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Evaluation of textural, sensorial and shelf-life characteristics of bread produced with mung bean sourdough and saffron petal extract

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ABSTRACT

The use of controlled fermented legumes along with the aqueous extract of aromatic plants is important in terms of improving the quality characteristics of wheat bread. In the present study, after spontaneous fermentation of mung bean sourdough containing saffron petal extract, the predominant lactic acid bacteria (LAB) isolate was used as a starter culture in controlled fermented sourdough, and then the characteristics of the produced wheat breads in terms of texture, surface expansion of fungi and overall acceptability were investigated. Sequencing results of PCR products led to the identification of *Levilactobacillus brevis* as the predominant LAB isolated from sourdough. Processing of wheat bread with mung bean sourdough containing mentioned bacteria and saffron petal extract not only improved the textural features and overall acceptability of the produced bread, but also increased their resistance towards the surface expansion of *Aspergillus niger*. Based on the results, wheat bread containing fermented mung bean and saffron petal extract showed the best textural features compared to other samples with crumb hardness of 10.21 N and porosity of 16.16%. Furthermore, the mentioned sample with 44.33% inhibition had the highest ability to inhibit the growth of the indicator fungus compared to other samples. Although the use of mung bean and saffron petal extract alone in the formulation of wheat bread did not show a great effect on reducing the surface expansion of fungi, but their combined application was significantly (P<0.05) effective in reducing the surface growth of fungus. Due to the appropriate techno-functional capabilities of controlled fermented mung bean sourdough containing saffron petal extract, it can be used as a natural improver in the bakery industries.

1. Introduction

Bread is one of the most important food products used by humans, which is considered the main component of many diets due to its nutritional properties and low price. Therefore, bread plays an essential role in feeding and providing daily energy to people in many parts of the world [1]. In recent years, there has been a lot of interest in using legumes in wheat bread formulations as an enrichment method because bread produced from grains such as wheat lacks some essential amino acids. Therefore, the use of a mixture of wheat flour and legumes can improve the composition of protein as well as the content of bioactive compounds in bakery products [2]. On the other hand, adding legumes weakens the gluten network and reduces the baking ability of wheat flour. Therefore, to solve this problem, sourdough fermentation (mixture of water and flour) is used. Sourdough fermentation delays the process of bread becoming stale and improves the characteristics of the dough, the texture of the bread, as well as its flavor [3]. In addition, fermentation can destroy some anti-nutritional compounds such as phytic acid and trypsin inhibitors [4]. The use of aromatic plants can also greatly affect the flavor and aroma of baked products [5]. Therefore, the combination of leguminous sourdough and aqueous extract of aromatic plants can be a suitable option for improving the sensory and technological characteristics of bakery products.

One of the widely used legumes is mung bean (Vigna radiata L; from the Fabaceae family), which has 20-25% protein and large amounts of essential amino acids such as phenylalanine, leucine, isoleucine, valine, tryptophan, arginine, methionine and lysine [6]. Saffron is also an autumn flowering herbaceous plant of the family Iridaceae [7], whose petals are produced in large quantities as a by-product, but are discarded after harvesting. Since saffron petals are cheaper and produced in large quantities compared to saffron stigmas, it can be used as a suitable source for bioactive compounds. Phytochemical studies have confirmed the presence of flavonoids and anthocyanins in saffron petals [8]. Various medicinal properties of saffron petals including its role as an immune system modifier, anti-diabetic and anti-hypertensive have also been proven [9]. According to these features, saffron petals can be used to improve the health of food consumers.

