

Beneficial effects of soaking and germination on nutritional quality and bioactive compounds of biofortified wheat derivatives

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ABSTRACT

The biotechnological advances and various crop improvement strategies have contributed to a great extent in nutritional improvement of important food crops, as an intervention toward alleviating the hidden hunger for the developing countries. The development, evaluation, and utilization of biofortified wheat are of great significance to achieve the above goal. In this study, biofortified wheat lines were subjected to soaking (12 hours) and germination (upto 96 hours) at room temperature. The soluble protein and starch contents were evaluated after 12 hours of soaking. Soluble protein, starch, total phenolics, and anthocyanin contents were determined in germinated seed samples at various time intervals (i.e., 12, 24, 48, 72, and 96 hours). In ungerminated wheat, soluble protein and starch contents were observed in the range of 6.96–8.04 mg/g and 0.110–0.147 mg/g, respectively, whereas, after 12 hours of soaking it was increased up to 7.15–8.18 and 0.119–0.150 mg/g, respectively. Moreover, the soluble protein content and starch were slightly improved by 26.8%–48.2% and 21.5%–50.8% of initial value, respectively, between 72 and 96 hours post-germination. In germinated wheat, the overall total phenolic and anthocyanin contents were substantially higher than that of non-germinated wheat. These results suggested that the germination has tremendous potential to improve the bioactive components and functional property of wheat flour. The improvement of the nutritional quality will help the consumers to get benefits of germinated wheat.

1. INTRODUCTION

The one of most important cereal crops as energy and nutrient source for humans and animals in developing countries is wheat. As a major food source, its nutritional properties are of great importance. The biofortified wheat derivatives with improved mineral contents and quality attributes have been reported recently [1–4]. An adequate amount of protein and amino acids are needed to maintain nitrogen concentration and body function. The cereal grain's average protein content varies from 8% to 15%, but is poor in some essential nutrients such as lysine, leucine, and threonine, decreasing its biological importance and nutritional value. The most widespread portion of dietary carbohydrates and

the main source of dietary energy is starch [5]. The eating quality of wheat flour-based products is also influenced by starch due to its major fraction. Starch properties are influenced by plant tissue physiological environments from which starch is isolated [6].

The most important contributors to cereal grain antioxidant properties are phenolic compounds [7]. In the pericarp, free phenolic compounds are mainly present and can be isolated with organic solvents [8]. These metabolites also contribute to the antioxidant activity of crops [9]. In the Mediterranean diets, these compounds constitute 30% of the total phenol [10]. In the plant kingdom, anthocyanins are water-soluble pigments which belongs to a class of compounds identified as flavonoids [11]. The anti-cancerous, anti-inflammatory, and free radical scavenging activity have been reported in Anthocyanins [12–15]. It is, therefore, important to examine the biochemical quality of wheat derivatives for food and nutritional purposes, and for greater nutritional consistency. The development of biofortified wheat derivatives have provided a

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boost to eradicate the problem of “Hidden hunger” caused by poor micronutrient availability in the developing countries, However, the newly developed wheat derivatives are not characterized for the other quality parameters under processing conditions.

The increased metabolic activities, due to rehydration during germination is supposed to enhance the digestibility, nutritional and functional properties of grains [16,17]. Soaking is followed by sprouting or germination, at which soaked seeds are allowed to improve their nutritional benefits in a humid condition as they germinate [18]. The increased metabolites and nutritional quality of cereals have been reported during germination [19]. The present study was conducted, where different biofortified wheat genotypes were evaluated for changes in various nutritional quality parameters in response to soaking and germination as compared to untreated material.

2. MATERIALS AND METHODS

2.1. Wheat Derivatives

Seven biofortified wheat genotypes used in this study were MB-64-1-1, 48-41-23-6-4-4, 75-1-4-16-3-5, 77-46-6-8-2-1, 1-1-7-18-5-5-18, 49-1-73-8-5, and 49-1-11-9-7-1 in addition to PBW343+Lr24+GPCB1 (PBW343LrP) as parental control non-biofortified wheat cultivar. The seed material was provided by the Department of Genetics, Plant Breeding and Biotechnology, Eternal University, Baru Sahib as an outcome of funded biofortification project.

