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ORIGINAL PAPER

Modification of einkorn flours via lactic acid fermentation: changes in techno-functional and rheological properties

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Abstract

Fermentation has emerged as a promising bioprocessing strategy to enhance the nutritional and functional qualities of cereal grains naturally. In this study, the powder properties, functional, and rheological properties of mature and immature einkorn wheat flours were evaluated for five days, based on the effects of lactic acid fermentation using *Lactiplantibacillus plantarum*. Viable cell count increased rapidly within the first 24 h of fermentation, accompanied by a sharp decrease in pH. Bulk and tapped densities decreased during fermentation, while Carr's index and Hausner ratio indicated a temporary reduction in flowability on the first day. Fermentation improved the water-holding capacity, emulsification capacity, and stability, foaming stability, solubility, and swelling index of flours, while the oil-holding capacity and foaming capacity decreased. In addition, temperature had a pronounced impact on the solubility characteristics of the flours. The rheological analysis revealed that both mature and immature einkorn flours exhibited pseudoplastic and viscoelastic solid-like behavior throughout the fermentation process, with MEF generally showing higher viscosity and G′ values. Fermentation and maturation induced substantial modifications in structural features, protein interactions, and gelatinization behavior, resulting in significant variations in viscosity, elasticity, and flow behavior with increasing temperature and frequency. Fermentation and grain maturity affected flour colour, with immature einkorn flour exhibiting lower a^* and higher b^* values. This study highlights the effectiveness of fermentation in improving the functional and technological properties of einkorn wheat flour, supporting its application in cereal-based food products.

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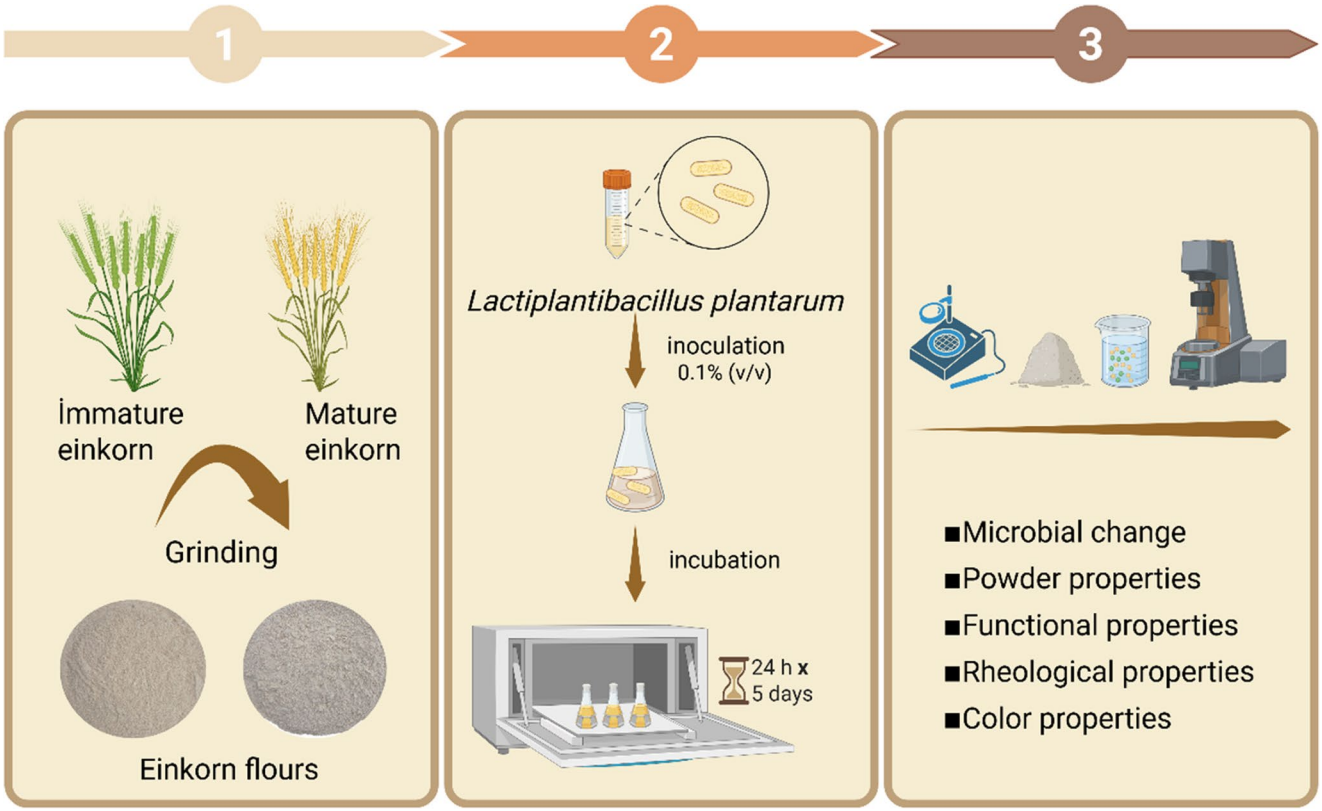
Graphical Abstract



Keywords *Lactiplantibacillus plantarum* · Wheat · Fermentation · Functional properties · Rheology

Introduction

Globally, wheat (*Triticum aestivum L.*) is one of the most important grains cultivated, and after rice and maize, it ranks third in terms of productivity [1, 2]. Wheat cultivation started about 10 thousand years ago with the primitive and cultivated form of einkorn wheat [3]. Durum (*Triticum turgidum* ssp. *durum*) and bread (*Triticum aestivum* ssp. *aestivum*) wheats are closely related to Einkorn (*Triticum monococcum* L. ssp. *monococcum*), a diploid (2n = 2x = 14) hulled wheat. Wheat flour is utilized to manufacture a wide variety of food products globally and is an essential source of energy, proteins, carbohydrates, phenolics, vitamins, fiber, minerals, and other bioactive substances for humans [4]. The high nutritional value of einkorn, especially its fertile nature suitable for low-input and organic farming, and the high nutritional value of its flour are of interest. Several recent studies have established a positive consensus on the dietary value of *T. monococcum*. Compared to durum and bread wheats, einkorn grains appear to be significantly richer in proteins, lipids (especially mono and polyunsaturated fats), and fructans, while some trace elements such as zinc and iron are present in high concentrations [3].

