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Evaluation of Wheat Germination for Making Gluten-Free Bread

Amany M. Sakr

Bread and Pastry Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

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ABSTRACT
This study included the germination process of the soft wheat (*Triticum aestivum* Giza 171). Wheat grains were soaked, germinated for seven days (at 20±2ºC).Also, protein fractions showed that the albumins and globulin increased until six days after that it decreased. As for the gluten, it decreased until sixth day after that it increased. The results from SDS-PAGE electrophoresis and extensogram confirmed these results. The results of chemical analysis reported that the wheat germination caused decrease in protein, wet and dry gluten, and total carbohydrates. However, ash, and crude fiber were increased after wheat germination for three and six days. Also, the mineral content of sodium, potassium, phosphorus, iron, and calcium increased after wheat germination for three and six days. Reduced-gluten wheat flour (after 6 days of germination) was used to prepare balady bread and was compared with bread made from raw wheat flour. Sensory evaluation, color measurements, and instrumental analyses of different bread were determined. Therefore, it is recommended to prepare bread from wheat germinated for six-day for celiac disease patients.

Key words: SDS-PAGE, germination**,** wheat flour, gluten protein, Sensory evolution, color measurements and instrumental analyses

Introduction

Wheat is a major component of most worldwide diets because of its nutritional quality, and the ability of its flour to produce a variety of tasty and satisfying foods. This is a consequence of the unique viscoelastic properties of wheat dough, which allow the entrapment of CO_2 during fermentation, enabling the preparation of leavened bread and other baked products. These wheat products make substantial contributions to the dietary intake of energy and protein, and supply dietary fiber, minerals, vitamins, and phytochemicals (Rosell, 2012)

Wheat flour cannot be tolerated by those who suffering allergies to gluten. Therefore, the germination of wheat, to make wheat gluten-free, is suitable for celiac patients and other gluten-intolerant individuals. Moreover, the low-gliadin flour has improved nutritional properties since its lysine content is significantly higher than that of normal flour. Conservative estimates indicate that celiac patients could safely consume 67 grams of bread per day that is made with low-gliadin flour. However, feeding trials with gluten-intolerant patients are still needed in order to determine whether or not the product can be consumed by the general celiac population, as well as the actual tolerated amount that can be safely ingested (Gil-Humanes *et al.,* 2014).

During germination, the main storage biopolymers of cereal grains, namely carbohydrates, proteins, and lipids are hydrolyzed to lower molecular weight compounds, because of the activity of the hydrolytic enzymes, which are inactive in raw seeds (Guardianelli *et al.,* 2019).

Celiac disease is a genetically intestinal disorder induced by proteins of wheat (gliadin), rye (secalin), and barley (hordein). The disease is fairly common; affecting approximately 1% of population in the US and in Europe (Fasano *et al.,* 2003).Ingestion of the above-mentioned cereal proteins provokes variable symptoms in susceptible subjects and induces small-bowel mucosal damage characterized by flattening of villi, crypt hyperplasia, and substantial inflammation. Gliadin, secalin, and hordein are rich in proline and glutamine residues and thus only poorly cleaved by digestive proteolytic enzymes, even in healthy subjects (Garcia-Horsman *et al.,* 2007).Incomplete degradation of these dietary proteins in the gastrointestinal tract leads to

Corresponding Author: Amany M. Sakr, Bread and Pastry Research Department, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
E-mail:amaysakr2009@yahoo.com

the appearance of peptide, of which some are toxic (de Riti *et al.,* 1988 & Sturgess *et al.,* 1994) and others immunogenic (Anderson *et al.,* 2000& Shan *et al.,* 2002) for celiac disease patients.

A preliminary study of Van Landschoot (2011) revealed that malting and brewing are able to reduce gluten epitopesmuch lower than the threshold of 20 ppm for food products. Since wheat bran is characterized by a high content of bioactive compounds such as dietary fiber, minerals, and folic acid (Walter and others 2014), several studies have focused on germinated wheat bran as a source for peptidase. Indeed, it has been indicated that there is CD-specific peptidase activity in the bran of sprouted cereals (Gessendorfer *et al.,* 2011 & Schwalb *et al.,* 2012).

