

Research Article

USE OF PROTEIN AND PHENOL CONTENTS AS BIOCHEMICAL MARKERS TO MEASURE MATURITY STATUS IN WHEAT CULTIVARS

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ABSTRACT

The determination of total phenolic compounds and total protein contents were measured with one day interval up to ten days in germinating wheat cultivars of *Triticum aestivum* cv. Abusbey (bread wheat) and *T. durum* cv Alibaba (durum wheat) to determine the growth stage. The above parameters were also measured in syrups of those cultivars to determine the optimum growth curve. The values were significantly higher in cultivar Alibaba cultivar as compared to Abusbey cultivar in both measurements. The mass accumulation of protein and phenol contents started in the 7th day of germination stage in Abusbey cultivar while those contents were accumulated earlier as the 4th day of germination in Alibaba cultivar. The similar pattern was also observed in cell wall-bound (insoluble) proteins of each cultivar. The mass protein and phenol accumulation in wheat germination stage was discussed for the possible use of wheat and its possible mechanisms to reduce the damage caused by oxidative and mutagenic agents on higher organisms.

Key Words: Wheat, wheat syrup, phenol contents, protein determination, DNA damage, DNA repair.

BUĞDAY ÇEŞİTLERİNDE PROTEİN VE PHENOL İÇERİĞİNİN BİTKİ OLGUNLUĞUNUN BELİRLENMESİNDE BİYOKİMYASAL BELİRLEYİCİ OLARAK KULLANIMI

ÖZET

Birinci günden onuncu güne kadar çimlenmekte olan *Triticum aestivum* cv. Abusbey (ekmeklik) ve *T. durum* cv Alibaba (makarnalık) buğday çeşitlerinde büyüme durumunu belirlemek için birer gün ara ile toplam fenol ve protein içerikleri ölçülmüştür. Ayrıca, optimum büyüme eğrisini elde etmek için adı geçen parametreler yukarıda bahsedilen çeşitlerin şuruplarından da ölçülmüştür. Değerler Alibaba'çeşidinde Abusbey'e kıyasla her iki metotda da istatistiki olarak yüksek bulunmuştur. Protein konsantrasyonu ve fenol içeriğindeki kümülatif artış ekmeklik çeşitte çimlenmenin 7. gününde gerçekleşirken, makarnalık çeşitte 4. gün gibi daha erken bir safhada gerçekleşmiştir. Benzer durum, her iki çeşit için hücre duvarına bağlı çözünmeyen proteinlerde de görülmüştür. Buğdayların çimlenme aşamasında meydana gelen protein ve fenol konsantrasyonlarındaki artışın, oksidatif ve mutajenik etkenlerden kaynaklanan yüksek organizmalara ait hücre zararlarının azaltılmasında muhtemel kullanımı ve mekanizmaları tartışılmıştır.

Anahtar Kelimeler: Buğday, buğday şurubu, fenol içerikleri, protein belirlenmesi, DNA hasarı, DNA tamiri.

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INTRODUCTION

Antioxidants are quite important to defend the organisms against biotic and abiotic stress agents. Therefore, they are important when added to the foods during processing to improve quality and stability. Due to negative consumer perception about synthetic antioxidants, natural antioxidants have gained more importance in recent years. These natural antioxidants may also provide additional health benefits to consumers.

Oxidative stress, because of biotic or abiotic stress factors, can cause severe damages to the organisms as a result of reactive oxygen species (ROS) such as O_2 , OH , and H_2O_2 which severely damage the lipid membrane, protein structure and eventually result in DNA damage (Kocyigit et al., 2005; Dikilitas et al., 2009). Under these conditions, all organisms have to protect and repair their genomic structures especially their double-stranded DNA molecules. In normal circumstances, there is a balance between DNA damage and repair. If this balance favours towards the damage, then the structure of DNA loses its form and cannot function properly. Recently, by means of natural antioxidants, the repair mechanisms have been enforced using plant extracts such as *Allium sativum* (garlic) and *Nigella sativa* extracts (Bektas, 2010). The pretreatment of mononuclear leukocyte cells with these extracts significantly reduced the DNA damage induced by H_2O_2 .

