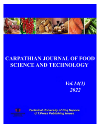

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# QUALITY CHARACTERISTICS OF MUFFINS PREPARED FROM REPLACEMENT OF WHEAT WITH BARLEY: NUTRITIONAL, ANTI-OXIDATIVE AND MICROBIAL POTENTIAL

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**ABSTRACT**

The objective of present study was to investigate the sensorial, nutritional and microbial value and acceptance of muffins prepared from barley flour as partial replacement of wheat flour. The barley flour was incorporated in the proportion of 100:0, 95:5, 90:10, 85:15, 80:20 and 75:25% of wheat flour for the formulation of muffins and found that muffins containing barley flour were nutritionally superior. The results revealed that with increase in incorporation of barley flour, a positive increase in protein, total phenolic compounds and scavenging activity toward $ABTS^+$ and DPPH of muffins was observed. However, baking led to a reduction in phenolic and antioxidant properties. The muffins were also found microbiologically safe for human consumption. This study suggested that partial replacement of wheat flour with barley flours rich in nutritional and bioactive compounds, diversify the utilization of barley flour in various bakery products.

## 1. Introduction

Barley (*Hordeum vulgare* L.) is an ancient and one of the most widely cultivated cereal grain possess functional components (β-glucan, B-complex vitamins, tocopherols and phenolic compounds) which provides associated with human health (Punia & Sandhu, 2015). Being rich in dietry fibres and bioactive compounds barley is still an underutilized cereal and used only in brewing industry and as animal feed. Wheat is a dominant portion of a standard diet and basic ingredients of bakery products. In bakery industries, before product formulation, wheat is processed into refined wheat flour. The refined wheat flour have poor quality protein deficient in essential amino acids. Therefore it is recommended that refined wheat flour may be fortified with essential nutrients to meet the need of humans. As reported by Baba et al. (2016) barley possess nutty flavour, better chewiness, good consistency and have potential to reduce blood glucose level and cholesterol.

Therefore, this may be an interesting opportunity to incorporate barley into bakery products to improve their nutritional behaviour.

Barley flour incorporated with wheat flour has been previously incorporated in the production of rusks (Punia et al., 2020); cakes (Yaqoob et al., 2018), chocolate chips cookies (Frost et al., 2011) and bread (Holtekjolen et al., 2008) have been studied previously. The previously reported studies have shown that addition of barley flour into nutritionally poor cereal is a successful attempt of improving their nutritional and antioxidant behaviour. There is a growing interest in developing novel bakery products supplemented with natural antioxidants.

Among breakfast products, muffin ranks 3rd and attract a broad range of consumers (Rosales-Soto et al., 2012) and consumed worldwide at all economic levels due to different varieties, ready-to-eat nature and reasonable cost. The supply of barley functional compounds through muffins may be an effective way to supply the bioactive compounds. To the best of our knowledge, studies regarding the incorporation of barley in muffins appear to be limited. This prompted us to investigate the effect of incorporation of barley at level of 5,10, 15, 20, and 25 % on the nutritional, antioxidant, microbial and sensorial properties of muffins.

## 2. Materials and methods

### 2.1. Materials

Wheat and barley cultivar were procured from local market of Sirsa, Haryana for preparing muffins. Grains were milled into fine powder using grinder for further evaluation. Wheat flour (WF) was incorporated with barley flour (BF) and the blends were reported as WF (100% wheat flour), BF-5%+WF-95%, BF-10%+WF-90%, BF-15%+WF-85%, BF 20%+WF-80%, and BF-25%+WF-75%, respectively.

### 2.2. Proximate Composition

WF and WF-BF blends were tested for their moisture, ash, fat, and protein contents by employing the standards methods of analysis (AOAC, 1990). The carbohydrate content was calculated by difference.

### 2.3. Muffin formulation and preparation

The muffins were prepared following the method as described by Nicol (1995). Wheat flour (WF) /Wheat flour-barley flour (WF-BF) blends (100g), butter (50g), sugar (50g), eggs (50g), milk (50g), baking powder (3.3g) and salt (0.4) were used as ingredients for muffins. Firstly, sugar, eggs, milk and butter were blended using a mixer and then baking powder, wheat flour and salt were added and mixed properly to produce uniform batter. Further, batter poured into muffin mould and baked at 200 °C for 25 min.

