30.2, 30.4, 30.5, 29.7, 29.9 and 30.1 cm in T3C1, T3C2, T3C3, T8C1, T8C2 and T8C3, respectively. In present study, as the level of protease increased, the width of cookies was also increased hence, cookies with highest width were produced from the dough formulation containing protease (600 mg).

Table 21 showed the width of cookies treated with potassium iodate (KIO3) at different (500, 1000, 1500 ppm) concentrations varying from 27.8 to 28.2 cm. Width of cookies was determined in different treatments i.e. T4C1, T4C2, T4C3, T9C1, T9C2 and T9C3 as 28.2, 28.1, 28.0, 28.2, 27.9 and 27.8 cm, respectively. Increasing levels of KIO3, reduced the width of cookies. These results are antithesis to findings by Pareyt et al. (2010) who rather noticed an increase in diameter of cookies with an addition of potassium iodate at high levels.

Varying concentrations (1000, 2000 and 3000ppm) of sodium metabisulfite impacted width of cookies ranging between 28.5 and 29.5 cm. T5C3 had highest width as 29.5cm followed by 29.2cm (T10C3), 29.1cm (T5C2), 29.0cm (T10C2), 28.9cm (T10C1) and 28.5cm (T5C1). Width of cookies increased progressively with an increase in SMS furthermore, cookies with largest width were made from dough containing 3000 ppm concentration of SMS. This may probably
due to the fact that reducing agent may have reduced gluten or inhibited gluten development. Present results support the findings of Kumar et al. (2013), who stated disulfide linkages in gluten protein were dilapidated through a chain of reactions with cysteine or glutathione, termed as disulfide interchange. However, in contrast to present results, Pareyt et al. (2010) reported that reducing compounds dwindled diameter of cookies but, increased cookie height. Diameter of cookies was significantly affected by protein content (Singh and Mohamed, 2007). In another study, Pareyt et al. (2008) described a decline in cookie width with an increase in gluten levels. Also, number and type of disulfide bridges between gluten have pronounced effect on characteristics of gluten structure and dough rheology (Wieser, 2007). Reducing agents support S-H/S-S interchange reactions resulted in a weaken dough, decreased mixing time and better dough machinability (Joye et al., 2009).

4.7.1.2. Thickness

Statistical data (Table 20) pertaining to thickness of cookies made with different treatments divulge that it differed significantly for all the combinations. Moreover, splitting statistical results further point out that treatments showed highly significant impact on cookies thickness. Similarly, the effect of concentration as well interaction of concentration and treatments was found to be significant regarding cookies thickness. In present study, it is worth mentioning that treatments were statistically significant as compared to control in terms of cookies thickness.

Mean values for thickness of cookies prepared with different treatments can be observed from Table 21 to be varied from 5.2 to 6.4 and 6.4 cm in control (T₀). Across treatments, thickness of cookies ranged as 5.2-5.4 cm (CMS), 6.0-6.4 cm (ascorbic acid), 5.2-5.5 cm (protease), 6.0-6.4 cm (KIO₃) and 5.6-5.9 cm (SMS). Taking all the treatments into account, cookies treated with ascorbic acid (15 ppm) and KIO₃ (1500 ppm) possessed highest thickness (6.4 cm) whereas, cookies made with CMS and protease gave lowest value (5.2 cm) of thickness at 15% level of substitution and 600 mg, respectively. Thickness of cookies made with the replacement of chemically modified starch varied from 5.2 to 5.4 cm. Highest thickness of cookies was measured in T₁C₁ (5.4 cm) followed by T₆C₁ (5.3 cm), T₁C₂ (5.3 cm), T₆C₂ (5.3 cm), T₆C₃ (5.2 cm) and T₁C₃ (5.2 cm). A significant reduction in thickness of cookies was observed as a result of replacement of wheat flour with chemically modified starch. The smallest value of thickness was found for cookies made from
flour replaced with CMS at 15% level. Chemical modification is the most common way to modify starch for its use as texture improver in different products. Physical properties of dough like pasting and rheological characteristics could be altered by modification (Liu et al., 2008). In contrast to present results, Pareyt et al. (2011) reported that substitution of sucrose with starch augmented cookie height but, decreased cookie diameter.

Treatment of flour with varying levels of ascorbic acid divulged thickness of cookies ranging between 6.0 and 6.4 cm. Regarding treatments, thickness of cookies was measured as T2C1 (6.0 cm), T2C3 (6.4 cm), T2C2 (6.1 cm), T7C1 (6.1 cm), T7C2 (6.2 cm) and T7C3 (6.4 cm). Ascorbic acid addition in cookie formulation resulted in a gradual increase in cookies thickness. Cookies containing 15ppm ascorbic acid had the highest value of thickness.

Thickness of cookies after the addition of protease presented in Table 21 ranged from 5.2 to 5.5 cm. Thickness of cookies was obtained in different treatments i.e. T3C1, T3C2, T3C3, T8C1, T8C2 and T8C3 as 5.5 cm, 5.3 cm, 5.2 cm, 5.5 cm, 5.4 cm and 5.4 cm, respectively. A significant decrease in thickness of cookies was observed by increasing the level of protease. In present case, the smallest value of thickness was recorded in cookies made with 600 mg protease.

Results regarding mean values of cookies thickness treated with KIO3 varied from 6.0 to 6.4 cm. With respect to treatments, values of thickness were measured as T4C1 (6.0 cm), T4C2 (6.1 cm), T4C3 (6.4 cm), T9C1 (6.2), T9C2 (6.2 cm) and T9C3 (6.3 cm). Increase in cookies thickness was noted as the concentration of KIO3 was increased. However, present results are in contrast to Pareyt et al. (2010) who reported that addition of potassium iodate at high levels reduced height of cookies.

In various treatments of dough formulation, addition of varying concentrations of sodium metabisulfite influenced thickness of cookies from 5.6 to 5.9 cm. As for as treatments are concerned, thickness of cookies was obtained as 5.8 cm, 5.6 cm, 5.6 cm, 5.9 cm, 5.7 cm and 5.7 cm in T3C1, T3C2, T5C3, T10C1, T10C2 and T10C3, respectively. Thickness of cookies treated with 3000 ppm sodium metabisulfite was lower than others. Present data suggests that thickness of cookies reduced with the addition of sodium metabisulfite to cookies dough. Lagrain et al. (2006) described that reduction of S-S linkages decrease gluten molecular weight (MW) thus, improving its molecular mobility and flexibility. Apart from that, it also increases the levels of free SH groups, which may cause SH/SS exchange reactions (Lagrain et al., 2008; Pareyt et al., 2010) particularly, at higher temperature. This study accede with outcome of
Masih et al. (2014) who measured 62.16 mm thickness of 100% wheat flour cookies (WFC) but, inconsistent with findings by Arshad et al. (2008) who found height of wheat flour cookies as 1.304 cm.

### 4.7.1.3. Spread factor

Statistical results related to spread factor of cookies prepared with different treatments presented in Table 20 showed highly significant variance among all experimental measures. Results further indicated the highly significant impact of treatments on spread factor of cookies however, concentration; interaction of concentration and treatments caused insignificant impact on spread factor. In this work, the treatments were statistically different as compared to control in view of spread factor of cookies.

Table 21 lists the mean values for spread factor of cookies made from different treatments ranged from 39.68 to 60.96 and 41.41 in T₀ (control). For the relative assessment of treatments, spread factor of cookies varied in response to all the chemical or modifying agents as 57.03-60.96 (CMS), 39.68-43.33 (ascorbic acid), 54.00-58.65 (protease), 43.75-47.00 (KIO₃) and 48.98-52.68 (SMS). Of all treatments, cookies made with replacement of wheat flour with CMS (85:15) produced cookies with more spread (60.96) whilst, cookies treated with 15 ppm ascorbic acid resulted in lower spread (39.68).

Substituting wheat flour with varying levels of chemically modified starch impacted spread factor and it varied from 57.03 to 60.96. Within treatments, T₀C₃ yielded highest spread factor (60.96) followed by T₁C₃ (60.00), T₀C₂ (59.43), T₁C₂ (58.49), T₀C₁ (58.30) and T₁C₁ (57.03). Substituting wheat flour with CMS (85:15) produced cookies with largest spread factor while (95:5) resulted in least. An ascent trend for spread factor was observed with a proportional increase of CMS in wheat flour. Kumar and Prabhasankar (2013) stated that effect of chemical modification on physicochemical, thermal and rheological characteristics of starch is determined by type of chemical modification.

A significant decline in spread factor of cookies after the treatment of flour with ascorbic acid was observed from 43.33 to 39.68. Regarding treatments, spread factor of cookies was calculated as T₂C₁ (43.33), T₂C₂ (42.46), T₂C₃ (39.84), T₇C₁ (42.29), T₇C₂ (41.29) and T₇C₃ (39.68). Hence, spread factor of cookies dwindled with ascorbic acid addition in cookie formulation. Hrušková and Novotná (2003) studied the effect of ascorbic acid on dough properties for bread quality and reported that it helps in a better dough development at
traditional mixing and improve bread quality by producing a higher oven spring leading to fine crumb grain and greater loaf volume.

Use of various levels of protease increased the spread factor of cookies from 54.00 to 58.65. Within treatments, spread factor of cookies was determined as 54.91, 57.36, 58.65, 54.00, 55.37 and 55.74 in T3C1, T3C2, T3C3, T8C1, T8C2 and T8C3, respectively. All the cookies varied in their response to protease addition hence, spread factor of cookies was increased. Addition of protease caused largest spread of cookies at the level of 600 mg. Present results commensurate with findings of Kara et al. (2005), who reported positive effect of protease on cookies spread may likely due to reduced gluten strength by protease.

High protease levels caused a progressive decrease in consistency of gluten on farinograph (Kruger, 1971). Proteases are added to modify quality of gluten protein and acts on inner peptide linkages of gluten. Though, reducing agents cause loss of resistance and heighten extensibility by breaking S-S linkages, proteolytic enzyme unveils same effect in a different way by breaking chains. In contrary to SMS, action of protease enzyme lasts with time and eventually, resulting in unmanageable dough (Manley, 2011). Li et al. (2016) studied the effect of six protease enzymes on the hydrolysis of allergenic protein (gliadin) in wheat and found that alcalase and papain were greatly effective in the reduction of gliadin content of wheat.

Physical properties of cookies showed an ascent trend regarding spread factor of cookies made with the addition of sodium metabisulfite from 48.98 to 52.68. Spread factor of cookies was obtained as 48.98, 51.96, 52.68, 49.14, 50.88 and 51.23 in T5C1, T5C2, T5C3, T10C1, T10C2 and T10C3, respectively. Mean values for the effect of SMS on spread factor of cookies presented a gradual declining trend. Largest spread factor of cookies was recorded in cookies made with formulation containing 3000 ppm of SMS. Present observations are in line with Kumar et al. (2013), who described that dough extensibility augmented by increasing the levels of reducing agents and cookie dough spread further increasing diameter of cookies. Reducing agents tend to cleave intermolecular and intramolecular disulfide (S-S) linkages in gluten, which led to
reduced molecular weight of protein while, increased dough extensibility (Stauffer, 1994). Values reported in present research are higher than those reported by various authors (Pasha et al., 2011; Masih et al., 2014; Akbar et al., 2016) who determined spread factor of wheat flour cookies in range of 42.99-44.82. Pareyt et al. (2015) studied the effect of gluten polymerization on properties of high sugar and fat, yeast-leavened developed dough system using redox compounds to selectively modify the quality of wheat gluten. Results showed that development of gluten during mixing and successive gluten polymerization during baking relates to expansion and shrinkage of dough thus, regulates product dimensions. Degree of polymerization controls spread rate and product dimensions. Also, greater polymerization produces firmer products.

4.7.1.4. Weight

Statistical analysis (Table 20) regarding weight of cookies prepared from different treatments point out that all combinations were statistically different with respect to weight. Data further showed that variations in weight of cookies were only due to treatments while, the effect of concentration and interaction of concentration and treatments was non-significant on cookies weight. In addition, the effect of control vs. treatments did not varied significantly for cookies weight.

Mean values for weight produced with different treatments given in Table 21 showed that it varied from 7.45 to 10.62 g/cookie and 9.93 g/cookie in control (T<sub>0</sub>). Across treatments, weight was ranged 9.23-10.62, 9.45-9.69, 7.45-10.26, 8.68-9.44 and 8.21 to 8.82 g in cookies made with CMS, ascorbic acid, protease, KIO<sub>3</sub> and SMS, respectively. Taking all the treatments into account, cookies made with the substitution of wheat flour with CMS had highest (10.62 g) weight at 5% level while cookies containing 200 mg protease exhibited lowest (7.45 g) weight. Weight of cookies made with replacement of wheat flour with different levels of chemically modified starch varied from 9.23 to 10.62 g.
Table 20: Mean squares for physical properties of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
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<td></td>
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<td>Thickness</td>
<td>Spread factor</td>
<td>Weight</td>
</tr>
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<td>Combination</td>
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<td>0.503**</td>
<td>105.446**</td>
<td>1.812**</td>
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<td>14.060**</td>
<td>1.873**</td>
<td>301.280**</td>
<td>2.163NS</td>
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<td>Conc.</td>
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<td>0.267*</td>
<td>5.193NS</td>
<td>1.475NS</td>
</tr>
<tr>
<td>Treat. (T)</td>
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<td>1.107**</td>
<td>311.002**</td>
<td>2.680**</td>
</tr>
<tr>
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<td>2.927NS</td>
<td>1.397**</td>
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<td>Total</td>
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</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Cont.: control; Treat: treatments; Conc.: concentration
Table 21: Mean values for physical properties of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Width (cm)</th>
<th>Thickness (cm)</th>
<th>Spread factor</th>
<th>Weight (g)</th>
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<td>41.41±1.07&lt;sup&gt;o&lt;/sup&gt;</td>
<td>9.93±0.63&lt;sup&gt;a-d&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₁C₁</td>
<td>30.8±0.76&lt;sup&gt;d-g&lt;/sup&gt;</td>
<td>5.4±0.16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>57.03±1.25&lt;sup&gt;lm&lt;/sup&gt;</td>
<td>9.55±0.77&lt;sup&gt;a-i&lt;/sup&gt;</td>
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<td>5.3±0.15&lt;sup&gt;e-h&lt;/sup&gt;</td>
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<td>5.2±0.16&lt;sup&gt;b-e&lt;/sup&gt;</td>
<td>60.00±1.37&lt;sup&gt;klm&lt;/sup&gt;</td>
<td>9.43±0.46&lt;sup&gt;a-h&lt;/sup&gt;</td>
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<tr>
<td>T₂C₁</td>
<td>26.0±0.71&lt;sup&gt;i&lt;/sup&gt;</td>
<td>6.0±0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>43.33±1.15&lt;sup&gt;o&lt;/sup&gt;</td>
<td>9.60±0.17&lt;sup&gt;a-g&lt;/sup&gt;</td>
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<tr>
<td>T₂C₂</td>
<td>25.9±0.72&lt;sup&gt;i&lt;/sup&gt;</td>
<td>6.1±0.17&lt;sup&gt;a-d&lt;/sup&gt;</td>
<td>42.46±1.20&lt;sup&gt;o&lt;/sup&gt;</td>
<td>9.64±0.74&lt;sup&gt;b-k&lt;/sup&gt;</td>
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<tr>
<td>T₂C₃</td>
<td>25.5±0.66&lt;sup&gt;i&lt;/sup&gt;</td>
<td>6.4±0.16&lt;sup&gt;a-d&lt;/sup&gt;</td>
<td>39.84±1.10&lt;sup&gt;o&lt;/sup&gt;</td>
<td>9.69±0.05&lt;sup&gt;a-g&lt;/sup&gt;</td>
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<tr>
<td>T₃C₁</td>
<td>29.7±0.80&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>5.5±0.13&lt;sup&gt;i&lt;/sup&gt;</td>
<td>54.00±1.61&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.45±0.18&lt;sup&gt;n&lt;/sup&gt;</td>
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<tr>
<td>T₃C₂</td>
<td>29.9±0.84&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>5.4±0.14&lt;sup&gt;i&lt;/sup&gt;</td>
<td>55.37±1.68&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.95±0.20&lt;sup&gt;i-m&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₃C₃</td>
<td>30.1±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.4±0.15&lt;sup&gt;cf-i&lt;/sup&gt;</td>
<td>55.74±1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.15±0.24&lt;sup&gt;b-j&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₄C₁</td>
<td>28.2±0.75&lt;sup&gt;c-g&lt;/sup&gt;</td>
<td>6.0±0.15&lt;sup&gt;b-e&lt;/sup&gt;</td>
<td>47.00±1.29&lt;sup&gt;im&lt;/sup&gt;</td>
<td>8.68±0.70&lt;sup&gt;d-m&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₄C₂</td>
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<td>6.1±0.13&lt;sup&gt;ghi&lt;/sup&gt;</td>
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<td>8.95±0.11&lt;sup&gt;b-j&lt;/sup&gt;</td>
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<tr>
<td>T₄C₃</td>
<td>28.0±0.76&lt;sup&gt;d-h&lt;/sup&gt;</td>
<td>6.4±0.15&lt;sup&gt;c-f&lt;/sup&gt;</td>
<td>43.75±1.33&lt;sup&gt;km&lt;/sup&gt;</td>
<td>9.06±0.31&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₅C₁</td>
<td>28.9±0.83&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>5.9±0.16&lt;sup&gt;e-h&lt;/sup&gt;</td>
<td>48.98±1.57&lt;sup&gt;dg&lt;/sup&gt;</td>
<td>8.21±0.56&lt;sup&gt;i-m&lt;/sup&gt;</td>
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<td>50.88±1.45&lt;sup&gt;dg&lt;/sup&gt;</td>
<td>8.50±0.34&lt;sup&gt;klm&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₅C₃</td>
<td>29.2±0.84&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.7±0.15&lt;sup&gt;b-e&lt;/sup&gt;</td>
<td>51.23±1.45&lt;sup&gt;dg&lt;/sup&gt;</td>
<td>8.82±0.22&lt;sup&gt;-l&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₆C₁</td>
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<td>5.3±0.16&lt;sup&gt;a-d&lt;/sup&gt;</td>
<td>58.30±1.26&lt;sup&gt;klm&lt;/sup&gt;</td>
<td>10.62±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5.3±0.16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>59.43±1.27&lt;sup&gt;ij-m&lt;/sup&gt;</td>
<td>9.80±0.49&lt;sup&gt;a-e&lt;/sup&gt;</td>
</tr>
<tr>
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<td>5.2±0.15&lt;sup&gt;dg&lt;/sup&gt;</td>
<td>60.96±1.31&lt;sup&gt;ij-m&lt;/sup&gt;</td>
<td>9.23±0.43&lt;sup&gt;b-i&lt;/sup&gt;</td>
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<td>42.29±1.22&lt;sup&gt;lmn&lt;/sup&gt;</td>
<td>9.45±0.80&lt;sup&gt;a-h&lt;/sup&gt;</td>
</tr>
<tr>
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<td>9.51±0.25&lt;sup&gt;a-h&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₇C₃</td>
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<td>6.4±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.68±1.17&lt;sup&gt;no&lt;/sup&gt;</td>
<td>9.52±0.14&lt;sup&gt;f-m&lt;/sup&gt;</td>
</tr>
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</tr>
<tr>
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<td>5.3±0.13&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>57.36±1.50&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>9.25±0.22&lt;sup&gt;e-m&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₈C₃</td>
<td>30.5±0.77&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>5.2±0.13&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>58.65±1.52&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>8.55±0.25&lt;sup&gt;b-i&lt;/sup&gt;</td>
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<tr>
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<td>6.2±0.15&lt;sup&gt;e-h&lt;/sup&gt;</td>
<td>45.48±1.38&lt;sup&gt;h-l&lt;/sup&gt;</td>
<td>9.03±0.64&lt;sup&gt;ei&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₉C₂</td>
<td>27.9±0.83&lt;sup&gt;cf&lt;/sup&gt;</td>
<td>6.2±0.16&lt;sup&gt;d-g&lt;/sup&gt;</td>
<td>45.00±1.47&lt;sup&gt;g-k&lt;/sup&gt;</td>
<td>9.25±0.37&lt;sup&gt;b-k&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₉C₃</td>
<td>27.8±0.82&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>6.3±0.14&lt;sup&gt;eh&lt;/sup&gt;</td>
<td>44.13±1.37&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>9.44±0.09&lt;sup&gt;lm&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₁₀C₁</td>
<td>28.5±0.70&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>5.8±0.13&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>49.14±1.37&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>8.31±0.64&lt;sup&gt;af&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₁₀C₂</td>
<td>29.1±0.76&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>5.6±0.14&lt;sup&gt;e-i&lt;/sup&gt;</td>
<td>51.96±1.44&lt;sup&gt;eh&lt;/sup&gt;</td>
<td>8.50±0.30&lt;sup&gt;g-m&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₁₀C₃</td>
<td>29.5±0.76&lt;sup&gt;d-g&lt;/sup&gt;</td>
<td>5.6±0.14&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>52.68±1.50&lt;sup&gt;d-g&lt;/sup&gt;</td>
<td>8.64±0.57&lt;sup&gt;h-m&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Selected wheat variety (SWV): T₀ to T₃

