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Hameed & Bayar

المجلة العراقية لبحوث السوق وحماية المستهلك

GERMINATION EFFECT ON GLIADIN DEGRADATION IN SOUR DOUGH BREAD.

Israa Majeed Hameed [1] , *Aswan H. Bayar* [2]
[1]Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, The General Company for Grain Processing, Baghdad, Iraq. majidesraa@yahoo.com.
[2]Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq. aswan@coagri.uobaghdad.edu.iq.

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ABSTRACT

The effect of the germination process on the decomposition of gliadins in Sour dough bread was studied. The use of sprouted wheat flour and sprouted Triticale flour in the manufacture of sour bread was tested and compared to unsprouted wheat and Triticale. Also, *Lactobacillus plantarum* starter was used in each of the four treatments, and the percentage of gliadins in each treatment was estimated using Elisa method so the percentage of gliadins in wheat flour before adding the starter culture was 17.83%, after adding the starter culture at a rate of 1%, there was a decrease in gliadins by 25%. However, when using sprouted wheat flour and when adding the starter culture, a 100% decomposition of the gliadins occurred. In the treatment of the Triticale flour, the gliadins percentage before adding the starter was 12.16%, while the gliadins decreased by 42.10% after adding the starter 85.32%. on the other side, the percentages of Vit A, Vit C, Vit K, Vit E and Vit B2 have raised with minerals, and a decrease in phytic acid was observed in sprouted wheat and triticale.

Key words: Gliadin, Germination, Celiac disease, Sour dough bread.

تأثير الإنبات في تحلل الكليادين في الخبز الحامضي

إسراء مجيد حميد[1] ،أسوان حمدالله عبود البيار[2]
[1]قسم علوم الاغذية ،كلية علوم الهندسة الزراعية،جامعة بغداد،الشركة العامة لتصنيع الحبوب ،بغداد،العراق. majidesraa@yahoo.com
[2]قسم علوم الاغذية،كلية علوم الهندسة الزراعية،جامعة بغداد،بغداد،العراق.aswan@coagri.uobaghdad.edu.iq

الخلاصة

درس تأثير عملية الانبات في تحلل الكليادين في الخبز الحامضي، إذ تم تجربة استعمال طحين الحنطة المنبتة وطحين الترتيكيلي المنبتة في تصنيع الخبز الحامضي ومقارنتها بالحنطة والترتيكيلي غير المنبتين، كذلك استخدم باديء *Lactobacillus plantarum* في كل معاملة من المعاملات الأربعة وتم تقدير نسبة الكليادين في كل معاملة بطريقة الايلايزا فكانت نسبة الكليادين في طحين الحنطة قبل اضافة البادئ 17.83%، بعد اضافة البادئ بنسبة 1% حصل انخفاض في الكليادين بنسبة 25% اما عند استعمال طحين الحنطة المنبتة وعند اضافة البادئ حصل تحلل للكليادين بنسبة 100% وفي المعاملة الخاصة بطحين الترتيكلي حيث كانت نسبة الكليادين قبل اضافة البادئ 12.16% بينما انخفض الكليادين بنسبة 42.10% بعد اضافة البادئ وعند اضافة البادئ الى طحين الترتيكيلي المنبتة حصل الانخفاض في الكليادين بنسبة 85.32%. ومن ناحية اخرى فقد ارتفعت نسب الفيتامينات(A,C,K,E,B2) والمعادن وانخفض حامض الفايتيك في طحين الحنطة والترتيكيلي المنبتين.

الكلمات المفتاحية: الكليادين، الانبات، حساسية الحنطة، الخبز الحامضي.

