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# RHEOLOGICAL, PHYSICAL AND SENSORY CHARACTERISTICS OF BREAD OBTAINED BY PARTIALLY REPLACING WHEAT FLOUR WITH HEN'S EGGSHELL POWDER

**Maria Quispe[1]✉, Karen Aquipucho[1], Omar Bellido[1], Juan Zegarra[1]**

[1]Faculty of Processes, Universidad Nacional de San Agustín de Arequipa – Calle Santa Catalina 117, Cercado, Perú

✉*mquispegut@unsa.edu.pe*

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**ABSTRACT**

Bread is one of the staple foods in many countries of the world. Currently, the consumption of bread made from wheat flour is of limited nutritional value. The aim of this investigation was to produce bread with high calcium content, performing different substitutions using eggshell powder in order to evaluate the effects it has on the product. The research began with the production of eggshell powder (HCH), and its proximal and microbiological composition. The eggshell powder was substituted in 3 different percentages (10%, 15% and 20%) in addition to a control group. The methodology included the conducting of a rheological study by evaluating parameters such as tenacity, extensibility, swelling index, dough's strength, tenacity/extensibility ratio and elasticity index. The rheological properties of the dough were evaluated with the Chopin alveograph. The physical characteristics (specific volume and height) were determined and the results showed no significant difference. On the other hand, the sensory evaluation was carried out using a hedonic scale of 5 points by 25 panelists; seven attributes were evaluated (color, smell, texture, taste, external appearance, internal appearance and overall appearance), in which there were significant differences.

## 1.Introduction

Nowadays, different food products for children are being developed. These are mainly fortified and nutritious products, within which we can find bakery products made by replacing wheat flour with flour of tubers, of cereals or of native grains, which increases their nutritional value (Obrego *et al*., 2013).

The World Health Organization (WHO) indicates that 80% of the world's population, that is, more than 2/3 (of 4 billion people) use traditional bread as a basic daily diet. The INEI - National Household Budget Survey (2009) – states that the average annual per capita consumption of bread is 24 kg/person, in comparison to the 2018 sectoral report of the Institute of Economic and Social Studies (IEES) which states that the average annual per capita consumption of bread in Peru is 35 kg/person, showing an increase of 11 kg/person in recent years.

A hen's eggshell is an excellent source of calcium. It is constituted in 96 % by calcium carbonate, and less by other minerals such as phosphorus and magnesium, which facilitate its absorption (Alais *et al.*, 1990).

In addition, fortification with calcium has been shown to be an economic way of obtaining additional calcium (Keller *et al.*, 2002). Moreover, the human body cannot produce adequate amounts of calcium without external support. Additionally, it is daily lost through hair, skin, nails, sweat, urine, and feces. This calcium loss must be replaced, or the body will take calcium from the bones to perform other functions (Kessenich, 2008).

On the other hand, in recent years environmental pollution rates have increased, causing a major problem for the planet and therefore for humanity. Large amounts of organic waste are discarded daily. The eggshell is classified as a waste material by the food industry, therefore it is not used despite having different properties, its calcium content being one of the most important of them.

For this reason, the present study aimed to investigate the rheological characteristics to evaluate the behavior of the dough, as well as the physical and sensory characteristics of the final product to determine its effect on it.

## 2. Materials and methods

### 2.1. Obtaining eggshell powder

Eggshells were collected from different sources such as restaurants, dealerships, bakeries, food trucks, etc. They were washed and disinfected with a concentration of 100 ppm for 5 min. Then they were dried at 120°C for 60 min. Afterwards, the shells were ground and passed through a mesh sieve for 10 min with an opening of 106 um.

### 2.2. Chemical and microbiological analysis

Proximal and microbiological analysis of the eggshell powder were determined by official methods such as moisture (NTP Method 209.085), ash (AOAC Method 2.173), fat (NTP Method 209.093), proteins (AOAC Method 2.057), fiber (NTP 209.074 method) and carbohydrates (AOAC Method 31.043) and salmonella (6579-1: 2017. Microbiology of food chain - Horizontal Method for the Detection, enumeration and serotyping of Salmonella. - Part 1: Detection of Salmonella spp).