In general, immature cereals have higher concentrations of valuable compounds. Due to its low starch and high fiber content, it has recently attracted more attention [5]. Although einkorn wheat has high nutritional value, this value is lost as it matures [6]. To enhance nutritional content and functional properties, wheat flour is modified industrially [7].

Various methods have been explored, such as chemical, physical, and biological modifications for this purpose. Fermentation represents a highly safe and effective method of biomodification. Enzymes produced by microorganisms during fermentation have the ability to break down macromolecules and anti-nutritional components in fermented substrates, transforming them into other useful products and enhancing their functional and sensory qualities [8]. The two most common methods, spontaneous fermentation (SF) and solid-state fermentation (SSF), are renowned for being economical, energy-efficient, and environmentally benign [9, 10]. Research has demonstrated that the physicochemical and functional characteristics of raw materials

and fermented raw material-based food products are significantly changed by the enzymes and organic acids generated during fermentation, mostly by lactic acid bacteria and yeast [11].

This study aimed to investigate the potential of fermentation as a natural and sustainable biomodification strategy to enhance the functional and rheological properties of einkorn wheat flours harvested at different maturation stages. Owing to its superior nutritional composition, particularly its elevated levels of protein, unsaturated fatty acids, dietary fiber, and essential micronutrients such as iron and zinc, einkorn wheat has emerged as a promising candidate for the development of functional foods. However, the nutritional and functional value of einkorn can significantly decline as the grain matures. Therefore, the present study was designed to address this limitation by applying fermentation with *Lactiplantibacillus plantarum*, a lactic acid bacterium widely recognized for its safe use in food systems and its capacity to improve the biochemical composition of cereal-based matrices. By comparing the effects of fermentation time on both mature and immature flours, this research aimed to provide novel insights into optimizing einkorn flour functionality for potential applications in clean-label food formulations.

Materials and methods

Materials

The immature einkorn (IE) and mature einkorn (ME) wheat were grown in Devrakani, Kastamonu, Türkiye. While IE wheat is harvested at the milk-formation period, ME wheat is harvested when fully mature. Einkorn flours were obtained by grinding the IE and ME wheat in a blade mill (Millsan, Turkey). The moisture, ash, and protein contents of IE flour were 13.0 ± 0.1%, 2.53 ± 0.08%, and 10.04 ± 0.18%, respectively, whereas those of ME flour were 13.0 ± 0.1%, 2.04 ± 0.02%, and 11.31 ± 0.35%, respectively [6]. *Lactiplantibacillus plantarum* 34BB10 isolated from the sourdough samples was provided by the Yildiz Technical University Food Engineering Department culture collection. All chemicals used in this study were obtained from Merck (Darmstadt, Germany).

Einkorn wheat flour fermentation

Einkorn flours were sterilized in an autoclave (HMC Hiclave HV-25 L, Gemini BV, Apeldoorn, Netherlands) at 121 °C for 15 min prior to fermentation. Einkorn flour was weighed into a Schott Duran bottle and mixed with sterilized distilled water at a ratio of 1:5 (w/v). The aqueous suspension was inoculated with 0.1% (v/v) *L. plantarum* culture containing 1×10^8 CFU/mL. Fermentation was carried out separately for 24 h, 48 h, 72 h, 96 h, and 120 h in an incubator shaker (WiseCube® WIS-10; Wisd Laboratory Instruments, Witeg, Germany) at 100 rpm and 37 °C. The samples (10 ml) were collected every day, and the count of viable cells was checked. The flour samples obtained at the end of fermentation were dried in a 37 °C oven for 24 h (Memmert UF 110, Memmert, Schwabach, Germany) [12, 13]. The obtained flours were used for the following analyses.

Determination of viable cell counts and pH value

Fermented einkorn wheat flour (10 g) was added to sterilized physiological saline (90 ml). The mixture was then diluted properly and spread on the de Man, Rogosa, and Sharpe (MRS) agar plate. Plates were incubated at 37 °C for 48 h, and the developing colonies were counted and expressed as colony-forming units per gram logarithmically (log CFU/g) [14]. The pH of fermented flour samples was measured using a pH meter (WTW Inolab 7710, Germany).

Bulk and tapped densities, Hausner ratio, Carr index

Some 25 g of sample was poured into a 50 ml measuring cylinder, and bulk density (BD) was calculated by dividing the flour mass (g) by the occupied flour volume (ml) in the measuring cylinder. Tapped density (TD) was calculated by dividing the flour mass (g) in the measuring cylinder by the occupied flour volume after tapping [15]. Hausner ratio (HR) was the ratio of the tapped density (TD) to the bulk density (BD) [16]. The Carr's index (CI) was calculated as follows (Eq. 1) [15]:

$$CI = 100 \times (TD - BD)/TD \quad (1)$$

Water and oil holding capacity

The water holding capacity (WHC) of the samples was determined by using the methods described by [17, 18]. 1 g of flour sample was mixed with 10 ml of distilled water in a centrifuged tube. Well-mixed suspension was allowed to stand for 30 min. The swallowed samples were centrifuged at 6500 rpm for 30 min. The sediments were weighed after the complete removal of the supernatant. The WHC (%) is calculated as (Eq. 2):

$$WHC\ (\%) = (w_1/w_2)\, w_0 \times 100 \quad (2)$$

w_0: the weight of sample, w_1: the weight of sediment with centrifuge tube, w_2: The weight of sample with centrifuge tube.

The oil holding capacity (OHC) of the samples was determined by using the methods described by [17, 18]. 0.5 g of flour sample was mixed with 5 ml of distilled sunflower oil in a centrifuged tube. A well-mixed suspension was allowed to stand for 30 min. The swallowed samples were centrifuged at 6500 rpm for 30 min. The sediments were weighed after the complete removal of the supernatant. The OHC (%) is calculated as (Eq. 3):

$$OHC\ (\%) = (w_1/w_2)\, w_0 \times d_{oil} \times 100 \tag{3}$$

w_0: the weight of the sample, w_1: the weight of the sediment with the centrifuge tube, w_2: the weight of the sample with the centrifuge tube, d_{oil}: the density of sunflower oil.

Swelling and solubility index

The analysis was performed by the method of Wani et al. [19]. Centrifuge tubes were weighed, and 0.2 g of flour samples were mixed with 10 ml distilled water in centrifuge tubes. The flour suspensions were incubated in a water bath for 30 min at 50, 70, and 90 °C with shaking after every 5 min. The suspensions were cooled immediately, and then the tubes were centrifuged at 6500 rpm for 20 min. Supernatants were decanted into pre-weighed aluminum pans. The gain in weight of the centrifuge tubes was recorded to express as a swelling index (g/g). Pre-weighted aluminum pans were dried in a drying oven at 110 °C for 12 h, and the gain in weights of pans was recorded to express as solubility index (g/g).