Many studies have shown that the consumption of grains, fruits, and vegetables might reduce the risk of aging-related diseases (Zielinski and Kozłowska, 2008). For example, several studies with the antioxidant properties of wheat and other cereals have shown that the free radicals produced by the organisms after the exposure of stress were suppressed (Onyeneho and Hettiarachchy, 1992; Baublis et al., 2000).

Apart from the use of wheat and its products on the effect of antioxidant capacities, the germinating sites of wheats have also gained popularity in recent years due to their high spectrum amino acid content and at least 30 enzymes. In recent years, the use of wheat grass juice, which is a complete food that can be taken orally without side effects, is an important nutritional supplement which contains most of the vitamins, minerals and enzymes in a bioavailable form. Since it contains vitamin C, E and carotene, it is able to

destroy free radicals. Wheatgrass or wheat syrup, which is a concentrated form of wheat juice (see Materials and Methods) are reported to have an apoptotic effects on cancer cells or it may lead to apoptosis of cancer cells, which is a way to kill the unhealthy cells in a controlled way.

Based on the above observation, the objective of this study was to determine the maturity status as assessed by the concentration of protein and phenol levels of bread and durum wheats in a germination period under unstressed conditions to evaluate the possible use of these extracts to prevent the possible formation of DNA damages caused by toxicity or high levels of oxidative stress in higher organisms or in plants. Results from this study may promote the use of wheat extracts and lead to the development of wheat varieties with enhanced antioxidant properties to prevent DNA and oxidative damages in organisms. Since this study is the first step of a series of studies carried out on stressed cells, the preliminary findings of protein and phenol levels on wheat cultivars would be tested on stressed cells to find out the optimum concentration of proteins which would prevent the occurrence of DNA damage.

MATERIALS and METHODS

Germination of seeds

Wheat seeds were obtained from GAP TSKTAEM. For each cultivar, 25 seeds were germinated on two layers of Whatman No.2 filter paper moistened with sterile distilled water in a Petri dish in the dark at room temperature. Germination rates were found over 95%. After the start of germination, the seedlings were kept for 16/8 hours in the light/dark period at room temperature for each consecutive day (regarded as treatment) up to 10 days. Each treatment was carried out in triplicates.

Preparation of wheat syrup

One hundred grams of seeds each from bread and durum wheat cultivars were incubated 24 hours at room temperature in 200 ml of tap water in semi-closed jars. Then, the colored water phase was filtered through a filter paper and discarded to remove the germination and enzyme inhibiting substances; then 200 ml of fresh tap water was added to the jars containing seeds from each cultivar.

Following further incubation for 48 hours in the same conditions as described above, the supernatant of each cultivar was designated as wheat syrups.

Protein extraction

Soluble protein determination

Five germinating seedlings, ca. 0.5 g, from each treatment of each cultivar were harvested; the seedlings were then ground to powder separately in a chilled mortar and pestle in the presence of liquid nitrogen and homogenized in double-distilled water. The homogenate was centrifuged at 10000g for 10 min at 4°C. The supernatant was saved for the measurement of soluble protein.

Insoluble protein determination

Insoluble protein extraction was performed by homogenizing the pellets from the above extraction. The pellets were incubated with 1M NaCl at 4°C for 24 h and centrifuged as above (Reuveni, 1998) and the supernatant was saved for the measurement of insoluble protein.

Protein determination was made by quantifying Coomassie Brilliant Blue G-250 according to the method of Bradford (1976). In this method; 100 mg of Coomassie Brilliant Blue G-250 (Sigma) was dissolved by agitation in 50 ml of 95% ethanol then the solution was mixed with 100 ml of 85% w/v phosphoric acid (H_3PO_4) then it was diluted with distilled water to 1 litre and filtered.

Sample (100 µl containing 10-100 µg of protein) was mixed with 5 ml of Coomassie blue reagent. The absorbance (595 nm) was measured after 10 min and before 1h in 3 ml cuvette against a reagent blank prepared from 0.1 ml of the appropriate buffer and 5 ml of protein reagent.

