# **ORIGINAL PAPER**



# **Use of fnger millet (***Eleusine coracana* **L.) as functional ingredient in wheat pan bread to promote sustainable agro‑food systems**

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# **Abstract**

Boiling, microwave-assisted hydration and ultrasonication-assisted hydration of eleusine four infuenced its chemical composition, leading to a reduction in total phenolics. The untreated eleusine four, with the highest total phenolics and antioxidant potential, was chosen for pan bread formulation. LC–ESI–MS analysis of eleusine four showed that favonoids, particularly quercetin-3-*O*-rhamnoside (484 µg/g extract), naringenin (326 µg/g extract) and epicatechin (312 µg/g extract) were the major compounds representing  $\sim$  70% of the total measured compounds. Substituting wheat by eleusine flour affected dough rheological properties and bread characteristics, notably at the highest substitution level. Compared to the control sample, a formulation with 50% eleusine flour (F5) showed that both dough extensibility was decreased by  $\sim$  70%. Simultaneously, there was a signifcant increase in dough tenacity value by 26%. In terms of the bread characteristics, the specifc volume was reduced by 35% aligning with a notable decline in springiness value by 26%. Although, the formulation F5 showed a signifcant decrease in alveoli number, there was no notable reduction in the density of the crumb cells. Despite these changes, the F5 bread maintained its acceptability on a 5-point hedonic scale, as seen by an overall acceptability mean score>3. Interestingly, the total phenolics and antioxidant capacity of the composite bread were signifcantly increased, suggesting that eleusine was a promising functional substitute for wheat in bakery products.

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# **Graphical Abstract**



**Keywords** Eleusine · Processing efects · Phenolic composition · Blended bread · Nutritional characteristics

# **Introduction**

Bread is a staple food consumed around the world, providing a signifcant portion of daily caloric intake and essential nutrients. Wheat is the cereal used in bread production due to its gluten content, which gives viscoelastic properties essential for dough formation and bread structure [\[1](#page-15-0)].

North Africa, a region characterized by its Mediterranean climate and historical dependence on agriculture, faces increasing challenges due to climate change [[2\]](#page-15-1). Wheat, an important crop for the region's food security, is particularly vulnerable to climate variations, raising concerns about its future sustainability and productivity. Unfortunately, in most dryland developing countries, bread has to be purchased with hard currency. Therefore, there is growing interest in incorporating alternative cereal grains into bread formulation to improve nutritional diversity and food security [[3](#page-15-2)]. Finger millet (*Eleusine coracana* L.) is a cereal crop that is resistant to harsh climatic conditions, which can be grown in regions where wheat cultivation is difficult. In addition, finger millet is often considered as efficient crop due to its long shelf life and resistance to insect and pest infestations [\[4](#page-15-3)]. In Tunisia, it is mostly grown in the semi-arid regions of the country, where it is known locally as "Bechna". Finger millet has interesting nutritional potential in terms of high levels of dietary fber, minerals (such as calcium, iron and magnesium) and antioxidants, which can supplement the nutrient profle of wheat-based products. In addition, it has several health-promoting attributes, including antidiabetic, anti-hypercholesterolemic, antioxidant and antimicrobial properties. Mainly, this cereal inhibits intestinal pancreatic amylase and  $\alpha$ -glucosidase, which helps to regulate postprandial hyperglycemia. Thus, regular consumption of fnger millet as a staple food will help to manage type 2 diabetes [[5\]](#page-15-4).

Shukla and Srivastava [\[6\]](#page-15-5) reported that a 30% blend of finger millet with refined wheat flour for noodles preparation has beneficial benefits for diabetic patients due to low glycemic response compared to the control. In addition, the use of 20% extruded finger millet improved the quality of composite bread in terms of texture, sensory properties, phenolics and antioxidant activity [\[7](#page-15-6)]. Another study showed that incorporation of 60% finger millet flour in muffins adversely affected their quality characteristics. However, when a combination of polysorbate-60 and hydroxypropyl methylcellulose was employed, it notably improved the texture of muffins containing 60% finger millet flour [[8\]](#page-15-7). Studies on the use of finger millet in the development of industrial food products are still limited. One approach to improve the consumption of these crops is to incorporate them into consumed foods, such as bread. The concept of blending wheat and finger millet flours in bread preparation offers a promising avenue for producing value-added composite bread with improved nutritional content and functional properties.

Processing technologies can be used to improve the availability of micronutrients and contribute to the overall improvement of the quality of food grains. Wang et al. [[5\]](#page-15-4) reported the influence of different processing methods on certain polyphenols and total polyphenols in millet, such as fermentation, malting, roasting, parboiling, ultrasonication and microwave. The polyphenol composition in millet changed positively or negatively depending on the processing method, subsequently influencing the antioxidant and hypoglycemic characteristics of the end products [[5\]](#page-15-4).

Therefore, the objective of this study was to develop a composite flour containing wheat and finger millet, suitable for bread production. The impact of certain processing methods such as boiling, microwave-assisted hydration and ultrasonication-assisted hydration on the chemical, antioxidant and hydration properties of finger millet was studied. Then, the characteristics of dough and bread produced by blending different levels of wheat and finger millet flours was measured, in terms of dough viscoelastic properties, bread characteristics and consumer acceptance.