2.2. Sample Preparation

Wheat seeds were sterilized at room temperature by soaking into 0.5% sodium hypochlorite at RT for 5 minutes. Thereafter, it was again soaked with 0.75% hydrogen peroxide for 5 minutes. After sterilization, the seeds were thoroughly washed and immersed in the ratio of 1:10 (*w/v*) distilled water) at RT for 12 hours. The soaked seed material was kept on a Petri plate under blotting paper, kept to germinate at RT up to durations of 12, 24, 48, 72, and 96 hours, respectively. During the germination period, the seeds were washed everyday using distilled water. At the specified intervals, the germinated seeds were collected, the roots and plumules were removed from the seed being germinated. The oven dried seeds were used for the estimation of soluble protein, starch content, total phenolics, and anthocyanin content using the standard protocols.

2.2.1. Soluble protein content

The amount of soluble proteins from the samples was determined by using Bradford assay [20]. 0.5 g of germinated and ungerminated wheat flours sample was mixed in 10 ml of 0.1 M, sodium acetate buffer of pH 5. (The mixture was kept on shaker incubator for 2 hours at RT and centrifugation was carried out at 8,000 rpm for 15 minutes. The supernatant was pooled in fresh tubes as crude extract and protein estimation was carried out at λ_{595} nm using BSA standard curve. The concentration of protein was expressed as mg/g.

2.2.2. Starch content

The extraction and estimation of starch content in germinated and ungerminated wheat samples was performed using protocol

of Hodge and Hofreiter [21]. The absorbance was taken at λ_{630} nm and a standard curve of glucose (Sigma-Aldrich) was used to determine the starch content (mg/g).

2.2.3. Total phenolic content

In both germinated and ungerminated samples, the extraction and estimation of total phenolic content (TPC) was performed in accordance to Chen et al. [22]. The 1 g sample of Wheat flour was mixed with 80% methanol (10 ml) and kept on shaker for 2 hours at RT followed by centrifugation for 15 minutes at 3,000 rpm and collected the supernatant for further analysis. Gallic acid (1 mg/ml) was taken as a standard. The absorption of sample was measured at 765 nm. The TPC (mg/g) was calculated using the formula as follows:

$$\text{Total phenolic} = C * V/m$$

where, *C* = concentration of sample (mg/ml)

V = Solvent volume (ml) used for extraction

m = Sample weight (g)

2.2.4. Total anthocyanin content (TAC)

In germinated and ungerminated samples, the amount of total anthocyanin was determined according to Abdel-Aal and Hucl [23]. Three gram wheat sample was mixed with 24 ml of acidified ethanol (ethanol and HCl, 85:15, *v/v*). The sample was mixed properly and pH was calibrated by using 4 N HCl and the solution was kept on shaker at 200 rpm for 30 minutes followed by centrifugation for 15 minutes at 4,000 rpm and collected the aliquot to a fresh centrifuge tube with a volume made up to 50 ml, with acidified ethanol solvent. The absorbance was measured against blank at 535 nm and anthocyanin concentration (mg/kg) was calculated using standard curve of cyanidin 3-glucoside (0–0.02 mmol or 0–27 μ g/3 ml). It was calculated using following formula:

$$C = (A/25,965) \times (50/1,000) \times 449 \times (1/3) \times 10^6 \text{ or } C = A \times 288.21 \text{ mg/kg}$$

where, *C* = TAC (mg/kg),

A = absorbance

2.2.5. Statistical analysis

All the experiments were carried out in triplicates and the data presented are mean \pm standard deviation. The standard deviation of average was calculated by XLSTAT program (<http://www.xlstat.com>)

3. RESULTS

3.1. Effect of Soaking and Germination on Soluble Protein Content

In germinated and ungerminated wheat sample, the values of soluble protein content (mg/g) are shown in Figure 1. In ungerminated wheat flour, it was found to be maximum in wheat genotype 1-1-7-18-5-5-18 (8.04 mg/g) followed by 77-46-6-8-2-1 (7.84 mg/g), 75-1-4-16-3-5 (7.32 mg/g), 49-1-11-9-7-1 (7.27 mg/g) and 48-41-23-6-4-4 (7.12 mg/g) as compared to control PBW343LrP (7.06