Mixed wheat flour (MWF): T₆ to T₁₀

<table>
<thead>
<tr>
<th>Treatments</th>
<th>5% CMS; T₂C₁: 1% CMS; T₃C₂: 15% CMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₅C₃: 5ppm AsA; T₆C₂: 10ppm AsA; T₇C₃: 15ppm AsA</td>
<td></td>
</tr>
<tr>
<td>T₆C₃: 200mg protease; T₇C₂: 400mg protease; T₈C₁: 600mg protease</td>
<td></td>
</tr>
<tr>
<td>T₇C₂: 500ppm KIO₃; T₈C₃: 1000ppm KIO₃; T₉C₂: 1500ppm KIO₃</td>
<td></td>
</tr>
<tr>
<td>T₉C₁: 1000ppm SMS; T₁₀C₂: 2000ppm SMS; T₁₀C₃: 3000ppm SMS</td>
<td></td>
</tr>
</tbody>
</table>

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Highest weight of cookies was measured in T6C1 (10.62 g) followed by T6C2 (9.80 g), T1C3 (9.55 g), T1C2 (9.45 g), T1C1 (9.43 g) and T6C3 (9.23 g). A slight increase in cookies weight was observed as the proportion of chemically modified starch was increased in wheat flour. Mean values for weight of cookies prepared from the treatment of flour with ascorbic acid tabulated in Table 21 were found in the range of 9.45-9.69 g. As for as treatments are concerned, weight of cookies was obtained in T2C1 (9.60 g), T2C2 (9.51 g), T2C3 (9.69 g), T7C1 (9.45 g), T7C2 (9.52 g) and T7C3 (9.52 g). As for as treatments are concerned, T2C3 possessed highest weight (9.69 g) and T7C1 had lowest (9.45 g).

Average measurement of cookies weight after the addition of protease varied between 7.45 and 10.26 g. Within treatments, weight of cookies was found as 7.45 g, 7.95 g, 9.15 g, 10.26 g, 9.25 g and 8.15 g in T3C1, T3C2, T3C3, T8C1, T8C2 and T8C3, respectively. Cookies containing 200 mg protease (T8C1) had maximum weight while, cookies (T3C1) were lighter in weight (7.45 g). It was noticed that cookie weight increased with the addition of protease to formulation. As stated earlier, cookies weight itself influenced by dough pieces weight (Pareyt et al., 2010).

Addition of KIO3 to cookie formulation revealed weight of cookies varied from 8.68 to 9.44 g. A significant increase in weight of cookies was observed from 8.68 g (T4C1) to 8.95 g (T4C2) to 9.06 g (T4C3) to 9.03 g (T9C1) to 9.25 g (T9C2) and 9.44 g (T9C3). Highest weight of cookies was measured in T9C3 (9.44 g) whereas lowest was observed in T4C1 (8.68 g).

Mean values for the effect of varying levels of sodium metabisulfite on cookies weight ranged between 8.21 and 8.82 g. Regarding treatments, weight of cookies was measured in T5C1, T5C2, T5C3, T10C1, T10C2 and T10C3 as 8.21 g, 8.50 g, 8.82 g, 8.31 g, 8.50 g and 8.64 g, respectively. In present work, increasing concentration of sodium metabisulfite caused a slight increase in cookie weight. Weight of cookies prepared from 100% wheat flour was reported 18.01-29.44 g earlier by Apotiola and Fashakin (2013) and Igbabul et al. (2015).

4.7.2. Color of cookies
4.7.2.1. L* values
Mean squares for L* values of cookies presented in Table 22 apprise that differences for all combinations were found to be highly significant. According to statistical analysis, treatments had highly significant effect on L* values of cookies while, L* values of cookies did not significantly changed due to concentration and interaction of concentration and treatments.
Likewise, the effect of control vs. treatments did not influence L* values of cookies. Table 23 represent mean comparison for L* values of cookies produced with different treatments varying over a wide range observed from 64.65 to 74.84 and 66.62 in T_o. In order to compare the treatments, highest L* values were reckoned in cookies made with SMS (73.10-74.84) followed by protease (71.19-72.65), KIO_3 (70.37-71.18), CMS (67.44-70.25) and ascorbic acid (64.65-67.04). Altogether, cookies treated with SMS (1000 ppm) gave highest (74.84) L* value while, cookies treated with ascorbic acid (5 ppm) had lowest (64.65).

L* values of cookies made from wheat flour blended with different levels of chemically modified starch ranged between 67.44 and 70.25. Highest L* value of cookies was obtained in T_6C_1 (70.25) followed by T_6C_2 (69.90), T_6C_3 (68.82), T_1C_1 (68.37), T_1C_2 (68.10) and T_1C_3 (67.44). In general, a decrease in L* values of cookies was pointed out as the rate of CMS substitution increased in wheat flour. Moreover, the cookies made with wheat flour substituted at higher levels by CMS were comparatively dark in color. Taking color of cookie into consideration, lower the L* values, darker was the surface appearance of cookie. Present findings are somewhat close to Pareyt et al. (2011) who observed L* values in cookies made with flour substitution by 10 to 40% starch (70.6-73.4).

Results evidence that L* values of cookies made from the treatment with ascorbic acid varied between 64.65 and 67.04. L* value was highest for T_7C_3 (67.04) followed by T_7C_2 (66.88), T_7C_1 (66.54), T_2C_3 (66.11), T_2C_2 (66.00) and T_2C_1 (64.65). A gradual increase in this trait was noticed with ascorbic acid. However, the cookies treated with ascorbic acid had dark color as designated by lower L* values as compared to their counterpart treatments. It has been reported that cookie quality is not only explicated by dough rheological properties, but as much of cookie assets are appraised during baking (Sciarini et al., 2013).

L* values representing lightness of samples ranged from 71.19 to 72.65 for cookies produced with the addition of protease. Within treatments, lightness of cookies was obtained as 72.25, 71.57, 71.19, 72.65, 71.58 and 71.48 in order of increasing the concentration of protease i.e. T_3C_1, T_3C_2, T_3C_3, T_8C_1, T_8C_2 and T_8C_3, respectively. It is known that, as the concentration of protease increase, the lightness values decrease. This can be justified by the fact that greater contribution of small peptides or amino acids released by the breakdown of protein due to the action of protease instigated Maillard reaction leading to color browning. Kara et al. (2005) found that L* value was significantly influenced by effect of interaction of protease type-
incubation time.
In Table 23, the mean values for lightness of cookies treated with KIO₃ can be observed to be varying from 70.37 to 71.18. Lightness was significantly higher in T₉C₃ (71.18) followed by T₉C₂ (71.15), T₉C₁ (71.10), T₄C₃ (70.78), T₄C₂ (70.69) and T₄C₁ (70.37). An assorted trend was observed for L* values of cookies with increasing the level of KIO₃.
L* values ranged between 73.10 and 74.84 for cookies treated with varying levels of sodium metabisulfite. Within treatments, lightness of cookies decreased from 73.42 to 73.16 and 73.10 with increasing the concentration of SMS i.e. from T₅C₁ to T₅C₂ and T₅C₃ and similarly, from 74.84 to 74.72 and 73.55 in T₁₀C₁, T₁₀C₂ and T₁₀C₃, respectively. L* values of cookies are known to be declined by increasing the level of SMS. However, values of cookies treated with sodium metabisulfite were higher than those produced with other treatments. Data here on, thus appears consonant with Saeed et al. (2012) and Mancebo et al. (2015) who reported L* value in wheat flour cookies ranged as 65.26-72.36.

4.7.2.2. a* values
Statistical data pertaining to a* values of cookies given in Table 22 portrayed highly significant variations within all combinations. Further exploring the results revealed that variations in a* values of cookies were only ascribed to treatments whereas, effect of concentration and interaction of concentration and treatments was non-significant on a* values of cookies. In this work, treatments were statistically significant for a* values of cookies as compared to control.
Table 23 shows that mean comparison for a* values in different treatments of cookies ranged from 1.22 to 3.75 and 3.31 in control (T₀). In order to compare treatments, a* values observed in cookies made with SMS, protease, KIO₃, ascorbic acid and CMS as 1.22-1.75, 2.02-2.25, 2.55-2.98, 3.15-3.33 and 3.47-3.75, respectively. Taken together, substituting wheat flour with CMS (85:15) produced cookies with highest (3.75) a* value whereas, cookies treated with CMS gave lowest (1.22) a* value at 1000 ppm.
Redness of cookies made from wheat flour blended with different levels of chemically modified starch varied from 3.47 to 3.75. Highest a* value of cookies was obtained in T₁C₃ (3.75) followed by T₁C₂ (3.69), T₁C₁ (3.62), T₆C₃ (3.55), T₆C₂ (3.52) and T₆C₁ (3.47). Replacing wheat flour with CMS increased redness of cookies. Pareyt et al. (2011) described a* values as 3.8-5.0 in cookies made from flour substituted with 10 to 40%. Saeed et al. (2012) reported that a* value decreased from 20.37 to 16.95 with an increase in the level of sweet
potato flour in cookies. Treatment of flour with ascorbic acid produced cookies with a* values ranging from 3.15 to 3.33. Highest a* values of cookies was obtained in T2C1 (3.33) followed by T2C2 (3.31), T2C3 (3.29), T7C1 (3.25), T7C2 (3.24) and T7C3 (3.15). The absolute decreasing of a* values of cookies was observed with the addition of ascorbic acid to cookies dough. Redness values of cookies treated with protease ranged between 2.02 and 2.25. In general, an increase in redness of cookies was observed from 2.03 to 2.15 and 2.25 in T3C1, T3C2 and T3C3 likewise, from 2.02 to 2.11 and 2.22 in T8C1, T8C2 and T8C3, respectively. The lowest a* value was observed in cookies containing 200 mg protease while, addition of different levels of protease increased a* values of cookies. Kara et al. (2005) investigated the effect of protease types and incubation time on color values of cookies and found that a* values were independent of protease type. Cookies containing KIO3 had a* values varying from 2.55 to 2.98. Within treatments, highest a* value of cookies was obtained in T9C1 (2.98) followed by T9C2 (2.95), T9C3 (2.90), T4C1 (2.66) T4C2 (2.60) and T4C3 (2.55). Increasing levels of KIO3 produced a significant reduction in a* values of cookies. However, these values were lower than control implying that their color was better comparing them to control. Sodium metabisulfite addition to cookie formulation impacted a* values and observed in range of 1.22-1.75. Redness of cookies was significantly reduced from T5C3 (1.75) to T5C2 (1.68) and T5C1 (1.38) similarly, from T10C3 (1.29) to T10C2 (1.25) and T10C1 (1.22). A high concentration of sodium metabisulfite induced an increasing trend for a* values of cookies. However, present observations were found to be incongruent with findings of past authors who reported a* value in wheat flour cookies vary widely from 3.94 to 20.37 (Saeed et al., 2012; Mancebo et al., 2015).

4.7.2.3. b* values

Statistical analysis regarding b* values of cookies point out highly significant variance for all combinations (Table 22). Further splitting results demonstrated that variations in b* values of cookies were only credited to treatments while, the effect of concentration and interaction of concentration and treatments was non-significant on b* values of cookies. In that way, the effect of control vs. treatments also did not varied significantly for b* values of cookies. Variations in means of b* values (Table 23) determined among cookies made with different
treatments and control (T₀) varied from 31.10 to 35.44 and 33.19, respectively. To have a better overview of present results, b* values in different treatments are summarized as 32.22-32.70 (CMS), 31.10-31.89 (ascorbic acid), 33.13-33.88 (protease), 32.99-35.44 (KIO₃) and 34.04-34.84 (SMS). On the whole, cookies treated with KIO₃ gave highest (35.44) b* value at level of 1500ppm whereas, ascorbic acid gave lowest (31.10) at 5ppm level.

Different replacement levels of chemically modified starch to wheat flour impacted b* values (yellowness) of cookies and a narrow range was observed from 32.22 to 32.70. Highest b* value of cookies was obtained in T₆C₁ (32.70) followed by T₆C₂ (32.60), T₆C₃ (32.44), T₁C₁ (32.33), T₁C₂ (32.27) and T₁C₃ (32.22). Cookies made with wheat flour: CMS (95:5) exhibited highest b* values whereas, cookies (85:15) had the lowest. There was a gradual decrease in b* values of cookies, as the level of replacement increased. Pareyt et al. (2011) reported b* values ranged as 29.1-30.4 in cookies from flour substituted with 10 to 40% starch.

As a result of flour treatment with ascorbic acid, b* values of cookies increased from 31.10 to 31.89. An ascent trend in b* values was observed from T₂C₁ (31.10) to T₂C₂ (31.31), T₂C₃ (31.57), T₇C₁ (31.58), T₇C₂ (31.84) and T₇C₃ (31.89). A significant rise in b* values along with ascorbic acid was observed however, b* values of cookies treated with ascorbic acid were lower as compared to control.

Addition of protease at different concentrations to dough formulation impacted b* values of cookies and found in the range of 33.13-33.88. Protease caused a slight reduction in b* values of cookies from T₃C₁ (33.41) to T₃C₂ (33.40) and T₃C₃ (33.13) similarly, from T₈C₁ (33.88) to T₈C₂ (33.75) and T₈C₃ (33.74). The absolute descent trend in b* values of cookies was noticed as the concentration of protease increased. However, b* values of cookies containing protease were higher as compared to control.

Yellowness (b*) values of cookies treated with KIO₃ are tabulated in Table 23 ranging from 32.99 to 35.44. Yellowness of cookies increased proportionally with KIO₃ from T₄C₁ (32.99) to T₄C₂ (33.85) and T₄C₃ (35.22) as well as from T₅C₁ (35.25) to T₅C₂ (35.31) and T₅C₃ (35.44). Also, b* values of cookies treated with KIO₃ were greater than control except T₄C₁ (32.99).
Table 22: Mean squares for color values of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>L* value</th>
<th>a* value</th>
<th>b* value</th>
</tr>
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<tbody>
<tr>
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<td>23.035**</td>
<td>1.933**</td>
<td>4.909**</td>
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<tr>
<td>Cont. vs treat.</td>
<td>1</td>
<td>39.230NS</td>
<td>1.309**</td>
<td>0.054NS</td>
</tr>
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<td>Conc.</td>
<td>2</td>
<td>1.245NS</td>
<td>0.003NS</td>
<td>0.174NS</td>
</tr>
<tr>
<td>Treat. (T)</td>
<td>9</td>
<td>70.556**</td>
<td>6.247**</td>
<td>15.366**</td>
</tr>
<tr>
<td>Conc. x T</td>
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<td>0.797NS</td>
<td>0.025NS</td>
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</tr>
<tr>
<td>Error</td>
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<tr>
<td>Total</td>
<td>92</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)
Cont.: control; Treat: treatments; Conc.: concentration
### Table 23: Mean values for color values of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L* value</th>
<th>a* value</th>
<th>b* value</th>
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<td>3.62±0.10abc</td>
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<tr>
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<tr>
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<td>34.43±0.94a-d</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

**Selected wheat variety (SWV):** T₀ to T₅
- T₁₀C₁: 5% CMS; T₁₀C₂: 10% CMS; T₁₀C₃: 15% CMS
- T₁₀C₁: 5ppm AsA; T₁₀C₂: 10ppm AsA; T₁₀C₃: 15ppm AsA
- T₁₀C₁: 200mg protease; T₁₀C₂: 400mg protease; T₁₀C₃: 600mg protease
- T₁₀C₁: 500ppm KIO₃; T₁₀C₂: 1000ppm KIO₃; T₁₀C₃: 1500ppm KIO₃
- T₁₀C₁: 1000ppm SMS; T₁₀C₂: 2000ppm SMS; T₁₀C₃: 3000ppm SMS

**Mixed wheat flour (MWF):** T₀ to T₁₀
- T₁₀C₁: 5% CMS; T₁₀C₂: 10% CMS; T₁₀C₃: 15% CMS
- T₁₀C₁: 5ppm AsA; T₁₀C₂: 10ppm AsA; T₁₀C₃: 15ppm AsA
- T₁₀C₁: 200mg protease; T₁₀C₂: 400mg protease; T₁₀C₃: 600mg protease
- T₁₀C₁: 500ppm KIO₃; T₁₀C₂: 1000ppm KIO₃; T₁₀C₃: 1500ppm KIO₃
- T₁₀C₁: 1000ppm SMS; T₁₀C₂: 2000ppm SMS; T₁₀C₃: 3000ppm SMS
Cookies made with the addition of sodium metabisulfite have shown b* values varying from 34.04 to 34.84. Increasing concentration of sodium metabisulfite caused a reduction in yellowness values of cookies from T5C1 (34.39) to T5C2 (34.13) and T5C3 (34.04), this was also the case for T10C1 (34.84) to T10C2 (34.75) and T10C3 (34.43). b* values of cookies tended to be decrease by increasing the level of sodium metabisulfite nevertheless, these values were greater than control.

