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# Effect of Lactic Acid, Ascorbic Acid, and Azodicarbonamide on Microstructure and Organoleptic Properties of Sangak Bread

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ABSTRACT: Nowadays, there are studies focusing on introduction and use of different types of improvers that affect the qualitative properties of baked goods with the outcome being higher quality, longer shelf life, and delayed staling of breads. The objective of this study was to analyze the effect of lactic acid, ascorbic acid and azodicarbonamide on some of the qualitative and sensory properties of Sangak bread. Sangak ingredients (flour, salt and sourdough) and improvers (lactic acid, ascorbic acid and azodicarbonamide) in Concentration of (zero, 0.25 and 0.5% of flour weight) were prepared and weighed. Data were analyzed using a completely randomized design with three replications. Means were compared through Duncan's multiple range tests at a significance level of $\alpha \leq 0.05$. The results showed that these improvers increased sensory properties (including flavor, color, aroma, and texture), energy, correlation and homogeneity, and reduced staling compared to the control samples. The study revealed that azodicarbonamide and ascorbic acid were capable of improving and modifying the structure of the bread core, and can enhance bread quality and improvers..

**Keywords**: Ascorbic acid, Azodicarbonamide, Improver, Lactic acid, Sangak Bread, Staling, Scanning Electron Microscopy.

## Introduction

Bread is the most consumed wheat product and is baked in different types based on taste, conditions and available means. They can be classified into three types: high-density breads (e.g. pan breads), medium-density breads (e.g. French bread), and low-density breads (e.g. flatbreads). Sangak is the most popular Iranian flatbread with special flavor and aroma, high nutritional value, high filling ability, easy digestion and high fiber content. Given the significance of bread, it is important to preserve its quality and curb its losses. Research findings are today focused on introduction and use of different types of improvers that affect the qualitative properties of baked goods with the outcome of higher quality, longer shelf life, and delayed staling of breads (Karimi & Mortazavi, 2014; Campo *et al.*, 2016; Movahhed *et al.*, 2021). In general, flour improvers are largely made of activating anion compounds that are classified as GRAS (Generally recognized as safe). In terms of appearance, they are white to cream powders with high solubility in cold water. Improvers play a significant role in preserving the texture of flour products as they can reinforce the gluten lattice of

