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Characterization of quinoa-wheat flour blend for the preparation of dry cake

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Abstract

The current study aimed at the quinoa-wheat composite flour's characterization (including total phenolics, total flavonoids, and antioxidant activity) as well as its effect on dry cake sensory quality. Findings revealed a rise in ash content, fat, protein, and crude fiber of composite-flour (0.56-1.23%, 1.13-1.76%, 10.14-11.02%, and 0.23-1.04%, respectively) with an increase of quinoa flour (5-20%). The addition of quinoa flour to the composite flour enhanced cake texture (0.26-.70 kg), but it also decreased L-value of cake crumb (55.29-50.73). Total flavonoids (56.44-59.48 mg QE/100 g) and antioxidant activity (4.66-9.76%) increased as quinoa flour was increased, whereas total phenolics (8.68-5.46 mgGAE/g) decreased. By mixing wheat flour with quinoa flour, the nutritional value of wheat flour was increased. During sensory evaluation, the cake made from composite-flour containing 10% quinoa-flour scored the highest overall acceptability. Sensory quality of last two treatments, which included 15% and 20% quinoa flour, was lower. Quinoa seeds had a better nutritional profile than wheat, with higher levels of amino acids, minerals, dietary fibers, and oil. Since wheat is a staple food in Pakistan, adding quinoa-flour would help to reduce malnutrition in Pakistan. Furthermore, no previous research on the suitability of quinoa-wheat flour for dry cake has been conducted on Pakistani wheat flour.

Keywords: dry cake; quinoa-wheat flour; sensory evaluation; total phenolics.

Practical Application: The nutritional content of a dry cake was boosted by mixing wheat flour with a small amount of quinoa flour, which was helpful to the consumer. Furthermore, it improves the cake's overall acceptability while having no effect on the cake's quality in other aspects.

1 Introduction

Wheat is one of the cereals that has dominated the world's temperate regions because of its compliance with global regions' environmental conditions and is widely used as a consumer product for humans (Tatham & Shewry, 2008). Gluten formation is also among the main features of wheat. In addition, the dietary dependency of people on wheat is considerably greater across the globe. Chemically, it may be because it consists of large concentrations of starch content (80% in white flour) (Shewry et al., 2013), along with it also has 10-15% (dry weight) of protein content, gluten as a major protein (Akhlaq et al., 2022; Tatham & Shewry, 2008). The bran portion of wheat is comparatively rich in phytochemical components, but are removed from white flour during milling (Shewry et al., 2013).

Likewise, quinoa is also a food plant belong to family "Amaranthaceae". Quinoa has remained an abundant crop in the Andean region compared to wheat and is highly resistant to high and unexpectedly varying environmental conditions. Maradini-Filho (2017) noted a higher content of protein (average 15%), and greater balance of essential amino acid distribution, high in tryptophan. Lysine content is also present in abundance, which exceeds many vegetables and many other food commodities in its nutritional profile (Belton et al., 2002). Quinoa is also a

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gluten free pseudo cereal. With the inclusion of vitamins and minerals, its gluten-free nature has also contributed much to its value in nutritional areas, which is why it can be used safely in the dietary cure and in gluten-tolerant celiac patients. It also contains a considerable quantity of dietary fibers (7 – 9% dry matter) and oil contents (2 – 9.5%) rich in certain essential fatty acids (Maradini-Filho, 2017). Quinoa seeds showed a better nutritional profile to satisfy the vitamins and mineral requirements for children between the age of 1-3 years (Ruales & Nair, 1993). But the presence of saponins is considered as a negative factor in the nutritional profile of quinoa. The bitter taste of quinoa goods is also an obstacle to the production of quinoa, thus it is better to remove saponins before processing, but it can lead to a reduction in nutritional value. The quantity of saponins in quinoa varies depending upon the color of the grains (Belton et al., 2002; Diaz-Valencia et al., 2018; Ruales & Nair, 1993).