# **Material and methods**

#### **Finger millet processing**

The fnger millet (*E*. *coracana* L.) grains were provided by a farmer from the area of Gabes (Tunisia). The impurities were removed by sifting grains. Subsequently, Fine four was produced using a laboratory hammer mill (Perten Instruments AB, Huddinge, Sweden) with a 0.8-mm screen. Then, the integral flour was uniformly dispersed in distilled water in a ratio of 1:10 (m/v), and subjected to the following processes:

- (i) boiling: using an oven (Memmert, Schwabach, Germany) at  $100^{\circ}$ C for 5 min;
- (ii) microwave-assisted hydration: using a convection microwave oven (Godrej GMX 20CA5 MLZ, Mumbai, India) at 120 W for 5 min;
- (iii) ultrasonication-assisted hydration: using an ultrasonic processor (20 kHz, JY 92-IIN Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) in a pulsed mode for 5 min.

After that, all processed eleusine flours were freeze-dried.

#### **Characterization of eleusine four**

#### **Chemical composition**

The moisture, lipids, ash and protein levels were measured using the official methods  $[9]$  $[9]$  $[9]$  and expressed as  $g/100$  g. The carbohydrates level was determined using the diference method: [total solids−(lipids+ash+protein)]. The dietary fber was determined using the enzymatic gravimetric method as previously described by Prosky et al. [[10\]](#page-15-9). The starch was measured using the anthrone–sulfuric acid method  $[11]$  $[11]$  $[11]$ . The gluten was extracted by eliminating starch and soluble components using washing a dough with excess water. The minerals concentrations were measured using the acid digestion of sample using a nitric acid/ perchloric acid solvent mixture (2:1, v/v). The potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn), and iron (Fe) were individually measured utilizing an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan).

#### **Phenolics and antioxidant activities**

The eleusine flours, subjected to the different treatments, were extracted in 95% ethanol (1/10, m/v) by maceration for 24 h with stirring. Subsequently, after centrifugation at 12,000 rpm for 30 min, the resulting supernatant was used for phenolic compounds and antioxidant activities measurements.

Total phenolics and favonoids were measured as previously described by Dewanto et al. [[12\]](#page-15-11) and they were presented as mg of gallic acid equivalent/100 g of eleusine flour (mg GAE/100 g) and mg of quercetin equivalent/100 g of eleusine flour (mg QE/100 g), respectively. The DPPH·-scavenging and β-carotene/linoleic acid bleaching activities were measured as previously described by Zouari et al. [[13\]](#page-15-12), and they were expressed by the  $IC_{50}$  value defined as the concentration of eleusine flour providing 50% inhibition. In the  $Fe<sup>3+</sup>$  reducing activity, the presence of antioxidants in the sample results in the transformation of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , observable by quantifying the formation of Perl's Prussian blue (Fe4[Fe(CN)6]3) at 700 nm. The efective concentration of eleusine four  $(EC_0, 5)$  required to achieve 0.5 absorbance at 700 nm was presented.

#### **Hydration properties**

The water retention capacity (g water/100 g of eleusine flour) and the swelling index (mL/100 g of eleusine flour) were determined as described by Jridi et al. [\[14](#page-15-13)].

#### **Phenolic compounds profle**

First, 25 g of untreated eleusine four were subjected to extraction using 250 mL of 95% ethanol in a sealed Erlenmeyer fask with continuous stirring at 250 rpm for 24 h. Subsequently, the solvent was eliminated using a rotary evaporator, and any remaining solvent residues were purged using nitrogen fushing. The concentration of phenolic compounds in the obtained extract (20 mg/mL) was measured using a Shimadzu quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The mass spectrometer was ftted with an electrospray ionization source and operated in the mode of negative ionization. Identifcation of phenolic compounds was done by comparing their mass spectra with those of high purity standards (Sigma Chemical Co., St Louis, MO, USA), as detailed in the previous study by Chahbani et al. [[15\]](#page-15-14).

#### **Color measurement**

The color parameters (lightness  $L^*$ , red index  $a^*$  and yellow index b<sup>\*</sup>) of eleusine-wheat composite flours were measured using a CR-400 chromameter (Konica Minolta Sensing, Osaka, Japan). The L\* value represents lightness on a scale from black (0) to white (100). The a\* index varies from red  $(+100)$  to green  $(-100)$ . The b<sup>\*</sup> index varies from yellow  $(+100)$  to blue  $(-100)$ . The saturation  $(C^*)$  and total color difference ( $\Delta E$ ) were determined using Eqs. [\(1](#page-3-0)) and [\(2\)](#page-3-1).

$$
C \vcentcolon= \sqrt{a^{*2} + b^{*2}} \tag{1}
$$

$$
\Delta E = \sqrt{(L^* - L_c^*)^2 + (a^* - a_c^*)^2 + (b^* - b_c^*)^2}
$$
 (2)

To determine the value of ∆E, the color parameters of F1–F5 are represented by  $L^*$ ,  $a^*$  and  $b^*$ , while the color parameters of F0 are denoted by  $L^*$ <sub>c</sub>,  $a^*$ <sub>c</sub> and  $b^*$ <sub>c</sub>.

