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Rheological and Textural Characteristics of Functional Breads Fortified with Different Eggshell Powders

Samiye ADAL[1]*, Nazlı SAVLAK[2]

Highlights:

- The highest level of eggshell fortification had no effect on the quality of bread
- The village eggshell-fortified bread had the greatest quality and the highest calcium content
- ESP can be used in bread to meet customer needs and bread manufacturing technologies

Keywords:

- Bread
- Eggshell fortification
- Rheology
- Texture

ABSTRACT:

This study explores the rheologic, textural, and sensory characteristics of bread that has been fortified with eggshell powder (ESP) obtained from various dietary and growing conditions (industrial, organic, free range, and village). The fortification of ESP led to a reduction in water absorbtion capacity, stability, softening degree, maximum resistance, energy, and resistance to extension, while the value of extensibility increased. The highest water absorption value was observed in bread flour with a percentage of 61.9, whereas among breads with ESP fortification, the highest water absorption value was 61.2% in S1 (23.5 g) and S2 (30 g) village ESP-added flours. Only the extensibility value increased when ESP was added, but the effects on water absorption, stability, softening degree, maximum resistance, energy, and resistance to extension declined. The addition of ESP results in significant changes in chewiness, springiness, resilience, and cohesiveness ($p \leq 0.05$), but the gumminess value is found to be insignificant ($p \geq 0.05$). Through sensory evaluation, all bread samples received scores of 3.77–4.46 on a 5-point scale. The rheological, textural and sensory analyses results obtained from the control and fortified bread samples demonstrated that the inclusion of calcium from egg shell powder improved the structural properties of the flour. In addition, the utilization of eggshell powder at its maximum concentration (30 g) did not yield any detrimental consequences for the dough or the bread production process. Based on a comprehensive analysis, it is concluded that including ESP in bread is an appropriate strategy for dealing with calcium deficiency and is in line with consumer acceptability and bread production technology.

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INTRODUCTION

In recent years, there has been a growing interest in food fortification due to the recognition that micronutrient deficiency contributes to the global burden of disease. The consumption of vitamins and minerals below the estimated recommended daily limit is frequently used to describe micronutrient deficiency. Micronutrients are essential for the body's generation of hormones, enzymes, and other necessary components for healthy growth and development. One of the biggest micronutrient concerns is calcium deficiency. Calcium has many structural and physiological roles in all species. It is an essential ion (Flammini et al., 2016). It is a key micronutrient needed for the development, maintenance, and health of bones and teeth. It additionally supports vital body processes like muscular contraction, blood clotting, and nerve conduction (Mann & Truswell, 2017). Micronutrient deficiencies account for 7.3% of the worldwide burden of disease, making them a substantial public health problem, especially with regard to iron and calcium deficits (Mattar et al., 2022). One effective approach to addressing micronutrient deficiencies is by including food additives as fortificants. The goal of food fortification is to deal with and avoid demonstrated nutrient deficiencies in specific populations or groups. Concerning food fortification and the development of novel products, nutritional value is a critical factor from this point of view (Gómez-Alvarez & Zapata, 2024). Effective utilization of food additives and fortifiers is crucial in contemporary food industry. Food additives serve other purposes in addition to food preservation. They also contribute to enhancing specific characteristics of food, like color, flavor, and flexibility, while simultaneously improving its nutritional value. For instance, it is possible to incorporate appropriate food nutrition additives to compensate for the loss of nutrients that occurs during food preparation. They effectively prevent malnutrition and dietary deficiencies while supporting nutritional balance (Wiley & Nee, 2020).

The most common method of adding calcium to a food product is by using wheat flour as the primary means of fortification (Khan et al., 2021). It can be consumed either as a component in other culinary preparations or as a snack (Ameh et al., 2013). There is a growing need for various combinations of flour, which involves replacing wheat flour with alternative flours like maize, sweet potato, and undefatted rice bran flours. Bread fortification is a health approach that can reach a larger population without necessitating changes to their current consumption habits. It is a viable method of supplementing daily calcium consumption to reduce the likelihood of various deficiencies. Additionally, it is a cost-effective approach to maintaining adequate calcium levels in the body (Alsuhaibani, 2018). The projected volume in the bread market is estimated to reach 216.70 billion kg by the year 2028. In 2025, the bread market is projected to experience a volume rise of 3.8%. In 2024, the projected average volume per individual in the bread market is estimated to be 24.8 kg (Statista, 2024). Turkey has the highest daily bread consumption per capita worldwide, at an average of 319 g/day per person. Denmark has a daily consumption of 195 g, which is second only to Turkey. Finland and England have a daily consumption of 140 g and 89 g, respectively (Yurdatapan & Güngör, 2014). Bread is widely recognized as the primary food source in our country, with individuals consuming an average of 350–400 g per day (ranging from 100–800 g). Additionally, around 40% of daily energy requirements are fulfilled through bread intake. Thus, bread is regarded as the optimal vehicle for fortification (Dursun, 2006). Given that each gram of bread yields 2.7 kilocalories (kcal), the total energy intake from bread per individual per day is calculated as (350 x 2.7) = 945 kcal. The proportion of this in the average daily energy consumption is calculated as (945 x (100/2200)) = 43%. It is established that a 350 gram serving of bread, which provides 43% of the daily calorie requirement, does not have nutrients in proportion to this value and does not fulfill 43% of the recommended daily allowance (Ekşi & Karadeniz, 1996). Bread is of great significance in the human diet because of its nutritious makeup. For example, a 100 gram serving of bread generally consists of roughly 59.8 grams of carbohydrate, 22.3 grams of moisture, 1.56 grams of total organic nitrogen, and approximately 8.9 grams of protein (Leung et al., 2012; Yusufoğlu et al, 2021). Therefore, it is believed that the widespread use of fortified bread will deal with the vitamin-mineral deficiencies linked to inadequate and imbalanced diets in our country and other countries around the world.

Eggshell is a biomaterial primarily composed of a mineral component and intercellular membranes. Eggshell is rich in bioavailable calcium, which is present in the form of calcium carbonate ($CaCO_3$) (Brun et al., 2013). The chemical composition of the substance is primarily calcium carbonate (94–95%), specifically in the form of calcite. Additionally, it comprises around 3% organic material, which corresponds to the protein structure of the membrane. The remaining material consists of magnesium carbonate and calcium phosphate (Baláž, 2018). Hence, the eggshell can function as a source of calcium for human nutrition by being added to calcium-fortified food. Furthermore, it has been demonstrated that powdered calcium carbonate ($CaCO_3$) generated from eggshell is more readily absorbed in the small intestine of rats compared to commercially available $CaCO_3$. Eggshells possess remarkable nutritional value as a source of calcium that is amenable to conversion and thus contributes positively to human nutrition (Zulkeflee, Chompoorat, & Siva, 2020). Although eggshells actually constitute a valuable source of calcium, they are unfortunately largely discarded as waste by the egg industry. The global production of hen eggs in 2021 amounted to 86 million tons, representing a decline of 0.8 percent compared to 2020. However, this figure reflects a significant growth of 69 percent since the year 2000, resulting in an additional 36 million tons produced throughout this period (FAO, 2023). In light of the current focus on sustainability, repurposing this waste for multiple uses would be a beneficial approach, serving as a value-added product for the food industry and contributing to waste recycling efforts for environmental concerns. In addition to its potential as a valuable food additive, eggshell powder (ESP) has been proposed for use in breads and pizzas. It has been observed that the powder has a negligible impact on the texture of these foods without altering their flavor (Brun et al., 2013).

The main objective of this study was to develop calcium-enriched bread by incorporating eggshell powders obtained from different dietary and growing conditions: industrial, organic, free-range, and village eggshells. This study differs from other research because it utilized chicken eggshells from four different feeding and growing conditions, which were then used at four different levels to cater to the specific health and age requirements of various groups, including children, pregnant or breastfeeding women, teenagers (13–19 years old), and adults (20–60 years old) following a typical diet. The bread-flour formulations were designed to include different amounts of calcium per 150 g of bread. These amounts were 800 mg, 1000 mg, and 1300 mg, which correspond to 23.5 g ESP (S1), 30 g (S2), and 38.5 g (S3) for the specific target groups mentioned. The rheological properties of flour and dough samples enriched with eggshell powder were assessed in this study, along with the textural characteristics of bread fortified with ESP. In addition, this study aimed to deal with the problem of food waste, which presents a substantial challenge in environmental management, by employing eggshell powder in the current approach to sustainability goals.

MATERIALS AND METHODS

Raw Materials

The eggshells used for producing ESP were collected from Keskinoglu Poultry and Breeding Companies Industry and Trade Inc. in a sterilized form. The bread flour used in the bread recipe was sourced from the Yüksel Tezcan Flour Factory, while the wet yeast (Pakmaya), salt (Salina), and water (Hayat) were acquired from a local market.