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flour, substantially improve the structure of weak flours, and create a desirable texture. Other advantages of improvers include easier processing conditions, improved dough drying time, increased dough stability during the drying period, and stable production formula. In products such as bread, improving agents can enhance dough development, reduce proofing time, and makes it robust to different weather and temperature conditions. They can also prevent dough from collapsing during final proofing and baking (Hrušková & Novotná, 2003; Mohammadi Golchin *et al.,* 2021). A number of oxidative improving agents are azodicarbonamide, bromate, potassium, potassium iodate, lactic acid and ascorbic acid. Dehydo ascorbic acid derived from oxidation of ascorbic acid appears as an oxidative compound in the bread industry. It can be carcinogenic as it destroys a number of bread vitamins. Azodicarbonamide is a chemical improving agent that is available as powder or crystals. It is yellow to reddish orange and is actually insoluble in water. It is used for conditioning and bleaching grain flours in order to obtain better condition doughs for lighter and more voluminous bread loaves. It can be also applied as a dough proofing agent in bakery flour and bread doughs (Zeng *et al.*, 2011). Dehydo ascorbic acid and azodicarbonamide are oxidative improvers that can enhance dough proofing, density, and the texture of the final product. Required ascorbic acid content, as a dough improver, is a function of flour type, ash content, gluten content, gluten quality, dough preparation conditions, mixing method, and mixing time (Sahlstream *et al.,* 2004). Lactic acid is another improving agent, which is a colorless organic acid with water and ethanol solubility. This acid is a result of lactose fermentation, which can be found abundantly in soured roots and milk fermented products such as yogurt and cheese. It is also naturally present in most food products. It is used as an acidifier, preserver, and pH regulator. In addition, it has no toxic effects and is classified as GRAS. Abdollahzadeh & Shahedi Baghkhandan (2001) studied the effect of ascorbic acid and diglycerides on improved dough rheological properties. They found that these compounds increase dough stability, dough extensibility, dough stability coefficient, and energy. It was also suggested that ascorbic acid had a fairly larger effect on dough rheology than mono- and di-glycerides. However, mono- and di-glycerides had a more substantial effect on delaying staling than ascorbic acid. In general, both ascorbic acid (60 mg per 1 kg flour) and mono- and di-glycerides (max. 0.5%) played a substantial role in improving quality of Tafton bread samples. Movahed *et al.* (2014) added different levels of compressed and active dry yeasts to improve the volume of Sangak, Barbari and Lavash breads. It was found that Sangak flour was stronger than Barbari and Lavash flours and had a higher water absorption. Aamodt *et al.* (2006) analyzed the effect of flour quality, ascorbic acid, and DATEM on the rheological properties of dough using high-performance liquid chromatography. Ascorbic acid and DATEM enhanced dough properties and improved the qualitative properties of dough rheology. Corsetti *et al.* (2011) studied the combined effect of ascorbic acid and commercial enzymes (amylase and xylanase) on dough rheology and bread quality. Dough rheology was characterized by moisture content, gluten and Farinograph testing. It was found that some of the rheological properties of dough and the gluten index were improved as a result of combined application of the improvers. Considering the role of all types of improving agents in texture preservation and improvement of flour-based goods and reinforcement of the gluten lattice, the present study aimed at analyzing the effect of different levels of ascorbic acid, lactic acid and azodicarbonamide (ADA) on the qualitative properties of treated Sangak bread in comparison with the control sample.

## Materials and Methods

The study was conducted in the laboratory of the Food Science Department of Tehran University in 2018.Whole grain wheat flour (Arddaran Co.) was used for baking Sangak breads. Lactic acid, ascorbic acid and ADA were bought from Pars Behbood Asia Co., and sourdough was purchased from Nanavaran Saboos Co. for sample preparation purposes The study treatments were: 1. Control (regular Sangak bread) (C); 2. Sangak bread with 0.25% ascorbic acid (A1); 3. Sangak bread with 0.5% ascorbic acid (A2); 4. Sangak bread with 0.25% lactic acid (L1); 5. Sangak bread with 0.5% lactic acid (L2); 6. Sangak bread with 0.25% ADA (Z1); 7. Sangak bread with 0.5% ADA (Z2); 8. Sangak bread with 0.25% ascorbic acid + 0.25% lactic acid (LA); 9. Sangak bread with 0.25% ADA + 0.25% lactic acid (LZ); and 10. Sangak bread with 0.25% ascorbic acid + 0.25% ADA (AZ).

### - *Sangak bread production*

Sangak ingredients—20 kg wheat flour, improvers (ascorbic acid, lactic acid and ADA) in three levels (zero, 0.25 and 0.5% of flour weight), 4 kg sourdough, and 2 kg NaCl—were prepared and weighed. The chemical assays including MC, ash, wet gluten, protein, fiber and pH were conducted on the study flour. The Sangak dough was then prepared in a mixer according the standards. For dough preparation, water (10–20 °C) was added to the mixer with all of the salt and one part of the flour. The mixer was turned on for 10 min, and then the dough was left to rest (proofing). At this point, the sourdough, the rest of the flour, and the improvers (lactic acid, ascorbic acid and ADA) were also added and were mixed for 7 to 8 min. The dough was left for proofing for 30 min. Sangak bread rolls were divided and spread for baking. The baked Sangak samples were kept in polyethylene bags after they were cooled down. The bread assays were then carried out on all the treated and control Sangak samples—*i.e.* texture analysis (staling), sensory profile and microstructure properties (Anonymous, 2002).