Addition of protease and SMS may have lowered yellowness values of cookies possibly due to their reducing effect on protein content releasing small peptides which prompted Maillard reaction. However, present results are inconsistent to the outcomes of earlier workers (Saeed et al., 2012; Mancebo et al., 2015). They rather reported b* value in wheat flour cookies was found to be 24.55-28.89.

4.7.3. Chemical properties of cookies

4.7.3.1. Moisture content

Statistical data for moisture content in wheat varieties presented in Table 24 divulge highly significant variations for all experimental measures. Statistical data further showed that treatments, concentration and their interaction caused highly significant variations in moisture content of cookies. Also, the treatments were significantly different from control with respect to moisture content of cookies.

Variations in mean values for moisture content of cookies prepared with various treatments and control shown in Table 25 varied from 1.50 to 3.68% and 3.20% in T0. In order to compare treatments, moisture content of cookies was recorded as 2.10-3.21% (CMS), 2.34-3.59% (ascorbic acid), 1.50-2.39% (protease), 3.09-3.68% (KIO3) and 2.09-2.59% (SMS). Of all, cookies treated with KIO3 had highest moisture (3.68%) at 1000 ppm while, lowest (1.50%) was found in cookies treated with protease at 400 mg.

Moisture content of cookies made with the replacement of chemically modified starch (CMS) at different (5, 10 and 15%) levels ranged between 2.10 and 3.21%. Within treatments, highest moisture content of cookies was find out in T1C2 (3.21%) followed by T6C2 (3.20%), T6C1 (2.99%), T1C3 (2.70%), T6C3 (2.60%) and T1C1 (2.10%). Highest moisture content of cookies was observed at 10% replacement of wheat flour with CMS (90:10). Pareyt et al. (2011) observed 2.4-2.6% moisture in cookies produced with flour substitution by 10 to 40%. Moisture content was decreased from 8.81 to 4.95 with addition of 10% crude African
breadfruit starch and slightly increased to 5.05 at 20% crude starch into cookies (Ojinnaka et al., 2013). Treatment of flour with ascorbic acid at different (5, 10 and 15 ppm) concentrations significantly increased moisture content of cookies from 2.34 to 3.59%. Moisture content of cookies was recorded as $T_2C_1$ (2.89%), $T_2C_2$ (3.05%), $T_2C_3$ (3.59%), $T_7C_1$ (2.34%), $T_7C_2$ (3.38%) and $T_7C_3$ (3.54%). An ascent trend is reported in moisture content of cookies, as the concentration of ascorbic acid was increased.

Addition of protease (papain) at different (200, 400 and 600 mg) concentrations to cookie dough had an effect on moisture content and it was found in the range of 1.50 and 2.39%. For treatments, moisture content of cookies was reckoned as 2.00, 1.50, 2.30, 2.00, 1.60 and 2.39% in $T_3C_1$, $T_3C_2$, $T_3C_3$, $T_8C_1$, $T_8C_2$ and $T_8C_3$, respectively. An assorted trend was observed for moisture content of cookies containing protease.

Table 25 show the moisture content of cookies treated with potassium iodate (KIO₃) at different (500, 1000, 1500 ppm) concentrations varying from 3.09 to 3.68%. Moisture content of cookies was established as $T_4C_1$ (3.26%), $T_4C_2$ (3.39%), $T_4C_3$ (3.19%), $T_9C_1$ (3.29%) $T_9C_2$ (3.68%) and $T_9C_3$ (3.09%). Likewise, an assorted trend was noticed for moisture content with increasing levels of KIO₃.

Moisture content of cookies made with varying concentrations (1000, 2000 and 3000 ppm) of sodium metabisulfite ranging between 2.09 and 2.59%. About treatments, moisture content was determined in $T_5C_1$, $T_5C_2$, $T_5C_3$, $T_{10}C_1$, $T_{10}C_2$ and $T_{10}C_3$ as 2.59, 2.40, 2.09, 2.58, 2.40 and 2.50%, respectively. Moisture content decreased progressively with an increase in sodium metabisulfite except $T_{10}C_2$ furthermore, cookies made from dough containing 1000 ppm concentration of sodium metabisulfite had highest moisture content. Present results support outcome of several publications related to moisture content in wheat flour cookies (WFC) (Mushtaq et al., 2010; Pasha et al., 2011; Masih et al., 2014; Qaisrani et al., 2014).

### 4.7.3.2. Ash content

Mean squares related to ash content of cookies (Table 24) apprised highly significant variations among all combinations. Statistical splitting further explained that treatments and concentration had highly significant effect on ash content of cookies however, their interaction have non-significant effect. In the same manner, the effect of control vs. treatments was insignificant on ash content.
Mean comparison for ash content of cookies prepared with different treatments can be observed from Table 25 to be varied from 0.333 to 0.547% and 0.450 % in mixed wheat flour ($T_o$). For the purpose of comparison among treatments, ash content of cookies ranged as 0.390-0.430% (CMS), 0.430-0.467% (ascorbic acid), 0.333-0.400% (protease), 0.480-0.513% ($\text{KIO}_3$) and 0.513-0.547% (SMS). Moreover, cookies after adding SMS at 3000 ppm contained maximum (0.547%) ash content while, lowest (0.333%) was found in cookies made with protease at 200 mg.

Substitution of wheat flour with chemically modified starch influenced ash content of cookies and it was observed in the range of 0.390 and 0.430%. Highest value for ash was reckoned in $T_1C_1$ and $T_6C_2$ (0.430%) followed by $T_1C_2$ (0.420%), $T_6C_3$ (0.417%), $T_6C_1$ (0.410%) and $T_4C_3$ (0.400%). An assorted trend was noticed in ash content of cookies as a result of replacement of wheat flour with CMS. Ojinnaka et al. (2013) observed that ash was decreased from 2.62 to 2.43 with addition of 10% crude African breadfruit starch and slightly increased to 2.81 at 20% crude starch into cookies.

From Table 25, it can be observed that ash content of cookies prepared from the treatment of flour with ascorbic acid was found in range of 0.430 and 0.467%. Ash content of cookies was recorded as $T_2C_1$ (0.430%), $T_2C_2$ (0.450%), $T_2C_3$ (0.467%), $T_7C_1$ (0.450%), $T_7C_2$ (0.460%) and $T_7C_3$ (0.467%). Accordingly, $T_7C_3$ possessed highest ash content (0.467%) than did others.

Addition of varying concentration of protease caused an increment in ash content of cookies from 0.333 to 0.400%. Ash content of cookies was figured as 0.333, 0.363, 0.397, 0.333, 0.367 and 0.400% in $T_3C_1$, $T_3C_2$, $T_3C_3$, $T_8C_1$, $T_8C_2$ and $T_8C_3$, respectively. $T_8C_3$ cookies had maximum ash content (0.400%) while minimum was recorded for $T_3C_1$ and $T_8C_1$ (0.333%). As stated earlier, an increase in ash content of cookies was observed by adding protease among treatments.

Results regarding mean values of ash content in cookies containing $\text{KIO}_3$ varied from 0.480 to 0.513%. Among treatments, ash content of cookies was established as $T_4C_1$ (0.480%), $T_4C_2$ (0.497%), $T_4C_3$ (0.500%), $T_9C_1$ (0.483), $T_9C_2$ (0.500%) and $T_9C_3$ (0.513%). Increase in ash content of cookies was noted as the concentration of $\text{KIO}_3$ was increased. $T_9C_3$ exhibited maximum ash content (0.513%) while, minimum was found in $T_4C_1$ (0.480%).

Variations in ash content observed among cookies containing various levels of sodium metabisulfite were observed from 0.513 to 0.547%. As for as treatments are concerned, ash
content ascertained in $T_5C_1$, $T_5C_2$, $T_5C_3$, $T_{10}C_1$, $T_{10}C_2$ and $T_{10}C_3$ as 0.513%, 0.527%, 0.533%, 0.517%, 0.530% and 0.547%, respectively. Ash content of cookies containing sodium metabisulfite at the concentration of 3000 ppm was highest as compared to others. Mean values for the impact of sodium metabisulfite on ash content of cookies portrayed an increasing trend. Present results are in accord with data obtained by various authors who reported ash content in 100% wheat flour cookies (WFC) in range of 0.45-0.65% (Mushtaq et al., 2010; Pasha et al., 2011; Masih et al., 2014). However, contrasting with results of earlier workers (Qaisrani et al., 2014; Igbabul et al., 2015). They rather reported ash content in WFC was found to be 1.17-2.40%.

4.7.3.3. Fat content
Statistical data (Table 24) regarding fat content of cookies point out highly significant differences within all combinations. Exploring the results further shown that treatments and concentration caused highly significant variations in fat content of cookies however, their interaction had non-significant impact on fat content. Likewise, the effect of control vs. treatments did not show significant variations in terms of fat content. Mean values for fat content of cookies prepared from different treatments can be observed from Table 25 to be varied greatly from 17.70 to 23.66% and 21.20% in mixed wheat flour ($T_o$). In order to make a comparison among treatments, fat content of cookies find out as 21.10-22.93% (CMS), 19.04-23.66% (ascorbic acid), 20.34-21.74% (protease), 17.70-23.30% (KIO$_3$) and 20.32-22.31% (SMS). Of all treatments, cookies treated with ascorbic acid had highest (23.66%) fat content at 5ppm while, lowest (17.70%) was found in cookies treated with KIO$_3$ at 500ppm.

Substituting wheat flour with varying levels of chemically modified starch impacted fat content of cookies and it was found in the range of 21.10-22.93%. Within treatments, fat content of cookies was reckoned as 21.66%, 22.50%, 22.65%, 21.10%, 22.70% and 22.93% in $T_1C_1$, $T_1C_2$, $T_1C_3$, $T_6C_1$, $T_6C_2$ and $T_6C_3$, respectively. Highest fat content was observed in cookies made with the supplementation of wheat flour by CMS (85:15) while lowest was found in cookies (95:5). This implies that fat content of cookies tend to increase with an increase of CMS level in wheat flour. Ojinnaka et al. (2013) observed fat content was increased from 11.25 to 18.33 with addition of 10% crude African breadfruit starch and further increased to 19.22 at 20% crude starch into cookies and so on.
Mean values showing variations in fat content of cookies in response to ascorbic acid varied from 19.04 to 23.66%. Further comparing the various treatments, fat content of cookies was examined as $T_2C_1$ (23.66%), $T_2C_2$ (21.95%), $T_2C_3$ (20.22%), $T_7C_1$ (19.04%), $T_7C_2$ (22.97%) and $T_7C_3$ (22.89%). Among treatments, $T_2C_1$ possessed highest fat content (23.66%) and $T_7C_1$ had lowest (19.04%). However, the effect of ascorbic acid on fat content of cookies is somewhat difficult to interpret since, it declined for SWV while, a random trend was observed for MWF.

The relative impact of varying concentration (200, 400 and 600 mg) of protease on fat content of cookies is presented in Table 25 and values ranged as 20.34-21.74%. Within treatments, fat content of cookies was determined as 21.74%, 20.92%, 20.34%, 21.48%, 20.90% and 20.63% in $T_3C_1$, $T_3C_2$, $T_3C_3$, $T_8C_1$, $T_8C_2$ and $T_8C_3$, respectively. $T_3C_1$ cookies had maximum fat content (21.74%) while minimum was recorded for $T_3C_3$ (20.34%). Addition of protease caused a gradual reduction in fat content of cookies.

Use of KIO$_3$ at various levels in dough formulation affected fat content of cookies positively from 17.70 to 23.30%. As shown, fat content was impacted by KIO$_3$ and reckoned as 17.90% ($T_4C_1$), 20.22% ($T_4C_2$), 23.30% ($T_4C_3$), 17.70% ($T_9C_1$), 20.14% ($T_9C_2$) and 22.67% ($T_9C_3$). Within treatments, highest fat content (23.30%) of cookies was determined in $T_4C_3$ while, lowest (17.70%) was observed in $T_9C_1$. Fat content of cookies tended to increase with a rise in the level of KIO$_3$.

Mean values for the effect of sodium metabisulfite on fat content of cookies showed a range of 20.32-22.31%. Highest fat content of cookies was recorded in $T_{10}C_3$ (22.31%) followed by $T_{10}C_1$ (22.29%), $T_{10}C_2$ (22.14%), $T_5C_1$ (21.65%), $T_5C_3$ (20.88%) and $T_5C_2$ (20.32%). Highest value of fat content of cookies was recorded in cookies containing 3000 ppm of sodium metabisulfite. An assorted trend for fat content was known with increasing levels of SMS. Present observations are assent with data obtained by various authors (Mushtaq et al., 2010; Olagunju and Ifesan, 2013; Masih et al., 2014; Qaisrani et al., 2014) who noticed fat content in wheat flour cookies (WFC) was found in range of 17.62-23.46%. But, in contradictory to findings by other authors (Ojinnaka et al., 2013; Okpala and Ekwe, 2013) who found fat content in cookies produced from 100% wheat flour in range of 5.64-11.25%.
4.7.3.4. Fiber content

Statistical analysis (Table 24) for fiber content of cookies portrayed that there were highly significant variations among all experimental measures. Results further showed that a highly significant effect caused by fiber content among all treatments while, effect of concentration was non-significant with respect to fiber content. However, fiber content of cookies was significantly affected by the interaction of concentration and treatments. Similarly, all the treatments were significantly different from control for fiber content.

Table 26 represent means for fiber content of cookies produced from different treatments varying over a wide range observed from 0.083 to 0.167% and 0.150% in mixed wheat flour (T0). In order of the importance of treatments, highest fiber content was reckoned in cookies made with KIO3 (0.150-0.167%) followed by protease (0.110-0.133%), ascorbic acid (0.110-0.130%), SMS (0.103-0.117%) and CMS (0.083-0.107%). Altogether, cookies treated with KIO3 possessed highest (0.167%) fiber content at level of 500 and 1000ppm while, lowest (0.083%) was observed in cookies treated with CMS at 5% level.

Fiber content of cookies made from wheat flour blended with chemically modified starch at different ratios (95:5, 90:10, 85:15) ranged from 0.083 to 0.107%. Within treatments, highest fiber content of cookies was found in T6C3 (0.107%) followed by T6C2 (0.097%), T1C3 (0.103%), T1C2 (0.093%), T6C1 (0.087%) and T1C1 (0.083%). It is obvious that highest value of fiber was pointed out in cookies (85:15) but, lowest was observed in cookies (95:5). In present study, fiber content of cookies was positively affected by increasing the replacement of wheat flour with CMS. According to Ojinnaka et al. (2013), fiber content was increased from 0.31 to 0.85 with addition of 10% crude African breadfruit starch and further increased to 1.01 at 20% crude starch into cookies.

In Table 26, the mean values for fiber content of cookies after the treatment with ascorbic acid can be observed to be varying from 0.110 to 0.130%. Highest fiber content of cookies was figured in T2C1 (0.130%) followed by T2C2 (0.127%), T2C3 (0.127%), T7C1 (0.117%), T7C2 (0.113%) and T7C3 (0.110%). A gradual decrease in this trait was noticed with increasing the level of ascorbic acid.

Varying the levels of protease (200, 400 and 600 mg) impacted fiber content of cookies and it was found in the range of 0.110 and 0.133%. About treatments, fiber content of cookies was ascertained as 0.117%, 0.113%, 0.110%, 0.133%, 0.133% and 0.130% in T3C1, T3C2, T3C3,
T_sC_1, T_sC_2 and T_sC_3, respectively. Maximum fiber content of cookies was recorded for T_sC_1 and T_sC_2 (0.133%) whereas, minimum was found in T_sC_3 (0.110%). It is established that addition of protease appeared to decrease fiber content of cookies.

Mean values describing the relative impact of different levels of KIO_3 on fiber content of cookies are given in Table 26 and varied from 0.150 to 0.167%. As for as treatments are concerned, fiber content of cookies was established in T_4C_1 (0.167%), T_4C_2 (0.167%), T_4C_3 (0.160%), T_9C_1 (0.150%), T_9C_2 (0.160%) and T_9C_3 (0.153%). Highest fiber content of cookies was reckoned in T_4C_1 and T_4C_2 (0.167%) whilst, lowest was exhibited in T_9C_1 (0.150%). A varied trend was observed for fiber content of cookies with increasing the level of KIO_3 however, values were greater than control.

