Effect of Soaking and Malting Process on Chemical Composition, Bioactive Compounds and Antioxidant Activity of some Egyptian Barley Varieties

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ABSTRACT

Recently, the demand for functional foods containing bioactive compounds with therapeutic and disease-preventing properties has increased. This work aimed to study the effect of soaking and malting processes on nutrient and bioactive compounds in selected Egyptian barley cultivars. Three barley varieties, namely, Giza128, Giza130, and Giza 2000, were investigated. Protein content in all raw and treated samples were ranged from 8.46 to 13.30g/100g on a dry weight basis. The crude fat and carbohydrate content were slightly decreased in all treated samples. Phenolic compounds content of raw barley seeds being, 336.2, 461.8, and 460.5 mg/100g in Giza128, Giza 130, and Giza 2000, respectively, while flavonoids content recorded 35.3, 58.1, and 31.3 mg/100g, respectively. Antioxidant activity in untreated samples were 29.45, 37.08, and 28.58% for Giza 128, Giza 130, and Giza 2000, respectively. Soaking of barely seed samples for 12 h increased total phenolic compounds, flavonoids, and antioxidant activity by 7.6-20.3, 11.7- 19.3, and 10.5- 21%, while the germination process for 48 h increased it by 37.3-69, 30.3- 62.6, and 36-65%, respectively. With germination time (48 h), the total phenolic compounds, flavonoids, and antioxidant activity have been gradually increased and subsequently decreased with the further of malting was occurred. It can be concluded from results that their nutritional properties and therapeutic properties with potential using treated barley flour to produce functional foods have hardly improved by soaking and germinating.

Keywords: Barley, Soaking, Germination, Chemical Composition Total Phenolic, Flavonoid and Antioxidant activity.

INTRODUCTION

Barley (Hordeum vulgare L.) is a plant of the Poaceae family and is an ancient and essential cereal crop, nowadays growing worldwide in demand and covering around 9.4% of the global cereal production area (Marwat et al., 2012; Mahmoudi et al., 2015). Barley was the fourth in terms of both production quantity (136 million tonnes) and cultivation area for the 2007 world cereal crop rankings, which amounted to 566,000 km² (FAOSTAT, 2009).

Two distinct types of barley, 2-rowed, and 6-rowed barley, are available. The key uses of barley as animal feed, in barley meal, and as malting grain (Gupta et al., 2010; Alazmani, 2015). Because of the high concentration of the bioactive compounds, the barley crop is considered to be generally one of the most important cereal crops of their possible use in the production of functional foods (Sharma and Gujral, 2010). Moreover, the high nutritional value of whole barley grain, including protein, fats, nutrient fibers, starches, minerals, β-glucans, and antioxidants such as polyphenols and vitamin E, is often used (Ju et al., 2007; Dvorakova et al., 2008).

The great content of bioactive compounds in barley is exciting as antioxidants because of the ability to act as free radical scavenging (Eksiri et al., 2014), hydrolytic and oxidative enzyme inhibition, reduction agents, pro-oxidant metals chelating, individual oxygen (Sharma and Gujral, 2010; Mahmoudi et al., 2015). The numerous possible chemo-preventive compounds called phytochemicals are partly the cause of these health benefits (Rui, 2007; Holtekjolen et al., 2008; Dauqan et al., 2011).

