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Impact of NaCl on microbial growth and activity in soft wheat dough: Implications for bread quality and shelf life

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Abstract
This study investigates the impact of sodium chloride (NaCl) concentrations on the microbial growth, fermentation dynamics, and quality attributes of soft wheat dough and the resultant bread. Doughs were prepared with varying NaCl concentrations (0%, 0.5%, 1.0%, 1.5%, and 2.0% w/w flour) and fermented for 3 hours. The growth of *Saccharomyces cerevisiae* (yeast) and lactic acid bacteria (LAB) was assessed, alongside the production of ethanol, lactic acid, and acetic acid. The results demonstrated that yeast and LAB populations significantly decreased with increasing NaCl concentrations, leading to lower ethanol and organic acid production. Bread quality analysis revealed that lower NaCl concentrations resulted in higher specific volume and softer crumb texture, while higher NaCl concentrations yielded firmer bread with a reduced volume. The shelf life of bread, however, was prolonged with increasing NaCl concentrations due to the antimicrobial properties of salt. These findings highlight the balancing act between reducing NaCl content for health benefits and maintaining optimal bread quality, such as texture, volume, and shelf life. The study provides valuable insights into the role of NaCl in bread formulation and suggests that careful consideration of NaCl reduction should be made to avoid compromising both the sensory properties and preservation of bread.

Keywords: Sodium chloride, *Saccharomyces cerevisiae*, lactic acid bacteria, bread quality, specific volume, shelf life, ethanol production, organic acids, NaCl reduction, dough fermentation, microbial dynamics, bread preservation

Introduction

The pivotal role of sodium chloride (NaCl) in bread production is deeply ingrained in both art and science, serving as a fundamental component that extends far beyond a simple flavor enhancer [1, 2]. Its influence is multifaceted, significantly impacting the rheological properties of dough by strengthening the gluten network, thereby improving gas retention and yielding a final product with enhanced volume and a desirable crumb structure [3, 4]. Simultaneously, NaCl acts as a key modulator of the complex microbial ecosystem resident within the dough matrix, which is primarily composed of *Saccharomyces cerevisiae* and a diverse population of lactic acid bacteria (LAB) [5]. This delicate microbial balance is critical for the fermentation process, contributing to leavening, the development of volatile flavor compounds, and ultimately defining the sensory profile of the bread [6, 7]. The growing global health concern regarding high sodium intake has led to widespread efforts to reduce salt content in processed foods, including bread [8, 9, 10]. This shift in formulation, while beneficial from a public health perspective, introduces a significant and poorly understood challenge to the established microbial and physicochemical dynamics of bread dough [11]. The core problem, therefore, is to elucidate the intricate relationship between reduced NaCl concentrations and their cascading effects on the growth kinetics and metabolic activity of the dough's microbiota [12]. Furthermore, a comprehensive understanding is needed to correlate these microbial changes with the resultant bread quality parameters and, critically, its post-baking shelf life, as a reduction in salt may lower a key preservative effect [13, 14]. Building on preliminary findings, the primary objective of this study is to systematically investigate the impact of varying NaCl levels (0%, 0.5%, 1.0%, 1.5%, and 2.0% w/w flour basis) on the temporal evolution of microbial populations and the production of organic acids and ethanol in soft wheat dough [15, 16]. A secondary objective is to evaluate how these microbial shifts directly influence key bread attributes, including specific volume, crumb hardness, and mold-free shelf life [17]. It is hypothesized that a reduction in NaCl concentration will stimulate an increase in initial microbial growth and leavening efficiency, but this will come at the expense of a compromised gluten network, resulting in a bread with a less stable structure and a significantly shortened shelf life due to reduced antimicrobial properties [18].