Bread is considered as a staple food in most of the countries. As a gluten-free pseudo-cereal, quinoa can be used in the bakery industry in combination with wheat flour to reduce the cost or produce gluten-free or enhance nutrition of the product (Moawad et al., 2019). So, composite flour is best an option, which is defined as a mixture of several different flours. The composition of composite flours depends upon the end-use, quality and required nutrition of products (Julianti et al., 2017). Previously, the combination of wheat-quinoa flour was studied for pan bread and biscuits at 5, 10 and 15% addition of quinoa flour. The researcher noted an acceptable sensory quality and reduction in bread staling prepared from 10% composite flour (Moawad et al., 2019). For the application of composite technology, cakes are found most suitable because the ratio of white-sugar and wheat flour is almost equal. Eggs and the quantity of lipids are both almost equivalent (Keppler et al., 2018). The specially treated wheat flour is widely used in cakes and the main reason behind such a prerequisite is to protein denaturation, control the holding ability of water and keep the sugar component (Delatte et al., 2019).

The goal of this study was to develop and evaluate the nutritional potential of quinoa-wheat composite flour. This research also focused on the development of composite flour of quinoa-wheat suitable for the baking of dry cake.

2 Materials and methods

2.1 Procurement of raw material

The research was conducted in institute of Agriculture Sciences, University of the Punjab, Lahore. Quinoa and wheat fine flour were procured from the University of Agriculture, Faisalabad and Rehmat Wheat Products Private Limited, respectively. Chemicals were purchased from RCI, Fluka, Labscan, Aldrich etc. All ingredients for cake including sugar, oil, baking powder were purchased from the Metro Cash and carry, Model town Lahore.

2.2 Preparation of raw material

The seeds of quinoa cleaned and milled in blender model number 718 (Brand name; National, Pakistan). Now this quinoa flour was blended with wheat fine flour in various ratios given in Table 1.

2.3 Proximate analysis

The moisture content was analyzed through the standard method no-44 15.02 (American Association of Cereal Chemists, 2009). The 10 g sample was taken in pre-weighed china dish and placed in the oven (Memmert, Germany). The temperature was maintained at 105 °C + 5 °C for 24 hours or until constant weight. After drying, the samples were placed in a desiccator and then weighed. The moisture content was calculated as follows (Equation 1),

$$Moisture(\%) = \frac{Sample\,wt - Dried\,sample\,wt}{Sample\,wt} * 100 \qquad (1)$$

The ash content was measured through method no. 08-01.01 (American Association of Cereal Chemists, 2009). 5 g samples were taken in a pre-weighed crucible and treated with the flame for charring. Then samples were placed in the muffle furnace (500-600 °C) for 5-6 hours till gray-whitish residues. Ash percentage can be calculated as (Equation 2)

$$Ash(\%) = \frac{Ash\,residues\,wt}{Sample\,wt} * 100 \qquad (2)$$

Method no 920.29 (Association of Official Analytical Chemists, 2010) was used for determining fat content. 5 g of moisture free sample was taken in Whatman's filter paper no 2 and placed in a Soxhlet apparatus containing hexane and run for 2-3 washings. The fat was calculated by following formula (Equation 3),

$$Fat(\%) = \frac{Sample\,wt - Defatted\,sample\,wt}{Sample\,wt} * 100 \qquad (3)$$

Protein content was determined by Kjeldahl apparatus through method number 920.87 (Association of Official Analytical Chemists, 2010). 2 g of sample was poured into a digestion tube along with digestion mixture and 25 mL concentrated H_2SO_4. The sample was digested till the clear whitish or light green color and then diluted up to 250 mL. The diluted sample was distilled with 10 mL 40% NaOH to liberate ammonia, which was collected in a beaker with the 4% boric acid solution and phenolphthalein indicator. The ammonium borate was titrated against 0.1N H_2SO_4 to measure the nitrogenous percentage, which was multiplied by 6.25 for the protein calculation. The formula used for this is as below (Equation 4),

$$N(\%) = \frac{Vol\,of\,0.1N\,acid\,used * 0.0014 * Vol\,of\,dilution}{Vol.of\,distillate\,taken * Sample\,wt} * 100 \qquad (4)$$

Table 1. Treatment plan for sample preparation.