The best techniques for improving the nutritional pattern of seed grains for digestibility and physiological functions, as used for the production of various foodstuffs, were generally accepted as cost-effectiveness and straightforward processing techniques such as soaking and malting seed (Warle et al., 2015; Senhofa et al., 2016). Desirable nutritional changes may have occurred during malting that was primarily due to the breakdown into a more straightforward type of complex compounds and their transformation into necessary components (Nonogaki et al., 2010). The period of germination increases enzyme activity and the number of bioactive seed compounds releasing energy from stored carbohydrates, lipids, and the degradation of starch, while starch degrading enzymes are synthesized (Lee et al., 2017). The total cereal protein content slightly increases during germination as other kernel components that become more intensively depleted for respiration. There was a significant decrease in carbohydrates and fat content that shows rapid degradation as alluded to in the study (Kaukovirta-Norja et al., 2004). The overall phenolic and antioxidant activity of germinated cereal grains are also more significant than that of ungerminated grain (Senhofa et al., 2016). As for (Yangcheng et al., 2016), it has been recommended as an incredibly healthy supplement for the prevention of various diseases. Malt exhibits therapeutic properties such as reduction of blood sugar levels, bowel disease control, enhancement of lactability, anti diarrheal, hair reinforcement, and prevents it from becoming gray (Namaghi and Ghaboos, 2010). Therefore, the research aimed to determining the effect of soaking and malting processes on
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nutrients and bioactive compounds in selected Egyptian barley varieties.

**MATERIALS AND METHODS**

**Materials**

The barley grains (*Hordeum vulgare* L.) included in this study were three Egyptian barley varieties; Giza128 (2 rows, hulled), Giza130 (6 rows, hull-less), and Giza 2000 (6 rows, hulled) were procured from Agricultural Research Center, Giza (ARC).

**Chemicals**

All chemicals were purchased from Sigma – Aldrich (St Louis, MO, USA).

**Preparation of samples**

**Raw seeds:** Whole dry barley seed was manually cleaned from broken seeds, dust, and other foreign materials and ground in a laboratory grinder to obtain fine flour. The powdered samples were then kept in sealed plastic pages and stored at -22 °C in a deep freezer until analysis.

**Soaking process:** Samples of cleaned barley seeds were soaked in distilled water for 12 h at room temperature (water was changed every six h). The seed/water ratio was used (1:5) (w/v).

The soaked seeds were washed twice with water, followed by rinsing with distilled water (Afify et al., 2011).

**Germination process:** Other part of the soaking seeds (12 h) were placed on wet laboratory paper and covered in germination trays, where water circulation was provided by capillarity. The trays were placed in the germinator (Model No. 549/A, Seedburo Equipment Company, Chicago, USA), the germination process occurred for (24, 48, 72, and 96 h) at temperature 20-25°C. The seeds were rinsed every 12 h with a solution of 0.3% sodium hypochlorite to inhibit microbial growth. The soaked and germinated seeds was dried in a hot-air oven at 55°C for 24 hours, then ground into a fine powder with a laboratory grinder at 71°C for the same time (a constant weight). The powdered samples were kept in sealed plastic pages and stored at -22°C in a deep freezer until further analysis (Abdel-Gawad, 1991; Cevallos-Casals and Cisneros-Zevallos, 2010).

**Analytical methods:**

**Gross chemical composition**

Moisture, crude fat, protein, fiber, and ash of the samples were determined according to (AOAC, 2012). The total carbohydrate was calculated by the difference. All determinations were in three replications, and the means were reported. The caloric value was calculated as follows: [(9 × fat) + (4 × carbohydrates) + (4 × protein)], as described by Nwabueze (2007).

**Determination of total phenolic compounds (TPCs)**

The Folin-Ciocalteu method (Taga et al., 1984), with some modification, was used for determining total crude phenolics. The phenolic concentrations were expressed as milligram of gallic acid equivalents (GAE) per100 gram of dry weight basis (mg GAE/100g) through the calibration curve with gallic acid. All samples were analyzed in three replications.

**Determination of total Flavonoids compounds**

The colorimetric method of aluminum chloride was used in evaluating flavonoids according to the procedure described by Prasad et al. (2010). The absorbance of the mixture reaction was measured at 510 nm on a UV/Visible 6850 spectrophotometer (UV/Visible 6850, Jenway, UK). The concentration of flavonoids was expressed in terms of mg quercetin (QE)/100g of dry weight basis.

**Determination of Antioxidant Activity**

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) test was performed according to the way described by Lee et al. (2003). Scavenging activity was then calculated as follows:

\[
\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Ab control} - \text{Ab sample})/\text{Ab control}]}{100}
\]

Where Ab is the absorbance value at 515 nm.