INTRODUCTION

Wheat and many other cereals include a class of proteins known as gliadin, which is a kind of prolamin. Gluten contains gliadin, which is required for bread to rise correctly during baking. The two primary parts of the gluten portion of wheat seeds are gliadin and glutenin. Wheat flour is one product that contains this gluten. Gliadin and glutenin, which make up an equal portion of gluten, are both insoluble in water; however, gliadins are soluble in 70% aqueous ethanol **(Ribeiro *et al*.,2013).**

Alpha, beta, and gamma are the three main kinds of gliadin that the body cannot handle well when celiac disease develops. **(Bethune *et al.*, 2008).**

A chronic immune-mediated intestinal illness called celiac disease occurs when the body develops an intolerance to gliadin, a protein found in gluten. Wheat, barley, and rye, which all contain prolamin, cause lifelong intolerance in people with celiac disease. In people with a hereditary predisposition, gliadin proteins may cause autoimmune enteropathy, which is brought on by an aberrant immune response. This action is caused by specific amino acid sequences in gliadin proteins. (**McGough *et al* ., 2005).**

Patients with gluten sensitivity may struggle to maintain a lifelong gluten-free diet because no wheat or related variety is safe to ingest. So, to lessen celiac disease and wheat gluten content, numerous molecular biology, genetic engineering, breeding, microbiological, enzymatic, and pharmacological treatments have been developed. (**Cohen *et al.*, 2019**).

According to (**Rico *et al*., 2020),** germination is a promising food engineering technique to improve the nutritional value of grains, with prospective applications in functional foods and pharmaceuticals, as well as their health advantages for the prevention of chronic diseases including heart disease, diabetes, and cancer.

According to (**Shao *et al*., 2019),** the goal of the germination process is to create and activate the enzymes that transform a significant portion of complex substances like starch into maltose, glucose into dextrin, and protein into peptones, peptides, amino acids, and other compounds. until germination, these complex chemicals maintain their most complex molecular form until being absorbed by the seed embryo after their disintegration **(Guardianelli *et al*., 2019),** through the action of lipolytic enzymes, fat degradation also takes place during germination to make up for energy needs during metabolic activities. Due to the removal of starch during germination, (**George ,2015),** observed that crude fibers—a significant component of cell walls—increase in terms of percentage and perceived value in seeds. According to (**Yang *et al*.,2011),** the germination procedure is an effective way to raise the vitamin content. The seeds, which can be germinated grains, may also synthesize vitamin C. excellent natural vitamin sources in diet foods.

Nkhata *et al*.(2018) claimed that variations in the phytate concentration of grains and legumes, as well as variations in the enzyme activity, may account for variations in the bioavailability of mineral elements after germination and over various periods. **Montemurro *et al.* (2019)** indicated that germinated wheat grains are more prone to microbiological damage, which restricts the expansion of the use of germinated grains in the manufacture of baked goods. As a result, controlling germination from enough humidity is important, a germination requires a specific temperature and the presence of oxygen. appropriate and controlled germination Steeping, germination, and drying are the three main steps in the germination process.

MATERIALS AND METHODS

Germination process

The Ministry of Science and Technology provided local wheat types (Tigris Al-Khair variety), which were harvested in 2022, while a farmer provided the Triticale variety. Before the study was done, wheat grains were cleaned, freed of contaminants, and stored in polyethylene bags at 4°C. As modified the procedure described by (**Yaqoob *et al*., 2018),** was to remove empty and broken seeds and foreign materials before washing the grains several times with distilled water at room temperature. The grains were then soaked for 24 h using the recommended water to grain ratio. To express the seeds' 40–44% moisture content, wheat is 1:3, or 3 times the weight of the seeds.

To encourage germination, the seeds were wrapped in a piece of muslin cloth. The seeds were then spread out in a single layer of steel trays after the trays had been covered with two layers of cotton cloth. Germination took place in an incubator at 25°C in complete darkness, the grains were dried with the sprouting sections at a temperature of 40°C to increase germination. This resulted in wheat grains with a moisture content of 10–11% and a good scent. Given that the length of the bud did not surpass the length of the grain, the germination process went on for 24 h **(American Association of Cereal Chemists. 2008).**

Measurement of phytic acid

Utilizing a method from 2015, Phytic acid determination for flour samples at the Ministry of Science and Technology/Department of Environment and Water/Food Research Center, using an HPLC device according to the method mentioned by **Lehrfeld. (1989).**

Sour dough processing.