Until now, the effect of controlled fermentation of whole wheat flour [10], oak flour [11], oat flour [12], cereal bran flour [13] and quinoa flour [14] containing the dominant lactic acid bacteria (LAB) isolate of sourdough as starter culture has been investigated with the aim of improving quality characteristics and mold control in bread. Research has also been reported on the use of legumes, fermented legumes and plant extracts in bread processing. For example, González-Montemayor et al. (2021) studied the nutritional and functional properties of chickpea, green bean, and mesquite flour, as well as their effect on the characteristics of sourdough bread. These researchers reported that the mixture of legumes and wheat flour had an effect on the protein content, fiber and physical properties of the produced bread, but with the addition of mesquite to wheat bread, its specific volume decreased by 7% [15]. Coda et al. (2016) also investigated the effect of adding bean flour and leaven to wheat bread and reported that adding bean leaven to wheat bread increased the texture hardness of the produced bread compared to the control sample and the addition of bean flour. The specific volume of the sample containing sour bean paste was significantly lower than the other two samples. In addition, the control sample and the samples containing bean flour had no significant difference in terms of specific volume despite the difference in tissue hardness.

Also, Nionelli et al. (2018) used hop extract as an antifungal compound to prepare sourdough bread in a research. The aforementioned researchers reported that sourdough containing hop extract has more phenolic compounds and antioxidant activity than sourdough produced under similar conditions but without hop extract. In addition, sourdoughs containing hop extract completely delayed the growth of the fungus for 7 days and improved the specific volume and firmness of the bread texture compared to the control sample [17]. Debone et al. (2018) also investigated the antifungal activity of sourdough bread containing thyme essential oil on *Aspergillus niger* and *Penicillium paneum* in laboratory conditions. The aforementioned researchers found that the use of sourdough leads to a longer shelf life and an increase in the quality characteristics of bread, but despite the interesting antifungal effect of thyme essential oil in laboratory conditions, an increase in the shelf life of manufactured bread was not observed [18].

Based on the review of sources, there has been no study on the use of mung bean sourdough containing saffron petal extract in the processing of wheat bread. Therefore, the purpose of this research was to determine the optimal formulation for the production of clean label bread (without artificial additives) and to examine the textural characteristics, acceptability and shelf life of the produced bread.

2.Materials and Methods

Raw materials and their properties

First, the mung beans purchased from the local market were disinfected using 1.25% sodium hypochlorite, and to remove excess sodium hypochlorite, they were washed again with sterile distilled water and dried in an oven (Binder, Germany). Then, the mung bean seeds were turned into flour using a mill (BEST, Iran) and passed through a mesh sieve. Also, wheat flour used in this research was prepared from Zahedi flour factory in Golestan province. After preparing wheat flour and mung bean flour, their characteristics include moisture (AACC 16-44), ash (AACC 08-01), fat (AACC 10-30), protein (AACC 12-46) and carbohydrates as [(moisture + ash + fat + protein) – 100] was determined according to documented methods [19]. Wheat flour and mung bean flour used in this research had 12.4% and 24.3% protein, 0.4% and 1.9% ash, 14.3% and 11.2% moisture, 1.1% and 3.3% fat, as well as 71.8% and 61.3% total carbohydrate, respectively. The saffron petal extract was also prepared by the Greek method after the combination of saffron petal powder and boiled water (100 °C) after 6 hours and finally filtered [20]. Consumed culture media including MRS Broth, MRS Agar (Merck, Germany) and other reagents were prepared from reputable brands.

Spontaneous fermentation of sourdough, isolation and identification of dominant LAB

Sourdough fermentation was done randomly by mixing mung bean flour and water with a dough yield (dough to flour ratio) of 500 for 24 hours at 37 °C. After the completion of fermentation, successive tenfold dilutions of sourdough were prepared and surface cultures were prepared from each dilution. Then, to obtain a pure colony of dominant LAB, a streak culture was prepared from them. Also, tests such as catalase and Gram staining were used for the primary identification of dominant LAB isolates.

Then, the dominant LAB DNA was extracted using a commercial kit (Bioneer, AccuPrep K-3032, South Korea) and amplified by PCR with F44 and R1543 primers, and then the PCR products were sequenced (Bioneer, South Korea). In the next step, in order to identify the lactic isolate, the PCR products after sequencing using the Blast method were aligned with the data in the NCBI database [21].