**RESULTS AND DISCUSSION**

**Gross Chemical Composition of Samples**

The effect of soaking and germination process on the proximate chemical composition of different barley varieties, namely (Giza128, Giza130, and Giza 2000), soaked and after germination for 1, 2, 3, and 4 days were studied. Table (1) and Fig. (1, 2, and 3) show the change percentages of chemical composition in untreated and treated samples. The crude fiber and protein contents were increased as a result of soaking and germination samples. In contrast, after soaking and germination, crude fat, total carbohydrate, and ash contents were decreased.

The data revealed that germinated barley flour (96h) Giza 130 (hull-less barley) has the highest level of protein content (13.3%), while raw barley flour of Giza 2000 (hulled barley) recorded the lowest percentage (8.47%). Similar results were recorded by Youssef et al., 2013; Warle et al., 2015. Table (1) and Fig. (1, 2 and 3) showed that the protein content during the germination was slightly increased compared to crude samples. The results obtained agree with those mentioned by (Youssef et al., 2013; Senhofs et al., 2016). The increase in protein could be attributed to the dry weight losses through respiration during malting; thus, the germinated seeds on a unit weight basis would contain more seeds and, therefore, more nitrogen than the ungerminated material Tian et al. (2010). The crude fat contents were 3.15, 3.86, and 2.15 % on a dry weight basis in Giza128, Giza130, and Giza 2000 barley flour, respectively. These findings correspond to those reported by (Makkeri et al., 2013; Youssef et al., 2013). Likewise, it could be seen from Table (1) and Fig. (1, 2 and 3) that soaking and germination for different periods recorded a slight decrease in crude fat content. The results were approved with (Youssef et al., 2013; Warle et al., 2015). The decrease occurred because fat and fatty acids are oxidized to carbon dioxide and water to generate energy for germination Hahn et al. (2008). In the present study, crude fiber, ash, and carbohydrates were ranged from 3.53 to 6.79 %, 1.63 to 2.71 %, and 77.57 to 81.15 %, respectively. The crude fiber content was higher (6.79%) in germinated barley flour (96h) in (Giza 2000) compared with other samples, as well as the fiber content was significantly higher in hulled barley (Giza128 and Giza 2000) than hull-less barley (Giza 130). Similar results occurred by (Biel and Jacyno, 2013; Ghafoor et al., 2015). Also, data in Table (1) and Fig. (1, 2 and 3) showed that the crude fiber was increased after soaking and germination, especially in finished malt compared with raw samples. These results agree with those reported by (Warle et al., 2015). The result indicated that the ash content of whole kernels was significantly higher in hulled barley than in hull-less barley varieties. The results were approved by Quinde et al. (2004). On the other hand, data showed that the highest ash
content has occurred in raw barley, then it was decreased during soaking and germination. These findings are in line with those (Youssef et al., 2013; Warle et al., 2015). The decrease in ash content represents the loss in minerals due to rootlet and washing of the barley in water to reduce the sour smell during the period of germination (Tatsadjieu et al., 2004). This reduction could be due to leaching of solid matter in soaking water (Ghavidel and Prakash, 2007). Data also showed a slight decrease in carbohydrates content (free nitrogen extract) in germinated barley varieties (96 h) (Giza 128, Giza 130 and Giza 2000), recording (77.88, 77.57, and 79.19), respectively if it compared with raw samples. The findings obtained correspond well to those of Youssef et al. (2013). There was a negative relationship between carbohydrates and protein content of barley grain. Such a relationship appeared in the present study, especially in raw barley flour (Giza 2000), which had the highest percentage of carbohydrates (81.15%) and the lowest percentage of protein (8.47%). Data showed that raw Giza 130 recorded the highest caloric value (395.77) followed by soaked Giza 130, which recorded (395.23) while germinated (Giza 2000) recorded the lowest caloric value (372.82 Kcal). In this respect, similar results were reported by Youssef et al. (2013).