Treatments	Wheat flour (%)	Quinoa flour (%)
T0	100	-
T1	95	5
T2	90	10
T3	85	15
T4	80	20

For crude fiber determination, 2 g of sample was placed in an extraction unit (Labconco Fibertech apparatus) (method # 962.09) (Association of Official Analytical Chemists, 2010). The sample was digested in 200 mL of 1.25% H_2SO_4 for 30 min and then washed with distilled water. In the next step, it was digested in 1.255% 200 mL NaOH solution for 30 min and then the sample was drained and washed with boiling water. After washing, the sample was dried, weighed, and ashes in the muffle furnace at 550 ˚C for 2 hours. Extracted fiber was calculated as follows (Equation 5),

$$Crude\ fiber(\%) = \frac{Digested\ sample\ wt - sample\ ash\ wt}{sample\ wt} * 100 \quad (5)$$

2.4 Preparation of dry cakes

Dry cakes were prepared by following the (Sedej et al., 2010). 160 g of eggs was poured and beaten in a bowl for 1 min. Then 150 g of icing sugar was added and mixed again for 1 min, after which the 135 g of oil was added and mixed properly. At the end, 2 g of baking powder and 150 g of flour was added and mixed the mixture. Now this better was poured into the pans and baked in oven for 40 min at 180 ˚C.

2.5 Sensory evaluation of dry cakes

Sensory quality of dry cakes was analyzed by the procedures mentioned in (Lawless & Heymann, 2010). A broad audience, including student, teachers, lab analysts, strangers were approached for sensory evaluation to balance the theory, methodology and practical application. The product application was also considered for the activity covering broad aspects in its composition. Therefore, the selection and competency of sensory evaluation were carried out as per suggested by (Kemp et al., 2009).

2.6 Texture analysis of dry cakes

The texture characterization of cake was carried out in the texture analyzer (TA-XT2, Plus, Stable Microsystems. Syrrey, UK). It was linked with a computer for a better efficiency in the results and the software used in this was named as texture expert program version 4.0.9.0. The results were expressed by the unit of kg (Piga et al., 2005).

2.7 Color analysis of dry cakes

The color of all dry cakes prepared from flours was checked through Color Reader CR-10 (Konica Minolta, Japan) following the manufacturer guidelines. Lightness is expressed by L* value, greenness and redness were expressed by a* i.e., –a and +a, respectively. Whereas blueness and yellowness were expressed by b* such as, -b and +b, respectively.

2.8 Determination of Total Phenolic Content (TPC)

The total phenolics were extracted from de-fated samples through 3 hours treatment in 80% methanol (1:5 weight by volume ratio) at room temperature. This extracted material was then filtered and further used for the estimation of total phenolic contents (Kim et al., 2006). 125 µL of this filtrate was diluted with 500 µL distilled water and 125 µL of folin-ciocalteu reagent and left for 6 min. After that, 1.25 mL of Na_2CO_3 (7%) and distilled water was added to make a final volume of 3 mL. Now left this mixture for 90 min to complete this reaction. The total phenolics were measured through UV-visible spectrophotometer with the wavelength of 760 nm and results were expressed as gallic acid equivalents (mg GAE/g dry weight). The benchmark used for the standard curve against the samples was the gallic acid with the absorbance at 760 nm. Solution preparation was carried out by adding 25 mL distilled water to 25 mg gallic acid. And this concentrate vary in composition from 0-450 µg/mL and standard curve was used to estimate the TPC in the samples (Gao et al., 2014).

2.9 Determination of Total Flavonoid Content (TFC)

500 µL of the sample-extract was poured into the test tubes having 2.5 mL distilled water, 5% solution of sodium nitrite (150 µL), and 300 µL of 10% aluminum chloride and left for 10 min. Then, 1M sodium hydroxide solution (1 mL) was added, and total volume of test tubes was made up to 5 mL by adding distilled water. Afterward, the absorbance was observed at 510 nm with the help of spectrophotometer. Final results of TFC were expressed as mg catechin equivalents QE/100 g (Gao et al., 2014).

2.10 Antioxidant activity by DPPH method

Principle of scavenging was used for the determination of antioxidant activity by following Yu et al. (2013). 1 g of sample was extracted with 10 mL of 8% methanol for 2 hours. 50 µL methanol extract was mixed in 2 mL of DPPH solution, prepared by dissolving 4 mg DPPH in 100 mL of methanol. Proper mixing of the solution was carried out by the strong agitation through external physical force and stay time was also given at normal lab temperature with dark surroundings. Now, the absorbance of the sample was observed at 515 nm wavelength. This scavenging was calculated as follows (Equation 6),

$$\%Radical\ scavenging = \left(1 - \frac{A\,sample}{A\,control}\right) * 100 \quad (6)$$

2.11 Statistical analysis

All the experiments were performed three times. One-way ANOVA and Post hoc comparison test was applied using Minitab-18 at a probability of 5%. Pairwise comparison of sensory quality of cake was also performed at probability of 5%.