### 2.4. Pasting properties of blends

Pasting properties of flours were studied using Modular Compact Rheometer (MCR 52, Austria). Parameters recorded were pasting temperature, peak viscosity, trough viscosity, final viscosity, breakdown viscosity and setback viscosity. All the measurements were replicated thrice.

### 2.5. Total phenolic content (TPC) of wheat flour- barley flour (WF-BF) blends and muffins

TPC of flours from different wheat cultivars were determined by following the Folin-Ciocalteau method as described by Gao et al. (2002). Gallic acid was used as the standard, and results are expressed as µg gallic acid equivalents (GAE)/g of flour.

### 2.6. Total flavonoids content (TFC) of wheat flour- barley flour (WF-BF) blends and muffins

TFC was determined by following the method described by Jia et al. (1998). Catechin was used as standard and the results were reported as µg catechin equivalents (CE)/g of sample.

### 2.7. Antioxidant activity (AOA) of wheat flour- barley flour (WF-BF) blends and muffins

AOA was measured using a modified version of the method described by Brand-Williams et al. (1995). Methanol was used as a blank, and antioxidant activity (AOA) was calculated as percent discoloration.

% AOA= (1- (A of sample$_{t=0}$ / A of control$_{t=30}$) x100

### 2.8. Metal chelating activity (MCA) of wheat flour- barley flour (WF-BF) blends and muffins

MCA of wheat extract was measured by following the method described by Dinis et al. (1994). The chelating activity of the extract for $Fe^{2+}$was calculated as follows:

Iron ($Fe^{2+}$) chelating activity (%) = {1-( Absorbance of sample /Absorbance of control)}x 100.

### 2.9. ABTS$^+$ scavenging capacity of wheat flour- barley flour (WF-BF) blends and muffins

ABTS$^+$ scavenging activity was measured by following the method described by Re et al. (1999). A standard curve was prepared by using different concentrations of vitamin C similar to DPPH assay. ABTS$^+$ scavenging property was expressed as vitamin C in µmol/g of wheat.

### 2.10. Textural parameters of wheat flour-barley flour (WF-BF) muffins

For textural properties, TA-XT$_2$ texture analyzer (stable micro systems, haslemeres, England) were used. From the force time curves of the texture profile analysis (TPA), hardness, firmness, chewiness, cohisiveness and springiness were calculated.

### 2.11. Sensory evaluation of wheat flour-barley flour (WF-BF) muffins

The muffins prepared were evaluated by a semi-trained sensory panel member who assessed the muffins for various sensory attributes such as color, flavor, texture, and overall acceptability, using a 9-point hedonic rating scale ranging from like extremely (9) to dislike extremely (1). Sensory evaluation was done by panel of 30 judges in the age group of 20–35 years, comprising postgraduate students, research scholars, and faculty members of the department.

### 2.12. Microbiological analysis of wheat flour-barley flour (WF-BF) muffins

Muffins were studied for total plate count (TPC) and mould count (MC) as the method described by de-Almeida Marques et al. (2016). A 10-fold dilution was prepared by homogenizing the sample in sterile saline and serially diluted in the same diluents. 1 ml of these dilutions was poured on agar plates for TPC and MC respectively. Plates were then incubated at 37 °C for 24 h and 24 °C for 48 h for determining TPC and MC respectively, prior to counting. The results were expressed as log of colony forming units per gram (log cfu/g) of sample.

### 2.13 Statistical analysis

The data reported in all the tables are expressed as mean ± standard deviation of three independent replications. Analysis of variance (ANOVA) was used to determine significant variations among the samples. When an effect was found to have significant effect by the ANOVA, Turkey's Multiple Comparison Test was used to determine which levels of the effect were significantly different at $P < 0.05$.

