Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Improving bulky Bread Quality with the Application of a Thermostable Amylase from *Bacillus safensis strain MT501806.1*

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ABSTRACT

Amylase improves the texture and sensory properties of bulky bread by degrading starch and producing dextrin in order to faster metabolism by bakery yeast. This study investigates the effect of thermostable α-Amylase 0, 1.9, 2.9 (U/ml), extracted from *Bacillus safensis*, and fermentation time at 35, 40 and 45 minutes on the quality of bulky bread baked in oven at 210°C for 20 min. The results of our study showed that adding filtered soup containing 1.9 (U/ml) and fermentation for 40 minutes was more acceptable than other samples in terms of volume, hardness, cohesiveness and overall acceptance, but adding more amounts of amylase enzyme at 2.9 (U/ml) level did not yield good results in terms of texture and sensory properties of bulky bread.

ARTICLE INFO

Article History:

Received:2023/5/5
Accepted:2023/7/11

Keywords:

Amylase,
Bulky Bread,
Texture,
Sensory properties

DOI: 10.22034/FSCT.20.138.41
DOR:20.1001.1.20088787.1402.20.138.4.6

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1- Introduction

Bread in various forms is one of the main food items consumed by humansAnd achieving its desired quality is a function of interconnected variables that must be designed and modeled in a cycle and as a system.. Humans have used wheat and other grains to produce bread since several thousand years ago. Baking bulky breads was first done by the ancient Egyptians, which is considered one of the oldest biotechnology processes. The use of enzymes in the preparation of bread also dates back to the same times. The first bakers unconsciously used enzymes found in grains or produced by yeasts and other microorganisms.With the increase of human knowledge, changes were made during mixing, fermentation and cooking of enzymes, which led to the introduction of new enzymes.Enzymes are one of the additives that are placed in the food category under the group of improvers. The use of these materials, in addition to improving the quality of the product, is also economical.Improvers are compounds that improve the quality of the final product by creating flexibility and increasing the endurance of the dough in all stages of bread production, including mixing, fermentation and baking, and help bakers to create a product with a better volume and shape and with the ability to maintain freshness. Produce more during the consumption period .Therefore, in recent years, the effect of additives in bread formulation in improving baking properties, organoleptic characteristics, nutritional value and increasing the shelf life has been highly considered [1, 2].On the other hand, in most regions, due to the type of soil and weather conditions, Iranian wheat does not have the right quality to produce flour suitable for bread, and because the control of weather conditions and other factors affecting the quality of wheat is difficult and often out of control. In many cases, the quality of the obtained flour is not very acceptable, and especially in the production of bread, deficiencies are observed. Among the problems of Iranian wheat for bread production is the inappropriate amylolytic enzyme activity of wheat flour, which is a problem Including the short-term shelf life of bread, production There is a high volume of waste and the creation of many economic costs [3].Adding amylase family enzymes to flour or bread dough is considered as a suitable solution. On the other handBreads made from wholemeal flour, made with yeast and without baking soda, and cooked with gentle, uniform heat and indirect flame, are considered the healthiest breads. The Ministry of Health, Medicine and Medical Education has banned the use of baking soda in bakery units due to the emergence of gastrointestinal complications and impaired absorption of salts such as iron. But unfortunately, with the growth of the population and the increase in the demand for bread consumption in the society, the need for industrial production of bread increased, as a result of which we see the use of chemical agents such as baking soda instead of baker's yeast and natural improvers such as enzymes. Since in an industrial bread factory, fermentation must be carried out for a long time to produce bulky bread, the addition of heat-resistant amylase enzyme, which is able to continue fermentation during baking, can produce better quality bread [1, 4, 5]. In general, baking bread is very effective in the quality of bread, as it turns the dough into a light, porous, digestible and flavorful product under the influence of heat, so the most obvious changes during baking can include volume expansion, crust formation, yeast activation and enzyme activities. protein cross-linking and starch gelatinization. Although enzymes such as amylase have been used in the preparation of bread for

some time, it should be noted that the more alpha-amylase has resistance to heat, the more starch it will break during baking. The results have shown that the amylase extracted from bacterial sources has good heat resistance and if it is used in bread, the enzyme can remain active after baking and makes the core of the bread remain soft and tender during storage. stay [1, 3, 6]. In order to improve the quality of Hajim, in this study, the effect of adding different concentrations of heat-resistant amylase produced by*Bacillus safensis* And we paid attention to the quality of the resulting bulky bread during fermentation.

