



# Utilization of germinated ancient wheat (Emmer and Einkorn) flours to improve functional and nutritional properties of bread

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## ABSTRACT

Germinated and untreated ancient (Einkorn and Emmer) and modern (Esperia) wheat flours (0, 5, 10, 15 and 20%) were used in bread dough to improve functional and nutritional properties of bread according to  $(3 \times 2 \times 5) \times 2$  factorial design. Utilization of wheat varieties in the germinated form increased the ash, total dietary fiber (TDF), total yellow pigment (TYP), total phenolic content (TPC), antioxidant activities, Ca, Fe and Mg content of bread, and also the most increments in those values (except TYP) were observed in Emmer flour usage. Germinated wheat flour decreased the mean phytic acid contents of bread samples from 313.32 mg/100 g to 291.81 mg/100 g compared to untreated wheat flour. The use of ancient wheat flour (einkorn and emmer) gave lower bread volume compared to modern bread wheat flour. The use of germinated wheat flour decreased the crust and crumb  $L^*$  values of the breads but increased the  $a^*$  and  $b^*$  values. As a result, increasing ratios of germinated ancient wheat flour increased the functional component and nutritional value of the bread, and at the same time, its usage at low ratio contributed positively to the technological quality of the bread.

## 1. Introduction

Ancient wheat, the ancestor of modern wheat, is the earliest cultivated wheat by humanity. The production of ancient wheat decreased drastically in the 1960s due to the development of agricultural processing techniques, nutrition habits, economic changes, and the use of high-yielding and easy harvesting of bread and durum wheat. Ancient wheat draws attention with its high protein digestibility and rich in essential amino acids (like phenylalanine, tyrosine and isoleucine), vitamins, minerals and bioactive components. It also contains low-toxicity gliadin and gluten forms that are more easily assimilated (Suchowilska, Wiwart, Kandler, & Krska, 2012).

Wheat is a crucial grain product that plays a significant part in the nutrition of 40% of the population around the world. However, the nutritional quality of wheat may lag behind some foodstuffs because of its low protein content, lack of some essential amino acids (lysine) and the availability of antinutritional factors such as phytic acid, tannins and polyphenols (Kaur & Gill, 2020). A wide range of food processing techniques like germination, cooking and fermentation can be used to enhance the nutritional qualities of wheat. Among them, germination is an effective and relatively inexpensive bioprocessing technology where enzymes are activated, breaking large-molecule nutrients into smaller

components. Biochemical reactions along with germination result in the biosynthesis and accumulation of a wide range of secondary metabolites such as flavonoids, vitamin C, tocotrienols,  $\gamma$ -aminobutyric acid, tocopherols, and phenolic substances while reducing anti-nutritional factors such as phytic acid (Azeke, Egielewa, Eigbogbo, & Ihimire, 2011). In Eastern countries, germinated grain consumption has been well-known for a long time; however, in Western countries, starting from the 1980s, consumption of germinated grain has become widespread due to consumer preference for exotic and dietetic healthy foods (Benincasa, Falcinelli, Lutts, Stagnari, & Galieni, 2019). Germinated grains, with their distinctive features such as rich flavor and remarkable bioactive substance content, are consumed as a snack after drying or roasting, to decorate salads, and to produce alcoholic beverages. In our previous study, we determined that the use of germinated ancient wheat in noodle formulation significantly increased the nutritional and functional properties of the noodle (Kömürcü & Bilgiçli, 2023). The latest trend is the fortification of wheat bread with germinated grains and pseudocereals (Falcinelli et al., 2018).

Bread is mainly produced from refined white flour, and a significant part of the functional components of the wheat grain is removed from the flour during refining process. Today, obesity, diabetes, constipation and high cholesterol are frequently seen as diseases. Enrichment of

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widely consumed products such as bread with bioactive components is important in terms of reducing the risk of such diseases (Yaver, 2021). In recent years, consumers' awareness of healthy nutrition has led to an advanced progress trend in the bread industry. Today, consumers pay attention to consuming natural and additive-free products with high nutritional value instead of bread varieties with low nutritional value (Gębski, Jezewska-Zychowicz, Szlachciuk, & Kosicka-Gębska, 2019). Whole wheat flours obtained from modern and ancient wheat and their germinated forms are among the potential ingredients to improve the nutritional feature of wheat bread.

According to our research, this is the first study to investigate the use of germinated ancient wheat (Einkorn and Emmer) flour in bread production. This study investigated the effect of wheat variety (ancient and modern), germination process and whole wheat flour ratio on the functional, physical and chemical properties of bread.

## 2. Material and methods

### 2.1. Material

Esperia, Einkorn and Emmer wheat were procured from Kastamonu, Kars and Konya, Turkey. White flour (0.54 g/100 g ash, 11.16 g/100 g protein, 0.89 g/100 g fat, 3.29 g/100 g TDF, 273.98 mg/100 g phytic acid, 1.92 µg/g (LE) TYP, 389.97 mg GAE/kg TPC, 242.36 mg TE/kg DPPH, 0.43 µmol TE/g FRAP and 3.16 µmol TE/g CUPRAC antioxidant activity), bakers' yeast and salt were purchased from a local market in Konya (Turkey).

### 2.2. Methods

#### 2.2.1. Germination of wheat

As described by Kömirçü and Bilgiçli (2023), after the wheat grains were disinfected with sodium-hypochlorite solution, they were kept in water for a certain period, and then they were germinated for 3 days by repeating the soaking process every 6 h in a climate cabinet at  $17 \pm 2^\circ\text{C}$  (80% RH). According to preliminary tests, 3 days of germination of wheat were found optimum time. The bread-making quality was adversely affected above 3 rd day germination due to excessively increased enzyme activity. On the other hand, the advantages of germination in terms of nutritional and functional components were found to be insufficient in samples with germination time shorter than 3 days. After the germination, the drying process ( $60^\circ\text{C}$  for 24 h) was applied via an air convection oven and grains were ground into whole wheat flour ( $< 500 \mu\text{m}$ ).