Table 1. Gross chemical composition and Caloric value of raw, soaked, and germinated barley.

<table>
<thead>
<tr>
<th>Vari.</th>
<th>Treat.</th>
<th>Moisture %</th>
<th>Protein%*</th>
<th>Ash%*</th>
<th>Fiber%*</th>
<th>Fat%*</th>
<th>Free Nitrogen Extract%**</th>
<th>Caloric value (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>10.73±0.34</td>
<td>9.68±0.31</td>
<td>2.65±0.01</td>
<td>4.37±0.05</td>
<td>3.15±0.21</td>
<td>80.15±0.54</td>
<td>387.64±0.83</td>
<td></td>
</tr>
<tr>
<td>Soaking</td>
<td>10.03±0.25</td>
<td>10.22±0.93</td>
<td>2.43±0.05</td>
<td>4.47±0.06</td>
<td>3.09±0.18</td>
<td>79.78±1.07</td>
<td>387.82±0.94</td>
<td></td>
</tr>
<tr>
<td>Giza 24h</td>
<td>9.04±0.13</td>
<td>10.94±0.69</td>
<td>2.27±0.24</td>
<td>4.68±0.12</td>
<td>2.98±0.3</td>
<td>79.13±0.72</td>
<td>387.11±2.46</td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>8.01±0.37</td>
<td>11.46±0.08</td>
<td>2.13±0.29</td>
<td>4.89±0.1</td>
<td>2.87±0.36</td>
<td>78.65±0.71</td>
<td>386.27±1.08</td>
<td></td>
</tr>
<tr>
<td>Germinated 24h</td>
<td>7.24±0.15</td>
<td>11.79±0.27</td>
<td>2.07±0.36</td>
<td>5.01±0.16</td>
<td>2.74±0.5</td>
<td>78.39±0.93</td>
<td>385.39±2.76</td>
<td></td>
</tr>
<tr>
<td>Giza 96h</td>
<td>5.92±0.27</td>
<td>12.07±0.24</td>
<td>1.98±0.13</td>
<td>5.53±0.11</td>
<td>2.55±0.43</td>
<td>77.88±0.16</td>
<td>382.71±2.63</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8.51±</td>
<td>11.03±</td>
<td>2.25±</td>
<td>4.83±</td>
<td>2.89±</td>
<td>78.99±</td>
<td>386.16±</td>
<td></td>
</tr>
</tbody>
</table>

The changes of gross Chemical composition in barley samples (Giza 128), after soaking and different periods of germination is shown in Fig. 1. The changes of gross Chemical composition in barley samples (Giza 130), after soaking and different periods of germination is shown in Fig. 2.
The effect of soaking and germination on phenolic compound contents in barley grain samples are shown in Table (2). Giza130 variety has higher phenolics contents than other varieties. Phenolic compounds contents of raw barley seeds were; 336.2, 461.8, and 460.5 mg GAE/100g in Giza128, Giza130, and Giza 2000, respectively. These results agreed with those mentioned by (Holtekjølen et al., 2011). The phenolic content was significantly higher in six-row barley (Giza 130 and Giza 2000) than two-row barley (Giza128) seeds. Similar results occurred in the study by (Gamal and Abdel-Aal, 2012; Lahour et al., 2014).

Soaking of barley samples 12 h led to increased total phenolic compounds content compared with control. The increment ratios were, 20.3, 7.6, and 10.7%. During the 48-hour germination process, the varieties Giza 128, Giza 130, and Giza 2000 increased by 69, 41.6, and 37%, respectively, of its initial control value. Similar results were reported by (Duenas et al., 2009; Lee et al., 2017). The synthesis of amylases, proteases, and α-glucanases which causes polymer degradation and other hydrolytic enzymes. Thus, these enzymes can lead to the release of bound phenolic compounds, mainly the phenolic acids associated with lignin and arabinoxylans (Maillard and Berset, 1995; Maillard et al., 1996). Total phenolic compounds were increased continuously with germination time (48h), then slightly decreased with the further of malting was occurred. Ha et al. (2016) mentioned that during the progress of germination, phenolic compounds are becoming available and are more easily extracted. After 48 h, lignification is initiated, resulting in the decreased total phenolic content and observed antioxidant and carbohydrate hydrolyzing enzyme inhibition activities.

