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Impact of Soaking and Germination Processes on Starch and Non-Starch Polysaccharides in some Egyptian Barley Cultivars

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ABSTRACT

The objective of this study was to evaluate the effect of soaking and germination processes on free sugars, starch and non- starch polysaccharides contents of selected Egyptian barley cultivars. Three barley grain cultivars, namely, Giza128, Giza130, and Giza 2000, were investigated. Reducing, non-reducing and total sugars content were increased in all treated barley samples as affected by soaking and germination processes. Starch content of raw barley grains being 59.42, 65.30, and 45.74 % in Giza128, Giza130, and Giza 2000, respectively, while β-Glucan content recorded 4.01, 4.76 and 3.54 %, respectively. Pentosans content in untreated barley samples was 5.09, 3.29 and 4.53% of the same varieties, respectively. Soaking (12 h) for these grains decreased starch, β-Glucan, pentosan content by 2.9-5.3, 5.0- 7.6 and 2.4-6.7%, respectively. Also, germination process had a great efficiency role on the starch and non- starch polysaccharides of selected barley grains. A large number of negatively valued components in the grains such as starch and, β-Glucan, pentosan contents of barley decreased prolonging germination time. After 96h of germination, starch, β-Glucan, pentosans content of barely grain samples was decreased by 27.3-32.0, 41.0- 42.7 and 33.6-36.0%, respectively. So this study recommended that, the produced flour from soaked and 48h-germinated barley grains increase their nutritional and therapeutic properties can be used to produce improved functional foods.

Keywords: Barley, Soaking, Germination, Starch, Reducing sugar, β-Glucan, Pentosan.

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 (158.98 million ton), with about 100.43 thousand ton annually Egyptian production, and cultivation area for the 2019 world cereal crop rankings, which amounted to 510,000 km^2 for year of the 2019 (FAO, 2021). Typically, barley is categorized as 2-rowed and 6-rowed barley are available and hulled or hull-less (Gangopadhyay *et al*., 2015).

Approximately 65% of cultivated barley is used for animal feed, 33% for malting, whereas only 2% is used directly for human consumption (Sullivan *et al.*, 2013; Alazmani, 2015). Barley is easily digestible (due to low gluten contents) and has superior nutritional qualities. Moreover, Barley is nutritionally rich because it has a high carbohydrate content, moderate protein level and high dietary fiber content especially β-glucan (Kumari and Kotecha, 2015). Starch is the main component in barley endosperm, being 62–77% of total grain weight also, it contains around 5–10% β- glucan (Asare *et al.*, 2011; Sullivan *et al.*, 2013).

The major structural component in barley endosperm cell walls is mixed linkage (1→3), (1→4)-β-D-glucan, generally referred to as β-glucan. Barley endosperm cell walls are composed of approx. 70–75% β-glucan, the remainder made up of 20% arabinoxylan and minor amounts of cellulose, glucomannan, protein, and phenolic constituents (Jamar *et al.*, 2011). Actually, Barley's non-starch polysaccharides (β-glucans and arabinoxylans) are the minor components of the barley grain (15 to 17%) (Cornejo, 2005). β-glucan reduce the risk of colon cancer, controls blood glucose better and lowers glycemic index as well as improve the insulin response (Östman *et al*., 2007). The United States Food and Drug Administration (FDA) have allowed whole-grain barley products that can supply β-glucan at levels of 0.75 g per serving or 3 g per day to carry a claim that they reduce the risk of coronary heart disease (Anonymous, 2005). The best techniques for improving the nutritional pattern of barley 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 germination grains (Warle *et al.*, 2015; Senhofa *et al.*, 2016). Desirable nutritional changes may have occurred during germination 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). During germination, the activation of dormant enzymes leads to significant changes in biochemical, nutritional and sensory characteristics. The α- and β-amylases rapidly degrade the starch, with a consequent surge in reducing form, while other enzymes degrade cell walls and enhance the availability of internal nutrients (Olaerts and Courtin, 2018). However, some compounds deemed beneficial, such as beta-glucans and

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pentosans might be degraded during long time of germination and adapt the germination process for the respective purpose (Hubner and Arendt, 2013). Beyond improving the health properties of the products, it is assumed that the β-glucans from barley in combination with arabinoxylans may improve some quality parameters of flour (Storsley *et al.*, 2003). Therefore, this study aimed to identify the effect of soaking and germination processes starch and non- starch polysaccharides as well as improving nutritional value of produced flour from Egyptian barley grains varieties.