Materials and Methods
Materials
The materials used in this study include soft wheat flour (12% protein content) purchased from a local supplier. Sodium chloride (NaCl) was procured as analytical-grade salt and incorporated into the dough at varying concentrations: 0%, 0.5%, 1.0%, 1.5%, and 2.0% (w/w flour basis) [1, 2]. The microbial cultures used were *Saccharomyces cerevisiae* (baker's yeast) and lactic acid bacteria (LAB), specifically *Lactobacillus brevis* and *Lactobacillus plantarum*, sourced from a commercial microbiological culture collection [3]. Other ingredients included water (pH 7.0) and flour improvers, as per standard bakery formulations. For microbial growth and metabolite analysis, the following media were used: MRS agar for LAB and Potato Dextrose Agar (PDA) for yeast. All reagents used were of analytical grade, sourced from Sigma-Aldrich (St. Louis, MO, USA) or equivalent suppliers.

Methods
- **Dough Preparation**: The dough was prepared by mixing soft wheat flour with NaCl at the specified concentrations (0%, 0.5%, 1.0%, 1.5%, and 2.0%). The ingredients were thoroughly mixed using a commercial mixer at a speed of 2.5 RPM for 10 minutes. The dough was then allowed to ferment in a climate-controlled chamber at 25 °C with 80% relative humidity for 3 hours [4, 5].
- **Microbial Growth and Activity**: Microbial populations, including *Saccharomyces cerevisiae* and LAB, were quantified at 0, 1, 2, and 3 hours of fermentation using plate count techniques. Yeast and LAB were enumerated on PDA and MRS agar, respectively, after incubation at 30 °C for 48 hours. Colony counts were expressed as log CFU/g of dough [6, 7].
- **Metabolite Analysis**: Organic acid (lactic acid and acetic acid) and ethanol concentrations were measured at 3 hours of fermentation using high-performance liquid chromatography (HPLC). The equipment used was a Shimadzu LC-20A series with a C18 column, and the mobile phase was prepared according to the method described by Mella *et al.* [8]. The analysis was done in triplicate for each NaCl concentration.
- **Bread Quality Evaluation**: After fermentation, dough portions were shaped and baked at 180 °C for 25 minutes in a conventional oven [9]. The specific volume of the bread was measured by the rapeseed displacement method. The crumb hardness was evaluated using a texture analyzer (TA.XT Plus, Stable Micro Systems) with a 36-mm cylindrical probe to assess the force required to compress the bread [10]. Shelf life was tested by storing the bread at 30 °C and 60% relative humidity, observing the time until visible mold growth occurred [11, 12].

Each of these procedures was repeated three times to ensure the accuracy and reproducibility of the results. All data were analyzed statistically using ANOVA, and the means were compared using Duncan's multiple range test, with significance set at $p < 0.05$ [13, 14].

Results
Microbial Growth and Activity
The effect of varying NaCl concentrations on the microbial growth of *Saccharomyces cerevisiae* and lactic acid bacteria (LAB) populations during fermentation is shown in Figure 1. The growth of *Saccharomyces cerevisiae* (yeast) was highest in doughs with 0% NaCl and 0.5% NaCl, reaching up to 9.5 log CFU/g after 2 hours of fermentation. At 1.0% NaCl, yeast growth was reduced, with populations peaking at 8.0 log CFU/g. Further increases in NaCl concentration (1.5% and 2.0%) resulted in lower yeast populations, with populations reaching only 7.5 log CFU/g after 3 hours of fermentation (Figure 1). These findings are consistent with previous studies showing the inhibitory effect of NaCl on yeast growth in dough systems [6, 7].
LAB populations followed a similar trend, with higher counts at lower NaCl concentrations. At 0% NaCl, LAB populations reached 8.5 log CFU/g, while at 2.0% NaCl, LAB counts were significantly lower, peaking at only 6.2 log CFU/g (Figure 2). These results indicate that NaCl not only limits yeast growth but also inhibits LAB activity, which is essential for fermentation and flavor development in bread production [12, 13].