It is obvious from data that fiber content of cookies treated with sodium metabisulfite varied from 0.103 to 0.117%. Mean values for fiber content among treatments were reckoned i.e. T_5C_1, T_5C_2, T_5C_3, T_10C_1, T_10C_2 and T_10C_3 as 0.117%, 0.113%, 0.113%, 0.110%, 0.107% and 0.103%, respectively. Maximum fiber content of cookies made from dough containing SMS was obtained in T_5C_1 (0.117%) whereas minimum was found in T_10C_3 (0.103%). It is known that, as the concentration of SMS increase, the fiber content of cookies decrease. In parallel, fiber in different treatments of cookies ranged from 0.123 to 0.134% (Mushtaq et al., 2010; Olagunju and Ifesan, 2013). In contrast to these results, fiber content in wheat flour cookies was found 0.39-0.40%, previously (Pasha et al., 2011; Qaisrani et al., 2014).

**4.7.3.5. Protein content**

Mean squares pertaining to protein content of cookies are given in Table 24. It is obvious from statistical results that there were highly significant variations for all experimental measures. Results further revealed that protein content of cookies had significantly more variability due to treatments while, it did not change significantly due to concentration and interaction of concentration and treatments. Data here on report that treatments were statistically different as compared to control in terms of protein content.

Table 26 display mean values for protein content of cookies produced by applying different treatments varied from 5.41 to 6.56% and 6.53% in mixed wheat flour (T_o). For the relative assessment of treatments, protein content of cookies was found as 6.39-6.56% (protease), 6.17-6.34% (SMS), 5.95-6.13% (KIO_3), 5.74-5.91% (ascorbic acid) and 5.41-5.69% (CMS). Taken together, cookies made from dough containing 600 mg protease had highest (6.56%) protein
content while, substituting wheat flour with CMS at 15% level produced cookies with lowest (5.41%) protein content.

Regarding this work, in various treatments of cookies, wheat flour was substituted with chemically modified starch and their protein content varied from 5.41 to 5.69%. Highest protein content of cookies was found in T6C1 (5.69%) followed by T6C2 (5.66%), T6C3 (5.63%), T1C1 (5.47%) T1C2 (5.44%) and T1C3 (5.41%). Cookies made with replacement of wheat flour with CMS (95:5) divulged highest protein content while lowest was observed in cookies (85:15). This implies that replacing wheat flour with CMS decreased protein content of cookies. Protein content was decreased from 9.51 to 7.59 with addition of 10% crude African breadfruit starch and slightly increased to 7.82 at 20% crude starch into cookies (Ojinnaka et al., 2013).

Treatment of flour with ascorbic acid at different concentrations produced cookies with protein content ranging from 5.74 to 5.91%. A significant rise in protein content of cookies was observed from T7C1 (5.74%) to T7C2 (5.77%), T7C3 (5.80%), T2C1 (5.85%), T2C2 (5.88%) and T2C3 (5.91%). The absolute ascent values of protein content of cookies was noted with increasing the level of ascorbic acid however, values were lower than control.

Protease addition at varying levels to cookie formulation increased protein content of cookies from 6.39 to 6.56%. Within treatments, protein content rose significantly from T3C1 (6.39%) to T3C2 (6.45%) and T3C3 (6.53%) that was also true for T8C1 (6.42%) to T8C2 (6.50%) and T8C3 (6.56%). Cookies containing protease at 600 mg (T8C3) possessed highest protein content (6.56%) while T3C1 had lowest (6.39%). This attribute increased progressively with the addition of protease to cookie dough.

Cookies containing KIO3 had protein content varying from 5.95 to 6.13%. As for as treatments are concerned, protein content of cookies was recorded as 5.95% (T4C1), 6.02% (T4C2), 6.09% (T4C3), 5.98 (T9C1), 6.06% (T9C2) and 6.13% (T9C3). Highest protein content of cookies was observed in T9C3 (6.13%) and lowest in T4C1 (5.95%). The absolute declining trend in protein content of cookies was observed with an increment in the level of KIO3.

Sodium metabisulfite addition to cookie formulation impacted protein content and observed in the range of 6.17 and 6.34%. Protein content of cookies was established as 6.17%, 6.23%, 6.31%, 6.20%, 6.28% and 6.34% in T5C1, T5C2, T5C3, T10C1, T10C2 and T10C3, respectively. Cookies containing sodium metabisulfite at the concentration of 3000 ppm had highest protein
content than others. This implies that a high concentration of SMS induced an increasing trend for protein content of cookies. Present results commensurate with findings of several researchers who reported protein content in wheat flour cookies in range of 5.40-6.47% (Siddiqui et al., 2003; Mushtaq et al., 2010; Pasha et al., 2011). However, in contrast, different authors observed higher protein content in 100% wheat flour cookies as 7.80-10.46% (Apotiola and Fashakin, 2013; Ojinnaka et al., 2013; Masih et al., 2014).

4.7.3.6. Nitrogen free extract (NFE) content

Mean squares regarding NFE content of cookies is demonstrated in Table 24. It is apparent from statistical results that that NFE content did not differ significantly within all statistical measures. Also, statistically splitting further shown that NFE content was not influenced by treatments, concentration and their interaction. Likewise, all the treatments were statistically similar to control with respect to NFE content.

Variations in means of NFE content (Table 26) determined among cookies made from different treatments ranged from 66.76 to 72.40% and 69.18% in mixed wheat flour (T0). To have useful interpretation of results, NFE content in different treatments are summarized as 67.92-70.28% (CMS), 67.04-72.32% (ascorbic acid), 69.42-70.64% (protease), 66.76-72.40% (KIO3) and 68.20-70.41% (SMS). Taken together, cookies treated with KIO3 had highest (72.40%) NFE content at 500 ppm while, lowest (66.76%) was found in cookies treated with KIO3 at 1500 ppm.

Substitution of wheat flour with chemically modified starch at different levels (95:5, 90:10, 85:15) have shown NFE ranged from 67.92 to 70.28%. About treatments, NFE content of cookies was obtained as 70.28%, 68.33%, 68.74%, 69.72%, 67.92% and 68.32% in T1C1, T1C2, T1C3, T6C1, T6C2 and T6C3, respectively. Replacing wheat flour with CMS (95:5) possessed highest NFE content while cookies (90:10) had lowest NFE content.

NFE content of cookies after the treatment with ascorbic acid is presented in Table 26 and it varied between 67.04 and 72.32%. Within treatments, NFE content of cookies was reckoned in T2C1 (67.04%), T2C2 (68.55%), T2C3 (69.70%), T7C1 (72.32%), T7C2 (67.30%) and T7C3 (67.20%). T7C1 possessed highest NFE content (72.32%) while T2C1 had lowest (67.04%). An assorted trend was noticed in NFE content of cookies with increasing the level of ascorbic acid.
<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Moisture</th>
<th>Ash</th>
<th>Fat</th>
<th>Fiber</th>
<th>Protein</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>30</td>
<td>1.024**</td>
<td>0.01051**</td>
<td>6.419**</td>
<td>0.00160**</td>
<td>0.3538**</td>
<td>6.648NS</td>
</tr>
<tr>
<td>Cont. vs treat.</td>
<td>1</td>
<td>0.154**</td>
<td>0.00001NS</td>
<td>0.112NS</td>
<td>0.00233**</td>
<td>0.7213**</td>
<td>0.028NS</td>
</tr>
<tr>
<td>Conc.</td>
<td>2</td>
<td>0.346**</td>
<td>0.00570**</td>
<td>8.586**</td>
<td>0.00001NS</td>
<td>0.0529NS</td>
<td>14.460NS</td>
</tr>
<tr>
<td>Treat. (T)</td>
<td>9</td>
<td>2.427**</td>
<td>0.03267**</td>
<td>5.427**</td>
<td>0.00490**</td>
<td>1.0776**</td>
<td>3.976NS</td>
</tr>
<tr>
<td>Conc. x T</td>
<td>18</td>
<td>0.446**</td>
<td>0.00054NS</td>
<td>7.025NS</td>
<td>0.00009*</td>
<td>0.0050NS</td>
<td>7.483NS</td>
</tr>
<tr>
<td>Error</td>
<td>62</td>
<td>0.017</td>
<td>0.00044</td>
<td>0.987</td>
<td>0.00005</td>
<td>0.0790</td>
<td>10.327</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Cont.: control; Treat: treatments; Conc.: concentration
Table 25: Mean values for chemical properties (%) of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture</th>
<th>Ash</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>3.20±0.07³i</td>
<td>0.450±0.012ghi</td>
<td>21.20±0.55³i</td>
</tr>
<tr>
<td>T₁C₁</td>
<td>2.10±0.05³nm</td>
<td>0.430±0.012ij</td>
<td>21.66±0.57³i</td>
</tr>
<tr>
<td>T₁C₂</td>
<td>3.21±0.09³cde</td>
<td>0.420±0.012hij</td>
<td>22.65±0.61³a-e</td>
</tr>
<tr>
<td>T₁C₃</td>
<td>2.70±0.08³hi</td>
<td>0.400±0.012jk</td>
<td>22.65±0.64³a-d</td>
</tr>
<tr>
<td>T₂C₁</td>
<td>2.89±0.08³gh</td>
<td>0.430±0.012hij</td>
<td>23.66±0.64³a</td>
</tr>
<tr>
<td>T₂C₂</td>
<td>3.05±0.08³fg</td>
<td>0.450±0.012ghi</td>
<td>21.95±0.62³b-h</td>
</tr>
<tr>
<td>T₂C₃</td>
<td>3.59±0.10³ab</td>
<td>0.467±0.015fg</td>
<td>20.22±0.53³ij</td>
</tr>
<tr>
<td>T₃C₁</td>
<td>2.00±0.05³b</td>
<td>0.333±0.009m</td>
<td>21.74±0.56³b-i</td>
</tr>
<tr>
<td>T₃C₂</td>
<td>1.50±0.04³o</td>
<td>0.363±0.009lm</td>
<td>20.92±0.54³e-i</td>
</tr>
<tr>
<td>T₃C₃</td>
<td>2.30±0.07³ln</td>
<td>0.397±0.009kl</td>
<td>20.34±0.54³nij</td>
</tr>
<tr>
<td>T₄C₁</td>
<td>3.26±0.09³cde</td>
<td>0.480±0.012ªfg</td>
<td>17.90±0.47³k</td>
</tr>
<tr>
<td>T₄C₂</td>
<td>3.39±0.10³bc</td>
<td>0.497±0.015ªc-f</td>
<td>20.22±0.54³jij</td>
</tr>
<tr>
<td>T₄C₃</td>
<td>3.19±0.08³c-f</td>
<td>0.500±0.012ªb-f</td>
<td>23.30±0.64³ab</td>
</tr>
<tr>
<td>T₅C₁</td>
<td>2.59±0.08³ijk</td>
<td>0.513±0.015ªa-e</td>
<td>21.65±0.58³c-i</td>
</tr>
<tr>
<td>T₅C₂</td>
<td>2.40±0.06³kl</td>
<td>0.527±0.015ªabc</td>
<td>20.32±0.55³ij</td>
</tr>
<tr>
<td>T₅C₃</td>
<td>2.09±0.05³a</td>
<td>0.533±0.015ªab</td>
<td>20.88±0.59³f-i</td>
</tr>
<tr>
<td>T₆C₁</td>
<td>2.99±0.08³ªg</td>
<td>0.410±0.012ªij</td>
<td>21.10±0.55³d-i</td>
</tr>
<tr>
<td>T₆C₂</td>
<td>3.20±0.08³ªc-f</td>
<td>0.430±0.012ªhij</td>
<td>22.70±0.59³a-d</td>
</tr>
<tr>
<td>T₆C₃</td>
<td>2.60±0.07³ªj</td>
<td>0.417±0.009ªij</td>
<td>22.93±0.61³abc</td>
</tr>
<tr>
<td>T₇C₁</td>
<td>2.34±0.07³l</td>
<td>0.450±0.012ªghi</td>
<td>19.04±0.50³jk</td>
</tr>
<tr>
<td>T₇C₂</td>
<td>3.38±0.09³bc</td>
<td>0.460±0.012ªgh</td>
<td>22.97±0.61³abc</td>
</tr>
<tr>
<td>T₇C₃</td>
<td>3.54±0.09³ªab</td>
<td>0.467±0.015ªfg</td>
<td>22.89±0.62³abc</td>
</tr>
<tr>
<td>T₈C₁</td>
<td>2.00±0.05³ªn</td>
<td>0.333±0.009ªm</td>
<td>21.48±0.58³c-i</td>
</tr>
<tr>
<td>T₈C₂</td>
<td>1.60±0.04³ªo</td>
<td>0.367±0.009ªklm</td>
<td>20.90±0.59³ªi</td>
</tr>
<tr>
<td>T₈C₃</td>
<td>2.39±0.06³ªkl</td>
<td>0.400±0.012ªjk</td>
<td>20.63±0.54³ªj</td>
</tr>
<tr>
<td>T₉C₁</td>
<td>3.29±0.08³ªcd</td>
<td>0.483±0.015ªªg</td>
<td>17.70±0.50³ªk</td>
</tr>
<tr>
<td>T₉C₂</td>
<td>3.68±0.10³ªa</td>
<td>0.500±0.012ªªª</td>
<td>20.14±0.53ªij</td>
</tr>
<tr>
<td>T₉C₃</td>
<td>3.09±0.08³ªªg</td>
<td>0.513±0.015ªªªe</td>
<td>22.67±0.59³ªd</td>
</tr>
<tr>
<td>T₁₀C₁</td>
<td>2.58±0.07³ªjk</td>
<td>0.517±0.015ªªªd</td>
<td>22.29±0.58³ªªf</td>
</tr>
<tr>
<td>T₁₀C₂</td>
<td>2.40±0.07³ªkl</td>
<td>0.530±0.012ªªabc</td>
<td>22.14±0.58³ªªg</td>
</tr>
<tr>
<td>T₁₀C₃</td>
<td>2.50±0.07³ªi</td>
<td>0.547±0.015ªªªa</td>
<td>22.31±0.59³ªªf</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Selected wheat variety (SWV): T₁ to T₅
- T₁C₁: 5% CMS; T₂C₁: 10% CMS; T₃C₁: 15% CMS
- T₁C₂: 5ppm AsA; T₂C₂: 10ppm AsA; T₃C₂: 15ppm AsA
- T₁C₃: 200mg protease; T₂C₃: 400mg protease; T₃C₃: 600mg protease
- T₁C₄: 500ppm KIO₃; T₂C₄: 1000ppm KIO₃; T₃C₄: 1500ppm KIO₃
- T₁C₅: 1000ppm SMS; T₂C₅: 2000ppm SMS; T₃C₅: 3000ppm SMS

Mixed wheat flour (MWF): T₆ to T₁₀
- T₆C₁: 5% CMS; T₇C₁: 10% CMS; T₈C₁: 15% CMS
- T₆C₂: 5ppm AsA; T₇C₂: 10ppm AsA; T₈C₂: 15ppm AsA
- T₆C₃: 200mg protease; T₇C₃: 400mg protease; T₈C₃: 600mg protease
- T₆C₄: 500ppm KIO₃; T₇C₄: 1000ppm KIO₃; T₈C₄: 1500ppm KIO₃
- T₆C₅: 1000ppm SMS; T₇C₅: 2000ppm SMS; T₈C₅: 3000ppm SMS