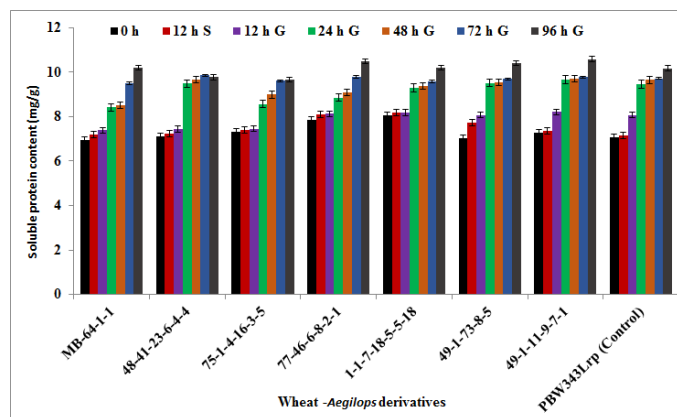


Figure 1: Effect of soaking and germination on soluble protein content of biofortified wheat-*Aegilops* derivatives.

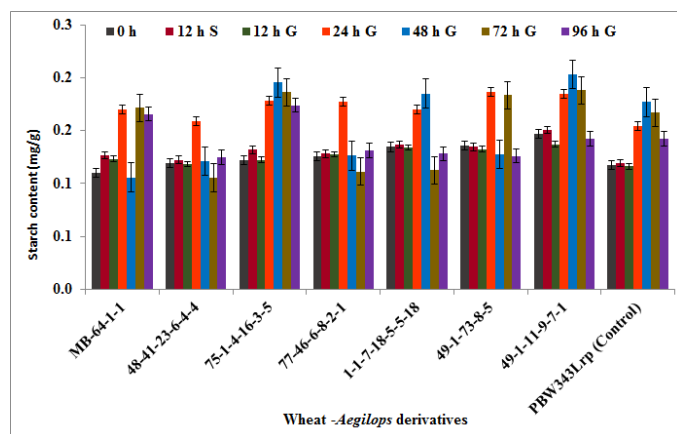


Figure 2: Effect of soaking and germination on starch content of biofortified wheat-*Aegilops* derivatives.

mg/g). At 12 hours soaking, there was an increase from 1.23% to 10.35% of the initial value and at 12 hours of germination, it was reportedly increased in the range of 1.70%–15.0 % of initial value. During 24–48 hours of germination, it was increased in the range of 16.1%–36.1% of initial value. The maximum increase was reported during 72–96 hours germination, where the protein content was increased from 26.8% to 48.2% as compared to initial values in selected wheat derivatives. The soluble protein content was increased maximum in wheat derivatives 49-1-73-8-5 (48.2%) followed by MB-64-1-1(46.6%) and 49-1-11-9-7-1 (45.6%) as compared to ungerminated samples.

3.2. Effect of Soaking and Germination on Starch Content

The starch content of germinated and ungerminated wheat derivatives was determined (Fig. 2). In untreated wheat flour, the starch content was found in the range of 0.110–0.147 mg/g. Although the starch content was between 0.119 and 0.150 mg/g, substantial variations were found in the selected samples during 12 hours of soaking. Starch content was decreased after 12 hours germination in few derivatives from approximately –0.88% to –2.65% of initial value, whereas it was increased slightly from the range 1.43% to 12.2% in others. At the stage of 24 hours

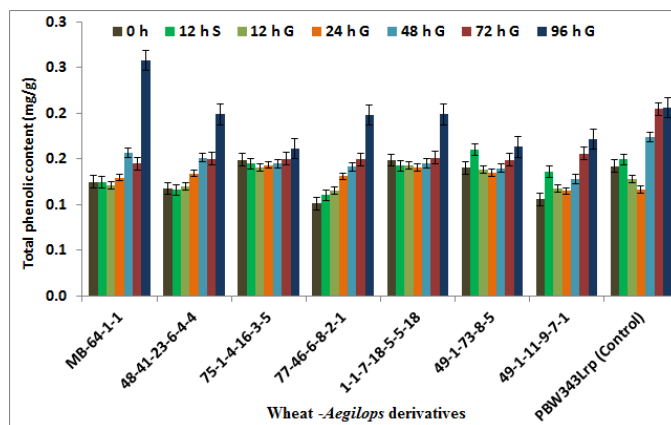


Figure 3: Effect of soaking and germination on total phenolic content of biofortified wheat-*Aegilops* derivatives.