#### 2.2.2. Preparation of bread

Control bread was prepared by mixing 100 g wheat flour, 3 g bakers' yeast and 1.5 g salt (NaCl) and 69 ml water. Water content adjusted according to farinograph water absorption values. In other bread formulations, wheat flour was replaced by untreated whole wheat flour (UTWF) and germinated whole wheat flour (GWF) of Esperia, Einkorn and Emmer at 0, 5, 10, 15 and 20% levels by applying a 3 (wheat variety)  $\times$  2 (processing type-germination)  $\times$  5 (whole wheat flour ratio) factorial design (Supplementary Material 1). Briefly, bread ingredients were kneaded with a mixer (Hobart N50, Offenburg, Germany) until a homogeneous and mature dough was obtained. The dough was put into a greased metal pan and allowed to ferment in a chamber (Fimak FMD16, Konya, Turkey) for 30 min at  $30^\circ\text{C}$  and 75–80% relative humidity. The dough was then thinned to 2 cm thick and folded, followed by a second fermentation for 30 min. The dough was then proofed for 60 min at  $30^\circ\text{C}$ . The proofed dough was baked in a rotary oven (Fimak Rokon Classic FRN10G, Konya, Turkey) at  $230 \pm 5^\circ\text{C}$ . The bread samples cooled for 1 h and were kept in polyethylene bags at room temperature until analysis.

#### 2.2.3. Chemical content and functional properties

Standard methods of AACC (1990) were used in the bread samples: 44–19 for moisture, 08–01 for ash, 46–12 for protein and 30–25 for fat content. The value of total dietary fiber (TDF) was determined by following the AOAC 991.43 method (AOAC, 2012). After mixing 1 g of the analysis sample with 40 ml of MES-TRIS buffer solution, it was treated with  $\alpha$ -amylase, protease and amyloglucosidase enzymes, respectively. Then, the organic components that were converted to free form, except dietary fiber, were removed by washing with ethyl alcohol (95% and 78%) and acetone. Afterwards, ash and protein analyses of the remaining residues were made and the amount of TDF was determined.

The amount of phytic acid in the bread samples was determined using the colorimetric method according to Haug and Lantzsch (1983). Bread samples, whose particle size was reduced by grinding, were extracted with 0.2 N hydrochloric acid solution. Briefly, after the extraction, 0.5 ml of extract and 1 ml of a solution of 0.4 Mm ammonium iron (III) sulfate were pipetted out into a test tube. Primarily, test tubes were kept in the boiling water bath for 30 min and then kept in an ice bath for 15 min. Later 2 ml of 2',2'-bipyridine solution was put into the test tube. The absorbance was measured at 519 nm by a UV/Visible spectrophotometer (Hitachi-U1800, Japan).

Total yellow pigment (TYP) content was determined according to the AACC method 14–50.01 with some modification (AACC, 1999). On a certain amount of bread sample, n-butanol saturated with water was added, the resulting mixture was vortexed for 1 min, then rested at room condition for 15 min and then vortexed again for 1 min. After resting for 16–18 h under refrigerator conditions ( $+4^\circ\text{C}$ ), the mixture was vortexed for 1 min, passed through Whatman No:1 filter paper and read at 445 nm in the spectrophotometer (Hitachi-U1800, Japan). Results were calculated using the absorbance coefficient specified for lutein by Lepage and Sims (1968) and Emeksizoglu (2016).

The Folin-Ciocalteu method described by Singleton and Rossi (1965) was used to determine the total phenolic content (TPC) of bread samples. 1% acidified (HCl) methanol:water solution (80:20, v/v) (10 ml) was added to the powdered bread samples (1 g). After shaking the mixture for 2.5 h in a water bath at  $24 \pm 1^\circ\text{C}$ , it was centrifuged (1056.51 g for 10 min). The resulting supernatant (0.1 ml) was mixed with 0.5 ml of Folin (10%, v/v, in water) and 0.4 ml of  $\text{Na}_2\text{CO}_3$  (7.5% w/v) and incubated for 2 h at room temperature in the dark. After incubation, the absorbance of the samples was read in a spectrophotometer at 760 nm and the TPC of the samples was calculated as mg GAE/kg.

For the antioxidant activity analysis of the samples, the samples were extracted with 80% methanol:water solution (80:20, v/v) as indicated in the TPC analysis method. Three methods were used in the antioxidant activity analysis of the samples.<sup>1</sup>For the analysis of the samples with the DPPH (2–2-Diphenyl-2-picrylhydrazil) antioxidant activity method, the method described by Beta, Nam, Dexter, and Sapirstein (2005) was used and the results were calculated as mg Trolox Equivalent/kg. <sup>2</sup>For the analysis of the samples with the FRAP (ferric reducing antioxidant power) antioxidant activity method, the method described by Gao, Björk, Trajkovski, and Uggla (2000) was used and the results were calculated as µmol Trolox Equivalent/g. <sup>3</sup>For the analysis of the samples with the CUPRAC (cupric ion reducing antioxidant capacity) antioxidant activity method, the method described by Apak, Güçlü, Özyürek, and Celik (2008) was used and the results were calculated as µmol Trolox Equivalent/g.

To determine the Ca, Fe, K, Mg, P and Zn amounts of the samples, 0.3 g dry sample was burned in the microwave system (Mars 5, CEM Corporation, USA) using 7 ml  $\text{HNO}_3 + \text{H}_2\text{SO}_4$ . The mineral content of the solutions was tested on the ICP-MS (Inductively Coupled Plasma Mass Spectrometer) instrument (Agilent Technologies - 7900 ICP-MS / ASX 500, Waldbronn, Germany) (Skujins, 1998).

#### 2.2.4. Color measurement

The crumb and crust color values of the bread samples were determined using Minolta CR-400 (Hunter Lab Chroma Meter, Osaka, Japan).

The  $L^*$  (brightness),  $a^*$  (red, green) and  $b^*$  (yellow, blue) values were determined in the samples. A white tile with color values  $L^* = 98.45$ ,  $a^* = -0.10$ ,  $b^* = -0.13$  was used as a reference in the analysis.

### 2.2.5. Weight, volume and specific volume

The bread sample was weighed after 60 min from baking which the bread reached room temperature. Then the bread volume was determined based on the rapeseed replacement method (Elgün, Türker, & Bilgiçi, 2001). The specific volume (ml/g) of bread samples was expressed as the ratio between volume and weight.