## 3. Results and discussions

### 3.1 Proximate composition and Physical and functional properties of WF-BF flour blends

The proximate composition of blends prepared from wheat flour (WF) and barley flour (BF) blends are presented in Table 1a. Incorporation with BF had a significant effect on proximate composition of flour blends. A significant ($p<0.05$) increase was observed in moisture content of flour blends was observed. Barley has been reported to have higher moisture content than wheat that has been associated to greater fiber content of barley (Haruna et al., 2011). For the WF, ash, protein and fat content of 1.83, 12.23 and 2.46 % was observed. A progressive increase in ash, protein and fat, contents of muffin with the increase in WF-BF blends was observed. Blending with BF significantly, ($P<0.05$) increased the protein content of WF-BF blends from 12.56 to 14.23 %. The increase in these nutritional compounds may be attributed to the presence of greater of these compounds in BF than WF. As reported by Yaqoob et al. (2018), an increase in moisture, ash, protein and fat content was observed for cake formulated from wheat-barley blends. Replacement of WF with BF significantly decreased the carbohydrate content to 72.55 to 68.22 %.

Bulk density of WF-WB flour blends decreased with the increase in level of BF from 5 to 25% (Table 1b). The wheat flour showed the highest value of bulk density. Functional properties of WF-BF flour blends increased with the increase in level of BF from 5 to 25%. Incorporation of BF to WF significantly ($p< 0.05$) increased both WAC and OAC (Table 1b) and was found the highest for WOF 25%. This increase in WAC and OAC may be attributed to the increase in the β-glucan level (Bhatty, 1986). Foaming capacity (FC) for WF was observed 32.2%. As the incorporation of BF was increased, FC was also observed and the values between 32.9 to 35.2 %. For WF and WF-BF flour blends, the emulsion activity was found in the range between 33.3 to 38.5%.

**Table 1a.** Proximate composition of WF-BF blends

| Blends | Moisture (%) | Crude protein(%) | Crude fat (%) | Ash (%) | Carbohydrates (%) |
|---|---|---|---|---|---|
| WF | 9.55$^{a}$±0.03 | 12.23$^{a}$±0.05 | 2.46$^{a}$±0.02 | 1.83$^{a}$±0.05 | 72.55$^{f}$±0.06 |
| BF-5%+WF-95% | 10.11$^{b}$±0.02 | 12.56$^{b}$±0.03 | 2.69$^{b}$±0.02 | 1.99$^{b}$±0.03 | 71.89$^{e}$±0.05 |
| BF10%+WF-90% | 10.36$^{c}$±0.01 | 12.98$^{c}$±0.05 | 2.94$^{c}$±0.03 | 2.11$^{c}$±0.03 | 70.21$^{d}$±0.03 |
| BF-15%+WF-85% | 10.57$^{d}$±0.02 | 13.11$^{d}$±0.04 | 3.35$^{d}$±0.01 | 2.56$^{d}$±0.02 | 70.37$^{c}$±0.04 |
| BF-20%+WF-80% | 10.78$^{e}$±0.01 | 13.90$^{e}$±0.02 | 3.68$^{e}$±0.01 | 3.07$^{e}$±0.02 | 69.03$^{b}$±0.05 |
| BF25%+WF-75% | 10.89$^{f}$±0.01 | 14.23$^{f}$±0.04 | 3.99$^{f}$±0.02 | 3.44$^{f}$±0.03 | 68.22$^{a}$±0.03 |