MATERIALS AND METHODS

Materials

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

Chemicals

All chemicals used in this study were purchased from Sigma – Aldrich (St Louis, MO, USA). Other chemicals were either of analytical grade or of the highest quality was purchased from El-Gomhoria Company, Egypt.

Preparation of barley samples

Raw seeds: Whole dry barley grains was manually cleaned from broken grains, 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 until analysis.

Soaking process: cleaned barley grains were soaked in distilled water (1:5) (w/v) for 12 h at room temperature (water was changed every six h). The soaked grains were washed twice with water, followed by rinsing with distilled water (Afify *et al.*, 2011).

Germination process: other part of the soaking grains (12 h) were placed on wet filter 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), in darkness for 24, 48, 72, and 96 h at temperature 20-25°C. The grains were rinsed every 12 h with a solution of 0.3% sodium hypochlorite to inhibit microbial growth. The soaked and germinated grains were dried in a hot-air oven at 55 °C for 24 h, then at 71 °C for the same time, and then ground into a fine powder with a laboratory grinder at 71 °C for the same time. The powdered samples were kept in sealed plastic pages and stored at -22°C until further analysis (Abdel-Gawad, 1991; Cevallos-Casals and Cisneros- Zevallos, 2010).

Analytical methods

Determination of starch, reducing and non-reducing sugars

Starch content of the samples were determined according to (AOAC, 2012).The water extract of the studied samples firstly was clarified by adding lead acetate to precipitate non sugars and then excess lead acetate was precipitate by adding potassium oxalate and filtrated to remove non sugars (AOAC, 2012). Reducing and total sugars were estimated by titration method according to Lane and Eynon method as mentioned in AOAC (2012). Non-reducing sugars was calculated by difference (Non-reducing sugars = total sugars- reducing sugars).

Determination of Beta-Glucan

β-Glucan was extracted from whole barley flour using procedure described by Temelli (1997). Extractable were milled to pass a 1.0 mm screen and hydrolysis with sulfuric acid according to McCleary and Draga (2016). The content of glucose in the solutions was analyzed by HPLC. The analysis was performed in National Research Center, Cairo, Egypt.

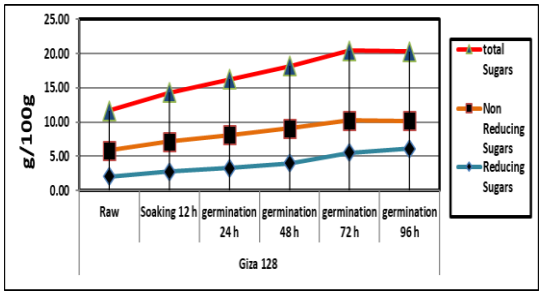
Determination of total pentosans

Total pentosan content was determined by colorimetric phloroglucinol method according to Douglas (1981).

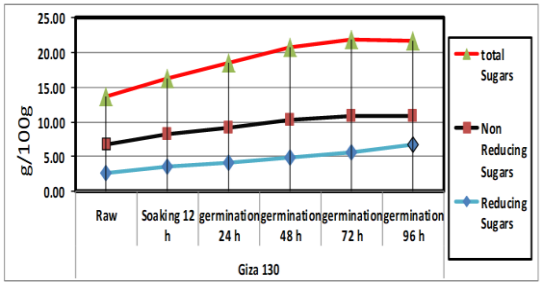
RESULTS AND DISCUSSION

Reducing, non-reducing and total sugars contents

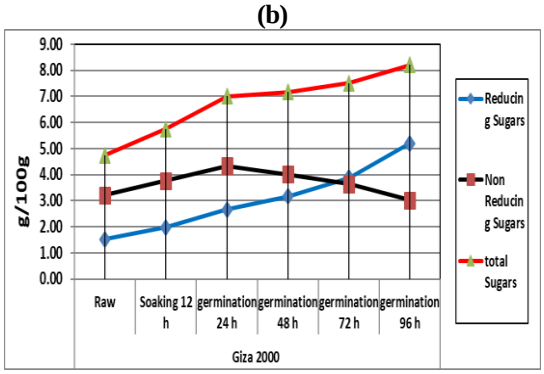
The effect of soaking and germination processes on reducing, non-reducing and total sugars contents in studied barley grains varieties are illustrated in Fig. (1). Reducing sugars contents in raw Giza128, Giza 130, Giza 2000 were 2.02, 2.69 and 1.52 g/100g, respectively. Non-reducing sugars were 3.8, 4.13 and 3.22%, while total sugars were 5.82, 6.83 and 4.74% of the same varieties, respectively. Giza 130 variety has higher reducing, non-reducing and total sugars content than other barley varieties. These results are in the line with those mentioned by Balcerek *et al.* (2016).