### 2.3. Rheological analysis

The Chopin alveograph was used to describe the dough's resistance to the extention caused during fermentation in the different substitutions (0%, 10%, 15% and 20%). The parameters obtained were: tenacity (P), extensibility (L), inflation index (G), strength (W), tenacity/extensibility ratio (P/L) and elasticity index (ie), following the AACC 54-30.02 standard *(Peña et al. 2008).*

### 2.4. Bread production

The method used for the production of the product was the direct method. The loaves were prepared according to the standardized formulation with a slight modification of Mesas and Alegre (2002). The wheat flour was replaced by eggshell powder (EP) in 3 different percentages which were 10% (F1), 15% (F2) and 20% (F3), and compared with a blank sample with 0% (F0) EP substitution. The kneading lasted 5 min. and 50 gr were weighed for each bread. The bread was baked at 180°C for 15 minutes. The loaves were then removed, cooled and stored for further study.

### 2.5.Determination of the physical characteristics of bread

The bread was evaluated at room temperature after 30 minutes of being removed from the oven based by preliminary tests. The weight was determined by means of a precision analytical balance with a maximum capacity of 300 g and sensitivity of 0.1 g (Henkel – model BQ1001). For the determination of the specific volume, data were collected using the 10-05 of the AACC (2000) method proposed by Lainez et al. (2008). Each parameter evaluated was measured with three repetitions.

## 2.6. Sensory analysis

Sensory evaluation was conducted with semi-trained judges consisting of 25 panelists, who evaluated the following sensory attributes: color, smell, texture, taste, external appearance, internal appearance and general appearance proposed by Larmond (1977).

## 2.7. Statistical Analysis

Data were statistically evaluated by a variance analysis (ANOVA) and the Tukey test with a reliability level of 95%, using the statistical software Minitab 18.

# 3.Results and discussions

## 3.1. Physical-chemical and microbiological analysis

The physical chemical characterization of the eggshell powder as a raw material used in the production of bread for cultivation is shown in Table 1.

**Table 1.** Proximal composition of eggshell powder

| Analyses | Results (%) |
|---|---|
| Moisture | 0.68 |
| Ash | 92.61 |
| Fat | 0.10 |
| Proteins | 5.34 |
| Fiber | 0.07 |
| Carbohydrates | 1.20 |

In the eggshell powder there was moisture (0.68%), ash (92.61%), fat (0.10%), proteins (5.34%), fiber (0.07%), and carbohydrates (1.20%). Similarly, Osonwa et al. (2017) states that eggshell powder has a humidity of 0.68 %, a value similar to that obtained in the present work. According to Velásquez and Obando (2017), humidity influences the conservation of quality since it is one of the factors of acceleration of chemical reactions, enzymes and growth of microorganisms.

On the other hand, the ash content in the eggshell powder was 92.61 %. This concentration is high due to the high content of minerals present, mostly calcium carbonate. Walton et al. (1973); Hassan (2015) and Ray *et al.* (2017) mention that the ash content in eggshell powder is 91.1 %, 90.2 %, 93.62 %. Burley and Vadhera (1989) and Shwetha *et al.* (2018) report that these differences may be related to nutrition and bird habitation.

**Table 2.** Microbiological analysis of eggshell powder

| Determination | Results | Units |
|---|---|---|
| Escherichia coli | <10 | UFC/g |
| Mohos | <10 | UFC/g |
| Salmonella spp. | Ausencia | En 25 g |

The table above shows that the count of Escherichia Coli and molds is less than 10 in UFC/g, this being a permissible parameter according to the Sanitary Standard that establishes the Microbiological Criteria of Health Quality and Safety Food and Beverages Human Consumption R.M. N°591-2008-MINSA. Detection of Salmonella spp. in the sample is negative showing to be a harmless product.

## 3.2. Moisture of substitutions by eggshell powder

Humidity is related to the water content necessary for the production of bread. As the humidity of the flour decreases, the absorption of water increases. This means that as wheat flour is replaced with eggshell powder, water absorption increases. This may be due to the fact that eggshell powder is a hydrophilic filling that can absorb more water (Shuhadah and Supri, 2009).

