**MAKERERE UNIVERSITY**


**COLLEGE OF NATURAL SCIENCES**

**SCHOOL OF BIOSCIENCES**

**DEPARTMENT OF PLANT SCIENCES, MICROBIOLOGY AND BIOTECHNOLOGY**


# PERFORMANCE OF ROSEMARY EXTRACT AND LEMON JUICE COMPARED TO SYNTHETIC CALCIUM PROPIONATE AS BREAD PRESERVATIVES.


**BY**

**ALEMA HAROLD**

**22/U/5733**

**SUPERVISOR**

**DR. ESTHER KATUURA**


**A REPORT SUBMITTED TO THE DEPARTMENT OF PLANT SCIENCES, MICROBIOLOGY AND BIOTECHNOLOGY, COLLEGE OF NATURAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY OF MAKERERE UNIVERSITY**

**JULY, 2025**

# ACADEMIC SUPERVISOR'S APPROVAL

This research report titled "Performance of rosemary extract and lemon juice compared to synthetic calcium propionate as bread preservatives" has been reviewed and approved by my supervisor.

ALEMA HAROLD

Signature....................................

Date..24th September 2025

**SUPERVISOR**

DR. ESTHER KATUURA

Signature....................................

Date.................................25/09/2025

# TABLE OF CONTENTS

Contents

ACADEMIC SUPERVISOR'S APPROVAL ..... ii

TABLE OF CONTENTS ..... iii

CHAPTER ONE ..... 1

1.0 INTRODUCTION ..... 1

1.1 BACKGROUND ..... 1

1.2 Problem Statement ..... 2

1.3 Objectives of the Study ..... 2

1.31 Specific objectives ..... 2

1.4 Justification ..... 3

1.5 Significance ..... 3

1.6 Hypothesis ..... 3

1.7 Scope of the study ..... 4

CHAPTER TWO ..... 5

2.0 LITERATURE REVIEW ..... 5

2.1 Bread shelf life ..... 5

2.2 The bread mold ..... 6

2.3 Calcium propionate as a synthetic preservative ..... 7

2.4 Natural preservatives ..... 8

2.4.1 Rosemary Extract as a bread preservative ..... 9

2.4.2 Lemon Juice as a bread preservative ..... 10

CHAPTER THREE ..... 11

3.0 MATERIALS AND METHODS ..... 11

3.1 Experimental Design ..... 11

3.2 Study Area ..... 11

3.3 Materials for the study ..... 11

3.4 Procedure ..... 12

3.5 Data Collection ..... 12

3.6 Data Analysis ..... 14

CHAPTER FOUR ..... 15

4.0 RESULTS ..... 15

4.1 Dough test results ........................................................ 15
4.12 Results for bread treated with Lemon ........................................................ 15
4.13 Results for bread treated with Rosemary extract ........................................................ 17
4.2 Sensory Evaluation Results Based on Panelist Preferences ........................................................ 18
4.3 Image J results ........................................................ 20
CHAPTER FIVE ........................................................ 21
5.0 DISCUSSION OF RESULTS ........................................................ 21
5.1 Discussion for Dough test results ........................................................ 21
5.2 Discussion for Sensory Evaluation Results ........................................................ 22
5.3 Discussion of Image J results ........................................................ 24
CHAPTER SIX ........................................................ 25
6.0CONCLUSION AND RECOMMENDATIONS ........................................................ 25
6.1 Conclusion ........................................................ 25
Recommendations ........................................................ 25
REFERENCES ........................................................ 27

# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 BACKGROUND

The quest for preserving food while maintaining its quality has led to the exploration of natural alternatives to synthetic preservatives. In the realm of bread production, calcium propionate has been a widely used synthetic preservative due to its effectiveness in inhibiting mold growth(Alam et al., 2010). However, with the rising consumer demand for clean-label products and natural ingredients, there is a growing interest in utilizing natural preservatives such as rosemary extract and lemon juice (Tzima et al., 2015). These natural alternatives not only extend the shelf life of bread but also align with health-conscious consumer preferences. Bread is a staple food consumed globally, and its preservation is crucial for reducing food waste and ensuring safety. Traditional preservatives like calcium propionate work by disrupting the metabolism of mold spores, thereby preventing spoilage (Garcia & Copetti, 2019). However, concerns regarding the long-term health effects of synthetic additives have prompted researchers and manufacturers to seek safer alternatives. Natural preservatives, derived from plant extracts and organic acids, offer a promising solution. For instance, rosemary extract contains carnosic acid, which has demonstrated antimicrobial properties effective against various spoilage organisms (Bakerpedia, n.d.). Similarly, lemon juice, rich in citric acid, not only enhances flavor but also lowers pH levels, creating an unfavorable environment for microbial growth (Chemsino, 2024). The use of rosemary extract and lemon juice as natural preservatives presents several advantages. These ingredients are not only effective at prolonging shelf life but also enhance the sensory qualities of bread. The incorporation of rosemary can impart a unique flavor profile while providing antioxidant benefits (Whole Grain 100, n.d.). Lemon juice contributes acidity that can improve the overall taste and texture of bread while acting as a natural preservative (Reddit AskCulinary, 2022). Moreover, the shift towards natural preservatives aligns with consumer trends favoring healthful eating habits and transparency in food labeling. In conclusion, as the food industry evolves towards more sustainable practices, the exploration of natural preservatives such as rosemary extract and lemon juice represents a significant step forward. By replacing synthetic calcium propionate with these natural alternatives, bread manufacturers can meet consumer demands for healthier options without compromising product quality or safety.

## 1.2 Problem Statement

The widespread use of synthetic preservatives like calcium propionate in bread production, while effective in inhibiting microbial spoilage and extending shelf life (Belz et al., 2012; Phechkrajang & Yooyong, 2016), faces increasing scrutiny due to consumer preference for natural and clean-label alternatives (Namanya Jonan, 2024). Concerns regarding potential health implications associated with synthetic additives (Ossenkopp et al., 2012; Arooj Attique, 2018) have driven the search for natural preservatives that can offer comparable efficacy without the perceived drawbacks. Rosemary extract, rich in antioxidant and antimicrobial compounds such as carnosic and rosmarinic acids (Ataman Kimya, n.d.), and lemon juice, containing citric and ascorbic acids known for their antimicrobial properties (Teixeira et al., 2019; EFSA, 2015), have emerged as promising natural alternatives. However, their performance in bread preservation, particularly in direct comparison to calcium propionate, requires thorough investigation to determine their optimal application levels and impact on bread quality attributes. Therefore, this study seeks to address the gap in knowledge regarding the comparative efficacy of rosemary extract and lemon juice against synthetic calcium propionate in preserving bread and maintaining its quality characteristics throughout its shelf life.