Mean ±SD- mean and standard deviation of triplicate analysis

Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

**Table 1b**. Functional properties of WF-BF blends

| Sample | Bulk density (g/ml) | WAC (%) | OAC (%) | FC (%) | EA (%) |
|---|---|---|---|---|---|
| WF | 0.534$^{f}$±0.02 | 135$^{a}$±0.01 | 107$^{a}$±0.02 | 32.2$^{a}$±0.02 | 33.3$^{a}$±0.01 |
| BF-5%+WF-95% | 0.523$^{e}$±0.01 | 145$^{b}$±0.02 | 123$^{b}$±0.01 | 32.9$^{b}$±0.01 | 35.23$^{b}$±0.01 |
| BF10%+WF-90% | 0.499$^{d}$±0.01 | 177$^{c}$±0.02 | 134$^{c}$±0.03 | 33.6$^{c}$±0.02 | 36.2$^{c}$±0.02 |
| BF-15%+WF-85% | 0.467$^{c}$±0.03 | 197$^{d}$±0.01 | 156$^{d}$±0.01 | 34.7$^{d}$±0.01 | 37.4$^{d}$±0.01 |
| BF-20%+WF-80% | 0.411$^{b}$±0.01 | 201$^{e}$±0.03 | 177$^{e}$±0.01 | 35.1$^{e}$±0.01 | 38.1$^{e}$±0.01 |
| BF25%+WF-75% | 0.356$^{a}$±0.01 | 205$^{f}$±0.01 | 194$^{f}$±0.02 | 35.2$^{f}$±0.03 | 38.5$^{f}$±0.01 |

Mean ±SD- mean and standard deviation of triplicate analysis

Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

### 3.2. Pasting properties of WF-WB flour blends

Pasting properties of WF and WB flour blends are summarized in Table 2. Viscosity (PV and FV) of WF was observed to be 945 cP and 1329 cP, respectively whereas incorporation of 5 to 25% BF in WF increased PV and FV and the range observed was from 998 to 1198 cP and 1357 to 1599 cP, respectively. Sullivan et al. (2011) reported that BF has higher PV as compared to WF and incorporation of BF into WF significantly ($p<0.05$) increased the pasting properties. SV also increased as the incorporation of BF increased in WB blends and the values ranged from 539 to 707 cP. As proportion of BF in WF increased, a progressive increase in PV, FV and BV was observed, which may be due to the soluble fibers in the BF.

Pasting temperature (PT) of wheat flour was 84.6°C and at increased level of incorporation of BF no significant increase was observed.

**Table 2.** Pasting properties of WF-BF blends

| Blends | Peak viscosity (cP) | Trough Viscosity (cP) | Breakdown Viscosity (cP) | Final Viscosity (cP) | Setback Viscosity (cP) | Pasting Temperature |
|---|---|---|---|---|---|---|
| WF | $945^{a}$±11 | $788^{a}$±31 | $157^{a}$±14 | $1329^{a}$±13 | $539^{a}$±22 | $84.6^{a}$±24 |
| BF-5%+WF-95% | $998^{b}$±15 | $799^{b}$±10 | $199^{b}$±21 | $1357^{a}$±11 | $560^{b}$±31 | $84.3^{a}$±19 |
| BF10%+WF-90% | $1035^{c}$±21 | $823^{c}$±12 | $212^{bc}$±25 | $1418^{b}$±15 | $595^{c}$±33 | $83.5^{b}$±17 |
| BF-15%+WF-85% | $1111^{d}$±26 | $825^{d}$±9 | $286^{c}$±28 | $1497^{c}$±17 | $672^{c}$±31 | $83.3^{b}$±25 |
| BF-20%+WF-80% | $1123^{e}$±18 | $829^{e}$±25 | $294^{d}$±33 | $1523^{d}$±21 | $703^{d}$±18 | $82.4^{b}$±31 |
| BF25%+WF-75% | $1198^{f}$±22 | $892^{f}$±27 | $306^{e}$±19 | $1599^{e}$±25 | $707^{e}$±15 | $82.1^{b}$±26 |

Mean ±SD- mean and standard deviation of triplicate analysis
Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

### 3.3. Textural parameters of WF-BF blends muffins

The textural parameters of the muffins were significantly altered by the replacement of wheat with barley flour (Table 3). The hardness of control WF muffins was 8.46 and as the incorporation of BF increased, an increase hardness was observed. Blending of WF with different proportions of BF, a significant ($P \leq 0.05$) increase in cohesiveness with values between 0.27 to 0.30 was observed when compared with WF muffin (0.25). A significant decrease in springiness values from 31.03 to 28.14 in comparison to WF- muffin (31.22) was observed with replacement of WF with BF.

