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Improving Effects of Lactic Acid, Ascorbic Acid, and Azodicarbonamide on the Qualitative Properties of Sangak Bread

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ABSTRACT: Given the significance of bread, it is important to preserve its quality and curb its losses. Nowadays, there are studies focusing on introduction and use of different types of improvers that affect the qualitative properties of baked breads with the outcome of higher quality, longer shelf life, and delay the staling. Ascorbic acid and Azodicarbonamide are oxidative improvers that can enhance dough proofing, density, and the texture of the final product. The objective of this study was to analyze the effect of Lactic acid, Ascorbic acid and Azodicarbonamide on the qualitative properties of Sangak bread. Sangak ingredients (flour, salt and sourdough) and improvers (Lactic acid, Ascorbic acid and Azodicarbonamide) in two levels (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 test at a significance level of $\alpha \leq 0.05$ in SPSS, and Excel 2010 was used for plotting charts and diagrams**.** The results showed that these improvers increased moisture content, ash, acidity, L^* color index, and reduced protein content, fat, pH, a^* and b^* color indices, and fiber content remained unchanged in treated samples as compared to the control samples. The study 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.

Keywords: *Ascorbic Acid, Azodicarbonamide, Improver, Lactic Acid, Sangak Bread.*

Introduction

Bread is the most consumed wheat product and is baked in different types based on taste, conditions and available means. Breads 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) (Qarooni, 1996). Sangak is the most popular Iranian flatbread with special flavor and aroma, high nutritional value, high filling ability, and easy digestion and thanks to its 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 being higher quality, longer shelf life, and delaye the staling of breads (Pourfarzad *et al.*, 2012). A number of oxidative improving agents such as Azodicarbonamide, bromate, potassium, potassium iodate, Lactic acid, and Ascorbic acid (Thiele, 2002) are available Dehydro ascorbic acid appears as an oxidative

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compound in the bread industry. It can damage and destroy a number of bread vitamins (Mortazavi *et al.,* 2015). 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 for better dough, condition for lighter and more voluminous bread loaves. It can be also applied as a dough proofing agent in bakery flour and bread dough (Zeng *et al.*, 2016). Ascorbic acid requirement, as a dough improver, is a function of flour type, ash content, gluten content, gluten quality, dough preparation conditions, mixing method, and mixing time (Sahlstrom *et al.*,2004). Lactic acid is another improving agent, that is a colorless organic acid with solability in water and ethanol. 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 and Shahedi Bagh Khandan, (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. 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. Yarmand *et al.*,(2008) added different levels of compressed and active dry yeasts to improve the volume of Sangak, Barbariand Lavash breads. It was found that Sangak flour was stronger than Barbari and Lavash flours and had higher water absorption. In a study on the effect of three acidic improving agents (Lactic acid, Acetic acid, and Citric acid) at three levels (0, 0.25 and 0.5%), Rahimi *et al.*,(2015) reported that sourdough bread samples had the lowest score, and treatments containing 0.25% Acetic acid had the lowest staling. The samples with 0.25% Lactic acid and the control sample reached a better staling score over time. Their results showed that sourdough treatment can be adjusted to obtain controlled levels of Lactic and Acetic acids in dough and bread. The combined effects of Ascorbic acid and commercial enzymes (amylase and xylanase) were studied 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 combin of the improvers (Hruskovaand Novotna, 2018).

Materials and Methods

The study was conducted in the Food Science Department laboratory of Tehran University, in 2017.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: Control (regular Sangak bread) (C); Sangak bread with 0.25% Ascorbic acid (A1); Sangak bread with 0.5% Ascorbic acid (A2); Sangak bread with 0.25% Lactic acid (L1); Sangak bread with 0.5% Lacticacid (L2); Sangak bread with 0.25% ADA (Z1); Sangak bread with 0.5% ADA (Z2); Sangak bread with (0.25% Ascorbic acid + 0.25% Lactic acid) (LA); Sangak bread with (0.25% ADA + 0.25% Lactic acid) (LZ); and 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 two levels (0.25 and 0.5% of flour weight), 4 kg sourdough, and 2 kg NaCl—were prepared and weighed. The chemical assays including moisture, ash, wet gluten, protein, fiber and pH were conducted on the study flour. The Sangak dough was then prepared in a mixer according to the standard methods. 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.*moisture, ash, protein, fiber, pH, acidity and color properties. The data were then statistically analyzed (Anonymous, 2002). Color analyses ofSangak breads were conducted after baking by measuring a^*, L^* and b^* parameters. The color analyses were carried in a HunterLab device to measure the above parameters (Anonymous, 2003).