Preparation of eggshell powders

The eggshells were obtained in the form of coarse powdered, sterilized, and packaged in hermetically sealed 5 kg containers. Eggshells were collected from the pasteurized egg production line, covering the whole production process. The eggshells were subjected to drying and sterilizing using a vibratory fluidized bed drying machine (Scolari LFV 250) at temperatures ranging from 160 to 180 ˚C. The eggshells were ground into a fine powder using a bead mill (Retsch PM 100) with operating conditions of 600 rpm/min for a duration of 10 min. In the end, the eggshells underwent screening using a 40 µm mesh prior to their utilization. Eggshell powder with a particle size of 40 µm or lower was used in the bread baking process. The eggshells, which had been ground into a fine powder, were stored in hermetically sealed containers at a temperature of +4 ˚C, until they were ready for utilization. Table 1 represents the bread formulations of all samples analyzed in the study.

Bread formulation and production

Breads were made in Manisa Celal Bayar University's Food Engineering Department lab. The KitchenAid mixer (5KSM150PS, KitchenAid, St. Joseph, Mich., USA) was used to blend bread formulations as shown in Table 1. Water and yeast were then added. The doughs were rotated at 40 rpm, and water at 30 ˚C was added until the appropriate development time was obtained. The dough was cut into 500 gram circles and placed on rectangular metal pans. For 30 min. at 30 °C and 80% humidity, the doughs were fermented in a fermentation cabinet (Inoksan FGM, Türkiye 100). After fermentation, the doughs were combined in a mixer for another minute to remove air. The final fermentation continued at 30 °C and 80% relative humidity until the specified time. The doughs were baked at 200 °C for 20 min. in a steam-heated oven (Inoksan FPE 110). After baking, the breads were cooled and stored for subsequent analysis.

Table 1: Bread formulations of control (S0) and ESP fortified flours

ESP type	ESP level	Flour (g)	Water (mL)	ESP (g)
Industrial	**S0**	1000.0	619.0	0.0
	S1	976.5	608.0	23.5
	S2	970.0	608.0	30.0
	S3	961.5	604.0	38.5
Village	**S0**	1000.0	619.0	0.0
	S1	976.5	612.0	23.5
	S2	970.0	612.0	30.0
	S3	961.5	611.0	38.5
Free range	**S0**	1000.0	619.0	0.0
	S1	976.5	607.0	23.5
	S2	970.0	607.0	30.0
	S3	961.5	599.0	38.5
Organic	**S0**	1000.0	619.0	0.0
	S1	976.5	608.0	23.5
	S2	970.0	603.0	30.0
	S3	961.5	602.0	38.5

* Yeast: 30.0 g Salt: 12.0 g for all samples, S0: Control; S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

Determination of dough rheological properties (physical dough tests)

Farinograph analysis

The flours' farinogram characteristics were assessed using a Brabender Farinograph, following the ICC method (ICC Standard, 2003). The farinographic water absorption value is the amount of water, measured in milliliters per 100 grams of flour at a moisture content of 14.0%, needed to achieve a dough with a maximum density of 500 Farinograph Units (FU) under the defined operating circumstances outlined in this standard (ICC Standard, 2003). The graph illustrates the measurement of the dough's resistance to the kneader paddles during the mixing process using a dynamometer. This test assesses the dough-forming characteristics of gluten proteins by quantifying the percentage of flour water absorption and analyzing the rheological properties of the dough during kneading, including stability, degree of softening, and valorimetry value.

Extensograph analysis

The extensogram characteristics of the flours that were prepared were assessed using a Brabender Extensograph, following the ICC method (ICC Standard, 1992). Extensographic water absorption refers to the amount of water, measured in milliliters per 100 grams of flour at a moisture content of 14.0%, needed to create a dough with a consistency of 500 FU after being mixed for 5 minutes under the conditions described in this standard (ICC Standard, 1992). The dough's capacity to hold the carbon dioxide gas generated during fermentation is linked to its stretching capacity and resistance to stretching. These are crucial factors that influence the bread qualities of flour. The extensograms were utilized to estimate the resistance to stretch (R5 resistance) Brabender Unit (BU), extensibility (mm), and the ratio values of the two (Ercili, 2004).

Color analysis

The color analysis of control and eggshell bread samples was conducted following the methodology reported in Ho et al. (2013) (Ho et al., 2013). The CIE $L*$ (lightness), $a*$ (redness/greenness), and $b*$ (yellowness/blueness) values of the inside surfaces of bread slices that were 2.5 cm thick were measured using the Konika Minolta CR5 Chromameter from Japan. Analyses were conducted by obtaining six measurements for each sample. The lightness (L*) is a measure of brightness, with 0° representing black and 100° representing white. The red/green value is denoted by a*, where a positive value indicates redness and a negative value indicates greenness. The yellow/blue value is represented by b*, with a positive value indicating yellowness and a negative value indicating blueness. The hue angle ranges from 0° to 360°, with 0° representing red, 90° representing yellow, 180° representing green, and 270° representing blue. The DE* value, which represents the total color difference between control and the fortified breads, is calculated using the formula DE*ab = $[(\text{difference in L* value})^2 + 283 * (\text{difference in a* value})^2 + (\text{difference in b* value})^2]^{1/2}$.

Texture profile analysis

The texture profile analysis was conducted utilizing a texture analyzer (TA-XT Plus Texture Analyzer, Stable Micro Systems, UK) with a 5 kg load cell using a 36 mm cylinder probe. Two slices of bread, each with a thickness of 2.5 cm, were placed on top and then subjected to two consecutive compressions. The test speed was adjusted to 100 mm/min (AACC, 1999). The gumminess, springiness, chewiness, cohesiveness, and resilience values of the bread samples were determined by the texture profile analysis.

Sensory analysis

A sensory analysis was conducted to evaluate the acceptability of the bread. The analysis was carried out by 16 educated panelists who were between 25 and 35 years old. The sensory analysis was conducted by adapting the sensorial score test developed by (Dursun et al., 2009). The sensory characteristics of crumb color, pore, texture, odor, chewing, flavor, aroma, and overall acceptance were assessed using a 5-point scale, with 1 indicating strong dislike and 5 indicating strong liking.

Statistical analysis

The bread baking experiments were replicated twice, with three parallels for each trial. The data were examined using a 4x4 factorial design. A completely randomized design was utilized to identify variances between the averages of the sample data. This was achieved through the application of one-way analysis of variance (ANOVA). The statistical analysis was performed using the SAS® System (SAS Institute Inc., Cary, NC, USA: SAS Proprietary Software Release 8.2) software, with a significance level set at $\alpha = 0.05$. The mixed and GLM techniques were employed. The variations among the samples were assessed utilizing the LSD multiple comparison test, while the associations between the outcomes were acquired by the PROC CORR approach (S.A.S., 1999).

RESULTS AND DISCUSSION

Farinograph Analysis

Rheology is of the utmost importance to cereal scientists when it comes to assessing the quality of flour. Rheological examinations are utilized to assess the mechanical properties and behavior of materials during processing, as well as to evaluate the quality of the end result throughout the production chain. The Brabender Farinograph is a prominent piece of equipment utilized for analyzing dough is mostly employed to ascertain the physical properties of dough (Khan et al., 2021). The resistance exhibited by the dough against the mixing paddles is recorded on a graph using a dynamometer. This test aims to gather information about the dough-forming properties of gluten proteins by measuring the water absorption capacity (%) of the flour and the rheological characteristics (stability) of the dough during kneading, including the degree of softening and development time. Several parameters can be taken into consideration while evaluating farinograms. The parameters commonly used by cereal chemists today are dough development time, stability, dough tolerance index, softening degree, and valorimeter value (Ercili, 2004). The characteristics of a flour's farinogram are related to the quantity and quality of gluten proteins. It is stated that the flours to be used for bread production should not have high water absorption, and the kneading time should not be too long considering the energy and time loss. However, flours with a short kneading time generally have low bread-making quality (Köksel, Sivri, Özboy, Başman, & Karacan, 2000).

From Figure 1 and Table 2, it can be observed that, the water absorption value of all the flours with the addition of ESP varies according to the control. The highest water absorption value is seen in bread flour with a percentage of 61.9, whereas among breads with ESP fortification, the highest water absorption value is 61.2% in S1 (23.5 g) and S2 (30 g) village ESP-added flours, and the lowest water absorption value is 59.9% in S3 (38.5 g) free-range ESP-added flour. The water absorbtion values of the flours with ESP levels S1 and S2 were the same, whereas there was a decrease in the flour with the addition of ESP level S3. As a result, the addition of ESP has caused a minimal decrease in the water absorption value of the bread.