For WF-muffin, chewiness was found to be 37.11 and the values increased from 38.11 to 45.66 as the BF incorporated upto 25%.Yaqoob et al. (2018) observed an increase in springiness and decrease in chewiness in barley incorporated cake. Replacement of WF with BF also increased the firmness of muffins with values between to 6.31 to 7.33 in comparison to WF muffins (6.01). Texture attributes of muffins made with WF was reported to improved with incorporation of BF. Consequently, WF-BF blends may be considered a functional source for quality improvement of wheat muffins by incorporating BF.

**Table 3**. Texture attributes of WF-BF blends muffins

| Blends | Hardness (N) | Cohisiveness | Springiness (mm) | Chewiness (Nmm) | Adhesiveness (Nmm) | Firmness (N) |
|---|---|---|---|---|---|---|
| WF | $8.46^{a}$±0.02 | $0.25^{a}$±0.01 | $31.22^{a}$±0.02 | $37.11^{a}$±0.02 | $1.19^{a}$±0.01 | $6.01^{a}$±0.02 |
| BF-5%+WF-95% | $8.63^{b}$±0.03 | $0.27^{b}$±0.02 | $31.03^{b}$±0.02 | $38.11^{b}$±0.03 | $1.35^{b}$±0.01 | $6.31^{b}$±0.03 |
| BF10%+WF-90% | $8.91^{c}$±0.01 | $0.29^{c}$±0.03 | $30.87^{c}$±0.03 | $38.44^{c}$±0.02 | $1.48^{c}$±0.03 | $6.62^{c}$±0.02 |
| BF-15%+WF-85% | $9.11^{d}$±0.01 | $0.29^{d}$±0.03 | $30.43^{d}$±0.01 | $42.11^{d}$±0.01 | $1.56^{d}$±0.02 | $6.87^{d}$±0.01 |
| BF-20%+WF-80% | $9.26^{e}$±0.01 | $0.29^{e}$±0.02 | $29.11^{e}$±0.01 | $43.67^{e}$±0.03 | $1.77^{e}$±0.02 | $7.06^{e}$±0.01 |
| BF25%+WF-75% | $9.41^{f}$±0.03 | $0.30^{f}$±0.03 | $28.14^{f}$±0.02 | $45.66^{f}$±0.02 | $2.01^{f}$±0.03 | $7.33^{f}$±0.03 |

Mean ±SD- mean and standard deviation of triplicate analysis
Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

### 3.4. Total Phenolics and flavonoids of WF-BF blends and muffins

Phenolic compounds have become the essentiality of food products for their remarkable antioxidant activities and ability to scavenge free radicals to prevent free radicals chain reactions (Gangopadhyay et al., 2016).The total phenolic content (TPC) of WF and BF was

1134 and 2699 µg(GAE)/g. Sandhu et al. (2016) reported TPC in wheat in the range between 974 and 1399 mgGAE/g. Incorporation of BF flour to WF led to a significant ($p < 0.05$) increase in TPC by 5.7, 8.8, 24.4, 42.2 and 61% when 5, 10, 15, 20 and 25% of WF was replaced with BF (Table 4a). After baking significant decrease in TPC was observed for muffins made from WF and flour blends. In case of muffin made from WF, TPC decreased by 9.8% whereas for flour blends the decrease was from 4.3 to 17.95. WOF-10 % had the largest decrease in TPC upon baking. Holtekjolen et al. (2008) reported similar decrease in TPC for bread made by incorporation of barley flour to wheat flour. Leenhardt et al. (2006) reported heat produced during baking may be reason of reduction of phenolic content. Molecular structure of phenolic compounds changes as a result of heating which leads to either reduced chemical reactivity or decreases their extractability due to certain degree of polymerization. TFC of WF was observed 121 µg CE/g and replacement of WF by BF at levels of 5, 10%, 15%, 20% and 25% exhibited TFC of 152, 197, 219, 335 and 398µg CE/g, respectively (table 5). Baking, however led to led to a significant ($p < 0.05$) decrease in TFC. Barley is a rich source of flavonoids as compared to wheat therefore, increasing its proportion in WF increased the TFC significantly ($p<0.05$). Baking led to a significant ($p<0.05$) decrease in TFC. The muffins prepared by WF exhibited TFC of 89 µg CE/g whereas those prepared by incorporating 5, 10, 15, 20 and 25% BF to WF showed TFC values of 132, 145, 166, 211 and 234µg CE/g, respectively. Holtekjolen et al., (2008) and Angioloni and Collar (2011) also observed a reduction in flavonoids content of breads during thermal processing, As reported by Xu and Chang (2008), flavonoids are heat sensible and during processing of food products, flavonoids are started to degrade, however, the extent of degradation of flavonoids depends upon the duration and processing conditions.