Controlled fermentation of sourdough, determination of optimal formulation and preparation of bread

For the controlled fermentation of sourdough, a mixture of mung bean flour and water/saffron petal extract was used with a dough yield of 500 along with a LAB isolate with a population of 10^8 CFU/g. The mixture was then fermented for 24 hours at 37 °C [22]. To determine the optimal formulation of sourdough bread containing saffron petal extract, different proportions of saffron petal extract (5, 10, 15 and 25%) were used in mung bean dough formulation. Then, the overall acceptance rate of the produced breads was determined in comparison with the control sample. The control bread contained a mixture of water (60% water absorption), wheat flour and 2% by weight of active dry yeast containing *Saccharomyces cerevisiae*. This dough did not have a sourdough and the first stage of fermentation was done at 30 °C for 30 minutes and the final fermentation was done after dividing into 250 g pieces at the same temperature for 90 minutes. Then, the produced dough was baked at 220 ± 5 °C for 15 minutes in an electric oven (Feller, Germany) [23]. In sourdough breads, 20% of sourdough was added to the same dough as the control sample, and then it was processed under the same conditions as the control sample. In order to investigate the effect of fermentation, saffron petal extract and mung bean different samples were prepared. The produced samples include wheat bread (C) and wheat breads containing mung bean (M), fermented mung bean (FM), saffron petal extract (E) and fermented mung bean mixed with saffron petal extract (FM+E).

Evaluation of textural characteristics of manufactured breads

For this purpose, rectangular pieces with a thickness of 20 ± 2 mm were separated from the geometric center of the bread. Then, in order to determine the characteristics of crumb texture using a texture analyzer device (Stable Microsystem, UK), the force required to create 50% compression in the initial thickness was measured. The starting point was 50 g and the speed of the probe used in the compression test was equal to 1 mm/s [24].

Determining the crumb porosity of manufactured breads

In order to evaluate the crumb porosity of manufactured breads, the method of processing scanned images using Image J software version 1.52v was used. In this method, the ratio of light to dark points was processed and calculated as an index of the porosity of the samples [25].

Evaluation of fungal growth rate on the surface of manufactured breads

For this purpose, 5 μl of *A. niger* PTCC 5012 spores (10^6 spores/ml) were inoculated on a sterile paper disc in the center of the bread. Then, the mentioned samples were kept for 7 days at a temperature of 25 °C. Finally, the amount of fungal growth in manufactured breads compared to the control sample was evaluated on a daily basis [26].

Investigating the sensory characteristics of manufactured breads

The sensory characteristics of the produced breads were evaluated by trained evaluators and by scoring the characteristics of aroma, taste, chewing, mouth feel and overall acceptability of the bread using a five-point hedonic method from very bad to very good (score 1 to 5). Also, during the test, the evaluators were asked to rinse their mouth with water after testing each sample for more accurate evaluation [27].

Statistical analysis of results

The results of this research were statistically analyzed in the form of a completely randomized design in three replications using SPSS software version 26, and comparison of means was performed using Duncan's test at a significance level of $P<0.05$. Also, the graphs were drawn using Microsoft Office Excel 2019 software.

3.Results and Discussion

Isolation and identification of dominant LAB

Examining on the culture of sourdough LAB isolates showed that the dominant isolate was a Gram-positive, catalase-negative and rod-shaped bacterium. Amplification of the target sequence of 1500 bp from the ribosomal locus of the dominant LAB isolate genome was also confirmed based on the results of gel electrophoresis of the PCR products (Figure 1). Also, comparing the sequence of PCR products with the data available in the NCBI database using the BLASTn method led to the identification of *Levilactobacillus brevis* (with 97% similarity) as the dominant LAB isolate.

Harth et al. (2016) studied the predominant LAB isolates of spontaneous fermentation of barley sourdough under different fermentation conditions. Accordingly, in fermentation with a dough yield of 400 and after 10 consecutive days of fermentation at 30°C, *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *L. brevis* were identified as the dominant LAB isolates, while by reducing the temperature (17 to 22 °C) and using sourdough with a dough yield of 200, *L. brevis* was eliminated among the dominant species [28]. Several factors such as fermentation temperature, dough yield and substrate quality affect the dominant type of sourdough microorganisms. Low fermentation temperature (25 °C) reduces the activity of LAB and promotes the growth and dominance of yeasts [29]. In addition to temperature, the ratio of LAB to yeasts in sourdough is also affected by the dough yield, so that the higher the dough yield, the more LAB prevail over the yeasts [30]. On the other hand, although sourdough is usually kneaded using the same type of basic flour, some changes (such as seasonal changes in grain) affects the content of nutritional compounds of the flour and even minor changes in the substrate quality. It can affect the final microbial flora of flour and sourdough [31].