#### **Dough alveographic characteristics**

The alveographic characteristics of the dough were evaluated using a Chopin alveograph (Tripette et Renaud, Villeneuve La Garenne, France) in accordance with the standardized method  $[16]$  $[16]$  $[16]$ . The studied flours were wheat flour, used as control, (formulation 0: F0) and wheat flour/eleusine flour blends in varied ratios based on mass: 90/10 (formulation 1: F1), 80/20 (formulation 2: F2), 70/30 (formulation 3: F3), 60/40 (formulation 4: F4), and 50/50 (formulation 5: F5). The maximum overpressure (P), necessary for infating the dough bubble, served as a measure of the dough's tenacity or resistance to deformation. This parameter was associated with both the quantity and quality of gluten, as well as its ability to absorb water. The average abscissa (L) at the

point of bubble rupture refected the dough's extensibility, representing the gluten's ability to hold gas. The confguration ratio (P/L) indicated the equilibrium between tenacity and extensibility in the dough. The deformation energy (W) served as an indicator of the dough's strength.

# **Bread formulation and characterization**

#### **Bread formulation**

The bread was prepared under industrial conditions in the BVM bakery company (Boulangerie Viennoiserie Méditerranéenne, Tunis, Tunisia). The pan bread, also known as loaf bread or sliced bread, was selected as a model for this study. It is distinguished by a soft crumb and absence of a crispy crust, commonly utilized for sandwich preparation owing to its convenient rectangular shape and soft texture. The bread formulation was developed by modifying the standard formulation, which consisted of: 500 g wheat four, 265 g water, 20 g dried, 20 g sucrose, 10 g salt, 2 g calcium propionate (as conservative), along with 1 g bean four and 0.75 g ascorbic acid (as oxidizing adjuvants). Bread formulations were prepared using wheat flour and blends of eleusine flour/ wheat four as described previously. The levels of the other ingredients remained constant for all formulations. The bread was produced using a straight dough method, which usually involves a direct mixing, in which all ingredients were mixed simultaneously to form the dough. The ingredients were blended in a mixer, initially at a low speed for 3 min and subsequently at a high speed for 5 min. Following the kneading, the dough was left to rest for 25 min. After relaxing, the dough was shaped into pieces and undergoes a fermentation for 90 min at 30 °C within a fermentation chamber. Finally, the diferent breads were baked at 260 °C for 20 min.

#### <span id="page-3-1"></span><span id="page-3-0"></span>**Physical properties**

The bread volume  $(cm<sup>3</sup>)$  was measured using the rapeseed displacement method, and the specifc volume of bread  $\text{cm}^3/\text{g}$ ) was measured by dividing the bread volume by its mass. The color characteristics of both the crust and crumb of the bread were measured using the same method as described earlier for the composite fours. The texture parameters of bread were measured using a texturometer (Lloyd Instruments, Ltd., West Sussex, United Kingdom). The bread sample (5 cm height) was placed between fat plates and a cylindrical probe (12 mm diameter). The bread sample, with a height of 5 cm, was positioned between flat plates. After that, the test was carried out by penetrating a cylindrical probe, with a diameter of 12 mm, at 50% of the sample height in a double deformation cycle, and at a constant speed of 40 mm/min. Texture profle parameters,

namely firmness  $(N)$ , springiness  $(\%)$  and chewiness  $(N \times mm)$  were calculated from the resulting force-strain curves [\[17](#page-15-16)].

#### **Analysis of bread crumb images**

The alveoli image analysis was done on bread slices from the formulations F0, F1, F3 and F5. For the same sample, the analysis was also done on three distinct time points designated as W0 (start), W1 (week 1) and W2 (week 2). The images of bread slices were captured  $(9280 \times 6944 \text{ px})$  with calibration values ranging from 23 to 24 microns/px using a Xiaomi 11 Lite 5G NE Smartphone. External calibration values were derived from several images with a properly positioned micro-ruler at the same distance from the lens as the alveoli of each sample. The images were acquired with almost tangentially oriented white light from a Neewer 10-inch RGB LED ring light (Neewer, Shenzhen, China), enhancing alveoli visibility within the slices. Post-capture, the images were processed using two software tools: Fiji (ImageJ 1.49q Software, National Institutes of Health, USA) and Nis Elements BR 3.2 software (Nikon Corporation, Tokyo, Japan). The frst software employed a local segmentation algorithm to generate binary images, followed by the second software to refne Fiji segmentation and perform Region of Interest (ROI) intersection of binary elements. The computed parameters included slice area  $(cm<sup>2</sup>)$ , sum of alveoli area (cm<sup>2</sup>), alveoli area (%), calculated as the sum of alveoli area divided by slice area, alveoli number and density of the crumb cells defined as alveoli number/ $\text{cm}^2$ .

#### **Total phenolics and antioxidant capacity of bread**

First, the bread was dried at 40 °C and then transformed into a fne powder using a pestle and mortar. Following this, 10 g of the powdered sample were homogenized with 100 mL 95% ethanol in a sealed Erlenmeyer fask with continuous stirring at 250 rpm for 24 h. The total phenolics (mg GAE/100 g of bread), DPPH· radical-scavenging activity (%) and Fe<sup>3+</sup> reducing power (OD<sub>700</sub>) were measured in the recovered extract using the same methods employed for eleusine fours.

#### **Sensory evaluation**

The sensory characteristics, including color, taste, aroma, frmness, and overall acceptability, of prepared breads were measured by sixty panelists as reported by Murray et al. [[18](#page-16-0)]. Evaluation of these attributes was done on a 5-point hedonic scale, where 1 corresponds to "dislike very much", 2 corresponds to "dislike moderately", 3 corresponds to "neutral", 4 corresponds to "like moderately", and 5 corresponds to "like very much". All samples were measured within 1 day of baking, and they were assigned three-digit random codes before being randomly distributed to the panelists. To ensure objectivity between samples, panelists cleansed their mouths with mineral water before each evaluation.