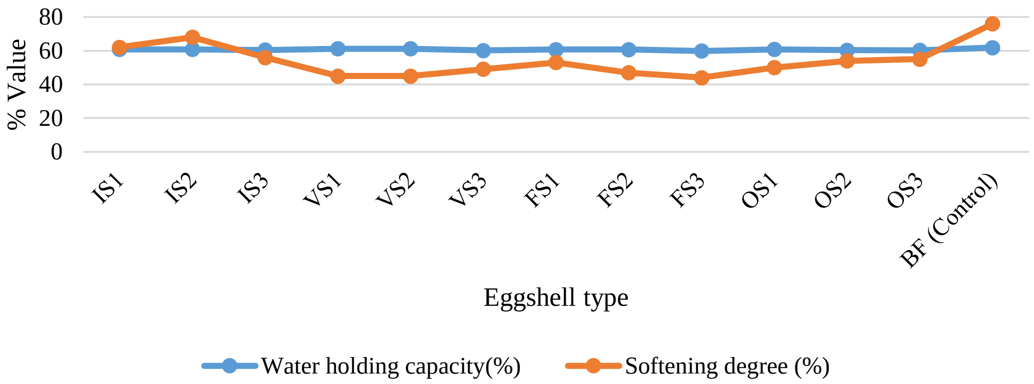

Figure 1. Changes in water holding capacity and softening degree of flours varying by type and level of ESP, I:Industrial, V: Village, O: Organic, F: Free range, BF:Control; S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

In contrast to our findings, the study conducted by Salem et al. (2012) examined the impact of adding ESP at concentrations of 10% and 20% on the rheology of cake dough in comparison to a control group. The researchers found that the addition of eggshell powder resulted in an increase in water absorption. However, there was a decrease in dough stability, dough development time, and time to breakdown (Salem, Ammar, & Habiba, 2012).

Table 2: Farinograph analysis results of bread flour and bread flour fortified with eggshell powder

ESP type	Water holding capacity(%)	Development time(min.)	Stability (min.)	Softening degree
IS1	60.8	1.5	1.7	62
IS2	60.8	1.5	1.5	68
IS3	60.4	1.5	1.8	56
VS1	61.2	1.8	1.9	45
VS2	61.2	1.8	1.1	45
VS3	60.1	1.7	1.4	49
FS1	60.7	1.4	1.8	53
FS2	60.7	1.4	2	47
FS3	59.9	1.7	2.2	44
OS1	60.8	1.5	1.8	50
OS2	60.3	1.7	1.9	54
OS3	60.2	1.4	1.7	55
Wheat flour (Control)	61.9	1.8	2.1	76

I:Industrial, V: Village, O: Organic, F: Free range, S0:Control; S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

Similarly, in a study where ESP was chemically extracted and used as a source of calcium for fortifying bread; water absorption ranged from 63.60 to 67.52. According to their study, the sample with 3% Ca fortification recorded the maximum water absorption rate of 67.52%, whereas the control group had the lowest value of 63.60%. Results indicate a positive correlation between the amount of fortificant added to wheat flour and the rate of water absorption (Khan et al., 2021). This phenomenon might be attributed to the utilization of ESP acquired through various extraction procedures or chemical treatments. The ESP utilized in our investigation is a natural source that has not undergone any chemical treatment.

In line with our results of farinograph, in the study conducted by (Kaur et al. 1994), the KCl, $MgCl_2$, and $CaCl_2$ were substituted with $MgSO_4$ and Na_2SO_3 to examine the impact of sodium chloride and other mineral salts on the rheology of dough. The objective was to produce bakery products with a reduced sodium content. $CaCl_2$ was shown to exhibit a unique attenuating impact after $MgCl_2$ among the salts utilized for substitution. It was shown that the water holding capacity of the flours decreased when salt was added, compared to the control sample without salt. The reduction in water absorption occurred as a result of the alteration in the gluten structure induced by the salt's binding to the areas where water was already bound (Srivastava, Patel, & Rao, 1994). Sudha & Leelavathi (2008) studied the influence of various iron and calcium salts, as well as their combination with vitamins, on bread quality for making and the rheological properties of wheat flour (Sudha & Leelavathi, 2008). According to their report, the inclusion of various iron salts in wheat flour had a minimal impact on the flour's ability to holding water. According to the study findings, the addition of iron in the form of ferrous sulfate and EDTA resulted in a progressive increase of 1–1.5% and 2% in the water holding value, respectively. However, the addition of iron in the form of ferrous fumarate did not have any impact on the water retention value. Similarly, the inclusion of several calcium salts at three different concentrations had minimal impact on the water retention value of the farinograph. According to their statement, the inclusion of a mineral-vitamin mixture resulted in a small increase in water absorbtion capacity, from 57.9% to 58.9%. Iron, in the form of ferrous sulfate, enhanced the stability of the dough, whereas both ferrous fumarate and ferrous EDTA had a modest negative impact on its stability. The incorporation of calcium salts had no impact on the duration of dough development, the stability of the dough, or the values of the mixing tolerance index. The inclusion of a vitamin-mineral mixture did not result in any notable alterations to dough development time, dough stability, or kneading tolerance index values.

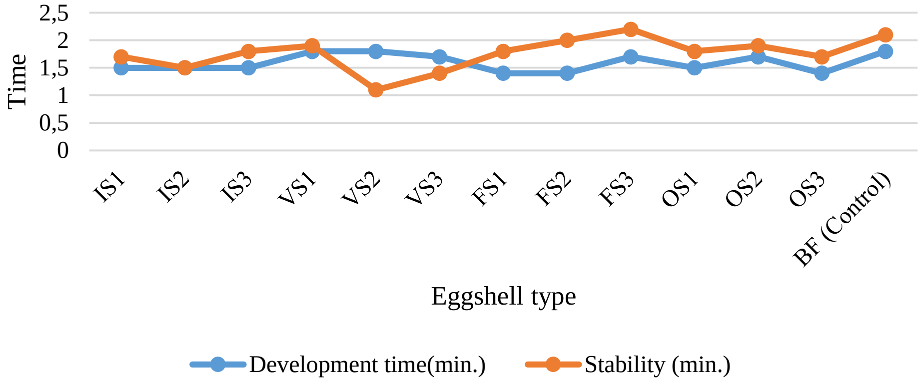

Figure 2. Changes in development time and stability of flours varying by type and level of ESP, I:Industrial, V: Village, O: Organic, F: Free range, BF:Control, S1: 23.5 g , S2: 30 g, S3: 38.5 g ESP fortification

Figure 2 illustrates that the control and village ESP-added doughs had the highest development time values, which were 1.8 minutes. On the other hand, the dough with free-range had the lowest development time value. ESP incorporated doughs with a duration of 1.4 minutes. Overall, the inclusion of ESP led to a reduction in the dough’s development time, similar to the line in previous studies. (Ranhotra et al. 1984), conducted a study on the impact of different mineral gluconates (Fe, Zn, Ca, and Mg) on the qualities of wheat and bread. They determined that, these minerals had no influence on rheological parameters, such as dough kneading time and development time

Figure 2 presents stability values that provide insight regarding the flour's kneading tolerance. The dough's resistance to the paddles during kneading remains constant at the 500 BU line for a period of time, depending on the protein content and quality of the flour. The stability value is determined by measuring the duration (in minutes) between the point at which the farinogram curve intersects the 500 BU line, and the point at which it separates from that line (D’Appolonia & Kunerth, 1984). According to Figure 2, the addition of ESP resulted in a reduction in the stability values of the flours. In comparison to the control, only the stability value of S3 free-range flour with ESP addition increased. In their study, Basset et al. explored stability values and the reduction in dough stability observed during a specific timeframe to gather insights regarding kneading tolerance. Control and 50% NaCl-replaced samples exhibited high stability values. The stability time of white bread decreased as the level of sodium chloride replacement rose (Bassett et al., 2014). Typically, the stability value serves as a measure of dough strength, reflecting its capacity to withstand strain at elevated levels. Upon analyzing the softening degree data, it was found that the doughs exhibited statistically significant differences (p<0.05). The control dough demonstrated a lower softening degree compared to the other experimental dough groups. The control dough exhibited the lowest softening degree, while the addition of calcium salts resulted in a progressive increase in the softening degree values. Nevertheless, there was no noticeable disparity between the doughs that had calcium salts added to them. According to the study, there is a correlation between low softening degree values and flours that have strong structure, require a long period for development, and have high stability values (Mohammed et al., 2012).

Figure 1 illustrates the variations in the degree of softening values for control and ESP-added flours. The degree of softening refers to the distance, measured in BU, between the point reached by the farinogram curve 20 minutes. after water is added and the 500-BU line (Köksel et al., 2000). A significant level of softening is indicative of the low strength of the flour (Ercili, 2004). Upon analyzing the softening degree values in Figure 1, it is evident that all of the flours with added ESP exhibit lower softening degree values compared to bread flour. The S2 industrial ESP-added flour exhibited the maximum degree of softening value, whereas the S3 free-range ESP-added flour showed the lowest degree of softening value. The lower softening degree values observed in the ESP-added flours compared to the control suggest that ESP enhances the structural integrity of the flour. Ercili's investigation revealed that an excessive level of softening in flour is indicative of its weakness. (Bassett et al. 2014) and (Mohammed et al. 2012) found that, flours with strong structure, an extended development period, and good stability values tend to have low softening degrees (Bassett et al., 2014; Mohammed et al., 2012). In their investigation on dough development times, Bassett et al. (2014) found that the addition of Ca salts did not alter the time it took for the dough to attain 500 BU stability. Furthermore, all doughs exhibited a consistent development time of 120 sec. The control and village ESP-added doughs exhibited the highest development time values of 1.8 min, as depicted in Figure 2.