The flour, salt, yeast, and starter were weighed before being put into the Japanese-made Topmatic kneading machine. To aerate the flour, aid in the growth and multiplication of the yeast, and make sure that the components are spread equally and uniformly, the ingredients were combined for one minute. Water was then added based on the absorbance data seen in the farinograph. After the appearance of evidence of the conclusion of the kneading stage, represented by the cleanliness of the kneading bowl walls, the ingredients were mixed for 3 min to produce a cohesive dough. the dough's pliability, elasticity, and lack of lumps after the gluten network construction. All the kneaders' pH and acidity levels were measured for all samples and after being coated with a small amount of fat, the dough is placed in standard Bowels and allowed to ferment for 30 min. After that, the dough is baked for 20 min at 220 C, followed by a sensory evaluation. **(Gül *et al*.,2005)**

The Tigris Al-Khair and Al-Triticale wheat flour was used according to the proportions in the following table:

The first treatment

Table (1): the proportions of the ingredients used in the production of sour bread.

The components	Quantity/gm
Wheat flour	100 gm
Sodium chloride	1.2gm
Yeast	1.5gm
Water according to the Farinograph records	65 ml

The second treatment
Using the Tigris Al-Khair wheat flour with the previously mentioned ingredients, with the addition of *Lactobacillus plantarum* starter at a rate of 1% (1×10^8).
Third treatment
Using the flour of the Tigris Al-Khair germinated wheat with the same ingredients mentioned in the table above, with the use of a starter culture of *Lactobacillus plantarum* 1%.(1×10^8).
Fourth treatment
Use whole-grain Triticale flour with the ingredients mentioned in the table above.
Fifth treatment
Use the whole grain Triticale flour with the ingredients mentioned in the above table and add 1%(1×10^8) *Lactobacillus Plantarum* starter.
Sixth treatment
Using sprouted Triticale flour with the ingredients mentioned in the table above, with the addition of *Lactobacillus Plantarum* at a rate of 1%(1×10^8)

Estimation of gliadin by Elisa gliadin test

The Gluten-Gliadin Elisa Kit was used to estimate the percentage of gliadin in the manufactured bread, as 1 gm of Sour dough bread was weighed in a test tube and 9 ml of ethyl alcohol solution at a concentration of 40% was added, and they were mixed with the homogenizer to obtain a homogeneous solution free of bread clumps, and then a procedure was carried out. Centrifuge at 3000rpm for 5 min to obtain a precipitate and a clear liquid. The sediment was thrown away, the liquid was taken, and ELISA assays were performed for it. The sample is withdrawn and placed in the ELISA pit, and then washed three times using 300 microliters of diluted washing solution in each well. After the last wash, the washing water is discarded, then 100 microliters of conjugated (anti_gliadin_peroxidase) is added for each pit, then incubation for 20 min at room temperature, the above-mentioned washing step is repeated, then 100 microliters of the substrate solution is added to each pit, the plate is left in a dark place for 20 min at room temperature, the reaction is stopped by adding 100 ml of stop solution 0.5M H_2SO_4 for all pits, color development is observed, then mixed quietly, and color stability is noted within only 30 min, using an ELISA reader at a wavelength of 450 nm to read results**. (Lacorn *et al*., 2022)**

$$\text{The rate of decrease in glaidin} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100$$

RESULTS AND DISSCUSSION

Chemical Composition of Wheat and Triticale:

Table (2) shows the effect of germination on the percentage of protein in flour, where the percentage of protein in sprouted wheat flour increased to 13.0%, while it was in the non-germinated whole wheat flour 11.7%. Likewise, the percentage of protein in the sprouted Triticale flour increased to 13.5%, while it was 12.5% in the non-sprouted whole grain flour.

Table (2): The chemical composition of sprouted and unsprouted wheat and Triticale flour.

Components	Whole grain wheat flour (%)	Sprouted wheat flour (%)	Whole Grain Triticale Flour (%)	Sprouted triticale flour (%)
Protein	11.7	13.0	12.5	13.5
Fat	1.89	1.4	1.74	1.48
Fiber	1.47	2.14	1.50	2.33
Ash	1.6	1.8	1.7	1.9
Moisture	14.0	11.7	13.7	11.4

This was supported by research by **(Muñoz -Llandes *et al*., 2019**), who found that germination is the only conventional method that increases protein content, as opposed to roasting and sterilization, which decreases it. The increase in protein content is attributed to a rise in protease activity. However, seed proteins are broken down into amino acids during germination, and storage protein breakdown is required for the production of amino acids and peptides to stimulate embryonic growth. The table also shows the effect of germination on the percentage of fat in the flour, as the percentage of fat in the germinated wheat flour decreased to 1.40%, while it was 1.89% in the whole grain wheat flour. Likewise, it decreased to 1.48% in the germinated Triticale flour, while it was in the whole grain Triticale flour 1.74%.