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### Table 26: Mean values for chemical properties (%) of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fiber</th>
<th>Protein</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>0.150±0.006ᵇ</td>
<td>6.53±0.17ᵃᵇ</td>
<td>69.18±1.80ᵃᵇ</td>
</tr>
<tr>
<td>T₁C₁</td>
<td>0.083±0.003ʲⁱ</td>
<td>5.47±0.14ᵖqr</td>
<td>70.28±1.99ᵃᵇ</td>
</tr>
<tr>
<td>T₁C₂</td>
<td>0.093±0.003ᵇⁱʲ</td>
<td>5.44±0.15⁴ʳ</td>
<td>68.33±1.78ᵃ</td>
</tr>
<tr>
<td>T₁C₃</td>
<td>0.103±0.003ᵍʰ</td>
<td>5.41±0.15ʳ</td>
<td>68.74±1.79ᵃ</td>
</tr>
<tr>
<td>T₂C₁</td>
<td>0.130±0.006ᶜ</td>
<td>5.85±0.16ᵗ</td>
<td>67.04±1.74ᵃ</td>
</tr>
<tr>
<td>T₂C₂</td>
<td>0.127±0.003ᵃᵈ</td>
<td>5.88±0.16ᵇᶜ</td>
<td>68.55±1.78ᵃ</td>
</tr>
<tr>
<td>T₂C₃</td>
<td>0.127±0.003ᵃᵈ</td>
<td>5.91±0.15ᵉᵖ</td>
<td>69.70±1.85ᵃ</td>
</tr>
<tr>
<td>T₃C₁</td>
<td>0.117±0.003ᵈᵉ</td>
<td>6.39±0.17ᵃ⁻ᵗ</td>
<td>69.42±1.80ᵃ</td>
</tr>
<tr>
<td>T₃C₂</td>
<td>0.113±0.003ᵉᶠ</td>
<td>6.45±0.18ᵃ⁻ᵈ</td>
<td>70.64±1.87ᵃ</td>
</tr>
<tr>
<td>T₃C₃</td>
<td>0.110±0.000ʳ⁻ᶠ</td>
<td>6.53±0.17ᵃᵇ</td>
<td>70.31±1.91ᵃ</td>
</tr>
<tr>
<td>T₄C₁</td>
<td>0.167±0.003ᵃ</td>
<td>5.95±0.15ᶠ⁻芊</td>
<td>72.25±1.92ᵃ</td>
</tr>
<tr>
<td>T₄C₂</td>
<td>0.167±0.003ᵃ</td>
<td>6.02±0.16ᵈ⁻芊</td>
<td>69.72±1.89ᵃ</td>
</tr>
<tr>
<td>T₄C₃</td>
<td>0.160±0.006ᵇ</td>
<td>6.09±0.16ᵇ⁻芊</td>
<td>66.76±1.89ᵃ</td>
</tr>
<tr>
<td>T₅C₁</td>
<td>0.117±0.003ᵈᵉ</td>
<td>6.17±0.16ᵃ⁻¹</td>
<td>68.96±1.87ᵃ</td>
</tr>
<tr>
<td>T₅C₂</td>
<td>0.113±0.003ᵉᶠ</td>
<td>6.23±0.16ᵃ⁻ʲ</td>
<td>70.41±1.99ᵃ</td>
</tr>
<tr>
<td>T₅C₃</td>
<td>0.113±0.003ᵉᶠ</td>
<td>6.31±0.17ᵃ⁻ʰ</td>
<td>70.08±1.82ᵃ</td>
</tr>
<tr>
<td>T₆C₁</td>
<td>0.087±0.003ⁱʲ</td>
<td>5.69±0.15ᵐ⁻ᵣ</td>
<td>69.72±1.85ᵃ</td>
</tr>
<tr>
<td>T₆C₂</td>
<td>0.097±0.003ˢʰⁱ</td>
<td>5.66±0.15ⁿ⁻ᵣ</td>
<td>67.92±1.84ᵃ</td>
</tr>
<tr>
<td>T₆C₃</td>
<td>0.107±0.003ᵉᶠ</td>
<td>5.63±0.15ᵃ⁻ʳ</td>
<td>68.32±1.93ᵃ</td>
</tr>
<tr>
<td>T₇C₁</td>
<td>0.117±0.003ᵈᵉ</td>
<td>5.74±0.15ᵃ⁻ʳ</td>
<td>72.32±1.96ᵃ</td>
</tr>
<tr>
<td>T₇C₂</td>
<td>0.113±0.003ᵉᶠ</td>
<td>5.77±0.15ᵏ⁻ᵣ</td>
<td>67.30±1.90ᵃ</td>
</tr>
<tr>
<td>T₇C₃</td>
<td>0.110±0.000ʳ⁻ᶠ</td>
<td>5.80±0.16ᵗ</td>
<td>67.20±1.75ᵃ</td>
</tr>
<tr>
<td>T₈C₁</td>
<td>0.133±0.003ᶜ</td>
<td>6.42±0.17ᵃ⁻ᵉ</td>
<td>69.63±1.97ᵃ</td>
</tr>
<tr>
<td>T₈C₂</td>
<td>0.133±0.003ᶜ</td>
<td>6.50±0.17ᵃᵇᶜ</td>
<td>70.50±1.83ᵃ</td>
</tr>
<tr>
<td>T₈C₃</td>
<td>0.130±0.006ᶜ</td>
<td>6.56±0.18ᵃ</td>
<td>69.89±1.82ᵃ</td>
</tr>
<tr>
<td>T₉C₁</td>
<td>0.150±0.006ᵇ</td>
<td>5.98±0.16ᵉ⁻ᵒ</td>
<td>72.40±1.88ᵃ</td>
</tr>
<tr>
<td>T₉C₂</td>
<td>0.160±0.006ᵇ</td>
<td>6.06±0.17ᶜ⁻ᵒ</td>
<td>69.47±1.80ᵃ</td>
</tr>
<tr>
<td>T₉C₃</td>
<td>0.153±0.003ᵇ</td>
<td>6.13±0.16ᵃ⁻ᵐ</td>
<td>67.45±1.79ᵃ</td>
</tr>
<tr>
<td>T₁₀C₁</td>
<td>0.110±0.006ᵈᶠ</td>
<td>6.20±0.18ᵃ⁻ᵏ</td>
<td>68.30±1.78ᵃ</td>
</tr>
<tr>
<td>T₁₀C₂</td>
<td>0.107±0.003ᵉᶠ</td>
<td>6.28±0.16ᵃ⁻¹</td>
<td>68.55±1.82ᵃ</td>
</tr>
<tr>
<td>T₁₀C₃</td>
<td>0.103±0.003ᵍʰ</td>
<td>6.34±0.16ᵃ⁻ᵍ</td>
<td>68.20±1.85ᵃ</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

**Selected wheat variety (SWV): T₀ to T₅**
- T₁C₁: 5% CMS; T₂C₂: 10% CMS; T₃C₃: 15% CMS
- T₁C₂: 5ppm AsA; T₂C₂: 10ppm AsA; T₃C₃: 15ppm AsA
- T₁C₃: 200mg protease; T₂C₃: 400mg protease; T₃C₃: 600mg protease
- T₁C₄: 500ppm KIO₃; T₂C₄: 1000ppm KIO₃; T₃C₄: 1500ppm KIO₃
- T₁C₅: 1000ppm SMS; T₂C₅: 2000ppm SMS; T₃C₅: 3000ppm SMS

**Mixed wheat flour (MWF): T₆ to T₁₀**
- T₆C₁: 5% CMS; T₇C₂: 10% CMS; T₈C₃: 15% CMS
- T₆C₂: 5ppm AsA; T₇C₂: 10ppm AsA; T₈C₃: 15ppm AsA
- T₆C₃: 200mg protease; T₇C₃: 400mg protease; T₈C₃: 600mg protease
- T₆C₄: 500ppm KIO₃; T₇C₄: 1000ppm KIO₃; T₈C₄: 1500ppm KIO₃
- T₆C₅: 1000ppm SMS; T₇C₅: 2000ppm SMS; T₈C₅: 3000ppm SMS
Addition of protease at different concentrations to cookie formulation have represented NFE content in the range of 69.42 and 70.64%. In different treatments, NFE content of cookies was found as 69.42, 70.64, 70.31, 69.63, 70.50 and 69.89% in T3C1, T3C2, T3C3, T8C1, T8C2 and T8C3, respectively. The highest NFE content (70.64%) was observed in T3C2 while, lowest value was calculated for T3C1 (69.42%). Cookies made with the addition of 400 mg protease showed a highest NFE content than did others.

From Table 26, the mean values for NFE content of cookies treated with KIO3 can be observed to be varied from 66.76 to 72.40%. Among treatments, highest NFE content of cookies was find out in T9C1 (72.40) followed by T4C1 (72.25%), T4C2 (69.72%), T9C2 (69.47%) and T9C3 (67.45%) while lowest was found in T4C3 (66.76%). NFE content of cookies tended to be decrease by increasing the level of KIO3.

Cookies made with the addition of sodium metabisulfite have shown NFE content to be varying from 68.20 to 70.41%. As for as treatments are concerned, NFE content of cookies was determined in T5C1, T5C2, T5C3, T10C1, T10C2 and T10C3 as 68.96, 70.41, 70.08, 68.30, 68.55 and 68.20%, respectively. Cookies made from formulation containing 2000 ppm sodium metabisulfite showed a highest NFE content than others. Values reported in this work are in good agreement with findings established by other researchers (Mushtaq et al., 2010; Okpala and Ekwe, 2013; Qaisrani et al., 2014) who observed NFE content in wheat flour cookies as 65.46-72.28%.

4.7.4. Hardness values of cookies

Statistical analysis pertaining to hardness of cookies presented in Table 27 divulge highly significant variations for all combinations. It is obvious from statistical results that storage duration had highly significant effect on hardness of cookies. Results further explored that variations in hardness of cookies were only ascribed to treatments whereas, the effect of concentration and interaction of concentration and treatments was non-significant. Likewise, the effect of interaction of days and combination was found non-significant. Also, the treatments were statistically similar to control in terms of hardness.

Variations in mean values for hardness of cookies during storage are shown in Table 28. Hardness of fresh cookies prepared from different treatments ranged from 0.86 to 3.55 kg whereas 2.29 kg was found in mixed wheat flour (T0). For the relative assessment of treatments, hardness of cookies varied in response to all the chemical or modifying agents as CMS (2.29-
2.71 kg), ascorbic acid (2.72-3.55 kg), protease (0.86-1.51 kg), KIO₃ (2.04-2.27 kg) and SMS (1.62-2.01 kg). Taking all the treatments into consideration at 0 day, cookies treated with ascorbic acid were harder at 15ppm while, cookies made from dough containing 600 mg protease were softest since these required minimum force to compress. Hardness of T₀ was significantly increased from 2.29 kg (0 day) to 3.24 kg (15 days) and 3.98 kg (30 days) storage interval.

Force required to break fresh cookies made with the replacements of wheat flour with chemically modified starch at different levels (95:5, 90:10, 85:15) ranged between 2.29 and 2.71 kg. At 0 day, force required to compress T₁C₃ was greater (2.71 kg) while smaller value was observed for T₆C₁ (2.29 kg). This implies that force required to compress cookies presented an ascent trend, as the rate of CMS substitution increased in wheat flour. During storage, hardness of cookies made with flour blended with CMS was significantly increased from 2.50-2.85 kg at 15 days to 3.40-3.88 kg after 30 days storage of cookies (Table 28). It is obvious from present results that storage did influence hardness of cookies. Pareyt et al. (2011) reported break strength of wheat cookies substituted with 10 to 40% starch (11.6-18.6N).

Peak force of fresh cookies prepared from the treatment with ascorbic acid ranged between 2.72 and 3.55 kg. At 0 day, maximum peak force of cookies was obtained in T₇C₃ (3.55 kg) and lowest in T₂C₁ (2.72 kg). A gradual rise in peak force was noticed with increasing the level of ascorbic acid. Difference in peak force of cookies treated with ascorbic acid at 15 days (2.95-3.68 kg) and after 30 days (3.17-4.15 kg) was considered as just noticeable. During storage, peak force gradually increased as the storage interval increased. Hardness of cookies may due to the strong linkages between particles after baking process or due to amalgamation of protein as a result of ascorbic acid addition. Faccio et al. (2012) reported the strengthening effect of ascorbic acid on dough system, as realized from rheological studies.

Breaking strength of fresh cookies prepared from the dough containing varying levels of protease (200, 400 and 600 mg) varied between 0.86 and 1.51 kg. At 0 day, T₈C₁ cookies had maximum breaking strength (1.51 kg) while minimum was recorded for T₃C₃ and T₈C₃ (0.86 kg). It is known that, as the concentration of protease increase, the cookies breaking strength decrease. During 15 days storage, breaking strength of cookies was significantly increased to 0.97-1.89 kg and 1.95-2.80 kg after one month storage of cookies.
Gaines and Finney (1989) found resistance to compression ranged from 5.2 to 6.8 kg for cookies treated with different selected enzymes. Treatment of dough with protease and reducing agents significantly reduced rheological properties (Manohar and Rao, 1997).

Measurement of peak force of fresh cookies treated with KIO$_3$ at different concentrations (500, 1000 and 1500 ppm) fall in the range of 2.04-2.27 kg. At 0 day, highest peak force of cookies was obtained in $T_9C_3$ (2.27 kg) and lowest in $T_4C_1$ (2.04 kg). An obvious ascent trend was found in peak force of cookies with an increase in concentration of KIO$_3$ in cookies dough. During storage, peak force of cookies was increased to 2.26-2.45 kg and 3.12-3.40 kg after 15 days and 30 days storage, respectively. Present data showed that storage had a great impact on hardness of cookies and it gradually increased as a function of storage. Present results are antithesis to findings by Pareyt et al. (2010) who reported that addition of potassium iodate at high levels reduced break strength of cookies.

Comparison of breaking strength of freshly baked cookies made with the dough containing varying concentrations of sodium metabisulfite (Table 28) presented a range between 1.62 and 2.01 kg. At 0 day, maximum force required to snap cookies prepared from dough treated with SMS was obtained in $T_{10}C_1$ (2.01 kg) whereas least force was required for $T_5C_3$ (1.62 kg). Mean values for the effect of SMS on breaking strength of cookies presented a gradual declining trend. During storage, hardness values of cookies were increased to 1.92-2.19 kg (15 days) and subsequently, to 2.81-3.09 kg (30 days storage). However, storage resulted in an increase in breaking strength of cookies made with the addition of SMS. Present results commensurate with findings of Kumar et al. (2013) who stated that hardness of cookies was decreased with increasing the concentration of reducing agents (L-cysteine and Glutathione) as compared to untreated cookies.

Values reported in this work are in good agreement with Mushtaq et al. (2010) who observed hardness in different treatments of cookies ranged from 1832 to 2302 g. However, values observed in this study are inconsistent with findings of Qaisrani et al. (2014) who described the hardness of cookies supplemented with psyllium husk was decreased with increase in storage period.
Table 27: Mean squares for hardness of cookies prepared from different treatments during storage

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hardness</td>
</tr>
<tr>
<td>Days (D)</td>
<td>2</td>
<td>30.7414**</td>
</tr>
<tr>
<td>Combination (C)</td>
<td>30</td>
<td>3.3798**</td>
</tr>
<tr>
<td>Cont. vs. Treat.</td>
<td>1</td>
<td>3.1436 NS</td>
</tr>
<tr>
<td>Conc.</td>
<td>2</td>
<td>0.0393 NS</td>
</tr>
<tr>
<td>Treat. (T)</td>
<td>9</td>
<td>9.9581**</td>
</tr>
<tr>
<td>Conc. x T</td>
<td>18</td>
<td>0.4749 NS</td>
</tr>
<tr>
<td>D x C</td>
<td>60</td>
<td>0.0839 NS</td>
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<tr>
<td>Error</td>
<td>186</td>
<td>0.0158</td>
</tr>
<tr>
<td>Total</td>
<td>278</td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Cont.: control; Treat: treatments; Conc.: concentration
Table 28: Mean values for hardness (kg) of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>( T_0 )</td>
<td>2.29±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.24±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.98±0.11&lt;sup&gt;abc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_1C_1 )</td>
<td>2.31±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.53±0.07&lt;sup&gt;defg&lt;/sup&gt;</td>
<td>3.40±0.10&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_1C_2 )</td>
<td>2.36±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.60±0.07&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.79±0.10&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_1C_3 )</td>
<td>2.71±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.65±0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.87±0.10&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_2C_1 )</td>
<td>2.72±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.95±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.20±0.08&lt;sup&gt;fg&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_2C_2 )</td>
<td>2.77±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.97±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.17±0.08&lt;sup&gt;fg&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_2C_3 )</td>
<td>3.04±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.21±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.01±0.11&lt;sup&gt;abc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_3C_1 )</td>
<td>1.46±0.04&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>1.85±0.05&lt;sup&gt;lm&lt;/sup&gt;</td>
<td>2.80±0.08&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_3C_2 )</td>
<td>1.24±0.03&lt;sup&gt;m&lt;/sup&gt;</td>
<td>1.36±0.04&lt;sup&gt;n&lt;/sup&gt;</td>
<td>2.66±0.07&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_3C_3 )</td>
<td>0.86±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97±0.03&lt;sup&gt;o&lt;/sup&gt;</td>
<td>2.02±0.05&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_4C_1 )</td>
<td>2.04±0.05&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>2.26±0.06&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>3.12±0.08&lt;sup&gt;gh&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_4C_2 )</td>
<td>2.09±0.05&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>2.43±0.06&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>3.20±0.09&lt;sup&gt;fg&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_4C_3 )</td>
<td>2.24±0.06&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2.44±0.07&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>3.27±0.09&lt;sup&gt;fg&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_5C_1 )</td>
<td>1.93±0.05&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>2.17±0.06&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>3.09±0.08&lt;sup&gt;gh&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_5C_2 )</td>
<td>1.86±0.05&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>2.01±0.05&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>2.83±0.08&lt;sup&gt;ij&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_5C_3 )</td>
<td>1.62±0.04&lt;sup&gt;jk&lt;/sup&gt;</td>
<td>1.92±0.05&lt;sup&gt;klm&lt;/sup&gt;</td>
<td>2.81±0.07&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_6C_1 )</td>
<td>2.29±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.50±0.07&lt;sup&gt;defg&lt;/sup&gt;</td>
<td>3.40±0.09&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_6C_2 )</td>
<td>2.33±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.56±0.07&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.65±0.10&lt;sup&gt;de&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_6C_3 )</td>
<td>2.38±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.61±0.07&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.88±0.11&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_7C_1 )</td>
<td>3.15±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.23±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.07±0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_7C_2 )</td>
<td>3.44±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.66±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.14±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>( T_7C_3 )</td>
<td>3.55±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.68±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.15±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_8C_1 )</td>
<td>1.51±0.04&lt;sup&gt;k&lt;/sup&gt;</td>
<td>1.89±0.05&lt;sup&gt;klm&lt;/sup&gt;</td>
<td>2.75±0.08&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_8C_2 )</td>
<td>1.32±0.03&lt;sup&gt;lm&lt;/sup&gt;</td>
<td>1.75±0.05&lt;sup&gt;m&lt;/sup&gt;</td>
<td>2.65±0.07&lt;sup&gt;j&lt;/sup&gt;</td>
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<tr>
<td>( T_8C_3 )</td>
<td>0.86±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15±0.03&lt;sup&gt;o&lt;/sup&gt;</td>
<td>1.95±0.05&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>( T_9C_1 )</td>
<td>2.06±0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.35±0.07&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>3.17±0.08&lt;sup&gt;fg&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_9C_2 )</td>
<td>2.09±0.06&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>2.41±0.06&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>3.18±0.08&lt;sup&gt;fg&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_9C_3 )</td>
<td>2.27±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.45±0.06&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>3.40±0.09&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_{10}C_1 )</td>
<td>2.01±0.06&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>2.19±0.06&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>3.07±0.08&lt;sup&gt;gh&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_{10}C_2 )</td>
<td>1.89±0.05&lt;sup&gt;ghi&lt;/sup&gt;</td>
<td>2.06±0.05&lt;sup&gt;ik&lt;/sup&gt;</td>
<td>2.88±0.08&lt;sup&gt;hij&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>( T_{10}C_3 )</td>
<td>1.73±0.05&lt;sup&gt;ij&lt;/sup&gt;</td>
<td>1.95±0.05&lt;sup&gt;kl&lt;/sup&gt;</td>
<td>2.83±0.08&lt;sup&gt;ij&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05)

**Selected wheat variety (SWV):** \( T_1 \) to \( T_5 \)
- \( T_1C_1 \): 5% CMS; \( T_1C_2 \): 10% CMS; \( T_1C_3 \): 15% CMS
- \( T_2C_1 \): 5ppm AsA; \( T_2C_2 \): 10ppm AsA; \( T_2C_3 \): 15ppm AsA
- \( T_3C_1 \): 200mg protease; \( T_3C_2 \): 400mg protease; \( T_3C_3 \): 600mg protease
- \( T_4C_1 \): 500ppm KIO<sub>3</sub>; \( T_4C_2 \): 1000ppm KIO<sub>3</sub>; \( T_4C_3 \): 1500ppm KIO<sub>3</sub>
- \( T_5C_1 \): 1000ppm SMS; \( T_5C_2 \): 2000ppm SMS; \( T_5C_3 \): 3000ppm SMS

**Mixed wheat flour (MWF):** \( T_6 \) to \( T_{10} \)
- \( T_6C_1 \): 5% CMS; \( T_6C_2 \): 10% CMS; \( T_6C_3 \): 15% CMS
- \( T_7C_1 \): 5ppm AsA; \( T_7C_2 \): 10ppm AsA; \( T_7C_3 \): 15ppm AsA
- \( T_8C_1 \): 200mg protease; \( T_8C_2 \): 400mg protease; \( T_8C_3 \): 600mg protease
- \( T_9C_1 \): 500ppm KIO<sub>3</sub>; \( T_9C_2 \): 1000ppm KIO<sub>3</sub>; \( T_9C_3 \): 1500ppm KIO<sub>3</sub>
- \( T_{10}C_1 \): 1000ppm SMS; \( T_{10}C_2 \): 2000ppm SMS; \( T_{10}C_3 \): 3000ppm SMS
4.7.5. Sensory evaluation of cookies from different treatments

Sensory evaluation of fresh cookies and cookies stored for one month was performed separately and sensory scores for cookies made from different treatments and mixed wheat flour (T₀) are given in Figure 1-6. Although, the present outcomes showed variations in all the cookies in response to different chemical or dough modifying agents. Overall, all cookies prepared from different treatments were usually well accepted (not rejected) since all the quality scores were greater than 5 that may be lowest acceptable score for nine point hedonic scale. Cookies prepared from MWF (T₀) were designated as control cookies for consumer test. Storage studies revealed a significant impact on sensory attributes which got comparatively lower color scores for all treatments. Overall acceptability of cookies was evaluated using the quality scores attained from color, flavor, taste, texture and crispiness. As a whole, highest score for overall acceptability was observed by freshly prepared cookies (at 0 day) which progressively reduced with storage period. During storage, a similar declining trend in overall acceptability scores was found in all the treatments.