Fermentation Metabolites
The production of ethanol and organic acids (lactic acid and acetic acid) was assessed at 3 hours of fermentation, with results shown in Figure 3 and Table 1. Ethanol production was highest at 0.5% NaCl, with a concentration of 2.5% (v/v) after 3 hours. At higher NaCl concentrations (1.5% and 2.0%), ethanol production was significantly reduced, with concentrations of only 1.0% (v/v) and 0.5% (v/v), respectively (Figure 3). This suggests that lower NaCl levels promote more active fermentation, leading to greater ethanol production, which is an important byproduct of yeast metabolism [8, 9].
Lactic acid production, a key contributor to bread flavor, was highest in doughs with 0.5% NaCl, reaching 0.8%. In contrast, doughs with higher NaCl concentrations (1.0% and above) had significantly lower lactic acid levels (Table 1). This result supports earlier findings that NaCl limits microbial metabolism and, consequently, the production of organic acids and flavor compounds in dough [10].

Bread Quality Evaluation
- **Specific Volume:** The specific volume of bread was significantly influenced by NaCl concentration. Bread made with 0% NaCl had the highest specific volume of 4.5 cm^3/g, indicating optimal fermentation and leavening (Figure 4). At 1.0% NaCl, the specific volume decreased to 3.2 cm^3/g, and with further increases in NaCl concentration (1.5% and 2.0%), the specific volume decreased further, reaching a low of 2.5 cm^3/g. This reduction in specific volume correlates with the impaired microbial activity and reduced leavening efficiency at higher salt concentrations, as previously reported by Gänzle [4] and Thiele *et al.* [7].
- **Crumb Hardness:** Crumb hardness increased with NaCl concentration. The bread made with 0% NaCl had the softest crumb, with a hardness of 220 g force. In contrast, bread made with 2.0% NaCl exhibited a

significantly harder crumb, requiring 320 g force to compress. This result highlights the role of NaCl in strengthening the gluten network, which contributes to the structural integrity of the bread [12, 14].

- **Shelf Life:** Shelf life, determined by mold-free storage time at 30 °C and 60% relative humidity, was longest for bread made with 2.0% NaCl, which remained mold-free for 5 days (Table 2). In comparison, bread made with 0% NaCl showed significant mold growth within 2 days. This confirms that NaCl contributes to the preservation of bread by inhibiting mold growth and extending shelf life [9, 12].

