T.R.
BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Biology

THE EFFECT OF POTATO PEELS ADDED TO YEAST GROWTH MEDIUM ON THE MAIN ENZYMES INVOLVED IN BREAD STALING

MASTER OF SCİENCE

HAWNAZ NAJMALDDIN

ACADEMIC SUPERVISOR
PROF.DR. SEYHUN YURDUGÜL

ACADEMIC CO-SUPERVIZOR
PROF. DR. HAIDER HAMZAH

BOLU, HAZİRAN - 2022

APPROVAL OF THE THESIS

The effect of potato peels added to yeast growth medium on the main enzymes involved in bread staling submitted by Hawnaz Othman NAJMALDDIN and defended before the below named jury in partial fulfillment of the requirements for the degree of Master of Science in Department of Biology**, The Graduate School of Natural and Applied Sciences of Bolu Abant Izzet Baysal University in 15.06.2022** by

Examining Committee Members	**Signature**
Supervisor Prof.Dr. Seyhun YURDUGÜL Bolu Abant İzzet Baysal University	
Member Assist.Prof.Dr.Elif Yolaçaner Hacettepe University	
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ABSTRACT

THE EFFECT OF POTATO PEELS ADDED TO YEAST GROWTH MEDIUM ON THE MAIN ENZYMES INVOLVED IN BREAD STALING
MSC THESIS
HAWNAZ OTHMAN NAJMALDDIN
BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF.DR. SEYHUN YURDUGÜL)
(CO-SUPERVISOR: PROF. DR. HAIDER HAMZAH)
BOLU, JUNE 2022
Number of pages in Roman + Arabic numerals (xi+ 51)

Bread staling is a term which is used to describe the physicochemical changes of bread during storage, resulting in undesirable changes in bread quality. It has always been one of the industrial burden throughout the history. At the same time, vegetable wastes, including potato peel (PP), is considered as a serious problem to environment. For this reason, PP has been used in this study to increase enzymatic activities of Saccharomyces cerevisiae, considered to have positive effects on bread quality. As a result, the highest growth rate of yeast was recorded in the treatment with 2% PP present in yeast growt medium. Moreover, treatments with 4% PP increased cellulase and invertase activity the most, in comparing to treatments with no peel and with 2% peel. While, amylase activity have been increased most in treatment with 2% peel, in contrast, lipase activity has been suppressed by adding the peel. Regarding bread quality, those which have been made from 4% peel treatment was the most acceptable ones in day 0 and 10, while few staling effects were recorded. Additionally, no effects of partially purified enzyme solutions, which 250 µL was used per 100 g of dough, on bacterial and fungal content of the breads were recorded in both days. The goal of this study is designing a microbial system for facilitating human activities in the industry, both joining the production of valuable enzymes by yeasts and recycling of PP at the same medium.

KEYWORDS: *Saccharomyces cerevisiae*, Potato peel, Enzyme activity, Bread staling.

ÖZET

MAYA BÜYÜME ORTAMINA EKLENEN PATATES KABUKLARININ EKMEK BAYATLAMASINDA YER ALAN BAŞLICA ENZİMLER ÜZERİNE ETKİSİ
YÜKSEK LİSANS TEZİ
HAWNAZ OTHMAN NAJMALDDIN
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
BİYOLOJİ ANABİLİM DALI
(TEZ DANIŞMANI: PROF.DR. SEYHUN YURDUGÜL)
(İKİNCİ DANIŞMAN: PROF.DR. HAIDER HAMZAH)
BOLU, HAZİRAN - 2022
Roma ve Arap rakamları ile tez sayfa sayıları (xi + 51)