2- Materials and methods

1-2-Preparation of heat-resistant amylase enzyme

From the previous study of thermostable amylase by bacterial isolate*Bacillus safensis* heat-resistant was isolated from the low-fat mayonnaise production line effluent of a canning factory.

2-2- Preparation of bread containing amylase

Bulky bread dough ingredients include white wheat flour (Zar Macaroon, Iran) 100 grams, salt 2 grams, active dry baker's yeast 2 grams, water 65 grams, suspension containing amylase filtered through 0.45 micrometer head filters and without filter on three levels. The activity was 0, 1.9 and 2.9 units per kilogram of flour. Enzyme activities based on previous study based on absorbance method and reagentDNS Obtained. All the components were mixed in a container for 20 minutes, then the resulting dough was kept for 45 minutes at 35 degrees Celsius, then the dough was divided into 70 grams of limes and stored at 35 degrees Celsius for 35, 40 and 45 minutes in a fermentation cabinet. became. Finally, the bulky bread was baked in the oven at a temperature of 210 degrees Celsius for 20 minutes [7].

2-3- Investigating the textural properties of bread

The properties of bread, including specific volume and height-to-width ratio, were measured with a caliper (marked ruler) after one hour of baking time, and the texture of the bread was checked with a texture analyzer [8].. from testTPA It was used to determine the texture of bread, this test is considered as a chewing simulator. In this way, the probe of the device is lowered and compresses the tissue to some extent. Then the probe goes up and repeats this work again, finally the graph of the force recorded in both times of lowering is recorded. To perform tissue analysis, first, the samples are cut using a knife, then the samples are placed under the probe of the device. The dimensions of the probe must be the same and clear for all samples so that the results can be compared under equal conditions. Also, the number of times the probe goes back and forth for the test was chosen as 2, and the speed of the probe was determined within the range of 1 mm/s. In each test, two slices in the middle of the bread were used to eliminate the effect of the sides of the bread. The indicators that were calculated in this test include stiffness (Hardness), consistency and elasticity (Cohesiveness), the power of the sample in reconstructing its structure (Springiness), chewing power of semi-solid sample (Gumminess), chewing power of solid sample (Chewiness), apparent modulus (Apparent modulus).

4-2- Examining the sensory properties of bread

After the preliminary training on the sensory evaluation method, 12 evaluators were selected. Then, using the hedonic test, 5 points of sensory properties including smell, taste, color, texture and general acceptance of the produced breads were checked and only overall acceptance was reported from very unfavorable (1) to very favorable (5).

5-2-Statistical analysis

addition effectFiltered and unfiltered amylase enzymein three activity levels of 0, 1.9 and 2.9 units per milliliter and fermentation timeon three levels35, 40 and 45 minutes on the sensory characteristics and texture of bread based on factorial statistical method and completely random statistical design and in three replications. Data variance analysis with softwareSPSS done. In case of significance, the comparison of means was checked based on Duncan's test at the probability level of 5%.

3-Results

3-1- Measuring specific volume of bread

In this volume studyspecialBread after baking It was measured with a caliper (Table 1). The results showedBreads grown from filtered microbial suspension*Bacillus sepensis* containamylaseIn three levels of activity(U/ml) 0, 1/9 and 2/9 and fermentation timeon three levels35, 40 and 45 minutes, was prepared compared to bulky breads without amylase enzyme in fermentation times of 35, 40 and 45 minutes were prepared, they had better quality and the addition of amylase enzymeon volume improvement which is one of the most important and significant factors in determining the quality of bulky breads has had an impact. The best volume of samples(U/ml) 2.9 filtered enzymes were obtained after 45 minutes of fermentation.