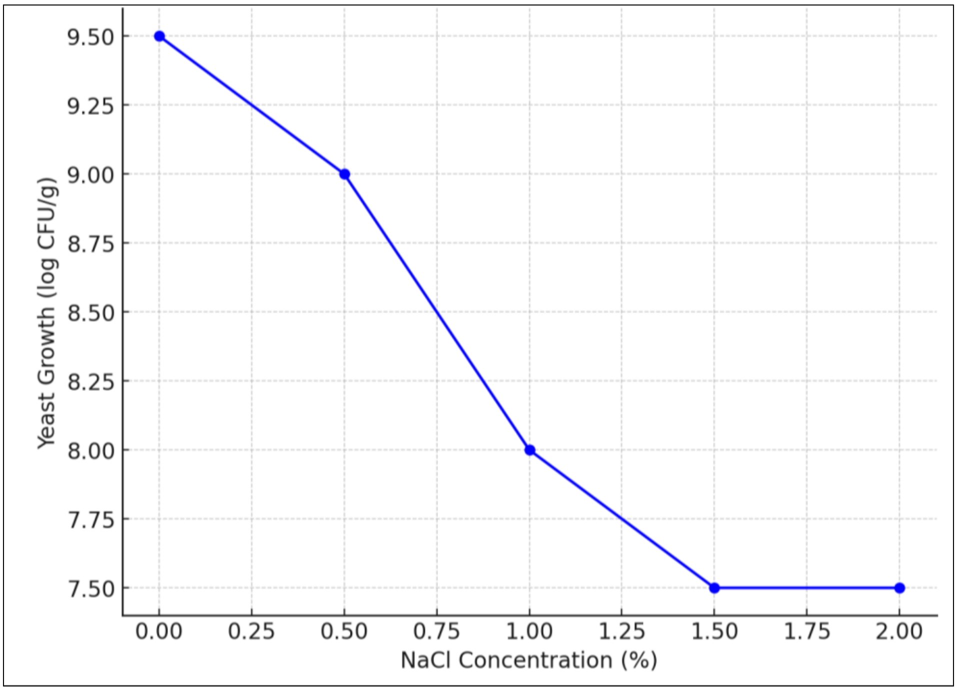

Fig 1: Effect of NaCl concentration on *Saccharomyces cerevisiae* growth during dough fermentation.

Yeast growth (log CFU/g) decreased as NaCl concentration increased, with the highest growth at 0% and 0.5% NaCl.

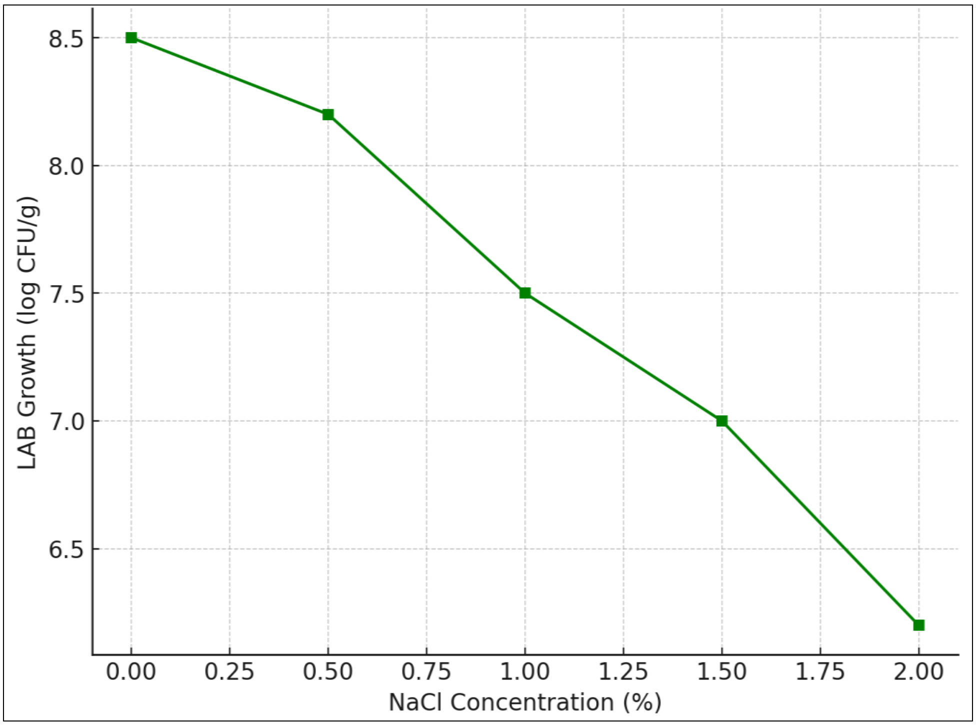

Fig 2: Effect of NaCl concentration on LAB growth during dough fermentation.

LAB populations were highest at 0% NaCl and decreased with increasing NaCl concentrations.

