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Effect of traditional sourdough on quality characteristics of loaf bread containing sprouted wheat and garlic extract

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ABSTRACT

Application of plant extracts in the formulation of sourdough can improve sensory characteristics and shelf-life of the produced bread. In the present study, effects of combined application of fermented sprouted wheat and garlic extract in the formulation of traditional sourdough containing yoghurt and vinegar were investigated on the quality characteristics of loaf wheat bread. Based on the results of texture analysis, among the produced breads the lowest crumb hardness was observed in the sample containing fermented sprouted wheat along with garlic extract (2.94 N), which was significantly ($p<0.05$) lower than those of the control sample. In addition, combined application of fermentation and sprouting increased the specific volume and reduced the gumminess of the product. Moreover, although the lowest surface growth of *Aspergillus flavus* was observed on breads containing yoghurt-vinegar and also garlic extract alone, there was no significant difference between the fungal growth rate on the sample containing fermented sprouted wheat along with garlic extract and bread containing calcium propionate. Bread containing fermented sprouted wheat and control sample had no significant difference in terms of overall acceptability. Accordingly, sprouted wheat sourdough containing garlic extract can be used as a biological preservative to improve the quality characteristics and shelf-life of wheat bread.

1-Introduction

Bread production is one of the oldest technologies known to humankind. Depending on cultural habits, bread is consumed in various types and forms around the world as a staple food. Wheat bread spoilage can occur due to various factors including bacterial and fungal contamination, with fungal spoilage being the primary concern [1,2]. One approach to controlling bread spoilage is the use of chemical preservatives such as propionic, sorbic, acetic acids, and their salts, which can slow down fungal spoilage. Although these compounds are classified as safe, their use has led to consumer dissatisfaction due to development of some resistant fungi [3,4]. An alternative to chemical preservatives is the use of natural compounds that can reduce or eliminate microbial populations while improving the quality of the produced product. These compounds can also serve as natural antifungal additives to prevent fungal growth and extend the bread's shelf-life, thus reducing public health risks. Among the most important biological preservatives in bread processing are fermented grains and plant extracts. Several studies have shown that plant extracts contain various bioactive components that can control fungal growth due to their phenolic compounds [5,6].

Studies have been conducted on using controlled fermentations as biological preservatives in bread. For example, the effect of controlled fermentation of oat containing *Pediococcus pentosaceus* starter culture on fungal growth inhibition, texture characteristics, and overall acceptability of wheat bread was evaluated, showing that controlled fermentation can improve these attributes [7]. Another study investigated the impact of controlled fermentation of acorns using *Pediococcus acidilactici* on the texture and antifungal activity in wheat bread. The results showed that this bacterium positively affected texture and reduced *Aspergillus flavus* growth on the surface of the produced bread [8]. Additionally, the effect of controlled fermentation of sprouted barley sourdough [9], amaranth [10], sprouted mung beans with ginger extract [11], sprouted clover [12], mung beans [13], quinoa [14], wheat bran [15], wheat germ [16], rice bran [17], and whole wheat flour [18] on technological-functionality, nutritional, and shelf-life characteristics of the produced bread has been confirmed.

There are also reports on using essential oils and plant extracts as biological preservatives in bread production. For instance, the antifungal effect of thyme essential oil on *Penicillium paneum* in partially baked bread was investigated. The results showed that this essential oil was able to inhibit fungal growth in the bread for up to 45 days [1]. Other researchers combined lactic acid bacteria fermentation with hop extract to improve antifungal properties and create a natural preservative in bread. These studies showed significant antifungal activity of hop extract, delaying fungal growth for up to 14 days [19]. The antifungal activity of essential oils from cinnamon, clove, pepper, camphor, star anise, lemon, and peppermint on three indicator fungi was also investigated, with

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cinnamon oil showing the greatest effect on all studied fungi [20].

Based on the literature reviewed, there has been no report on the combined use of garlic extract and sprouted wheat in sourdough wheat bread. Thus, the aim of this study was to evaluate the impact of traditional sourdough containing sprouted wheat and garlic extract on the textural, shelf-life, and overall acceptability characteristics of wheat bread.

2-Materials and Methods

2.1. Preparation of raw materials and their evaluation

The characteristics of wheat flour, including moisture, ash, protein, and fat contents, were determined using standard methods from AACC [21]. The carbohydrate percentage was calculated using the formula: 100 - (fat + protein + ash + moisture). Accordingly, the wheat flour used in this study had 12.4% protein, 0.4% ash, 14.3% moisture, 1.1% fat, and 71.8% carbohydrates. Microbiological culture media and chemical reagents were obtained from reputable commercial brands for laboratory use.