Foaming capacity and stability

Foaming capacity and stability of the flours were determined by the method of Wani et al. [19]. Aqueous suspensions (2% w/v) were prepared with 1 g of flour and 50 ml of distilled water and homogenized using an ultra-turrax at 750 rpm for about 2 min. Aqueous solutions were transferred into a 100 ml measuring cylinder. The total volumes of the suspensions were recorded after 30 s. The solutions were allowed to stand for 60 min at room temperature, and the total volumes of suspension after standing were again recorded. The foam capacity (FC) and foam stability (FS) were calculated as follows (Eq. 4 and Eq. 5):

$$FC\ (\%) = (Volume\ after\ whipping - Volume\ after\ whipping) / Volume\ after\ whipping \times 100 \tag{4}$$

$$FS\ (\%) = (Foam\ volume\ after\ standing\ 60\ min / Initial\ foam\ volume) / \times 100 \tag{5}$$

Emulsifying activity and emulsion stability

Emulsifying activity (EA) and emulsion stability (ES) were performed according to the method reported by Cyrille et al. [20]0.1 g of flour was homogenized in 3 ml of water and 3 ml of sunflower oil. The resulting emulsion was centrifuged at 3500 g for 30 min at 25 °C. For emulsion stability, emulsions were heated in a water bath at 80 °C for 30 min. It was then centrifuged at 3500 g for 30 min at 25 °C. EA and ES were calculated as follows (Eq. 6):

$$EA\ or\ Es\ (\%) = V_1/V_2 \times 100 \tag{6}$$

Where: V_1 = Height of the emulsified layer in cm; V_2 = Total height of the liquid in the tube in cm.

Rheological measurements

Rheological measurements were performed using a rheometer (Anton Paar, MCR-302, Austria) equipped with a 50 mm parallel plate geometry and a 1 mm gap at 25 °C. Fermented flour samples were prepared as aqueous suspensions (1:5, w/v) prior to analysis. Steady shear measurements were conducted over a shear rate range of 0.1–100 s^{-1} to determine viscosity.

An amplitude sweep test (0.1–100% strain) was initially performed to identify the linear viscoelastic region (LVR), and a strain value of 0.5% was selected for subsequent oscillatory measurements. Frequency sweep tests were carried out over an angular frequency range of 0.1–100 rad/s at 25 °C. In addition, temperature sweep analysis was conducted at a constant strain (0.5%) and angular frequency (10 rad/s), with the temperature increased from 25 to 95 °C at a rate of 3 °C/min.

Dynamic rheological parameters, including storage modulus (G′) and loss modulus (G″), as well as the loss factor (tan δ) and complex shear modulus (G*), were automatically calculated by the rheometer software. The loss factor is defined as the ratio of G'' to G' (tan δ = G″/G′), while the complex shear modulus is expressed as $G^* = \sqrt{(G'^2 + G''^2)}$ [21].

Color characteristics

A chroma meter (CR-400, Konica Minolta, Japan) was used to measure the color characteristics of fermented flour samples. The terms L^* (whiteness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) were used to represent the color values.

Statistical analysis

Statistical tests were performed using JMP version 6 software (SAS Institute, Inc., Cary, USA). One-way ANOVA (Analysis of Variance) was used to assess significant differences among means, followed by Duncan's multiple range test as a post hoc procedure to determine homogeneous groups at a 5% significance level ($\alpha \leq 0.05$). All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation ($n = 3$).

Results and discussion

Viable cell counts and pH value

Figure 1 displays the pH and viable cell count results. The flour and water mixture was mixed with 1×10^8 CFU/mL of LAB at the start of fermentation. The first day of fermentation, the concentration of *L. plantarum* in the fermented MEF and IEF was increased and reached a peak concentration of 9.55 and 9.20 log CFU/g, respectively. Longer fermentation, however, resulted in a lower concentration of *L. plantarum*, which was linked to an excessive concentration of lactic acid, the primary metabolite, which prevented *L. plantarum* from growing [22]. Although the initial increase and decrease trend was the same between IEF and MEF, the concentration of *L. plantarum* in MEF was found to be lower than that in IEF as of day 3. A similar trend, characterized by an initial increase in LAB counts followed by a subsequent decline, has also been reported in a previous study on *Lb. amylovorus* fermentation [13]. These findings corroborate the results of the present study, demonstrating a similar fermentation behavior.

The pH value of fermented samples decreased from 6.13 to 4.28 and from 6.32 to 4.40 for MEF and IEF, respectively. Also, the pH of the IEF was higher than that of the MEF during fermentation. During fermentation, lactic acid and other organic acids are synthesized, resulting in a decrease in pH and inhibition of the growth of undesirable microorganisms. The decrease in pH observed in this study is consistent with the findings of Zhang et al., [13], who reported a similar pH range (3.27–3.60) in starch derived from fermented millet flour, indicating a comparable acidification behavior. The difference between IEF and MEF is thought to be due to the loss of valuable components with maturation. So, there are more valuable compounds present in immature grains [5]. In the part of the fermentation until the end of the first day, the viable cell count of IEF was higher for this reason, and the pH decrease observed at the end of the first day is thought to inhibit *L. plantarum* growth in the IEF sample. pH and viable cell counts are consistent with each other.

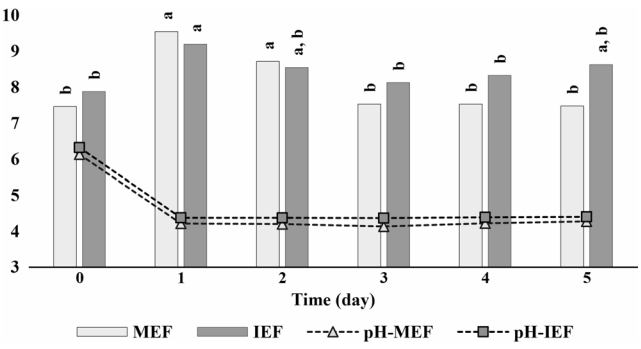


Fig. 1 Viable cell count and pH value in fermented einkorn wheat flours. [1]MEF: mature einkorn wheat flour. IEF: immature einkorn wheat flour. D = fermentation days