### 2.2.6. Texture profile analysis

Bread texture was examined on days 1, 3 and 5 of storage. The bread was cut into 25 mm thick slices for the texture profile analysis (TPA) with a Texture Analyser (Stable Micro Systems TA-XT.Plus, Surrey, UK). It was according to the method AAC method 74–09 (AACC, 2000). The samples were compressed up to 50% of their height with a cylindrical probe of 36 mm diameter at a speed of 1 mm/s, and a time interval of 5 s was left between the two increments. The values obtained by the TPA software were selected to define the crumb textural parameters of the bread samples: hardness, springiness, cohesiveness, chewiness and resilience. The measurements were performed on days 1, 3 and 5.

### 2.2.7. Statistical analysis

The statistical analysis of data was analyzed using the statistical software SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The significant difference between the means was analyzed by Tukey HSD tests. All measurements were made in triplicate unless specifically described.

## 3. Results and discussion

### 3.1. Chemical properties of bread

The moisture, ash, protein, fat and total dietary fiber contents of bread samples are shown in Table 1. Chemical properties were discussed separately in terms of three main factors (wheat variety, processing type and WWFR). The ash, protein, fat and TDF content of some raw materials were found as 1.63 g/100 g, 11.99 g/100 g, 1.73 g/100 g and 12.78 g/100 g for untreated Esperia wheat flour, 1.84 g/100 g, 12.82 g/100 g, 1.98 g/100 g and 8.90 g/100 g for untreated einkorn wheat flour, 2.04 g/100 g, 12.91 g/100 g, 2.13 g/100 g and 8.35 g/100 g for untreated emmer wheat flour, 1.69 g/100 g, 11.86 g/100 g, 1.66 g/100 g and 12.99 g/100 for germinated Esperia wheat flour, 1.87 g/100 g, 13.13 g/100 g, 1.93 g/100 g and 8.95 g/100 g for germinated einkorn wheat flour and 2.08 g/100 g, 13.56 g/100 g, 1.98 g/100 g and 8.58 g/100 g for germinated emmer wheat flour respectively (Supplementary Material 2). When the results were evaluated in terms of the wheat variety factor, it was determined that the bread prepared with Emmer flour addition had the highest moisture, ash, protein and fat content. The rich ash, protein and fat content of Emmer wheat compared to Einkorn and Esperia may be effective in obtaining this result. Bread containing modern Esperia wheat flour had higher TDF contents than bread prepared with ancient Emmer and Einkorn wheat flour addition. Richer TDF content found in contemporary bread wheat compared to ancient wheat has been reported in the literature (Kulathunga, Reuhs, Zwinger, & Simsek, 2021). Similarly, in this study, the high TDF content of modern wheat was reflected in the bread samples. When the results were evaluated in terms of processing type factor, GWF addition into bread formulation decreased the moisture and fat content, but increased ash and TDF content compared with UTWF addition. The increase in the ash content of bread with GWF usage is probably due to the proportional increase in ash content as a result of dry matter loss (especially as starch) that occurs with the germination of wheat. On the other hand, the reason for the increase in the amount of TDF in bread may be the increment of soluble dietary fiber amount with the germination of wheat (Koehler, Hartmann, Wieser, & Rychlik, 2007). The addition of 10% or more

**Table 1**

Tukey HSD test results of moisture, ash, protein, fat and TDF content of bread samples.<sup>1</sup>

Factor	n	Moisture (%)	Ash (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	TDF <sup>2</sup> (g/100 g)
<i>Wheat variety</i>						
Esperia	20	38.50 ± 0.37c	1.26 ± 0.15b	12.88 ± 0.32b	1.36 ± 0.07b	4.15 ± 0.78a
Einkorn	20	39.06 ± 0.58b	1.30 ± 0.16b	13.15 ± 0.55ab	1.40 ± 0.07ab	3.79 ± 0.43b
Emmer	20	39.59 ± 0.80a	1.35 ± 0.22a	13.41 ± 0.69a	1.41 ± 0.09a	3.70 ± 0.39b
<i>Processing type</i>						
UTWF <sup>3</sup>	30	39.22 ± 0.64a	1.28 ± 0.16b	13.09 ± 0.55a	1.41 ± 0.09a	3.81 ± 0.57b
GWF <sup>4</sup>	30	38.88 ± 0.82b	1.34 ± 0.20a	13.21 ± 0.60a	1.36 ± 0.07b	3.94 ± 0.61a
<i>WWFR<sup>5</sup> (%)</i>						
0	12	39.77 ± 0.57a	1.09 ± 0.01e	12.40 ± 0.12e	1.28 ± 0.01d	3.07 ± 0.03e
5	12	39.30 ± 0.65ab	1.18 ± 0.02d	12.53 ± 0.13 cd	1.35 ± 0.04c	3.56 ± 0.12d
10	12	39.09 ± 0.55b	1.27 ± 0.05c	13.16 ± 0.21bc	1.38 ± 0.03bc	3.95 ± 0.22c
15	12	38.86 ± 0.60b	1.42 ± 0.07b	13.53 ± 0.37ab	1.43 ± 0.04b	4.22 ± 0.26b
20	12	38.23 ± 0.54c	1.57 ± 0.11a	13.84 ± 0.45a	1.50 ± 0.05a	4.58 ± 0.50a

<sup>1</sup> Means followed by the different letters within a column are significantly ( $P < 0.05$ ) different. Chemical properties are based on dry matter. Tukey HSD comparison test results are according to three ways analysis of variance. Values are the average of triplicate measurements on the duplicate samples. n: number of samples analyzed according to  $(3 \times 2 \times 5) \times 2$  factorial design.

<sup>2</sup> Total dietary fiber.

<sup>3</sup> Untreated wheat flour.

<sup>4</sup> Germinated wheat flour.

<sup>5</sup> Whole wheat flour ratio.

whole wheat flour ratio (WWFR) in bread production caused a decrease in the moisture content of bread samples, but ash, protein, fat, and TDF content of the bread increased with the increasing amount of WWFR. Substitution of all wheat varieties as whole flour instead of refined white wheat flour in the bread formulation revealed these increases.