Adrianos *et al.* (2017) reported that proteases from germinated cereals can significantly reduce the amounts of toxic gluten proteins or peptides. Therefore, they may be used in a variety of areas: food supplements that help the body digest gluten without allergic reactions, as well as in the production of special foods for CD patients. Ding *et al.* (2018) examined how germination time affected the functionality of whole-wheat flour (WWF) and enhancement of γ-aminobutyric acid (GABA) content through ultra sonication. The findings of this study demonstrated that controlled germination for 5–15 h produced WWF with improved flour functionality i.e., increased glucose content, less starch retrogradation during gelatinizing. Boukid *et al.* (2017) studied the effectiveness of germination on wheat protein degradation, with a specific focus on proteins involved in adverse reactions to wheat. The effects of 8 days of germination at 25 °C did not have a significant effect on starch, lipid, and ash contents. General protein profile, as indicated by SDS-PAGE analysis, revealed that germination induced a relevant degradation in protein fraction. Regarding gluten peptides related to celiac disease, germination enabled an average reduction of 47% in peptides eliciting an adaptive immune response.

Germination is already a well-accepted process by consumers with many products made from sprouted seeds or containing limited amounts of flour from sprouted grains. Therefore, it was assessing the usefulness of germination in reducing gluten peptides associated with celiac disease, at the same time evaluating some technical features of the obtained germinated wheat (Boukid *et al.*, 2018).

The aim of this study was to produce gluten-free wheat bread as an alternative to normal wheat bread for consumers suffering from gluten-related celiac diseases.

Materials and Methods

1.Materials

Wheat **(***Triticum aestivum* L) Giza 171 variety**,** was provided from Field crops Institute, Agricultural Research Center, Giza, Egypt. Instant active dry yeast and sodium chloride were purchased from the local market, Cairo, Egypt and also carboxyl methyl cellulose (CMC) was purchased from El- Gomhouria Co., Egypt.

2. Methods

2.1. Germination of wheat

One kilogram of wheat grains was weighed, sorted, steeped in distilled water for 12h at room temperature, and then completely drained of steep water using sieves. The drained wheat grain was then spread on a moistened jute sack and allowed to germinate at room temperature for seventh days at 20±2 °C. After during germination period, tissues were collected daily up to seventh day and dried at 60±5 °C for 48 hours.

2.2. Milling of raw and germinated wheat

The dried wheat germinated and raw grain were milled using Hummer mill to obtain whole meal flour according to AACC (2000).Whole meal wheat flour was sieved through a 50 mm sieve to obtain flour extraction rate and packed in polyethylene nylon; also, it was stored at room temperature to make gluten-free bread.

2.3. Determination of Chemical composition in raw and germinated wheat flour

Chemical composition; protein, fat, crude fibers and ash content, total carbohydrates were determined in raw and germinated wheat flour according to AOAC (2010). Total carbohydrates were determined by difference.

2.4. Determination of wet and dry gluten in raw and germinated wheat flour:

About 25 g of wheat flour was taken into a bowl and made a dough ball by water and put stands in water for 20-60 min at room temperature. After soaking knead the dough ball gently under running tap water until all starch matter removed and the web-like gluten network structure formed. Let allow the isolated gluten to stand in water for 1 hour, press the gluten ball in between the hands to dry as much as possible, weigh the moist gluten ball paced in a Petri plate. Transfer to oven, maintain at 100° (24 hr.), after cooling dry weight of gluten was calculated according to AACC (1999).

2.5. Determination of minerals content in raw and germinated wheat flour

Minerals content (Mg, Na, K, Zn, P, Fe and Cu) in raw and germinated wheat flour were determined according to the method of the AOAC. (2010) using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300). Phosphorus was determined by spectrophotometer according to the AOAC. (2010).

2.6. Determination of protein fractions (albumin, globulin and gluten) in raw and germinated wheat flour

Raw Wheat and germinated flour (0.25 g) were suspended in 2.5 mL 10 % NaCl, stirred for 45minutes and centrifuged at 4000 xg for 10 minutes. The extraction was repeated three times. The combined supernatants contained albumins and globulins were analyzed along. The pellet remaining after the NaCl extraction was extracted with 2.5 mL 70 % (v/v) ethanol to separate gliadins. The extraction was carried out three times for 45 minutes and the obtained mixture was centrifuged at 4000xg for 10 minutes. The pellet remaining after the extraction of gliadins was treated with 2.5 mL 0.2 % NaOH, stirred for 45 minutes, and centrifuged. Alkaline extraction was repeated three times. The combined supernatants were used to obtain the glutenin fraction (Karamaćet *al.,* 2007). The gliadin and gluten infraction extracted from raw and germinated wheat flour present together gluten fractions. The protein content of every the fraction was determined by Kjeldahl method according to AOAC (2010).