Powder properties

The bulk (BD) and tapped density (TD), Hausner ratio (HR), and Carr index (CI) of fermented einkorn flour samples were measured, and the results are presented in Table 1. The fill volume, compaction profile, and particle arrangement of a powder determine its packing behavior, which is described by its bulk and tapped densities [23]. The degree of compactness of flour is described by the bulk density. During fermentation, the decrease in BD and TD was significant for the MEF sample ($p < 0.05$) but not for the IEF sample ($p > 0.05$). During the fermentation process, the breakdown of cellular structures and the modification of biopolymers by microbial enzymes cause particles to become more porous, irregular, and low-density. These structural changes can lead to a decrease in BD and TD values. The decrease in bulk density with increasing fermentation time observed in this study is consistent with the findings of Rahayu et al. [24], who also reported a reduction in bulk density of sorghum flour modified with *L. brevis*, which was associated with an increase in microbial concentration. The protein and fat content are thought to contribute to the reduction of bulk and tapped density of MEF. Carr's ındex indicates the powder's resistance to consolidation, related to its bridge strength and stability, whereas the Hausner ratio reflects the degree of interparticulate friction. Both parameters vary inversely with powder flowability [25]. According to Carr [25], CI values ranging between 5 and 10, 12–16, 18–21, and 23–28 correspond to excellent, good, fair, and poor flow properties, respectively. While the CI value increased and the flow decreased from good to fair on the first day of fermentation, the flow properties of the samples were evaluated as good on the second day ($p > 0.05$). The increase in IEF was more pronounced. Similarly, for the Hausner Ratio, values below 1.20 are indicative of good flowability, whereas values equal to or greater than 1.50 suggest poor flow characteristics due to increased interparticle friction

Table 1 The techno-functional properties of fermented einkorn wheat flours

Sample ID	BD (g/ml)	TD (g/ml)	CI	HR	WHC (%)	OHC (%)	FC (%)	FS (%)	EA (%)	ES (%)
MEFD0	0.73±0.03^{a}	0.87±0.01^{a}	16.70±2.40^{a}	1.20±0.03^{a}	86.15 ± 0.97^{b}	120.60 ± 2.53^{b}	45.33 ± 1.15^{a}	48.55 ± 1.26^{b}	25.73±0.39^{a}	22.82±1.16^{b}
MEFD1	0.68±0.00^{a}	0.82±0.01^{b}	17.08±1.82^{a}	1.21±0.03^{a}	89.90 ± 1.86$^{a, b}$	114.32 ± 0.76^{c}	30.00 ± 2.00^{b}	51.11 ± 1.92^{b}	30.01±0.85^{a}	26.99±0.65$^{a.b}$
MEFD2	0.69±0.01^{a}	0.81±0.01^{b}	14.84±1.64^{a}	1.17±0.02^{a}	89.46 ± 2.00$^{a, b}$	125.43 ± 0.93^{a}	20.00 ± 0.00^{c}	50.00 ± 0.00^{b}	30.38±0.54^{a}	27.46±0.76$^{a.b}$
MEFD3	0.67±0.02$^{a, b}$	0.78±0.01$^{b, c}$	14.00±2.83^{a}	1.16±0.04^{a}	90.92 ± 1.98$^{a, b}$	119.69 ± 0.53^{b}	22.67 ± 1.15^{c}	82.32 ± 0.87^{a}	32.65±1.74^{a}	29.69±2.38^{a}
MEFD4	0.70±0.00^{a}	0.78±0.01$^{b, c}$	11.00±1.41^{a}	1.12±0.02^{a}	86.30 ± 1.90^{b}	122.39 ± 0.21$^{a.b}$	22.00 ± 0.00^{c}	82.22 ± 0.00^{a}	33.00±1.41^{a}	32.00±0.00^{a}
MEFD5	0.62±0.02^{b}	0.75±0.02^{c}	18.14±1.45^{a}	1.22±0.08^{a}	92.10 ± 1.78^{a}	124.42 ± 1.85^{a}	10.67 ± 1.15^{d}	81.11 ± 1.92^{a}	32.74±0.78^{a}	28.89±1.57^{a}
IEFD0	0.69±0.01^{a}	0.79±0.02^{a}	13.10±3.80^{a}	1.15±0.05^{a}	94.82 ± 0.24^{d}	140.01 ± 2.31^{b}	66.00 ± 2.00^{a}	24.26 ± 0.74^{d}	29.71±0.42^{b}	27.73±0.39^{b}
IEFD1	0.62±0.01^{a}	0.76±0.01^{a}	18.00±0.00^{a}	1.22±0.00^{a}	107.77 ± 0.76^{a}	133.23 ± 2.44^{c}	41.33 ± 1.15^{b}	70.95 ± 0.82^{b}	39.63±0.36^{a}	37.63±0.53^{a}
IEFD2	0.69±0.01^{a}	0.79±0.05^{a}	13.10±4.10^{a}	1.15±0.05^{a}	103.71 ± 0.21^{c}	125.23 ± 1.19^{d}	21.33 ± 1.15$^{c, d}$	71.82 ± 1.57^{b}	41.91±0.56^{a}	38.10±0.51^{a}
IEFD3	0.68±0.01^{a}	0.80±0.06^{a}	14.10±5.51^{a}	1.17±0.07^{a}	103.39 ± 0.84^{c}	126.14 ± 3.03^{d}	23.33 ± 1.15^{c}	65.66 ± 1.75^{c}	41.41±0.84^{a}	37.37±0.89^{a}
IEFD4	0.63±0.01^{a}	0.76±0.05^{a}	17.50±3.54^{a}	1.21±0.05^{a}	105.47 ± 0.54^{b}	133.58 ± 0.87^{c}	18.67 ± 1.15^{d}	64.44 ± 3.85^{c}	40.37±0.02^{a}	35.81±1.03^{a}
IEFD5	0.65±0.04^{a}	0.76±0.01^{a}	15.00±4.24^{a}	1.18±0.06^{a}	106.45 ± 0.87$^{a.b}$	146.91 ± 1.47^{a}	8.00 ± 0.00^{e}	100.00 ± 0.00^{a}	40.61±1.79^{a}	37.63±0.53^{a}

[1] *MEF* mature einkorn wheat flour, *IEF* immature einkorn wheat flour. D = fermentation days

[2] *BD* Bulk density, *TD* Tapped density, *CI* Carr index, *HR* Hausner ratio, *WHC* Water holding capacity, *OHC* Oil holding capacity, *FC* Foaming capacity, *FS* Foaming stability, *EA* Emulsifying activity, *ES* Emulsifying stability

[3] Values with different superscript lowercase letters within the same column are significantly different p E < 0.05

and cohesion. HI was below 1.20 for MEF and IEF after the second day of fermentation, which shows good flowability and less cohesive characteristics ($p > 0.05$). Jan et al. [15] reported CI and HR values of 15.480 and 1.183, respectively, in commercial rice flour, which are similar to those in einkorn flour (Table 1).