## 1.3 Objectives of the Study

The primary objective of this study is to evaluate the performance of rosemary extract and lemon juice compared to synthetic calcium propionate as bread preservatives.

### 1.31 Specific objectives

1. To determine the optimal concentrations of rosemary extract and lemon juice for the best dough quality.

2. To assess the impact of rosemary extract and lemon juice on the texture, aroma, taste and tenderness of bread compared to calcium propionate.

3. To Compare Shelf Life of bread treated with rosemary extract and lemon juice against that of bread treated with calcium propionate.

## 1.4 Justification

The growing consumer demand for natural and clean-label food products, coupled with the need for effective and safe alternatives to synthetic preservatives in the baking industry is a key driver for the study. While calcium propionate is a widely used and effective synthetic preservative, concerns regarding its potential health implications and consumer preference for natural ingredients necessitate the exploration of plant-based alternatives. Rosemary extract and lemon juice possess inherent antimicrobial and antioxidant properties, suggesting their potential as natural preservatives in bread. Evaluating their performance against a benchmark synthetic preservative like calcium propionate will provide valuable insights into their efficacy in extending shelf life, maintaining bread quality, and meeting consumer expectations for more natural food options. This research contributes to the development of healthier and more sustainable food preservation strategies.

## 1.5 Significance

Firstly, the study addresses the increasing consumer preference for natural and minimally processed foods by investigating plant-derived alternatives to synthetic preservatives in a staple food like bread. Successful identification of rosemary extract and/or lemon juice as effective preservatives could offer the baking industry natural solutions to extend product shelf life and reduce food waste, aligning with sustainability goals. Secondly, the study contributes to scientific knowledge by comparatively evaluating the preservative efficacy of these natural extracts against a well-established synthetic compound, providing valuable data on their antimicrobial and antioxidant mechanisms in a food matrix. This information can inform future research and development of other natural food preservation strategies. Ultimately, the findings could lead to the production of bread with a cleaner ingredient list, potentially appealing to a wider consumer base and contributing to healthier dietary choices without compromising food safety or quality.

## 1.6 Hypothesis

Null Hypothesis 1 (HO): There is no significant impact of rosemary extract and lemon juice on the texture, aroma, taste and tenderness of bread.

Null Hypothesis 2 (HO): There is no significant difference in the shelf life of bread treated with rosemary extract and lemon juice compared to bread treated with synthetic calcium propionate.

## 1.7 Scope of the study

The scope of this study is limited to evaluating the effectiveness of rosemary extract and fresh lemon juice as bread preservatives in comparison to commercially available synthetic calcium propionate. The investigation will focus on assessing the impact of these preservatives on the shelf life of bread, specifically by monitoring changes in microbial load (mold), and selected quality parameters such as texture, color, and potentially aroma over a defined storage period under specific environmental conditions.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 Bread shelf life

The shelf life of bread, defined as the period during which it remains acceptable to consumers in terms of safety, nutritional value, and sensory attributes, is a critical factor in the baking industry, influencing production planning, distribution, and ultimately, food waste reduction (Cauvain & Young, 2007). Several intrinsic and extrinsic factors contribute to the deterioration of bread quality over time. The inherent composition of bread, including the type of flour, water activity, pH, and the presence of specific ingredients, plays a significant role in its susceptibility to spoilage (Ribotta et al., 2004). For instance, higher water activity levels provide a more favorable environment for microbial growth, while the nutritional content offers substrates for their proliferation (Legan, 1993).

Microbial spoilage is a primary cause of bread deterioration, significantly limiting its shelf life. Molds, particularly those belonging to the *Penicillium*, *Aspergillus*, and *Rhizopus* genera, are commonly found on stale bread, leading to visible growth, off-flavors, and potential health hazards due to mycotoxin production (Pitt & Hocking, 2009; Dal Bello et al., 2007). Bacteria, including *Bacillus* species, can also contribute to spoilage, causing ropiness, a slimy and sticky texture in the crumb, and unpleasant odors (Thompson et al., 1998). The initial microbial load of the raw materials and the hygiene practices during production and packaging significantly influence the rate and extent of microbial spoilage (Ledenbach & Marshall, 2009).

Beyond microbial spoilage, physicochemical changes also contribute to the perceived staleness of bread. Starch retrogradation, the recrystallization of amylopectin, is a major factor leading to crumb firming and a loss of elasticity (Gray & Bemiller, 2003). This process is time and temperature-dependent, occurring most rapidly at refrigeration temperatures (Baik & Chinachoti, 2000). Moisture migration within the loaf and between the bread and its environment also contributes to staling, leading to a dry crust and a firm, less palatable crumb (Heenan et al., 2004). Furthermore, lipid oxidation can occur, particularly in breads containing higher fat content or bran, resulting in rancid off-flavors and a reduction in overall quality (Frankel, 2005).

Various strategies are employed to extend the shelf life of bread, including the use of preservatives, modified atmosphere packaging, freezing, and improvements in hygiene and processing techniques (Smith et al., 2004). Chemical preservatives, such as calcium propionate, potassium sorbate, and sodium benzoate, are widely used due to their effectiveness in inhibiting the growth of molds and bacteria (Branen et al., 2002). However, as previously discussed, consumer demand for natural alternatives is increasing, prompting research into plant-derived compounds with antimicrobial and antioxidant properties (Carocho & Ferreira, 2013). Understanding the complex interplay of factors affecting bread shelf life is crucial for developing effective and consumer-acceptable preservation strategies.

### 2.2 The bread mold

Bread mold, a common cause of spoilage in baked goods, primarily refers to filamentous fungi that thrive on the nutrient-rich and moist environment of bread (Pitt & Hocking, 2009). While various mold species can colonize bread, the most frequently encountered genera include *Rhizopus*, *Penicillium*, *Aspergillus*, and *Mucor* (Legan, 1993; Samson et al., 2014). These ubiquitous microorganisms are naturally present in the air, soil, and even on surfaces within food processing environments, making their contamination of bread a significant challenge in maintaining product quality and extending shelf life (Filtenborg et al., 1996). The visible growth of mold on bread not only renders it unappetizing but can also pose health risks due to the potential production of mycotoxins, secondary metabolites with toxic effects upon ingestion (Bennett & Klich, 2003).