### - *Microstructure of Sangak breads*

The core of the treated and control bread samples was sampled in slices by a cutter, which were freeze dried for 24 hours. A scanning electron microscopy (SEM) (XL 30, Philips, the Netherlands) was used in this study. Thin slices of the samples were secured on a special aluminum bases by liquid adhesive. Then the metal beads were sprayed with argon on a sample for 20 min. Then the samples fully coated with gold were inserted into the SEM (20 kV). Images with 30x (for bread core porosity analysis) and 1000x (for starch and protein compounds in cavity walls) magnification were captured (Anonymous, 2016).

### - *Texture analysis of Sangak samples*

A texture analyzer (Instron) was used to determine bread staleness. A plate probe was used in this study. Tests were conducted at 24, 48 and 72 hours after baking of Sangak breads. To this end, the samples were kept separately in plastic bags at room temperature. Slices (2cm x 2cm) from their core were cut for testing in the texture analyzer. The required force—the force imposed by the upper jaw on the sample—was set at 40% of the sample thickness to compress the samples for 8 mm. The movement rate of the upper jaw was 30 mm/s (Anonymous, 2003).

- ***Organoleptic analysis of Sangak breads***

The five-point hedonic approach was adopted to evaluate the sensory profile of Sangak bread samples by analyzing their properties. The samples were cut and coded once cooled down and were assessed by panelists. Assessments were based on bread appearance (aroma, color, flavor and texture) each with a specific weighted score. The panelists then gave an overall score within the range in the evaluation form (Movahed *et al.*, 2014).

- ***Statistical analysis methods***

Experimental data were analyzed using a completely randomized design with three replications. Means were compared through Duncan's multiple range test at a significance level of $\alpha \leq 0.05$ in SPSS 16.

**Results and Discussion**

Table 1 shows the results for chemical profile of wheat samples used in Sangak breads.

- ***SEM tests***

Table 2 and Figure 1 show the results for SEM imaging of sangak bread samples.
According to the means comparison results for the effect of different improver levels on the energy content of Sangak breads from SEM analysis (Table 2), it was found that C had the lowest energy content. The highest energy content was found in AZ followed by LA and LZ compared to other treatments ($p \leq 0.05$).

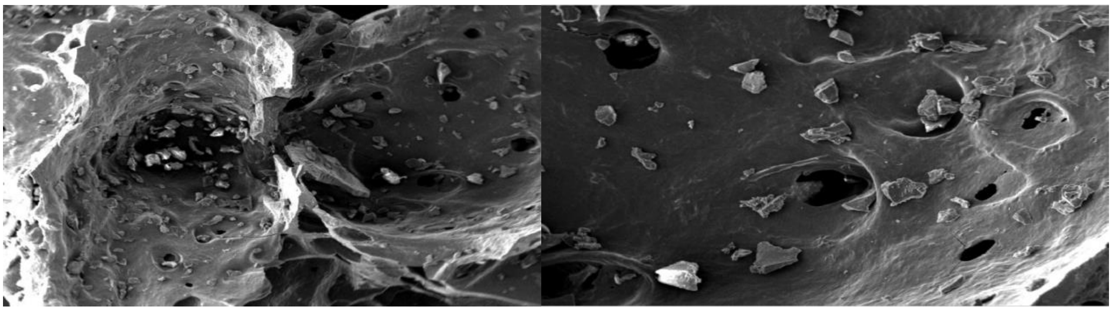
**Table1.** Results of chemical assays on wheat flour used in Sangak bread

| | Moisture (%) | Ash (%) | Protein (%) | Fiber (%) | Wet gluten | pH |
|---|---|---|---|---|---|---|
| Wheat flour | 12.53 | 1.1 | 11.52 | 1.1 | 0.26 | 6.21 |