### 3.2. Functional properties of bread

The results of phytic acid, TYP, TPC and DPPH, FRAP and CUPRAC values of bread samples are demonstrated in Table 2. The phytic acid, TYP, TPC and DPPH, FRAP and CUPRAC content of untreated Esperia, untreated einkorn, untreated emmer, germinated Esperia, germinated einkorn and germinated emmer wheat flour were 1326.1 mg/100 g, 3.39 µg/g (LE), 480.9 mg GAE/kg, 396.2 mg TE/kg, 2.38 µmol TE/g and 5.06 µmol TE/g, 1594.7 mg/100 g, 13.61 µg/g (LE), 521.6 mg GAE/kg, 647.6 mg TE/kg, 2.49 µmol TE/g and 5.3 µmol TE/g, 1863.1 mg/100 g, 4.75 µg/g (LE), 551.7 mg GAE/kg, 801.0 mg TE/kg, 2.64 µmol TE/g and 6.20 µmol TE/g, 1024.7 mg/100 g, 7.76 µg/g (LE), 785.8 mg GAE/kg, 978.1 mg TE/kg, 4.64 µmol TE/g and 20.27 µmol TE/g, 1194.6 mg/100 g, 20.79 µg/g (LE), 830.08 mg GAE/kg, 131.4 mg TE/kg, 5.09 µmol TE/g and 25.1 µmol TE/g and 1485.0 mg/100 g, 9.87 µg/g (LE), 1164.3 mg GAE/kg, 1123.7 mg TE/kg, 6.80 µmol TE/g and 29.53 µmol TE/g respectively (Supplementary Material 2). When the results were evaluated in terms of the wheat variety factor, it was found that the bread prepared with Emmer flour addition had a higher phytic acid content than Esperia flour substituted bread samples. When the phytic acid results are compared numerically, it was seen that the bread containing modern wheat flour was lower than the bread formulated with ancient wheat flour. The low phytic acid content of bread is very important in

**Table 2**Tukey HSD test results of phytic acid, TYP, TPC and antioxidant activity (DPPH, FRAP and CUPRAC) of bread samples.<sup>1</sup>

Factor	n	Phytic acid (mg/100 g)	TYP <sup>2</sup> µg/g (LE)	TPC <sup>3</sup> (mg GAE/ kg)	DPPH <sup>4</sup> (mg TE/kg)	FRAP <sup>5</sup> (µmol TE/g)	CUPRAC <sup>6</sup> (µmol TE/g)
<i>Wheat variety</i>							
Esperia	20	271.79 ± 81.60b	1.57 ± 0.30b	399.11 ± 73.98b	161.56 ± 64.24c	0.49 ± 0.34b	2.80 ± 0.98c
Einkorn	20	308.23 ± 88.53ab	1.81 ± 0.39a	418.09 ± 74.49b	179.84 ± 75.27b	0.69 ± 0.43a	2.92 ± 1.04a
Emmer	20	327.68 ± 105.16a	1.56 ± 0.28b	456.11 ± 85.41a	199.23 ± 72.46a	0.76 ± 0.48a	3.02 ± 1.11a
<i>Processing type</i>							
UTWF <sup>7</sup>	30	313.32 ± 96.39a	1.58 ± 0.30b	379.59 ± 22.26b	167.09 ± 72.46b	0.55 ± 0.37b	2.58 ± 0.72b
GWF <sup>8</sup>	30	291.81 ± 92.36b	1.72 ± 0.37a	469.66 ± 82.36a	193.33 ± 86.08a	0.75 ± 0.48a	3.25 ± 1.21a
<i>WWFR<sup>9</sup> (%)</i>							
0	12	176.59 ± 5.63c	1.23 ± 0.05d	350.16 ± 4.78d	64.21 ± 2.47e	0.12 ± 0.01e	1.24 ± 0.01e
5	12	239.73 ± 32.25b	1.41 ± 0.10d	393.91 ± 34.74c	131.36 ± 20.61d	0.37 ± 0.08d	2.70 ± 0.21d
10	12	294.35 ± 30.14b	1.66 ± 0.21c	427.45 ± 62.80bc	192.62 ± 32.75c	0.59 ± 0.16c	3.04 ± 0.18c
15	12	377.63 ± 40.83a	1.86 ± 0.22b	452.08 ± 68.41b	238.11 ± 22.82b	0.89 ± 0.23b	3.65 ± 0.71b
20	12	424.52 ± 32.98a	2.08 ± 0.17a	499.52 ± 93.77a	274.74 ± 43.99a	1.26 ± 0.32a	3.95 ± 0.65a

<sup>1</sup> Means followed by the different letters within a column are significantly ( $P < 0.05$ ) different. Chemical properties are based on dry matter. Tukey HSD comparison test results are according to three ways analysis of variance. Values are the average of triplicate measurements on the duplicate samples. n: number of samples analyzed according to  $(3 \times 2 \times 5) \times 2$  factorial design.

<sup>2</sup> Total yellow pigment (LE, lutein equivalent).

<sup>3</sup> Total phenolic content (GAE, gallic acid equivalent).

<sup>4</sup> 2,2-diphenyl-1-picrylhydrazyl radical scavenging (TE: Trolox equivalent).

<sup>5</sup> Ferric reducing antioxidant power.

<sup>6</sup> Cupric ion reducing antioxidant capacity.

<sup>7</sup> Untreated wheat flour.

<sup>8</sup> Germinated wheat flour.

<sup>9</sup> Whole wheat flour ratio.

terms of mineral bioavailability. Phytic acid is an antinutritional factor that limits the bioavailability of minerals. As stated above, phytic acid amounts of untreated Esperia, Einkorn and Emmer wheat flours used as raw materials were 1326.06, 1594.56 and 1863.06 mg/100 g, respectively. The phytic acid amounts of ancient wheat were found higher than modern wheat. The mean phytic acid content of bread prepared with Esperia, Einkorn and Emmer wheat flours addition were found as 271.79, 308.23 and 327.68 mg/100 g, respectively. When compared with the raw material, it is understood that a significant loss of phytic acid occurs with the bread-making process. It has been reported in many studies that there is a significant decrease in the content of phytic acid in the bread-making process, especially at the fermentation stage. The decrease in phytic acid along with the fermentation was most probably due to the action of the activity of phytase, yeast amount and fermentation time (Türk & Sandberg, 1992). In this study, the high TYP amount of bread samples with Einkorn flour was found remarkable. In general, the lowest TPC and antioxidant activity values were found in bread produced with the addition of modern Esperia wheat flour. When ancient wheats were compared among themselves, bread prepared using Emmer exhibited higher TPC and DPPH antioxidant activity than Einkorn breads. When the findings were compared in terms of processing type factor; the use of GWF in the bread formulation improved TYP, TPC and antioxidant activity values (DPPH, FRAP and CUPRAC) compared to UTWF usage. On the other hand, the use of GWF supported the increase of mineral bioavailability by reducing phytic acid. Germination not only accumulates biologically active substances such as gamma-aminobutyric acid, polyphenols and vitamins but also enhances the level of antioxidants and carotenoids. On the other hand, germination contributes greatly to the reduction of phytic acid by causing a significant increase in phytase activity (Thompson & Serraino, 1985). In this study, the use of GWF in bread production improved the TYP, TPC and antioxidant activity and decreased the phytic acid content of the bread. Phenolic compounds are in the form bound to the grain cell walls, and these bound phenolic compounds are released with the increase of the activities of the enzymes that break down the cell wall during germination and contribute to the increase in the amount of TPC (Sharma, Saxena, & Riar, 2015). The WWFR factor was found to be