#### **Statistical analysis**

Each analytical determination was carried at least in duplicate for three samples  $(n=3)$ . A one-way analysis of variance was executed utilizing SPSS software for Windows™ (version 17, SPSS Inc., Chicago, IL, USA). Duncan's multiple range test ( $p \le 0.05$ ) was employed to compare the average responses among diferent treatments.

# **Results and discussion**

#### **Characterization of processed eleusine fours**

The results of the nutrient composition  $(g/100 g)$  of processed eleusine fours were presented in Table [1.](#page-5-0) The carbohydrates (76–80.06) followed by protein (7.28–11.8) were the most abundant macronutrients, whereas the lipids  $(1.2-1.54)$  and ash  $(1.35-1.65)$  were the less compounds. The dietary fber ranged from 8.99 to 10.5 with the majority being comprised of insoluble fber. The major minerals, expressed in mg/100 g, included potassium (ranging from 277.2 to 326) and calcium (ranging from 270.9 to 310.2). The lipids, protein and minerals were comparable to other varieties of fnger millet or cereals. However, the dietary fber was notably higher than that observed in other cereals, such as wheat and rice [[4\]](#page-15-3).

Table [1](#page-5-0) shows that processing of eleusine four infuenced its chemical composition. The used treatments resulted in a decrease in protein and thereby a corresponding increase in carbohydrates. In addition, swelling index and water retention capacity were reduced following the used treatments (Table [1\)](#page-5-0). In the same context, Wu et al. [[19\]](#page-16-1) observed significant alterations in the structure, nutrient composition and functional characteristics of sorghum grains following thermal treatment, steaming and soaking. Batariuc et al. [[20\]](#page-16-2) reported that thermal treatment of sorghum grains positively infuenced the water retention capacity, as well as the carbohydrates, fat and ash, whereas the oil absorption capacity, protein and dietary fber showed an opposing trend. The observed variation in chemical composition could be explained by several factors. The intense mechanical forces created during sonication could lead to the reduction in the size of protein aggregates, as well as to the breaking of weak non-covalent bonds that maintain the protein structure. Thus, this mechanical stress could result in unfolding or fragmentation of the proteins [\[21](#page-16-3)]. In addition, the high temperatures obtained by boiling or microwaves could cause <span id="page-5-0"></span>**Table 1** Chemical, antioxidant and hydration properties of processed eleusine fours



a,b,c,dDifferent lower case letters in the same row within the same treatment indicate significant differences (*p*≤0.05)

1 Total penolics and favonoids were expressed as gallic acid equivalent (GAE) and quercetin equivalent (QE), respectively

<sup>2</sup>The EC<sub>0.5</sub> (effective concentration resulting in an absorbance of 0.5 at 700 nm) and IC<sub>50</sub> (50% inhibitory concentration) of eleusine four are determined based on the curves representing the respective antioxidant activities

protein degradation, resulting in the release of peptides into the solution, which might explain the reduction in protein as well as hydration properties.

Table [1](#page-5-0) also shows that total phenolics (240–480 mg GAE/100 g) and flavonoids  $(78.4–114.2 \text{ mg QE/100 g})$  were comparable to the values previously reported by Wang et al. [\[5](#page-15-4)], and which varied depending on the millet species. However, the used treatments resulted in a decrease of total phenolics and favonoids (Table [1\)](#page-5-0), and thereby a reduction in the antioxidant potential as evaluated by many complementary antioxidant activities, which showed a dose-dependent antioxidant effect (data not shown). The processed eleusine flours showed a notable reduction in  $EC_{0.5}$  for (Fe<sup>3+</sup>) reducing power, as well as a significant decrease in  $IC_{50}$ values for both DPPH·-scavenging activity and β-carotene/ linoleic acid bleaching activity, when compared to untreated flour (Table [1](#page-5-0)). Similarly, Onyango et al. [\[22\]](#page-16-4) reported that hydrothermal treatment of fnger millet resulted in a decrease in the phenolics from 305 to 83 mg GAE/100 g, attributed to the formation of complexes with protein molecules and other polyphenolic compounds. Furthermore, microwave treatment reduced the phenolics of barnyard millet, but increased it in foxtail millet and proso millet, potentially due to changes in protein fractions, development of conjugates with free radicals, and the formation of a complex matrix with proteins and polyphenols [[5](#page-15-4)]. However, Wu et al. [[19\]](#page-16-1) reported that dry heat treatment (133 °C, 15 min) led to an increase in the phenolic compounds, probably due to the destruction of internal tissues and dissociation of phenolic compounds attached to cellular structures.

On the other hand, Jayawardana et al. [[23](#page-16-5)] studied the antioxidant activities for ethanolic and methanolic extracts of three varieties of fnger millet grown in Sri Lanka. These authors showed that these extracts had the potential to

scavenge radicals and to reduce metal ions, highlighting the promising antioxidant potential of fnger millet. Interestingly, compared to commonly consumed grains such as rice, wheat, corn, barley and oats, fnger millet has the highest antioxidant properties [\[23](#page-16-5)]. These fndings suggest the potential role of fnger millet in reducing oxidative stress and preventing or alleviating various diseases associated with free radical damage. It is therefore important for the food industry to eleusine four as a practical substitute or enhancement to wheat four. However, it's worthy to note that the antioxidant potential of eleusine may fuctuate based on factors, such as the variety, growing conditions, and processing methods. Following the current study, the untreated eleusine four was chosen for further analyses and use into bread formulation due to its higher antioxidant potential compared to the processed fours.