A standard curve was prepared using Bovine Serum Albumin fraction V (Sigma) and absorbance was measured at 595 nm using UV 1700 Spectrophotometer (UV-Vis Spectrophotometer, Shimadzu). The response was linear over the range 10 to 100 µg protein. All protein concentrations from wheat leaves were determined by this method. The results were expressed mg protein/g fresh weight. All measurements were made in triplicate.

Determination of total phenolic contents

The amount of total phenolics in leaf

extracts was determined according to the Folin-Ciocalteu procedure with slight modifications (Shetty et al., 1995). Four hundred µg of leaf sample from each treatment of each cultivar was extracted with 5 ml of 80% methanol in a boiling water bath (95 °C) for 30 min and the extract was centrifuged at 10,000g for 10 min. Three hundred µl of previously diluted leaf extract was reacted with 1.5 ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and after 5 min 1.2 ml of 7.5% of Na_2CO_3 was added and the mixture was allowed to stand for 30 min in the dark. Solutions were heated in a 40 °C water bath for 30 min. The color was developed and the absorbance against reagent blank was determined at 765 nm with an UV-visible spectrophotometer (UV 1700, Shimadzu). The standard curve was prepared using 0, 0.05, 1.0, 1.5, 2.0 and 2.5 ml of gallate stock solution (8 mg/100 ml) in 25 ml reaction mixture. Total phenolic content of leaves were estimated from a standard curve of gallic acid and the results were expressed as mg gallic acid equivalents (GAE) g⁻¹ fwt.

Statistical Analysis

All results reported here were the means of three replicates with standard errors. Data were analyzed with one way ANOVA using SPSS (10.0).

RESULTS

Accumulation of protein in living organisms is of great importance to monitor the stage of the organisms. It actually shows the growth progression (Li et al., 2010). In this study, bread and durum wheat cultivars were monitored with respect to protein accumulations during germination stages over a 10-day period. Both wheat cultivars gradually accumulated increasing soluble protein contents. However, the increase in protein content was more marked in durum wheat than that of bread wheat cultivar Abusbey (Fig 1a). The durum wheat cultivar Alibaba accumulated proteins just after following germination and the increase in protein content carried out until the end of experiment. However, bread wheat cultivar accumulated significant amount of proteins in the 7th day of germination, and the increase in protein content lasted in a slow trend until the end of experiment.

When the insoluble protein contents of those cultivars were examined, both cultivars did not show statistically significant differences from each other with respect to accumulation of insoluble protein contents (Fig 1b).

When the phenol contents were measured with respect to both soluble and insoluble fractions, the differences among cultivars were not significantly different (Fig 1 c & d). Although the phenol contents of soluble fractions were higher than those of insoluble fractions in both cultivars, the difference between cultivars in phenol contents was not

wheat cultivar Alibaba accumulated just a bit higher phenol contents than those of cultivar Abusbey.

Protein and phenol contents of both cultivars were also determined in their respected syrup solutions to find out if protein accumulation was changed and showed a different pattern. In this case, the protein contents of durum wheat were significantly higher than those of bread wheat ($P < 0.01$, Fig 2a). When phenol contents were measured, their concentration was again higher in durum wheat cultivar than that of bread wheat cultivar although the difference was not statistically