2.2. Preparation of sprouted wheat flour

For sprouting wheat grains, the grains were initially disinfected using 1.25% sodium hypochlorite and then rinsed after 30 minutes [22]. Subsequently, the disinfected grains were hydrated in the dark at 25 °C with 12-hour intervals. The sprouted grains were then dried at 50 °C in an oven (Binder, Germany) for 25 h, and after grinding, sprouted wheat flour was prepared.

2.3. Fermentation using traditional sourdough

The sourdough used in this study was prepared from a mixture of sprouted wheat flour (68%), yogurt (3.5%), vinegar (1.5%), and water (27%) according to the Iranian national standard [23] with minor modifications. After 24 h of fermentation at 37 °C, the mixture was added to a mix of water and wheat flour (60% water absorption) and baker's yeast (2% w/w). The dough was then fermented for 2 h at 37 °C before baking. The sourdough containing garlic aqueous extract was prepared using the method described by Irakli et al. [24], with the optimal garlic extract amount determined for fermentation. The optimal amount of garlic extract in the bread formulation was determined in the range of 5 to 20% based on overall acceptability scores.

2.4. Production of leavened wheat bread

The control sample (containing wheat flour, water, and *Saccharomyces cerevisiae*) was fermented for 2 h at 37 °C and then baked in an electric oven (Feller, Germany) at 180 °C for 20 minutes. Bread samples containing calcium propionate (0.3% w/w), sprouted wheat, garlic extract, yogurt and vinegar mixture, sourdough (sprouted wheat, yogurt, and vinegar), and sourdough with garlic extract were processed similarly to the control sample. To determine the optimal sourdough amount in the wheat bread formulation, sourdough amounts ranging from 5 to 20% were used, and the optimal amount was determined based on overall acceptability and texture analysis results [25].

2.5. Evaluation of quality characteristics of produced bread

2.5.1. Crumb hardness and specific volume measurement

Hardness of the bread crumb was measured using a texture analyzer (Stable Micro System, UK) with a cylindrical probe at a speed of 50 mm/min and a force of 500 N to achieve 50% compression from the initial height of the sample. The crumb hardness was determined one hour after

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baking using texture profile analysis [25]. The specific volume of the bread was measured using the rapeseed displacement method [17].

2.5.2. Surface mold growth assessment

To assess the expansion of *A. flavus* on the surface of the produced bread samples, 3 μL of fungal spore suspension (10^6 spores/mL) were inoculated onto a sterile paper disk placed at the center of the bread. The samples were incubated for one week at 25 °C. Mold surface growth was compared to the control sample over time by daily photography and analysis using Image J software [26].

2.5.3. Overall acceptability of the produced bread

Sensory evaluation of the produced bread samples was conducted by trained evaluators using a 5-point hedonic scale. This evaluation included assessing the crust color, taste, chewiness, bread aroma, and mouth-feel. Scores were reported from very undesirable (1) to very desirable (5). Overall acceptability of the bread samples was calculated as the average score of these attributes [27].

2.6. Statistical analysis of results

The results were analyzed using a completely randomized design with one-way ANOVA. Mean comparisons were performed using the Least Significant Difference (LSD) test at $p<0.05$. All tests were conducted in triplicate, and data analysis was performed using SPSS software (version 16) and Microsoft Office Excel 2013 for plotting graphs.

3-Results and Discussion

3.1. Selection of optimal sourdough formulation

Initially, adding more than 20% sourdough led to excessive stickiness in the bread's texture. Therefore, lower percentages of sourdough were used to achieve desirable textural and sensory characteristics in the produced bread. As shown in Table 1, the crumb hardness and gumminess of the bread containing 10% sprouted wheat sourdough were significantly ($p<0.05$) lower than those of the samples with 5% and 15% sourdough. Additionally, there was no significant difference in overall acceptability between the samples containing 5% and 10% sourdough. Therefore, a 10% sourdough formulation was selected as the optimal for further stages of the research. The optimal garlic extract amount in the bread formulation, based on overall acceptability scores, was selected as 7.5% as a substitute for water in the sourdough formulation.

Table 1. Textural and sensorial properties of the produced breads containing different amounts of sprouted wheat sourdough. The different letters in each column show significant difference at $p<0.05$.