Extensograph analysis

The extensograph is a device used to measure the dough's resistance to stretching and its ability to stretch. The dough's capacity to hold carbon dioxide gas generated during fermentation, its ability to stretch, and its resistance to stretching are crucial factors that influence the bread characteristics of flour. Extensograms provide insight into the overall quality of flour and its reaction to additives (AACC, 2000). The physical characteristics of dough can be determined using different extensograph values. The frequently used parameters include Rm, which represents the maximum resistance (measured at the point when the extensogram curve reaches its highest point, expressed in BU); R5, indicating the resistance at 5 cm extension (also in BU); E, representing the extensibility (the total length of the curve, measured in cm); Rm/E, which is the ratio of maximum resistance to extensibility (known as the viscoelastic ratio); and A, which denotes the area under the curve (measured in cm^2). The resistance to stretch and area values are regarded as indications of dough strength, while the ratio of resistance to extensibility can be seen as an indicator of the viscoelastic stability of the dough (Ercili, 2004).

Studies have shown that, extensograph parameters can be correlated to the qualitative attributes of bread. It is commonly accepted that there is a positive association between the area or resistance to stretching in the extensogram and the volume of bread (Preston & Hoseney, 1991). Indeed, it was established that the breads exhibiting the greatest resistance to stretching also had the highest volume. The extensograph analysis of bread flour is presented in Figure 3. The resistance values measured from the flours range between 529 and 749 BU. The bread flour (control) had the greatest resistance value. Upon analyzing the data in the figures, it becomes evident that the flours containing village ESP had a behavior most similar to the resistance value of the control flour. The resistance to stretch value decreased in all of the flours containing ESP compared to the control. Furthermore, the resistance to stretch value exhibited a negative correlation with the ESP level. A study was conducted to examine the impact of various iron and calcium salts, along with certain vitamins, on the quality of bread made with wheat flour. The study found that calcium levels ranging from 800 to 1.600 ppm did not affect the mixing properties of the dough.

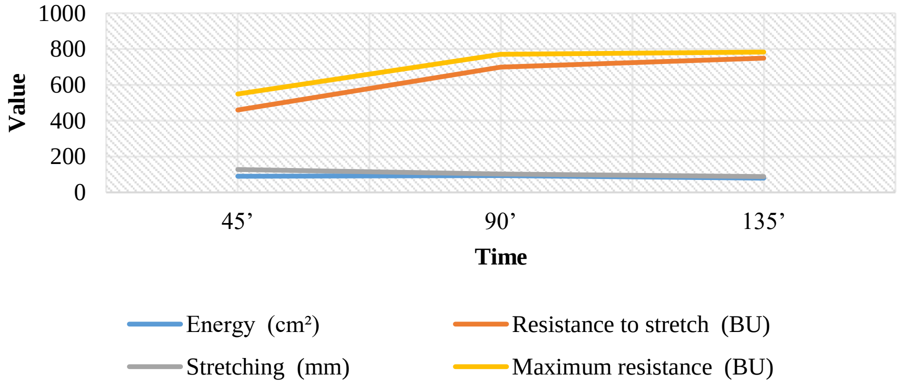

Figure 3: Bread flour (control) extensograph analysis results (S0)

The inclusion of a micronutrient premix containing vitamins such as ferrous sulfate, calcium carbonate, thiamine (thiamine hydrochloride), riboflavin, niacin, and folic acid at varying concentrations had a minimal impact on the rheological characteristics. The addition of a vitamin mineral premix to flour did not have any impact on the rheological qualities and bread manufacturing quality, even after storage (Sudha & Leelavathi, 2008). Upon analyzing the extensibility values, it was seen that the addition of ESP to the flours resulted in an increase in extensibility compared to the control. The control flour, characterized by its strongest resistance to stretching, consequently exhibited the lowest extensibility value. The S3 industrial ESP-added flour formulation exhibited the maximum extensibility value, as seen in Figure 4. In a study investigating the effects of reducing NaCl content in bread and adding Ca, it was found that, the control group exhibited higher values compared to the experimental group. The addition of Ca to the dough had a significant ($p<0.05$) impact on dough extensibility, rising, and deformation energy. Furthermore, the values of these parameters exhibited a tendency to diminish as the NaCl substitution rate increased.

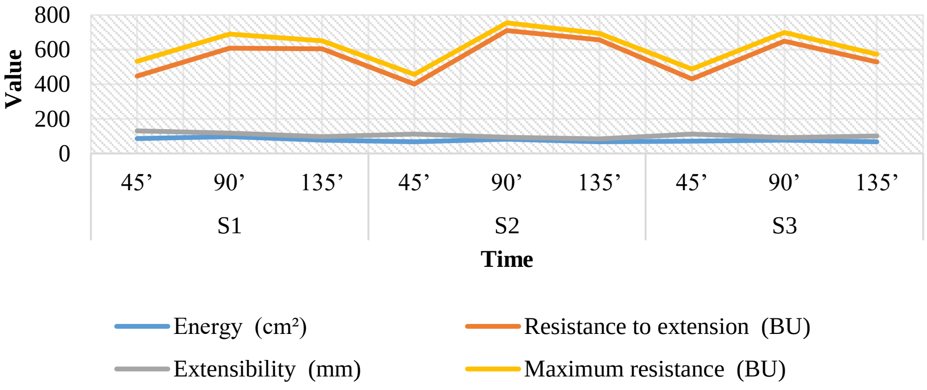

Figure 4. Industrial ESP-added bread flour extensograph analysis results, S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

The dough with a 50% NaCl substitution exhibited the highest similarity to the control in comparison to the other doughs. The dough’s high extensibility values were found to correspond with high stretchability values when formed into a thin film. According to reports, a significant amount of deformation energy causes in the creation of larger bubbles in dough. This is because of the balanced values of elasticity and extensibility (Bassett et al., 2014).

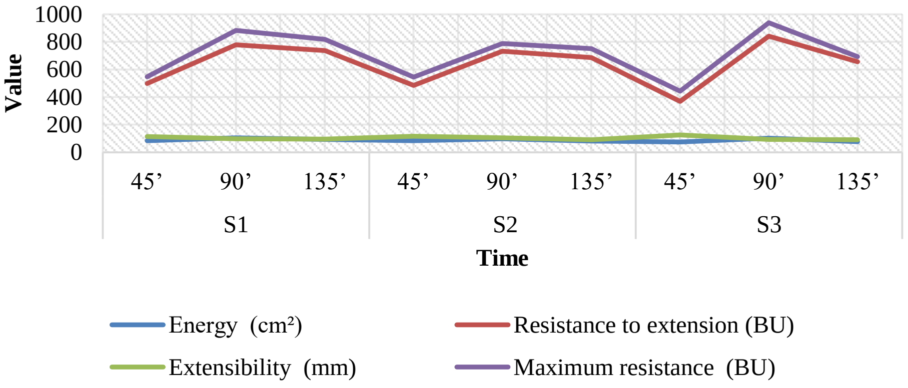

Figure 5. Village ESP-added bread flour extensograph analysis results, S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

(Ercili 2004) reported that, the resistance values observed in flours enriched with minerals varied from 550 to 770 BU. Similar patterns were noted in the enriched flours compared to the control over the 12-week assessments. During storage, it was noted that the resistance to stretching of all flours increased. The study found that enriching flour with iron and zinc at the given levels did not have a statistically significant influence on extensibility values. The flours exhibited comparable extensibility patterns to the control over a period of 12 weeks (Ercili, 2004). Upon analyzing the maximum resistance values, it was found that the flours with S1 village ESP addition exhibited the highest maximum resistance value. Nevertheless, the highest resistance value exhibited a tendency to decline with a rise in the ESP level. The energy values of the dough exhibited a declining pattern with the increase in ESP level. Figure 5 indicates that, the S1 village flours had the highest energy value, whereas the S3 industrial flours had the lowest energy value.

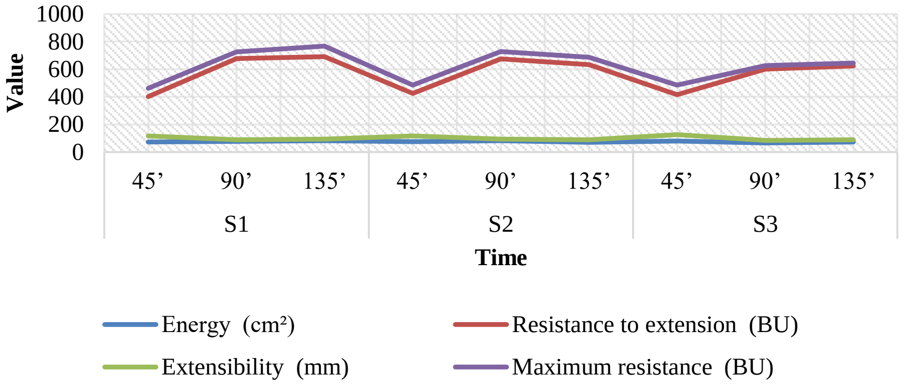

Figure 6. Free range-ESP-added bread flour extensograph analysis results
S1: 23.5 g; S2: 30 g, S3: 38.5 g ESP fortification

A study examining the impact of adding micronutrients to flour on its rheological properties and quality found that the inclusion of calcium in the form of calcium carbonate, calcium citrate, and calcium phosphate did not result in any notable alteration in maximum pressure. Nevertheless, it was discovered that the addition of calcium lactate resulted in a slight reduction in the maximum resistance value, decreasing it from 70 mm (in the control flour) to 63 mm (in the enriched flour). Similarly, the addition of calcium salts did not have an impact on any of the other alveographic indices, with the exception of calcium lactate (Sudha & Leelavathi, 2008).