**Table 3.** Moisture of flour according to the substitution

| WF (%) | EP (%) | Moisture (%) |
|---|---|---|
| 100 | 0 | 14.25 |
| 90 | 10 | 13.71 |
| 85 | 15 | 13.62 |
| 80 | 20 | 13.24 |

Legend: Where, WF= Wheat Flour; EP= Eggshell Powder

In the table above, it is noted that the humidity decreased as the substitution increased, presenting a behavior inversely proportional. These values are within the parameters as indicated in CODEX STAN 152 (1985). The moisture requirement for bread's wheat flour should not exceed 15.5 %. The moisture data in the table are necessary for the determination of the rheological parameters shown below.

## 3.3. Rheological characterization

**Table 4.** Mass parameters obtained from Chopin's alveograph

| Parameters | Partial substitution of WF by EP | | | |
|---|---|---|---|---|
| | **F0** | **F1** | **F2** | **F3** |
| P (mm $H_2O$) | 158 | 121 | 113 | 106 |
| L (mm) | 52 | 59 | 52 | 42 |
| G | 16 | 17.1 | 16 | 14.4 |
| W ($10^{-4}$ J) | 343 | 277 | 206 | 196 |
| P/L | 3.04 | 2.05 | 2.17 | 2.52 |
| Ie (%) | 59.4 | 56.3 | 39.5 | 54.2 |

Legend: Where, F0= White group, F1= Experimental group of 10% substitution, F2= Experimental group of 15% substitution, F3= Experimental group of 20% substitution, P= tenacity, L= extensibility, G= Inflation rate, W= Mass strength, P/L= tenacity/extensibility ratio, ie= elasticity index.

The substitution of flour influences the rheological characteristics of the dough and this is mainly due to the weakening of the gluten net (Ho et al., 2013) which is due to the substitution of wheat flour with eggshell powder. The results of the dough's parameters obtained from the Chopin alveograph based on the substitution of eggshell powder in different percentages are shown in Table 3.

### *3.3.1. Tenacity (P)*

The tenacity of the dough refers to the dough's resistance to rupture and deformation. In addition, the value of P serves as a reference to the ability of the dough to retain gas (De la Vega, 2009; Vázquez, 2009; Wang *et al*., 2002).

Toughness depends on the glutenin content of wheat flour (De la Vega, 2009; Perego *et al*., 2002; Wang et al., 2002). Wheat flour, when partially replaced with eggshell powder, decreased the amount of these storage proteins, thus reducing the resistance to dough breaking (tenacity). This is because eggshell powder has a high content of ash, due to its composition of calcium carbonate. Ferreras (2009) indicates that ash is composed mostly of fiber, and consequently fiber is not composed of proteins, therefore, the higher ash content, the less amount of proteins, affecting the formation of gluten.

According to the requirements of the Uruguayan Institute of Technical Standards (UNIT), standard 951:94 (1994), the recommended value of tenacity for a baking flour should be in the range of 100 to 130 mmH2O. The tenacity value for F0 (100 % WF) shows that it is outside the recommended range, possibly because of the dietary additives of wheat flour used as raw material for this study. For example, the dough's tenacity increases through the use of glucose oxidase enzyme (Steffolani *et al*., 2011).

The tenacity of the different substitution percentages (10 %, 15 % and 20 %) is within the range set out in standard 951:94 (UNIT), showing that a good dough's resistance to rupture and deformation is obtained.

### *3.3.2. Extensibility (L)*

Extensibility refers to the dough's ability to be stretched (Banu *et al*., 2012) and depends on the gliadins present in wheat flour (De la Vega, 2009; Gómez, 2011; Perego *et al.,* 2002). According to the requirements of the Uruguayan Institute of Technical Standards (UNIT), standard 951:94 (1994), the recommended extensibility value for a bakery flour should be in the range of 100 to 130 mm.

The results obtained show that the values are below the recommended and this can be attributed to the wheat flour's additives used as raw material. For example, the enzyme glucose oxidase decreases the dough's extensibility (Preedy et al., 2011; Steffolani et al., 2011).

According to the study carried out by Kajishima et al. (2001), which produced French bread with calcium sulphate enriched flour, the extensibility of wheat flour without substitution was 38 mm, flour with 50 % IDR (Recommended Daily Intake) of Ca sulfate was 47 mm and flour with 100 % of Ca sulfate IDR was 38 mm. This shows that its results are also below the recommended value according to Standard (UNIT) 951: 94 (1994).