Bread sample	Overall acceptability	Gumminess	Crumb hardness (N)
5% Sourdough	2.92 ± 0.16 a	1934.20 ± 77.35 a	a 0.37 ±4.00
10% Sourdough	2.48 ± 0.43 a,b	842.59 ± 128.53 b	b 0.33 ±1.93
15% Sourdough	2.16 ± 0.29 b	1474.60 ± 288.50 a,b	0.19 a ±3.43

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3.2. Textural characteristics of the produced bread

As shown in Table 2, the addition of yogurt and vinegar significantly ($p<0.05$) increased the crumb hardness while also increasing its specific volume. Additionally, sprouting significantly reduced both crumb hardness and specific volume. The inclusion of sourdough did not result in a significant reduction in crumb hardness, but it significantly increased the specific volume. Moreover, adding garlic extract to the bread significantly increased both crumb hardness and specific volume, but incorporating garlic extract into the sourdough formulation significantly reduced crumb hardness and specific volume of the produced bread.

Table 2. Overall acceptability, pH, specific volume and crumb hardness of the produced bread samples including control bread (C), bread containing sprouted wheat (SW), fermented sprouted wheat (FS), garlic extract (E), calcium propionate (CP), yoghurt and vinegar (YV) and fermented sprouted wheat containing garlic extract (FS+E). The different letters in each column indicate significant difference at $p<0.05$.

Bread sample	Overall acceptability	pH	Specific volume (cm^3/g)	Crumb hardness (N)
C	4.40 ± 0.21 a	6.15 ± 0.02 a	2.42 ± 0.02 d	3.83 ± 0.02 d
SW	3.68 ± 0.34 b	6.13 ± 0.01 a	1.72 ± 0.06 e	2.92 ± 0.04 e
FS	4.20 ± 0.17 a	5.93 ± 0.03 c	3.56 ±0.03 b	3.55 ± 0.20 d
E	4.22 ± 0.43 a	6.04 ± 0.01 b	2.94 ±0.09 c	6.45 ± 0.12 b
CP	4.14 ± 0.21 a	6.13 ± 0.02 a	2.52 ± 0.02 d	4.70 ± 0.16 c
YV	0.21 ±4.14 a	6.00 + 0.00 b	3.86 ± 0.16 a	7.63 ± 0.16 c
FS+E	0.24 ± 3.52 b	5.94 ± 0.01 c	1.93 ± 0.03 e	2.94 ± 0.24 e

As observed in the study by Nionelli et al. (2018), the specific volumes of the control bread, the bread with hop extract, and the sourdough bread with hop extract were reported as 2.71, 2.69, and 2.99 cm³/g, respectively, with the sourdough bread containing hop extract showing a significantly higher specific volume compared to the control sample. Additionally, the crumb hardness of these samples was reported as 2819, 2826, and 2706 g, respectively, indicating that the sourdough bread with hop extract had significantly lower crumb hardness compared to the control. However, the use of hop extract alone increased crumb hardness and reduced the specific volume of the produced bread [19].

In the research by Irakli et al. (2019), the specific volumes of the control bread, the sourdough with hop extract, and the sourdough with hop extract and rice bran were reported as 3.8, 3.1, and 3.4 cm³/g, respectively, with significant differences among them [24]. Generally, in samples containing sprouted substrate, the intensification of enzymatic activity and the breakdown of gluten and starch, which ultimately leads to less gas retention, result in a reduced specific volume. In the sample with garlic extract, a higher density mixture likely formed more stable bubbles, which, despite the increased volume, resulted in a firmer texture. The type and nature of the starter culture used in sourdough and the pH of the sourdough have also different effects on the bread's characteristics. During

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fermentation, metabolites such as organic acids, exopolysaccharides, and enzymes are produced, positively affecting bread texture [3]. Overall, the sample with sprouted wheat, which had less crumb hardness and a lower specific volume, was not considered suitable in terms of textural characteristics. However, the sourdough sample with less crumb hardness and higher specific volume was considered more desirable texturally.

3.3. Overall acceptability of the produced bread

The lowest overall acceptability was observed in the sample containing sprouted wheat, which showed a significant difference ($p<0.05$) compared to the control sample. The best sample in terms of chew-ability and mouth-feel was the one containing garlic extract, which did not differ significantly from the control. The bread samples did not show significant differences in taste and color, although the sample containing yogurt and vinegar differed in aroma from the others. Moreover, fermentation did not significantly impact the overall acceptability of the produced bread, but adding garlic extract to the sourdough formulation decreased the overall product acceptability.