#### **Phenolic profle**

The liquid chromatography-electrospray ionization coupled with mass spectrometry (LC–ESI–MS) was used to quantify the phenolic compounds in eleusine ethanolic extract (Table [2\)](#page-7-0). Seven phenolic acids and 9 favonoids were measured based on a comparison of their mass spectra to those of 32 authentic standards. However, if the extract contained compounds diferent from the standards, their identifcation was not possible. Flavonoids were the major group, which represent 97% of the total measured compounds. Quercitrin (quercetin-3-*O*-rhamnoside) (484 µg/g), naringenin (326  $\mu$ g/g) and epicatechin (312  $\mu$ g/g) were the major compounds representing ∼70% of the total measured compounds. Furthermore, approximately half of the total favonoids consisted of various quercetin derivatives, such as quercetin-3-*O*-rhamnoside, quercetin-3-*O*-galactoside, and quercetin 3-*O*-rutinoside. The majority of millet phenolic compounds were concentrated in the seed coat and consisted mainly of phenolic acids and favonoids. Phenolic acids observed in millet exist primarily in bound form, including hydroxybenzoic acids, such as protocatechuic, gentisic, vanillic, and syringic acids, as well as hydroxycinnamic acids, such as *p*-coumaric, sinapic, ferulic, and cinnamic acids. In addition, millet favonoids were present in free form and essentially include quercetin, catechin, gallocatechin, taxifolin and various apigenin derivatives [[5\]](#page-15-4).

Flavonoids have attracted considerable interest from both food scientists and consumers due to their impact on human health [[24\]](#page-16-6). Quercetin 3-*O*-rhamnoside, which was the major compounds measured, has promise potential for treating various diseases, due to its broad spectrum of pharmacological efects. Multiple functions of this compound were reported, such as antioxidant, antimicrobial, analgesic, anti-infammatory, wound-healing, immunomodulatory and vasodilatory properties [[25](#page-16-7)]. Naringenin was also studied for its pharmacological efects and health benefts. It was reported that naringenin may have positive impacts on dyslipidemia in humans and animal models. When administered to mice with cardiovascular disease, naringenin treatment reduced atherosclerosis by addressing dyslipidemia, hyperinsulinemia and obesity [[26\]](#page-16-8). Besides, many studies reported the antioxidant and antiandrogenic propeties of naringenin, in addition to its ability to defend against infammation and cancer, using a series of in vitro and in vivo studies. This compound might infuence various cell signaling pathways, inhibit the production of cytokines and growth factors, and stop the cell cycle [\[27](#page-16-9)]. Qu et al. [\[28](#page-16-10)] also reported that epicatechin, preserved human well-being, by displaying notable antioxidant and anti-inflammatory effects. In addition, it improved muscle function, alleviated symptoms associated with cardiovascular and cerebrovascular diseases, prevented diabetes and protected the nervous system.

Currently, bioactive compounds from derived from plants play an important role in the formulation of functional foods and nutraceuticals. Therefore, several studies focused on exploring the antioxidant potential of diverse bio-resources [\[29](#page-16-11), [30\]](#page-16-12) and integrating them into the formulation of various cereal products [\[31](#page-16-13), [32](#page-16-14)].

# **Characteristics of eleusine‑wheat composite four and alveographic properties of dough**

The physicochemical composition and color of the composite four were measured (Tables [3](#page-10-0) and [4](#page-11-0)). Substituting wheat flour with eleusine flour did not result to a significant diference in lipid and moisture for the composite four. However, a signifcant increase in fber, accompanied by a decrease in starch and gluten, was observed. This change could be attributed to eleusine four's signifcant dietary fiber  $(10.5 \pm 0.5 \text{ g}/100 \text{ g})$ , which contributed to increase the dietary fiber from  $3.9 \pm 0.2$  g/100 g (in wheat flour) to  $7.3 \pm 0.12$  g/100 g in F5. The higher levels of dietary fiber and reduced starch in composite four imply that bread produced from such four could potentially support digestion and alleviate constipation [[33](#page-16-15)].

Additionally, Table [4](#page-11-0) shows the color properties of the resultant composite fours. The composite four showed a slightly darker color compared to the control. Notably, as the level of enrichment increased, the L\* values decreased, while the a\* values significantly increased. The shift in color was mainly a result of the infuence of the reddish hue of eleusine seed coats [[4\]](#page-15-3). The chroma  $(C^*)$  values, indicative of color saturation or "vivacity", showed a slight rise as the level of eleusine incorporation increased. Nevertheless, a notable increase in the total color diference (∆E) between the control and enriched fours was observed (Table [4](#page-11-0)). This signifcant increase implies a substantial disparity in perceptual capacity between the samples. Therefore, the resulting



<span id="page-7-0"></span>



### **Table 2** (continued)



**Table 2** (continued)

# Compounds<sup>a</sup> Molecular mass [M–H]− m/z Content  $(\mu g/g)$ extract) Apigenin O  $\cap$ H OH O HO 270 269 3.2 Luteolin O OH OH O HO OH 286 285 26.9

a The compounds are presented in their elution order from an Aquasil C18 column; identifcation was confrmed using 32 authentic commercial standards

<span id="page-10-0"></span>

F1, F2, F3, F4 and F5 represent the formulations with eleusine substitution level of 10, 20, 30, 40 and 50%, respectively. F0 represents wheat four, used as control

a,b,c,d,eDifferent letters in the same column within different formulations indicate significant differences (*p*≤0.05)

change in color for composite fours has the potential to notably infuence the bread's color.