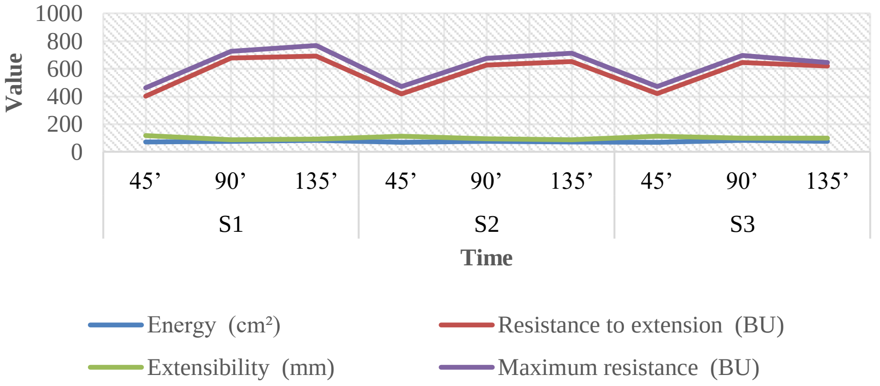

Figure 7: Organic ESP-added bread flour extensograph analysis results, S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

Texture profile analysis results

Table 4 presents the results of the texture profile analysis conducted on the bread that was fortified with the addition of ESP, as well as the control bread. Through the evaluation of Table 3, it was determined that the interaction between the type of ESP and the level of ESP has a statistically significant effect on the chewiness value ($p \leq 0.05$). It was found that, the breads made with industrial ESP type and S2 level exhibited the highest chewiness value among the breads that had ESP fortified. The addition of higher levels of ESP resulted in increased chewiness values. Zulkeflee et al. (2020) found that, adding eggshell powder to bread has a significant effect on its textural characteristics, specifically hardness, gumminess, and chewiness. The addition of 6% eggshell powder greatly increased the chewiness of the bread compared to other formulations. Gumminess refers to the amount of energy required to break down a semisolid substance into smaller pieces that can be easily swallowed. Chewiness, on the other hand, is the amount of energy needed to chew the substance until it is ready to be swallowed. The addition of natural calcium disrupts the interaction between proteins or their solubility, thereby impacting the texture of gumminess and chewiness (Zulkeflee et al., 2020).

Salinas et al. (2015) investigated the rheology of calcium carbonate and inulin-fortified wheat dough. The dough's hardness, gumminess, and elasticity improved as calcium and inulin concentrations increased, with inulin having the greatest effect. Calcium did not affect structural elasticity in the absence of inulin. However, high inulin levels (6.5 g per 100 g) increase calcium levels while decreasing suppleness. Hardness and viscosity acted similarly. Calcium has no effect on crispness in the absence of inulin. In calcium carbonate samples, the bread crumb returned to its original shape with greater immediate flexibility. This demonstrates that calcium salts improve bread crumb structure (Salinas & Puppo, 2015).

Increased calcium levels can enhance bread chewiness by resulting in a softer bread crust. Upon examining Table 4, it is evident that the chewiness value we obtained exhibited a tendency to increase as the addition of ESP increased, in line with the findings of Salinas et al. (2015). This phenomenon can be elucidated by the correlation between elevated ESP levels and heightened calcium concentrations, resulting in the attainment of a more tender bread crust. Cohesiveness is the quality of the internal forces that bind the product together as a single unit. Cohesiveness is quantified by calculating the ratio of the areas under the graphs of the first and second compressions of the sample. In their study, Chilek et al. (2018) reported that the addition of eggshell does not impact the cohesiveness of the bread. The statistical analysis in Table 4 revealed that the interaction between ESP type and ESP level had no significant influence on the cohesiveness value of fortified breads generated with ESP ($p \geq 0.05$). When the effects of ESP type and ESP level were examined individually, ESP level was shown to be statistically significant ($p \leq 0.05$). Also, the type of ESP was determined to have a significant statistical impact ($p \leq 0.05$). The cohesiveness of bread is determined by the combination of ingredients used, and it is preferable for this characteristic to have a high value in freshly baked bread (Salinas & Puppo, 2015). Upon analyzing Table 4, it becomes evident that breads produced at industrial ESP-S1 level exhibit the highest cohesiveness value.

In a study in which NaCl was reduced and substituted with Ca in bread samples, at an 80% substitution rate, the cohesiveness value was the highest compared to the other samples. When the top crust qualities were examined, all texture data revealed substantial variances. When compared to the other bread samples, the 80% substitution showed reduced elasticity and higher hardness, chewability, and cohesiveness ratings. However, it has been shown that, replacing NaCl with Ca at 70% and 50% has less of an effect on bread (Bassett et al., 2014). In our study, increased chewiness and cohesiveness values were obtained in bread samples fortified with eggshell powder, with Ca content increasing in direct proportion to ESP addition.

Springiness is a measure of how well a material returns to its original shape after being deformed. It is calculated by dividing the deformation of the material during the second compression by the deformation during the first compression (Chilek et al., 2018). The statistical analysis in Table 4 indicates that the interaction between the type of ESP and the level of ESP was found to be statistically significant ($p \leq 0.05$). Upon analyzing the springiness values of the breads with the addition of ESP, it is evident that the springiness values of the breads with the addition of village ESP are nearly the same, whereas the springiness values of the breads with the addition of village ESP surpass those of the other breads. (Krupa-Kozak et al. 2012) found that, breads enriched with calcium exhibited increased softness and springiness, compared to breads without calcium enrichment. Furthermore, it was indicated that substituting 80% of NaCl resulted in different effects on the upper and lower crust of bread, leading to a more tender bottom crust and a more rigid top crust composition (Krupa-Kozak, Altamirano-Fortoul, Wronkowska, & Rosell, 2012). Overall, our investigation found that the springiness value generally rose when ESP was added, indicating a positive correlation with increasing Ca levels. It was determined that, the impact of the interaction between ESP type and ESP level on the resilience value of the fortified breads when ESP was added was not statistically significant ($p \geq 0.05$). When the impacts of different types of ESP and different levels of ESP were examined individually, it was shown that the impact of the ESP level was not statistically significant ($p \geq 0.05$). However, the ESP type showed a statistically significant result ($p \leq 0.05$). The statistical analysis in Table 4 revealed that the interaction between ESP type and ESP level had no significant influence on the chewiness value of fortified breads made with ESP ($p \geq 0.05$). When the impacts of ESP type and ESP level were examined individually, it was determined that both the effects of ESP level and ESP type were statistically negligible ($p \geq 0.05$). Khan et al. (2021) reported that an increase in the level of calcium fortificant in wheat flour led to a noteworthy decrease in hardness values. In addition, the fortificant had a substantial impact on the chewiness of the bread, whereas the springiness was shown to be statistically similar. (Khan et al., 2021)

The correlations between the calcium level and texture profile analysis of the bread with the addition of ESP are shown in Table 3. There is a positive correlation between chewiness and elasticity ($r^2 = 0.40$). There is a negative correlation between Ca level and elasticity ($r^2 = -0.41$) and chewiness ($r^2 = -0.42$) values.

Table 3. Corralations between texture values and Ca in eggshell

	Chewiness	Resilience	Springiness	Gumminess
Chewiness			0.40 0.0212	
Ca		-0.41 0.0191		-0.42 0.0156

Table 4: Texture profile analysis results of eggshell powder fortified breads

ESP type	Chewiness	Springiness	Resilience	Gumminess	Cohesiveness
IS0	541.60±0.414^{ae}	0.591±0.414^{a}	1.710 ±0.017^{ac}	484.22±0.101^{a}	0.853±0.013^{ac}
I S1	375.77±0.093^{f}	0.5605±0.093^{bc}	1.060 ±0.019^{e}	354.11±0.336^{ac}	0.8705±0.011^{a}
IS2	624.24±0.034^{ab}	0.5605±0.344^{bc}	1.340±0.024^{ade}	446.90±0.421^{ab}	0.858±0.020^{ac}
IS3	575.75±0.023^{ad}	0.5695±0.234^{ac}	1.265±0.021^{de}	431.94±0.460^{ab}	0.856±0.009^{ac}
VS0	477.51±0.027^{bf}	0.5670±0.278^{ac}	1.260±0.027 ^{de}	407.58±0.534^{ac}	0.857±0.017^{ac}
VS1	479.49±0.043^{bf}	0.565±0.210^{bc}	1.760±0.012^{a}	270.33±0.311^{c}	0.861±0.018^{ab}
VS2	533.99±0.270^{ae}	0.5535±0.472^{bd}	1.750±0.024^{a}	318.49±0.358^{bc}	0.853±0.011^{ac}
VS3	497.71±0.021^{bf}	0.5675±0.250^{ac}	1.600 ±0.023^{ad}	334.62±0.316^{ac}	0.8565±0.010^{ac}
OS0	590.514±0.046^{ad}	0.5645±0.467^{bc}	1.415±0.008^{ade}	456.60±0.071^{ab}	0.840±0.015^{cd}
OS1	396.40±0.018 ^{ef}	0.5295±0.186^{d}	1.120±0.015^{e}	356.80±0.426^{ac}	0.8275±0.010^{d}
OS2	440.24±0.222^{cf}	0.5495^{c}±0.222^{d}	1.180±0.009^{de}	365.41±0.412^{ac}	0.8385±0.011^{cd}
OS3	435.92±0.034^{df}	0.5585±0.344^{bc}	1.280 ±0.020^{ce}	394.50±0.357^{ac}	0.8385±0.006^{cd}
FS0	580.98±0.155^{a}	0.575±0.155^{ab}	1.330±0.054^{ade}	479.93±0.072^{a}	0.854±0.015^{ac}
FS1	530.54±0.041^{abf}	0.5525±0.413^{bd}	1.405±0.026^{ade}	425.35±0.590^{ac}	0.857±0.018^{ac}
FS2	583.64±0.051^{ad}	0.549 ±0.321^{cd}	1.730±0.012^{ab}	364.39±0.322^{ac}	0.841±0.014^{cd}
FS3	591.74±0.031^{ac}	0.547±0.317^{cd}	1.305±0.009 ^{be}	388.23±0.387^{ac}	0.850±0.010^{bc}
LSD	155.28	0.0249	0.4372	159.49	0.0199
***p* value**	**0.0333**	**0.0269**	**0.0371**	0.3182	**0.0308**