The life cycle of bread mold, characteristic of filamentous fungi, involves both asexual and sexual reproduction, although asexual reproduction is the primary mode of propagation responsible for rapid colonization on bread surfaces (Alexopoulos et al., 1996). Asexual reproduction typically begins with the germination of spores, microscopic propagules that are easily dispersed through air currents. Upon landing on a suitable substrate like bread, and under favorable conditions of temperature, humidity, and nutrient availability, these spores germinate to form hyphae, thread-like filaments that represent the vegetative growth of the fungus (Griffin, 1994). The hyphae elongate and branch, forming a network called a mycelium, which spreads across the bread surface and penetrates its structure to absorb nutrients.

Asexual reproduction continues through the formation of new spores. This process varies depending on the specific mold genus. For instance, *Penicillium* species produce conidia, asexual spores, borne externally on specialized hyphal structures called conidiophores, often giving the mold a characteristic powdery or brush-like appearance (Samson et al., 2014). *Aspergillus* also produces conidia from conidiophores, which can be arranged in a more complex structure, sometimes forming a distinct head. In contrast, *Rhizopus* species reproduce asexually through sporangiospores, which are formed within sac-like structures called sporangia, held aloft by sporangiophores. When the sporangia mature, they rupture, releasing numerous spores into the environment, facilitating further contamination (Pelczar et al., 1993).

While less frequently observed on the surface of visibly moldy bread, sexual reproduction can occur under certain environmental or nutritional stress conditions. This process involves the fusion of compatible nuclei from two different mating types, leading to the formation of sexual spores with genetic recombination (Moore et al., 2011). For example, *Rhizopus* species can undergo sexual reproduction to produce zygospores, thick-walled resting spores that can survive unfavorable conditions. *Penicillium* and *Aspergillus* species also have sexual stages, although they may not be commonly encountered in the context of bread spoilage. The resulting sexual spores can contribute to the genetic diversity of the mold population, potentially influencing their ability to adapt to different environmental conditions or preservation strategies (Bennett & Klich, 2003). Understanding the life cycle of bread molds, particularly their efficient asexual reproduction through spore dispersal and germination, is crucial for developing effective strategies to prevent their growth and extend the shelf life of bread products.

## 2.3 Calcium propionate as a synthetic preservative

Calcium propionate ($Ca(C_3H_5O_2)_2$), the calcium salt of propionic acid, is a widely employed synthetic preservative in the baking industry, recognized for its effectiveness in inhibiting the growth of molds and certain bacteria in bread and other baked goods (Sofos, 1989; коды пищевых добавок, n.d.). Its efficacy stems from the propionate ion, which at the acidic pH typically found in bread dough and the early stages of spoilage, exists predominantly in its undissociated form (propionic acid). This undissociated form can readily penetrate the cell membranes of microorganisms, disrupting their internal pH, interfering with enzyme function, and ultimately inhibiting their growth and reproduction (Russell & Chopra, 1996).

The primary mechanism of action of calcium propionate against molds involves the inhibition of key metabolic pathways. Propionic acid is thought to interfere with the citric acid cycle (Krebs cycle), a central metabolic pathway for energy production in fungi (Dziezak, 1991). By disrupting this cycle, calcium propionate effectively prevents the mold from obtaining the energy necessary for growth and spore germination. While calcium propionate is generally more effective against molds and some bacteria, its activity against yeasts is less pronounced, which is advantageous in leavened baked goods where yeast activity is essential for dough development (De Lacey et al., 2000).

Calcium propionate is typically added to bread dough during the mixing stage, and its effectiveness is influenced by factors such as the dosage level, the pH of the dough, and the overall formulation of the bread (Kabaca, 2007). Regulatory bodies worldwide, including the Food and Drug Administration (FDA) in the United States and the European Food Safety Authority (EFSA) in Europe, have generally recognized calcium propionate as safe for use in food at permitted levels (EFSA, 2011; FDA, 2023). These evaluations consider toxicological studies and establish acceptable daily intakes to ensure consumer safety.

Despite its widespread use and recognized efficacy, concerns regarding the use of synthetic food additives, including calcium propionate, have been raised by some consumers who prefer foods with natural ingredients (Namanya Jonan, 2024). While scientific evidence regarding significant adverse health effects at typical usage levels is limited, this consumer sentiment drives the ongoing research into natural alternatives for food preservation. Furthermore, the effectiveness of calcium propionate can be somewhat pH-dependent, requiring relatively acidic conditions for optimal activity, which might not always be ideal for certain bread formulations or storage conditions. Understanding the mechanisms of action, regulatory status, and consumer perceptions of calcium propionate is crucial when evaluating potential natural alternatives for bread preservation.

## 2.4 Natural preservatives

Driven by consumer demand for cleaner labels and concerns regarding synthetic additives, the exploration of natural preservatives for food applications has gained significant momentum (Carocho & Ferreira, 2013; Decker, 2009). These natural alternatives, often derived from plants, animals, or microorganisms, possess inherent antimicrobial and antioxidant properties that can contribute to food preservation (Davidson & Branen, 2005). Examples include essential oils from herbs and spices like rosemary, thyme, and oregano, which contain bioactive compounds with broad-spectrum antimicrobial activity (Burt, 2004).

### 2.4.1 Rosemary Extract as a bread preservative

Rosemary (*Salvia rosmarinus* L.) extract has garnered significant attention as a promising natural preservative for various food products, including bread, due to its potent antioxidant and antimicrobial properties (Botsoglou et al., 2003; Moreno et al., 2006). These beneficial effects are primarily attributed to its rich composition of phenolic compounds, particularly carnosic acid and rosmarinic acid, which are known for their strong radical-scavenging and antimicrobial activities (Schwarz et al., 1992; Valeriano et al., 2005). Carnosic acid, a diterpene, is considered one of the most potent natural antioxidants, capable of inhibiting lipid oxidation, a major cause of quality deterioration and off-flavor development in bread, especially those containing fats or whole grains (Frankel, 1996). Rosmarinic acid, a phenolic acid ester, also contributes significantly to the antioxidant capacity and exhibits antimicrobial activity against a range of foodborne pathogens and spoilage microorganisms, including molds and bacteria commonly found in bread (Oboh et al., 2010).

The application of rosemary extract in bread production aims to extend shelf life by inhibiting both oxidative rancidity and microbial growth. Studies have investigated the effectiveness of rosemary extract in maintaining the sensory quality and reducing microbial load in bread during storage. For instance, some research has shown that the incorporation of rosemary extract can significantly delay the onset of mold growth and reduce the levels of total aerobic bacteria and fungi in bread compared to control samples without preservatives or even those with lower concentrations of synthetic preservatives (Martínez et al., 2012). The antioxidant properties of rosemary extract also help to preserve the color and flavor of the bread by preventing the oxidation of lipids and other susceptible compounds (Hsu et al., 2003).