### *3.3.3. Rate of swelling (G) Inflation rate*

Ferreras (2009) maintains that the rate of swelling is represented by the volume of air necessary to cause the rupture of the balloon dough. Morales (2014) indicates that G provides a value proportional to extensibility.

Hassan (2015) mentions that replacing wheat flour with sources of calcium reduces the amount of amylose and amylopectin, which are the main inflating components. The calcium particles found in EP are deposited within the structure of the starch gel causing changes in the structure. So higher levels of calcium substitution lead to smaller sizes of pores or air cells.

### *3.3.4. Dough's strength (W)*

Dough's strength refers to the work necessary to deform the bread dough (Fálder, 2002). Polymeric proteins (glutenins) give strength to the wheat dough (Shewry *et al.*, 1992; Belton, 1999), meaning that there is a directly proportional relationship between the glutenin content and the dough's strength (Islands *et al.*, 2005). The gluten network is responsible for forming a viscoelastic dough, but by decreasing proteins the development of this network is hindered, and the dough's strength decreases (Mohamed *et al.,* 2010) according to the data obtained in Table 3.

Shewry and Halford (2002) and De la Vega (2009) mention that the dough's strength (W) must be between 200 and 300 10-4 J. Unlike Monleón and Collado (2008) who say that for fermented baked products, flour should be used between 180 and 200.

The substitution rates were found within the range recommended by Shewry and Halford (2002) for F1 (10 % EP) and F2 (15 % EP), while F3 (20 % EP) was found within the value recommended by Monleón and Collado (2008).

According to Quaglia (1991), if baking flour has W greater than 250, it is recommended to be used in mixtures with other flours. According to research work F0 (100 % EP) submitted 343 10-4 J, which confirms that eggshell powder can work with wheat flour in bread production.

The study carried out by Kajishima *et al.* (2001) obtained that the dough's strength was 250 (10-4 J) with 50 % IDR of calcium sulphate and 280 (10-4 J) with 100 % IDR of calcium sulphate, showing that its results are also within the recommended value according to Shewry and Halford (2002).

### *3.3.5. P/L: tenacity/extensibility ratio*

The tenacity/extensibility ratio depends on the relationship between glutenins/glycine (De la Vega, 2009). It also indicates the balance of the dough (Ferreras, 2009).

The P/L scale is dimensionless and can range from 0,1 to 6 (Espitia *et al.,* 2003) or 0,5 to 1,5, with a balanced value of 1,1 for baking (Banu *et al.,* 2012; Martínez *et al.,* 2014; Vázquez, 2009).

The P/L values obtained were higher than recommended. This can be attributed to food additives used in wheat flour, such as the enzyme glucose oxidase that catalyzes the oxidation of glucose by generating o-D-gluconolacone and hydrogen peroxide. The latter oxidises the thiol groups of gluten and forms disulfide bonds that make up the dough (Steffolani *et al*., 2011). With the values obtained in Table 3 the flour was classified as strong and tenacious according to Espitia *et al.* (2003).

According to the study carried out by Kajishima et al. (2001) the values obtained from the tenacity/extensibility ratio were 3.45 (standard flour), 2.72 (flour with 50% of the Ca sulfate IDR) and 3.47 (flour with 100% of the Ca sulfate IDR) for French bread, showing that these values are also outside the recommended range in greater proportion in comparison to the results obtained experimentally.

***3.3.6. Ie: Elasticity index.***

The elasticity index is expressed as a percentage, which means that some of the curves, after reaching the maximum pressure, have a very abrupt fall and show a dough with little elasticity, On the contrary, a curve with a little steep fall will give us a more elastic dough, optimal for baking according to Ferreras (2009).