2.7. Extraction of total proteins

About 0.1 g of fine powder from raw and germinated wheat flour then 400 µL of Sample Buffer, 5µl of 10% SDS and 5µl of β–mercaptoethanol was added then boiling the mixture for 5 min and was centrifuged at room temperature at 10,000 rpm for 10min.The extracted protein were collected as supernatant and stored at 20°C according to method by Laemmli, (1970).

2.8. SDS-PAGE electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of total protein was performed in a discontinuous buffer system according to the method of Laemmli, (1970). Protein profiling of extracted samples during germination period (seventh day) were analyzed using 15 % polyacrylamide gel. A protein marker (Protein Molecular weight marker, thermo) was loaded as standard along with samples. Gels were vidualized by staining with Coomassie brilliant blue R-250 and distained using 40% methanol and 7% acetic acid for overnight followed by gel scanning and photography.

2.9. Extensogram of raw and germination wheat flour

Extensogram tests were carried out to determine the elasticity, extensibility, proportion number and energy of raw and germinated wheat flour according to the method described in AACC (2000).

2.10. Bread Making

The dough was prepared on a flour weight basis: for 300 g raw and germinated wheat flour for sex day, 3.6 g baker's yeast, 4.8 g table salt and 0.1% carboxyl methyl cellulose (CMC) as alternative gluten according to Wongklom *et al.,* (2016). Ingredients were mixed in a mixture for 4 min and rested for 10 min with a plastic film cover to avoid drying. The dough was divided manually and dough pieces were rolled mechanically in a ball homogenizer. Dough pieces were placed on aluminum trays and fermented for 45 min at 30°C. Dough pieces were baked in an electric convection oven. The baking process was performed at a fixed oven temperature of 400-450°C for 3-5 min. After baking, bread loaves were rested for 30 min to cool down. The control sample was prepared from raw wheat flour to make control bread.

2.11. Sensory evaluation of different bread

A panel of 10 judges' research staff members of Bread and Pasta Dept. at the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt evaluated the sensory characteristics of prepared bread. The assessment involved the consideration of (aroma, taste, texture, and color, using 5-point Hedonic rating scale (5-like extremely, 4.5-like very much, 4-like moderately, 3.5-like slightly, 3-neither like nor dislike, 2.5-dislike slightly, 2- dislike moderately, 1.5-dislike very much, 1- dislike extremely) according to Bhat *et al.,* (2015).

2.12. Color of different bread

Crust and crumb color was determined by a Chroma Meter CR-400colorimeter (Konica Minolta Sensing Inc., Japan), and expressed in a CIE-*L* a* b** color scale (CIE-Lab). The CIE-Lab color species composed by three perpendicular axes: *L*, a** and *b**.These three coordinates indicate the lightness of the color (*L*;* where L = 100 indicates white color and L = 0 black color), and its position between green and red (*a*;* where negative values indicate green and positive values indicate red), and between blue and yellow (*b*;* where negative values indicate blue and positive values indicate yellow). Two independent measurements were made to each of the three loaves to determine crust and crumb color according to Choudhury (2014).

2.13. Instrumental analyses of different bread:

Texture Profile Analysis (TPA) indices of different bread were determined using a Brookfield CT3 instrument (Brookfield Engineering Laboratories, Inc., MA 02346-1031, USA). The conditions of texture analyzer were provided with software, 35 mm diameter compression disc was used. Two cycles were applied at a constant crosshead velocity of 1 mm/s, to 30% of sample depth, and then returned. From the resulting force-time curve the values for texture attributes, hardness, resilience, cohesiveness, springiness and chewiness were calculated from TPA graphic according to Bourne (2003).

Statistical analysis

The data generated in this research were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan multiple range tests to compare the mean values at $P<0.05$ level of significance (SAS, 2004).

Results and Discussion

1. SDS-PAGE analysis

Gluten is fractionated into alcohol-soluble prolamins and alcohol insoluble glutelins. The wheat prolamins, gliadins, are monomeric proteins with molecular weight ranging from 30 to 50 kDa and can be classified into α/β, γ, and ω-type. The wheat glutelins, glutenins, can be divided into high molecular weight (HMW) glutenins with molecular weights of 66–88 kDa, and low molecular weight (LMW) glutenins with molecular weights falling in the range of the gliadin proteins, ~32–45 kDa(Vensel*et al.,* 2014).The primary allergic proteins are prolamins of wheat grain with a relatively low molecular weight of about 30 to 80 kDa (Van Eckert *et al.,* 2010). Results showed that a gradual degradation of glutens started after three days and the lowest concentration was measured at the sixth

day of germination of wheat grain with molecular weight 66 – 32 kDa, shown in Figure (1). Similar results were obtained with Bigiarini *et al.,* (1995) & Michalcová *et al*., (2012).