Nionelli et al. (2018) reported that the bread samples containing hop extract had a bitter and herbal taste compared to the control. However, these samples were still considered acceptable by evaluators [19]. According to Irakli et al. (2019), the sourdough bread with hop extract did not differ significantly from the control bread. Additionally, the sourdough bread with hop extract and rice bran had the highest overall acceptability among the tested samples and did not show a significant difference from the control bread. The acceptability scores for the control bread, the sourdough bread with hop extract, and the sourdough bread with hop extract and rice bran were reported as 3.8, 3.3, and 4.2, respectively [24]. Flavor, taste, and aroma are crucial factors in bread acceptance. It has been generally established that metabolites produced during fermentation can positively affect these factors and increase acceptability. The use of plant extracts has also a significant impact on each of these factors [3].

3.4. Inhibition of surface fungal growth

As shown in Figure 1, the highest antifungal effect was observed in the samples containing yogurt and vinegar, as well as in the sample with garlic extract alone. However, there was no significant difference ($p<0.05$) in the inhibitory effect of these samples compared to the bread containing calcium propionate and the sprouted wheat sourdough with garlic extract. Additionally, the greatest fungal growth among the produced breads was observed in the control and the sprouted wheat sample. According to the results, both fermentation and garlic extract reduced fungal growth, with garlic extract showing a more pronounced inhibitory effect compared to fermentation alone. Furthermore, the sprouted wheat sourdough with garlic extract provided a suitable antifungal effect compared to calcium propionate. Therefore, it can be used as a biological preservative in wheat bread processing.

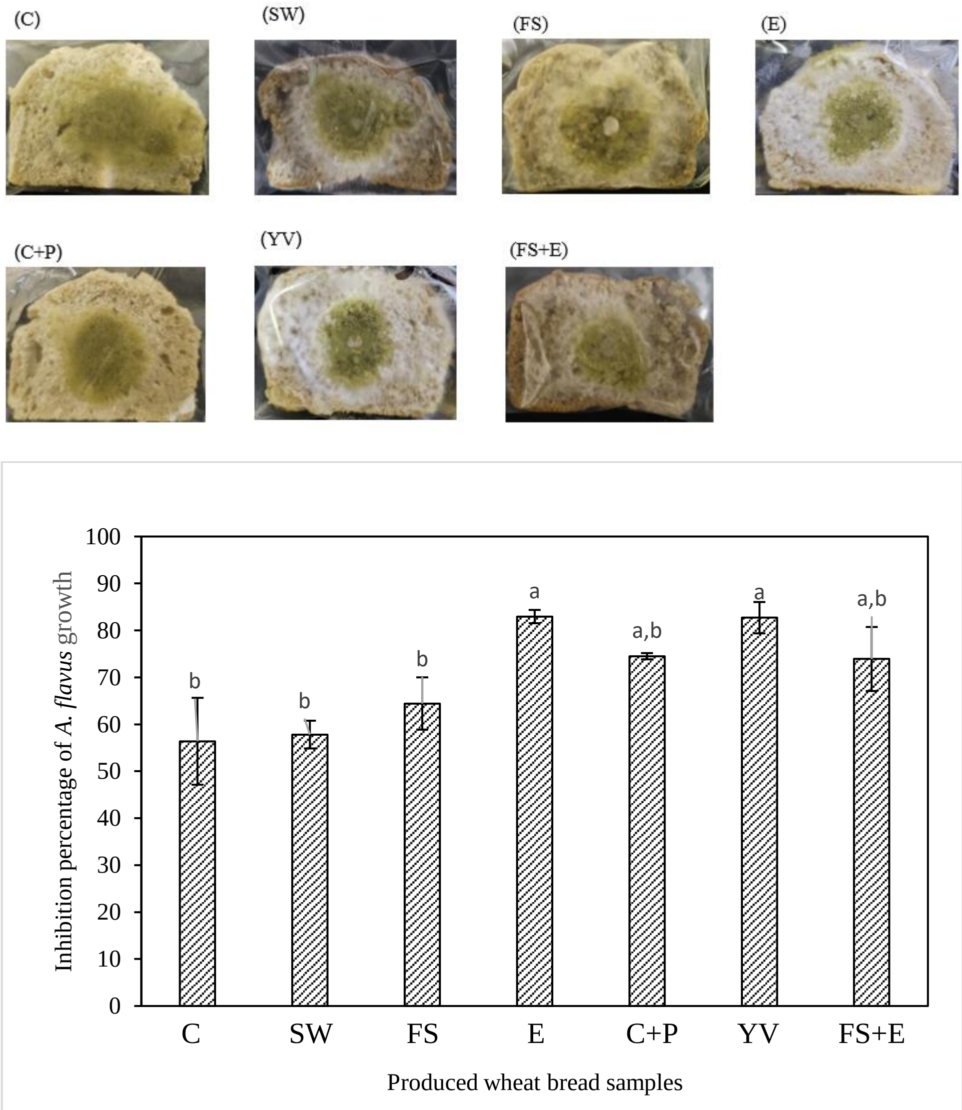

Fig. 1. A: surface expansion of *A. flavus* after five days of storage on the produced bread samples. B: inhibition percentage of *A. flavus* growth on the produced bread samples including control wheat bread (C), bread containing sprouted wheat (SW), fermented sprouted wheat (FS), garlic extract (E), calcium propionate (CP), yoghurt and vinegar (YV) and fermented sprouted wheat containing garlic extract (FS+E). The different letters indicate significant difference at $p<0.05$.