3 Results

3.1 Physicochemical quality of flour

The results of physicochemical analysis showed that the different treatments varied significantly (Table 2). The ash content, fat content, protein, and crude fiber content were all lower in control sample (T0) (0.56%, 1.13%, 10.14%, and 0.23%, respectively), and gradually increased as quinoa flour content was increased. T4 (1.23%, 1.76%, 11.02%, and 1.04%, respectively), which contained 20% quinoa flour, had the highest values.

3.2 Color and texture analysis of cake

The texture property, as well as the a and b values of cake color (Table 3) were found to be lower in T0 (0.26 kg, 2.06, and 32.25, respectively) and higher in T4 (0.26 kg, 2.06, and 32.25, respectively) (0.7 kg, 3.8, and 33.65, respectively). In comparison, the L-value was higher in the control sample (T0) (55.29) and lower in the T2 sample (50.73).

3.3 Sensory evaluation of cake

The sensory quality of the cake, such as taste, flavor, texture, appearance, color, and overall acceptability, was significantly influenced by the composite flour. T0 had the best taste (7.3), flavor (7.2), and texture (7.5), with T2 coming in second (7.2, 7.0, and 7.1, respectively). The highest overall acceptability score (7.5) was given to T2, followed by T0 and T1 (7.3) (Table 4).

3.4 Total phenolic content, total flavonoid content and antioxidant activity

TFC (56.44 mg QE/100 g) and antioxidant activity were lower in the control sample (T0), which had a higher moisture content (13.53%) and TPC (8.68 mg GAE/g) (4.66%). T4 sample had less moisture (12.83%), TPC (5.46 mg GAE/g), but had more TFC (58.51 mg QE/100 g) and antioxidant activity (9.76%) (Table 5).

4 Discussion

4.1 Physicochemical quality of flour

Previously, the nutritional profiles of various colored quinoa seeds were examined, and all were found to be a good source of nutrition (Diaz-Valencia et al., 2018). In the current study, the moisture content of composite-flour and control-flour was found to be within the appropriate range (14%). Food spoilage is linked to bound and unbound water, either directly or indirectly. Because of its high percentage, it is susceptible to external environmental and microbiological factors (Rehman et al., 2007).

Previously, beefburgers made with quinoa flour had a higher fat content than those made with soy flour or buckwheat flour. Quinoa flour also had a higher mineral content than wheat flour (Bahmanyar et al., 2021). The current study found that increasing quinoa-flour supplementation increased total fat, crude protein and ash content in composite flour (Demir & Kilinç, 2017) previously published similar findings. An rise in protein, lipid, and ash content was also observed in gluten-free ladyfinger biscuits when quinoa flour was supplemented to rice flour (Cannas et al., 2020). Quinoa flour had a higher quality of protein, lipids, crude fiber, and ash than wheat flour (72% extraction) (Moawad et al., 2019), which led to an increase in the nutritional quality of the composite flour and commodity (Demir & Kilinç, 2017).

Table 2. Physico-chemical properties of different blends of wheat flour and quinoa flour.

Treatments	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fiber (%)
T0	13.53 ± 0.03^{a}	0.56 ± 0.01^{e}	1.13 ± 0.03^{e}	10.14 ± 0.01^{d}	0.23 ± 0.01^{e}
T1	13.44 ± 0.01^{b}	0.74 ± 0.01^{d}	1.23 ± 0.02^{d}	10.23 ± 0.01^{d}	0.47 ± 0.01^{d}
T2	13.11 ± 0.00^{c}	0.89 ± 0.01^{c}	1.46 ± 0.31^{c}	10.48 ± 0.03^{c}	0.62 ± 0.02^{c}
T3	13.01 ± 0.02^{d}	1.05 ± 0.01^{b}	1.56 ± 0.03^{b}	10.75 ± 0.06^{b}	0.91 ± 0.00^{b}
T4	12.83 ± 0.04^{e}	1.23 ± 0.01^{a}	1.76 ± 0.01^{a}	11.02 ± 0.04^{a}	1.04 ± 0.00^{a}

Means in same columns followed by different letters are significantly different ($p < 0.05$).

Table 3. Texture and color value of cake prepared from quinoa-wheat composite flour.