The optimal concentration of rosemary extract required for effective preservation in bread can vary depending on factors such as the specific formulation of the bread, the processing conditions, and the desired shelf life (Sánchez-Escalante et al., 2003).

### 2.4.2 Lemon Juice as a bread preservative

Lemon juice, the aqueous extract of lemon fruit (*Citrus limon*), has been explored as a potential natural preservative in food systems, including bread, due to its inherent antimicrobial and antioxidant properties. The primary active components responsible for these effects are citric acid, a weak organic acid that can lower the pH of the food environment, and ascorbic acid (vitamin C), a well-known antioxidant (Penniston et al., 2008). The acidic nature of lemon juice, primarily due to citric acid, creates an unfavorable environment for the growth of many spoilage microorganisms, particularly molds and some bacteria, by disrupting their cellular functions and enzymatic activities (Lambert et al., 2001).

The antimicrobial action of citric acid involves the permeabilization of microbial cell membranes and the inhibition of key metabolic enzymes, ultimately leading to cell death or growth inhibition (Raybaudi-Massilia et al., 2009). Ascorbic acid, while also contributing to a lower pH, acts primarily as an antioxidant by scavenging free radicals and inhibiting oxidative reactions that can lead to the deterioration of food quality, including flavor and color changes in bread (Davey et al., 2000). The combination of these compounds in lemon juice offers a dual mechanism for preservation, addressing both microbial spoilage and oxidative degradation.

However, the use of lemon juice as a bread preservative also presents certain considerations. The high acidity of lemon juice can potentially affect the rheological properties of the dough and the final texture and flavor profile of the bread (Akhtar et al., 2008). Therefore, optimizing the concentration of lemon juice is crucial to achieve effective preservation without negatively impacting the sensory quality of the bread. Furthermore, the effectiveness of lemon juice might be enhanced when used in combination with other preservation methods or natural antimicrobials to provide a broader spectrum of protection against spoilage microorganisms. Future research could focus on optimizing the application methods of lemon juice in bread production, exploring its synergistic effects with other natural preservatives, and assessing its impact on the overall consumer acceptability of the final product.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Experimental Design

The study employed a three-phase approach to evaluate the effectiveness of natural preservatives rosemary extract and lemon juice in comparison to calcium propionate. For dough optimization, a central composite design was used to test varying concentrations of rosemary extract and lemon juice. Response surface methodology was applied to identify optimal combinations based on measurements of dough characteristics including softness/firmness/stickiness, gluten development, first proof, percentage rise, texture after proofing, final proof, rise time, oven spring, rise after baking, visual volume, crumb texture, crust color, and flavor. Formulations that achieved ≥85% sensory acceptance in preliminary taste panels were prioritized.

Shelf-life assessment was conducted by storing bread samples under standardized conditions (25°C, 65% RH) with daily monitoring. Mold visibility thresholds were established using digital image analysis to quantify surface mycelium coverage.

Sensory evaluation was carried out using triangle tests with 10 panelists to identify significant differences between experimental and control groups. A Likert scale was used to quantify consumer acceptance across aroma, taste, and texture attributes. Statistical analysis was performed using mixed-effects models to account for batch variations, with significance determined at $p<0.05$.

## 3.2 Study Area

The study was conducted in a home-based kitchen environment. This setting provided a controlled, albeit non-industrial, space for the preparation and initial storage of bread samples treated with different preservatives. Subsequent storage and analysis of the bread samples were carefully managed to maintain consistency across treatment groups within this home-based setting.

## 3.3 Materials for the study

The materials and equipment utilized in this study included all-purpose or whole wheat flour, water, active dry yeast or instant yeast, salt, optional sugar, and optional fat (such as butter or oil). Key preservatives tested were rosemary extract, lemon juice, and synthetic calcium propionate.

The preparation and baking processes were carried out using an oven, mixing bowls, measuring cups and spoons, a kitchen scale, a dough scraper, and baking pans.

### 3.4 Procedure

A standard white bread recipe was prepared and the dough was divided into four equal portions following primary fermentation. The first portion served as the control, with no added preservative. The second portion was treated with a predetermined concentration of commercially available rosemary extract, the third with a specific volume of freshly squeezed and filtered lemon juice, and the fourth with a calculated amount of synthetic calcium propionate. All preservatives were incorporated during the final mixing stage. Each dough portion was then proofed, baked under consistent oven conditions, and allowed to cool completely. Once cooled, the bread loaves from each treatment group were sliced and packaged in sterile polyethylene bags, then stored under controlled ambient conditions, mimicking typical household storage. Samples from each treatment group were collected daily for analysis. These analyses included visual assessment for mold growth, microbial analysis, and instrumental evaluation of texture (firmness) and color for both the crust and crumb. The data collected was statistically analyzed to compare the effectiveness of rosemary extract and lemon juice in preserving bread quality and inhibiting microbial spoilage relative to the synthetic calcium propionate and the control group over the storage duration of 1 week.

### 3.5 Data Collection

For objective one, to determine the optimal concentrations of rosemary extract and lemon juice to use in bread making, making four replicates of dough per preservative. Firstly, for lemon juice, first replicate having no preservative, second replicate having 1.6% of lemon juice, third replicate having 3% and fourth having 6% of lemon juice. Then also make four replicates of dough with rosemary extract. First replicate being the control sample with no preservative, second replicate with 2.4% of rosemary extract, third with 3.6% and the fourth with 8%. To determine effects after baking. Then make an equal number of replicates of dough treated with each preservative with the control group having no preservative and then bake.

For objective two, to assess consumer preferences, a sensory evaluation was conducted involving ten panelists who rated the texture, aroma, taste, and tenderness of bread samples. Four groups were assessed: bread without any preservative (control), bread treated with calcium propionate, bread treated with lemon juice, and bread treated with rosemary extract. Ratings were recorded using a Likert scale ranging from 1 to 10, where 1 indicated the least preferred and 10 the most preferred. All data were compiled and organized in an Excel sheet for subsequent analysis.

**A. Sensory evaluation**

Tick (✓) your choice [1 (Strongly Disagree; 5 (Strongly Agree)]

1. The bread has a desirable aroma

1 2 3 4 5 6 7 8 9 10

2. The bread has a good taste

1 2 3 4 5 6 7 8 9 10

3. The bread is tender.

1 2 3 4 5 6 7 8 9 10

4. The bread has a good texture.

1 2 3 4 5 6 7 8 9 10

For objective three, to compare the shelf life of bread treated with rosemary extract and lemon juice against bread treated with calcium propionate, four bread samples per preservative were baked. The first group consisted of control samples with no preservative, the second group contained four bread samples treated with calcium propionate, the third group included four samples treated with lemon juice, and the fourth group comprised bread samples treated with rosemary extract. All bread samples were cut down the middle to expose the inner crumb and stored for one week under ambient conditions. After the storage period, high-resolution photographs of all samples in each group were captured for mold analysis using ImageJ software.