Techno-functional properties

Table 1 shows the WHC and OHC of *L. plantarum* fermented MEF and IEF. Lactic acid fermentation significantly alters the functional properties of various flours. Yang et al. [26] reported that fermentation generally decreases water-holding capacity. They also stated that centrifugation makes it easier to remove water from fermented corn flour because of its fluffy structure and higher number of broken starch particles. In contrast to these findings, the WHC of MEF and IEF was increased during the first day of fermentation as a result of fewer broken starch particles. Because the effects of fermentation can vary depending on the specific lactic acid bacteria used, fermentation time, and pH conditions [27, 28]. However, fermentation increased water-extractable arabinoxylans, total and soluble dietary fiber, and enhanced water holding and retention capacities [29].

It was clear that fermentation time had a significant effect on the OHC of both MEF and IEF samples ($p < 0.05$). Overall, OHC increased from 120.60% to 124.42% for MEF, while it increased from 140.0%1 to 146.91% for IEF. The proteins partially unravel during fermentation, revealing hidden hydrophobic groups that can attract oil [27]. Periago et al. [30] reported that an increase in the degree of hydrolysis (by enzymatic modification) of pea flour was associated with a corresponding increase in OHC. However, the surface properties of dietary fiber particles, such as composition, surface area, porosity, surface site activity, and structural looseness, were frequently mentioned as having a strong correlation with OHC [31].

Swelling and solubility index of fermented mature and immature einkorn flours are shown in Fig. 2. Swelling index of MEF between the 50, 70, and 90 °C were 1.31–1.16 (g/g), 3.82–3.53 (g/g) and 8.48–6.83 (g/g) respectively, while those of IEF were 1.24–1.41 (g/g), 3.93–3.24 (g/g) and 7.78–7.08 (g/g). The gelatinization of starch, which reflects the breakdown of intramolecular hydrogen bonds in the crystalline areas, and the uptake of water by hydrogen bonding, as well as water absorption by non-starch polysaccharides and proteins, are associated with the flour's swelling index [32]. Generally, the swelling index increased

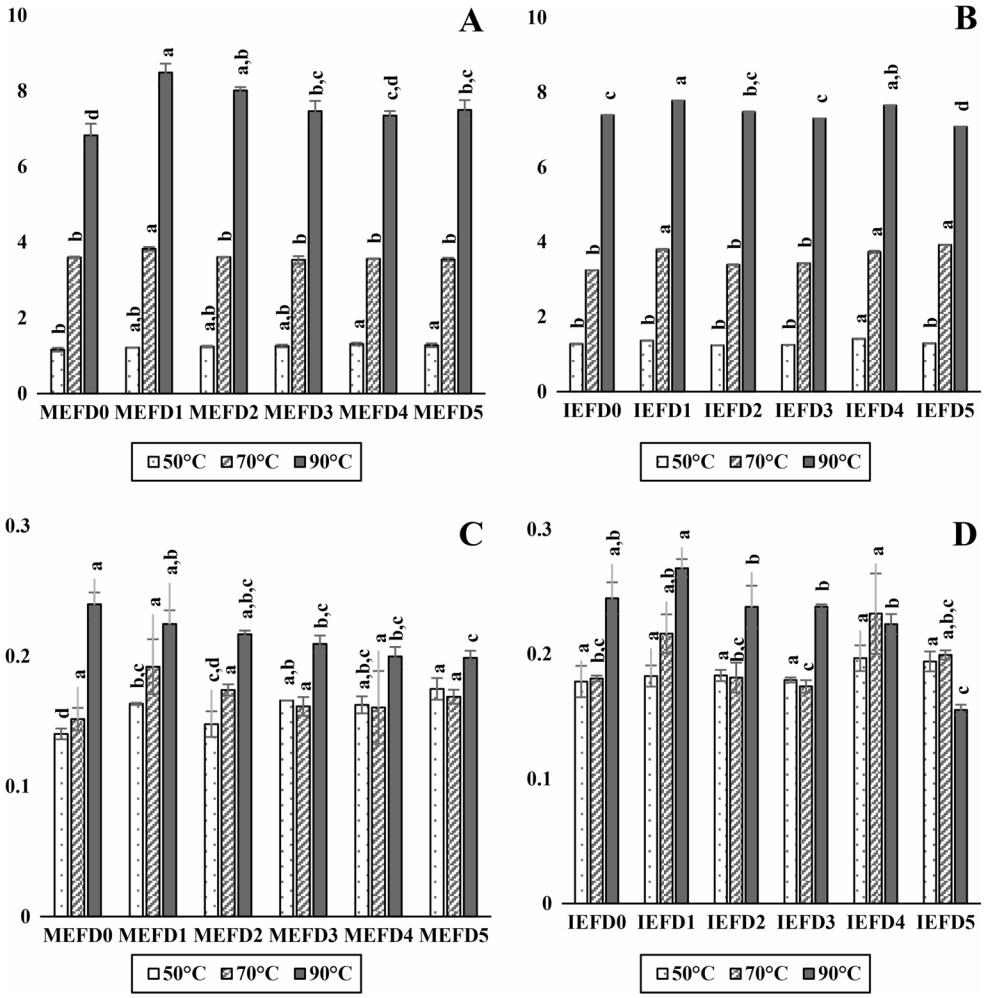


Fig. 2 Swelling (**A**, **B**) and solubility index (**C**, **D**) of fermented einkorn wheat flours. [1]MEF: mature einkorn wheat flour. IEF: immature einkorn wheat flour. D = fermentation days. [2]Different letters at the selected temperature levels indicate that the samples are significantly different from each other ($p < 0.05$)

during fermentation in both MEF and IEF samples ($p < 0.05$). It was observed that the maximum value was reached at the end of the first day. These increases are probably the result of the proteins surrounding the starch granules on the surface breaking down biochemically during fermentation. Proteins in the flour matrix usually provide a barrier that prevents water from penetrating, hence limiting the expansion of starch granules [33].