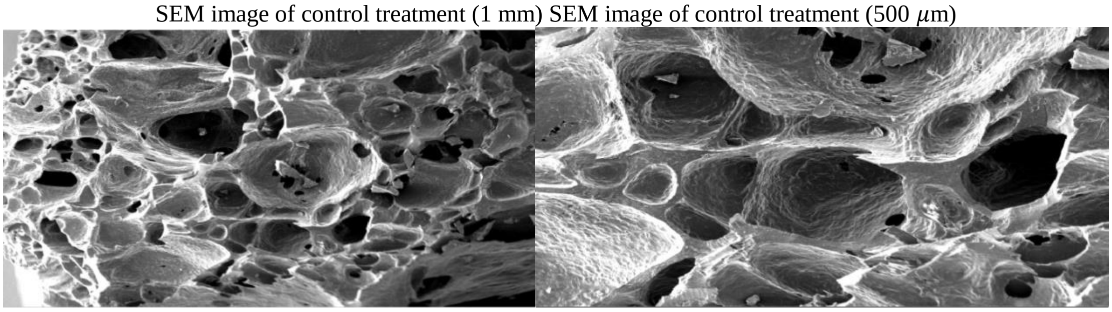
**Table 2.** Mean comparison results of data from SEM imaging of sangak bread samples

| Treatment | Energy (cal) | Contrast | Correlation | Homogeneit |
|---|---|---|---|---|
| AZ | 0.069±0.02[a] | 0.059±0.01[f] | 1.43±0.02[a] | 0.96±0.05[a] |
| LA | 0.065±0.01[b] | 0.060±0.02[ef] | 0.93±0.03[b] | 0.88±0.03[b] |
| LZ | 0.060±0.01[c] | 0.065±0.03[de] | 0.90±0.03[b] | 0.85±0.03[b] |
| A2 | 0.057±0.01[c] | 0.069±0.02[d] | 0.80±0.04[c] | 0.73±0.02[c] |
| Z2 | 0.052±0.01[d] | 0.072±0.01[cd] | 0.78±0.03[cd] | 0.73±0.02[c] |
| L2 | 0.050±0.01[de] | 0.073±0.01[c] | 0.75±0.02[d] | 0.70±0.02[c] |
| A1 | 0.048±0.02[e] | 0.083±0.02[b] | 0.68±0.01[e] | 0.65±0.01[d] |
| Z1 | 0.043±0.01[f] | 0.084±0.03[b] | 0.68±0.01[e] | 0.63±0.01[d] |
| L1 | 0.040±0.01[fg] | 0.0869±0.03[b] | 0.58±0.01[f] | 0.63±0.01[d] |
| C | 0.038±0.02[g] | 1.063±0.02[a] | 0.58±0.01[f] | 0.56±0.03[e] |

In each column, means with at least one common letter show no significant difference ($p \leq 0.05$).
C = Control; A1 = Sangak bread containing 0.25% ascorbic acid from the total weight; A2 = Sangak bread containing 0.5% ascorbic acid from the total weight; L1 = Sangak bread containing 0.25% lactic acid from the total weight; L2 = Sangak bread containing 0.5% lactic acid from the total weight; Z1 = Sangak bread containing 0.25% ADA from the total weight; Z2 = Sangak bread containing 0.5% ADA from the total weight; LA = Sangak bread containing 0.25% lactic acid + 0.25% ascorbic acid from the total weight; AZ = Sangak bread containing 0.25% ascorbic acid + 0.25% ADA from the total weight; and LZ = Sangak bread containing 0.25% lactic acid + 0.25% ADA from the total weight.