Table 1. Parameters of Specific Volume of Bulk Bread in different formulations affected by Amylase Activity

2-3- Evaluation of bread texture by test TPA

Weight	to rise	an offer	the length	parameters
g	mm			Unit
58.2	4.8	4.5	12.9	2.9Filter-45Min
58.0	4.8	4.5	12.7	2.9Filter-40Min
57.9	4.5	4.5	12.7	2.9Filter-35Min
55.9	4.4	4.1	12.5	2.9-45Min
55.7	4.2	4.0	12.2	2.9-40Min
55.6	4.2	3.9	12.2	2.9-35Min
57.4	3.6	3.8	11.8	1.9Filter-45Min
57.3	3.5	3.8	11.6	1.9Filter-40Min
57.0	3.5	3.5	11.6	1.9Filter-35 Min
56.9	3.4	3.5	11.3	1.9-45 Min
56.5	3.4	2.8	11.3	1.9-40 Min
56.3	3.2	2.8	11.3	1.9-35 Min
55.7	3.0	2.5	10.8	0-45 Min
55.7	3.0	2.5	10.8	0-40 Min
55.5	3.0	2.3	10.5	0-35 Min

The results showedBreads withamylaseIn three levels of activity(U/ml) 0, 1.9 and 2.9 and fermentation time 35, 40 and 45 minutes were prepared compared to bulky breads without amylase enzymein fermentation times of 35, 40 and 45 minutes were prepared, they had differences that include the following:

3-2-1-hardness of bread

Due to the The results of analysis of variance of the effect of fermentation time and different levels of amylase enzyme activity on the hardness of bulk bread at the 99% confidence levelshowed a significant difference.By increasing the fermentation time up to 40 minutes, the hardness of the bread decreased in all treatments, but after this period up to 45 minutes, the hardness level only in the samples containingAmylase enzymeIncreased. among which the highest difficulty is related to the sample containing enzyme filtered soup(U/ml) 2.9 after 45 minutes and the least hardness to the sample containing enzyme filtered soup(U/ml) It was 1.9 after 40 minutes, which can be seen in Figure 1, the numbers of each column represent the average of each parameter and the error bar is displayed on each column.

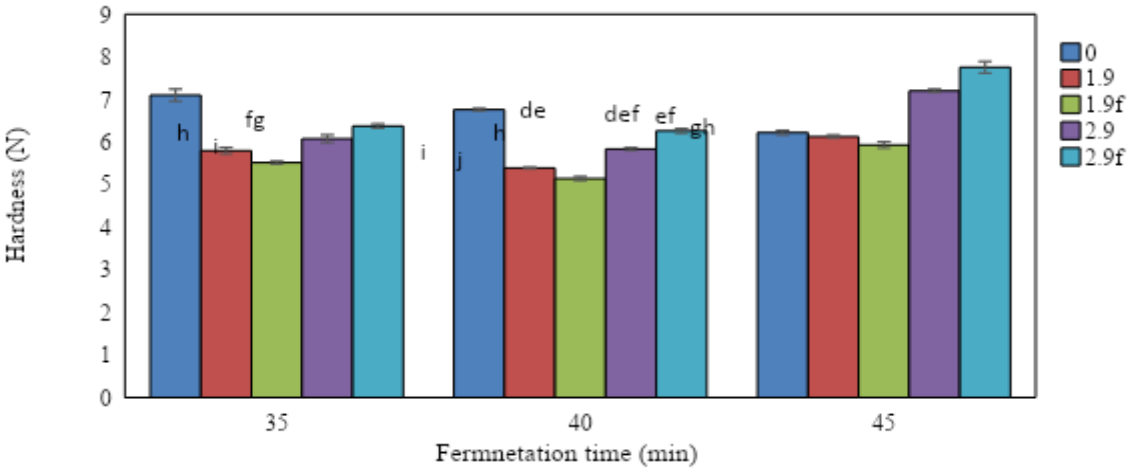

Fig 1. The effect of fermentation time and enzyme activity level on the hardness of bulk breads containing filtered (f) and unfiltered amylase soup at three levels of enzyme activity 0, 1.9 and 2.9 (Unit/ml) and three fermentation times of 35, 40 and 45 min. The non-identical letters indicate a significant difference between samples.