**Table 2. Effect of soaking and germination process on phenolic content in barley seeds (mg/100g,dw).**

<table>
<thead>
<tr>
<th></th>
<th>Raw</th>
<th>Soaking 12h</th>
<th>Germination</th>
<th>Mean of Vari.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Giza 128</td>
<td>336.2±8.95</td>
<td>404.32±2.21</td>
<td>482.56±1.06</td>
<td>505.41±3.41</td>
</tr>
<tr>
<td>Giza 130</td>
<td>461.8±0.67</td>
<td>497.12±1.48</td>
<td>569.07±1.01</td>
<td>589.61±3.46</td>
</tr>
<tr>
<td>Giza 2000</td>
<td>460.51±1.28</td>
<td>509.04±8.02</td>
<td>541.05±3.97</td>
<td>632.24±5.55</td>
</tr>
<tr>
<td>Mean of Treat.</td>
<td>419.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>470.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>530.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>618.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>L.S.D 5%</td>
<td></td>
<td></td>
<td></td>
<td>2.845</td>
</tr>
<tr>
<td>vari.</td>
<td>Treatment</td>
<td>4.109</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vari.</td>
<td>*Treatment</td>
<td>7.116</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±: standard deviation (SD) on a dry weight basis.

<sup>**</sup> Values in the same column and the same row with different superscript letters different significantly at a 5% level of significance.

**Flavonoids compounds:**

The effect of soaking and germination on flavonoid content in selected Egyptian barley cultivars is shown in Fig. (5). In untreated samples, Giza130 has higher in flavonoids contents than other investigated samples. Flavonoids content was 35.31, 58.11, and 31.31 mg QE/100g for raw Giza128, Giza130, and Giza 2000, respectively. The flavonoid contents were significantly higher in hull-less barley than hulled barley. Results obtained are in the same line with those reported by (Abidi et al., 2015; Lee et al., 2017). Results indicated that soaking for 12 h could increase the level of flavonoid contents below the control value. Soaking for 12h showed a significant increase in flavonoid contents of samples by 19.3, 11.7, and 17% while, germination process for 48 h increased it significantly by 62.6, 30.3, and 43% for Giza128, Giza130, and Giza 2000, respectively of the control. The results agree with (Lee et al., 2017; Duenas et al., 2009) and similar to those reported by Kim et al. (2012a). During the germination of cereal, the contents of bioactive compounds are known to increase because of the activation of hydrolytic enzymes (Tian et al., 2005).

**Fig. 5. Effect of soaking and germination process on flavonoids content in barley seeds**

**Total Antioxidant Activity (DPPH %):**

Data in Fig. (6) show the effect of soaking and the germination process on antioxidant activity in different Egyptian barley varieties. Giza130 variety has higher in...
antioxidant activity contents than other investigated samples. Antioxidant activity in raw Giza128, Giza130, and Giza 2000 varieties were; 29.45, 37.08, and 28.58 %, respectively. Similar results were given by (Sharma and Gujral, 2010; Abid Aljanabi and Al Abdullah, 2018). Fig. (6) summarizes the changes in total antioxidant activity during soaking and germination processes. Soaking of samples in distilled water for 12 h increases antioxidant activity compared with control. Antioxidant activity was increased by 13.3, 21and 10.5% of its initial values of control in Giza128, Giza130, and Giza 2000, respectively. While the germination process of samples led to a significant increase in antioxidant activity and the significantly increasing was during germination for 48h, then a slightly decreased with the further of malting process has occurred. The increasing level was 36, 65, and 39 % for Giza128, Giza130, and Giza 2000 of its initial value in control after 48 h of germination, respectively. These results are in line with those mentioned by (Sharma and Gujral, 2010; Ha et al., 2016; Abid Aljanabi and Al Abdullah, 2018).