SEM image of control treatment (1 mm) SEM image of control treatment (500 $\mu$m)



SEM image of AZ treatment (1 µm) SEM image of AZ treatment (500 µm)

**Fig. 1.** Results for SEM imaging of sangak bread samples (C and AZ).

This shows that the collective use of improvers had a better effect on the energy content than their separate application. The combined application of A and Z (0.25%) had a larger effect on energy content. At the same time, by increasing the concentration of improvers from 0.25 to of the 0.5% for separate application, the energy content was increased for all improvers. Accordingly, ADA and ascorbic acid led to an increase in the energy content of the treated Sangak breads. The energy parameter explains how smooth or flat is the sample surface. To this end, the frequency of pixel pairs is measured, and higher frequencies represent smoother sample surface and vice versa. The texture of SEM images of ADA and ascorbic acid was higher than lactic acid as the highest energy content was recorded at the core of the bread samples containing ADA and ascorbic acid. The increased energy content as a result of adding improvers is probably due to formation of a regular molecular structure and lattice similar to the improvers. By increasing ADA and ascorbic acid, given their molecular structure, the contrast and cavity size were reduced whereas parameters such as energy and porosity were increased. The results were in agreement with Karami & Safiyari (2018) who studied bread core with image processing techniques and found that alyssum homolocarpum and gum and xanthan gums improved the microstructure of the breads by reducing the cell sizes and increasing porosity and energy. The contrast resulted from the SEM-revealed microstructure of Sangak samples showed that AZ and then LA and LZ had the lowest contrast, and the C sample had the highest result compared to other treatments ($p \leq 0.05$). In other words, ADA and ascorbic acid reduced contrast in treated Sangak breads. Since contrast is suggestive of spatial scattering in the SEM texture, the improvers had a direct effect on the contrast with their different structure. The results showed that contrast declined as both improvers were increased. The addition of ADA and ascorbic acid reduced spatial scattering leading to a more uniform image texture. By increasing

ADA and ascorbic acid, given their molecular structure, the contrast and cavity size were reduced whereas parameters such as energy and porosity were increased. Karimi & Mortazavi (2014) reported similar results, where higher levels of alyssum homolocarpum and xanthan gums led to reduced entropy, contrast, and cavity size while increasing energy, homogeneity, number of pores, total pore area, and porosity. The correlation resulted from the SEM micro-structure of Sangak samples showed that AZ and then LA and LZ had the highest correlation, and the C sample had the lowest result compared to other treatments ($p \leq 0.05$). This shows that the collective use of improvers had a better effect on correlation than their separate application. By increasing the concentration of improvers from 0.25 to of the 0.5% for separate application, correlation showed an increase for all improvers. SEM images revealed that starch granules of baked breads had lost their natural shape through displacement. Demirkesen *et al.* (2013) used image processing to study the porosity of gluten-free breads. They reported that it can successfully determine the sphericity and the surface ratio of starch granules and pores. The homogeneity resulted from the SEM micro-structure of Sangak samples showed that AZ and then LA and LZ had the highest homogeneity, and the C sample had the lowest result compared to other treatments ($p \leq 0.05$). By increasing the concentration of improvers from 0.25 to of the 0.5% for separate application, homogeneity showed an increase for all improvers. This means that application of ADA and ascorbic acid improved homogeneity in treated bread samples. This is due to the stronger gluten lattice and better qualitative properties of the Sangak breads made of a strong flour than those made of medium and weak flours. The other reason is the gelatinization phenomenon occurring in the microscopic structure of Sangak breads. As a necessary condition of baking Sangak breads, proofing for a proper time allows more starch granules to enter the medium and develop sufficient amount of protein–starch complexes in the dough. As a result, a uniform homogeneous structure is formed, which is the result of coagulation within the gluten lattice. Gelatinization also occurs uniformly and at the same time. Therefore, fewer starch granules are seen separately in Sangak bread's structure. In addition, the heating medium of Sangak bread is different from other breads during baking, and thus the former has a more homogeneous and uniform micro-structure, according to the SEM images. The results were not in agreement with Jaldani *et al.* (2018) who reported that increased levels of whole-grain quinoa flour led to reduced homogeneity and increased contrast and entropy in treated samples compared to the control. Higher levels of quinoa flour were also translated into higher hardness and lower elasticity during the storage period. In other words, addition of this ingredient accelerated staling.