Ekmek bayatlaması, ekmekte saklama sırasındaki fizikokimyasal değişiklikleri tanımlayan bir terim olup ekmek kalitesinde arzu edilmeyen değişikliklerle sonuçlanır. Bu durum tarih boyunca endüstriye bir yük oluşturmuştur. Aynı zamanda patates kabukları dahil sebze atıklarının, çevre için ciddi bir sorun olduğu düşünülmektedir. Bu nedenle, patates kabukları ekmek kalitesinde pozitif etkileri olacağı düşünülerek *Saccharomyces cerevisiae*'nin enzimatik aktivitelerini arttırmak için bu çalışmada kullanılmıştır. Sonuç olarak maya büyüme ortamındaki %2 patates kabuğu ilavesinde en yüksek maya büyümesi kaydedilmiştir. Dahası, %4 patates kabuğu muamelesi selülaz ve invertaz aktivitesini, kabuksuz ve %2 kabuk ilavesi ile karşılaştırıldığında en fazla arttırmıştır. Amilaz aktivitesi %2 kabuk muamelesi ile fazlasıyla arttığı halde, lipaz aktivitesi kabuk ilavesi ile tam tersine baskılanmıştır. Ekmek kalitesi esas alındığında, %4 kabuk muamelesi ile yapılanlar 0. ve 10. günde en kabul edilebilenler olup az sayıda bayatlama etkisi kaydedilmiştir. Ek olarak her iki günde 250µL enzim /100 g hamur başına kullanıldığında, kısmi saflaştırılmış enzim çözeltilerinin bakteri ve küf miktarına etkisi kaydedilmemiştir. Bu çalışmanın amacı, değerli enzimlerin mayalarca üretimi ile patates kabuklarını aynı ortamda geri dönüştürerek, endüstride insan faaliyetlerini kolaylaştırmak için mikrobiyal bir sistem tasarlamaktır.

ANAHTAR KELİMELER: *Saccharomyces cerevisiae*, Patates kabukları, Enzim aktivitesi, Ekmek bayatlaması.

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LIST OF ABBREVIATIONS AND SYMBOLS

PP : Potato peel

BS : Bread staling

EA : Enzyme activity

TA : Texture analysis

MAs : Microbiological assays

WHC : Water holding capacity

OHC : Oil holding capacity

U : Unit

mL : Mililiter

µL : Microliter

OD : Optical density

pNPP : p-NitroPhenyl Palmitate

pNP : P-Nitrophenyl

ACKNOWLEDGEMENTS

The author wishes to express his deepest gratitude to his supervisor Prof. Dr. Seyhun Yurdugül and co-supervisor Prof. Dr. Haider Mousa Hamzah for their guidance, advice, criticism, encouragements and insight throughout the research.

The author would also like to thank Asst. Prof. Dr. Sirwan Muhsin Muhammed Amin for his guidance, help, and consideration during the strict time of Corona pandemic and Assist. Prof. Dr. Hastyar Hama Rashid Najmuldeen for his suggestions and comments.

The assistance of my husband, Shad Arif Mohammed, in statistical analysis is gratefully acknowledged.

This study was supported by the Islamic Development Bank (IsDB) scholarship.

1. INTRODUCTION

Bread staling (BS) is termed as the physicochemical changes of bread during storage allowing it undesirable by the consumer (1,2). It has always been one of the serious problems for bread industry and consumers, moreover, it is one of the most important problems of the poor in the world (1). Various attempts have been carried out to solve such an issue along with other physicochemical changes of bread, including aroma, flavor, texture, crunchiness, and many others. For example, increasing bread shelf life was successful by using certain hydrocolloids (3,4). Additionally, several enzymes were used for the same purpose, including alpha amylase (1,2), and xylanases (1). Amylases work by obstructing recrystallization of amylopectin by partially degrading it, and prevent rearrangement of amylose during aging (2) . Also, several controlled processing factors, such as storage temperature, sourdough fermentation, time and temperature of baking and fermentation, hydrostatic pressure are tested for their influence on staling rate (1).