Treatments	Texture (kg)	L-value (crumb)	a	b
T0	0.26 ± 0.01^{c}	55.29 ± 0.3^{c}	2.06 ± 0.03	32.25 ± 0.04
T1	0.26 ± 0.02^{c}	50.84 ± 0.4^{c}	2.95 ± 0.03	33.17 ± 0.03
T2	0.27 ± 0.02^{c}	50.73 ± 0.4^{c}	3.29 ± 0.04	33.42 ± 0.04
T3	0.44 ± 0.01^{b}	52.06 ± 0.3^{b}	3.42 ± 0.02	33.55 ± 0.04
T4	0.7 ± 0.03^{a}	50.96 ± 0.4^{c}	3.8 ± 0.03	33.65 ± 0.03

Means in same columns followed by different letters are significantly different ($p < 0.05$).

Table 4. Impact of different treatments on sensory quality of cake.

Treatments	Taste	Flavor	Texture	Appearance	Color	Overall Acceptability
T0	7.3 ± 0.06^{a}	7.2 ± 0.12^{a}	7.5 ± 0.11^{a}	7.1 ± 0.08^{a}	7.0 ± 0.06^{a}	7.3 ± 0.11^{a}
T1	7.1 ± 0.08^{a}	6.7 ± 0.11^{a}	7.1 ± 0.06^{ab}	6.8 ± 0.08^{ab}	6.7 ± 0.08^{ab}	7.3 ± 0.06^{a}
T2	7.2 ± 0.11^{a}	7.0 ± 0.09^{a}	7.1 ± 00.08^{ab}	7.1 ± 0.08^{a}	7.2 ± 0.11^{a}	7.5 ± 0.06^{a}
T3	6.3 ± 0.13^{ab}	6.3 ± 0.13^{ab}	6.9 ± 0.08^{ab}	6.8 ± 0.10^{ab}	6.6 ± 0.08^{ab}	6.8 ± 0.08^{ab}
T4	5.7 ± 0.13^{b}	5.8 ± 0.14^{b}	5.7 ± 0.09^{b}	7.0 ± 0.08^{a}	7.1 ± 0.10^{a}	5.8 ± 0.14^{b}

Means in same columns followed by different letters are significantly different ($p < 0.05$).

Table 5. Antioxidant activity, total phenolic contents, and total flavonoid contents in quinoa-wheat composite flour.

Treatments	TPC (mg GAE/g)	TFC (mg QE/100 g)	DPPH (%)
T0	8.68 ± 0.26[a]	56.44 ± 0.18[d]	4.66 ± 0.06[e]
T1	6.84 ± 0.06[b]	57.01 ± 0.01[d]	6.13 ± 0.03[d]
T2	6.23 ± 0.03[c]	57.71 ± 0.21[c]	7.02 ± 0.03[c]
T3	5.88 ± 0.09[c]	59.48 ± 0.15[a]	8.84 ± 0.05[b]
T4	5.46 ± 0.03[d]	58.51 ± 0.40[b]	9.76 ± 0.11[a]

Means in same columns followed by different letters are significantly different ($p < 0.05$). TPC = Total phenolic contents, TFC = Total flavonoid contents, DPPH = Antioxidant activity.

In the current study, TPC was decreased, whereas TFC and antioxidant activity were increased in composite flour with an increase in quinoa-flour. Increased quinoa-flour addition, on the other hand, was previously correlated with an increase in TPC in cookies and snacks. The researcher also noted that Quinoa flour has a higher TPC content than wheat flour, resulting in a TPC rise (Acosta et al., 2022; Bahmanyar et al., 2021; Demir & Kilinç, 2017). Significant reductions in TPC have previously been documented, and have been linked to the type of treatment used, similar to the results of this research (Valenzuela-González et al., 2022).

Similar to the present study, one of the researchers reported a higher antioxidant activity (31.33%) in quinoa flour compared to breadcrumbs (0.99 mg GAE/g and 21.64%, respectively) (Bahmanyar et al., 2021). The addition of quinoa flour to rice flour also improved total flavonoids and antioxidant activity in gluten-free biscuits (Cannas et al., 2020). Increase in bioavailability of TPC, and flavonoids with microwave heat treatment was also reported previously (Valenzuela-González et al., 2022).