significant on all bioactive components. The increasing ratio of WWFR in bread formulation also increased TYP, TPC and antioxidant activity values together with phytic acid. The richest bioactive substance content in bread was obtained with the use of 20% WWFR. One of the reasons for this result is that the bioactive component content of whole wheat flour used in bread formulation by replacing refined wheat flour is higher than refined flour. Another reason is that ancient wheats and their germinated forms are also found in whole wheat flour samples. As stated before, in general, the bioactive component value of ancient wheat is higher than modern wheat, and the content of bioactive components further increases with germination. In addition, Yu and Nanguet (2013) reported that Maillard reaction products formed during the baking of bread also contribute to antioxidant activity.

### 3.3. Mineral content of bread

The mineral contents of the bread samples are given in Table 3. The Ca, Fe, Mg, K, P and Zn content of untreated Esperia, untreated einkorn, untreated emmer, germinated Esperia, germinated einkorn and germinated emmer wheat flour were 30.19, 4.01, 131.0, 370.7, 526.2 and 4.37 mg/100 g, 32.54, 6.13, 134.7, 382.2, 678.4 and 6.07 mg/100 g, 31.25, 5.30, 132.4, 389.1, 690.3 and 5.77 mg/100 g, 30.69, 4.30139.8, 341.0, 430.8 and 4.59 mg/100 g, 33.24, 7.09, 145.29, 346.3, 589.5 and 7.37 mg/100 g and 31.87, 6.14, 143.6, 349.7, 618.0 and 5.80 mg/100 g, respectively (Supplementary Material 2). Wheat variety factors significantly affected the Fe, P and Zn contents of the bread samples. The amounts of F, P and Zn of bread produced using ancient wheat flours were higher than those produced using modern wheat. It has been reported that ancient wheat generally has richer mineral content than modern wheat (Suchowilska et al., 2012). Contrary to the use of UTWF, higher Ca, Fe and Mg values but lower K content were obtained with the use of GWF in bread production. As stated, before, in the ash section, this increase may be a proportional increase due to dry matter loss during germination. As a result, the utilization of mineral-rich raw materials in bread production also increased the mineral content of the final product, bread. As expected, increased WWFR in the bread formulation resulted in a rise in all mineral substances. The existence of the bran layer (rich in

**Table 3**

Tukey HSD test results of mineral contents (mg/100 g dry matter) of bread samples.<sup>1</sup>

Factor	n	Ca	Fe	Mg	K	P	Zn
<i>Wheat variety</i>							
Esperia	20	29.46 ± 2.48a	2.88 ± 0.53b	60.71 ± 6.37a	194.46 ± 12.29a	328.20 ± 41.71c	2.44 ± 0.75c
Einkorn	20	30.79 ± 2.51a	3.54 ± 1.00a	59.84 ± 6.28a	193.31 ± 10.45a	376.62 ± 46.84b	3.05 ± 1.10a
Emmer	20	28.98 ± 2.37a	3.20 ± 0.69a	61.66 ± 6.08a	196.70 ± 13.21a	418.83 ± 71.79a	2.55 ± 0.84b
<i>Processing type</i>							
UTWF <sup>2</sup>	30	29.09 ± 2.11b	3.06 ± 0.77b	60.08 ± 5.89b	196.20 ± 13.81a	388.05 ± 70.63a	2.59 ± 0.90a
GWF <sup>3</sup>	30	30.40 ± 2.81a	3.36 ± 0.83a	61.40 ± 6.60a	192.77 ± 10.34b	361.05 ± 58.72a	2.77 ± 0.99a
<i>WWFR<sup>4</sup> (%)</i>							
0	12	26.04 ± 0.54d	2.21 ± 0.04c	52.30 ± 0.83e	179.54 ± 3.32d	299.27 ± 20.92d	1.31 ± 0.06d
5	12	28.54 ± 1.04c	2.69 ± 0.16bc	56.28 ± 0.90d	184.79 ± 1.84d	348.26 ± 40.20cd	2.12 ± 0.26c
10	12	30.10 ± 1.17bc	3.18 ± 0.39b	60.41 ± 1.31c	193.87 ± 3.95c	370.38 ± 37.11bc	2.85 ± 0.25b
15	12	31.48 ± 1.51ab	3.85 ± 0.58a	64.89 ± 1.37b	202.89 ± 5.21b	410.97 ± 56.60ab	3.33 ± 0.31ab
20	12	32.56 ± 1.33a	4.10 ± 0.58a	69.81 ± 1.38a	211.36 ± 5.34a	443.87 ± 53.48a	3.81 ± 0.59a

<sup>1</sup> Means followed by the different letters within a column are significantly ( $P < 0.05$ ) different. Chemical properties are based on dry matter. Tukey HSD comparison test results are according to three ways analysis of variance. Values are the average of triplicate measurements on the duplicate samples. n: number of samples analyzed according to  $(3 \times 2 \times 5) \times 2$  factorial design.

<sup>2</sup> Untreated wheat flour.

<sup>3</sup> Germinated wheat flour.

<sup>4</sup> Whole wheat flour ratio.

minerals) in whole wheat flour was effective in obtaining this result. [Ranhotra, Loewe, and Lehmann \(1977\)](#) found the Ca, Fe, Zn and Mg contents of bread samples prepared with refined wheat flour, 20% whole wheat flour and 20% germinated wheat flour as 19.4, 2.17, 0.81 and 34.4 mg/100 g, 34.9, 4.12, 2.79 and 169.4, and 35.7, 3.50, 2.90 and 160.23 mg/100 g, respectively. They stated that the mineral content of the bread samples increased with the use of whole wheat flour and germinated wheat flour.