- ***Staleness analysis***

According to the means comparison results for the effect of different improver levels on staleness of Sangak breads (Table 3), it was found that C had the highest staleness whereas its lowest scores were observed in AZ followed by LA and LZ compared to other treatments. The mentioned treatments also had significant differences with other treatments ($p \leq 0.05$). The lower staleness of treated samples compared to the control was due to the lower water loss during the storage period, complete proofing process, lower pH and higher acidity (Movahhed *et al*., 2019; Movahhed & Ahmadi Chenarbon, 2018). In other words, the treated breads had lower staleness that can be attributed to the applied improving agents—*i.e.* these compounds had an effect on flour components, particularly starch, which reduced core hardening in these breads. The results were in agreement with Movahhed & Rajabzadeh (2004) who studied the effect of acidic improving agents (lactic acid, acetic acid and citric acid) on bread staling. It was reported that sourdough can be processed to produce controlled levels of lactic and acetic acids in dough and bread to ensure the maximum effect on staleness reduction of breads.

- ***Organoleptic analysis***

Table 4 shows the results of organoleptic properties of Sangak breads.

**Table 3.** Mean comparison results of data from staleness analysis of sangak bread samples (N)

| Treatment | Time (h) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| C | 2.80±0.05$^{k}$ | 9.57±0.05$^{f}$ | 24.21±0.02$^{a}$ |
| L1 | 2.73±0.05$^{kl}$ | 9.55±0.05$^{f}$ | 22.45±0.02$^{b}$ |
| Z1 | 2.64±0.05$^{l}$ | 9±0.02$^{g}$ | 19.84±0.03$^{c}$ |
| A1 | 2.59±0.02$^{lm}$ | 8.47±0.05$^{h}$ | 18.84±0.03$^{c}$ |
| L2 | 2.55±0.02$^{m}$ | 7.65±0.05$^{i}$ | 15.60±0.01$^{d}$ |
| Z2 | 1.65±0.05$^{n}$ | 7.60±0.05$^{i}$ | 13.52±0.02$^{e}$ |
| A2 | 1.63±0.05$^{n}$ | 7.57±0.05$^{i}$ | 13.49±0.02$^{e}$ |
| LZ | 1.61±0.04$^{n}$ | 5.42±0.05$^{j}$ | 9.62±0.03$^{f}$ |
| LA | 1.60±0.05$^{n}$ | 5.35±0.05$^{j}$ | 9.57±0.03$^{f}$ |
| AZ | 1.55±0.05$^{n}$ | 5.30±0.05$^{j}$ | 7.66±0.05$^{i}$ |

Means with at least one common letter show no significant difference ($p \leq 0.05$).
C = Control; A1 = Sangak bread containing 0.25% ascorbic acid from the total weight; A2 = Sangak bread containing 0.5% ascorbic acid from the total weight; L1 = Sangak bread containing 0.25% lactic acid from the total weight; L2 = Sangak bread containing 0.5% lactic acid from the total weight; Z1 = Sangak bread containing 0.25% ADA from the total weight; Z2 = Sangak bread containing 0.5% ADA from the total weight; LA = Sangak bread containing 0.25% lactic acid + 0.25% ascorbic acid from the total weight; AZ = Sangak bread containing 0.25% ascorbic acid + 0.25% ADA from the total weight; and LZ = Sangak bread containing 0.25% lactic acid + 0.25% ADA from the total weight.