2-2-3- Consistency and elasticityBread

Due to the The results of analysis of variance of the effect of fermentation time and different levels of amylase enzyme activity on consistency and elasticity of bulk breadIt shows a significant difference. By increasing the fermentation time up to 40 minutesConsistency and elasticityBread in samples containing enzymes(U/ml) 9/1 and 9/2The amount increased slightly, but after this period up to 45 minutesConsistency and elasticityIt did not show a noticeable change. among which the most Consistency and elasticityRelated to the sample containing enzyme filtered soup(U/ml) 1/9 with 40 minutes of fermentation and minimumConsistency and elasticityTo the sample containing enzyme filtered soup(U/ml) 2.9 belonged to 45 minutes of fermentation (Figure 2).

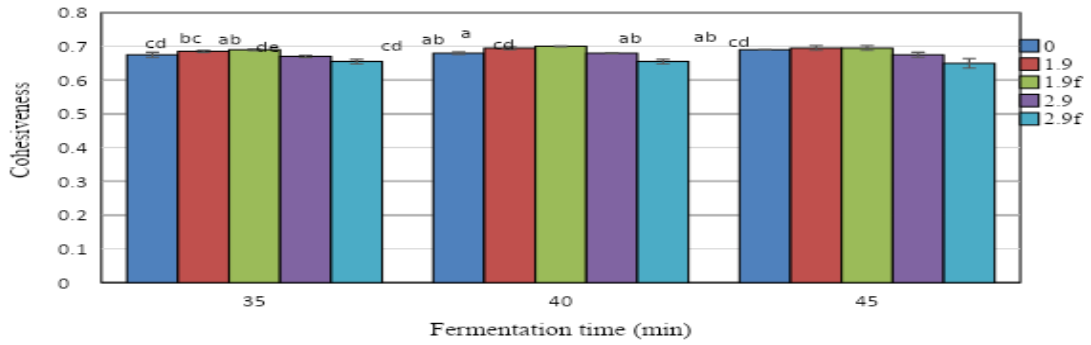

Fig 2. The effect of fermentation time and enzyme activity level on the cohesiveness of bulk breads containing filtered (f) and unfiltered amylase soup at three levels of enzyme activity 0, 1.9 and 2.9 (Unit/ml) and three fermentation times of 35, 40 and 45 min. The non-identical letters indicate a significant difference between samples.

3-2-3- The strength of the sample in rebuilding the structure after removing the force

Due to the The results of analysis of variance of the effect of fermentation time and different levels of amylase enzyme activity on the characteristics of bulk bread at the 99% confidence level.showed a significant difference.By increasing the fermentation time up to 40 minutesPhenrite featureBread increased in all treatments, but after this period up to 45 minutesProperty Phenrite in control samples and samples containing enzyme(U/ml) 9.1 increase in the sample containing the enzyme(U/ml) 2/9 decreased. among which the mostPhenrite featureRelated to the sample containing enzyme filtered soup(U/ml) 1/9 with 45 minutes of fermentation and minimumPhenrite featureTo the sample containing enzyme filtered soup(U/ml) 2.9 belonged to 45 minutes of fermentation.

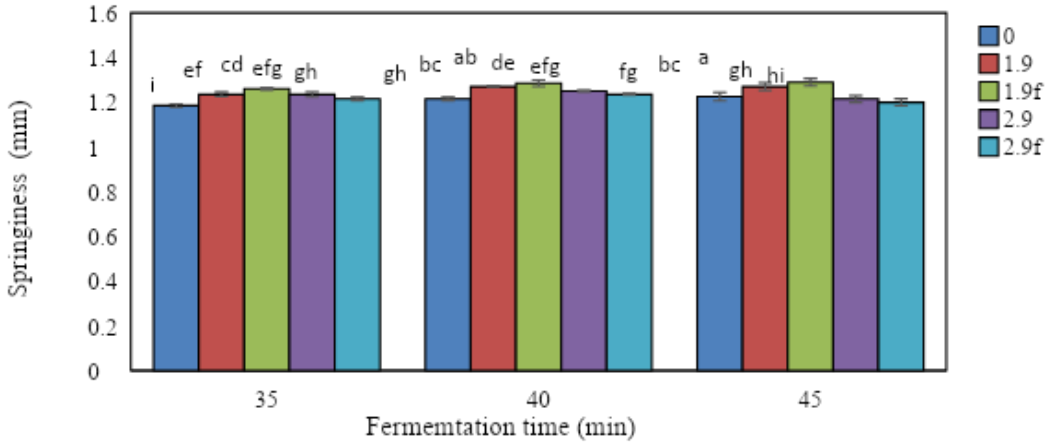

Fig 3. The effect of fermentation time and enzyme activity level on the springiness of bulk breads containing filtered (f) and unfiltered amylase soup at three levels of enzyme activity 0, 1.9 and 2.9 (Unit/ml) and three fermentation times of 35, 40 and 45 min. The non-identical letters indicate a significant difference between samples.