This was supported by the studies demonstrated that the production of energy to support metabolic processes such as the synthesis of structural proteins, enzymes, DNA, and RNA, as well as being used as a major source of carbon for seed growth, is responsible for the decrease in the percentage of fats during germination **(Megat Rusydi *et al*., 2011; Al-Haidari *et al*., 2019).**

The table also showed the effect of germination on the percentage of fiber in flour, as it increased to 2.14%, while its percentage was 1.47% in non-germinated whole grain wheat flour, as well as in germinated triticale flour, it increased to 2.33%, while its percentage in non-germinated Triticale flour was 1.50%.

According to researcher (**George, 2015).** germination increases crude fibers in germinated grains because starch disappears. Crude fibers, a significant component of cell walls, also rise in terms of proportion and perceived value. Like whole wheat flour, whole grain flour is a healthy and abundant source of soluble and insoluble fiber, which has a favorable and helpful impact on the health of the person (**Hammood & Nasir, 2018),** It also showed an increase in the percentage of ash in the germinated wheat flour, as it rose to 1.8%, while it was 1.6% before germination in the whole grain wheat flour, as well as in the germinated triticale flour, where the percentage increased to 1.9%, while it was 1.7% before germination, and this was confirmed by the researchers (**Uduwerella *et al*., 2021),** where they indicated as a result of the germination process, which enhances the bioavailability of minerals like iron, zinc, and calcium in the germinated seeds, demonstrated that the reason for the increase in ash is due to the increase in the percentage of mineral elements in the germinated wheat flour. As mentioned the internal enzyme of complex organic substances is hydrolyzed, which is why the amount of ash has increased. It causes the antifoods to seep into the germination media and the phytase enzyme to be active during germination, which causes the disintegration of the bonds between protein and minerals to become free. This results in the release of more nutrients.

The table also shows a decrease in the percentage of moisture in the germinated wheat flour, as the moisture percentage reached 11.7%, while it was 14% before germination. The same applies to the germinated triticale flour, where the moisture percentage reached 11.4%, while it was 13.7% before germination. This was confirmed by the researcher (**Ersedo, 2019),** who explained that the drying of the grains serves to halt the microbial activity of the grains and extends the shelf life of food products made from flour and wheat by reducing the humidity after germination in order to stop the enzyme action.

Table (3) Percentage of vitamins in sprouted and non-sprouted flour.

Name	Vit A (IU)	Vit C (ppm)	Vit E (ppm)	Vit K (ppm)	Vit B 2 (ppm)
Whole grain wheat flour	9.22	0.47	8.25	1.11	2.55
Sprouted wheat flour	17.14	1.10	13.22	2.47	4.11
Whole grain Triticale flour	11.58	0.88	10.24	1.52	2.98
Sprouted Triticale flour	21.56	1.98	15.44	3.58	6.00

Table (3) shows that germination leads to an increase in the flour content of some vitamins, as the percentage of vitamin A in germinated wheat flour increased to 17.14[IU], where it was before germination 9.22[IU]. As for the germinated Triticale flour, the percentage of vitamin A increased to 21.56[IU], while it was 11.58[IU] before germination. The same applies to vitamins (C, E, K, and B2), where the increase occurred clearly in the sprouted wheat flour compared to what it was in other flours. Ungerminated the whole wheat This was confirmed by researchers (**Zilic *et al*., 2015; Abas**,**2012),** that it's probable that the biosynthesis carried out by the newly formed seedling as a result of the germination process is what led to the buildup of specific vitamins.

Table (4) Percentage of minerals and phytic acid in sprouted and unsprouted wheat and Triticale flour.