Figure [1](#page-11-1) shows that partial substitution of wheat flour with eleusine flour, ranging from 10 to 50%, resulted in signifcant changes in dough rheological parameters (P, L and W). With the increase in substitution level, there was a signifcant reduction in dough extensibility (L) and deformation energy (W), which indicated that elevated substitution levels delayed the development of the dough. The formulation F5, which contained 50% eleusine four, showed that L and W values decreased by  $\sim 83\%$  and 70%, respectively, compared to control sample. However, the dough tenacity (P) increased by around 26% for the F5 probably due to insufficient gluten hydration. Furthermore, the observed impact on (P) and (L) parameters was refected in the increased (P/L) ratio, suggesting an inextensible dough. In a similar context, numerous investigations studied the production of bread using combinations of wheat four and various vegetable fours known for their abundance in starch, protein and other essential nutrients. Olaoye and Ade-Omowaye [[34\]](#page-16-16) reported that a minimum of 70% wheat flour was necessary to ensure optimal dough formation in wheat-composite four for effective bread production. Similarly, previous studies reported an important increase in the tenacity (P), and a reduction in the deformation energy (W) and extensibility (L) of the dough enriched with mallow leaf powder [[31\]](#page-16-13) or cactus pear cladodes powder [[35\]](#page-16-17). The obtained results could be explained by the poor development of the gluten network. Specifcally, substituting wheat four with eleusine four led to a notable decline in gluten (Table [3](#page-10-0)). In

<span id="page-11-0"></span>**Table 4** Color analysis of composite eleusine-wheat fours and corresponding bread



F1, F2, F3, F4 and F5 represent the formulations with eleusine substitution level of 10, 20, 30, 40 and 50%, respectively. F0 represents wheat four, used as control

a,b,c,d,eDifferent letters in the same column for the same parameter within different formulations indicate significant differences ( $p \le 0.05$ )

<span id="page-11-1"></span>**Fig. 1** Alveographic properties of dough made from eleusinewheat composite flours. P: tenacity (**A**); L: extensibility (**B**); P/L ratio (**C**); W: deformation energy (**D**). F1, F2, F3, F4 and F5 represent the formulations with eleusine substitution level of 10, 20, 30, 40 and 50%, respectively. F0 represents wheat flour, used as control. a,b,c,d,e,fDiferent letters above the bars for the same parameter within diferent formulations indicate signifcant diferences  $(p ≤ 0.05)$ 



addition, the interactions between dietary fiber or phenolic compounds present in eleusine four and gluten might also infuence the gluten network's structure. The presence of fber can physically interrupt the alignment and bonding of gluten proteins, resulting in a less cohesive gluten network. While, the phenolic compounds could potentially reduce the disulfde bonds present in certain proteins, making them less structurally stable [\[36](#page-16-18)]. On the other hand, eleusine flour

with a swelling index of  $312 \pm 23$  $312 \pm 23$  $312 \pm 23$  g/100 g (Table 1) could limit the available moisture for gluten hydration, leading to a less-developed and weaker gluten structure. Consequently, the gluten proteins were not adequately hydrated and didn't undergo sufficient development to establish a viscoelastic network with the ability to retain the gas obtained during fermentation.

## **Bread quality evaluation**

#### **Physical properties**

Table [5](#page-12-0) shows that as the substitution level of eleusine flour increased, there was a signifcant decrease in the bread specifc volume. At the 10 and 50% substitution levels, the specific volume decreased by  $\sim$  15 and 35%, respectively, compared to the control. Likewise, a signifcant decrease in the specific volume of bread when enriched with 3–9% lemon fiber  $[38]$  $[38]$  or 5% mallow leaf powder  $[31]$  was reported. Nevertheless, other studies showed that incorporating vegetable powders into bread led to an increase in specifc volume at substitution levels below 10% [[35,](#page-16-17) [37\]](#page-16-20). The decrease in specifc volume observed might be attributed to the interference of insoluble fber with the formation of the gluten network, causing a destabilizing efect at the interfaces of the dough gas cells and consequently reducing the gas retention capacity [[31\]](#page-16-13). This suggests that higher levels of eleusine substitution negatively afected the physical characteristics of the bread, resulting in smaller and less airy loaves.

Following this, the texture profle analysis of the bread was done. This evaluation included frmness, indicating the force required to modify the shape or structure of the crumb; springiness, representing the degree of recovery after the force was removed; and chewiness, which refers the energy required to chew the bread into a texture suitable for swal-lowing (Table [5\)](#page-12-0). The decline in the specific volume of bread coincided with a signifcant reduction in springiness and an increase in both hardness and chewiness. These changes suggest a weakening of the gluten network. Similar trends, such

Formulations Specific volume  $\text{cm}^3\text{/g}$ )

as an increase in hardness and a decrease in springiness, were observed in lemon fber-enriched bread as reported by Fu et al. [[37](#page-16-20)]. This implies that the densifcation of these breads may be attributed to the interaction between dietary fber and gluten. Specifcally, the dietary fber present in eleusine four could disrupt the formation of a continuous gluten network, exerting a destabilizing infuence at the interfaces of the dough gas cells, as reported by Fakhfakh et al. [\[31\]](#page-16-13).