4-2-3-Chewing

Due to the The results of analysis of variance of the effect of fermentation time and different levels of amylase enzyme activity on the chewability of bulky bread at the 99% confidence levelshowed a significant difference.By increasing the fermentation time up to 40 minutesChewable featureBread decreased in all treatments, but after this period up to 45 minutesProperty Ability to chew It increased in enzyme-containing samples and decreased in control samples. among which the mostChewable featureRelated to the sample containing enzyme filtered soup(U/ml) 2/9 after 45 minutes and the lowestChewable featureFor example, enzyme filtered soup(U/ml) 1/9 belonged after 40 minutes.

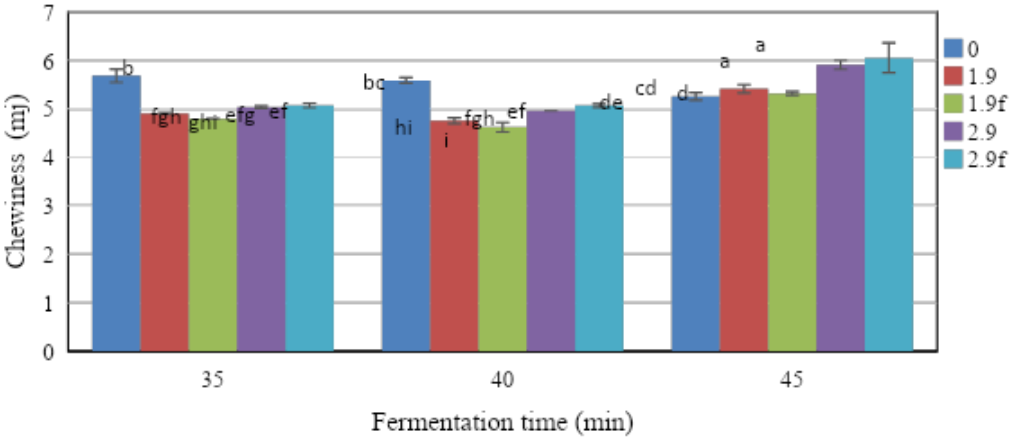

Fig 4. The effect of fermentation time and enzyme activity level on the chewiness of bulk breads containing filtered (f) and unfiltered amylase soup at three levels of enzyme activity 0, 1.9 and 2.9 (Unit/ml) and three fermentation times of 35, 40 and 45 min. The non-identical letters indicate a significant difference between samples.

3-3- Examining the sensory properties of bread

Descriptive sensory test was performed using a linear scale of 0 to 5, the number 0 for the minimum and the number 5 for the maximum intensity of an attribute. Here, only the results of the comparison of the overall acceptance of the samples are displayed as a diagram in Figure 5. The results of sensory evaluation showed that by addingAmylase enzyme to the formulation of bulky bread, the overall acceptance score of the bread increased, but in a higher doseU/ml 9/2 This scoredecreases so that samples containing amylaseIN/ml 9/1 They got the most points among the evaluators.In general, in terms of overall acceptance, there is a significant difference bYto n sampleYmade from Shahid dough with tYSnakes containing anzYM amylase was observed.