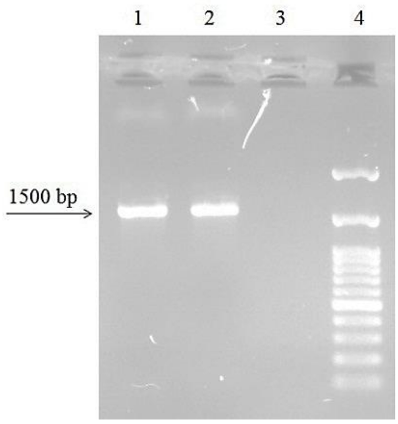

Figure 1 Gel electrophoresis of the PCR products to confirm the amplification of a 1500 bp target sequence of the predominant LAB isolated from mung bean sourdough. Line 1: DNA extracted from the predominant LAB isolate, Line 2: positive control containing DNA of the collection strain, Line 3: negative control, Line 4: 100 bp DNA Ladder.

Optimal formulation

The optimal formulation of production samples is reported in Table 1. By substituting saffron petal extract up to a concentration of 10% instead of water in the sourdough formulation, no unpleasant taste was observed in the produced breads, so 10% saffron petal extract was used for preparation sourdough bread.

Table 1 Formulation of the produced breads including wheat bread (C) and wheat breads containing mung bean (M), fermented mung bean (FM), saffron petal extract (E), combination of fermented mung bean and saffron petal extract (FM+E).

Formulation	C	E	M	FM	FSM+E
wheat flour (g)	151	151	141	141	141
mung bean flour (g)	-	-	10	10	10
water (mL)	94	54	94	94	54
saffron extract (mL)	-	40	-	-	40
baker's yeast (g)	5	5	5	5	5
loaf weight (g)	250	250	250	250	250

Texture characteristics of manufactured breads

Based on the results obtained from texture analysis (Table 2), the addition of saffron petal extract (E) or mung bean flour (M) alone increases hardness, gumminess and reduces the porosity of bread texture, compared to the control sample (C). Controlled fermentation also had a significant effect on improving the textural characteristics of the produced samples, so that the sample containing fermented mung beans (FM) did not show significant difference ($P>0.05$) in terms of crumb porosity, hardness and gumminess. Compared to the control sample, the sample containing the mixture of fermented mung bean and saffron petal extract (FM+E) was significantly ($P<0.05$) harder, gummy and more porous than other produced samples. However, other textural characteristics such as springiness, resilience, and cohesiveness were not significantly different from each other in the production samples.

Bojnanská et al. (2012) investigated the characteristics of wheat bread containing 10-50% chickpea and lentil flour. According to the results of the aforementioned research, with the increasing in the amount of legume flour, the hardness of the bread texture increased [32]. This increase in hardness can be due to the higher moisture requirement of legume proteins for hydration [33] and also the presence of fiber [28]. Perri et al. (2021) also investigated the effect of the addition of fermented lentils on the textural properties of wheat bread. According to the report of these researchers, the bread containing fermented lentils had lower hardness than the control sample. Also, these researchers reported that the degree of springiness and cohesiveness of the produced samples do not differ significantly from each other [34]. At acidic pH (below 4) during sourdough fermentation, a significant net positive charge is created, and with the increase of electrostatic repulsion, the solubility of proteins increases and the formation of new bonds is effectively prevented [36, 35]. Therefore, the reduction of intermolecular and intramolecular disulfide bonds increases the ability of gluten proteins to dissolve, potentially providing more access to proteolytic enzymes and thus more efficient proteolysis [37], which makes bread texture softer. The lower porosity and increased hardness in the sample containing saffron petal extract (E) compared to the control sample can be related to the insufficient time for the adaptation of *S. cerevisiae* and the decrease in the fermentation ability of yeast.