Name	Whole grain wheat flour	Sprouted wheat flour	Whole Triticale grain flour	Sprouted Triticale flour
Fe (µg / gm)	33.6	40.5	36.9	39.8
Zn (µg / gm)	29.3	36.5	31.5	39.5
Cu (µg / gm)	3.5	4.8	4.0	5.6
Mn (µg / gm)	34.5	41.2	37.9	44.6
Na (µg / gm)	50.1	56.9	53.6	60.3
Ca (µg / gm)	310.5	345.8	325.9	355.9
Phytic acid	5022	77.7	395	49.6

Table (4) shows that germination increases the percentage of minerals in the germinated flour, where it was observed that the percentage of (Fe, Zn, Cu, Mn, Na, and, Ca) increased clearly than it was in the whole grain ungerminated wheat flour, and this was also confirmed by the researchers (**Luo *et al*., 2014),** where they stated that the germination process is a way to improve the nutritional value of some crops like wheat by improving the bioavailability of mineral elements and increasing their percentage. Germinated seeds contain a number of mineral elements, including iron, zinc, calcium, magnesium, copper, and sodium.

The table also shows decrease in the percentage of phytic acid in the sprouted wheat flour, where the acid percentage in the whole-grain wheat flour was 5022ppm, while it decreased in the sprouted wheat flour to 77.7ppm and also in the whole-grain Triticale flour was 395ppm, while it decreased in the sprouted Triticale flour to 49.6 ppm, and this was also confirmed by the researchers (**Samia *et al*., 2013; Al-Timimi *et al.*, 2006) ,** they said the soaking and emergence of the seedling improved the nutritional value of the chosen seeds in terms of the high concentration of nutrients and the reduction of phytic acid contents during germination, as demonstrated by the decrease in phytic acid contents due to the enzymatic changes during the duration of soaking and germination in the seeds, and the lack of effect of the presence or absence of light during the germination process on the achieved results. Additionally, the endogenous phytase enzyme's activity during the process of reducing phytic acid is primarily responsible for that.

Baking results

Table (5) shows the bread treatments before and after adding the starter and the extent of the effect of the starter on the percentage of gliadins, which is one of the components of gluten and is considered one of the causes of gluten sensitivity disease, where the percentage of gliadins in the first treatment before adding the starter was 17.83%, while in the second treatment after adding the starter, There was a decrease in the gliadins by 25%, but in the third treatment when using sprouted wheat flour and when adding the starter culture there was a decomposition of the gliadins by 100%. In the fourth treatment of Triticale flour, where the percentage of gliadins in the whole-grain Triticale flour before adding the starter culture was 12.16%, while in the fifth treatment, after adding the starter, there was a decrease in gliadins by 42.10%, while in the sixth treatment, when the starter was added to the sprouted Triticale flour, there was a decrease in gliadins by 42.1%. 85.32, and this was confirmed by the researchers (**Gerez, *et al*., 2009),** that the protease enzyme secreted by the lactic acid bacteria *Lactobacillus plantarum* was used to assess gliadin units. Protease enzymes are also activated during the germination phase, and this also leds to an increase in the rate of gliadin degradation (**Ohm *et al.*, 2016).**

Table (5) includes the six treatments with the ratio of gliadins.

Name	Concentration of Gliadins, ppm	Percentage decrease in gliadin %	pH	Acidity%
Wheat control	17.83	_	5.6	0.63
Wheat Control+1% *Lactobacillus Plantarum*	13.33	25	4.3	1.08
Sprouted wheat flour + 1% *Lactobacillus plantarum*	Zero	100	4.6	0.81
Triticale control	12.16	_	5.4	0.72
Triticale control+1% *Lactobacillus plantarum*	7.04	42.10	4.4	0.99
Sprouted Triticale flour+1% *Lactobacillus plantarum*	1.51	85.32	4.5	0.90

CONCLUSION

The germination process leads to an increase in the percentage of protein, fiber, ash, mineral elements (iron, zinc, copper, manganese, sodium, calcium) and vitamins (A, C, E, K, B2), and a decrease in fats, moisture, carbohydrates, as well as phytic acid for the two types of Degla Khair wheat. It is also possible to produce the glutinase enzyme from the local bacteria Bacillus subtilis IHB3, which decomposes gliadin, gliadin, and gliadin, in addition to eliminating the gliadin protein by germination, as its percentage decreased by 100% when using the gliadin measurement by ELISA test.

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