These observations are expected because during the germination process, the plant is producing defense components against environmental stress (Antioxidant Activity) and phenolic phytochemicals have such characteristics Kaukovirta-Norja et al. (2004). These observations are not surprising since non-germinated extract had the lowest phenolic content, while 48 h germinated extract had the highest total phenolic and flavonoid contents. It is very well demonstrated that these bioactive compounds have antioxidant activity and our findings agree with this fact. The 48 h water extract of barley germination had significantly higher total phenolic and flavonoid contents, resulting in a higher total antioxidant activity.

![Fig. 6. Effect of soaking and germination process on antioxidant activity in barley seeds](image)

**CONCLUSION**

It could be concluded that the changes in the chemical composition, bioactive compounds, and antioxidant activity in barley flour from different cultivars have been affected by the soaking and malting process.

The soaking and germination process caused a considerable increase in the level of antioxidant compounds and other nutrients in the Egyptian barley cultivars under investigation. The germination process for 48h of barley grain proved to have high levels of bioactive compounds, nutrient composition, and antioxidant capacity, which recorded higher value compared with the control. The antioxidant activity of barley seems to be somewhat related to the total phenolic and flavonoid contents. Therefore, a barley cultivar that shows a bioactive compound profile with the most antioxidative compounds may be more desirable for choice. After 48 h of germination, it can be observed a decrease in the phenolic content and a subsequent decrease in flavonoid compounds. Our results suggested that phenolic compounds up to 48 h germination may play a role in increasing antioxidant activities of barley grain cultivars. The content of the bioactive compound depended significantly on the variety of barley and corresponding malt. Also, this process was essential to improve the nutritional properties of barley and effectively utilize their full potential as human food.

**REFERENCES**


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تأثير عمليات النقع والانبات على التركيب الكيميائي والمركبات الفعالة ومضادات الأكشمة في بعض أصناف الشعير المصري

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في الأونة الأخيرة ازداد الطلب على الأغذية الطازجة مثل الشعير، وتعتبر عمليات النقع والانبات من العمليات الغذائية التي تؤثّر على التركيب الكيميائي والمركبات الفعالة. هذه الدراسات تدعم ذلك، حيث أن عمليات النقع والانبات تؤثّر على تركيب الشعير، مما يؤدي إلى تغيير في المركبات الفعالة والمضادات الأكشمة. 

في هذه الدراسة، تم تضمين ثلاثة أصناف من الشعير وهي جيزة مصر، أسيوط، أسوان، وشغلت هذه الدراسة سبعة عينات. الأصناف تم معالجتها بطرق مختلفة، بما في ذلك تشغيل، مرطبًا، ونواع. تم تحليل كل عينة باستخدام تقنيات متعددة، بما في ذلك مسح ترقيق الحمضات، والتفاعلات الكيميائية في المونوجل، والتفاعل الكيميائي في الخلايا. 

النتائج تظهر أن العمليات الكيميائية تؤثّر على تركيب الشعير، حيث أن التغيرات في المركبات الفعالة والمضادات الأكشمة تؤثّر على خصائص الشعير. بالإضافة إلى ذلك، يتم تدفق المواد الكيميائية من الخلايا إلى الخلايا المجاورة، مما يؤدي إلى تغيير في التركيب الكيميائي والمركبات الفعالة. 

توصيات الحالة: من المهم تحسين الطرق والتقنية المستخدمة في عمليات النقع والانبات للحد من تأثيره على خصائص الشعير، وتقليل تغيرات المركبات الفعالة والمضادات الأكشمة. من أجل ذلك، يوصى بتطوير الأساليب التي تساعد على الحفاظ على خصائص الشعير وتحسين جودته.

References