(a)



(b)



(c)

(a) Giza 128 (b) Giza 130 (c) Giza 2000

Fig. 1a,b,c. Effect of soaking and germination process on Reducing, Non-reducing and Total Sugars contents in barley grains varieties.

There were increases in reducing, non-reducing and total sugars content in soaked barley varieties (12 h), recording (2.77, 3.47 and 1.97%)for Giza 128, (4.36,4.68 and 3.77%) for Giza 130 and (7.13, 8.15 and 5.75) for Giza 2000), respectively. The reduction of starch content was due to the activity of α–amylase enzymes during germination process and causes an increase in total and reducing sugars (Tian *et al*., 2010).

A significant increase was observed in reducing and total sugars content of barley grains varieties due to germination compared with un-germinated grains. Reducing sugars content increased from 2.02 to 6.11%; 2.69 to 6.72 and 1.52 to 5.20% for Giza128, Giza130, and Giza 2000 of its initial value after 96h of germination, respectively. Total sugars content increased from 5.82 to 10.15%; 6.83 to 10.83 and 4.74 to 8.21% for Giza128, Giza130, and Giza 2000 of its initial value after 96h of germination, respectively. The germination process of barley grains led to a slightly increase in non-reducing sugars in germinated for 48h, then decreased with the further of germination process has occurred. Similar results were given by Warle *et al.* (2015). During germination, the reducing sugar content increased gradually with a marked decrease in starch contents, these changes probably were caused, in part, by the increased enzymatic activity during germination (Arif *et al.*, 2011).

Starch contents in barley grains

The results in Table (1) shows the effect of soaking and germination processes on the content of starch in barley grain varieties. Starch content was 59.42, 65.30 and 45.74% for Giza128, Giza 130, Giza 2000, respectively. It can be observed that, hull-less barley (Giza 130) grains was significantly higher level of starch compared with the other hulled varieties. Similar finding was reported by Abdel-Gawad *et al.* (2016).

Soaking in distilled water for 12h of barley grain varieties under investigation lowered starch content in all samples compared with control (Table 1). The reductions of starch contents were 5.25, 2.9 and 3.8 % for Giza128, Giza 130, Giza 2000, respectively. The loss in starch level during soaking may be attributed to leaching of the water-soluble inhibitor into the soaking medium.

Moreover, There was a significant ($P<0.05$) decrease in barley starch content during germination periods progress up to 96h (Table 1). The reduction of starch content was 10.87- 30.64, 8- 27.27 and 9-32 % for Giza128, Giza 130, Giza 2000, respectively of its initial value in raw grains. Decreasing starch content is due to the activation of starch-degrading enzymes during germination, primarily α-amylase and β-amylase (Pinkaew *et al.*, 2017). Typically, there is an inverse relationship between crude protein and starch content. The significant increase in crude protein content in the early germination stages could be led to the losses of non-protein dry matter as a consequence of intensive imbibition (Yu *et al.*, 2019).

Table 1. Effect of soaking and germination processes on starch contents in barley grain varieties. (%, DW):

Barley sample	Raw grains	Soaking process 12 h	Germination process 24 h	48 h	72 h	96 h
Giza 128	59.42^{bc}± 3.09	56.3 ^{cd}±2.58	52.96 ^{d}±2.80	47.19 ^{e}±2.42	42.56 ^{fgh}±1.90	40.02 ^{gh}±2.78
Giza130	65.30 ^{a}±1.53	63.4 ^{ab}±2.65	60.08 ^{bc}±4.22	56.37 ^{cd}±1.82	52.89 ^{d}±1.78	47.49 ^{e}±2.94
Giza 2000	45.74 ^{ef}±3.08	44.00 ^{efg}±3.41	41.59 ^{fgh}±1.91	38.85 ^{h}±2.51	33.99 ^{i}±1.93	31.18 ^{i}±2.95
LSD 5%			4.3900			

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

$^{a-i}$Values in the same table with different superscript letters represent significant differences between results at a 5% level of significance.