Nionelli et al. (2018) observed that wheat bread containing hop extract weakly inhibited fungal growth in the produced bread. However, the addition of hop extract delayed the growth of *Penicillium roqueforti* for at least 14 days, which was similar to bread with 0.3% calcium propionate. They attributed the antifungal effect to the presence of weak acids in the hop extract. Nonetheless, the use of sourdough containing hop extract was more effective, primarily due to the acidification by lactic acid bacteria and the synthesis of a wide range of low molecular weight compounds such as antifungal peptides [19]. Jin et al. (2021) also noted that lactic acid bacteria could produce various organic acids with antifungal properties, although the mechanisms of some are not yet fully

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understood. Studies have shown that acetic acid, one of the most effective organic acids, might have antifungal effects due to its low pKa [28]. Given the antimicrobial properties of the intrinsic microorganisms in sourdough [29-32], using sourdough as a biological preservative in bakery industries could be a suitable alternative to conventional synthetic preservatives. Moreover, combining antifungal plant extracts with sourdough can significantly enhance mold control in sourdough breads while modulating the sour taste [33-35].

4- Conclusion

The use of sprouted grain sourdough in wheat bread processing is of technological significance. These ingredients not only affect the textural and sensory properties of the product but can also serve as natural additives, improving the nutritional properties and shelf-life of the bread. Additionally, enzymatic activity during sprouting facilitates the breakdown of more complex substrates into simpler compounds, which can then be utilized by microorganisms during fermentation. Therefore, sprouted grains are suitable substrates for sourdough fermentation. In this study, fermented sprouted wheat with garlic extract positively impacted the texture, overall acceptability, and mold inhibition of the produced wheat bread, with the least crumb hardness observed in the bread containing fermented sprouted wheat with garlic extract. This sourdough also effectively prevented mold growth and showed no significant difference in antifungal activity compared to calcium propionate. Based on these results, using garlic extract alone or sprouted wheat alone did not significantly impact the texture of the produced samples. However, the combined use of both in sourdough formulation proved to be a simple and cost-effective method to improve the quality attributes of the wheat bread.

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چکیده

استفاده از عصاره‌های گیاهی در فرمولاسیون خمیرترش می‌تواند سبب بهبود ویژگی‌های حسی و زمان ماندگاری نان تولیدی گردد. در پژوهش حاضر، تاثیر استفاده توام از گندم جوانه‌زده تخمیر شده و عصاره سیر در فرمولاسیون خمیرترش سنتی حاوی ماست و سرکه بر ویژگی‌های کیفی نان گندم حجیم مورد بررسی قرار گرفت. بر اساس نتایج بافت‌سنجی از بین نمونه‌های تولیدی، کمترین سفتی بافت در نان حاوی گندم جوانه‌زده تخمیر شده و عصاره سیر (۲/۹٤ نیوتن) مشاهده شد که به شکل معنی‌داری (p<۰/۰۵) از سفتی بافت نمونه شاهد کمتر بود. استفاده توام از تخمیر و جوانه‌زنی نیز سبب افزایش حجم مخصوص و کاهش چسبندگی بافت نان تولیدی گردید. علاوه بر این، اگر چه کمترین توسعه سطحی قارچ *Aspergillus flavus* در نان‌های حاوی ماست و سرکه و همچنین عصاره سیر به تنهایی مشاهده شد اما تفاوت معنی‌داری بین میزان توسعه سطحی قارچ در نمونه حاوی گندم جوانه‌زده تخمیر شده و عصاره سیر با نان حاوی پروپیونات کلسیم وجود نداشت. همچنین نمونه حاوی گندم جوانه‌زده تخمیر شده با نمونه شاهد به لحاظ پذیرش کلی تفاوت معنی‌داری نشان نداد. بر این اساس، خمیرترش گندم جوانه‌زده حاوی عصاره سیر به عنوان یک نگهدارنده زیستی می‌تواند جهت بهبود ویژگی‌های کیفی و زمان ماندگاری نان گندم مورد استفاده قرار گیرد.