## 3.4. Physical Characteristics of bread

**Table 5.** Physical Characteristics of bread

| Substitution | Weight (g) | Volume ($cm^3$) | Specific Volume ($cm^3$/g) | Height (cm) |
|---|---|---|---|---|
| F0 | 41.70 | 307.39 | 7.36 | 4.11 |
| F1 | 39.66 | 289.74 | 7.31 | 4.05 |
| F2 | 38.86 | 275.91 | 7.10 | 3.83 |
| F3 | 38.62 | 270.03 | 7.01 | 3.81 |

***3.4.1. Bread's weight***

The results, with three repetitions, indicated that the average weights of the bread of the different formulations decreased as the substitution was greater; however, they did not show significant difference ($p > 0.05$). The average weight of F0 (100 % WF, 0 % EP) is 41.70 g and F3 (80 % WF, 20 % EP) is 38.62 g, these two values represent the maximum and minimum bread weight respectively, the difference between these formulations' ranges around 7.37 %. A similar result was obtained by Martinez et al. (2014) who used banana flour in two percentages 0 and 10 %. This type of dough does not allow gas retention because the dough shows resistance to expansion, reflecting the bread's low weight.

***3.4.2. Volume of the bread***

As the substitution level of wheat flour with eggshell powder increased, the bread decreased in volume; however, it did not show a significant difference ($p > 0.05$). This is due to the eggshell's lack of gluten. Previous research, such as that carried out by Pineda (1977) where tarwi flour was used in a panification trial, concluded that there was a descending variation in the volume of the replaced bread. The volume of the center of the bread is a very important parameter for consumers, because it is related to the perception of a light but not dense product, which means that the center of the bread's density and volume characteristics are associated with a specific bakery product (Hathorn *et al.*, 2008).

***3.4.3. Specific volume***

The results showed that the specific volume decreased according to the degree of substitution; however, they did not show any significant difference ($p > 0.05$). Seguchi *et al.* (2007), Lorenz and Coulter (1991) found that the specific volume decreases as the amount of wheat decreases, which was also observed in the present study.

Kajishima *et al.* (2001) analysed the effects of the addition of calcium sulphate on the physical characteristics of bread. It showed that, although the specific volume was not significantly modified, the addition of the highest dose (100 % of the Recommended Daily Intake) caused a slight decrease in its external characteristics. The specific volume of bread is one of its most important visual characteristics, strongly influencing the consumer's choice. It is therefore a fundamental parameter when assessing bread's quality (Hager and Arendt, 2013).

***3.4.4. Bread's height***

The bread that obtained the highest height was F0 (100 % WF, 0 % EP) with 4.11 cm while F3 (80 % WF, 20 % EP) obtained the lowest height with 3.81 cm. Statistical analysis revealed that there is no significant difference ($p > 0.05$). The height of the bread decreased as the percentage of substitution with eggshell powder was increased, showing an inversely proportional ratio.

The height depends mainly on the gluten network and the production of CO2 during the dough's fermentation stage (Chinma *et al.*, 2014). Based on the results obtained from Table 4, it was observed that the gluten network was weakened as the percentage of replacement with eggshell powder was increased, thus affecting gas retention and the bread's height.

### 3.5. Sensory evaluation

The seven attributes evaluated (color, smell, texture, taste, external appearance, internal appearance and general appearance) showed significant difference (p 0,05) compared to the formulation. In other words, the substitution of eggshell powder influenced the sensory characteristics of the bread.

#### *3.5.1. Color*

Color, particularly color uniformity, has an important effect on the consumer's acceptability (Vasquez, 1982).

The possible cause of the significant difference could be due to the baking process, because it causes color changes. This is associated with a complex phenomenon commonly known as 'browning' or the Maillard reaction (Toaquiza, 2012).

#### *3.5.2. Odor*

The presence of a difference of smell in the bread could be due to the characteristic smell of the eggshell. The toasted smell of bread depends on the formation of active flavour compounds in the crust during the cooking process. The compound with the greatest impact on the smell of bread crust is the 2-Acetyl-1-pyrroline (Belitz and Grosch, 2009). It is an aroma and flavor compound that gives the usual smell to white bread.

Odor is a very important parameter in the acceptability of any product, but this characteristic is influenced by the raw materials used such as flour, flakes and bran. These inputs are not food that transmit strange or unpleasant odors to the product, as Coloma points out (2000).

#### *3.5.3. Texture*

Bradauskiene et al. (2017) found that to a higher content of eggshell powder, the texture of the bread is considerably reduced, since the granulometry was perceptible when chewing.