β-Glucan contents

Figs. (2 and 3) illustrate the concentrations of Extractable β-Glucan and β-Glucan in untreated and treated barley grains varieties.

Among untreated barley samples, Giza130 has higher in extractable β-glucan and β-Glucan contents than other investigated varieties. Extractable β-Glucan content were 3.68, 3.96 and 3.07 %, While, β-Glucan concentrations were 4.01, 4.76 and 3.54% in raw Giza128, Giza130, and Giza 2000, respectively. The Extractable β-Glucan and β-Glucan contents were significantly higher in hull-less barley than hulled barley. Results obtained are in the same line with those reported by Sharma and Gujral (2010); Waly (2017).

The extractable β-glucan and β-glucan content of barley grains significantly decreased by soaking process (Figs. 2 and 3). The reduction of the extractable β-glucan content was 5.4, 7.6 and 6.2%, while, it was 5.0, 7.6 and 6.5 % for β-glucan of Giza128, Giza130, and Giza 2000, respectively. The decrease in β-glucan content depends on the synthesis and distribution of endo-(1→3), (1→4)-β-glucanase activity during germination (Wang *et al.*, 2004).

Germination time progress caused a significant decrease in extractable β-glucan and ß-glucan contents continuously with germination time if compared with that of raw grains samples (Figs 2 and 3).

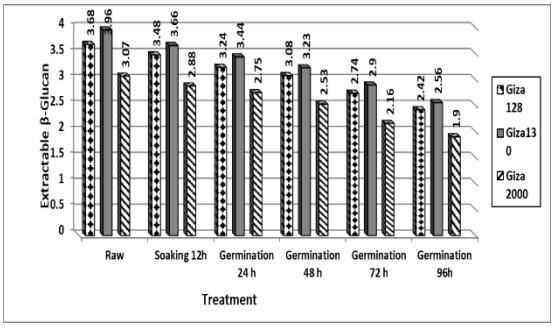


Fig. 2. Effect of soaking and germination process on Extractable β-Glucan contents in barley grains.

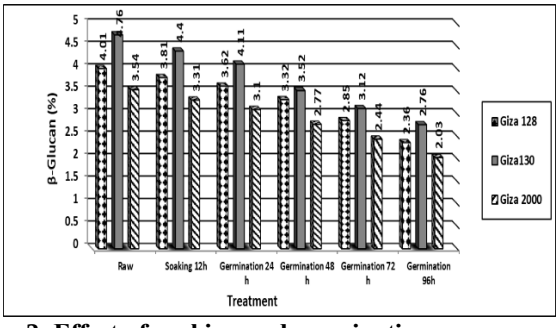


Fig. 3. Effect of soaking and germination process on β-Glucan contents in barley grains.

The decrement ratio of extractable β-glucan was 12.00- 34.24, 13.13-35.35 and 10.42- 38.11 % in Giza128, Giza130, and Giza 2000, of its initial content in raw materials after 24- 96h of germination; respectively, however, the reduction of β-glucan content was 9.7-41, 13.7- 42.0 and 12.4- 42.7%, respectively. From these results it can be observed that, a sharp decrease in this compound occurred during the 96h- germination. β-glucan levels in barley grains decreased to a short extent during germination for short times, but longer germination times cause greater losses. The breakdown and degradation of β-glucan is due to activation of β-Glucanase, which are responsible for breaking down cereal endosperm cell walls during soaking and germination processes.

On the contrary, larger reductions of β-glucan content (50%) was found after 48–120 h of germination in barley grains (Farzaneh *et al.,* 2017; Rico *et al.,* 2020).

Pentosans content:

Pentosan content was 5.09, 3.29 and 4.53% of Giza128, Giza 130, Giza 2000 raw barley grains, respectively (Fig 4). Results indicated that, hull-less barley (Giza 130) grains was significantly lower level of pentosan compared with the other hulled varieties. Similar finding was reported by Abdel-Gawad *et al.* (2016). Soaking process (12h) caused a slight decrease in the pentosan content of barley samples compared with control. The decrement ratio was 2.4, 6.7, and 5.1%; for Giza128, Giza130, and Giza 2000, respectively. Besides, data revealed that, with the germination time increase, the pentosan content of these samples significantly decreased, reached up to 3.29, 2.11 and 3.01 %, respectively, after 96h-germination, of its initial value in control. These results agree with those reported by (Kołodziejczyk and Michniewicz, 2004; Donkor *et al.* (2012).