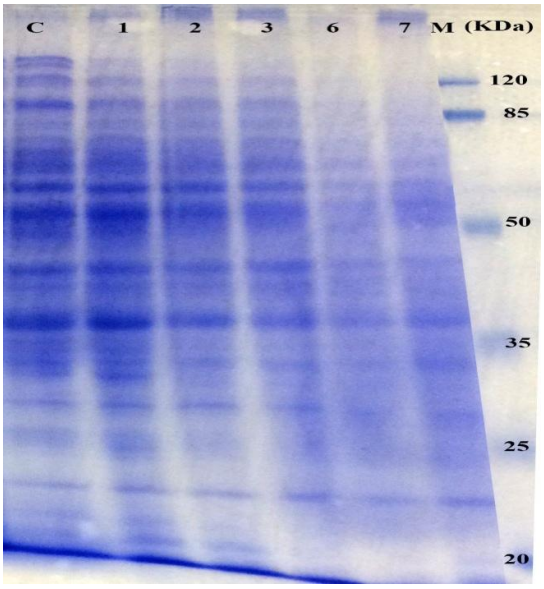

Fig. 1: SDS-PAGE analysis of gluten degradation of wheat during germination for 7 days.

During germination, besides starch, storage proteins are used to nourish the embryo. Because these proteins are insoluble in water, their utilization by the growing embryo is possible only after their degradation to soluble products (Capocchi *et al.,* 2000). The proteolytic activity increase was due to the synthesis and secretion of endoproteases (Dominguez and Cejudo 1996). Gluten hydrolysis by endoprotease might result in an alteration of gluten epitopes immunogenic potential. According to Hartmann *et al.,* (2006), germinated cereals led to α-gliadin degradation into nonallergenic small peptide fragments. Later, in vitro cell trials showed that protease isolated from naturally germinating wheat did not stimulate T-cell proliferation to the same extent as unprocessed gliadin, concluding that germinating wheat enzymes are able to alter gliadin immunological potential (Stenman *et al*., 2009)

Panda and Garber, (2019) indicated that the Codex Standard and the European Commission states that the gluten level of gluten-free foods must not exceed 20 ppm. The FDA requires food bearing the labeling claim "gluten-free" to contain <20 ppm gluten.

2.Quantifications of protein in raw and germinated wheat

The concentration of albumins, globulins, and gluten was determined in raw and germinated wheat and the results are reported in Table (1). From the results, it could be noticed that the albumins and globulins increased from 7.51mg/g (dry weight) in raw wheat to 9.45, 10.38, 11.23, and 14.75 mg/g (dry weight) in germinated wheat after one, two, three, and six days (at 20 °C). Meanwhile, concentrations of these proteins decreased on the seventh day of germination (at 20 °C) to12.50 mg/g. This increase in concentrations of albumins and globulins may be due to the germination process of grain which is accompanied by increasing hydrolytic enzyme activities (Michalcová *et al.,* 2012). When the hydrolytic enzyme activity was lowered after six days, albumins and globulins were increased (Mikola and Jones, 2000).

From the results in the same table, it is found that the started concentration of gluten was 32.12 mg g and after three days of germination (at 20 °C) the concentration decreased to 20.28 mg g Meanwhile, the lowest concentration of gluten was 13.19 mg after six days of germination (at 20 °C). These results agree with work by Koehler *et al.* (2007). The concentration of gluten increased after seven days of germination to be 15.38 mg g. These results agree with work by Michalcová *et al.* (2012). These results pointed out that germination of the wheat grains until the

sixth day is a significant tool for decreasing gluten in wheat grains, which makes it suitable for use in the production of gluten-free or gluten low loaves for celiac diseases. Wheat gluten is the viscoelastic mass that remains when starch and other water-soluble components are washed out of the wheat dough (Scherf *et al.,* 2016). Wheat gluten consists of >80% protein, 5–10% lipids, and residues of starch and non-starch polysaccharides (Codex Alimentarius Standard, 2001).