### 3.6 Data Analysis

For Objective One, dough samples were evaluated for handling characteristics, including softness, firmness, and stickiness, as well as gluten development, first proof, percentage rise, texture after proofing, final proof, rise time, oven spring, rise after baking, visual volume, crumb texture, crust color, and flavor under each preservative concentration. Comparative analysis was carried out and final notes were deduced to identify the concentration that produced the best dough quality.

For Objective Two, sensory evaluation results from ten panelists were analyzed using One-way ANOVA in R Studio to determine the mean scores for texture, aroma, taste, and tenderness across the four bread treatment groups.

For Objective Three, mold coverage on each bread sample replicate was measured after one week using ImageJ software. The data was subsequently entered into Excel, and R Studio was used to calculate the mean percentage of mold growth observed on the bread surfaces under each preservative treatment.

**Analysis of Results using Image J.**

1. Launch ImageJ: Open ImageJ on your computer.

2. Open Your Bread Image: Go to File > Open… and select the high-resolution image of your bread sample.

3. Convert to Grayscale: Go to Image > Type > 8-bit to simplify color into grayscale.

4. Adjust Image Contrast:

Go to Image > Adjust > Brightness/Contrast.

Use the sliders to make the mold areas more visually distinct from the bread surface.

Click apply when mold is visually distinct.

5. Apply Threshold:

Go to Image > Adjust > Threshold.

A red overlay will appear on the selected areas (mold growth).

Adjust the minimum and maximum sliders until only mold areas are highlighted.

Choose Light background and then click Apply to binarize the image (convert to black and white).

6. Analyze Particles (Measure Mold Area)

Go to Analyze > Analyze Particles.

Set the Size to exclude small noise.

Check the boxes for: Display Results and Summarize.

Click "OK"

ImageJ will then output: Individual particle areas and a summary with total area and % coverage

7. Record Total Mold Coverage

Use the Total Area (mold) and Image Area (whole bread surface) to compute:

% Mold Coverage = (Total Mold Area / Bread Surface Area) × 100

# CHAPTER FOUR

# 4.0 RESULTS

## 4.1 Dough test results

### 4.12 Results for bread treated with Lemon

**Dough Formula Per Sample**

Four dough samples were kneaded with control group having 0% of lemon juice then samples 1, 2 and 3 having 1.6%, 3% and 6% respectively. A dough test was then performed to assess the optimum lemon concentration required.

| Ingredient | Control | Sample 1 (1.6%) | Sample 2 (3%) | Sample 3 (6%) |
|---|---|---|---|---|
| Flour | 500g | 500g | 500g | 500g |
| Water | 300g | 292g | 285g | 270g |
| Yeast (dry) | 10g | 10g | 10g | 10g |
| Salt | 8g | 8g | 8g | 8g |
| Sugar | 20g | 20g | 20g | 20g |

| | | | | |
|---|---|---|---|---|
| Fat (e.g., oil) | 20g | 20g | 20g | 20g |
| Lemon Juice | 0g | 8g | 15g | 30g |

### Table showing results from dough test for samples containing lemon juice

| Stage | Control | Sample 1 (1.6%) | Sample 2 (3%) | Sample 3 (6%) |
|---|---|---|---|---|
| Dough Handling (mixing & kneading) | Smooth, elastic | Slightly softer, easy to knead | Soft, a bit sticky | Very soft, slightly sticky |
| Soft/Firm/Sticky? | Firm and well-structured | Soft and smooth | Soft, slightly sticky | Sticky and weak structure |
| Gluten Development (windowpane test?) | Good stretch | Good stretch | Slightly weaker | Weak, tears easily |
| First Proof (1hr) | Doubled in size | Slightly more rise | Moderate rise | Slower rise |
| Rise (%) | 100% | 110% | 90% | 70% |
| Texture after proofing | Puffy and airy | Puffier | Less airy | Dense and soft |
| Final Proof (after shaping) | Normal rise | Good rise | Slightly reduced | Weak rise |
| Rise time (min) | 45 | 45 | 50 | 60 |
| Oven spring (during baking) | Excellent | Good | Moderate | Poor |
| After Baking | Well risen | Slightly taller | Slightly smaller | Low volume |
| Volume (visual estimate) | High | High | Medium | Low |
| Crumb texture | Open and even | Open and light | Tighter crumb | Very tight, slightly gummy |
| Crust color | Golden brown | Slightly darker | Even, lighter | Pale crust |
| Flavor | Balanced | Bright, slightly tangy | Noticeably tangy | Overly sour, less balanced |

| Final Notes | Standard performance | Lemon improved yeast action slightly | Flavorful but affected rise | Too much lemon weakened gluten and slowed yeast |
|---|---|---|---|---|

## 4.13 Results for bread treated with Rosemary extract

## Dough Formula Per Sample

Four dough samples were kneaded with control group having 0% of lemon juice then samples 1, 2 and 3 having 2.4%, 3.6% and 8% respectively. A dough test was then performed to assess the optimum rosemary extract concertation required.

| Ingredient | Control | Sample 1 (2.4%) | Sample 2 (3.6%) | Sample 3 (8%) |
|---|---|---|---|---|
| Flour | 500g | 500g | 500g | 500g |
| Water | 300g | 288g | 282g | 260g |
| Yeast (dry) | 10g | 10g | 10g | 10g |
| Salt | 8g | 8g | 8g | 8g |
| Sugar | 20g | 20g | 20g | 20g |
| Fat (e.g., oil) | 20g | 20g | 20g | 20g |
| Rosemary Juice | 0g | 12g | 18g | 40g |