The solubility of MEF increased during fermentation at 50 °C ($p < 0.05$). On the other hand, no change was observed at 70 °C ($p > 0.05$), whereas it decreased at 90 °C ($p < 0.05$). Nevertheless, the highest solubility value was obtained at 90 °C. The observed variations in the solubility index may be primarily attributed to the activation of endogenous proteolytic systems during lactic fermentation. This enzymatic activity results in the hydrolysis of native proteins into low-molecular-weight peptides and amino acids, thereby increasing the exposure of hydrophilic functional groups and enhancing solubility. Furthermore, fermentation-associated acidification may induce structural rearrangements in protein molecules, including partial unfolding and the disruption of non-covalent interactions, which collectively contribute to the observed changes, particularly under elevated temperature conditions [34, 35]. A more significant reduction in protein content, underscoring the modulatory influence of protein content on starch solubility, is the reason for the increased solubility. The dispersion of amylose and other starch molecules into the surrounding media is probably facilitated by the weaker starch granule structure brought on by protein breakdown, which improves the solubility of flour overall [33, 36]. Just like MEF, different trends were observed in the solubility values of IEF at different temperatures. Solubility of IEF was increased on the first day of fermentation at 70 °C and 90 °C, while no significant changes were observed at 50 °C ($p > 0.05$). As the fermentation period progressed, more starch granules were destroyed, which led to an increase in solubility [1]. However, swelling and solubility are affected by granule size. For instance, little barley and wheat granules hydrolyze more quickly than large granules when exposed to acid or enzymes, presumably due to the small granules' larger surface area per unit weight of starch. The larger proportion

of hydrolysis is also a result of the starch granules' smaller size [3].

Flour can produce foam because of its surface-active proteins [37]. The amount of protein and its solubility are positively correlated with foaming capacity [38]. Three variables control the development of foam: the movement, penetration, and reorganization of molecules at the air-water interface [19]. Foaming capacity of MEF decreased from 45.33% to 10.67% during fermentation ($p < 0.05$). A similar decrease was observed for IEF, and FC was reduced from 66% to 8% ($p < 0.05$). The ability of a protein to move to the air-water interface, reduce surface tension, and then realign its hydrophobic and hydrophilic groups toward the apolar and polar phases, respectively, is what gives it its foam-forming qualities [27]. It was assumed that too many hydrophobic groups were seen during fermentation, decreasing the capacity of the protein to move to the interface. The same phenomenon appears to be valid for the IEF. It is concluded that the high FC value of IEF is due to the high protein content in immature einkorn wheat [6].

Foaming stability is crucial because the effectiveness of foaming agents is dependent on their capacity to maintain the foam for as long as possible. It was determined that the foam stability of MEF and IEF samples increased as the fermentation time increased ($p < 0.05$). FS of MEF was higher than that of IEF in all fermentation days. This difference is thought to be caused by changes in protein structure. It is thought that the compositions of einkorn that differentiate with maturation cause different changes in protein structures with fermentation. High levels of protein, starch, and other nutrients build up as the grains mature [39].

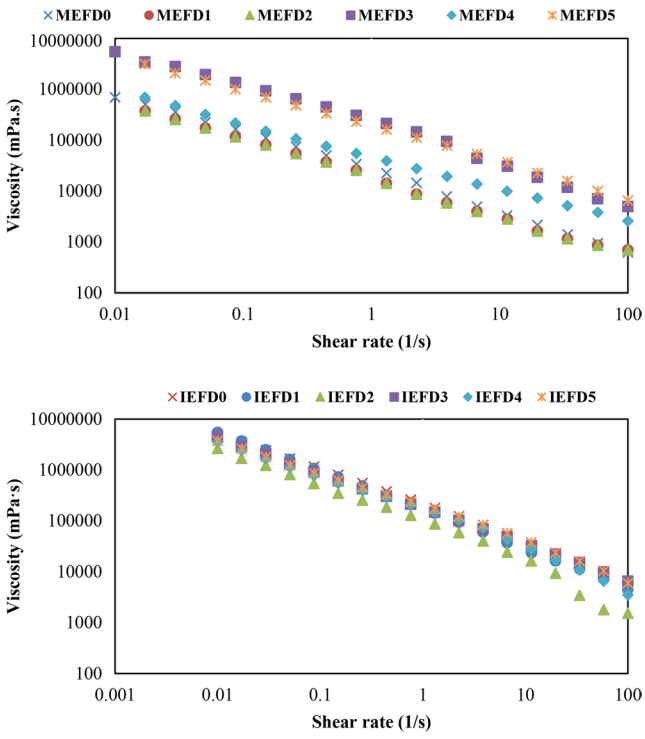


Fig. 3 Viscosity versus shear rate of fermented einkorn wheat flours. [1]MEF: mature einkorn wheat flour. IEF: immature einkorn wheat flour. D = fermentation days

Emulsion activity refers to the largest quantity of oil that may be emulsified by a protein dispersion, while emulsion stability denotes the capability of an emulsion to maintain its integrity [40]. The emulsion activity of IEF and MEF is given in Table 1. EA of MEF was between 25.73% and 32.74%, and fermentation day had no significant effect ($p > 0.05$). On the other hand, the emulsion activity of IEF increased on the first day and did not show a significant change afterwards ($p > 0.05$). Çabuk et al. [27] observed that fermentation time decreased the emulsion activity of pea protein-enriched flour. The protein's capacity to migrate to the oil-water interface to reduce interfacial tension, promote emulsion formation, and potentially encourage the aggregation of released peptides and unhydrolyzed proteins may have been hampered by its enhanced hydrophobicity [41]. Our result in this study doesn't agree with these reports, but Oloyede et al. [42] reported the significant increase in the EA of *Moringa oleifera* seed flour with an increase in fermentation time.

ES of MEF and IEF were increased on the first day of fermentation, and no significant change was observed as fermentation progressed ($p > 0.05$). While some types of polysaccharides can help stabilize the emulsion by increasing the system's viscosity, proteins can emulsify and stabilize the emulsion by lowering the oil droplet's surface tension and providing electrostatic repulsion on the oil droplet's surface [32, 43].