### 3.4. Color values of bread

The crust and crumb color values of bread samples are shown in [Table 4](#). Emmer flour addition into bread formulation revealed lower crumb and crust lightness compared to other wheat types. The high carotenoid pigment content of Einkorn, especially lutein, affected the crumb  $b^*$  value of the final product, bread. Lutein is the primary carotenoid in wheat and is responsible for the yellow color of the wheat and its product ([Brandolini & Hidalgo, 2011](#)). [Brandolini and Hidalgo \(2011\)](#) used einkorn, durum and bread wheat flours in bread formulation and found that einkorn bread has the highest crumb  $b^*$  color value in all bread samples. Compared to UTWF, the use of GWF in bread production decreased the  $L^*$  value but increased the  $a^*$  and  $b^*$  values of

**Table 4**

Tukey HSD test results of color values of bread samples.<sup>1</sup>

Factor	n	Crust			Crumb		
		$L^*$	$a^*$	$b^*$	$L^*$	$a^*$	$b^*$
<i>Wheat variety</i>							
Esperia	20	49.31 ± 4.81a	10.91 ± 0.77c	31.03 ± 0.84b	68.03 ± 3.20a	-0.37 ± 0.95b	16.36 ± 1.09b
Einkorn	20	49.62 ± 5.77a	13.76 ± 1.05a	34.56 ± 1.28a	68.29 ± 3.42a	-0.36 ± 0.97b	17.37 ± 0.87a
Emmer	20	47.43 ± 7.21b	13.04 ± 0.92b	33.70 ± 1.69a	66.38 ± 4.73b	-0.02 ± 1.12a	16.34 ± 1.39b
<i>Processing type</i>							
UTWF <sup>2</sup>	30	52.33 ± 2.81a	12.23 ± 1.44b	32.79 ± 1.80b	69.06 ± 3.03a	-0.33 ± 1.00b	16.15 ± 1.40b
GWF <sup>3</sup>	30	45.25 ± 6.41b	12.91 ± 1.53a	33.40 ± 2.13a	66.07 ± 4.16b	-0.18 ± 1.05a	17.23 ± 1.53a
<i>WWFR<sup>4</sup> (%)</i>							
0	12	56.23 ± 0.75a	11.16 ± 0.83d	31.45 ± 1.27d	72.70 ± 0.29a	-1.79 ± 0.01e	14.80 ± 0.20d
5	12	50.61 ± 3.44b	12.38 ± 1.56c	32.19 ± 1.44±	69.19 ± 1.66b	-0.93 ± 0.17d	15.74 ± 0.65c
10	12	47.29 ± 5.03c	12.75 ± 1.43bc	33.12 ± 1.58bc	67.73 ± 1.81b	-0.03 ± 0.28c	17.12 ± 1.30b
15	12	45.83 ± 4.90c	13.14 ± 1.32ab	33.89 ± 1.73ab	65.14 ± 2.39c	0.54 ± 0.33b	17.71 ± 1.06ab
20	12	43.98 ± 5.55d	13.42 ± 1.25a	34.82 ± 1.92a	63.08 ± 3.26d	0.96 ± 0.34a	18.06 ± 1.16a

<sup>1</sup> Means followed by the different letters within a column are significantly ( $P < 0.05$ ) different. Tukey HSD comparison test results are according to three ways analysis of variance. Values are the average of triplicate measurements on the duplicate samples. n: number of samples analyzed according to  $(3 \times 2 \times 5) \times 2$  factorial design.

<sup>2</sup> Untreated wheat flour.

<sup>3</sup> Germinated wheat flour.

<sup>4</sup> Whole wheat flour ratio.

crust and crumb. The reason for the increase in darkness and redness of the crust is probably due to the Maillard reaction. Increasing amylase and protease enzyme activity with germination causes the Maillard reaction by increasing the amount of free sugar and amino acids ([Goesaert, Slade, Levine, & Delcour, 2009](#)). [Mounjouenpou et al. \(2020\)](#) explained the darker color of the bread with the addition of germinated cereal flour with the amount of phenolic compounds. Many researchers have reported that the germination process is responsible for the increase in the phenolic compound content in grains. Phenolic compounds impart a darker color to the flour and indirectly to the final product ([Atudorei, Atudorei, & Codină, 2021](#)). In the formation of bread crust color, especially Maillard reaction and caramelization are effective due to the high temperatures reached during baking. On the other hand, the raw material color properties have an effect on the color of the bread crumb rather than the Maillard and caramelization reaction due to crumb temperature during baking does not reach high degrees. [Marti, Cardone, Nicolodi, Quaglia, and Pagani \(2017\)](#) researched the effects of malt flour, enzyme additives and germinated wheat flour on various features of bread, and they reported that the use of germinated wheat flour increased the crumb  $a^*$  value but decreased the bread crumb  $L^*$  value. In the same study, germinated wheat flour usage increased the darkness, yellowness and redness of the crust color values. They reported that raw

material properties may be the dominant responsible factor in bread crumb color changes while the increase in Maillard reaction was the probable cause of bread crust color changes. Baranzelli et al. (2018) used 24, 48 and 72 h of germinated wheat flour in bread production, and they reported that the darkness and the  $a^*$  and  $b^*$  color values increased with germinated wheat flour. In the present study, increasing the usage ratio of WWFR also increased darkness, redness and yellowness in both crust and crumb color. This color change in bread crumbs with the addition of WWFR is due to the different color characteristics of the additives (whole wheat) from refined wheat flour. The color change in the crust of the bread occurred both with additive (whole wheat) and with the effect of the Maillard reaction as stated above.