- *Statistical analysis methods*

Experimental 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 $\alpha \leq 0.05$ in SPSS 16, and Excel 2010 was used for plotting charts and diagrams.

Results and Discussion

- *Chemical analysis*

Table 1 shows the results for chemical profile of wheat samples used in Sangak breads. According to the means comparison results (Table 2), addition of the improving agents increased moisture, ash, and acidity of Sangak bread samples compared to the control.

Table 1. Results of chemical assays on wheat flour used in Sangak bread

Material	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 chemical assays for Sangak bread samples

Treatment	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Acidity (mg/100)	pH
AZ	36.26 ± 0.07^{a}	1.80 ± 0.01^{b}	8.88 ± 0.01^{c}	0.48 ± 0.01^{d}	1.21 ± 0.05^{a}	0.20 ± 0.01^{bc}	6.29 ± 0.01^{c}
LA	34.50 ± 1.12^{ab}	1.78 ± 0.01^{c}	8.88 ± 0.01^{c}	0.48 ± 0.01^{d}	1.23 ± 0.04^{a}	0.22 ± 0.01^{b}	6.19 ± 0.01^{d}
LZ	33.65 ± 0.81^{b}	1.80 ± 0.01^{b}	8.88 ± 0.01^{c}	0.48 ± 0.01^{d}	1.23 ± 0.04^{a}	0.20 ± 0.01^{bc}	6.27 ± 0.01^{c}
A2	32.93 ± 1.33^{b}	1.78 ± 0.01^{c}	8.89 ± 0.01^{c}	0.45 ± 0.01^{c}	1.25 ± 0.07^{a}	0.27 ± 0.01^{a}	5.92 ± 0.04^{f}
Z2	32.68 ± 0.79^{bc}	1.83 ± 0.01^{a}	8.89 ± 0.01^{c}	0.45 ± 0.01^{c}	1.25 ± 0.07^{a}	0.25 ± 0.01^{a}	6.05 ± 0.02^{e}
L2	32.59 ± 0.93^{bc}	1.78 ± 0.01^{c}	8.89 ± 0.01^{c}	0.45 ± 0.01^{c}	1.28 ± 0.05^{a}	0.27 ± 0.01^{a}	5.85 ± 0.04^{f}
A1	32.55 ± 0.46^{bc}	1.78 ± 0.01^{c}	8.93 ± 0.01^{b}	0.53 ± 0.01^{b}	1.20 ± 0.05^{a}	0.18 ± 0.01^{c}	6.32 ± 0.01^{b}
Z1	31.04 ± 0.78^{cd}	1.80 ± 0.01^{b}	8.93 ± 0.01^{b}	0.54 ± 0.01^{b}	1.20 ± 0.05^{a}	0.18 ± 0.01^{c}	6.32 ± 0.01^{b}
L1	30.84 ± 0.82^{d}	1.78 ± 0.01^{c}	8.93 ± 0.01^{b}	0.54 ± 0.01^{b}	1.19 ± 0.04^{a}	0.20 ± 0.01^{bc}	6.29 ± 0.01^{c}
C	30.35 ± 0.21^{d}	1.76 ± 0.01^{d}	8.97 ± 0.02^{a}	0.58 ± 0.02^{a}	1.18 ± 0.06^{a}	0.15 ± 0.01^{d}	6.36 ± 0.01^{a}

In each column, means with at least one common letter show no significant difference ($p \leq 0.05$).