**Table 4a.** Total phenolic content and total flavonoids content of WF-BF blends and muffins

| Sample | TPC (µgGAE/g) | | TFC (µgCE/g) | |
|---|---|---|---|---|
| | Before baking | After baking | Before baking | After baking |
| WF | $1134^{a}\pm23$ | $743^{a}\pm25$ | $121^{a}\pm20$ | $89^{a}\pm24$ |
| BF-5%+WF-95% | $1199^{b}_{\uparrow5.7}\pm32$ | $887^{b}\pm19$ | $152^{b}_{\uparrow25.6}\pm16$ | $132^{b}\pm31$ |
| BF10%+WF-90% | $1234^{c}_{\uparrow8.8}\pm15$ | $801^{c}\pm20$ | $197^{c}_{\uparrow62.8}\pm12$ | $145^{c}\pm33$ |
| BF-15%+WF-85% | $1411^{d}_{\uparrow24.4}\pm25$ | $887^{d}\pm34$ | $219^{d}_{\uparrow80.9}\pm24$ | $166^{d}\pm 17$ |
| BF-20%+WF-80% | $1613^{e}_{\uparrow42.2}\pm11$ | $934^{e}\pm37$ | $335^{e}_{\uparrow193}\pm36$ | $211^{e}\pm 29$ |
| BF25%+WF-75% | $1826^{f}_{\uparrow61}\pm26$ | $1122^{f}\pm19$ | $398^{f}_{\uparrow228}\pm15$ | $234^{f}\pm31$ |
| Barley | $2699^{g}\pm17$ | - | $1568^{g}\pm26$ | - |

Mean ±SD- mean and standard deviation of triplicate analysis

Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

### 3.5. Antioxidant potential of WF-BF blends and muffins

Various antioxidant assays are currently being used by the researchers to detect the antioxidant potential in natural extracts. Owing to the complex nature of bioactive compounds in natural extracts, single assay is not enough to quantify the antioxidant potential. In the present study DPPH, ABTS, and metal chelating activity assays were used to assess the antioxidant activity in the extracts. The results obtained from antioxidant analysis of different extracts revealed that BF incorporated muffins significantly ($p<0.05$) improve the antioxidant potential (Table 4b). For WF muffins, DPPH antioxidant activity and Metal chelating activity was observed to be 10.3 and 22.4%, respectively. Incorporation of BF proportion in WF increased the antioxidant activity in terms of DPPH and ABTS$^{+\cdot}$ ABTS$^{+}$ scavenging activity of WF was 7.22%. Increasing the level of BF in the WB blends progressively increased the scavenging activity from 8.11 to 11.78 µmol/g. The increase in antioxidant activities may be due to the higher scavenging activity of barley as compared to wheat. Baking resulted in a decrease in AOA for wheat and WF-BF muffins. Antioxidative compounds are very heat sensitive and reported a reduction in TPC as well as loss of AOA in products during the heat treatment.