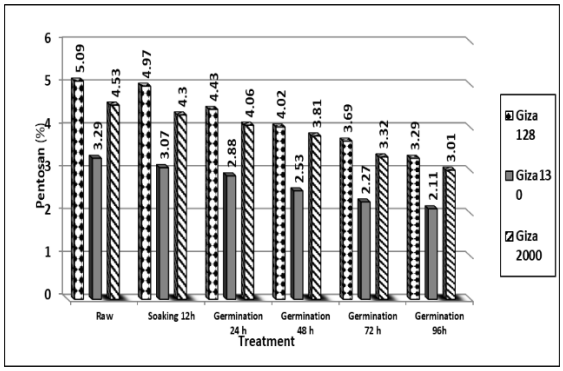


Fig. 4. Effect of soaking and germination processes on pentosan content in barley grains.

CONCLUSION

The soaking and germination process caused a considerable decrease in the level of starch and non-starch polysaccharides and increasing the values of reducing, non-reducing and total sugars content in the Egyptian barley cultivars under investigation. Soaking and germination processes for 48 h led to a slight decrease in the most of these compounds. A major breakdown and degradation occurred with further of germination time. After 48 h of germination, it can be observed high decrement in starch and non-starch polysaccharides. These processes were essential to improve the nutritional properties of barley and effectively utilize their full potential as human food.

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تأثير النقع والإنبات على النشا والسكريات العديدة الغير نشوية في بعض اصناف الشعير المصري

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تهدف الدراسة الى تقييم تاثير معاملات النقع والانبات على السكريات الحرة والنشا والسكريات العديدة الغير النشوية فى بعض اصناف الشعير المصرى حيث تمت الدراسة علي ثلاثة أصناف من حبوب الشعير وهي (جيزة ١٢٨ ، وجيزة ١٣٠ ، وجيزة ٢٠٠٠) . وقد لوحظ زيادة فى محتوى جميع العينات من السكريات المختزلة وغير المختزلة والكلية حيث تأثرت بعمليات النقع والإنبات. فقد بلغ محتوى النشا في حبوب الشعير الخام ٥٩,٤٢ و ٦٥,٣٠ و ٤٥,٧٤٪ في أصناف جيزة ١٢٨ و جيزة ١٣٠ و جيزة ٢٠٠٠ على التوالي بينما سجل محتوى البيتا جلوكان ٤,٠١ و ٤,٧٦ و ٣,٥٤٪ على التوالي. وكان محتوى البنتوزان في عينات الحبوب غير المعاملة ٥,٠٩ و ٣,٢٩ و ٤,٥٣٪ من نفس الأصناف على التوالي. وقد أدت عملية النقع لمدة ١٢ ساعة من هذه الحبوب إلى انخفاض في محتوى النشا والبيتا-جلوكان والبنتوزان بنسبة ٢,٩-٥,٣ و ٥,٠- ٧,٦ و ٢,٤-٦,٧٪ على التوالي. و كان لعملية الإنبات دور كبير في رفع الفاعلية للسكريات النشوية وغير النشوية لحبوب الشعير المختارة.ولقد تلاحظ انخفاض كبير فى قيمة المكونات ذات التاثير السلبى في الحبوب مثل النشا والبيتاجلوكان و البنتوزان من محتويات الشعير مع إطالة مدة الإنبات إلي ٩٦ ساعة ، و ايضا انخفض محتوى النشا والبيتا-جلوكان والبنتوزان لعينات الشعير بنسبة ٢٧,٣ - ٣٢,٠ و ٤١,٠ - ٤٢,٧ و ٣٣,٦ - ٣٦,٠٪ على التوالي. ولذا توصى الدراسة الى استخدام الدقيق المنتج من حبوب الشعير المنقوعة والمنبت لمدة ٤٨ ساعة لما له من خصائص غذائية وعلاجية لإنتاج أغذية وظيفية مرتفعة القيمة الغذائية والحيوية.