Previously, lots of research have been performed to retard BS rate. To mention few, the effect of alpha-amylases on BS was investigated and its positive effects on BS retardation was concluded. This is because of the production of short chain dextrins (5). Also, bacterial and cereal alpha-amylases stimulate reduction of amylopectin recrystallization (6). Adding commercial alpha-amylases have showed significant changes in crust darkness (7). Additionally, purified alpha-amylase from *Rhizopus oryzae* FSIS4 showed more improvements in the quality of baked breads when used in concentration of 1.936 U per kg of flour, compared to commercial counterpart (8). Also, effects of alpha-amylase and hemi-cellulase have been investigated on bread qualities, which have been made by whole wheat flour. The article concluded that both of these enzymes have the ability to improve gas retention of dough, specific loaf volume, increasing bread softness, and elongating bread shelf-life as the result of starch and hemicellulose degradation, respectively (9). Moreover, beneficial effects of transglutaminase was studied on retaining crumb moistness over time (10).

Regarding potato peel (PP), many experiments have been conducted for dealing with such a vegetable waste. This is because of their availability, biological convertibility, availability of fermentable carbohydrate, and their presence in bulk

as the result of industrial activities. Leaving this kind of agro-industrial waste will negatively affect environment by the act of microbial spoilage, green gas emission, and carbon footprint. Thus, incorporating such a waste in a value-added process will be an effective contribution to both economy and environment (11).

While the two previous paragraph is about enzymes and PP waste, respectively, this paragraph is about using such a waste in increasing the amount of microbial enzymes. Some articles reported that PP have the greatest effect on increasing the amylase gene expression in *Bacillus sp.* in comparison to bagasse and kitchen waste. This is because of the fact that PP contains large amount of starch, nearly 51%. In contrast, substrate which contain high glucose level repress amylase gene expression (12). Also, PP have increased both quality and amount of certain enzyme of *Bacillus amyloliquefaciens*, in particular, compared to other many other fruit samples. The strains of *Bacillus licheniformis* and *Bacillus subtilis*, which have been grown on PP by solid state fermentation show amylase enzyme activity(EA) of 270 Unit (U)/mililiter (mL) and 600 U/mL, respectively (13).

Additionally, increasing baker yeast's performance on bread quality has been another golden research interest area by many researchers. For example, Al-Eid and others concluded that date syrup substrate can be used for producing excellent quality baker's yeast. In another words, bread that has been made with yeast grown on date syrup, showed no significant decrease in bread's crust smoothness and shape among different used yeast strains in comparisons to the yeasts that have been grown on molasses (14). While the above-mentioned factors are about dealing with BS problem throughout history, almost all researchers stopped in a point which is performing the same work with less amount of resources and more elongated bread shelf life.

Several techniques are used for solving aging complications. One way of retarding aging in bread is using potato fibers, because of their water retention capacity (15). Also, *Saccharomyces cerevisiae* is used as one of the most beneficial microorganisms in food industries as it improves certain food qualities of bread (16).

The strength point of this study is designing a microbial system for facilitating human activities in the industry, both by the production of valuable

enzymes by yeast and recycling of PP at the same system. Considering bio-economy in the innovation of biological commercial product manufacturing is a critical issue for industries (17). It means that a huge amount of products are needed to be produced without wasting a minimum amount of raw materials and the maximum selling rate (17). This can be gained by a continuous improvement in the flow of bioprocess (17). So a robust interrelated biological system can make a great contribution to any industrial process (17) by upgrading industrialized bioprocess (18).

Today improving food industries is an urgent need to improve the quality and quantity of products by recycling waste material in the same process, using a biological system (17). Based on this reason, we put the aim of our research to determine the EA of yeast that may extend bread freshness based on our data. Also, this research is a novel attempt for changing certain bread characteristics, such as hardness, gumminess, volume, texture, moisture contents, and many others with using different yeast enzymes and PP. Besides, we collected some data about enzymes in yeast. This is because yeast bioinformatics is one of the valuable and vibrant aspects of microbial bioinformatics nowadays (19). Moreover, we tested PP as a waste recycling material for yeast culturing (20). In other words, through our research we made the expectation of the engineered bio-system behavior based on experimental data, thus controlling the biological system for industrial purposes would be easier (18). We are going to do such work after incorporating PP into yeast culture media, getting enzyme crude solutions, treating bread with certain enzymes from yeast, comparing treated bread with controls, feeding our data to R programing language, and analyzing and plotting our data using some specific packages of R language.