germination, it was significantly increased in all derivatives from 25.3 to 54.6%, while it was reduced in MB-64-1-1 (–3.75%), 49-1-73-8-5(–6.24%) at 48 hours of germination and its content was increased in other genotypes from 1.29% to 59.7%, respectively. At 72 hours germination, starch content was found to increase from 27.8% to 56.2% of initial value. A significant positive correlation was observed between 48 to 72 hours germination. Starch content was significantly increased up to 56.2%, and highest increased starch content was observed during 96 hours germination in wheat derivative MB-64-1-1 (50.8%) and 75-1-4-16-3-5 (42.2%), respectively.

3.3. Effect of Soaking and Germination on Total Phenolic Content

Based on the analysis of wheat flour, the TPC was reported in the range of 0.101–0.149 mg/g in selected wheat derivatives before germination as shown in Figure 3. At the stage of 12 hours soaking, the TPC was increased in derivatives 49-1-11-9-7-1 (28.8%), 49-1-73-8-5 (14%), 77-46-6-8-2-1 (8.86%), PBW343 *LrP* (5.09%), whereas, it was reduced in derivatives MB-64-1-1(–0.69 %),48-41-23-6-4-4 (–1.31%), 75-1-4-16-3-5 (–2.91%), 1-1-7-18-5-5-18 (–4.09%). After 24-, 48-, 72-, and 92-hour germination, there was an increase in TPC in the range of 3.47%–29.4%, 21.1%–39.4%, 1.95%–47.7%, and 7.96%–106%, respectively, as compared to initial value in untreated samples. At the 96 hours of post germination stage, maximum increase in TPC was noticed in derivatives MB-64-1-1 (106%) followed by genotypes77-46-6-8-2-1 (95.7%) and 48-41-23-6-4-4 (69%) as compared to ungerminated sample.

3.4. Effect of Soaking and Germination on TAC

The changes of the TAC (mg/kg) during germination are shown in Figure 4. In ungerminated wheat flour, TAC was noticed in the range of 31.2–78.6 mg/kg, with maximum content in sample 49-1-73-8-5 (78.6 mg/kg) followed by 48-41-23-6-4-4 (58.9 mg/kg), 1-1-7-18-5-5-18 (56.1 mg/kg), MB-64-1-1 (51.7 mg/kg), 75-1-4-16-3-5 (47.7 mg/kg) and least content was found in 77-46-6-8-2-1 (29.2mg/kg) as compared to control genotype PBW343 *LrP* (31.2 mg/kg). Analysis of 12 hours soaking samples revealed that TAC

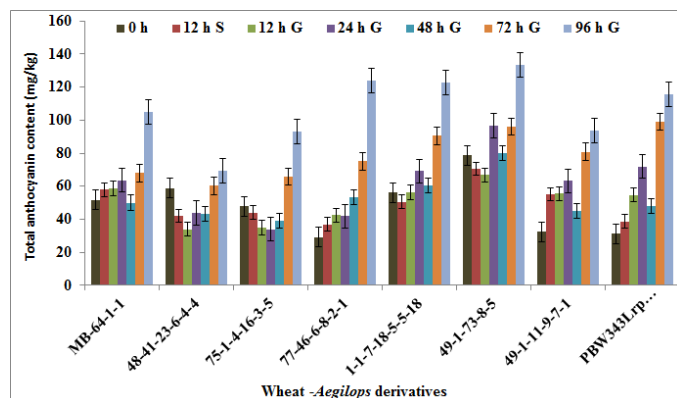


Figure 4: Effect of soaking and germination on TAC of biofortified wheat-*Aegilops* derivatives.

reported a mixed response with few samples reported increased content in the range 11.9%–70%, whereas, there was a decrease from –7.8 to –28.7% in some wheat derivatives. Similar trends in responses were obtained at 12–48 hours germination stages. However, at 72 hours and 96 hours germination stages, TAC was increased from 156.9% to 216.9% and 189.3% to 324.7% over ungerminated sample, respectively. The highest increase in content of total anthocyanin was observed in derivatives 77-46-6-8-2-1 (324.7%) followed by PBW343 + GPC + Lr24 (271.1%) and 49-1-11-9-7-1 (183.9%), respectively.