### 3.5. Physical properties of bread

The physical properties of the bread samples are tabulated in Table 5. When the results were evaluated in terms of the wheat variety factor; it was found that the bread prepared with Esperia flour addition had a higher volume and specific volume but lower weight values than Einkorn and Emmer flour substituted bread samples. When ancient wheats are compared among themselves, it was seen that the volume of the bread with Emmer wheat flour was higher than the bread with the addition of Einkorn flour. In a study, the technological properties of ancient and modern wheats were compared with micro-cooking tests; the volume values of the bread samples obtained from modern bread wheat were found to be the highest, followed by the volume values of the bread obtained from spelt, durum, emmer and einkorn wheat, respectively (Geisslitz, Wieser, Scherf, & Koehler, 2018). Also, it has been reported that the dough obtained from einkorn flour is sticky and difficult to handle, and the volume of the bread is small. The results are examined in terms of processing type; the use of GWF in bread production decreased the weight and increased the volume and specific volume value. Increased  $\alpha$ -amylase activity during germination may have played an important role in increasing bread volume especially at low utilization ratios of GWF. At high GWF usage ratios, the volume decreased significantly compared to the control bread. Olaerts and Courtin (2018) explained the higher bread volume obtained with the use of germinated wheat flour, with higher  $\alpha$ -amylase activity, increasing the amount of fermentable sugars and consequently increasing CO<sub>2</sub> production. Marti et al. (2017) stated that the germinated wheat flour

**Table 5**  
Tukey HSD test results of physical properties of bread samples.<sup>1</sup>

Factor	n	Weight (g)	Volume (ml)	Specific volume (ml/g)
<i>Wheat variety</i>				
Esperia	20	148.28 ± 1.38b	689.00 ± 69.63a	4.76 ± 0.49a
Einkorn	20	149.97 ± 1.60a	649.51 ± 85.78c	4.54 ± 0.43b
Emmer	20	150.43 ± 1.28a	669.50 ± 51.01b	4.43 ± 0.42b
<i>Processing type</i>				
UTWF <sup>2</sup>	30	150.59 ± 1.47a	637.34 ± 54.60b	4.33 ± 0.32b
GWF <sup>3</sup>	30	148.53 ± 1.11b	701.33 ± 73.24a	4.82 ± 0.46a
<i>WWFR<sup>4</sup> (%)</i>				
0	12	148.98 ± 1.00b	710.02 ± 10.86b	4.77 ± 0.03ab
5	12	148.89 ± 1.73b	726.88 ± 85.65a	4.98 ± 0.51a
10	12	149.48 ± 1.43ab	679.38 ± 63.65c	4.70 ± 0.40b
15	12	150.03 ± 1.60ab	643.33 ± 33.53d	4.33 ± 0.27c
20	12	150.50 ± 1.83a	578.08 ± 29.03e	4.10 ± 0.30c

<sup>1</sup> Means followed by the different letters within a column are significantly (P < 0.05) different. Tukey HSD comparison test results are according to three ways analysis of variance. Values are the average of triplicate measurements on the duplicate samples. n: number of samples analyzed according to (3 × 2 × 5) × 2 factorial design.

<sup>2</sup> Untreated wheat flour.

<sup>3</sup> Germinated wheat flour.

<sup>4</sup> Whole wheat flour ratio.

included in the bread formulation increased the gas production amount during fermentation and contributed to the production of bread with a high specific volume. When the results were examined in terms of WWFR factor, the use of 15–20% WWFR reduced both volume and specific volume. The fact that the bran fraction in the whole flour dilutes the gluten amount and damages the gluten network may have been effective in reducing the bread volume and specific volume. Van Hung, Maeda, Fujita, and Morita (2007) used 0, 10, 30, 50 and 100% whole wheat flour in bread production and reported that the specific volume value of the bread samples decreased as the WWFR increased in the bread formulation compared to the control group. In the study, the specific volume value of the control group samples was determined as 3.3 ml/g, while the specific volume value was reported as 2.9 ml/g and 2.2 ml/g at 30% and 100% WWFR, respectively.

### 3.6. Textural properties of bread

The textural characteristics of bread are one of the most important criteria for its acceptability by the consumer. Textural properties (hardness, springiness cohesiveness, chewiness and resilience) of bread samples are shown in Table 6. According to the texture profile analysis (TPA) result, significant differences were determined in the textural properties of the bread samples produced from different wheat varieties. The hardness and chewiness values of all bread increased, while cohesiveness and resilience values decreased during the shelf life. Emmer bread samples had a harder crumb, while Esperia bread had a softer texture on day 1. This may be related to the weak gluten structure of Emmer and Einkorn wheat and the inability to keep the gas formed during fermentation in the bread sufficiently. The hardness, springiness cohesiveness, chewiness and resilience value of the bread samples with the addition of GWF was determined to be lower than the bread with the UTWF. Storage caused a further increase in the hardness value of untreated wheat flour added bread samples. This may be related to the lower expansion rate and lower specific volumes of bread samples containing untreated whole wheat flour (Scheuer, Luccio, Zibetti, de Miranda, & de Francisco, 2016). Especially at high utilization ratios of GWF, an increase in  $\alpha$ -amylase and protease activity with germination may have been effective in the decrease in the springiness value of bread. In the literature, it has been indicated that increased amylase activity causes changes in starch structure responsible for cohesiveness (Olaerts & Courtin, 2018). In the early stages of cooking, gelatinized starch is severely broken down before the enzymes ( $\alpha$ -amylases) increased by the germination process are thermally inactivated. This causes the starch network in the bread to weaken. A high rate of starch hydrolysis adversely affects the water-binding and gel-forming capacities of starch. (Goesaert et al., 2009). It is thought that the increased content of reducing sugar and dextrin, together with the water not sufficiently bound by the starch gel, forms a sticky mass. As a result, it can lead to reduced hardness and less resilient bread properties. The average hardness and chewiness values of the bread samples showed a gradual increase due to increasing WWFR rates and storage days. The increase in bread hardness during storage is generally related to staling. The factors affecting bread staling are amylopectin aggregation and amylose retrogradation. Amylose retrogradation occurs within a few hours, creating oven exit hardness, while amylopectin aggregation occurs within a few days and is considered to be the primary cause of staling (Goesaert et al., 2009). The present results revealed that chewiness was positively related to hardness, that is, the chewiness value increased as the hardness increased. Scheuer et al. (2016) reported that this may be related to the fact that the bran fragments from whole wheat flour weaken the gluten network of the bread dough, and the bran, which is rich in albumin and globulin, has weaker dough-forming properties compared to gluten. The obtained results reflect that springiness, cohesiveness and resilience values decrease with increasing whole wheat flour content.