**Table 4b.** Antioxidant potential of WF-BF blends and muffins

| Blends | DPPH (%) | | ABTS$^{+}$ (µmol/g) | | Metal chelating activity(%) | |
|---|---|---|---|---|---|---|
| | Before baking | After baking | Before baking | After baking | Before baking | After baking |
| WF | $10.3^{a}\pm0.03$ | $6.7^{a}\pm0.03$ | $7.22^{a}\pm0.01$ | $5.11^{a}\pm0.01$ | $22.4^{a}\pm0.02$ | $17.4^{a}\pm0.03$ |
| BF-5%+WF-95% | $12.9^{b}\pm0.02$ | $8.4^{b}\pm0.01$ | $8.11^{b}\pm0.03$ | $5.23^{b}\pm0.03$ | $23.9^{b}\pm0.01$ | $17.6^{b}\pm0.03$ |
| BF10%+WF-90% | $14.9^{c}\pm0.01$ | $9.7^{c}\pm0.02$ | $8.75^{c}\pm0.02$ | $6.03^{c}\pm0.01$ | $26.8^{c}\pm0.02$ | $18.1^{c}\pm0.01$ |
| BF-15%+WF-85% | $17.4^{d}\pm0.01$ | $12.5^{d}\pm0.01$ | $9.95^{d}\pm0.01$ | $6.46^{d}\pm0.01$ | $32.3^{d}\pm0.02$ | $18.4^{d}\pm0.02$ |
| BF-20%+WF-80% | $19.3^{e}\pm0.02$ | $14.6^{e}\pm0.03$ | $11.21^{e}\pm0.01$ | $7.13^{e}\pm0.03$ | $36.6^{e}\pm0.01$ | $19.3^{e}\pm0.01$ |
| BF25%+WF-75% | $21.4^{f}\pm0.03$ | $16.4^{f}\pm0.02$ | $11.78^{f}\pm0.02$ | $7.28^{f}\pm0.02$ | $39.2^{f}\pm0.03$ | $20.2^{f}\pm0.03$ |

Mean ±SD- mean and standard deviation of triplicate analysis

Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

### 3.6. Sensorial attributes of muffins

The sensorial scores reported by the test panel by following 9 point hedonic scale are shown in Table 5. Statistically significant ($p<0.05$) variations were observed for organoleptic quality (color, flavour, aroma, taste and overall acceptability) of muffin by semitrained panel of judges. The sensory results showed that the chewing properties, taste, aroma and overall acceptability of muffin were best for muffin made from only from wheat flour (WF). Increasing levels of barley flour (BF) slightly decreased the sensorial scores. The results showed that BF supplementation at different levels (5, 10, 15, 20 and 25%) into formulation had considerable effects on the muffins quality. Upto 15% incorporation of BF into WF did not affect sensory attributes of muffin considerably, after that color of muffins became more darker. Gumminess increased for muffins made by BF incorporation to WF. Incorporation more than 20% resulted in low score and the muffin were not acceptable. Incorporation of BF to WF at levels of 25% decreased the sensory scores drastically and muffin were unacceptable due to their irregular shape, dilution of gluten, gummy mouthfeel and barley characteristic flavor/aroma.

According to sensory analysis, overall acceptance of muffin were found the best for control sample. Muffin prepared with BF addition to WF were liked moderately and like slightly by panelists. Although as the incorporation of BF in WF for making muffin has increased, bioactive potential and antioxidants, but addition upto 15% BF in flour gave satisfactory sensorial results in terms of overall acceptability.

**Table 5.** Sensory analysis of WF-BF blends muffins

| Blends | Color | flavor | Taste | Aroma | Overall acceptability |
|---|---|---|---|---|---|
| WF | 9.1$^{f}$±0.02 | 8.4$^{f}$±0.02 | 9.1$^{f}$±0.03 | 9.2$^{c}$±0.03 | 9$^{f}$±0.03 |
| BF-5%+WF-95% | 8.7$^{e}$±0.02 | 8.1$^{e}$±0.02 | 8.8$^{e}$±0.01 | 8.8$^{c}$±0.02 | 8.7$^{e}$±0.02 |
| BF10%+WF-90% | 8.3$^{d}$±0.02 | 7.7$^{d}$±0.01 | 8.6$^{d}$±0.01 | 8.5$^{c}$±0.02 | 8.2$^{d}$±0.02 |
| BF-15%+WF-85% | 7.8$^{c}$±0.02 | 7.3$^{c}$±0.02 | 8.3$^{c}$±0.02 | 8.3$^{c}$±0.01 | 8.1$^{c}$±0.02 |
| BF-20%+WF-80% | 6.8$^{b}$±0.03 | 6.4$^{b}$±0.01 | 7.2$^{b}$±0.01 | 7.3$^{b}$±0.02 | 6.3$^{b}$±0.01 |
| BF25%+WF-75% | 6.1$^{a}$±0.01 | 5.9$^{a}$±0.01 | 6.1$^{a}$±0.01 | 6.3$^{a}$±0.02 | 5.9$^{a}$±0.02 |