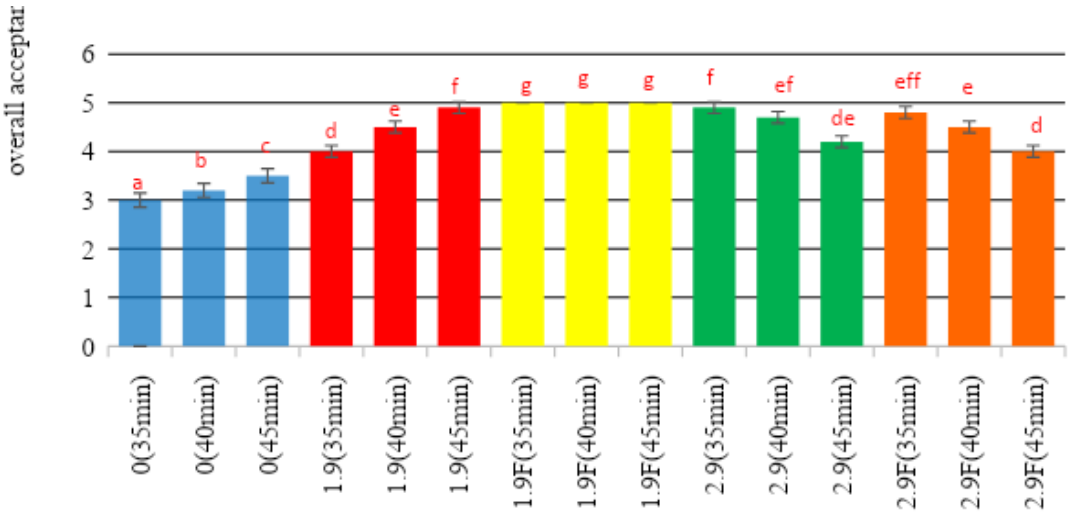

Fig 5. The effect of fermentation time and enzyme activity level on overall acceptance of bulk breads containing filtered (f) and unfiltered amylase soup at three levels of enzyme activity 0, 1.9 and 2.9 (Unit/ml) and three fermentation times of 35, 40 and 45 min. The non-identical letters indicate a significant difference between samples.

4- Discussion

Since most of the industrial processes are usually carried out at high temperatures, the use of thermophilic amylases of microbial origin can help to advance these processes as much as possible due to their ability to maintain their activity and structure at high temperatures.. Today, the most useful application of amylases has been proven in various industries such as textiles, detergents, food processes and grain processing, especially bread.In bakery systems, enzymes are used as softeners, strengthening and increasing dough resistance to the fermentation process and anti-staleness agents. This enables bakers to remove undesirable chemical improvers and produce products labeled chemical free by replacing enzymes. Alpha-amylases occur naturally in wheat, but their natural levels are usually too low and variable to produce optimal bread. For this reason, in this research from addingAnzYAmylase has been used to produce bulk bread with good quality. In the year1387 , naYOr originY et al., ThothYR. AnzYespecially amylaseYet rheologyYKY bendYThey made a special volume of bread and concluded that the addition of amylase increased the specific volume of bread, but it did not have a favorable effect on the rheological characteristics of the dough [9].As for FYZY Pour et alYafter adding itYcompared to the control sample and found that the volume of the sample increasedYShYIt is consistent with the results of our research [10]. heartYL AfzaYThe volume of the samples, after the addition of anzYM alpha-amylase, tolYGas DioxideYCarbon bYcamel mYbe In our study, the positive effect of amylase enzyme on the texture of bread was shown, the reason for the improvement of the texture of bulky breads under the influence of alpha-amylase enzyme is the breaking down of starch and the increase of fermentable monosaccharides by yeasts, and as a result, the number of voids in the bread.Breads with high porosity increase in volume and the bread has a softer and more desirable texture. Mahdavian et al., 2013, in evaluating the results of bread tissue porosity, stated that the increase in porosity can be attributed to the addition of amylase enzyme and its effect in creating a more uniform size of gas cavities, and on the other hand, the increase in the number of these cells per unit area with a smaller average cell size [11]