Rheological properties

The viscosities of MEF and IEF are shown in Fig. 3. As the shear rates increased, the viscosities of both MEF and IEF decreased, confirming pseudoplastic or non-Newtonian shear thinning behavior. MEF displayed slightly higher viscosity values across the entire shear rate range. Aguilar et al. [44] have shown that ungerminated cowpea paste has a lower viscosity compared to germinated cowpea paste. They attribute these differences to changes in protein structure, such as conformational changes. Higher viscosity was observed in IEFD0 and IEFD1. Starch-based food viscosity is closely associated with fermentation intensity and α-amylase activity. The structure of starch changes during fermentation; the apparent amylose concentration and gelatinization enthalpy increased, but the starch granule size, solubility, and swelling power decreased. These changes contribute to a decrease in viscosity [45]. Karow et al. [46] also reported that fermentation reduced the viscosity of high-amylose potato starches.

The plot of G′ and G″ as functions of angular frequency is shown in Fig. 4. The variations in the elasticity and viscosity of the sample within the scanning frequency range are represented by G′ and G″. Frequency sweep analysis showed that for both MEF and IEF, G′ was greater than G″ over the entire frequency range, showing viscoelastic behavior with dominance of solid-like characteristics [47]. G′ and G″ of MEF decreased after the first day of fermentation. The decrease in pH may be responsible for this reduction, as acidification modifies chemical components and alters the interactions between water molecules and structural elements such as proteins and starch, thereby affecting their functional behavior [48]. Also, it is seen that maturation affects the G′ and G″ values of einkorn flours. Hadnadev et al. [49] concluded that the rheological properties of flours obtained from ancient cereals are affected largely by the amount and quality of wheat gluten. The extended fermentation of einkorn flour may promote partial gluten modification, indicating potential for reduced or pre-digested gluten applications.

Figure 5 depicts the temperature sweep of the mature and immature einkorn wheat, showing the G′ and G″ modulus as a function of temperature. For this analysis as well, G′ was always higher than G″ for MEF and IEF, demonstrating that elastic-like behavior predominates from the initial stage of measurement. While MEFD0 had the highest G′ for MEF, IEFD0 had the highest G′ for IEF. A similar curve

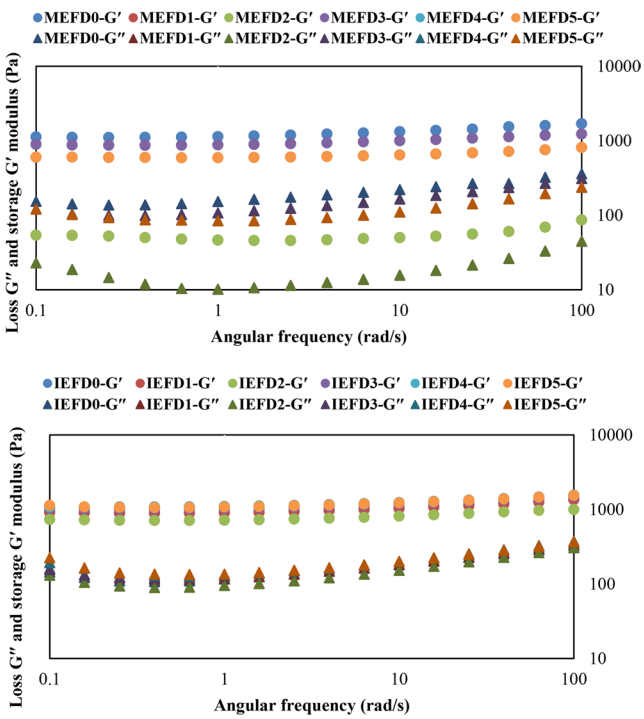


Fig. 4 Frequency sweep dependence of fermented einkorn wheat flours. [1]MEF: mature einkorn wheat flour. IEF: immature einkorn wheat flour. D = fermentation days

was observed for the complex shear modulus (G*) of the samples. According to Aprodu et al. [50], starch swelling is the main factor causing this rheological behavior, and it outweighs the phenomena of protein weakening (i.e., destabilization of protein structure). However, the breakdown of protein structures during fermentation is the cause of the lower G′ values seen for other samples [51]. It is thought that the difference between MEF and IEF may be due to the difference in starch content. Çetin-Babaoğlu et al. [5] stated that because free sugars are transformed into starch during maturation, immature grains have less starch than mature grains.

Previous studies have connected lower G′ values to the strong swelling power of starches, which are a significant component of grains, legume pulses, and tubers [21, 50, 52]. Additionally, our research found a relative tendency in which the G′ increase was highest for MEFD0 and IEFD0, which had the lowest swelling index. As heating increased to about 80 °C, MEFD1 G′ surpassed MEFD0 G′, with the G′ values of the MEFD0 being slightly greater than those of the MEFD1 [51]. Consequently, the proteins denatured at the higher temperatures of the temperature range (80 °C to 95 °C), which triggered the start of starch gelatinization and increased MEFD1 G′ values. The same trend was also observed in IEFD0 and IEFD1.

The material mostly exhibits gel or solid-like behavior when G′ > G″ (tan δ < 1), while G′ < G″ (tan δ > 1) indicates a more liquid-like behavior. Across all temperature ranges, G′ was found to be greater than G″, indicating that the samples' elastic rather than viscous characteristics predominated [53]. Both MEF and IEF samples showed similar curve loss factors versus temperature (Fig. 5). The viscoelastic properties of the samples changed because of starch gelatinization and gluten protein denaturation brought on by rising temperatures. Samples acted more elastically (lower tan δ) below the temperature at which gelatinization occurred, and viscous behavior increased when gelatinization began, raising tan δ. The sample was more elastic prior to gelatinization because the starch granules resisted deformation. The swelling of starch granules during gelatinization increased viscosity and viscous dissipation, which increased tan δ [54].