Table 1: Quantifications of protein fractions in raw and germinated wheat flour (dry weight)

Treatments (mg/g^{-1})	Albumins and globulins	Gluten
Raw wheat	7.51 ^{e}±0.04	32.12 ^{a} ±1.26
Germinated wheat after 1 day	9.45 ^{d} ±0.14	29.50 ^{ab}±0.90
Germinated wheat after 2 days	10.38 ^{c}±0.25	25.24^{b}±1.53
Germinated wheat after 3 days	11.23^{bc}±0.28	20.28 ^{c} ±0.92
Germinated wheat after 6 days	14.75 ^{a}±0.39	13.39 ^{e} ±0.15
Germinated wheat after 7 days	12.50^{b}±0.28	15.38^{d}±0.37

Values are means ± SD (n = 3). Means followed by different letters in the same column are significantly different (P ≤0.05)

3. Chemical composition of raw and germination wheat flour

The proximate composition of raw wheat and germinated wheat flour after 3 and 6 days are presented in Table (2). The moisture content of germinated wheat flour after 6 days was the highest (12.80%), while that of raw wheat flour was the lowest (12.58%). These values were within the range reported by Amagloh *et al.,* (2012). However, investigations have shown that low moisture content of food samples is a desirable phenomenon since the microbial activity is reduced. Meanwhile, low moisture content in food samples increased the storage periods of the food products (Alozie *et al.,* 2009), while high moisture content in foods encourages microbial growth; hence, food spoilage (Temple *et al.,* 1996).

Moreover, the results for protein in raw and germinated wheat flour was 12.45% in raw wheat, meanwhile, in germinated wheat flour after 3 and 6 days there were a decrease to 12.11 and 11.76%, respectively. Protein losses during germination have been attributed to their degradation by proteases. Furthermore, this observation agreed with other scientific findings that processing techniques such as germination improved the nutritional quality of the food products, particularly in terms of protein content (Enujiugha *et al.,* 2003 and Fasasi, 2009).

Results showed that the highest wet and dry gluten was in raw wheat flour 25.42 (10.95%). While, the wet and dry gluten in germinated wheat after three days was decreased to 22.38 (8.13%) and the lowest gluten was that recorded after 6 germination days 18.54 (6.44%) respectively. These results were reported by Balamurugan *et al.,* (2018) who found that in raw wheat, the gluten ball was formed by the continuous kneading of raw wheat flour , it contain 27.52% in wet and 10.96% in dry gluten. The highest gluten content obtained after 12 h soaked wheat 37.4% in wet and 13.84% in dry gluten. When, after 12 h soaked + 24 h sprouted wheat, the gluten content lowered to 20.08% in wet gluten and 6.44% in dry gluten. Increased germination decreased the gluten content of wheat substantially by degrading the gliadin peptides into nontoxic fragments by proteases may benefit the gluten sensitive and celiac patients (Ali *et al.,* 2016).

Crude fiber and ash content were increased during germination from 0.71 and 0.61% in raw wheat flour to 2.14 and 1.89% in germinated wheat after six days. Germination could be an effective way of improving the fiber content in foods (Jan *et al.,* 2017).The increase in fiber is desirable because dietary fiber slows down glucose release from food which could be beneficial for people with diabetes. Moreover, fiber forms gels in the stomach that slows down starch digestion and gastric emptying which subsequently increase satiety (Yu *et al.,* 2014).

Carbohydrate values and total lipids of the raw wheat flour were decreased from 84.16 and 2.07 to 82.87 and 1.34% in germinated wheat after six days, respectively. Decrease in carbohydrates in germinated grains may be attributed to increase in alph-amylase activity which breaks down complex carbohydrates into simpler and more absorbable sugars as previously reported by Hung *et al.,* (2011).In addition, the carbohydrate and lipids contents of germinated samples were lower than that of raw wheat flour samples this observation could be due to the

utilization of fat and carbohydrate for biochemical activities of the germinating seeds (Wang *et al.,* 1997).

Table 2: Chemical composition of raw and germinated wheat flour on dry weight basis

Chemical Composition%	Raw wheat flour	Germinated wheat flour After 3 days	Germinated wheat flour After 6 days
Moisture	12.58[a]±0.84	12.61[a]±1.04	12.80[a]±1.08
Crude protein	12.45 [a]±0.91	12.11 [b]±0.95	11.76 [c]±0.76
Wet gluten	25.42 [a]±1.39	22.38 [b]±1.57	18.54 [c]±1.21
Dry gluten	10.95 [a]±0.92	8.13 [b]±0.84	6.44 [c]±0.38
Total lipids	2.07 [a]±0.04	1.84 [b]±0.07	1.34 [c]±0.08
Ash content	0.61 [c]±0.02	1.25 [b]±0.03	1.89 [a]±0.04
Total carbohydrates	84.16 [a]±3.19	83.35 [b]±3.18	82.87 [c]±2.97
Crude fiber	0.71 [c]±0.03	1.45 [b]±0.04	2.14 [a]±0.09