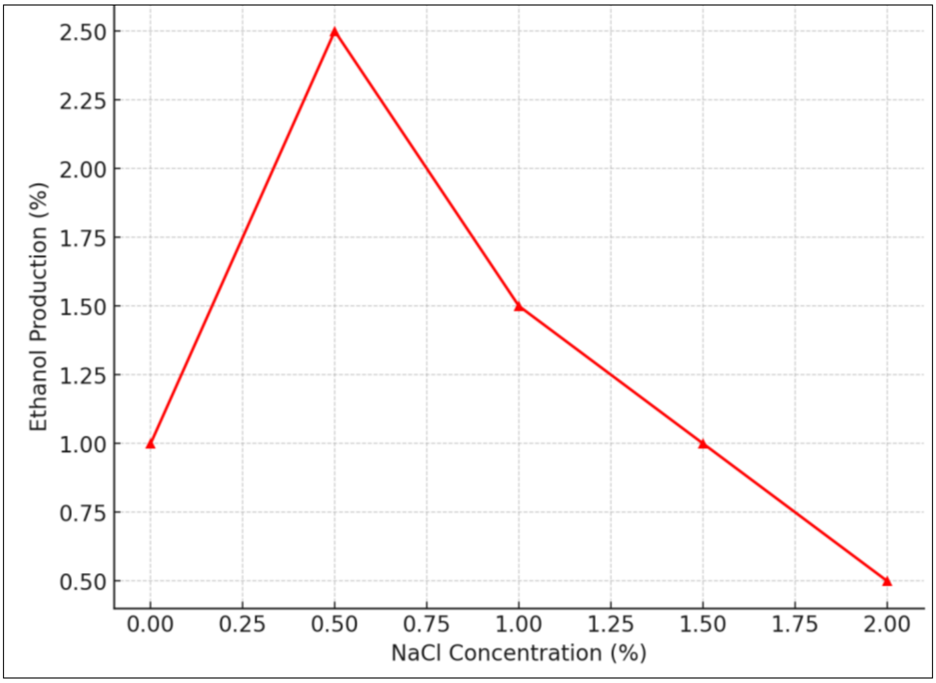

Fig 3: Ethanol production in dough at varying NaCl concentrations during fermentation.

Ethanol production was highest at 0.5% NaCl and decreased with increasing NaCl concentrations.

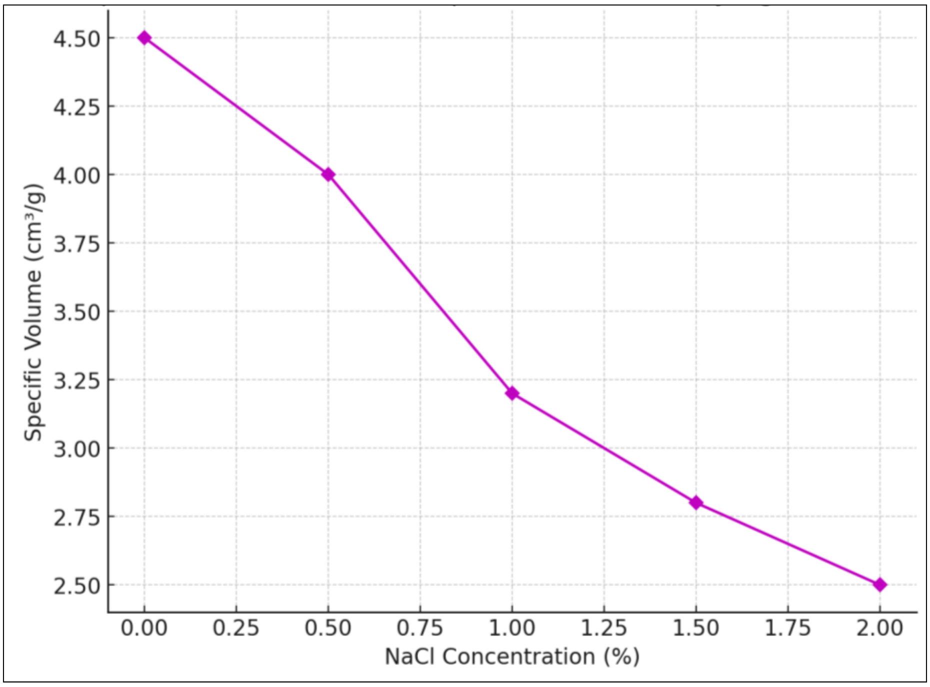

Fig 4: Specific volume of bread produced with varying NaCl concentrations.