The impact of substituting wheat flour with eleusine flour on the color of bread crust and crumb was measured (Table [4](#page-11-0)). A noticeable distinction was observed in the crumb, between the control bread and those with high levels of eleusine, as evaluated by the ∆E measurement. Moreover, there was a signifcant decrease in the C\* value, particularly noticeable in the crust compared to the crumb. The breads enriched with elevated eleusine four levels showed a darker tone compared to the control, as indicated by the lower  $L^*$ values. The decline in the b\* values suggested a decrease in the initial yellowish color. The rise in a\* values could be attributed to the shift in the bread color towards a more brownish hue.

### **Image analyses of crumb structure**

The analyses of alveoli images of composite bread crumb were done at three distinct time points: initially, at week 1 and at week 2 (Fig. [2](#page-13-0) and Table [6](#page-13-1)). The substitution of wheat four with fnger millet led to a decrease in the surface area of the bread slices, particularly noticeable at the highest substitution level (F5), which also showed the lowest specifc volume (Table [5\)](#page-12-0). In the case of the control bread (F0), the total alveoli area was  $15.46 \text{ cm}^2$  at the initial phase, constituting 31.32% of the entire surface area of the bread slice (Table [6\)](#page-13-1). There was no important change in the percentage of alveoli area or alveoli number across all formulations. However, the alveoli number showed a considerable reduction in the formulation F5 compared to the control. Although, the formulation F5

Firmness (N) Springiness (%) Chewiness (N $\times$ mm)

<span id="page-12-0"></span>**Table 5** Volume and textural properties of bread made from composite eleusine-wheat fours



F1, F2, F3, F4 and F5 represent the formulations with eleusine substitution level of 10, 20, 30, 40 and 50%, respectively. F0 represents wheat four, used as control

a,b,c,d,eDiferent letters in the same column within diferent formulations indicate signifcant diferences (*p*≤0.05)

<span id="page-13-0"></span>**Fig. 2** Images of bread slices (F0 as A, F1 as B, F3 as C, and F5 as D) captured during time. The frst column corresponds to week 0, the second column to week 1 and the third column to week 2. The black line on the right image serves as a scale reference across all images



<span id="page-13-1"></span>



F1, F3 and F5 represent the formulations with eleusine substitution level of 10, 30 and 50%, respectively. F0 represents wheat four, used as control. The initial phase is indicated as W0, followed by week 1 (W1) and week 2 (W2)

showed a signifcant decrease in alveoli number, there was no notable reduction in density of the crumb cells (alveoli number/ $\text{cm}^2$ ) that could be explained by the reduction on the total area of the bread slice (Table [6\)](#page-13-1). These results align with those observed in the chemical properties (Table [3](#page-10-0)) and alveographic characteristics (Fig. [1](#page-11-1)) of composite fours, indicating a potential infuence on the bread texture. At higher substitution levels, the notable decline in gluten, coupled with increased dietary fber and the lowest dough extensibility in F5, likely contributed to the substantial reduction in the total bread slice area compared to the formulations with lower substitution levels.

The obtained results highlighted the important role of ingredient composition in the bread-making process and emphasizes how variations in composition could impact the ultimate structure and texture of the end product. Variations in bread structure were evident when using starch from different sources or incorporating diverse ingredients, as previously reported [[38,](#page-16-19) [39\]](#page-16-21). For example, the formulations with mesquite (*Prosopis pallida*) flour up to a substitution level of 10%, resulted in crumbs featuring a higher number of small-sized alveoli compared to the wheat flour bread as control. Notably, when combined with wheat four T65, the alveoli were both smaller and more numerous than when mixed with wheat four T55. However, the incorporation of 15% mesquite four in wheat four T65 yielded crumbs with larger cell sizes than in mixture with wheat four T55, which suggest that small alveoli might no longer be retained in the matrix and tend to merge or coalesce [\[39\]](#page-16-21). Alencar et al. [\[38](#page-16-19)] assessed image analysis on gluten-free bread formulated using non-wheat starch and enriched with pseudo-cereals, such as amaranth and quinoa fours. These authors reported a reduced number of alveoli but with larger surface areas, in contrast to the control gluten-free bread.

Table [6](#page-13-1) also showed that for the same bread sample the sum of alveoli area decreased during storage, while density of the crumb cells increased across all formulations. The evolution of bread crumb structure or staling during storage was infuenced by various factors, primarily starch retrogradation, involving a rearrangement of starch chains, and moisture change from crumb to crust [[40\]](#page-16-22). Over time, starch in bread could undergo retrogradation afecting the bread's texture and altering the size and distribution of alveoli. Moreover, moisture mobility during storage could lead to the contraction of the bread crumb, increasing density in existing alveoli and potentially forming new ones, as observed by the increase in the alveoli number/cm<sup>2</sup> (Table [6](#page-13-1)). Recently, the processing potential of various proteins and their hydrolysates has garnered attention for slowing down the staling of starch products. These ingredients showed the ability to reduce the formation of hydrogen bonds in gelatinized starch during storage or to reduce water mobility, while increasing gluten's water retention [\[40\]](#page-16-22).

The alveoli structure of bread, important for sensory attributes, is primarily impacted by the quality of the gluten network, dough fermentation and the inclusion of additional ingredients. A well-developed porous crumb structure tends to retain more leavened gas, resulting in a product with increased volume and reduced crumb hardness [\[41](#page-16-23)].