The analytical determinations were performed in triplicate, and the means ± Standard Deviation were reported. Means followed by different letters in the same raw are significantly different (P≤0.05)

4. Mineral content of raw and germination wheat flour

The mineral composition of raw and germinated wheat flour (Table 3) show that the mineral composition of sodium, potassium, phosphorus, iron and calcium in raw wheat flour was 20.58, 140.21, 210.0, 6.75 and 18.25 mg/100g, respectively, on dry weight. After six-day germination recorded 50.49, 220.12, 240.12, 8.43 and 25.46 mg/100g dry weight, respectively. Meanwhile, Magnesium, zinc and copper were significantly decreased from 12.94, 0.48 and 1.75 mg/100g dry weight in raw wheat flour to 9.56, 0.16, and 0.14 mg/100g dry weight, respectively after six germination days. It was observed in this study that the germination processing techniques improved the mineral composition of the flour samples except in Magnesium, zinc and copper. This observation could be attributed to bio-synthesis and activities of microorganisms during germination processes (Gabriel and Akharaiyi, 2007).

Table 3: Mineral content in raw and germination wheat flour (on dry weight basis)

Mineral Content(mg/100g)	Raw wheat flour	Germinated wheat flour after 3 day	Germinated wheat flour after 6 day
Magnesium	12.94 [a]±0.11	11.14 [b]±0.12	9.56 [c]±0.29
Sodium	20.58 [c]±0.43	35.13 [b]±1.24	50.49 [a]±1.97
Potassium	140.21 [c]±3.26	190.39 [b]±4.27	220.18 [a]±5.31
Zinc	0.48 [a]±0.04	0.35 [b]±0.02	0.16 [c]±0.03
Phosphorus	210.0 [c]±5.13	220.38 [b]±5.39	240.12 [a]±6.28
Iron	6.75 [c]±0.08	7.51 [b]±0.06	8.43 [a]±0.06
Calcium	18.25 [c]±0.18	20.23 [b]±0.27	25.46 [a]±0.63
Copper	1.75[a]±0.01	0.50 [b]±0.03	0.14 [c]±0.02

The analytical determinations were performed in triplicate, and the means ± Standard Deviation were reported. Means followed by different letters in the same raw are significantly different (P≤0.05)

5. Extensogram of raw and germinated wheat flour

Rheologaical measurements are considered the most valuable method to assess the quality of flour; their parameters are designed to monitor the molecular structure, mechanical properties, material composition and to anticipate the quality of end product (Bockstaele *et al.,* 2008). Extensogram represented gluten do extensibility resistance to extension, ratio of resistance to extensibility and dough deformation energy (Hadnadev *et al.,* 2011).

Figures (2 and 3) and Table (4) showed that the extensogram test as dough extensibility significantly decreased when increasing in wheat germination time to 6 days (50.0 mm) compared with raw-wheat (160.0 mm). In addition, the raw-wheat showed a decrease in dough elasticity by 80 B.U. and elevation in germinated wheat after 6 days to 170 B.U., respectively. Singh *et al.,* (2001) studied the effect of germinating conditions on the functional and dynamic rheological properties of wheat. They also, stated that the falling number and water absorption index (WAI) decreased and the water solubility index (WSI) increased with the increase in germination. The elastic modulus

decreased with the increase in germination duration and also, the viscous modulus showed a decrease with the increase in germination duration.

The proportion number indicated that the ratio between the resistance to extension and extensibility were increased with the germination wheat after 6 days to 3.4 and reduction to 0.5 in raw- wheat. Finally, the energy required for deformation was decreased by the germination wheat for 6 days to 10.0 cm^2 and elevated to 20 cm^2 in raw- wheat.