4.7.5.1. Color

Mean squares (Table 29) regarding color of cookies showed highly significant variations for all combinations. It can be observed from statistical results that storage showed highly significant effect on color of cookies. Statistical splitting further explored that there were highly significant variations in color of cookies due to treatments. But, the effect of concentration did not influence color of cookies. In this work, interaction of concentration and treatments as well as the interaction of days and combination was found highly significant. Also, treatments vs. control had significantly different effect on color of cookies. Figure 1 is the representative of mean comparison for color of cookies prepared from different treatments. Color of cookies prepared from mixed wheat flour (T₀) was found to be 7.25 at 0 day while, decreased from 6.75 at 15 days to 6.25 after 30 days storage interval. Whereas, color of cookies prepared from different treatments ranged from 6.13 to 7.75. In order to compare treatments, color of cookies was recorded as CMS (6.13-7.13), ascorbic acid (6.50-7.00), protease (7.00-7.75), KIO₃ (6.13-7.38) and SMS (7.19-7.7.50). Of all, cookies made from dough containing 600 mg protease had highest color score while cookies made with CMS and KIO₃ had lowest score at 15% and 1500 ppm, respectively.

Sensory evaluation of fresh and cookies stored for 30 days was performed separately and have
shown that cookies made by substituting wheat flour with CMS at various (95:5, 90:10, 85:15) levels resulted in a variation of cookie color from 6.13 to 7.13. At 0 day, highest sensory score for color was obtained in T₁C₁ and T₆C₁ (7.13) followed by T₆C₂ (7.06), T₁C₂ and T₆C₃ (6.50) and T₁C₃ (6.13). Highest color was observed in cookies from wheat flour: CMS (95:5) while lowest was found in cookies (85:15). This infers that color of cookies showed lower score with increasing level of CMS. Also, T₁C₁ and T₆C₁ were preferred by panelists because, CMS at 5% level contributed desired color to cookies than others. During storage, there was a declining trend for color of cookies substituted with CMS and color score is reported as 6.06-7.00 (15 days) and 5.38-6.88 (30 days). As obvious, storage revealed a significant decline for color score of cookies and darkening in color was observed with increase in storage period.

Quality scores of freshly prepared cookies in response to ascorbic acid addition at different (5, 10 and 15 ppm) concentrations ranged from 6.50 to 7.00. At 0 day, T₂C₃ had maximum color score was obtained by (7.00) which was considerably declined as storage increased. Range of quality score of cookies between 15 days and 30 days was 6.13-6.88 and 5.50-6.38, respectively. Storage days showed most expressed effect on color scores and a declining trend was observed during storage period.

Organoleptic properties of fresh cookies prepared from dough formulation containing protease at different (200, 400 and 600 mg) concentrations showed color of cookies in the range of 7.00-7.75. At 0 day, maximum color score was obtained by T₅C₃ (7.75) while minimum was found in T₃C₁ (7.00) as shown in Figure 1. An ascent trend is reported regarding color of cookies, as the concentration of protease increased. Color of cookies stored for 15 days and 30 days were assessed from 6.25-7.25 to 6.13-6.88, respectively. Present observations report a significant declining trend in cookie color scores due to storage. However, these cookies had somewhat satisfactory color after one month storage as compared to others. High degree of Maillard reaction may due to proteases activity may be responsible of the release of a greater level of small peptides and free amino acids (Kara et al., 2005).

Quality assessment in terms of sensory attributes of fresh cookies treated with KIO₃ at different (500, 1000 and 1500 ppm) concentrations varied from 6.13 to 7.38. At 0 day, T₄C₁ exhibited maximum color while, minimum was found in T₉C₃ (6.13). A significant reduction in color
Table 29: Mean squares for organoleptic properties of cookies prepared with different treatments

<table>
<thead>
<tr>
<th>SOV</th>
<th>DF</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Color</td>
</tr>
<tr>
<td>Days (D)</td>
<td>2</td>
<td>17.9530**</td>
</tr>
<tr>
<td>Combination (C)</td>
<td>30</td>
<td>4.1310**</td>
</tr>
<tr>
<td>Cont. vs. treat.</td>
<td>1</td>
<td>4.8500*</td>
</tr>
<tr>
<td>Conc.</td>
<td>2</td>
<td>1.7930NS</td>
</tr>
<tr>
<td>Treat. (T)</td>
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<td>7.6634**</td>
</tr>
<tr>
<td>Conc. x T</td>
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<td>2.5847**</td>
</tr>
<tr>
<td>D x C</td>
<td>60</td>
<td>0.4957**</td>
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<td>1.0093</td>
</tr>
<tr>
<td>Total</td>
<td>743</td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant (P<0.01); * = Significant (P<0.05); NS = Non-significant (P>0.05)

Cont.: control; Treat: treatments; Conc.: concentration
Figure 1: Color of cookies prepared with different treatments (a) at 0 day, (b) at 15 days, (c) at 30 days
score of cookies is established with an increase in $\text{KIO}_3$. Storing cookies treated with $\text{KIO}_3$ for 15 days and 30 days had a significant effect on color and ranged as 6.13-7.25 and 6.13-7.06, respectively. Mean color scores of cookies were progressively declined with passage of time. Individual assessment of sensory attributes of freshly baked cookies made from formulation containing varying concentrations of sodium metabisulfite ranging between 7.19 and 7.50. At 0 day, maximum color was obtained by $\text{T}_3\text{C}_3$ and $\text{T}_{10}\text{C}_3 (7.50)$ while, minimum was found in $\text{T}_3\text{C}_1 (7.19)$. Sensory evaluation show that color score of cookies tended to be increased progressively with an increase in SMS. Color of cookies stored for a period of one month was evaluated in the range of 7.13-7.50 (15 days) and 7.06-7.25 (30 days) storage. This data presented a slight decrease in color scores of cookies. However, these cookies had comparatively acceptable color after one month storage as compared to others.

Results regarding color of cookies in present study are akin to earlier findings of Pasha et al. (2002) who observed a similar decreasing pattern in color during 60 days storage. These observations are further justified by results of Saeed et al. (2012) who found a gradual decrease from 8.6 to 6.4 in color of cookies from wheat: sweet potato composite flour during three months storage. Reduction in color of biscuit may attributed to moisture absorption from air and may also consequence of Maillard’s reaction (Mebpa et al., 2007). According to study of Sharif et al. (2003), color in different treatments of cookies ranged from 6.32 to 7.77.

4.7.5.2. Crispiness

Statistical analysis (Table 29) for crispiness of cookies showed highly significant differences for all experimental measures. It is apparent from statistical results that storage had highly significant effect on crispiness of cookies. Also, treatments were statistically different from control regarding crispiness of cookies. Further exploring statistical results revealed that treatments caused highly significant effect on crispiness of cookies while, concentration and interaction of concentration and treatments caused non-significant effect on crispiness of cookies. Likewise, the interaction of days and combination was found non-significant.

Figure 2 illustrates the mean values for crispiness of cookies prepared from different treatments varied in the range of 6.25 and 7.75. Crispiness of cookies prepared from mixed wheat flour ($\text{T}_0$) was found to be 6.44 at 0 day while, decreased from 6.31 at 15 days to 6.25 after 30 days storage interval.
For the purpose of comparison among treatments, crispiness of cookies ranged as 6.88-7.75 (CMS), 6.38-7.25 (ascorbic acid), 7.13-7.50 (protease), 6.25-7.00 (KIO\textsubscript{3}) and 7.13-7.63 (SMS). Moreover, cookies after substituting wheat flour with CMS (85:15) were highly crispy while those made of KIO\textsubscript{3} at 500 ppm had lowest crispiness.

Quality scores regarding crispiness of freshly baked cookies made by the supplementation of CMS in wheat flour represented a range of 6.88 and 7.75. About treatments, T\textsubscript{6}C\textsubscript{3} got highest score (7.75) whereas, T\textsubscript{1}C\textsubscript{1} obtained lowest score (6.88). Taking storage into consideration, cookies showed maximum crispness score (7.75) at 0 day which tend to decrease with storage while, minimum score (5.88) was found at 30 days. As for as the storage effect is concerned, crispiness of cookies ranged between 0 day (6.88-7.75) and 30 days (6.25-7.25) implying that this trait was decreased as a function of storage.

From Figure 2, it can be observed that crispiness of freshly prepared cookies in response to ascorbic acid varied between 6.38 and 7.25. Results revealed that at 0 day, T\textsubscript{7}C\textsubscript{1} exhibited maximum score for crispiness (7.25) while, T\textsubscript{7}C\textsubscript{3} had least (6.38). Present data reported a decreasing trend in crispiness of cookies, as the concentration of ascorbic acid was increased. Decreasing trend was also observed for storage, crispiness of cookies decreased during 15 days (6.00-7.00) and subsequently after 30 days (5.88-6.38) storage.

Organoleptic evaluation of fresh cookies containing protease can be observed from Figure 2 varying from 7.13 to 7.50. For fresh cookies, T\textsubscript{8}C\textsubscript{3} showed maximum crispiness (7.50) while, T\textsubscript{3}C\textsubscript{1} minimum (7.13). Protease addition at high concentration resulted in an increment for sensory scores of cookies crispiness. A significant reduction in crispiness of cookies was observed from 15 days (6.88-7.25) and 30 days (6.19-7.19) storage period. Present data report a decreasing trend during storage among treatments.

As a result of dough treatment with KIO\textsubscript{3}, crispiness scores of cookies fall in the range of 6.25 and 7.00. For fresh cookies, T\textsubscript{4}C\textsubscript{3} exhibited maximum score of crispiness (7.00) while, T\textsubscript{9}C\textsubscript{1} had minimum (6.25). There was a rising trend in crispiness scores of cookies with the addition of KIO\textsubscript{3}.

Means for the cookies stored for 15 days and 30 days were evaluated as 6.25-6.88 and 6.13-6.56, respectively. Storing of cookies made with KIO\textsubscript{3} exhibited a most expressed impact on crispiness of cookies. Figure 2 unveiled mean comparison for the effect of sodium metabisulfite on crispiness of freshly baked cookies observed varying from 7.13 to 7.63.
Figure 2: Crispiness of cookies prepared with different treatments (a) at 0 day, (b) at 15 days, (c) at 30 days
For fresh cookies, T\textsubscript{10}C\textsubscript{3} exhibited maximum crispiness (7.63) while, T\textsubscript{10}C\textsubscript{1} minimum (7.13). In various treatments, increasing concentrations of SMS positively influenced crispiness of cookies. During storage, crispiness of cookies decreased during 15 days (7.00-7.38) and 30 days (6.63-7.38) storage. This trait was affected by storage and cookies were less crispy in relation to their fresh counterparts. This work is consistent with Sharif et al. (2003) who found crispness of cookies ranged from 6.28 to 7.08 which was decreased significantly with storage.

4.7.5.3. Flavor

Mean squares regarding flavor of cookies given in Table 29 showed highly significant variations for all combinations. It can be observed from statistical data that storage had highly significant impact on flavor. Results further explored that variations in flavor of cookies were only due to treatments while, effect of concentration and interaction of concentration and treatments was non-significant. Likewise, the effect of interaction of days and combination was found non-significant. Also, the treatments were statistically similar to control for flavor of cookies.

As shown by Figure 3, mean values for flavor of cookies produced with different treatments ranged from 6.13 to 7.50. Flavor of cookies prepared from mixed wheat flour (T\textsubscript{o}) was found to be 7.13 at 0 day while, decreased from 6.88 at 15 days to 6.50 after 30 days storage interval. In order to make a comparison among treatments, flavor of cookies find out as 6.88-7.25 (CMS), 6.13-6.88 (ascorbic acid), 7.13-7.50 (protease), 6.63-7.25 (KIO\textsubscript{3}) and 7.00-7.44 (SMS). Of all treatments, cookies made with the addition of protease (600 mg) had pleasant flavor while cookies treated with ascorbic acid at 5 ppm had lowest score for flavor.

Flavor of freshly baked cookies from the replacement of chemically modified starch ranged between 6.88 and 7.25. At 0 day, highest flavor of cookies was evaluated for T\textsubscript{6}C\textsubscript{1} (7.25) while, lowest in T\textsubscript{1}C\textsubscript{3} and T\textsubscript{6}C\textsubscript{3} (6.88). It is obvious that highest flavor of cookies was observed at 5% replacement of wheat flour with CMS. A significant reduction in flavor of cookies was observed as a result of replacement of wheat flour with CMS. Storage trend exhibited flavor scores of cookies were dropped from 15 days (6.13-7.00) to 30 days (5.50-6.75).

Mean values showing variations in flavor of fresh cookies in response to ascorbic acid varied from 6.13 to 6.88. At 0 day, highest flavor of cookies was obtained in T\textsubscript{2}C\textsubscript{3} (6.88) and lowest in T\textsubscript{2}C\textsubscript{1} (6.13). Further comparing various treatments, addition of ascorbic acid increased flavor of cookies but, values were lower than control.
Figure 3: Flavor of cookies prepared with different treatments (a) at 0 day, (b) at 15 days, (c) at 30 days.
These were least accepted as compared to control. Results regarding storage indicated reduction in flavor score as average determined at 15 days was 6.00-6.63 which decreased to 5.88-6.13 at 30 days.

The relative impact of protease on flavor scores of fresh cookies was found as 7.13-7.50. At 0 day, highest flavor of cookies was obtained in T₃C₃ and T₈C₃ (7.50) while, lowest in T₃C₁ and T₈C₁ (7.13). In this work, addition of protease caused a gradual increase in flavor of cookies. To get the effect of storage regarding flavor, mean values were found 6.75-7.38 during 15 days and 6.44-7.13 after 30 days.

Use of KIO₃ in dough formulation affected flavor of fresh cookies positively from 6.63 to 7.25. At 0 day, highest flavor of cookies was obtained in T₄C₃ (7.25) and lowest in T₉C₁ (6.63).

Storage revealed a significant influence on flavor scores such as during two week storage, sensory scores were reduced to 6.19-6.75 and further decreased to 6.13-6.75 after one month storage.

Individual assessment of organoleptic properties have shown flavor of freshly baked cookies by adding sodium metabisulfite (reducing agent) varied from 7.00 to 7.44. At 0 day, highest flavor of cookies was obtained in T₁₀C₃ (7.44) while, lowest was found in T₁₀C₁ (7.00). Present study report the outcome that addition of SMS inclined to increase flavor of cookies.

Comparison of flavor scores of cookies stored for 15 days and 30 days were evaluated as 6.75-7.25 and 6.63-7.25, respectively. Results showed that flavor scores decreased slightly as a function of storage as compared to other treatments and was higher at start of storage. Storage study of cookies revealed a gradual decrease in flavor might due to moisture absorption which caused fat oxidation (Sharif et al., 2003; Saeed et al., 2012).

4.7.5.4. Taste

Statistical data (Table 29) regarding taste of cookies portrayed highly significant variations for all combinations. From statistical results, a highly significant impact of storage on taste of cookies can be observed. Moreover, treatments shown highly significant influence on taste while, concentration did not show any effect on taste however, the interaction of concentration caused highly significant effect on taste of cookies. Furthermore, the effect of control vs. treatments and interaction of days and combination was found non-significant.

From Figure 4, mean values for taste of cookies from different treatments can be observed varying from 6.13 to 7.63. Taste of cookies prepared from mixed wheat flour (Tₒ) was found
to be 7.06 at 0 day while, decreased from 6.75 at 15 days to 6.31 after 30 days storage interval.

In order of the importance of treatments, highest score for taste was reckoned in cookies made
with SMS (7.13-7.63) followed by protease (7.00-7.50), ascorbic acid (6.13-7.50), KIO₃ (6.75-
7.38) and CMS (6.69-7.38). Altogether, cookies treated with SMS (3000ppm) possessed
highest taste score whereas cookies blended with CMS (15%) had lowest taste score.

Assessment of sensory attributes regarding taste of fresh cookies made by substituting wheat
flour with chemically modified starch varied from 6.69 to 7.38. Data showed that maximum
taste score was gained by freshly baked cookies (0 day) and that was progressively declined
with storage interval. Judges appraised T₆C₂ (7.38) maximum point scores while, T₆C₃ (6.69)
was given least points. In present study, taste score of cookies was decreased by increasing the
replacement of wheat flour with CMS. Regarding storage, range of taste of cookies was 6.25-
7.00 and 5.88-6.75 during 15 days and 30 days, respectively.