Values are the means of 2 replicates (with 3 parallels)± SD; samples in same column with the same subscripts are not significantly different at ($p \leq 0.05$). Mean values with different superscript letter(s) within each row differ significantly ($p < 0.05$). * In dry matter, I:Industrial, V: Village, O: Organic, F: Free range, S0:Control; S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

Table 5. Sensory evaluation results of ESP-fortified bread samples

Sample	Crumb colour	Pore	Texture	Odor	Chewing	Flavour	Aroma	Overall acceptance
IS0	4.50 ± 0.054^{a}	4.12 ± 0.381^{ab}	4.04 ± 0.054^{c}	4.00 ± 0.000^{ab}	4.19 ± 0.054^{ac}	4.08 ± 0.109^{ab}	4.04 ± 0.054^{ad}	4.16 ± 0.218^{ab}
I S1	4.39 ± 0.109^{ac}	4.31 ± 0.326^{a}	4.00 ± 0.326^{c}	4.12 ± 0.163^{ab}	4.27 ± 0.054^{ac}	4.31 ± 0.109^{ab}	4.00 ± 0.326^{ad}	4.35 ± 0.381^{a}
IS2	4.42 ± 0.054^{ac}	4.24 ± 0.544^{ab}	4.19 ± 0.054^{bc}	4.04 ± 0.054^{ab}	4.12 ± 0.163^{ac}	4.27 ± 0.381^{ab}	4.16 ± 0.109^{ad}	4.31 ± 0.109^{a}
IS3	4.66 ± 0.054^{a}	4.31 ± 0.109^{a}	4.62 ± 0.109^{a}	4.04 ± 0.054^{ab}	4.31 ± 0.109^{ab}	4.19 ± 0.381^{ab}	4.39 ± 0.109^{a}	4.46 ± 0.000^{a}
VS0	4.39 ± 0.326^{ac}	4.08 ± 0.109^{ab}	4.16 ± 0.218^{bc}	4.12 ± 0.054^{ab}	4.20 ± 0.163^{ac}	4.12 ± 0.054^{ab}	4.31 ± 0.109^{ab}	4.23 ± 0.109^{a}
VS1	4.47 ± 0.218^{ac}	3.39 ± 0.054^{ab}	4.23 ± 0.109^{ac}	4.00 ± 0.435^{ab}	4.16 ± 0.435^{ac}	4.12 ± 0.490^{ab}	4.12 ± 0.490^{ad}	4.27 ± 0.490^{a}
VS2	4.43 ± 0.163^{ac}	4.20 ± 0.163^{ab}	4.39 ± 0.326^{ac}	4.12 ± 0.163^{ab}	4.27 ± 0.163^{ac}	4.38 ± 0.000^{a}	4.31 ± 0.109^{ab}	4.27 ± 0.054^{a}
VS3	4.00 ± 0.109^{cd}	3.89 ± 0.163^{ab}	4.08 ± 0.109^{c}	3.92 ± 0.326^{ab}	4.16 ± 0.109^{ac}	4.04 ± 0.054^{ab}	3.77 ± 0.109^{d}	4.04 ± 0.054^{ab}
OS0	4.16 ± 0.218^{cd}	3.97 ± 0.163^{ab}	4.15 ± 0.000^{bc}	4.16 ± 0.109^{a}	4.15 ± 0.000^{ac}	4.23 ± 0.218^{ab}	4.12 ± 0.163^{ad}	4.04 ± 0.054^{ab}
OS1	4.54 ± 0.000^{ab}	4.12 ± 0.163^{ab}	4.35 ± 0.163^{ac}	4.12 ± 0.272^{ab}	4.42 ± 0.054^{a}	4.04 ± 0.054^{ab}	4.19 ± 0.054^{ac}	4.28 ± 0.073^{a}
OS2	4.62 ± 0.109^{a}	4.00 ± 0.000^{ab}	4.19 ± 0.272^{bc}	3.96 ± 0.054^{ab}	4.27 ± 0.272^{ac}	4.00 ± 0.109^{ab}	3.93 ± 0.109^{bd}	4.04 ± 0.054^{ab}
OS3	4.44 ± 0.272^{ac}	3.77 ± 0.000^{b}	4.35 ± 0.054^{ac}	4.04 ± 0.163^{ab}	4.15 ± 0.000^{ac}	4.04 ± 0.054^{ab}	3.96 ± 0.054^{bd}	4.15 ± 0.000^{ab}
FS0	4.50 ± 0.054^{ab}	3.96 ± 0.381^{ab}	4.27 ± 0.054^{ac}	4.19 ± 0.272^{a}	4.39 ± 0.218^{a}	4.16 ± 0.218^{ab}	4.23 ± 0.326^{ac}	4.27 ± 0.163^{a}
FS1	4.70 ± 0.109^{a}	4.08 ± 0.109^{ab}	4.50 ± 0.054^{ab}	4.16 ± 0.109^{a}	4.39 ± 0.109^{a}	4.16 ± 0.109^{ab}	4.23 ± 0.218^{ac}	4.42 ± 0.054^{a}
FS2	4.27 ± 0.054^{bd}	$4.04 \pm 0.054^{a b}$	4.04 ± 0.381^{c}	3.96 ± 0.272^{ab}	3.96 ± 0.272^{bc}	$3.97 \pm 0.163^{a b}$	3.85 ± 0.109^{cd}	3.77 ± 0.435^{b}
FS3	4.16 ± 0.218^{cd}	3.97 ± 0.163^{ab}	4.23 ± 0.109^{ac}	3.73 ± 0.054^{b}	3.93 ± 0.109^{c}	3.85 ± 0.326^{ab}	4.00 ± 0.000^{ad}	4.08 ± 0.005^{ab}
LSD	0.3384	0.4915	0.4009	0.4225	0.3794	0.4780	0.4143	0.4442
***p* value**	**0.0237**	0.5951	0.1712	0.7827	0.3532	0.7309	0.1804	0.2694

Values are the means of 2 replicates (with 3 parallels)± SD; samples in same column with the same subscripts are not significantly different at *($p \leq 0.5$)* Mean values with different superscript letter(s) within each row differ significantly *($p < 0.05$)*. I:Industrial, V: Village, O: Organic, F: Free range, S0:Control; S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

Sensory evaluation of ESP fortified bread samples

The sensory evaluation results for the ESP-fortified breads are presented in Table 5. The sensory characteristics of ESP-fortified breads varied as follows: The crumb color ranged from 4.00 to 4.70, the pore values from 3.39 to 4.31, the texture values from 4.00 to 4.62, the odor values from 3.73 to 4.19, the chewing values from 3.93 to 4.39, the flavor values from 3.85 to 4.38, the aroma values from 3.77 to 4.39, and the overall acceptability values ranged from 3.77 to 4.46. The combination of ESP type and ESP level had a significant impact on the color of the crumb ($p \leq 0.05$). The bread with the greatest crumb color score was found to be at the free-range ESP-S1 level. However, this bread was statistically similar in crumb color score to the industrial ESP-S3, organic ESP-S2, and industrial ESP-S0 fortified bread. Quispe et al. (2021) suggest that, the notable variations may be attributed to the baking procedure, as it has an impact on the color. This is linked to an intricate phenomenon widely referred to as 'browning' or the Maillard reaction (Quispe et al., 2021).