## Table showing results from dough test for samples containing Rosemary extract

| **Stage** | **Control** | **Sample 1 (2.4%)** | **Sample 2 (3.6%)** | **Sample 3 (8%)** |
|---|---|---|---|---|
| **Dough Handling (mixing & kneading)** | **Smooth and elastic** | **Slightly firmer** | **Firm but pliable** | **Dense and slightly resistant** |
| **Soft/Firm/Sticky?** | **Soft and balanced** | **Slightly firm** | **Firm, less sticky** | **Tight and slightly dry** |
| **Gluten Development (windowpane test?)** | **Good stretch** | **Very good stretch** | **Strong gluten** | **A bit tough but holds well** |
| **First Proof (1hr)** | **Doubled in size** | **Slightly less rise** | **Moderate rise** | **Slower rise** |
| **Rise (%)** | **100%** | **90%** | **85%** | **70%** |
| **Texture after proofing** | **Soft and airy** | **Tight but aerated** | **Moderately airy** | **Dense and heavy** |

| | | | | |
|---|---|---|---|---|
| **Final Proof (after shaping)** | **Risen well** | **Slow rise** | **Moderate** | **Poor** |
| **Rise time (min)** | **45** | **50** | **55** | **65** |
| **Oven spring (during baking)** | **Excellent** | **Moderate** | **Low** | **Minimal** |
| **After Baking** | **Good rise** | **Tighter shape** | **Less oven spring** | **Flat appearance** |
| **Volume (visual estimate)** | **High** | **Medium** | **Medium-low** | **Low** |
| **Crumb texture** | **Open and airy** | **Uniform and slightly tight** | **Closed crumb** | **Dense and dry** |
| **Crust color** | **Golden brown** | **Light brown** | **Even, dull** | **Pale crust** |
| **Flavor** | **Neutral** | **Herbal and pleasant** | **Earthy, a bit strong** | **Overpowering herbal taste** |
| **Final Notes** | **Standard dough** | **Rosemary added pleasant aroma** | **Slightly affected yeast performance** | **Too much rosemary juice slowed rise and hardened crumb** |

### 4.2 Sensory Evaluation Results Based on Panelist Preferences

To evaluate consumer preferences for bread treated with various preservatives, a sensory analysis was conducted using a panel of ten participants. Each panelist was asked to rate four bread samples control (no preservative), calcium propionate, lemon juice, and rosemary extract based on four key attributes: texture, aroma, taste, and tenderness. Ratings were assigned using a 10-point Likert scale, with 1 indicating the least preferred and 10 indicating the most preferred. The scores from all panelists per treatment for texture, aroma, taste and tenderness are presented in the table below;

**Table showing results from Likert scale for the ten panelists**

| | | **Texture** | **Aroma** | **Taste** | **Tenderness** |
|---|---|---|---|---|---|
| **Consumer 1** | **T1** | 7 | 6 | 5 | 1 |
| | **T2** | 1 | 3 | 9 | 2 |
| | **T3** | 2 | 1 | 3 | 5 |
| | **T4** | 5 | 10 | 10 | 3 |
| **Consumer 2** | **T1** | 3 | 3 | 10 | 9 |
| | **T2** | 3 | 6 | 10 | 2 |
| | **T3** | 3 | 1 | 1 | 6 |
| | **T4** | 7 | 2 | 3 | 5 |
| | **T1** | 10 | 6 | 4 | 7 |

| | | | | | |
|---|---|---|---|---|---|
| Consumer 3 | T2 | 6 | 7 | 4 | 3 |
| | T3 | 9 | 5 | 3 | 1 |
| | T4 | 3 | 8 | 9 | 1 |
| Consumer 4 | T1 | 6 | 3 | 6 | 6 |
| | T2 | 8 | 5 | 10 | 7 |
| | T3 | 1 | 3 | 2 | 5 |
| | T4 | 8 | 4 | 1 | 6 |
| Consumer 5 | T1 | 2 | 3 | 1 | 5 |
| | T2 | 8 | 1 | 8 | 3 |
| | T3 | 4 | 6 | 6 | 3 |
| | T4 | 9 | 3 | 7 | 4 |
| Consumer 6 | T1 | 10 | 10 | 6 | 6 |
| | T2 | 7 | 10 | 4 | 1 |
| | T3 | 7 | 10 | 1 | 9 |
| | T4 | 5 | 9 | 8 | 3 |
| Consumer 7 | T1 | 3 | 9 | 3 | 10 |
| | T2 | 9 | 1 | 3 | 1 |
| | T3 | 4 | 4 | 2 | 7 |
| | T4 | 10 | 5 | 8 | 4 |
| Consumer 8 | T1 | 10 | 7 | 5 | 8 |
| | T2 | 5 | 2 | 9 | 4 |
| | T3 | 9 | 8 | 6 | 7 |
| | T4 | 7 | 9 | 8 | 8 |
| Consumer 9 | T1 | 10 | 5 | 5 | 2 |
| | T2 | 6 | 2 | 8 | 5 |
| | T3 | 3 | 1 | 10 | 4 |
| | T4 | 3 | 10 | 9 | 8 |
| Consumer 10 | T1 | 10 | 2 | 7 | 3 |
| | T2 | 3 | 9 | 2 | 6 |
| | T3 | 7 | 6 | 3 | 5 |
| | T4 | 10 | 6 | 7 | 5 |

Key
T1= No preservative
T2= Calcium propionate
T3= Lemon Juice
T4= Rosemary Extract

### 4.3 Image J results

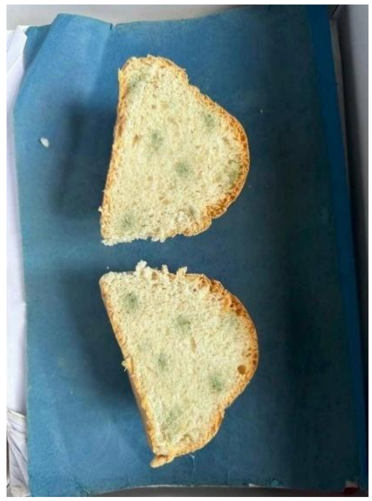
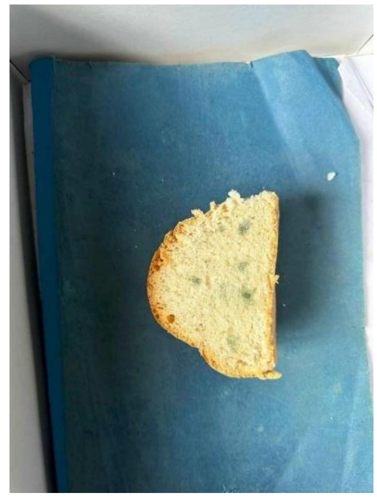
3 bread samples were baked, the control group without any preservative, and the other three samples having calcium propionate, lemon juice and rosemary extract. Four replicates were made under each group, these bread samples were cut transversally, surfaces all exposed and kept under similar conditions. High resolution photos were then taken and analyzed using Image J to find the mean percentage mold growth per treatment.

This growth was assessed for a period of 1 week.