4. DISCUSSION

The evaluation of quality attributes of cereals and other foodstuff is of great importance for its promotion and utilization. The increasing demand and trends of processed food consumption realizes the importance of better evaluation methods and multifaceted analysis. In the present study, the analysis of mineral improved wheat derivatives using sprouting and germination-based approaches has provided an insight of associated changes under current experimental conditions. In the present investigation, soluble protein content was increased slightly during early period of germination, whereas, after 96 hours of germination, 26.8%–48.2% increase was reported. This increase might be attributed to the impact of protein hydrolysis by protease enzyme activity throughout germination of the seeds. Protein was increased in germinated flour which may be due to the synthesis of enzymes during germination, and production of certain amino acids during protein synthesis [24]. Inyang and Zakari [25] also reported that the content of protein can be increased by germination. According to Hung *et al.* [26], protein content of the waxy wheat sprouts did not substantially change from that of the seeds over the 48-hour germination period. The protein content might also changes depending on the equilibrium of protein denaturation and protein biosynthesis during germination [27]. During germination, soluble proteins and total protein content were examined by Veluppillai *et al.* [28], and showed that soluble protein was increased on the 5th day until germination of rice grains cultivated in Sri Lanka. The nutrient compositions as well as the quality of bioactive substances from cereal grains are highly affected by germination [29]. Therefore, to enhance the quality of germinated seeds, increase in germination time is necessary.

In the present study, the content of starch increased in the range of 1.72%–15.5% within 12 hours of soaking, however after 12–96 hours of germination, its content was increased by 21.4%–50.8% in all the selected samples. In another hand, the initial value of the sample was slightly reduced in a few samples. The solubility of starches improved in wheat after 24 hours of germination relative to non-germinated wheat. Germination may have led to improved solubility by degrading starch to smaller sugars, rendering it more soluble [30]. In another side, throughout the initial stages of germination, there was a dramatic drop in starch content in barley varieties, accompanied by a slight decrease within that final stage [31]. High solubility of starch was reported in mung bean after germination for 12 hours [32]. During the germination, limited information is available on changes in the structural and physicochemical properties of starch [33]. Many other studies have proposed that germination has greatly improved the nutritional content of starch granules [6,34].

The increased TPC in this study is also in relevance to previous reports. During buckwheat germination, phenolic accumulation increased with a rise in flavonoid accumulation [35]. The overall phenol in wheat was reportedly increased by germination [8]. In sorghum, germination led to several fold increase in total phenolic content [36]. Barroga *et al.* [37], reported that total phenolic content in Arhar was reduced by 23.39% and 13.61%, while germinated for 12 and 24 hours, respectively. In the first 12 hours of germination, a huge reduction for 48.7% was recorded in masur [38].

TAC was increased in some wheat derivatives during soaking and germination and was reduced in few samples. The study showed that TAC content in germinated wheat flours was significantly higher as compared to ungerminated flour. Similar to this study, relatively increased TAC in coloured wheat flours relative to white wheat flours was reported earlier [39–42]. Similarly, higher antioxidant activity and TAC in sprouts of colored wheat grains over white wheat was reported by Sytar *et al.* [43]. There was a significantly higher anthocyanin content in the sprouts of the purple wheat derivatives than in the blue or yellow wheat derivatives [44].

5. CONCLUSION

In conclusion, the observations in the current study suggested a variation in quality parameters of wheat derivatives due to soaking and germination process is useful for their nutritional significance in human diet. However, sensory evaluation of germinated or soaked material will give additional information about its acceptability. The outcome of the study may be validated by *in vivo* studies for beneficial impact on human health. Moreover, molecular characterization of altered responses may be carried out for a greater insight and further validation with inclusion of more parameters will be the emphasis in future studies.

6. LIST OF ABBREVIATIONS

S: Soaking; G: germination; WS: wheat seeds; TPC: Total phenolics content; TAC: Total anthocyanin content.

7. ACKNOWLEDGMENTS

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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