Table 2 Textural characteristics of the produced breads including wheat bread (C) and wheat breads containing mung bean (M), fermented mung bean (FM), saffron petal extract (E), combination of fermented mung bean and saffron petal extract (FM+ E).
Different letters in each column indicate significant difference at P<0.05.

Sample	C	E	M	FM	FM+E
Hardness (N)	13.08 ± 0.72^{c}	14.86 ± 0.88^{b}	18.12 ± 0.08^{a}	11.63 ± 0.56^{cd}	10.21± 0.42^{d}
Porosity (%)	14.86 ± 0. 40^{b}	13.53 ± 0.15^{c}	11.86 ± 0.40^{d}	15.40 ± 0.43^{b}	16.16 ± 0.25^{a}
Resilience	0.3 ± 0.00^{a}	0.24 ± 0.07^{a}	0.24 ± 0.07^{a}	0.30 ± 0.03^{a}	0.34 ± 0.04^{a}
Gumminess	895.31 ± 39.95^{b}	914.01 ± 10.21^{b}	1115.16 ± 73.52^{a}	790.17 ± 87.93^{bc}	711.58 ± 18.4^{c}
Springiness	0.93 ± 0.02^{a}	0.90 ± 0.01^{a}	0.89 ± 0.05^{a}	0.91 ± 0.04^{a}	0.93 ± 0.01^{a}
Cohesiveness	0.67 ± 0.01^{a}	0.60 ± 0.03^{a}	0.60 ± 0.04^{a}	0.66 ± 0.04^{a}	0.68 ± 0.01^{a}
Crumb pH	6.18 ± 0.02^{a}	6.15 ± 0.00^{a}	6.19 ± 0.01^{a}	5.60 ± 0.02^{b}	5.49 ± 0.02^{c}

Fungal growth rate in produced samples

In general, the use of mung bean, fermented mung bean and saffron petal extract effectively reduced the surface growth of *A. niger* in manufactured wheat bread compared to the control sample, but the combined use of fermented mung bean and saffron petal extract was more effective (Figure 2). So that the wheat bread containing a mixture of fermented mung bean and saffron petal extract (FM+E) with 44.33% inhibition of the growth of the mentioned fungus compared to the control was the best sample in terms of inhibition of the surface growth of the fungus. Also, the mentioned sample had the longest shelf life compared to other samples with 4 days of shelf life without mold.

Algboory et al. (2021) studied the shelf life of wheat bread containing aqueous extract of *Cyperus rotundus* rhizome and containing identified antimicrobial compounds. Based on the results of these researchers, it was determined that the aqueous extract of the mentioned rhizomes is a potential natural alternative to artificial preservatives to increase the shelf life of wheat bread with high acceptability [38]. Nionelli et al. (2018) also reported that substituting hop extract for water in wheat sourdough and part of the original dough delayed the growth of *Penicillium roqueforti* DPPMAF1 for 7 days, similar to wheat bread containing hop extract (without sourdough). This was despite the fact that the surface growth of the mentioned fungus in the sample containing sourdough and hop extract was significantly lower than the sample containing hop extract (without sourdough) [17]. Regardless of the presence of antifungal metabolites, sourdough greatly increases the activity of weak organic acids by lowering pH, allowing for the intensification of their antifungal activity [39]. On the other hand, plant extracts have good antifungal activity due to their phenolic compounds and antioxidant capacity. Also, when plant extracts/essential oils are combined with other inhibitory compounds, they can show promising results in inhibiting fungal growth [40].