#### **Antioxidant activities**

The total phenolics,  $Fe^{3+}$  reducing and DPPH $\bullet$  radicalscavenging activities of composite breads were measured (Table [7\)](#page-14-0). Bread made with 100% wheat four (F0) showed the lowest total phenolics (56.6 mg GAE/100 g of bread), DPPH· radical-scavenging activity (34.9%) and  $Fe<sup>3+</sup>$  reducing power ( $OD_{700}$ =0.21). Interestingly, substituting wheat flour with eleusine flour led to a significant increase in the total phenolics, consequently enhancing the antioxidant activities of the composite breads. Table [7](#page-14-0) also shows that at a 50% substitution level (F5), the bread showed the highest total phenolics (70 mg GAE/100 g of bread), DPPH· radical-scavenging activity (95.15%) and  $Fe<sup>3+</sup>$  reducing power  $(OD<sub>700</sub>=1.94)$ . A similar pattern was observed in previous studies on wheat bread enriched with plant extracts/fours, such as grape seed extract, sorghum extract, green coffee bean flour, pomegranate seed powder, apple seed flour, cactus cladodes four, hazelnut and walnut fours, showing improved antioxidant potential [\[35,](#page-16-17) [36\]](#page-16-18). The presence of strong antioxidant compounds, such as favonoids measured in eleusine four (Table [2](#page-7-0)), might individually or synergistically contribute to the antioxidant activity of the composite bread, thereby enhancing its nutraceutical effect. Nevertheless, substituting wheat with integral eleusine four could pose sensory challenges, mainly due to the alterations in the texture of the end product and/or distinctive favor notes

<span id="page-14-0"></span>**Table 7** Total phenolics and antioxidant properties of the bread

	Formulations Total phenolics <sup>1</sup>	Antioxidant activities	
		$Fe3+$ reduc- ing power (OD $700 \text{ nm}$ )	DPPH scaveng- ing activity $(\%)$
F <sub>0</sub>	$52.6 \pm 0.2$ <sup>d</sup>	$0.21 \pm 0.01$ <sup>t</sup>	$34.9 \pm 1.02^f$
F1	$56.4 + 0.6^{\circ}$	$0.32 + 0.02^e$	$46.1 + 0.68^e$
F2	$59.4 + 0.6^{\circ}$	$0.49 + 0.02$	$53.9 \pm 1.01$ <sup>d</sup>
F3	$65.1 \pm 0.2^b$	$0.69 + 0.07^c$	$72.0 \pm 0.47$ <sup>c</sup>
F4	$68.7 + 0.9^a$	$1.23 \pm 0.11^b$	$83.7 + 2.36^b$
F5	$70.0 + 0.23$ <sup>a</sup>	$1.94 + 0.07a$	$95.15 + 1.71^a$

a,b,c,d,fDifferent letters in the same column within different formulations indicated significant differences ( $p \le 0.05$ )

1 Total penolics was expressed as mg gallic acid equivalent (GAE)/100 g of bread



<span id="page-15-17"></span>**Fig. 3** Sensory evaluation of the bread prepared with eleusine-wheat composite fours. F1, F2, F3, F4 and F5 represent the formulations with eleusine substitution level of 10, 20, 30, 40 and 50%, respectively. F0 represents wheat flour, used as a control. The attributes were evaluated on a 5-point hedonic scale, ranging from 5: "like very much" to 1: "dislike very much"

of eleusine. As a result, it is important to evaluate a balance between nutraceutical and sensory properties.

#### **Sensory properties**

Sensory evaluation involved the examination of color, taste, aroma, frmness, and overall acceptability of freshly prepared breads (Fig. [3](#page-15-17)). The formulations F1, F2, and F3 showed higher average scores across all assessed attributes compared F0. While, F4 and F5 showed slightly lower overall acceptability scores than F0. Notably, the bread incorporating 50% eleusine flour (F5) maintained its acceptability, as seen by an overall acceptability mean score  $>$  [3](#page-15-17) (Fig. 3). In the same context, Singh et al. [\[42\]](#page-16-24) studied the sensory characteristics of bread made from composite fours, comprising 61.8 g/100 g barnyard millet, 31.4 g/100 g wheat and 6.8 g/100 g gluten. These authors reported that the acceptability of the composite bread was nearly equivalent to the wheat bread. In another study, the sensory evaluation of yam/wheat composite bread showed that a substitution level of 25% yam flour produced bread that was acceptable to consumers, whereas levels of 50% and above were unacceptable [[43\]](#page-16-25).

# **Conclusions**

This study highlights the potential of untreated eleusine four as a functional ingredient in wheat pan bread, promoting sustainable agro-food systems. With higher phenolic content and antioxidant potential compared to processed fours, the untreated eleusine four presented a rich profle of phenolic compounds, particularly favonoids. Its incorporation in the formulation of bread improved the antioxidant properties and the dietary fber content, although it modifed the texture of the bread, thus reducing the specifc volume and increasing the chewiness. Despite these changes, sensory evaluation showed that bread containing 50% eleusine four remained acceptable to consumers. Overall, the study contributed to elucidate eleusine four's specifc efects on pan bread quality, highlighting its potential as a valuable functional component in bread manufacturing. Future studies could explore optimization techniques to mitigate texture changes while maximizing nutraceutical benefts.

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**Author contributions** MM and AS: conceptualization, methodology and writing—original draft; CM: formal analysis and writing; OA and LM: methodology and formal analysis; MJ: design experiment and Investigation; NZ: writing—review and editing, and validation; NF: supervision.

**Data availability** All authors confrm that the data supporting the fndings of this study are available within the article.

#### **Declarations**

**Conflict of interest** The authors declare that they have no confict of interest.

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