The possible cause may have been the characteristic texture of the eggshell powder, as Li-Chan and Kim (2008) indicate. They mention that the proteins in the eggshell matrix influence the crystalline growth process by controlling the size, the shape and orientation of the calcite crystals, thus affecting the texture and biomechanical properties of the eggshell.

In addition, Brun *et al.* (2013) point out that the most appropriate way to use the eggshell as a source of calcium is as powder added to pizza, bread or spaghetti, since it presents small changes in texture and no changes in flavor.

#### *3.5.4. Taste*

The result evaluated in taste showed a significant difference, possibly due to the texture provided by the eggshell powder that is perceived at the time of chewing the bread. Bradauskiene *et al.* (2017) made bread with added eggshell powder, finding that in all calcium concentrations the bread's taste score decreased in comparison to the control.

#### *3.5.5. External and internal aspects*

The external aspect was influenced by the color attribute, due to Maillard's reaction, presenting different appearances such as very light and dark colors in the bread.

#### *3.5.6. Internal aspects*

The internal aspect is reflected in the center of the bread, which is related to the amount of water added to the dough and the possible use of special flours in the process. But the most determining factors are the quantity and quality of the protein (Kihlberg, 2004).

In the process of making bread, when increasing the percentage of substitution, the center changed its sensory characteristics due to its low protein content.

#### *3.5.7. General appearance*

Salem *et al.* (2012) studied the influence of the addition of eggshell powder at 10 % and 20 % as a source of calcium fortification on the sensory properties of butter cake. They reported that no statistically significant differences were detected between the unfortified cake and the fortified cake with 10 % and 20 % eggshell powder for color and overall acceptability, while significant differences were found in texture, smell, taste and appearance.

Piscoya (2002) mentions in his research that bread fortified with calcium had a good acceptability.

**Table 6.** Average score of sensory characteristics by attribute of processed bread

| Sensory characteristics | Partial substitutin of WF by EP | | | |
|---|---|---|---|---|
| | F0 | F1 | F2 | F3 |
| Color | 4.6 | 3.4 | 3.36 | 2.4 |
| Smell | 4.16 | 3.4 | 3.4 | 3.04 |
| Texture | 4.16 | 3.28 | 3.52 | 2.52 |
| Taste | 4.36 | 3.44 | 3.84 | 3.12 |
| External aspect | 4.8 | 3.72 | 3.52 | 2.6 |
| Internal aspect | 4.48 | 3.52 | 3.32 | 3.16 |
| General appearance | 4.72 | 3.8 | 3.52 | 3 |
| **Average** | 4.47 | 3.51 | 3.50 | 2.83 |

In Table 6, the results showed the averages of each sensory characteristic of a total of 25 panelists out of a total of 5 points per attribute. The different formulations (F0, F1, F2 and F3) obtained an average score of 4.47, 3.51, 3.50 and 2.83 respectively.

According to the averages shown by F1, F2 and F3 (Fig. 1), the values are lower than the formulation F0 (100 % wheat flour). However, it is necessary to consider that bread with a 10 % substitution had similar characteristics of color, smell, texture, taste, external appearance, internal appearance and overall appearance in relation to F0 (the control test). Finally, it was shown that bread made from 10 % eggshell powder is accepted by the consumer.

In their research, Ali and Badawy (2017) carried out the sensory evaluation of bread strips, where they demonstrate and recommend using eggshell powder as a dietary calcium supplement to strengthen the bread in homes to a level of 10%. In Cuba, salted and sweet bread fortified with calcium was produced in such a way that each bread contained 477 and 538,80 mg, with an acceptability (over the target population) of 100 % and 96 % respectively (Riera *et al.*, 1999).

In Chile the bread was also fortified with calcium with a concentration of 120 mg of calcium per unit of bread. Later, the acceptability test obtained results of "liked" or "liked it a lot" in 80% of the population **(Toop *et al.*, 1994)**.

## 4. Conclusions

According to the results, the formulation of bread with partial substitution of wheat flour (HT) with eggshell powder (HCH) at 10% (F1) has the highest calcium assimilation compared to bread with conventional formulation (F0). It is worth mentioning that this product rich in calcium and generated from an organic residue would contribute to solve calcium deficit in the body.

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