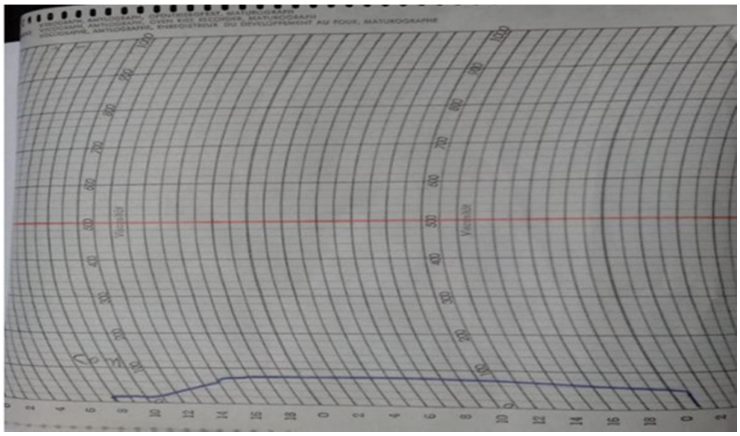

Fig. 2: Extensogram of raw wheat **flour**

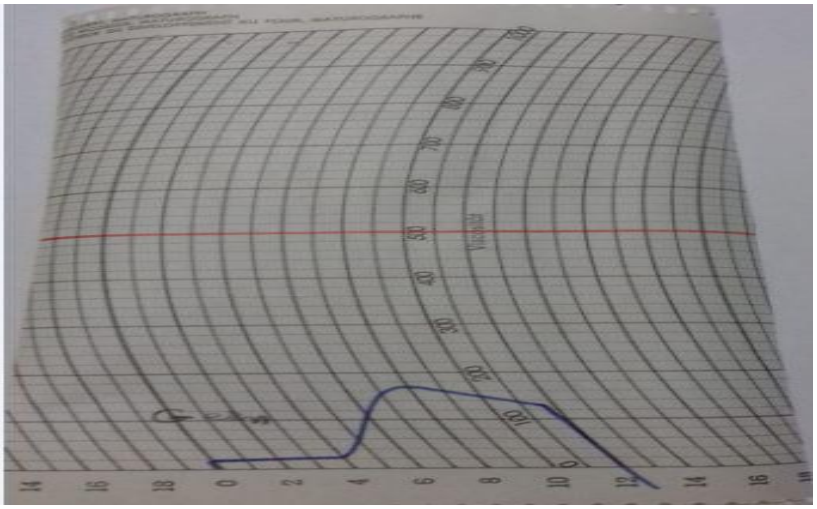

Fig. 3: Extensogram of germination wheat flour for 6 days

Table 4: Extensogram of raw and germinated wheat flour after 6 days

Wheat treatment	Elasticity B.U.	Extensibility mm	Proportion number	Energy cm^2
Raw wheat flour	80.0	160.0	0.5	20.0
Germination wheat flour after 6 day	170.0	50.0	3.4	10.0

5. Sensory evaluation of bread made from raw and germination wheat flour

The assessment of bread made from raw and germinated wheat flour after 3 and 6 days involves aroma, taste, texture, and color, using 5-point Hedonic rating scale and the finding are indicated in Table (5).The results showed that the taste and aroma were decreased from 4.5 in bread made from raw wheat flour to 3.0 in bread prepared from germinated wheat flour for 6 days. Thus, this difference in taste and aroma may be attributed to the reduction of gluten during germination of wheat flour resulting in lowering down the taste and odor values (Zubaidi, 2009).

The results for color in raw and germinated wheat flour were parallel to the above results and this different color may be due to the increase in the sugar content in germinated wheat after

6 days resulting in non-enzymatic browning during the baking process. Fadel and Al – Mudhafar, (2020) found that the difference between raw and germinated wheat is in color, as the color of the germinated wheat flour gives a darker color than the raw wheat flour. This is due to the drying process that takes place after the wheat germination process ending.

As for the texture the bread from raw wheat flour was superior to the bread made from germinated wheat; this was due to the high fiber content of the germinated wheat flour which led to water retention and reduction in its evaporation rate. Thereby, not allowing the pulp cells to expand, causing tissue values to be reduced (Solaka and ZainAlAbidin, 1995). Moreover, the decreases in the mean values of texture may be due to an increase in the fiber and sugar content may be able to be resulting in the hard crust.

While the changes that occur during germination can improve then nutritional value of the resulting flour, they may lower the mixing and baking (end-use) properties of flour due to increased amylolytic and proteolytic activities (Baranzelli *et al.,* 2018).This can result in an increase in dough stickiness, difficulty in dough handling, and a decrease in dough strength (Olaerts *et al.,* 2018).Furthermore, bread made from extensively sprouted flour resulted in sticky and gummy crumbs and poor texture (Olaerts *et al.,* 2018).