C: Control sangak bread; 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.

According to the mean comparison results for the effect of different improver levels on moisture content (MC) of Sangak breads, it was found that AZ had the highest MC whereas the lowest MC was observed in C compared to the other treatments. In other words, the simultaneous effect of Ascorbic acid and ADA on MC was higher than their cumulative effect with Lactic acid. This is because ADA is an improving agent that absorbs moisture, and Ascorbic acid is a reducing agent that is convated to Dehydroascorbic acid in the presence of oxygen. This is the reason behind the increased MC of the product (Movahed, 2012). The ash content of Z2 samples was highest, and C samples had the lowest ash content and all of the treatments had allow significant differences with to gethers ($p \leq 0.05$). ADA (NCONH2) is an oxidative improver that can increase the ash content. In terms of functional and physicochemical properties, ADA can enhance dough proofing, density, and the texture of the final product. ADA required amount, as a dough improving agent, is a function of flour type, ash content, gluten content, gluten quality, dough preparation conditions, mixing method, and mixing time (Svec and Hruskova, 2004). In general, the individual or collective use of ADA, Ascorbic acid, and Lactic acid led to an increase in ash content of all treated breads compared to the control. The results were consistent with those reported by (Movahed, 2012) that suggested improving agents (*e.g.* gums, sourdough, and yeasts), either individually or collectively, can increase MC and ash content, improve dough rheology, and reduce staling of treated samples compared to the control. The protein content of control was the highest, and AZ, LA and LZ treatments, followed by A2, Z2 and L2 with no significant difference, had the lowest protein content than other treatments. Moreover, there was a significant difference between these treatments and C, L1, Z1, and A1 ($p \leq 0.05$). In other words, application of 0.5% wt. improving agent (individually or collectively) can reduce protein content when compared to the 0.25% level. The lower protein content of treated samples was due to the fact that a portion of flour weight was replaced with these compounds, which in turn reduced the protein content of treated breads by reducing the gluten content of their dough. The results were consistent with Bechtel *et al.*,(1954) that studied breads with different (weight based) levels of starch as a replacement. It was found that, by increasing the starch level (wt. %), the protein content of the breads was reduced and their staling was also increased. The results showed that the C treatment had the highest fat content, and A2, L2 and Z2 (with no significant difference) had the lowest score in this parameter, followed by LA, LZ and AZ (with no significant difference). These treatments had a significant difference with the control ($p \leq 0.05$). In other words, application of 0.05% Lactic acid, Ascorbic acid and ADA separately and then collectively reduced fat content of Sangak treatments compared to the control. This is because these improving agents work synergistically when used in their maximum concentration alone or in combination with each other and act as a fat-absorption inhibitor and a fat substitution, which reduced the fat content of treated breads compared to the control (Svec and Hruskova, 2004). The fiber content of Sangak breads had no difference between different treatments and control ($p \leq 0.05$). The applied improving agents had no effect on the fiber content of Sangak breads. The results were not in agreement with (Movahed, 2012) that studied the effect of gram flour on the qualitative and quantitative properties of oil and sponge cakes. The chemical assays showed that the ash and moisture contents of both cakes were increased but the results showed negligible changes in fiber content.The acidity of L2 samples was highest followed by A2 and Z2, whereas C samples had the lowest acidity compared to other treatments. These treatments had a significant difference with the control ($p \leq 0.05$). This means that Lactic acid led to the highest rise in acidity levels from control levels. The next highest increases in acidity were seen in 0.5% Ascorbic acid and 0.5% ADA treatments—*i.e.*Sangak breads with 0.5% Lactic acid had the highest acidity than other treatments. Lactic acid has free carboxyl groups that raise acidity in breads. Lactic acid is a desirable acid that induces a good flavor in fermentation products such as Sangak bread and at the same time, it is also produced during fermentation that further increases H^+, reduce pH, and increase the acidity. It also causes reduced microbial load, better flavor, reduced staling, baker's yeast growth, and increased product size (Hruskova and Novotna, 2018). The highest pH was recorded in control samples, and L2 had the lowest pH followed by A2 and Z2. These treatments also had significant differences with each other and other treatments ($p \leq 0.05$). The addition of 0.5% Lactic acid, 0.5% Ascorbic acid and 0.5% ADA had the highest effect in reducing pH, respectively. This is due to the presence of lactic acid with a lower pH domain than other applied improving agents. The result of fermentation is also lactic acid and acetic acid, which in turn further increase H^+ and reduce pH in bread samples treated with Lactic acid than other treatments. This means that Sangak breads with maximum level of Lactic acid had the highest pH than other applied treatments. Lactic acid has free carboxyl groups that reduces the pH in breads. Lactic acid is a desirable acid that induces a good flavor in fermentation products such as Sangak bread. It also reduces the pH, microbial load, staling, and baker's yeast growth, and improves flavor and volume through its H^+ content (Movahed, 2017).