1.1 PP Waste

Generally, 50% of by-product wastes come from fruits and vegetable industrial activities, which negatively affect environment. Fruits and vegetable wastes can be used, such as in baking goods, as they contain high nutritional values

and bioactive compounds (21). For example, in a review (published during this thesis (22)), we concluded that vegetable wastes are increasingly produced in a considerable amount in Turkey and Iran. Potato is one of the agricultural products that comprise a huge portion of daily food consumption in many countries in many forms, such as, for starch extraction, like processed food, table food (20), and potato chips (23). For example, in Japan 2.5 million tons of potato is produced each year (20). Using a large number of potato results in increasing potato wastes as peels worldwide. 70- 140 thousand tons of PP are generated per year globally as a result of industrial activities (23). Based on this fact, the peels should be recycled in many ways, otherwise, they have detrimental effects on environment due to their spoilage by the act of microorganisms (23). They can be recycled in many ways, for example using them as fertilizer, biogas production, and animal feeds (23). The recycling process can be done through many processes, for example, lactic acid fermentation, ultrasonic, and phenolic acid extraction (23). In our study, we use it as an extra food source for yeast growth, assuming that the peel affects yeast growth and some enzyme expression rates.

1.2 Yeast

Yeast is an oval, unicellular, asexual, eukaryotic cell, belonging to the kingdom of fungi (16). Yeasts, especially *Saccharomyces cerevisiae,* have been used in bread making process throughout history as leaving agents (16). This is because of the effects that yeast has on bread, such as bread volume, texture, structure, aroma, color, and staling (16). Nowadays, yeasts and their enzymes are manipulated for getting a better bread quality by extracting their enzymes, genetically modifying yeasts, and many other state-of-art techniques (16). Yeast is a good alternative to chemical leavener as chemical ones produce bad flavor and cause browning of the bread (16). So, yeast positively affects the nutritional value, quality, and staling of bread (24).

1.3 Fresh Yeast vs. Instant Yeast

Generally, baker's yeast can be found in many forms e.g. instant active dry, fresh, compressed, and active. They differ mainly in moisture contents. In other

words, moisture is removed or reduced in some forms of yeast to prolong its shelf life as a preservation method. However, reducing the moisture content of the yeast adversely affects the yeasts in many ways, for example retarding their activity, metabolism, and tolerance to acids, osmosis, and temperature (16).

1.4 Yeast Growth

The growth of yeast during the bread-making process results in some changes of dough, thus the final bread quality as they release CO_2 and some other metabolites during the fermentation process (25). Both external and internal factors influence yeast growth. Examples of external factors are storage and fermentation temperature. To mention some other external factors, available sugars, minerals, vitamins, salts, and many others affect yeast growth (25). Also, the strain of the yeast and the pre-growth condition of the yeast, as internal factors, affect the bread quality (25).

1.5 Bread Staling

BS is a physicochemical change in the quality of bread according to time. This aging process has been studied throughout history and still is an ongoing research area of interest. The changes occur in the texture, aroma, flavor, and water content (26). These changes occur on two main parts of bread which are crust and crumb. During BS, water molecules move from crumb to crust, leading to a rubber texture of the two parts or redistribute between gluten and starch (27). Many factors are determined as a result of such changes. Many researchers agreed that starch retrogradation is the main factor of BS. During such a process, changes in the structure of gluten occur moisture content of the crumb is redistributed (26). Additionally, some articles explain the change in bread firmness, during the aging of bread, as a result of starch –starch interaction (28). However, some articles have been concluded that plasticization of networks of bread structure, as a function of water, is another reason for BS. So, rearrangement and modification of a starch portion of bread is the most important basis of staling (27).