protrudeYesThe result of comparing the average of samples by Duncan's method showed that the addition of anzI am Adding amylase to the dough increases the consistency, elasticity and volume of the bulky bread and reduces the stiffness of the bread, but it did not have much effect on the regeneration power of the samples. Of course, with an increase in the doseI am Amylase, firmness is increased. Samples containing unfiltered amylase soup containing bacteria producing this enzyme ie*Bacillus saphinensis* with its amylolytic and proteolytic activities, during fermentation and early cooking, it can break down the starch and protein structure of bread and cause softness and decrease the hardness of bread compared to samples containing filtered enzyme soup. Several studies of amylolytic and proteolytic activities of the genus*Bacillus* have reported [12, 13]. The comparison between samples containing amylase with the control shows that the control sample with a fermentation time of 40 minutes has a higher hardness than the treatments and with increasing the fermentation time up to 45 minutes, this hardness increases in the treatments. It is obvious that in the early stages of fermentation, due to the breakdown of starch by amylase in the treatments, we expect a decrease in the stiffness of the bread due to a greater loss of cohesion compared to the control, but the increase in this stiffness with increasing fermentation time can be attributed to the retrogradation of amylose molecules, which The event after baking can also continue.Chen et al. (2021) showed that breads containing amylase have a slight hardness compared to control bread. Because after baking, the amylose molecules in fresh bread start to rearrange and retrograde during the cooling time of the bread.The rapid crystallization of amylose is believed to be responsible for the hardness of the initial bread structure. This result is strongly related to the final viscosity of the bread. They showed that the hardness of bread containing amylase increases at a faster rate from day 1 to day 4 of storage and at a slower rate from day 4 to day 14, and they stated that the increase in hardness in the early stages of storage of bread containing amylase can probably be due to the retrogradation kinetics of amylopectin. be[14, 15]. In general, samples that have less stiffness, the bread has a higher volume, which means that increasing the volume causes Bread production has become smoother. In breads that are less firm, the water in the diffusion system is less, and this increases water absorption and maintains the moisture of the final product during the baking and storage process, and as a result, staleness is delayed. In the results of this research, it was found that in the bulk breads in which amylase enzyme was used, they have more volume and the hardness of the bread is less, but as mentioned before, by adding more amounts of amylase enzyme, the hardness of the bread increases and the chewability decreases. The results showed that by reducing the hardness of the bread, the sensory score obtained for the texture was higher, which means that by reducing the hardness, a more desirable and softer bread was obtained and it had a higher score among the consumers. In the comparative study of sensory evaluation methods and instrumental measurement of bread texture, it was shown that the instrumental method of texture measurement can well predict the sensory characteristics of bread core tissue such as softness, elasticity, chewiness and firmness. As a result, evaluation of bread texture using a device method can replace a part of the sensory evaluation method in order to evaluate the quality of the texture of the product [16].Since an increase in hardness can lead to a decrease in elasticity, therefore, according to the results of the hardness section and the reasons explained

for hardness changes under the influence of enzyme values, these results from the elasticity examination were expected. If we check the changes in the tissue in 45 minutes of fermentation time, which was enough time to perform enzymatic reactions, it is better to compare the changes made in the samples. On the other hand, an increase in hardness can lead to a decreaseThe strength of the sample in the reconstruction of the structure after removing the force Therefore, according to the results of the hardness section and the reasons that were explained for the hardness changes under the influence of enzyme amounts, these results are obtained from the investigationReconstruction of the structureIt was to be expected. Because microbial proteolytic and amylolytic activity in addition to amylase activity in samples containing unfiltered enzymatic soup leads to the breakdown of the structure and the loss of cohesion of the sample tissue compared to the filtered sample, and as a result, the strength of the sample structure reconstruction after removing the force is reduced. In the samples with higher amylase activity, due to the phenomenon of starch retrogradation, compared to the sample with lower amylase levels, which was mentioned earlier, we see more stiffness and as a result, a decrease in the strength of the structure of the sample after removing the force. Chewiness To*The energy required to chew a solid food is called readiness to swallow*[17]. Kim et al. (2020) showed that by adding amylase to the dough, the resulting breadHardness AndChewiness Less and vice versaCohesiveness More than the control sample lacks amylase[18] Similar results were obtained in our study. Several studies have investigated the effects of amylase on bread texture, which reported results almost similar to ours [15, 19].Examining the chewability of bread shows that the addition of amylase improves this feature. The reason for this problem is the hydrolysis of starch, which has caused more gas production by yeasts, and finally, due to the increase in the number of gas bubbles in the dough and the increase in surface area per unit volume, the core of the bread has softened, and as a result, the ability to chew the bread due to the use of this enzyme. has improved The results of our study are consistent with other similar studies, with the difference that if we increase the amount of amylase enzyme to 2.9 units, the bread becomes hard and the chewability decreases. There was a significant difference between the control sample and the treatments containing amylase enzyme in terms of bread aroma. The reason for this difference is the hydrolysis of starch polysaccharide under the influence of amylase enzyme and its conversion into mono and disaccharides, which causes the Maillard reaction to intensify during bread baking and the aroma of bread to increase. The result of the research done by previous researchers was consistent [20].The taste testers agreed that adding amylase enzyme to the dough improvesYlifeYThis is a feelingand texture Bulky bread, including size, crust color, flavor, smell, and shape of breadYbut Breads that have more amounts than thisDue to the higher amylase activity, the enzyme produces more gas, which emits and creates with increasing temperatureIt cracks on the surface and creates an asymmetric appearance. These cracks probably cause heat to penetrate into the inner parts and cook the internal tissue more and as a resultThe hardness of the bread increases and its chewiness decreases.As the results showed by addingAmylase enzyme to the formulation of bulky bread, the overall acceptance score of the bread increased, but in a higher doseU/ml9/2 Due to the negative effects of high amylase activity, including on texture (chew), color (darkness resulting