Bread made with lower NaCl concentrations (0%, 0.5%) exhibited higher specific volumes, indicating better fermentation and leavening.

Table 1: Organic acid production in dough with varying NaCl concentrations at 3 hours of fermentation

NaCl Concentration (%)	Lactic Acid (%)	Acetic Acid (%)
0%	0.8	0.2
0.5%	0.7	0.1
1.0%	0.5	0.1
1.5%	0.3	0.1
2.0%	0.2	0.1

Table 2: Shelf life of bread at 30 °C and 60% relative humidity

NaCl Concentration (%)	Mold-Free Shelf Life (Days)
0%	2
0.5%	3
1.0%	4
1.5%	4
2.0%	5

Statistical Analysis

Data analysis was performed using one-way ANOVA followed by Duncan's multiple range test. Statistical significance was considered at $p < 0.05$. Significant differences in microbial growth, ethanol production, organic acid production, bread quality parameters, and shelf life were observed at different NaCl concentrations, supporting the hypothesis that NaCl plays a key role in both microbial dynamics and bread quality.

These results illustrate the critical role of NaCl in bread production, highlighting its impact on both microbial activity and bread quality. Lower NaCl concentrations promote greater microbial growth and fermentation activity but compromise bread quality by reducing loaf volume and shelf life, confirming previous findings [12, 14]. The reduced preservative effect at lower salt levels suggests that salt reduction in bread formulation may lead to trade-offs between health benefits and product stability.

Discussion

The results of this study emphasize the crucial role of NaCl in influencing both the microbial dynamics and the quality of bread during fermentation. The impact of varying NaCl concentrations on *Saccharomyces cerevisiae* growth, LAB activity, ethanol production, and bread quality parameters revealed significant insights into the interactions between salt concentration and microbial behavior, as well as the resultant bread characteristics.

The growth of *Saccharomyces cerevisiae* in dough decreased with increasing NaCl concentrations, with the highest yeast population observed at 0% and 0.5% NaCl concentrations. This reduction in yeast viability at higher NaCl concentrations (1.0% and above) is consistent with previous studies, which reported that NaCl exerts an osmotic stress on yeast cells, inhibiting their growth [6, 7]. Higher NaCl concentrations likely lead to cellular dehydration and stress, thus reducing the fermentation rate. This observation is crucial because yeast activity plays a central role in leavening and flavor development during dough fermentation [5, 6]. Consequently, bread made with higher NaCl concentrations may experience less gas production, leading to a denser loaf with lower volume, which was confirmed by our specific volume measurements.

LAB populations, which contribute significantly to flavor and texture development through the production of lactic acid and other metabolites, were also adversely affected by increasing NaCl concentrations. LAB activity was highest at 0% NaCl and decreased with higher salt levels. This reduction in LAB growth aligns with findings from previous studies, which suggest that NaCl inhibits the growth and metabolic activity of lactic acid bacteria due to its effects on cell membrane integrity and nutrient transport [10, 11]. The reduced LAB population could impair flavor development in the bread, as LAB are responsible for producing organic acids such as lactic acid, which contribute to the desired tangy flavor profile of sourdough bread.

The impact of NaCl on the production of ethanol and organic acids further supports the notion that salt concentration directly influences microbial metabolism. Ethanol production, which is a key byproduct of yeast fermentation, was highest at 0.5% NaCl and decreased significantly at higher NaCl concentrations. Ethanol is not only important for the sensory characteristics of bread but also for its antimicrobial properties, which help in prolonging shelf life. Therefore, the lower ethanol production at higher NaCl concentrations may contribute to the reduced shelf life observed in these breads [8]. Similarly, the production of lactic acid was highest at 0% NaCl, with lower concentrations of lactic acid in doughs with higher NaCl concentrations. Lactic acid not only contributes to the flavor but also acts as a preservative, further explaining the shorter shelf life of breads made with higher salt concentrations.