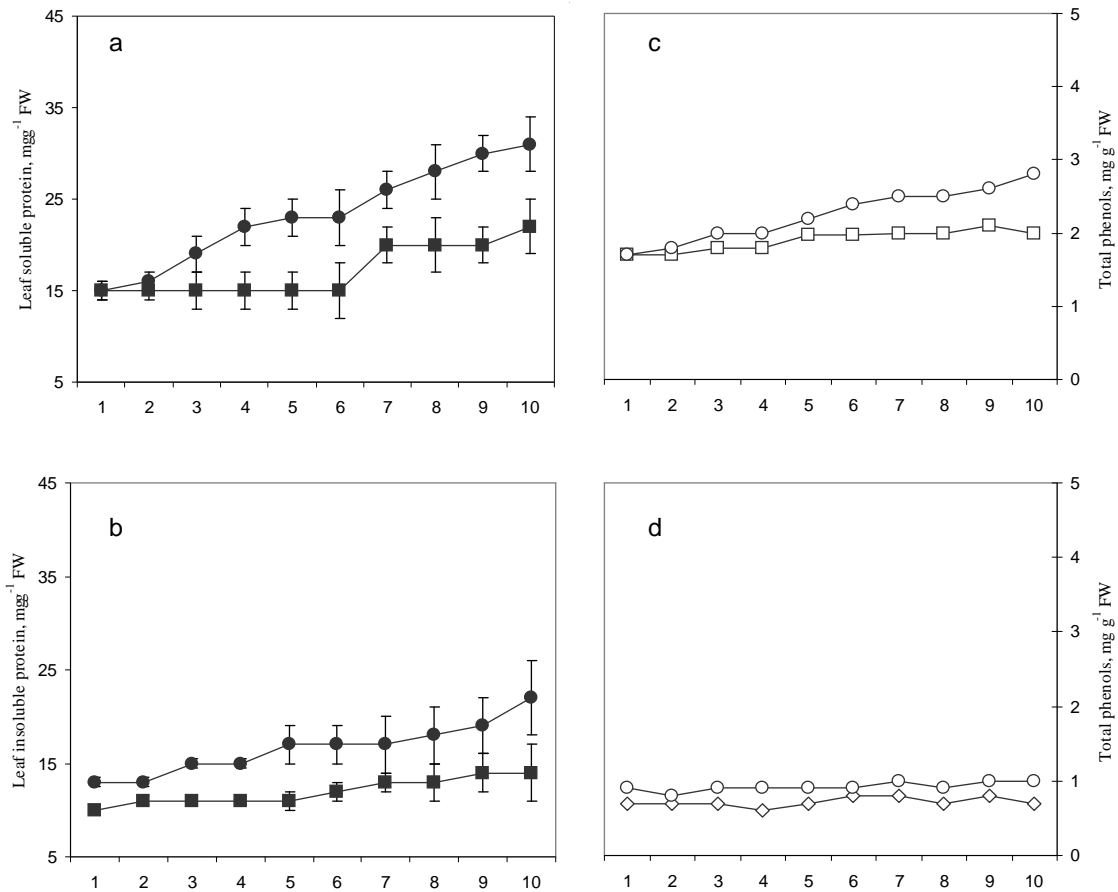


Fig 1 a & b. (a) Determination of soluble and (b) insoluble protein contents in *Triticum durum* cv. Alibaba (●—●) and in *T. aestivum* cv. Abusbey (■—■).

Fig 1 c & d. Total phenol contents in (c) soluble and (d) insoluble fractions of *Triticum durum* cv. Alibaba (○—○) and *T. aestivum* cv. Abusbey (□—□).



Fig 2 a & b. (a) Determination of protein and (b) phenol contents of *Triticum durum* cv. Alibaba and *T. aestivum* cv. Abusbey in prepared syrup solutions.

DISCUSSION and CONCLUSION

Proteins are the most important components of wheat grains. Variations in protein contents and composition significantly modify flour quality and thus nutrition quality. Although grain protein composition depends primarily on genotype, it is significantly affected by environmental factors (Zhu and Khan, 2001). In this study, accumulation of protein and phenol contents were evaluated under non-stressed conditions, therefore, it is not expected that the cultivars should give different induced responses. In fact, their different responses should arise from their genetical backgrounds. From the results of protein contents in both cultivars, durum wheat cultivar Alibaba accumulated increasing amount of protein through the germination period and the contents of protein in each day

was higher than that of cultivar Abusbey, which also accumulated increasing amount of protein as the germination period. Since the soluble protein contents reflect the growth period (Li et al., 2010), it is expected to find out the highest protein contents in a peak time of maturity. Under stress conditions such as drought or salinity, soluble leaf protein content may increase in plant tissues. This may result from the decrease of water content in plant tissues, as well as the increase in osmotic substances when plants suffered from the stress agents (Triboi et al., 2003; Li et al., 2010). In fact, when plants suffered drought or salt stress, we might expect that the synthesis rate of total protein would decrease, but the synthesis of some original proteins such as stress-induced proteins may be induced to adjust osmotic potential of cells in order to keep a certain turgor and normal physiological

processes (Zhu and Zhang, 2003; Xie et al., 2005). For example, in the study of Li et al. (2010) the soluble proteins of stem leaves and fruit branch leaves increased when encountering certain drought stress.