Color properties

Color characteristics of MEF and IEF were presented in Table 2. Fermentation had no significant effect on *L* and *a** values of MEF samples at the end of 4 days ($p > 0.05$). On the other hand, an increase in *b** value with fermentation (until day 4) ($p > 0.05$). IEF samples show the same trend until the third day of fermentation. On the other hand, *L* values of IEF did not change until the first day of fermentation

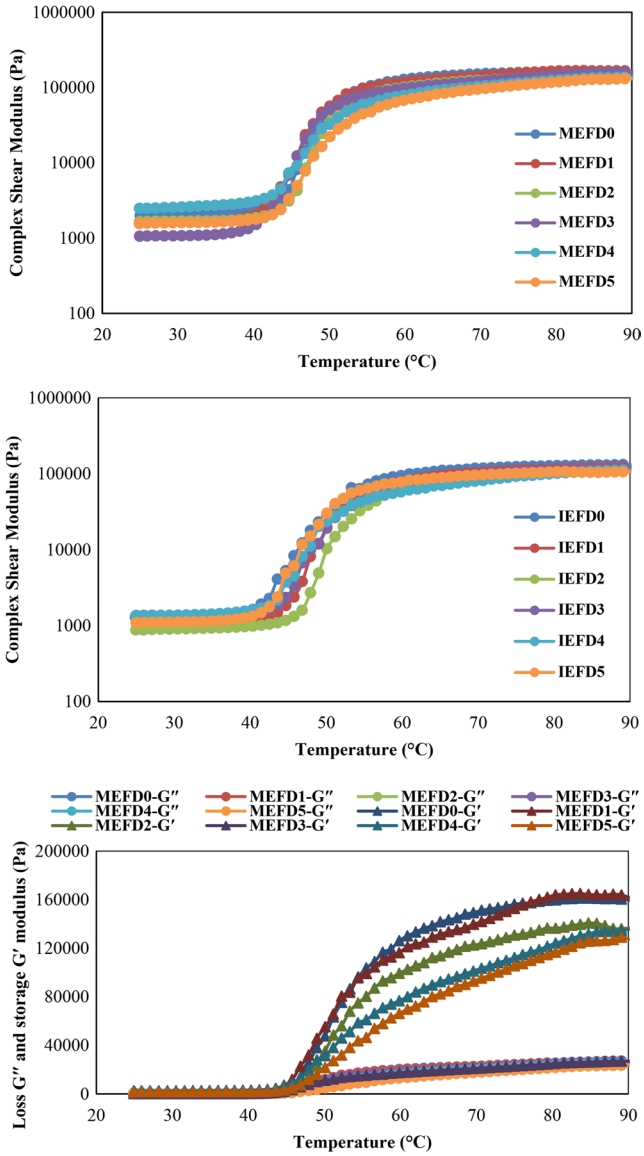


Fig. 5 Temperature sweep dependence of complex shear modulus (G^*), the storage modulus (G'), loss modulus (G''), and loss factor ($tan\ \delta$). [1]MEF: mature einkorn wheat flour. IEF: immature einkorn wheat flour. D = fermentation days

($p > 0.05$). Afterwards, it decreased until the third day and then increased ($p < 0.05$). a^* values of IEF increased during the 3-day fermentation period ($p > 0.05$), then decreased ($p < 0.05$). Gong et al. [55] reported that increasing fermentation time led to higher L values and lower a^* and b^* values in potato flours, indicating improved whiteness; however, in the present study, while L increased and a^* decreased similarly, b^* values increased, suggesting a different trend in yellowness development. Silva et al. [56] naturally fermented sweet potato flour for 8, 12, and 16 days and reported that the increase in L values resulted in a lighter final product. The observed fluctuations in color values can be attributed to pH-induced alterations in pigment stability, particularly

Table 2 The colour properties of fermented einkorn wheat flours

Sample ID	L	a*	b*
MEFD0	80.86±0.79^{c}	2.64±0.14^{a}	12.89±0.16^{c}
MEFD1	83.04±0.55$^{a, b}$	2.55±0.11^{a}	14.56±0.23^{b}
MEFD2	82.10±0.39$^{b, c}$	2.66±0.06^{a}	15.20±0.30^{a}
MEFD3	82.40±0.44^{b}	2.59±0.07^{a}	14.80±0.13$^{a, b}$
MEFD4	80.98±0.27^{c}	2.76±0.10^{a}	15.31±0.27^{a}
MEFD5	83.81±0.10^{a}	2.24±0.07^{b}	13.45±0.07^{c}
IEFD0	81.21±0.11^{b}	1.97±0.02$^{b, c}$	15.27±0.13$^{c, d}$
IEFD1	81.17±0.23^{b}	2.15±0.14$^{a, b}$	15.72±0.33$^{b, c}$
IEFD2	79.61±0.48^{c}	2.32±0.10^{a}	16.31±0.12^{a}
IEFD3	80.63±0.19^{b}	2.13±0.06$^{a, b}$	15.94±0.08$^{a, b}$
IEFD4	82.77±0.21^{a}	1.81±0.06^{c}	15.10±0.19^{d}
IEFD5	82.07±0.28^{a}	1.89±0.07^{c}	14.95±0.22^{d}

[1]MEF: mature einkorn wheat flour. IEF: immature einkorn wheat flour. D = fermentation days

[2]Values with different superscript lowercase letters within the same column are significantly different $p < 0.05$)

toward the end of the first day of fermentation [57]. The fact that similar trends were not observed between MEF and IEF was interpreted as the changes in pigments with maturation may affect the color stability.

Conclusion

Lactic acid fermentation with *Lactiplantibacillus plantarum* significantly alters the physicochemical, functional, and rheological characteristics of mature (MEF) and immature (IEF) einkorn wheat flours. The fermentation-induced acidification and microbial growth cause a marked reduction in bulk and tapped densities, with transient impairment in powder flowability as indicated by Carr's index and Hausner ratio. Fermentation led to improvements in key functional properties of einkorn flour, including water holding capacity, emulsification capacity and stability, solubility, swelling index, and foam stability. The observed pseudoplastic and viscoelastic solid-like behavior suggests good processability and structural stability, while the higher viscosity and G′ values of mature einkorn flour indicate a stronger network formation and improved textural potential. Additionally, colorimetric variations, particularly reduced a^* and elevated b^* values in IEF samples, underscore maturity-dependent compositional changes. These findings emphasize that fermentation is an applicable bioprocessing strategy for tailoring einkorn flour characteristics, as prolonged fermentation enhances its functional performance, processing behavior, and industrial applicability, making it suitable for value-added clean-label bakery applications, including natural flavor development and dehydrated dough improver production. Since the chemical composition was not evaluated in this study, future research should focus on

these aspects to achieve a more comprehensive understanding of the underlying mechanisms affecting these properties.

Author Contribution Burcu Mine Kıymaz: Formal analysis, Investigation, Methodology, Software, Visualization, Data curation, Writing – review and editing. Eylül Ozturk: Investigation, Data curation. Yilmaz Ozcan: Investigation, Writing – original draft. Omer Said Toker: Conceptualization, Investigation, Supervision, Project administration. Muhammet Arici: Investigation, Resources, Writing – review and editing.

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Data availability All data generated or analyzed during this study are included in this published article and comply with research standards. The data supporting this study's findings are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare no conflict of interest.

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