**Table 6**  
Texture analysis values of bread samples.1

Factor	Days	n	Hardness (g)	Springiness (%)	Cohesiveness (%)	Chewiness (g)	Resilience (%)
<i>Wheat variety</i>							
Esperia	1	20	542.67 ± 163.23	0.88 ± 0.11	0.69 ± 0.09	341.52 ± 146.08	0.33 ± 0.09
	3	20	873.67 ± 279.03	0.90 ± 0.05	0.61 ± 0.07	468.97 ± 132.78	0.24 ± 0.02
	5	20	1073.94 ± 234.02	0.90 ± 0.06	0.56 ± 0.06	548.61 ± 114.70	0.23 ± 0.01
Einkorn	1	20	567.96 ± 164.62	0.91 ± 0.08	0.67 ± 0.09	354.10 ± 134.53	0.31 ± 0.08
	3	20	801.34 ± 250.69	0.92 ± 0.07	0.57 ± 0.08	427.14 ± 179.98	0.24 ± 0.05
	5	20	1060.29 ± 377.67	0.91 ± 0.06	0.56 ± 0.06	548.45 ± 189.80	0.22 ± 0.04
Emmer	1	20	596.20 ± 289.40	0.89 ± 0.09	0.66 ± 0.08	365.81 ± 203.52	0.30 ± 0.09
	3	20	965.06 ± 435.57	0.91 ± 0.06	0.61 ± 0.07	533.09 ± 257.14	0.25 ± 0.06
	5	20	1304.19 ± 572.36	0.89 ± 0.05	0.53 ± 0.05	611.97 ± 234.38	0.21 ± 0.03
<i>Processing type</i>							
UTWF <sup>2</sup>	1	30	705.81 ± 226.89	0.95 ± 0.02	0.73 ± 0.03	483.25 ± 128.49	0.37 ± 0.03
	3	30	1086.80 ± 358.28	0.94 ± 0.02	0.62 ± 0.04	621.06 ± 183.92	0.26 ± 0.03
	5	30	1387.52 ± 482.54	0.92 ± 0.04	0.56 ± 0.06	705.97 ± 173.54	0.23 ± 0.03
GWF <sup>3</sup>	1	30	432.08 ± 60.66	0.83 ± 0.10	0.62 ± 0.09	224.37 ± 63.90	0.26 ± 0.08
	3	30	673.25 ± 124.05	0.88 ± 0.07	0.58 ± 0.09	331.74 ± 74.45	0.22 ± 0.05
	5	30	904.76 ± 160.20	0.88 ± 0.06	0.55 ± 0.05	433.38 ± 62.71	0.22 ± 0.03
<i>WWFR<sup>4</sup> (%)</i>							
0	1	12	446.48 ± 34.15	0.97 ± 0.01	0.75 ± 0.02	326.78 ± 27.64	0.40 ± 0.02
	3	12	627.72 ± 17.86	0.96 ± 0.03	0.66 ± 0.02	395.94 ± 19.59	0.29 ± 0.03
	5	12	842.19 ± 86.81	0.95 ± 0.03	0.60 ± 0.05	476.11 ± 24.42	0.26 ± 0.02
5	1	12	472.48 ± 126.94	0.92 ± 0.05	0.72 ± 0.06	309.67 ± 113.68	0.34 ± 0.06
	3	12	714.87 ± 209.98	0.94 ± 0.05	0.63 ± 0.07	409.92 ± 139.48	0.25 ± 0.03
	5	12	935.84 ± 206.51	0.92 ± 0.03	0.57 ± 0.06	502.22 ± 122.19	0.24 ± 0.02
10	1	12	540.44 ± 133.07	0.88 ± 0.08	0.65 ± 0.09	327.06 ± 147.31	0.29 ± 0.08
	3	12	871.92 ± 244.66	0.90 ± 0.05	0.58 ± 0.05	469.55 ± 177.14	0.23 ± 0.04
	5	12	1119.51 ± 287.76	0.91 ± 0.03	0.55 ± 0.06	576.90 ± 180.06	0.22 ± 0.02
15	1	12	626.56 ± 181.10	0.85 ± 0.10	0.64 ± 0.08	371.58 ± 173.55	0.28 ± 0.08
	3	12	986.34 ± 219.66	0.88 ± 0.06	0.57 ± 0.08	512.11 ± 208.67	0.23 ± 0.04
	5	12	1317.64 ± 465.09	0.87 ± 0.04	0.53 ± 0.04	614.97 ± 201.61	0.20 ± 0.02
20	1	12	758.75 ± 315.13	0.82 ± 0.11	0.62 ± 0.08	433.98 ± 244.15	0.25 ± 0.08
	3	12	1199.26 ± 449.83	0.86 ± 0.06	0.55 ± 0.05	594.48 ± 286.61	0.21 ± 0.04
	5	12	1515.51 ± 533.98	0.85 ± 0.04	0.51 ± 0.05	678.19 ± 249.45	0.19 ± 0.02

<sup>1</sup> Values are the average of triplicate measurements on the duplicate samples. n: number of samples analyzed according to (3 × 2 × 5) × 2 factorial design.

<sup>2</sup> Untreated wheat flour.

<sup>3</sup> Germinated wheat flour.

<sup>4</sup> Whole wheat flour ratio.

#### 4. Conclusion

In this study, the effect of germinated ancient and modern wheat flours on bread properties was examined. The obtained findings were evaluated about the main factors (wheat variety, germination process and WWFR). Among the wheat varieties, Emmer wheat the most improved the nutritional and functional properties of bread. The use of GWF in bread production increased ash, TDF, TWP, TPC, Ca, Fe, Mg amounts, DPPH, FRAP and CUPRAC antioxidant activity values. Increasing WWFR increased all nutritional and functional properties as predicted, but high usage of germinated or untreated whole wheat flour negatively affected the bread properties such as volume, specific volume, hardness and color. The decrease in bread volume and specific volume with the use of whole flours of ancient wheat was compensated by the utilization of low levels of germinated wheat flour.

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#### CRediT authorship contribution statement

**Tekmile Cankurtaran-Kömürcü:** Investigation, Resources, Formal

analysis, Writing – original draft. **Nermin Bilgiçi:** Methodology, Project administration, Funding acquisition, Conceptualization, Supervision, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

Data will be made available on request.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ifset.2023.103292>.

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