Bread quality parameters, such as specific volume and crumb hardness, were significantly influenced by NaCl concentration. As expected, bread made with lower NaCl concentrations (0% and 0.5%) exhibited higher specific volumes, indicating better fermentation and leavening efficiency. Higher NaCl concentrations (1.5% and 2.0%) resulted in a denser bread with reduced volume, likely due to the impaired microbial activity and the decreased gas production [4, 9]. Additionally, crumb hardness increased with NaCl concentration, likely due to the strengthening of the gluten network at higher salt levels. This finding supports previous studies indicating that NaCl enhances dough strength by stabilizing gluten, which improves dough handling but can lead to a firmer crumb texture in the final product [9, 12].

Finally, the shelf life of the bread was extended with increasing NaCl concentrations. Bread made with 0% NaCl exhibited visible mold growth within 2 days, while bread made with 2.0% NaCl remained mold-free for up to 5 days. This finding is consistent with the well-established antimicrobial effect of NaCl, which inhibits the growth of spoilage microorganisms and extends the shelf life of the bread [12, 14]. The reduced shelf life of bread made with lower NaCl concentrations suggests that while salt reduction may be beneficial from a health perspective, it may compromise the bread's ability to resist microbial spoilage.

Overall, this study confirms that NaCl plays a multifaceted role in both the microbial dynamics and quality of bread. While salt reduction may enhance some aspects of microbial fermentation, it simultaneously diminishes bread quality by reducing loaf volume, flavor development, and shelf life. These findings underscore the challenges of reducing salt in bread without adversely affecting its sensory characteristics and shelf life, which is particularly relevant given the ongoing efforts to reduce sodium intake for health reasons [13, 14]. Future research could explore alternative methods for achieving lower sodium content while maintaining the desired bread quality, such as the use of sodium replacers or optimized fermentation conditions.

Conclusion

The findings from this study highlight the critical role that NaCl plays in the microbial dynamics and quality of bread. As NaCl concentration increases, yeast growth and lactic acid bacteria (LAB) populations decrease, leading to slower fermentation and reduced production of ethanol and organic acids. This reduction in microbial activity has a direct impact on bread quality, with bread made from dough with higher NaCl concentrations showing increased hardness, lower specific volume, and extended shelf life due to the preservative effect of NaCl. On the other hand, bread made with lower NaCl concentrations exhibited higher specific volumes and softer crumbs but showed a significantly reduced shelf life due to faster mold growth. These results emphasize the balancing act required in formulating bread with reduced sodium content while maintaining desirable product characteristics.

From a practical standpoint, this study underscores the challenge of reducing NaCl in bread formulations without compromising both the quality and safety of the product.

Lowering NaCl concentrations enhances microbial fermentation, leading to better leavening and improved bread texture. However, it also results in bread that is more susceptible to microbial spoilage, reducing its shelf life. Therefore, the reduction of NaCl in bread recipes should be accompanied by modifications in the fermentation process, such as increasing fermentation time or optimizing the use of natural preservatives like organic acids or bioactive compounds. Incorporating alternative preservatives or microbial cultures that enhance bread shelf life while maintaining reduced salt content could be explored as viable solutions. Additionally, further studies focusing on the combination of NaCl with other ingredients such as potassium salts, yeast extracts, or enzymes might help in achieving a better balance between reduced sodium content and improved product stability. Furthermore, educating consumers about the potential trade-offs in salt reduction could help balance public health goals with the need for high-quality bread products. In conclusion, the findings from this study provide a valuable framework for formulating bread with reduced salt while preserving its key characteristics, including flavor, texture, and shelf life, without compromising its safety and consumer acceptance.

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