4.2 Color and texture analysis of cake

In the current study, the addition of quinoa-flour in wheat flour also showed a great impact on the color of composite flour. Previously, cookies made from composite wheat-flour and quinoa-flour showed a similar decrease in L and an increase in a* and b* (Demir & Kilinç, 2017). In bread, a similar reduction in lightness (L) and an increase in redness (a*) were observed, but the researcher also found that yellowness (b*) had the opposite effect (Moawad et al., 2019). Increased supplementation of quinoa-flour in rice-flour produced similar results in gluten-free biscuits, with a decrease in L and an increase in a* (Cannas et al., 2020). Carotenoids, chlorophyll, and lignin are pigments found in quinoa seeds, and these pigments affect the color of flour, crumb, and crust (Kurek & Sokolova, 2020).

With an increase in quinoa-flour supplementation, the texture of the cake improved in the current study. Similar findings have previously been published in cookies, with an improvement in hardness due to increased quinoa-flour supplementation (Demir & Kilinç, 2017). The addition of quinoa flour to wheat flour, on the other hand, resulted in a decrease in cookie hardness (Bick et al., 2014). The addition of quinoa flour to gluten-free biscuits also reduced the hardness of the biscuits (Cannas et al., 2020).

4.3 Sensory evaluation of cake

In this research, quinoa-flour supplementation had a major effect on sensory quality of cake i.e., taste, flavor, texture, color, appearance, and overall acceptability. Previously, adding quinoa flour to wheat flour improved overall acceptability, crust color, pore size, and uniformity of pore size of bread, but had no effect on taste, contrary to the findings of this research. The researcher also discovered that increasing the proportion of quinoa flour (5, 10 and 15%) in wheat flour increased loaf weight while decreasing bread loaf volume and specific volume. According to the researcher, bread made with a composite flour containing 5 and 10% quinoa flour had an overall acceptable sensory quality, which matched the results of the current study (Moawad et al., 2019).

Increased quinoa-flour addition to rice-flour improved the color and texture of gluten-free biscuits, according to Cannas et al. (2020). Previously, beefburgers made with quinoa flour were found to have improved sensory qualities and shelf-life stability than beefburgers made with soy protein and breadcrumbs. In the formulation of beef burgers, the researcher also suggested substituting quinoa flour for soy protein and bread crumbs (Bahmanyar et al., 2021). No improvement in sensory characteristics was recorded in cookies made with wheat flour supplemented with quinoa flour in the literature (up to 30%) (Bick et al., 2014).

Because of its superior chemical composition, the researchers proposed that quinoa-flour supplementation be considered (Bahmanyar et al., 2021; Bick et al., 2014; Cannas et al., 2020; Moawad et al., 2019). However, the overall rate of quinoa-flour supplementation should not exceed 50%, as this increases the bitterness of the product, which decreases customer acceptance (Cannas et al., 2020).

5 Conclusion

As compared to wheat flour, quinoa flour had a superior nutritional profile. The current study looked at how much quinoa flour could be combined with wheat flour to produce nutritionally superior and palatable cakes. Increased supplementation of quinoa flour increased TFC, antioxidant activity, Ash, protein, fat, and crude fiber, while TPC and moisture content decreased. The addition of quinoa flour improved the texture and increased a* and b* while decreasing the lightness of the cake crumb. Supplementation had a significant impact on the sensory quality of cake ($p < 0.05$). In terms of overall acceptability, the cake made with 10% quinoa-flour supplementation was good. As a result, given the current state of health and the need to improve dietary nutrition, quinoa supplementation might be a useful source to employ on a commercial scale in the production of cakes, particularly in undernourished areas.

Availability of data and material

The data that support the findings of this study are available from the corresponding author, [Dr. Shinawar Waseem Ali], upon reasonable request.

Author contributions

Muhammad Umer Farooq conceived and carried out the experiment. Mamoona Amir, Farzana Siddique, Muhammad inam Afzal, Muhammad Umer, Ahmed Mujtaba, Mateen Ahmad, Muhammad Awais, Ayesha Murtaza and Muhammad Imran contributed in designing the study and facilitated different analysis. Shinawar Waseem Ali supervised the whole research work. Munawar Iqbal evaluated the data statistically. Muhammad Akhlaq wrote the manuscript. Muhammad Arshad Javed, Muhammad Riaz, Rai Muhammad Amir and Aftab Ahmed edited the manuscript. Muhammad Mubashar Munir performed different assignments during this study.

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