Upon analyzing the results of sensory metrics including pore, texture, odor, chewing, flavor, aroma, and overall acceptance evaluation, it was shown that the interaction of ESP type and ESP level and the separate effects of type and level had no statistically significant effect on these parameters ($p \geq 0.05$). The addition of ESP to bread had no detrimental impact on the sensory characteristics of the bread, which is noteworthy. Consistent with our sensory results, Arnold et al. (2022) reported that the inclusion of 3% ESP had no significant impact on the visual appearance, aroma, texture, or taste of the gingerbread samples, as assessed by various descriptors (Arnold et al., 2022). In their study, Ali and Badaway (2017) found that, the inclusion of ESP in bread strips did not result in any notable differences in the hedonic appraisal of several qualities when compared to the control group (Ali & Badawy, 2017). Kobus-Cisowska (2020) reported that there were no notable disparities in the intensity of the sensory characteristics (Kobus-Cisowska et al., 2020). According to Bradauskiene et al. (2017), bread containing ESP exhibited superior characteristics in terms of crust color, flavor, and overall acceptance when compared to the control bread (Bradauskiene et al., 2017).

For overall acceptance, it was determined that the industrial, village, organic, and free-range ESP breads exceeded the overall acceptance score of 3.50/5 and were liked by the consumers. The highest overall acceptance score (4.46) was obtained by the industrial ESP-S3 fortified breads, and the lowest (3.77) was obtained by the breads of free-range ESP-S2, but no statistically significant difference was observed with the others. In their study, Bradauskiene et al. (2017) investigated the potential to enhance bread baking by adding chicken eggshell powder. The sensory evaluation of the bread showed enhanced overall acceptability compared to the control; however, the taste and flavor remained the same or worse. Out of the many concentrations that were examined, the bread that was baked with the addition of 5 g of ESP was considered to be the best (Bradauskiene et al., 2017). From the obtained results, it can be concluded that, the use of ESP in bread as a functional food ingredient, which is a natural source of mineral substances, may be possible from a sensory point of view.

Color analysis results

The color of food products is a significant factor that influences customer choices. It is a crucial quality indicator that significantly impacts the acceptability of bread. The color analysis of bread involved assessments based on the CIE L^* a^* b^* scale. The L^* scale measures the level of brightness, the a^* scale measures the level of redness or greenness, and the b^* scale measures the level of yellowness or blueness. The color of the crust is a significant factor in consumers' bread preferences; excessively pale or excessively dark colors are undesirable. The crust color of a loaf of bread is determined by various elements, including the type of flour, the quality and quantity of additions (Komlenić et al., 2010), the baking time (Shittu et al., 2007), and temperature (Salinas et al., 2012). The results of the investigations conducted for the crumb color of ESP-fortified breads are presented in Table 6.

Upon analyzing the brightness value in Table 6, it was determined that the interaction between ESP type and ESP level had no significant statistical effect ($p \geq 0.05$). The separate effects of ESP type and ESP level were determined to be statistically significant ($p \leq 0.05$). The fortified breads made with organic ESP exhibited the highest brightness value, while the brightness value of the breads made with S1, S2, and S3 ESP was higher than that of the control. Upon examination of the a^* and b^* values, it is evident that the values are highly close to each other. After analyzing the a^* and b^* data, it was determined that the interaction between ESP type and ESP level had no significant effect ($p \geq 0.05$). Upon performing an analysis of the separate impacts of ESP type and ESP level, it was determined that both factors were statistically insignificant ($p \geq 0.05$).

Table 6. Color analysis results of ESP-fortified bread samples

Sample	***L****	***a****	***b****
IS0	73.300^{g}	2.250^{ac}	20.805^{a}
IS1	75.715^{bd}	2.150^{bc}	20.940^{a}
IS2	76.265^{ac}	2.065^{bc}	20.755^{a}
IS3	75.555^{be}	2.015^{c}	21.055^{a}
VS0	72.740^{g}	2.050^{c}	20.380^{a}
VS1	75.320^{ce}	2.065^{bc}	20.110^{a}
VS2	75.495^{be}	2.070^{bc}	19.965^{a}
VS3	74.350^{f}	2.230^{ac}	20.265^{a}
OS0	74.860^{df}	2.010^{c}	20.230^{a}
OS1	76.715^{a}	2.260^{ac}	20.595^{a}
OS2	76.440^{ab}	2.285^{ac}	20.430^{a}
OS3	76.265^{ac}	2.405^{ab}	20.765^{a}
FS0	72.670^{g}	2.010^{c}	20.230^{a}
FS1	75.300^{df}	2.355^{ac}	20.985^{a}
FS2	75.245^{df}	2.250^{ac}	20.160^{a}
FS3	74.605^{ef}	2.500^{a}	20.290^{a}
LSD	0.9557	0.3464	1.225
***p* value**	<.0001	0.1345	0.7395

Values are the means of 2 replicates (with 3 parallels)± SD; samples in same column with the same subscripts are not significantly different at ($p \leq 0.05$). Mean values with different superscript letter(s) within each row differ significantly ($p < 0.05$) I:Industrial, V: Village, O: Organic, F: Free range, S0:Control; S1: 23.5 g; S2: 30 g; S3: 38.5 g ESP fortification

In a study that was undertaken to investigate the effect of adding chicken eggshell to bread, the results showed that there was no significant difference in the crumb color, as measured by the $b*$ value, between the control group and the group that had 2% eggshell powder added to it. Nevertheless, the $b*$ values exhibited an increase when 4% of eggshell powder was incorporated, as opposed to the 2% addition. Specifically, the $b*$ value rose from 13.05 to 15.00. Furthermore, the addition of 6% eggshell powder resulted in a $b*$ value of 18.6 in the formulation. The higher $b*$ values suggested the crumb was towards yellowness in color (Zulkeflee et al., 2020). According to Chilek et al. (2018), there was a notable difference in lightness between the control and eggshell-added bread samples. The addition of the eggshell affected the $L*$ value, but did not have an effect on the $a*$ and $b*$ values. They stated that this was because white bread, being an opaque product, exhibits a high level of lightness. Nevertheless, there are numerous additional parameters that impact the whiteness of bread, including wheat pigment, grain content, and grain fineness. Among these various factors, the size of the flour particles stands out as the most crucial. The variation in lightness could be attributed to the varying quantities of eggshell utilized, with the sample with the highest amount of eggshell powder exhibiting the lowest lightness (Chilek et al., 2018). Another study conducted on injera found that the lightness of injera decreased as the amount of eggshell powder increased. There was a 3% reduction in the brightness of injera samples, indicating no significant differences (Fekadu et. al., 2022).

CONCLUSION

The rheological, textural and sensory analyses results obtained from the control and fortified bread samples indicated that the incorporation of calcium derived from egg shell powder enhanced the structural characteristics of the flour. Furthermore, the incorporation of egg shell powder at its highest concentration did not result in any adverse effects on the dough or the bread production process. The study has determined that the most effective combination of ESP types is village ESP-S1 (23.5 g of ESP added). This ESP type and combination do not have any adverse impact on the technological and qualitative attributes of the bread's structure. Furthermore, it improves the overall quality of bread making. Nevertheless, the highest degree of ESP-S3 fortification did not adversely affect the physical and sensory characteristics of the ESP-fortified bread. The integration of ESP had no detrimental effect on the color of the bread. For overall acceptance, it was determined that the industrial, village, organic, and free-range ESP breads exceeded the overall acceptance score of 3.50/5 and were liked by the consumers. After a comprehensive investigation, it has been established that adding ESP in bread is a feasible approach to addressing calcium deficiency. This method is also compatible with customer preferences and bread manufacturing technology.

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Conflict of Interest

The article authors declare that there is no conflict of interest.

Author's Contributions

Formal analysis; Methodology, Conceptualization, Investigation Writing-original draft: Samiye ADAL**.** Conceptualization; Funding Acquisition; Methodology; Writing, Rewieving, and Editing: Nazlı SAVLAK

REFERENCES

AACC. (1999). Method 74-09.01. Measurement of Bread Firmness by Universal Testing Machine. In: Cereals & Grains Association St. Paul, MN, USA.

AACC. (2000). Approved methods of the American association of cereal chemists.

Ali, M., & Badawy, W. (2017). Utilization of eggshells by-product as a mineral source for fortification of bread strips. Journal of Food and Dairy Sciences, 8(11), 455-459.

Ali, M., & Badawy, W. Z. (2017). Utilization of eggshells by-product as a mineral source for fortification of bread strips. Journal of Food and Dairy Sciences, 8(11), 455-459. https://dx.doi.org/10.21608/jfds.2017.38960

Alsuhaibani, A. M. (2018). Rheological and nutritional properties and sensory evaluation of bread fortified with natural sources of calcium. Journal of Food Quality, 2018, 1-7.

Ameh, M. O., Gernah, D. I., & Igbabul, B. D. (2013). Physico-chemical and sensory evaluation of wheat bread supplemented with stabilized undefatted rice bran. Food and Nutrition Sciences, *4*(09), 43.

Arnold, M., Rajagukguk, Y. V., Sidor, A., Kulczynski, B., Brzozowska, A., Suliburska, J., Wawrzyniak, N., & Gramza-Michalowska, A. (2022). Innovative Application of Chicken Eggshell Calcium to Improve the Functional Value of Gingerbread. International Environmental Research and Public Health, 19(7). https://doi.org/10.3390/ijerph19074195

Baláž, M. (2018). Ball milling of eggshell waste as a green and sustainable approach: a review. Advances in colloid and interface science, 256, 256-275.