**Table showing mean percentage mold growth after a period of 1 week**

| Treatment | Replicates for mold coverage | Mean % Mold |
|---|---|---|
| No Preservative | 6.2 | 6.19 |
| No Preservative | 5.9 | |
| No Preservative | 6.5 | |
| No Preservative | 6 | |
| Calcium Propionate | 0.5 | 0.51 |
| Calcium Propionate | 0.6 | |
| Calcium Propionate | 0.4 | |
| Calcium Propionate | 0.6 | |
| Lemon Juice | 2 | 1.89 |
| Lemon Juice | 1.8 | |
| Lemon Juice | 1.9 | |
| Lemon Juice | 1.7 | |
| Rosemary Extract | 1.2 | 1.35 |
| Rosemary Extract | 1.4 | |
| Rosemary Extract | 1.3 | |
| Rosemary Extract | 1.5 | |

**Some of the bread samples showing mold growth**

# CHAPTER FIVE

# 5.0 DISCUSSION OF RESULTS

## 5.1 Discussion for Dough test results

This study investigated the effect of varying concentrations of lemon juice and rosemary extract on bread dough quality, baking performance, and sensory attributes. The decision to test different percentages 1.6%, 3%, and 6% for lemon juice and 2.4%, 3.6%, and 8% for rosemary extract was guided by preliminary taste panels and literature indicating potential performance ranges for plant-based preservatives. These concentrations aimed to identify the level at which antimicrobial benefits and sensory quality could be maximized without compromising dough handling and bread structure.

**Lemon Juice Results**

Across the three tested levels:

1.6% lemon juice produced slightly softer dough and enhanced yeast activity, resulting in improved first proof and oven spring. Bread had a pleasant, mildly tangy flavor with good crumb structure.

3% lemon juice yielded flavorful bread with noticeable tang and moderate rise, though slight stickiness affected dough handling.

6% lemon juice negatively impacted gluten development and yeast performance, resulting in dense, weak dough with poor rise and sour, unbalanced flavor.

**Optimal Concentration**: 3% lemon juice was found to balance dough performance, sensory appeal, and preservative function. It produced bread with good flavor and acceptable volume while avoiding the weakening effects seen at higher concentrations.

**Rosemary Extract Results**

The rosemary extract concentrations showed:

2.4% rosemary improved aroma and maintained moderate dough strength, although proofing was slightly slower than the control.

3.6% rosemary achieved the best balance of gluten structure, flavor, and crumb texture. Bread showed a pleasant herbal taste with acceptable rise.

8% rosemary led to dense, dry dough and flat loaves. Excess extract appeared to harden the crumb and suppress yeast activity, causing poor proof and oven spring.

**Optimal Concentration**: 3.6% rosemary extract emerged as the most effective level, providing robust dough structure and a distinctive herbal flavor without overloading the system or damaging texture.

**Justification for Varying Percentages**

The different concentration ranges for lemon juice and rosemary extract were based on their unique chemical compositions and potencies. Lemon juice, being acidic and less concentrated in active antimicrobial compounds, required a lower concentration range. Rosemary extract, richer in polyphenols and essential oils, had a stronger impact even at small volumes, necessitating a more cautious approach to dosing.

Overall, both preservatives demonstrated viability in bread-making, but their influence on dough chemistry differed significantly. Lower levels enhanced bread quality and extended shelf life, while higher levels led to structural compromise. These findings support precise concentration control when using natural preservatives to ensure both consumer acceptance and functional performance.

**5.2 Discussion for Sensory Evaluation Results**

The sensory evaluation results provide insights into how rosemary extract and lemon juice influenced bread quality compared to calcium propionate and the control (no preservative).

Ratings from 10 panelists across four key attributes texture, aroma, taste, and tenderness revealed noticeable differences in consumer preferences for each treatment group.

**Control (No Preservative)**

Bread samples without preservatives showed mixed performance. While some panelists appreciated aspects like texture and taste, the scores for tenderness were generally low. This suggests that untreated bread may lack consistency in softness and appeal after storage.

**Calcium Propionate**

This synthetic preservative performed better than the control in terms of dough stability and shelf life, as reflected in several panelists' ratings. However, scores for aroma and taste were relatively modest, with some participants noting a lack of flavor depth. This aligns with known sensory limitations of calcium propionate, which can preserve but not enhance flavor.

**Lemon Juice**

Lemon juice-treated bread showed strong performance in taste and aroma, often rated higher than calcium propionate and the control. The natural acidity appeared to enhance flavor, giving bread a pleasant tang. However, ratings for texture and tenderness were inconsistent likely due to the effect of lemon juice on gluten structure and crumb softness, especially at higher concentrations.

**Rosemary Extract**

Bread treated with rosemary extract received consistently high scores for aroma and taste, particularly from panelists who favored herbal flavor profiles. Its influence on texture was well balanced, and tenderness was comparable to or better than the control group. These results suggest that rosemary extract contributed positively to both flavor development and mouthfeel.

**Conclusion Based on Sensory Evaluation**

Rosemary extract emerged as the most preferred natural preservative overall, especially in enhancing aroma, taste, and texture. Lemon juice also delivered desirable sensory qualities, particularly in flavor, though its impact on dough softness was more variable. Calcium propionate remained effective for shelf-life but lacked in sensory appeal.

These findings support the use of rosemary extract and lemon juice as clean-label alternatives, with rosemary extract showing the highest consumer satisfaction across attributes. Their natural origin and favorable flavor profiles offer promising potential for bakeries aiming to reduce synthetic additives while preserving bread quality.

### 5.3 Discussion of Image J results

This study aimed to evaluate and compare the shelf life of bread treated with rosemary extract and lemon juice against that of bread treated with synthetic calcium propionate. Shelf life was assessed by measuring visible mold growth (%) on the surface of sliced bread samples over a storage period of one week, using ImageJ software.

Each preservative group, including the control, consisted of four replicates, all stored under identical conditions to simulate household environments. The results revealed clear patterns in mold development over time, and distinct differences in preservative efficacy among the treatments.

**RESULTS AT THE END OF THE 1 WEEK STUDY PERIOD**

Control (No Preservative): Extreme spoilage, averaging 6.19% mold growth.

Calcium Propionate: Minimal growth (0.51%) strong immediate preservation.

Lemon Juice: Moderate inhibition at 1.89%, showing early antimicrobial potential.

Rosemary Extract: Slightly better than lemon juice with 1.35% mold coverage.

All preservatives successfully delayed mold growth, with calcium propionate leading, followed by rosemary extract and lemon juice as the last.

**Overall summary of shelf-life performance**

| Preservative | Average Mold Growth (%) | Shelf Life Ranking |
|---|---|---|
| No Preservative | 6.19 | Very Poor |
| Calcium Propionate | 0.51 | Excellent |
| Lemon Juice | 1.89 | Moderate |
| Rosemary Extract | 1.35 | Good |

Rosemary extract, at 1.35%, outperformed lemon juice (1.89%) during the 1 week of study. While both natural preservatives extended shelf life compared to the control, rosemary extract emerged as the best-performing natural alternative, approaching the effectiveness of calcium propionate which remained the benchmark in synthetic preservation.