The increase in protein contents are a good source for enzymatic activities and therefore, it gives rise to better protection of the organism. In our case, accumulation of earlier and higher proteins could be useful since we plan to use this type of cultivar for the alleviation of damaged cells in higher organisms. For example, a study of Bektas (2010) showed that the extracts of *Allium sativum* (garlic) and *Nigella sativa* were found quite effective in reducing the DNA damages in mononuclear leukocyte cells. However, the repair of the cells with these extracts were not achieved, but, the protection of cells, in advance, against stress agents were remarkably high. From the various literature, prophylactic effect of wheat extracts were also reported (Kumar et al., 2010).

When insoluble protein fraction was determined, the protein contents among cultivars did not show any significant differences. In a study of Lutts et al. (1996) showed that the insoluble protein fraction was not affected by salinity after a short term stress in rice genotypes.

When phenol contents were measured in insoluble and soluble fractions, the phenol contents of insoluble fractions did not show any significant differences among cultivars indicating that their responses were similar under normal conditions. Phenol contents of soluble fractions of both cultivars were also similar although durum wheat synthesized a little more phenols during germination stage.

When the syrups of those cultivars were used for protein and phenol measurements, durum wheat cultivar Alibaba synthesized more proteins and phenols as compared to the bread wheat cultivar Abusbey. Syrups of both cultivars under normal conditions produced more proteins and phenols as compared to that of media in Petri plates. This clearly indicates that the available water as concentrated substrate in syrup solution facilitated the growth of cultivars and resulted in a higher production of protein contents.

Since this study is the first of a series studies which would be carried out on higher organisms, it is important to determine the

optimum concentration of proteins which would reflect the maturity of plants and also make comparisons between cultivars. Another important parameter is to find out the best method which brings about the accumulation of the highest amount of proteins in a very short time.

Wheat extracts have free radical scavenging properties (Yu, 2001). From this characteristic, wheat bran enhanced the protective potential and induced molecular alteration in colonic cells during carcinogenesis of *Acidophilus -casei dahi* rats (Kumar et al., 2010). Since wheat sprouts contain a very high level of organic phosphates and a powerful cocktail of different molecules such as enzymes, reducing glycosides and polyphenols, it has been documented that they are able to protect DNA against free-radicals mediated oxidative damage (Amici et al., 2008). Kamran et al. (2008) also suggested that breakfast diet with wheat was useful for the prevention and treatment of constipation, cardiovascular diseases and hypertension.

In recent years, several companies proposed and took place in the food market suggesting wheat syrups or wheat grass solutions or even suggesting their pills prepared with various ingredients. They have been commonly reporting that wheat grass contained apigenin and chlorophyll, flavonoids, vitamin A, vitamin E, vitamin C, iron calcium, magnesium, and aminoacids. These preparations have also antioxidant and antibiotic properties and effects on higher organisms (www.ayurvedicure.com).

With this work, we established that the optimum protein concentration of germinating wheat is in 5th and 7th days in cultivars Alibaba and Abusbey cvs., respectively. However, the protein content of Abusbey was much lower than that of Alibaba throughout the germination period. We also established that the syrups of wheat cultivars could accumulate the highest protein contents in 3rd day of the start of the preparation of syrup. We could also say the phenol contents of those cultivars were not high throughout the germination period, however, it could easily be said that the production of phenols were quite helpful for us to determine the characteristics of resistance of those cultivars under stress conditions if we have to grow them in such conditions. Since DNA damages are caused by either toxic or mutagenic agents, it is important to tackle with the unpleasant consequences such as cancer or

other diseases. Therefore, we have to try every other option to minimize the impact of those health problems with natural ways due to the evasive approaches to the chemotherapy which has many side effects.

We suggest that the wheat sprouts or syrups has good advantageous to diminish the DNA damages by preventing the higher organism from such effect and enhancing the cell resistance through its life cycle.

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