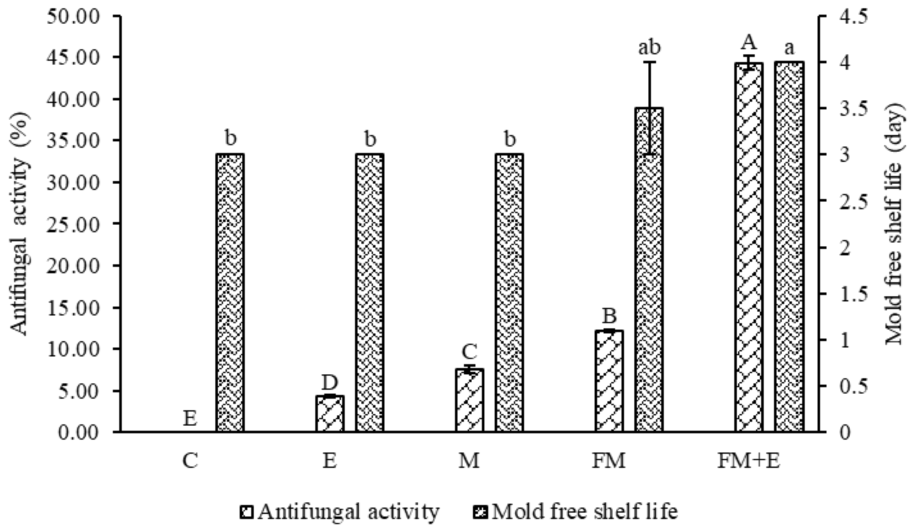

Figure 2 Mold free shelf life and percentage of inhibition from surface growth of the indicator fungus on the produced breads. The different lowercase and uppercase letters indicate significant difference at $P<0.05$ among the produced samples in terms of mold free shelf life and percentage of inhibition, respectively.

Overall acceptability of the produced samples

According to the evaluators, there was no a significant difference (P>0.05) among the produced samples with each other in terms of aroma, taste, chew ability, mouth feel and overall acceptability, and all samples received an acceptance score higher than 4. In terms of color, the samples containing saffron petal extract had an unusual color (gray violet), which was considered a negative factor by the evaluators and received a lower score compared to other samples (Figure 3).

Salim-ur-Rehman et al. (2007) reported in a study that the addition of citrus peel essential oil to bread can affect the sensory characteristics such as crust characteristics, taste, texture and aroma of bread [41]. The presence of aromatic compounds and pigments in plant extracts can have a significant effect on the aroma, color and taste of the produced samples. In addition, it should be noted that excessive use of plant extracts in bakery products may adversely affect their sensory characteristics.

In Aryashad et al. (2023) research, there was no significant difference in terms of sensory characteristics and overall acceptance between the control sample and the sample containing fermented sprouted mung beans [42]. By the activity of LAB during sourdough fermentation, flavor compounds including non-volatile compounds and volatile compounds are produced, which affect flavor and aroma of the produced sourdough bread [43, 44].