**Table 4.** Mean comparison results of data from sensory analysis of sangak breads

| Treatment | Aroma | Color | Flavor | Texture |
|---|---|---|---|---|
| AZ | 4.9±0.1$^{a}$ | 5±0.2$^{a}$ | 4.5±0.1$^{a}$ | 4.8±0.1$^{a}$ |
| LA | 4.8±0.1$^{a}$ | 5±0.2$^{a}$ | 4.5±0.1$^{a}$ | 4.8±0.1$^{a}$ |
| LZ | 4.8±0.1$^{a}$ | 5±0.2$^{a}$ | 4.5±0.1$^{a}$ | 4.5±0.2$^{b}$ |
| A2 | 4±0.1$^{b}$ | 4±0.1$^{b}$ | 4±0.2$^{b}$ | 4±0.1$^{c}$ |
| Z2 | 4±0.1$^{b}$ | 4±0.1$^{b}$ | 4±0.2$^{b}$ | 4±0.1$^{c}$ |
| L2 | 3.5±0.2$^{c}$ | 3.5±0.1$^{c}$ | 4±0.2$^{b}$ | 4±0.1$^{c}$ |
| A1 | 3.5±0.2$^{c}$ | 3.5±0.1$^{c}$ | 3.5±0.1$^{c}$ | 3.5±0.2$^{d}$ |
| Z1 | 3±0.1$^{d}$ | 3±0.2$^{d}$ | 3.5±0.1$^{c}$ | 3.5±0.2$^{d}$ |
| L1 | 3±0.1$^{d}$ | 3±0.2$^{d}$ | 3.5±0.1$^{c}$ | 3.5±0.2$^{d}$ |
| C | 2.5±0.2$^{e}$ | 3±0.2$^{d}$ | 3.5±0.1$^{c}$ | 3.5±0.2$^{d}$ |

In each column, means with at least one common letter show no significant difference ($p \leq 0.05$).
C = Control; A1 = Sangak bread containing 0.25% ascorbic acid from the total weight; A2 = Sangak bread containing 0.5% ascorbic acid from the total weight; L1 = Sangak bread containing 0.25% lactic acid from the total weight; L2 = Sangak bread containing 0.5% lactic acid from the total weight; Z1 = Sangak bread containing 0.25% ADA from the total weight; Z2 = Sangak bread containing 0.5% ADA from the total weight; LA = Sangak bread containing 0.25% lactic acid + 0.25% ascorbic acid from the total weight; AZ = Sangak bread containing 0.25% ascorbic acid + 0.25% ADA from the total weight; and LZ = Sangak bread containing 0.25% lactic acid + 0.25% ADA from the total weight.

As shown in Table 4, AZ had the highest sensory scores in color followed by LA and LZ. The lowest color score was, however, found in the control sample ($p \leq 0.05$). The collective use of improvers had a better effect on the color score. At the same time, by increasing the concentration from 0.25 to 0.5%, the color score was increased for all improvers. Color changes occur during bread baking, which are largely due to maillard and caramelization reactions. The result of such reactions is a brown–golden color and increased sensory color score, which are intensified in the presence of ascorbic acid and ADA (Lazaridou *et al*., 2007). The combined application of ADA and ascorbic acid increased the aroma, flavor and texture scores in treated bread samples. Ascorbic acid and ADA increased aroma by playing a role in the proofing rate. Therefore, the maillard reaction during baking was intensified that improved the aroma of treated samples compared to the control (Movahed *et al*., 2014). The results were in agreement with Movahhed *et al.* (2013) that studied the effect of adding DATEM emulsifier on the sensory profile and staling of gluten-free Barbari breads. Moreover, the flavor score was higher in Sangak breads treated with higher levels of these improvers. This can be due to the formation of more aromatics during the proofing process by these improvers (Movahed *et al*., 2014). ADA and ascorbic acid improved the sensory texture score, this can be due to the better form and shape of the breads during the proofing process in the presence of the improving agents.

## Conclusion

This study and its findings revealed that azodicarbonamide and ascorbic acid were capable of improving and modifying the structure of the bread core, and can enhance bread quality and improvers. Finally, considering the results of all tests, AZ (containing 0.25% ADA and 0.25% ascorbic acid) was selected as the best study treatment.

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