from Maillard's browning reaction and carmelization [21] more of the skin due to more sugar), this scoredecreases so that samples containing amylaseU/ml9/1 They got the most points among the evaluators.

5- General conclusion

Due to the growing demand for natural bread improvers such as amylases, the aim of this study was to use heat-resistant amylase to improve the quality of bulk bread.In this context The degree of resistance of amylase to heat is very important, amylase in dough attacks the damaged starch granules and breaks the starch molecules. By increasing the temperature, especially during cooking, swelling and gelatinization of starch, more amount of substrate will be prepared for the enzyme to work, and the enzyme will work faster and more intensively with increasing temperature. In this way, with the simultaneous formation of a flexible gluten network, gas retention is improved, which can be considered a positive aspect of the reaction, but on the other hand, the formation of a significant amount of dextrin, which has a sticky state, can be considered as a negative aspect. Therefore, excessive use of amylase is undesirable due to these changes in the bread texture.The results of our study showed that the addition of filtered soup containing heat-resistant amylase on the surface (U/ml) 1.9 and fermentation for 40 minutesCompared to other samples, it was more acceptable in terms of parameters such as volume, softness, elasticity and chewing ability, and overall acceptance, but by adding more amounts of amylase enzyme on the surface (U/ml)2.9 In terms of textural and sensory properties, good results were not obtained.

5- Resources

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مقاله علمی_پژوهشی

بهبود کیفیت نان حجیم با بکارگیری آمیلاز مقاوم به حرارت تولید شده از *Bacillus safensis strain MT501806.1*

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۲/۱۵

تاریخ پذیرش: ۱۴۰۲/۴/۲۰

کلمات کلیدی:

آمیلاز،

نان حجیم،

بافت،

خواص حسی.

DOI: 10.22034/FSCT.20.138.41

DOR:20.1001.1.20088787.1402.20.138.4.6

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چکیده

آمیلاز با تجزیه نشاسته آرد و تولید دکسترین با قابلیت متابولیسم سریع‌تر توسط مخمر نانوایی باعث بهبود بافت و خواص حسی نان حجیم می‌شود. این مطالعه به بررسی اثر آمیلاز مقاوم به حرارت استخراج شده از *Bacillus safensis* در سه سطح (U/ml) ۰، ۱/۹، ۲/۹ و مدت زمان تخمیر در سه سطح ۳۵، ۴۰ و ۴۵ دقیقه بر کیفیت نان حجیم که در فر با دمای ۲۱۰ درجه سانتی-گراد به مدت ۲۰ دقیقه پخته شده بود می‌پردازد. نتایج مطالعه ما نشان داد که افزودن سوپ فیلتر شده حاوی آمیلاز مقاوم به حرارت در سطح (U/ml) ۱,۹ و تخمیر به مدت ۴۰ دقیقه نسبت به سایر نمونه‌ها از نظر پارامترهایی از قبیل میزان حجم، نرمی، قابلیت ارتجاع و پذیرش کلی دارای مقبولیت بیشتری بود اما با افزودن مقادیر بیشتر آنزیم آمیلاز در سطح (U/ml) ۲,۹ از نظر خواص بافتی و حسی نتایج خوبی حاصل نشد.