Bassett, M. N., Pérez-Palacios, T., Cipriano, I., Cardoso, P., Ferreira, I. M. P. L. V. O., Samman, N., & Pinho, O. (2014). Development of Bread with NaCl Reduction and Calcium Fortification: Study of Its Quality Characteristics. Journal of Food Quality, 37(2), 107-116. doi:10.1111/jfq.12079

Bradauskiene, V., Montrimaite, K., & Moscenkova, E. (2017). Facilities of bread enrichment with calcium by using eggshell powder. https://doi.org/10.22616/foodbalt.2017.014.

Brun, L. R., Lupo, M., Delorenzi, D. A., Di Loreto, V. E., & Rigalli, A. (2013). Chicken eggshell as suitable calcium source at home. International Journal of Food Sciences and Nutrition, 64(6), 740-743.doi:10.3109/09637486.2013.787399

Chilek, T. Z. T., Kairuaman, N. A., Ahmad, F. I. S. A. L., Wahab, R. A., Zamri, A. I., & Mahmood, A. Z. I. Z. A. H. (2018). Development of white bread fortified with calcium derived from eggshell powder. Malaysian Applied Biology, 47(6), 29-39.

D'Appolonia, B. L., & Kunerth, W. H. (1984). Farinograph handbook: American association of cereal chemists. Inc. St. Paul, Minnesota, USA.

Dursun, S. (2006). Ekmek Zenginleştirmede Protein Kaynağı Olarak Balık Etinin Kullanımı. Pamukkale Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı Yüksek Lisans Tezi, Denizli.

Dursun, S., Yapar, A., & Çelik, İ. (2009). Kadife balığı (*Tinca tinca L.*, 1758) etiyle zenginleştirmenin hamurun reolojik özellikleri ve ekmeğin duyusal özellikleri üzerine etkisi. Gıda Teknolojileri Elektronik Dergisi, 4(3), 44-58.

Ekşi, A., & Karadeniz, F. (1996). Gıda zenginleştirme yaklaşımı ve Türkiye'de uygulanma olanağı. Beslenme ve Diyet Dergisi, 25(2), 47-51.

Ercili, D. (2004). Ekmeğin aminoasit şelatlı demir ve çinko bileşikleri ile zenginleştirilmesi. İstanbul Teknik Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 86 s.

FAO. 2023. World Food and Agriculture – Statistical Yearbook 2023. Rome.

Flammini, L., Martuzzi, F., Vivo, V., Ghirri, A., Salomi, E., Bignetti, E., & Barocelli, E. (2016). Hake fish bone as a calcium source for efficient bone mineralization. International journal of food sciences and nutrition, 67(3), 265-273.

Gómez-Alvarez, L. M., & Zapata, J. E. (2024). Effect of fortification with $CaCO_3$ nanoparticles obtained from eggshell on the physical and sensory characteristics of three food matrices. Heliyon.

Ho, L. H., Abdul Aziz, N. A., & Azahari, B. (2013). Physico-chemical characteristics and sensory evaluation of wheat bread partially substituted with banana (*Musa acuminata* X *balbisiana* cv. Awak) pseudo-stem flour. Food Chemistry, 139(1-4), 532-539. doi:10.1016/j.foodchem.2013.01.039

ICC Standard, N. (1992). 114/1 Method for Using Brabender Extensograph. International Association for Cereal Science and Technology: Vienna, Austria.

ICC Standard, N. (2003). 115/1; Method for Using the Brabender Farinograph. International Association for Cereal Science and Technology: Vienna, Austria.

Khan, F. A., Ameer, K., Qaiser, M. A., Pasha, I., Mahmood, Q., Anjum, F. M., Amir, R. M. (2021). Development and analysis of bread fortified with calcium extracted from chicken eggshells of Pakistani market. Food Science and Technology, 41, 14-20. https://doi.org/10.1590/fst.07220

Kobus-Cisowska, J., Szymanowska-Powalowska, D., Szymandera-Buszka, K., Rezler, R., Jarzebski, M., Szczepaniak, O., Marciniak, G., Jedrusek-Golinska, A., & Kobus-Moryson, M. (2020). Effect of fortification with calcium from eggshells on bioavailability, quality, and rheological characteristics of traditional Polish bread spread. Journal of Dairy Science, 103(8), 6918-6929. https://doi.org/10.3168/jds.2019-18027

Köksel, H., Sivri, D., Özboy, Ö., Başman, A., & Karacan, H. (2000). Hububat laboratuarı el kitabı. Hacettepe Üniversitesi Mühendislik Fakültesi Yayınları, 47.

Komlenić, D. K., Ugarčić-Hardi, Ž., Jukić, M., Planinić, M., Bucić-Kojić, A., & Strelec, I. (2010). Wheat dough rheology and bread quality effected by Lactobacillus brevis preferment, dry sourdough and lactic acid addition. International Journal of Food Science & Technology, 45(7), 1417-1425. https://doi.org/10.1111/j.1365-2621.2010.02282.x

Krupa-Kozak, U., Altamirano-Fortoul, R., Wronkowska, M., & Rosell, C. M. (2012). Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts. European Food Research and Technology, 235(3), 545-554. doi:10.1007/s00217-012-1782-z

Leung, C. C. J., Cheung, A. S. Y., Zhang, A. Y.-Z., Lam, K. F., and Lin, C. S. K. (2012). Utilisation of waste bread for fermentative succinic acid production. Biochemical Engineering J. 65, 10–15. doi: 10.1016/j.bej.2012.03.010

Mann, J., & Truswell, A. S. (2007). Essentials of human nutrition (No. Ed. 3, pp. 599-pp).

Mattar, G., Haddarah, A., Haddad, J., Pujola, M., & Sepulcre, F. (2022). New approaches, bioavailability and the use of chelates as a promising method for food fortification. Food chemistry, 373, 131394. https://doi.org/10.1016/j.foodchem.2021.131394

Mohammed, I., Ahmed, A. R., & Senge, B. (2012). Dough rheology and bread quality of wheat-chickpea flour blends. Industrial Crops and Products, *36*(1), 196-202. https://doi.org/10.1016/j.indcrop.2011.09.006.

Preston, K. R., & Hoseney, R. C. (1991). Applications of the extensigraph. The Extensigraph Handbook, 13-19.

Quispe, M., Aquipucho, K., Bellido, O., & Zegarra, J. (2021). Rheological, Physical and Sensory Characteristics of Bread Obtained by Partially Replacing Wheat Flour with Hen's Eggshell Powder. Carpathian Journal of Food Science and Technology, 13(2), 128-139. https://doi.org/10.34302/crpjfst/2021.13.2.12

Ranhotra, G., Gelroth, J., Dreese, P., & Blockcolsky, D. (1984). Effect of Mineral Gluconates on Quality Characteristics of Bread and Cookies. Journal of Food Science, 49(3), 913-915. https://doi.org/10.1111/j.1365-2621.1984.tb13240.x.

Salem, I. S., Ammar, A., & Habiba, R. A. (2012). Effect of eggshell powder addition as a source of calcium fortification on butter cake quality. Journal of Agricultural and Veterinary Sciences, *5*(2), 109-118.

Salinas, M. V., & Puppo, M. C. (2015). Optimization of the formulation of nutritional breads based on calcium carbonate and inulin. LWT-Food Science and Technology, 60(1), 95-101. doi:10.1016/j.lwt.2014.08.019

Salinas, M. V., Zuleta, A., Ronayne, P., & Puppo, M. C. (2011). Wheat Flour Enriched with Calcium and Inulin. Food and Bioprocess Technology, 5. https://doi.org/10.1007/s11947-011-0691-7

Shittu, T. A., Raji, A. O., & Sanni, L. O. (2007). Bread from composite cassava-wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. Food research international, 40(2), 280-290. https://doi.org/10.1016/j.foodres.2006.10.012

Srivastava, A. K., Patel, V. R., & Haridas Rao, P. (1994). Effect of common salt substitution on the dough characteristics and bread quality. Journal of Food Science and Technology, 31(1), 15-18.

Statista, (2024), Market Insights, Bread-Worldwide, (2024).

Sudha, M. L., & Leelavathi, K. (2008). Influence of micronutrients on rheological characteristics and bread-making quality of flour. International Journal of Food Sciences and Nutrition 59(2), 105-115. doi:10.1080/09637480701497170

Wiley, D., and C. N. Y. Nee. 2020. Food ingredients. In Food and Society, 377–91.Elsevier: UK.

Yurdatapan, S., Güngör G., Türkiye'de Ekmek Sanayi ve Ekmek Tüketim Eğilimleri: Edirne İli Merkez İlçe Örneği. Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü, Tarım Ekonomisi Anabilim Dalı, (Yüksek Lisans Tezi) Tekirdağ, 2014, 78 s.

Yusufoğlu, B., Yaman, M., & Karakuş, E. (2021). Glycemic evaluation of some breads from different countries via in vitro gastrointestinal enzymatic hydrolysis system. Food Science and Technology, 42, e34920. doi: 10.1590/fst.34920

Zulkeflee, W., Chompoorat, P., & Siva, R. (2020). Physicochemical and Sensory Properties of Bread Added with Chicken Eggshell Powder. Malaysian Journal of Analytical Sciences, 24(6), 873-881.