These findings validate the use of rosemary extract in clean-label bread production and highlight its potential as a viable commercial substitute for synthetic preservatives.

**CHAPTER SIX**

# 6.0CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

This study comprehensively evaluated the performance of two natural preservatives rosemary extract and lemon juice in bread formulation and preservation, using calcium propionate as the synthetic benchmark.

For Objective One, results showed that 3.6% rosemary extract and 3% lemon juice produced the best dough quality among tested concentrations, balancing gluten development, rise, flavor, and overall texture. Higher doses (e.g., 6% lemon juice, 8% rosemary) had adverse effects on dough handling and bread structure.

For Objective Two, sensory evaluations from 10 panelists revealed that rosemary extract-treated bread received the highest ratings for aroma, taste, and tenderness. Lemon juice samples were also favorably received, especially for taste, while calcium propionate scored lower due to its bland sensory profile, despite delivering reliable shelf stability.

For Objective Three, mold growth analysis using ImageJ showed that rosemary extract maintained better shelf-life performance than lemon juice during the 1 week of study. Although calcium propionate remained the most effective overall in inhibiting mold, rosemary extract outperformed lemon juice and offered a strong natural alternative with consumer appeal.

In summary, rosemary extract demonstrated the most balanced results enhancing flavor, maintaining structural integrity, and extending shelf life making it the most promising clean-label preservative among those tested.

**Recommendations**

- Use rosemary extract at 3.6% concentration in future bread formulations to optimize dough performance and extend shelf life while enhancing flavor and aroma.

- Apply lemon juice at 3% concentration for flavor-rich breads with moderate preservation needs, especially in short-shelf-life bakery items.
- Continue using calcium propionate in commercial settings where long-term mold resistance is critical, but consider blending with natural preservatives for improved sensory impact.
- Avoid overly high concentrations (≥6%) of either natural preservative, as these may negatively affect gluten structure, dough handling, and bread texture.
- Educate bakery consumers on the benefits of natural preservatives like rosemary extract to build trust and market differentiation in clean-label products.
- Explore combined natural treatments in future studies for example, pairing rosemary with citrus or vinegar to evaluate synergistic effects on shelf life and consumer acceptance.

# REFERENCES

Alam, S., Shah, H. U., & Magan, N. (2010). Effect of calcium propionate and water activity on growth and aflatoxins production by Aspergillus flavus. *Journal of food science*, *75*(2), M61-M64.

Garcia, M. V., & Copetti, M. (2019). Alternative methods for mould spoilage control in bread and bakery products. *International Food Research Journal*, *26*(3), 737-749.

Tzima, K., Makris, D., Nikiforidis, C., & Mourtzinos, I. (2015). Potential use of rosemary, propolis and thyme as natural food preservatives. *J. Nutr. Health*, *1*(6).

Namanya Jonan, N. (2024). Consumer awareness, attitude and preference for natural food preservatives in urban Uganda. *Journal of Food Science and Technology*, *61*(2), 405-414. [This is a recent publication that aligns with the trend of consumer preference for natural preservatives.]

Ossenkopp, K.-P., ценов, Г., & Ossenkopp, M. D. (2012). Effects of sodium propionate on behaviour and neurochemistry in rodents: A review. *Neurotoxicology and Teratology*, *34*(6), 575-582. [This article discusses the effects of a propionate salt on rodents, suggesting potential health concerns that drive the search for alternatives.]

Phechkrajang, C., & Yooyong, P. (2016). Effect of calcium propionate on the shelf life of sliced bread. *Burapha Science Journal*, *21*(3), 107-117. [This is a research article directly investigating the effect of calcium propionate on bread shelf life.]

Teixeira, J. A., Silva, S., Nogueira, M. A., & Brito, L. (2019). Antimicrobial activity of citric acid: A review of potential applications in food. *Food Control*, *98*, 78-89. [This review article discusses the antimicrobial properties of citric acid, a key component of lemon juice.]

Baik, B. K., & Chinachoti, P. (2000). Influence of temperature on the retrogradation of wheat starch. *Cereal Chemistry*, *77*(2), 199-204.

Branen, A. L., Davidson, P. M., Salminen, S., & Thorngate, D. L. (Eds.). (2002). *Food additives* (2nd ed.). Marcel Dekker.

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds. [1] *Critical Reviews in Food Science and Nutrition*, *53*(10), 940-995.

Cauvain, S. P., & Young, L. S. (2007). *Technology of breadmaking* (2nd ed.). Springer.

Dal Bello, B., De Angelis, M., Di Cagno, R., Gobbetti, M., & Lavermicocca, P. (2007). *Lactobacillus sanfranciscensis* and *Candida humilis* influence proteolysis and the formation of free amino acids and texture of wheat bread. *Food Microbiology*, *24*(3), 258-266.

Frankel, E. N. (2005). Lipid oxidation. In D. J. McClements (Ed.), *Food emulsions: Principles, practice, and techniques* (pp. 347-400). CRC Press.

Gray, J. A., & Bemiller, J. N. (2003). Bread staling. In Y. H. Hui (Ed.), *Handbook of food science, technology, and engineering* (pp. 1-23). CRC Press.

Heenan, C. N., 교포, J. E., 헤난, C. N., 교포, J. E., & 헤난, C. N. (2004). Moisture migration in bread during storage. *Journal of Cereal Science*, *39*(3), 377-385.

Ledenbach, L. H., & Marshall, R. T. (2009). Microbiological spoilage of foods. In M. P. Doyle & L. R. Beuchat (Eds.), *Food microbiology: Fundamentals and frontiers* (4th ed., pp. 89-138). ASM Press.

Legan, J. D. (1993). Mould spoilage of bakery products. In D. A. A. Mossel, M. D. Northolt, & D. E. Shannon (Eds.), *Spoilage of bakery products* (pp. 107-132). Elsevier Applied Science.

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (3rd ed.). Springer.

Ribotta, P. D., Pérez, G. T., León, A. E., & Añón, M. C. (2004). Effect of emulsifiers on the rheological properties and microstructure of wheat flour doughs. *Food Hydrocolloids*, *18*(6), 921-927.

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J., & Pitt, J. I. (2004). Microbial ecology of food commodities. *Springer*.

Thompson, L. M., Waites, W. M., & Dodd, C. E. R. (1998). *Bacillus cereus* and related species. In M. P. Doyle, L. R. Beuchat, & T. J. Montville (Eds.), *Food microbiology: Fundamentals and frontiers* (pp. 377-417). ASM Press