Mean ±SD- mean and standard deviation of triplicate analysis

Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

### 3.7. Microbiological analysis of WF-BF blends muffin

Regarding the shelf life of bakery products, microbiological spoilage is a major limiting factor. Spoilage from microorganism by bacteria, yeast and moulds is the concern in high moisture products which causes manufacturer's financial loss and a cause of threat to consumer's health. Improper handling in packaging, storage conditions, sanitary practices may be the possible reasons of such loses. Result of microbiological analysis of the samples is shown in Table 6. Total plate count reflects the conditions in which the food is manufactured, and stored, this count may be used as a tool to check the keeping quality of the finished product. An increased in incorporation of BF in WF, did not show a significant ($p<0.05$) increase in total plate count and mould count of muffin till 10th day .The microbial content of all WF-BF blends muffins suggested that barley incorporated muffins are microbiologically safe for human consumption.

**Table 6.** Microbial analysis of WF-BF blends muffins

| | TPC (log10cfu) | | | | Mold (log10cfu) | | | |
|---|---|---|---|---|---|---|---|---|
| Blends | 0 day | 5th day | 10th day | 15th day | 0 day | 5th day | 10th day | 15th day |
| WF | - | 0.632$^{a}$± 0.05 | 1.432$^{a}$± 0.03 | 3.454$^{a}$± 0.02 | - | 0.211$^{a}$± 0.03 | 1.032$^{a}$± 0.02 | 3.001$^{a}$± 0.05 |
| BF-5%+WF-95% | - | 0.624$^{b}$ ±0.03 | 1.445$^{b}$± 0.02 | 3.789$^{b}$± 0.02 | - | 0.221$^{b}$± 0.02 | 1.078$^{b}$± 0.05 | 3.022$^{b}$± 0.03 |
| BF10%+WF-90% | - | 0.597$^{c}$± 0.02 | 1.478$^{c}$± 0.05 | 3.997$^{c}$± 0.02 | - | 0.256$^{c}$± 0.03 | 1.103$^{c}$± 0.03 | 3.156$^{c}$± 0.03 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BF-15%+WF-85% | - | 0.571$^{d}$ ±0.02 | 1.501$^{d}$± 0.03 | 4.235$^{d}$± 0.03 | - | 0.288$^{d}$± 0.02 | 1.143$^{d}$± 0.01 | 3.212$^{d}$± 0.02 |
| BF-20%+WF-80% | - | 0.521$^{e}$± 0.02 | 1.536$^{e}$± 0.05 | 4.457$^{e}$± 0.02 | - | 0.312$^{e}$± 0.05 | 1.167$^{e}$± 0.02 | 3.245$^{e}$± 0.02 |
| BF25%+WF-75% | - | 0.593$^{f}$± 0.03 | 1.578$^{f}$± 0.03 | 4.657$^{f}$± 0.03 | - | 0.324$^{f}$± 0.02 | 1.898$^{f}$± 0.02 | 3.567$^{f}$± 0.05 |

Mean ±SD- mean and standard deviation of triplicate analysis

Values followed by the same superscript within the column do not differed significantly ($p < 0.05$)

## 4.Conclusions

The nutritional, sensorial and antioxidant properties of muffins were enhanced by progressive enhancement of barley flour in wheat flour. Results showed that substituting WF with BF at level upto 15% produced muffins with better properties almost similar to the control muffins. Baking tests showed that BF addition with <15% significantly impaired the color, taste, aroma and overall acceptability of muffins. Therefore, muffins made from WOF-15% was found to be acceptable in terms of sensory, nutritionally and microbiologically. From health point of view, development of such functional bakery products would be beneficial to improve the nutritional status of consumer.

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## ETHICAL STATEMENTS

Conflict of interest: The authors declare that they do not have any conflict of interest.