1.6 Yeast As A Source Of Enzymes

Recently, many enzymes are used as additives, such as amylase, hemicellulase, protease, and many others in making bread for improving its quality (16,26). Generally, enzymes are added during the bread-making process as EA is low in wheat flour (26). Microorganisms are largely used as a source of enzymes for food industrial activities. This is because they can produce different enzymes and biologically active substances at a rapid rate (29). In the bread-making process, microbial enzymes are used as they contain an important nutritious value, they have high digestibility property, they affect other endogenous enzymes of the flour (29). One of the microorganisms that its enzymes are utilized mostly in food biotechnology is yeast (30).

1.6.1 Invertase

Invertase is one of the enzymes that is produced by yeast cells and play an important role in making bread (25). This is because of its ability for converting sucrose to glucose and fructose (31). During this fermentation process, gases are released and result in forming bubbles in the dough, thus bread has an open crumb structure (31). In addition to sucrose, fructans is fermented by the activity of invertase. Several studies investigated that nearly all sucrose is fermented by the act of yeast invertase during mixing stage of the dough. Additionally, 30-80% of fructans is degraded in mixing and fermentation stage of the dough. Yeast invertase is encoded by closely related families of *SUC* genes. These genes is regulated by glucose repressor and they are responsible for rapid change of fructans and sucrose in the first hour of dough fermentation process and resulting glucose and fructose are used by the yeast cells. When glucose, fructose, fructans, and sucrose are about to deplete, then maltose will be chosen by the yeast for degradation (25).

1.6.2 Amylase

Amylase is the most commonly used enzyme for retarding bread aging (32,33) as it has been investigated for having antistaling activity, especially alpha-amylases (28). It can perform such action by enhancing bread's crumb elasticity (34). Moreover, bread firmness is reduced as the result of the number of maltodextrins that are produced by the enzyme or changes in starch-gluten interactions (28), the fragrant aroma is increased, bread loaf volume is improved, and starch retrogradation is retarded by the act of amylase enzyme (34). Also, they can positively affect bread quality by reducing starch levels which is prone to retrogradation (32). This enzyme elongates the shelf life of bread by many modes of action. To mention a few, endo-acting amylases can degrade starch internal bonds, while exo-acting amylases break outer amylopectin bonds and, in this way, control amylopectin recrystallization (34).

On the other hands, some other articles explain the effect of amylase on BS as a way of interfering with protein-protein and protein-starch interaction as the function of low molecular weight dextrin (32,35,36). Another doubt on amylase negative effect is producing osmotic effect on yeast cells. This may occur due to massive maltose production which produce osmotic stress on yeast cells, causing decrease in their viability, thus fermentation rate (25).

To sum up, if amylase is added to dough in appropriate amount, it will positively affect the quality of the resulting bread by improving its volume as the result of their effects on gelatinized starch. Another point which must be considered is the fact that yeast cells prefer using glucose and fructose (products of Invertase activity) at early stages of dough fermentation. After depleting these products in the later stages of fermentation, the role of amylase will be observed and maltose will be produced (Figure 1) (25).