Table 5: Sensory properties of bread made from raw and germinated wheat flour

Treatments	Taste	Aroma	Texture	Color
Control (raw wheat)	4.5^{a}±0.14	4.5^{a}±0.11	4.5^{a}±0.21	4.5^{a}±0.23
Bread after 3days	3.5^{b}±0.18	3.5^{b}±0.14	3.5^{b}±0.09	3.5^{b}±0.08
Bread after 6days	3.0^{c}±0.13	3.0^{c}±0.12	3.0^{c}±0.24	3.0^{c}±0.07

Values are means ± SD (n=3). Means followed by different letters in the same column, significantly different (P ≤0.05)

7.Color analysis of bread made from raw and germinated wheat flour

The *L*, a** and *b** values of bread made from raw wheat flour as control, comported with treatment bread made from germinated wheat flour after three and six days of germination (Table 6) show that the brightness results (*L**) showed the highest values for the control flour, which corresponded to lighter flour, whereas the bread made from germinated wheat flour after six days had the lowest value due to the reduction of gluten and brown coloring was dependent on the rate of Millard reaction, and which depend on the quality and quantity of reducing sugars and the presence of amino groups residual free in dough and the baking temperature (Fenemma, 1999). In relation to the chromaticity coordinate *a**, it was observed that the bread made from germinated wheat flour after six days had the highest value tending to a red hue. The induce germination caused an increase in *a** value. The high *a** values are associated with the decrease in *L** that causes browning, as occurred in bread made from germinated wheat flour after six days. The germination did not cause changes in the *b**values, with the exception of bread made from germinated wheat flour after six-day treatment. According to Ohm *et al.,* (2008), the flours that have the higher water absorption and higher fiber content is less bright and redder, which explains what happened in pre-harvest sprouting flour, according to the values found for water absorption.

Table 6: Color analysis of bread made from raw and germinated wheat flour

Treatments	*L**	*a**	*b**
Control raw wheat	84.23^{a}±1.84	1.12^{c} ±0.02	17.5^{a} ±0.25
Bread after 3days	80.48^{b}±2.04	1.51^{b} ±0.01	17.0^{b} ±0.46
Bread after 6days	75.25^{c}±1.65	2.32^{a}±0.02	16.35^{c} ±0.39

Values are means ± SD (n = 3). Means followed by different letters in the same row, significantly different (P ≤0.05)

8. Texture profile analysis for bread

The results from Table (7) showed that the effect of germination of wheat on bread texture profile analysis. From the results, it could be noticed that the hardness was between 1.54 and 2. 35 N from bread made from germinated wheat, this could be due to the high fiber content of the germinated wheat flour, which led to water retention and reduced its evaporation rate, thereby, causing tissue values to be reduced (Solaka and Zain Al Abidin, 1995). Meanwhile, resilience

and cohesiveness were decreased from 0.56 and 1.12 in raw wheat bread to 0.32 and 0.80 in bread prepared from wheat germinated for six days. The reduction of resilience value during germination may be attributed to a decrease in protein and increases in crude fiber (Bhol and Bosco, 2014).As well as, springiness was decreased from 5.12 to 3.38 mm, respectively. Meanwhile, chewiness was increased from 4.38 mj in control raw wheat bread to 6.83 mj in bread prepared from wheat germinated for six days, this resulted from the bread from germinated wheat had increased in hardness due to reduction of gluten and increased in natural fibrous (Kumar and Kumar, 2011).

Table 7: Texture profile characteristics of different bread

Treatments	Hardness (N)	Resilience	Cohesiveness	Springiness (mm)	Chewiness (mj)
Control raw wheat	$1.23^{c} \pm 0.01$	$0.56^{a} \pm 0.01$	$1.12^{a} \pm 0.04^{2}$	$5.12^{a} \pm 0.02$	$4.38^{c} \pm 0.15$
Bread after 3days	$1.54^{b} \pm 0.02$	$0.40^{b} \pm 0.01$	$0.90^{b} \pm 0.04$	$4.16^{b} \pm 0.01$	$5.89^{b} \pm 0.31$
Bread after 6days	$2.35^{a} \pm 0.01$	$0.32^{c} \pm 0.00^{2}$	$0.80^{c} \pm 0.03$	$3.38^{c} \pm 0.01$	$6.83^{a} \pm 0.42$

Values are means ± SD (n = 3). Means followed by different letters in the same column, significantly different ($P \leq 0.05$)

Conclusion

The process of germinating wheat has the ability to degrade gluten interestingly, the hydrolysis of the gluten started after the three days of germination of wheat grains and the lowest concentration of protein (wet and dry gluten) and insoluble dietary fiber were achieved on the six days of wheat germinated grains. Meanwhile soluble dietary fiber and mineral contents were increased at the end period of wheat germination. Sensory evaluation, color measurement and texture analysis profile in bread made from the germinated wheat flour confirmed the reduction of gluten. Therefore, it could be used to making bread for celiac diseases**.**

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