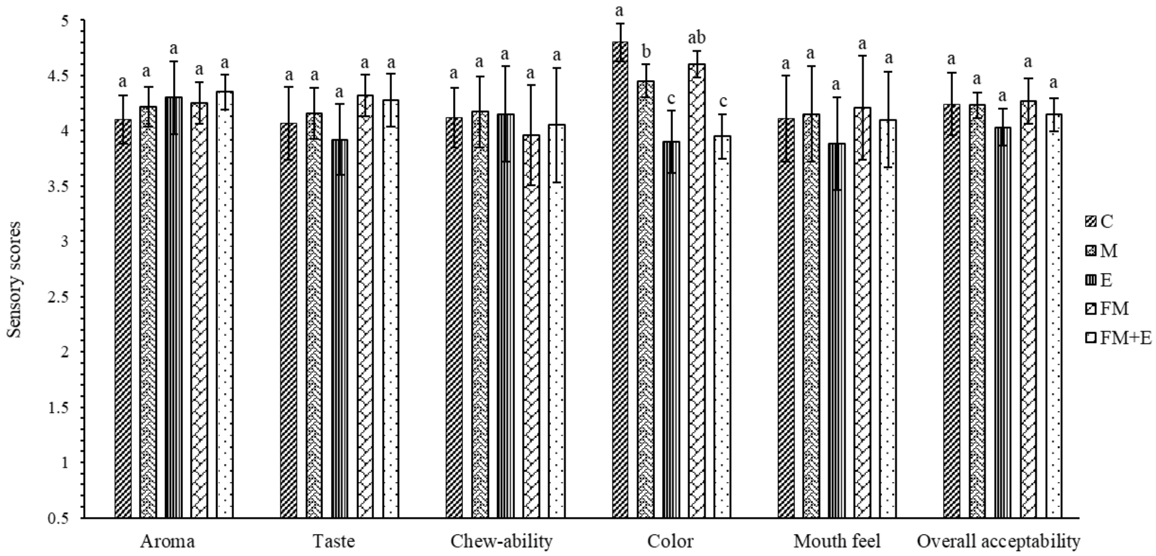

Figure 3 Sensory characteristics of the produced breads. Different letters in each characteristic indicate significant difference at $P<0.05$ among the produced samples.

4.Conclusions

Although wheat bread provides carbohydrates and energy needed by the human body, it lacks some essential amino acids and bioactive components. Therefore, the use of legumes rich in vitamins and essential amino acids is considered a suitable solution to improve the properties of wheat bread. On the other hand, extracts of aromatic plants can also be used to improve acceptance and shelf life of bread. In the present research, mung bean sourdough containing saffron petal extract was used to process wheat bread and control mold in it. After isolating dominant LAB from mung bean sourdough containing saffron petal extract, dominant LAB strain was used as starter culture in wheat bread processing. Based on the results of this research, the combination of fermentation and saffron petal extract had a significant effect on reducing crumb hardness and *A. niger* growth compared to the control sample. Accordingly, mung bean sourdough containing saffron petal extract can be used as a natural additive in formulation of wheat bread as the staple food, and it has a positive effect on its quality and functional characteristics.

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ارزیابی ویژگی‌های بافتی، حسی و ماندگاری نان تهیه شده با خمیرترش ماش حاوی عصاره گلبرگ زعفران

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چکیده

استفاده از تخمیر کنترل شده حبوبات به همراه عصاره آبی گیاهان معطر به لحاظ بهبود ویژگی‌های کیفی نان گندم حائز اهمیت است. در این پژوهش پس از تخمیر تصادفی خمیرترش ماش حاوی عصاره گلبرگ زعفران، باکتری اسید لاکتیک غالب جدا شده به عنوان کشت آغازگر در تخمیر کنترل شده خمیرترش مورد استفاده قرار گرفت و سپس ویژگی‌های نان‌های گندم تولیدی از نظر بافت، توسعه سطحی قارچ و پذیرش کلّی بررسی شد. توالی‌یابی محصولات PCR منجر به شناسایی *Levilactobacillus brevis* به عنوان جدایه لاکتیکی غالب خمیرترش گردید. فرآوری نان گندم با خمیرترش ماش حاوی باکتری مذکور و عصاره‌گلبرگ زعفران نیز نه تنها ویژگی‌های بافتی و پذیرش کلّی نان‌های تولیدی را بهبود بخشید بلکه ماندگاری آنها را در برابر توسعه سطحی *Aspergillus niger* افزایش داد. بر اساس نتایج حاصل، نان گندم حاوی ماش تخمیر شده و عصاره گلبرگ زعفران با سفتی بافت ۱۰/۲۱ نیوتن و تخلخل ۱٦/۱٦٪، مناسب‌ترین ویژگی‌های بافتی را نسبت به سایر نمونه‌ها نشان داد. همچنین نمونه مذکور با ٤٤/۳۳٪، بیشترین قابلیت ممانعت از رشد قارچ شاخص را نسبت به سایر نمونه‌ها دارا بود. علیرغم اینکه استفاده از ماش و عصاره گلبرگ زعفران به تنهایی در فرمولاسیون نان گندم تاثیر زیادی در کاهش توسعه سطحی قارچ نداشت اما استفاده توام از عصاره گلبرگ زعفران و ماش تخمیر شده به شکل معنی‌داری ($P < ۰/۰۵$) بر کاهش رشد سطحی قارچ موثر بود. با توجه به قابلیت‌های فناوری-عملکردی مناسب تخمیر کنترل شده خمیرترش ماش حاوی عصاره گلبرگ زعفران، می‌توان از آن به عنوان یک بهبود دهنده طبیعی در صنایع نانوایی استفاده نمود.