In Figure 4, mean comparison for taste of freshly baked cookies prepared from the treatment
of ascorbic acid varied greatly from 6.13 to 7.50. At 0 day, highest score (7.50) was found in
T₇C₁ while, T₇C₃ had lowest score (6.13). Results showed that all cookies attained varying
scores for taste and a declining trend was noticed with the addition of ascorbic acid. In order
to assess the storage effect, means for taste of cookies stored between 15 days and 30 days
were determined independently and observed as 5.63-6.75 and 5.38-6.50, respectively. A
similar decreasing trend for this trait was also noticed with increasing the storage interval.

Mean values describing the relative impact of different levels of protease on taste of freshly
prepared cookies were observed from 7.00 to 7.50. At 0 day, highest taste of cookies was
obtained in T₃C₃ (7.50) while, minimum was found in T₃C₁ and T₈C₁ (7.00). As for as
treatments are concerned, addition of protease to cookie dough resulted in an increment for
taste of cookies. Comparison of taste scores of cookies stored for 15 days and 30 days showed
a decreasing trend from 6.88-7.13 and 6.50-7.00, respectively.

Fresh cookies prepared from the treatment with KIO₃ were evaluated for sensory attributes and
their taste scores varied widely from 6.75 to 7.38. Highest taste of fresh cookies was obtained
in T₉C₃ (7.38) while, minimum value (6.75) was recorded T₄C₁ and T₄C₃ at 0 day as shown in
Figure 4. During 15 days storage, mean values for taste of cookies ranged 6.25-7.31 while, after 30 days storage, taste scores of cookies were reduced to 6.25-7.25.
Figure 4: Taste of cookies prepared with different treatments (a) at 0 day, (b) at 15 days, (c) at 30 days
Quality scores regarding taste of cookies in response to sodium metabisulfite were evaluated in the range of 7.13-7.63. At 0 day, highest score of taste was obtained in $T_{10}C_3$ (7.63) while, lowest in $T_{10}C_1$ (7.13). Regarding this work, in various treatments, taste scores of cookies were increased with the addition of SMS to dough formulation. Difference between cookies stored for two weeks (7.06-7.63) and one month (6.50-7.50) was just noticeable. It is known that, the taste scores of cookies decrease, as the storage time increase. This study accede with outcome of Elahi (1997) who found a decrease in taste of biscuits prepared from composite flour from 6.62 to 5.81 after 90 days storage. Outcome of this work also endorse the findings of Sharif et al. (2003) who found taste of cookies ranged from 6.62 to 7.36 and progressively decreased with storage.

4.7.5.5. Texture

Mean squares related to texture of cookies presented in Table 29 point out highly significant variance for all experimental measures. Data apprised highly significant effect of both treatments and storage on texture of cookies. Statistically splitting further indicated that concentration did not influence texture while, highly significant variations in texture were observed due to interaction of concentration and treatments. Also, the effect of control vs. treatments and interaction of days and combination was almost insignificant on cookies texture.

Figure 5 represents mean values for texture of cookies prepared from different treatments vary from 5.88 to 8.00. Texture of cookies prepared from mixed wheat flour ($T_o$) was found to be 6.50 at 0 day while, decreased from 6.38 at 15 days to 6.25 after 30 days storage interval. For the relative effect of treatments, texture of cookies was found in order of preference as 6.88-8.00 (protease), 7.13-7.75 (SMS), 6.19-7.50 (CMS), 6.00-7.25 ($\text{KIO}_3$) and 5.88-7.00 (ascorbic acid). Taken together, cookies made from dough containing protease (400 mg) exhibited highest texture score while, cookies treated with ascorbic acid (15 ppm) had lowest.

Sensory scores of fresh cookies made with wheat flour substituted with chemically modified starch showed texture scores of cookies ranged between 6.19 and 7.50. At 0 day storage, cookies prepared from flour substituted with CMS exhibited maximum texture 6.19-7.50. Highest texture of cookies was obtained in $T_6C_2$ (7.31) and lowest in $T_1C_3$ (6.19). It is obvious that enhancement in level of CMS decreased texture score of cookies. Highest texture score of cookies observed in $T_6C_2$ indicated that best level for substituting the wheat flour with CMS in
Figure 5: Texture of cookies prepared with different treatments (a) at 0 day, (b) at 15 days, (c) at 30 days
order to improve the cookie making quality was 10% and above (90:10) CMS in formulation, cookies revealed a hard and unacceptable texture. To assess the impact of storage, cookies had texture 6.13-6.75 (at 15 days) which decreased up to 5.75-6.63 after 30 days of storage.

Figure 5 is the representative of mean comparison for texture of fresh cookies after the treatment of ascorbic acid varying from 5.88 to 7.00. At 0 day, highest value of texture of cookies was obtained in T_2C_1 (7.00) and lowest in T_7C_3 (5.88). A significant decrease in texture of cookies was observed with addition of ascorbic acid. The absolute descent trend for texture of cookies was observed from 5.50-6.88 (at 15 days) to 5.38-6.63 after 30 days of storage duration.

Protease addition to cookie formulation caused an increase in texture of cookies at 0 day from 6.88 to 8.00. For fresh cookies, highest texture of cookies was obtained in T_8C_2 (8.00) and lowest T_3C_1 (6.88). As discussed earlier, this attribute increased progressively with the addition of protease to cookie dough. However, texture score ranged as 6.63-7.25 was noted at 15 days which reduced up to 6.50-7.25 after 30 days of storage. A decreasing trend was observed during storage however, cookies treated with protease were acceptable at end of storage.

Quality assessment regarding texture of freshly baked cookies containing KIO₃ ranged from 6.00 to 7.25. Highest texture of cookies was obtained in T_9C_1 (7.25) and lowest in T_9C_3 (6.00) at 0 day. A descent trend is reported regarding for texture of cookies, as the concentration of KIO₃ increased. Regarding storage, range of quality score for texture of cookies between 15 days and 30 days was 6.00-6.75 and 6.00-6.69, respectively. Storage days showed most expressed effect on texture scores and a declining trend was observed during storage period.

Organooleptic properties of fresh cookies prepared from dough formulation containing sodium metabisulfite showed texture in the range of 7.13-7.75. At 0 day, highest texture of cookies was obtained in T_{10}C_3 (7.75) and lowest in T_{10}C_1 (7.13). An ascent trend is reported regarding texture of cookies, as the concentration of SMS increased. Regarding storage, texture scores of cookies were recorded as 6.75-7.25 (at 15 days) and 6.50-7.25 (at 30 days) storage period. Present data report a significant declining trend in cookie texture due to storage. Sharif et al. (2003) evaluated sensory attributes of wheat flour cookies and found the texture of cookies ranged from 6.26 to 7.08.
4.7.5.6. Overall acceptability

Statistical analysis pertaining to overall acceptability of cookies is presented in Table 29. It is evident from statistics that all experimental measures had significantly more variability for overall acceptability of cookies. Statistical results revealed that overall acceptability of cookies was highly significantly affected by treatments and storage. While, concentration did not influence overall acceptability of cookies alone however, the interaction of concentration and treatments caused highly significant variations in overall acceptability. In present research, the effect of control vs. treatments and interaction of days and combination was insignificant.

Figure 6 represents mean comparison for overall acceptability scores of cookies produced from different treatments ranged from 5.75 to 8.00. Overall acceptability scores of cookies made from mixed wheat flour (T₀) was found to be 7.00 at 0 day while, decreased from 6.50 at 15 days to 6.31 after 30 days storage interval. To have useful interpretation of results, overall acceptability of cookies in different treatments are summarized as CMS (6.75-7.50), ascorbic acid (5.75-7.39), protease (7.13-8.00), KIO₃ (6.13-7.13) and SMS (7.13-7.7). Above all, cookies treated with protease exhibited highest overall acceptability at 400 mg however, cookies treated with ascorbic acid (15 ppm) had lowest.

Quality assessment in terms of overall acceptability scores of fresh cookies made with the replacement of wheat flour with chemically modified starch varied from 6.75 to 7.50. At 0 day, highest overall acceptability of cookies was obtained in T₆C₁ (7.50) and lowest in T₆C₃ (6.75). In various treatments, cookies replacement of wheat flour with CMS (90:10) were highly acceptable. This implies that wheat flour may possibly be substituted with up to 10% for cookie preparation without affecting overall quality. Storing cookies made with CMS for 15 days and 30 days had a significant influence on overall acceptability and ranged as 6.13-7.25 and 5.88-7.00, respectively. Overall acceptability of cookies was progressively declined with passage of time.

Individual assessment of overall acceptability of freshly baked cookies made from formulation containing ascorbic acid varied between 5.75 and 7.39. At 0 day, T₇C₁ exhibited maximum (7.39) while T₇C₃ exhibited minimum score (5.75) for overall acceptability of cookies. Sensory evaluation show that overall acceptability score of cookies tended to be decreased progressively with addition of ascorbic acid. Overall acceptability score of cookies stored for a period of one month was evaluated in the range of 5.63-6.75 (15 days) and 5.25-6.63
days) storage. This data presented a significant decreasing trend in overall acceptability scores of cookies as a result of storage.

Figure 6 represents mean comparison for overall acceptability of freshly prepared cookies varied from 7.13 to 8.00. Highest overall acceptability score (8.00) was observed in T8C2 at start of storage which reduced up to 6.69 at the end of storage while, minimum was found as 7.13 at 0 day in T3C1 which reduced to 7.00 after 30 days of storage. During two week storage, overall acceptability score significantly reduced from 6.88-7.38 to 6.25-7.00 after one month storage. Storage had effect on overall acceptability and a declining trend was observed during storage period.

Overall acceptability of fresh cookies prepared from the treatment with KIO3 ranged between 6.13 and 7.13. At 0 day, highest overall acceptability of cookies was obtained in T4C1 (7.13) and lowest in T9C3 (6.13). Overall acceptability of cookies treated with KIO3 decrease, as the concentration of KIO3 increased. Difference between overall acceptability of cookies stored for two weeks (6.00-6.88) and one month (5.75-6.75) was noticeable.

Individual assessment regarding overall acceptability of freshly baked cookies made from formulation containing sodium metabisulfite varied from 7.13 to 7.75. At 0 day, highest overall acceptability of cookies was obtained at 0 day in T10C3 (7.75) and lowest T3C1 (7.13). Overall acceptability of cookies tended to be increased progressively with an increase in SMS. During 15 days storage, overall acceptability score ranged 7.06-7.38 and 6.75-7.37 after one month storage. In the same manner, overall acceptability declined as a function of storage. In work of Pasha et al. (2002) and in agreement with Elahi (2006), a gradual reduction in overall acceptability of cookies was observed during storage period. Millard reaction and fat oxidation might be main cause for this type of deterioration (Butt et al., 2007). This may likely due to moisture absorption, increment in peroxide value and free fatty acid. Overall acceptability of different treatments of cookies ranged from 6.33 to 7.44 (Sharif et al., 2003).
Figure 6: Overall acceptability of cookies prepared with different treatments (a) at 0 day, (b) at 15 days, (c) at 30 days
4.8. Correlation studies

All the parameters in present study were evaluated for their interdependence using Pearson’s correlation coefficients. Correlation matrix (Table 30) revealed a significant and positive relationship of test weight with peak time (r= 0.903*), setback viscosity (r= 0.958*), taste (r= 0.957*) and texture (r= 0.979*). Similarly, data further divulged a highly significant and positive correlation of test weight with fat content (r= 0.987**) and LASRC (r= 0.969**). It is obvious that thousand kernel weight and degree of softening were positively linked to each other (r= 0.907*).

Ash content portrayed a significant and positive association with fiber (r= 0.954*), peak viscosity (r= 0.891*), trough viscosity (r= 0.896*), final viscosity (r= 0.888*) and thickness of cookies (r= 0.951*). Likewise, a highly significant and positive correlation of ash content was also determined with SOCSRC (r= 0.968**) and hardness of cookies (r= 0.962**). A highly significant and negative correlation (r= -0.977**) between ash content and spread factor of cookies confirmed the importance of ash being indicative of cookie baking quality.

From Table 30, it is evident that fat content was found to be significantly associated with setback viscosity (r= 0.937*), SUCSRC (r= 0.890*) and cookies texture (r= 0.942*). Likewise, a highly significant and positive correlation of fat content was found with LASRC (r= 0.985**). A similar correlation of fat content was observed with taste of cookies (r= 0.987**).

Significant and positive associations of fiber content established with respect to protein (r= 0.935*), damaged starch (r= 0.917*), WSRC (r= 0.929*), SOCSRC (r= 0.951*) and cookies thickness (r= 0.913*) are of statistical significance. However, present study adduce a significant and negative relationship of fiber with nitrogen free extract (r= -0.953*). In parallel, a highly significant and negative relationship was appraised between fiber content and spread factor of cookies (r= -0.979**).

From correlation matrix, it is established that fiber and hardness of cookies were intimately correlated to each other (r= 0.994**). These correlation point out that fiber content of wheat flour significantly contributed to water absorption of wheat flour and may also possibly affect baking quality of cookies. In Table 30, a significant and positive correlation can be ascertained between protein content and degree of softening (r= 0.934*) as well as SUCSRC value (r= 0.919*). Figure 7 illustrates that protein was well correlated to hardness of cookies (r= 0.943*). In this work, the best correlation was find out between protein content and damaged starch (r
Table 30: Correlation matrix for different parameters

| TW | TKW | Ash | Fat | Fiber | Prot | NFE | Starch | DS | WA | DDT | DoS | FQN | PT | PV | TV | FV | SV | WS | SOC | LA | SF | H | Color |
|----|-----|-----|-----|-------|------|-----|--------|----|----|-----|-----|-----|----|----|----|----|----|----|----|----|----|    |     |
| Fat |     |     |     |       |      |     |         |    |    |       |     |     |    |    |    |    |    |    |    |    |    | 0.997*** | 0.934* |
| Prot |     |     |     | 0.915* |      |     |         |    |    |       | 0.906** | 0.909** | 0.907** | 0.905* |
| NFE |     |     |     |      | 0.915* |      |         |    |    | 0.906** | 0.909** | 0.907** | 0.905* | 0.925* |
| DS |     |     |     |      |     | 0.917* |         |    |    |       | 0.906** | 0.909** | 0.907** | 0.905* |
| DSt |     |     |     |      |     |     |         |    |    | 0.907** | 0.904* | 0.908* | 0.905* | 0.925* |
| DoS |     |     |     |      |     |     |         |    |    |       | 0.907** | 0.904* | 0.908* | 0.905* |
| FQN |     |     |     |      |     |     |         |    |    |       | 0.907** | 0.904* | 0.908* | 0.905* |
| PT |     |     |     |      |     |     |         |    |    | 0.903* | 0.891* | 0.894* | 0.880* | 0.887* |
| PV |     |     |     |      |     |     |         |    |    |       | 0.891* | 0.894* | 0.880* | 0.887* |
| TV |     |     |     |      |     |     |         |    |    |       | 0.894* | 0.897* | 0.894* | 0.897* |
| FV |     |     |     |      |     |     |         |    |    | 0.894* | 0.897* | 0.894* | 0.897* | 0.925* |
| WV |     |     |     |      |     |     |         |    |    |       | 0.903* | 0.906* | 0.909** | 0.910* |
| SOC |     |     |     |      |     |     |         |    |    |       | 0.906** | 0.909** | 0.910* | 0.910* |
| SUC |     |     |     |      |     |     |         |    |    |       | 0.909** | 0.910* | 0.910* | 0.910* |
| LA |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| W |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| T |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| SF |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| H |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| Taste |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| Texture |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |
| OA |     |     |     |      |     |     |         |    |    |       | 0.910* | 0.910* | 0.910* | 0.910* |

TW: Test weight; TKW: Thousand kernel weight; Prot: Protein; NFE: Nitrogen free extract; DS: Damaged starch; WA: Water absorption; DDT: Dough development; DSt: Dough stability; DoS: Degree of softening; FQN: Farinograph quality number; PT: Peak time; PV: Peak viscosity; TV: Trough viscosity; FV: Final viscosity; BV: Breakdown viscosity; SV: Setback viscosity; WS: Water solvent retention capacity; SOC: Sodium carbonate solvent retention capacity; SUC: Sucrose solvent retention capacity; LA: Lactic solvent retention capacity; W: Width; T: Thickness; SF: Spread factor; H: Hardness; Text: Texture; OA: Overall acceptability

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0.970**). While, protein showed a highly significant (r= -0.968**) negative correlation with NFE content. As anticipated, a significant (r= -0.892*) and negative correlation between protein and spread factor (Figure 8). Present study corroborate that spread factor of cookies is affected by protein content. Present results support outcome of Xiao et al. (2006) who found protein content was strongly related to four SRC tests in hard winter wheat. Similar relationship was reported formerly by Miller and Hoseney (1997) further supports understanding of present results. This is attributed to gluten content, as reported by Chung et al. (2014) that larger cookies spread was reported with an increase in non-wheat protein level. In present work, it seems possible to inter-relate NFE content with spread factor of cookies (r= 0.914*). Further, NFE content was observed to be negatively associated with damaged starch (r= -0.923*), degree of softening (r= -0.908*) and hardness (r= -0.949*).

Table 30 divulge that starch content had a significantly negative association with trough viscosity (r= -0.926*), final viscosity (r= -0.917*), WSRC (r= -0.948*), SOCSRC (r= -0.894*) and LASRC (r= -0.911*). A positive association was evaluated between damaged starch and protein content (r= 0.970**), in agreement with Colombo et al. (2008) and Keskin et al. (2012). Likewise, damaged starch exhibited significant positive association with degree of softening (r= 0.905*), setback viscosity (r= 0.906*) and hardness (r= 0.905*). Figure 9 illustrates a highly significant and positive relationship between damaged starch with SUCSRC (r= 0.985**). Hence, it is put forward that SRC test may predict level of damaged starch in flour approximately. Therefore, an important correlation established in present study between damaged starch and SUCSRC commensurate with several researchers (Barrera et al., 2007; Colombo et al., 2008; Duyvejonck et al., 2011).