- *Color analysis*

According to the means comparison results presented in Table 3, the improving agents (Lactic acid, Ascorbic acid and ADA) increased *L** color parameter of treated Sangak bread samples ay compared to the control.

Table 3. Mean comparison results of data from color analysis of Sangak bread samples

Treatment	*L**	*a**	*b**
AZ	68.05±0.05[a]	-0.59±0.06[e]	11.38±0.05[h]
LA	67.19±0.07[b]	-0.31±0.03[d]	11.44±0.05[h]
LZ	67.07±0.07[b]	0.12±0.05[c]	11.87±0.07[g]
A2	66.91±0.08[c]	0.12±0.05[c]	12.12±0.05[f]
Z2	66.77±0.10[cd]	0.16±0.05[c]	12.20±0.05[f]
L2	66.68±0.08[d]	0.16±0.05[c]	12.49±0.05[e]
A1	66.65±0.08[d]	0.35±0.04[b]	12.81±0.05[d]
Z1	66.61±0.09[d]	0.54±0.05[a]	13.08±0.08[c]
L1	66.37±0.05[e]	0.56±0.05[a]	13.49±0.05[b]
C	66.30±0.05[e]	0.63±0.05[a]	14.18±0.08[a]

In each column, means with at least one common letter show no significant difference ($p \leq 0.05$).

C: Control sangak bread; 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.

LA, AZ and LZ had the highest *L**, and C showed the lowest value in this parameter. The mentioned treatments also had significant differences with other treatments ($p \leq 0.05$). In other words, *L** showed a declining trend from L1, Z1, A1, L2, Z2, A2, LZ to LA. This shows that the collective use of improvers had a better effect on *L** than their separate application. This is because these compounds allow the complete dough proofing and increase the rate of browning reactions during baking. The combined application of A and Z (0.25%) had a larger effect on *L**. The bread crust undergone color changes during baking, which were mainly related to the Maillard and caramelization reactions. The improving agents enhanced these reactions giving a brown–gold color to the crust and increased its brightness *L** (Lazaridou*et al.*, 2007; Movahhed*et al.*, 2014). The levels of *a** and *b** in treated Sangak samples was reduced. This is related to the crust color changes during baking that, as a result of Maillard and caramelization reactions, brighter colors are developed on the crust, and red and yellow (*a** and *b**) are reduced (Lazaridou*et al.*, 2007). The results were consistent with Jahandideh *et al.*,(2015) that suggested improvers, acids and yeasts can increase *L** and reduce *a** and *b**.

Conclusion

The results of chemical assays on Sangak breads treated with Lactic acid, Ascorbic acid and Azodicarbonamide (ADA) revealed that the moisture content was highest in AZ (0.25% Ascorbic acid and 0.25% ADA), ash content in Z2 (0.5% ADA), and acidity in L2 (0.5% Lactic acid) whereas C samples had the lowest levels in all three parameters.In addition, the protein content, fat content and pH showed their highest values in C samples whereas their minimum values were in AZ.In terms of *L**, the AZ treatment had the highest and C had the lowest scores. However, the results were opposite for *a** and *b** parameters (C highest and AZ lowest). 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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