From Figure 10, it can be observed that WSRC explored a significant and positive association with SOCSRC (r= 0.952*). In the same manner, a significant and positive correlation was find out between WSRC values and hardness of cookies (r= 0.891*). As shown by scatter plot in Figure 11, a significant and negative correlation was ascertained between WSRC values and spread factor (r= -0.892*). Correlations of WSRC and SOCSRC determined in present work are in harmony with Colombo et al. (2008) as well as of Pasha et al. (2009b) who stated that SOCSRC was affected by damaged starch and WSRC furthermore, damaged starch is the main factor responsible for water absorption in wheat flour. In present investigation, SOCSRC values correlated quite differently with cookie quality.
Figure 7: Correlation between protein and hardness

Figure 8: Correlation between protein and spread factor
Figure 9: Correlation between damaged starch and SUCSRC values

Figure 10: Correlation between SOCSRC and WSRC values
Here, SOCSRC was observed to be significantly and positively related to thickness ($r= 0.947*$) and hardness of cookies ($r= 0.934*$).

Scatter plot in Figure 12 shows a significant and negative ($r= -0.952*$) relationship of SOCSRC with spread factor of cookies, which is consistent with Ram et al. (2005). It has been reported that spread factor is associated with SOCSRC values, which consequently increase with damaged starch (Zhang et al., 2007). Therefore, present study accede with outcome of earlier researchers (Barrera et al., 2007; Colombo et al., 2008), who observed that cookie factor was negatively related to SOCSRC and WSRC values. Results established the negative effect of hydrophilic constituents on cookie baking quality (Roccia et al., 2006). Associations of WSRC and SOCSRC with cookie factor indicate the ability of damaged starch, pentosans to hold aqueous solution thus, affecting flour cookie quality whereas, protein had no significant role (Moiraghi et al., 2013).

Positive correlations determined between LASRC and taste ($r= 0.974**$) as well as texture ($r= 0.897*$) were statistically significant. Likewise, LASRC showed a significant and positive relation to SUCSRC ($r= 0.898*$), in compatible with the findings of Ram et al. (2005) and Pasha et al. (2009b). But, this correlation was antithesis to the finding of Duyvejonck et al. (2011), who did not observe any relationship between LASRC and other SRC values in 19 European commercial flours. Similarly, Colombo et al. (2008) reported that LASRC determined in 18 Argentinean wheat varieties were also independent of SRC values.

Figure 13 is the representative of a surprisingly highly significant and positive correlation of water absorption with SDS sedimentation ($r= 0.912*$). This correlation underlines the importance of SDS sedimentation test to predict water absorption of wheat flour. From correlation matrix, a significant and inverse correlation ($r= -0.883*$) was observed between water absorption and width of cookies. However, contrary to present results Kaur et al. (2013) stated that water absorption was found to be negatively associated with sedimentation volume ($r= -0.437; p \leq 0.05$).

A highly significant and positive association observed between dough development time and dough stability ($r= 0.967**$) implies that wheat varieties with greater dough development time also revealed a higher dough stability. A significant and positive correlation can be determined between degree of softening and SUCSRC ($r= 0.882*$). A negative correlation between farinograph quality number and width of cookies ($r= -0.910*$) was of statistical significance.
From Table 30, peak time can be observed to have positive relations to breakdown viscosity.
Present data show that peak viscosity had significant and positive relationship with setback viscosity (r= 0.879*) and SOCSRC (r= 0.914*). Similarly, it was highly significantly and positively linked to trough viscosity (r= 0.976**) and thickness (r= 0.980**). A highly significant and positive association reported between peak viscosity and breakdown viscosity (r= 0.985**), is endorsed by Kaur et al. (2013) who also found a positive relationship between breakdown and peak viscosities. It is obvious from Table 30 that trough viscosity and final viscosity were positively inter-related (r= 0.974**). Likewise, trough viscosity exhibited a significant and positive relation to WSRC (r= 0.890*), SOCSRC (r= 0.955*) and thickness of cookies (r= 0.953*). Final viscosity revealed a highly significant and positive relation to thickness (r= 0.978**). Likewise, final viscosity showed a significant and positive relation to setback viscosity (r= 0.920*), WSRC (r= 0.887*), SOCSRC (r= 0.933*) and LASRC (r= 0.913*). In this work, setback viscosity was observed to be significantly and positively associated with SUCSRC (r= 0.941*), LASRC (r= 0.946*), thickness (r= 0.898*) and texture (r= 0.918*).

Figure 14 is the representative of a highly significant and negative correlation between spread factor and hardness of cookies (r= -0.990**). Spread factor revealed a highly significant and negative correlation with thickness of cookies (r= -0.888*). In Table 30, it can be observed that hardness and thickness of cookies were positively inter-related (r= 0.894*). In present study, both width and overall acceptability were significantly and positively correlated to color of cookies (r= 0.916* and r= 0.917*, respectively).

This work may support the fact that water absorption can also be reliably predicted by SDS-sedimentation volume. A strong and positive correlation between damaged starch and SUCSRC indicate that SRC test may predict level of damaged starch in flour approximately. Based on these correlations, width of cookies was negatively affected by water absorption of wheat flour. Likewise, spread factor of cookies was influenced by ash, fiber, protein, WSRC and SOCSRC values. Hardness of cookies was positively related to damaged starch, WSRC and SOCSRC values. Present results establish that SRC test particularly, WSRC and SOCSRC values could be used with higher confidence to predict cookie making quality of wheat varieties in addition to other physicochemical characteristics of flour.
Figure 13: Correlation between SDS-sedimentation and water absorption

Figure 14: Correlation between spread factor and hardness
CHAPTER 5

SUMMARY

Wheat being the largest grain crop and staple food of Pakistan has great usefulness in a variety of products. It is the basic ingredient in most of the baked products (cookies) and cookies quality is primarily related to soft wheat. Physicochemical characteristics of flour are important to assess flour quality. Technological problems encountered in cookie making are hard texture and inconsistency in surface appearance of cookies. In Pakistan, mixed wheat is delivered to miller that is main source of varying flour properties like protein and gluten content. These variations in flour characteristics considerably influence product (cookies) quality.

Since, baking industry do not have access to particular wheat varieties which are suitable for cookies so, mixed wheat flour resulted in hard texture cookies; also, addition of high levels of fat and leavening agent is practiced somehow to compensate these variations as compared to standard (regular) cookie formulation for dough softening but, it leads to economical loss. Problem in reaching the desired consistency for cookie quality is the challenge for baking industry which needs more attention. So, a practical approach was necessary to improve quality of mixed wheat flour using different modifying agents.

In order to avoid overlapping of results, present study was divided into different steps to have better insight. In first phase, five wheat varieties (Punjab and Sindh) were characterized for numerous physico-chemical and rheological parameters. On the basis of these results, the best wheat variety was selected and it was further utilized for the preparation of cookies along with application of different treatments i.e. chemically modified starch (CMS), ascorbic acid, protease, potassium iodate (KIO\(_3\)) and sodium metabisulfite (SMS). Moreover, their cookie baking quality was also evaluated to study the effect of treatments and their concentrations, which is the basis of research. In the last part, relative dependence of different physicochemical and rheological parameters with cookie quality was evaluated. The compendium of present results is herein.

In first phase of research, results pertaining to physico-chemical parameters evidence that a wide range of test weight and thousand kernel weight was observed as 67.00-81.00 kg hl\(^{-1}\) and 32.82-48.70g, respectively. According to results, wheat varieties exhibited moisture (8.50-10.78%), ash (0.33-0.83%), fat (1.36-1.49%), fiber (0.38-0.52%), protein (9.85-13.75%) and NFE (72.82 to 79.58%) content. Mineral composition of wheat varieties varied as 2.20-2.64, 20.25-28.45, 2.12-4.71, 1.24-2.31, 0.54-0.85 and 0.45-0.57 mg/100g for Na, Ca, Fe, Zn, Mn.
and Cu content, respectively. Wheat varieties divulge 24.68-36.40% wet gluten, 7.12-11.84% dry gluten, 68.88-72.73% starch and 6.28-10.97% DS content. SDS-sedimentation and AWRC values were reckoned in wheat varieties with varying levels from 18.20 to 30.50ml and from 43.91 to 66.85%, respectively. SRCs evaluated in terms of WSRC, SOCSRC, SUCSRC and LASRC values varied greatly from 50.83 to 69.64%, 56.66 to 82.03%, 75.79 to 106.97% and 79.43 to 121.20%, respectively.

Rheological properties of wheat varieties were determined as water absorption (51.00-56.00%), dough development time (1.20-4.20 min), dough stability (2.90-18.20 min), degree of softening (85.00-174.00 BU) and farinograph quality number (48.00-200.00). Pasting properties of wheat varieties indicated variability from 65.25 to 67.80°C (pasting temperature), 3.22 to 6.00 min (peak time), 1330 to 1784 cP (peak viscosity), 645 to 1002 cP (trough viscosity), 1799 to 2565 cP (final viscosity) 685 to 782 cP (breakdown viscosity) and 1154 to 1563 (setback viscosity).

Cookie baking quality of wheat varieties was also evaluated and compared with MWF tested as control. Physical analyses of cookies prepared from wheat varieties indicated width (25.20-26.60 cm), thickness (5.80-6.40 cm), spread factor (39.38-45.86) and hardness (2.17-3.76 kg). Comparing these values to MWF (control) showed width (26.50 cm), thickness (6.40 cm) and spread factor (41.41) and hardness (3.50 kg). Sensory evaluation of cookies produced from wheat varieties showed that cookies made with AARI-11 were highly acceptable as compared to others. On the basis of different physico-chemical, rheological and sensory parameters, AARI-11 was selected as best wheat varieties for cookie making.

In phase two, AARI-11 as selected wheat variety along with mixed wheat flour were further utilized for the preparation of cookies along with application of different treatments (chemical and enzymes). These treatments were applied to cookie formulation to improve quality of mixed wheat flour. In addition, their cookie baking quality was also evaluated in order to assess the effect of treatments on wheat flour. Therefore, in phase two results pertaining to physical properties of cookies prepared from different treatments indicated width of cookies ranged as 30.8-31.7 cm (CMS), 25.4-26.0 cm (ascorbic acid), 29.7-30.5 cm (protease), 27.8-28.2 cm (KIO₃) and 28.5-29.5 cm (SMS). On the whole, cookies made with the replacement of chemically modified starch (CMS) produced largest width at 15% level of substitution.

Across treatments, thickness of cookies ranged as 5.2-5.4 cm (CMS), 6.0-6.4 cm (ascorbic
acid), 5.2-5.5 cm (protease), 6.0-6.4 cm (KIO₃) and 5.6-5.9 cm (SMS). Taking all the treatments into account, cookies made with CMS and protease gave smallest value of thickness at 15% level of substitution and 600 mg, respectively.

For the relative assessment of treatments, spread factor of cookies varied in response to all the chemical or modifying agents as 57.03-60.96 (CMS), 39.68-43.33 (ascorbic acid), 54.00-58.65 (protease), 43.75-47.00 (KIO₃) and 48.98-52.68 (SMS). Of all treatments, cookies made with replacement of wheat flour with CMS (85:15) produced cookies with more spread as compared to others.

Physical properties of cookies prepared with different treatments indicated width (25.4-31.7 cm), thickness (5.2 to 6.4), spread factor (39.68 to 60.96). Taking all the treatments into account, cookies made with the replacement of wheat flour with CMS (85:15) produced largest width, smallest thickness more spread. Cookies made with the addition of protease gave lowest value of thickness at 600 mg.

Color of cookies produced from different treatments determined in terms of L*, a* and b* values of cookies varying over a wide range observed as 64.65-74.84, 1.22-3.75 and 31.10-35.44, respectively. Of all, L* (lightness) value of cookies treated with SMS was highest at level of 1000ppm. On the whole, cookies treated with KIO₃ possessed highest b* value at level of 1500ppm whereas, ascorbic acid gave lowest at 5ppm level. Likewise, substituting wheat flour with CMS (85:15) produced cookies with highest a* value whereas, cookies treated with SMS resulted in lowest a* value at 1000 ppm.

Chemical properties of cookies showed moisture (1.50-3.68%), ash (0.333-0.547%), fat (17.70-23.66%), fiber (0.083-0.167%), protein (5.41-6.56%) and NFE (66.76-72.40%) content. Hardness of cookies prepared with different treatments ranged from 0.86 to 3.55 kg at 0 day. For the purpose of treatments comparison, hardness of cookies varied in response to all the modifying agents as CMS (2.29-2.71 kg), ascorbic acid (2.72-3.55 kg), protease (0.86-1.51 kg), KIO₃ (2.04-2.27 kg) and SMS (1.62-2.01 kg). Amongst, cookies made with ascorbic acid were harder at 15ppm while, cookies made from dough containing 600 mg protease were soft as evident from their minimum breaking strength.

Sensory evaluation of freshly baked cookies and cookies stored for 30 days was achieved separately. Though, present results exhibited variations for all the cookies in response to different dough modifying agents. Storage studies revealed a significant impact on sensory
attributes for all treatments. To get an overview of results regarding treatments, overall acceptability of cookies in different treatments ranged as CMS (6.75-7.50), ascorbic acid (5.75-7.39), protease (7.13-8.00), KIO₃ (6.13-7.13) and SMS (7.13-7.7). On the whole, cookies treated with protease were highly acceptable at 400 mg as compared to other treatments. The correlations of all the parameters were evaluated and significant relationships were observed between protein and damaged starch, spread factor and hardness of cookies. Moreover, spread factor of cookies was affected by WSRC and SOCSRC values. A significant and positive association of damaged starch with SUCSRC values highlight the importance of SRC test to predict the level of damaged starch. Present study establish that water absorption can also be reliably projected by SDS-sed. volume.
CONCLUSION

In present study, wheat varieties point out significant variations in most of physico-chemical, rheological and sensory parameters. This work come to some general conclusions such as wheat variety; AARI-11 was remarkably well suited for cookie making on the basis of physico-chemical, rheological and sensory characteristics. Nonetheless, wheat varieties from Sindh were found unsuitable (being strong) for cookies and may have great potential for bread making. Present study report that physicochemical characteristics may help in the selection of specific wheat varieties and flour according to intended use.

Across treatments, significant variations were found for all combinations and treatments. During storage, significant decline was observed for all quality parameters in response to treatments. Across treatments, substituting wheat flour with CMS (85:15) produced cookies with largest spread, protease (400 mg) had highest overall acceptability and lower hardness (600 mg). However, substitution of flour with chemically modified starch at level above 10% level had adverse impact on cookies characteristics. Therefore, in order to compare treatments, addition of protease and SMS in dough formulation was effective to maximize cookie making potential in terms of spread, hardness and sensory properties.

Based on correlations evaluated in present study, it can be inferred that flour components had a strong impact on cookie quality as evident from the significant relationships of spread factor with ash, fiber and protein content. Likewise, spread factor and hardness values of cookies were influenced by WSRC and SOCSRC values. In present work, some important correlations were observed between water absorption and SDS-sed. volume as well as of damaged starch and SUCSRC values. This implies that SDS-sed. and SUCSRC test can predict water absorption and damaged starch of wheat flour, respectively.

It seems almost impossible to acquire a specific wheat quality and thus, problematic for baking industry regarding cookie quality. This piece of work is thus an effort to curtail the requirement of a particular wheat variety for cookies production. It is worth noting that the application of different modifying agents to cookie formulations positively affected the cookie making quality of mixed wheat flour. It can also be deduced that different processing aids can be used to successively deal with the problems resulting from variations in wheat flour without compromising cookie quality.
RECOMMENDATIONS

On the basis of present study, following recommendations have been devised for researchers:

- Quality assessment of wheat flour is diligent, laborious and time consuming task hence, a combination of rapid and small scale tests as SRC and SDS-sedimentation can also be used to reliably anticipate water absorption and DS in wheat flour.

- Execution of these rapid tests in quality control laboratories of milling and baking industry must be pragmatically ensured.

- It is recommended that vigilant use of these physicochemical parameters of wheat could become a wise choice in the selection of suitable variety or flour for intended use.

- Enzymes carrying GRAS status can become a legitimate approach for texture or quality improvement of baked products over other chemical additives.

- There is need to study further the effect of protease enzyme on various proteins present in wheat flour.

- Further research should be conducted to stabilize color, flavor and freshness of cookies and these could be stored for a long period of time without quality deterioration.

- It is of commercial interest particularly for cookie manufacturing where it is imperative to transform or alter the quality of protein usually by physical and chemical methods.

- Therefore, extending the requirement to explore many biochemical agents (enzymes and chemical additives) which can be employed to improve the product quality.

- Present study recommend that government organization must ensure the implementation of a system to supply best or suitable varieties to the millers and bakers, which would consequently reduce the need to depend on chemical additives to improve functionality of bakery products.
Limitations of Present Study

- In current scenario, major stakeholders like Federal and Provincial departments may be on board to play their part and ensure the provision of individual wheat varieties to the millers and subsequently, baking/biscuit industry.

- In present study, no attention was paid to the application of physical treatment such as heat treatment of wheat grains and optimization or setting of milling conditions to modify the levels of protein and damage starch content.
Future Research Directions

- Future studies will be directed to the use of physical methods including microwave and irradiation on the properties of wheat flour particularly, denaturation of gluten protein and their impact on cookies.
- Also, more emphasis of research work will be based on the preparation of cookies from the sprouted and heat-treated wheat flour.
- Damage starch produced during milling and has a negative impact on the cookie quality. Hence, further studies are required to practically reduce the damaged starch content during flour milling.
- Inevitably, the gluten protein of wheat is detrimental to cookie quality and celiac disease patients. In future, the application of protease enzymes will be exploited to produce gluten-free cookies in addition to high spread factor to achieve technical, nutritional and safety benefits.
- Exploration will be based on improving the baking quality of cookies by the use of various dough modifying agents.
- Although, further researches based on the modification of wheat flour may be challenging but, beneficial from industrial point of view with special reference to cookies. More research should be focused on the search and application of new treatments associated with cookie quality.
- Since, present study was industry-oriented to seek the solution of varying wheat quality so, nutritional aspects of cookies were not considered much. Future studies could be anticipated to depend on the use of various enzymes to improve the nutritional value and shelf stability of cookies.


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