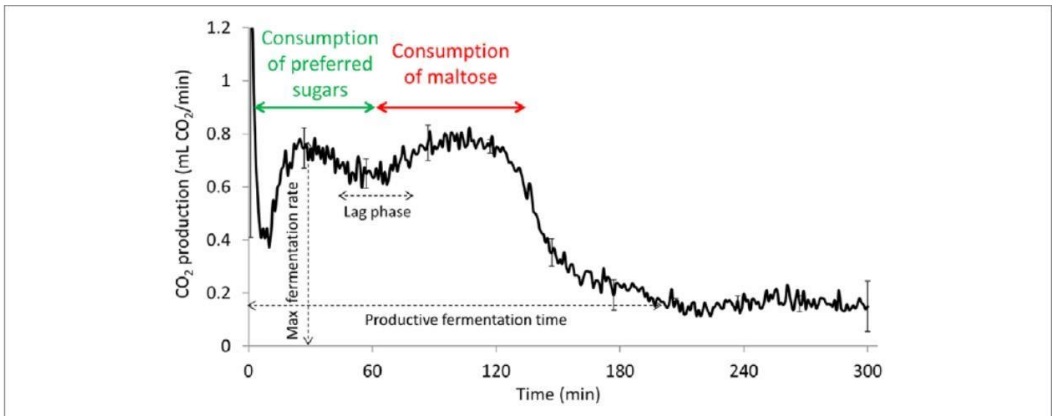

Figure 1: interaction of bread dough and yeast. At the beginning (from 0 to 60 min) yeast consume glucose and fructose (products of sucrose and fructans respectively) as the result of Invertase activity. Afterwards (from 60 to 120 min), yeast cells turn to maltose utilization as the result of starch degradation by amylase action (Struyf et al., 2017).

1.6.3 Protease

Protein quality and quantity are among the significant factors that affect bread quality. In the light of this fact, various proteolytic enzymes affect the quality of bread as time is going, for example, glucose oxidase (37). Proteases are used in the baking process because they reduce mixing time, provide a good dough consistency, control the texture of bread, keep the gluten strength of the bread, enhance flavor, reduce crumb hardness and chewiness, and expand bread volume and thickness. They reduce gluten's disulfide bonds. In this way, the increase in the softness and extensibility of the dough (29).

1.6.4 Cellulase

Cellulase is another important enzyme that is used in many industrial processes, including the bread-making process. This is because the enzyme converts cellulose to smaller molecules, for example, oligosaccharides, cellobiose, and

glucose (29). As a result, these small molecules positively affect the properties of dough handling, for example, they soften the dough, lessen water absorption capacity, and minimize baking time. Cellulose fibers and pentosans are a substrate for cellulases (29).

1.6.5 Lipase

Lipase is one of the most important enzymes that have been investigated for its amazing effects on retarding staling rate (38). Based on this fact, we tested for lipase activity to know how to increase its activity.

1.7 Using Enzymes From Yeast Source

Enzymes are catalysts that speed up biochemical reactions. They are present in all biological systems in different types and concentrations (39). Furthermore they can be extracted from living cells and use them in different industrial fields (40).

Different types of microorganisms, such as bacteria, fungi, yeast, and plant, produce different attractive, commercially important enzymes which may be extracellular or intracellular enzymes. An example of intracellular enzymes are catalase which present in *S. cerevisiae* and *A. niger*. Laccase, amylase, pectinase, protease, xylanase, lipase, and cellulase are examples of microbial extracellular enzymes (41).

In this study, we investigated yeast enzymes for the following reasons. Firstly, microbial enzymes are ecofriendly, and highly specific biocatalysts with an efficient acceleration rate (10^5-10^8). Secondly, 50% of industrial enzymes are made by yeast and fungi, while 35% and 15% of them are produced by bacteria and plants. Thirdly, microbial enzymes are more stable and active than animal and plant counterparts. Producing well-characterized and purified enzymes on large scales is easier. Fourthly, manipulation of microbial genome is easier for product optimization as they have biochemical diversity and susceptibility to genetic

engineering. Fifthly, through many state-of-art techniques, such as genome mining, metagenome screening, and extremophiles diversity exploration, discovery of new of microbial enzymes would be more possible (42). Sixthly, yeast have the ability to grow on wide range of substrates (43).

1.8 Using Enzymes For Improving Bread Quality

Making bread is one of the biochemical processes that are globally used in every nation (16), and bread has been one of the main human food throughout the history in nearly all nations (44). Many factors affect bread quality such as, yeast strain, pre-treatment to the yeast, type and amount of fermentable sugars and carbohydrates in the dough, and type of the flour that is been used (25).

Moreover, different enzymes are used to improve bread qualities (7) for example, amylases, lipases, hemicellulases, and many others (45). They elongate the shelf life of bread in many ways. For example, they work by increasing bread volume (7), retarding starch retrogradation (34), and enhancing endogenous flour enzymes by the act of exogenous enzymes addition (46). Choosing enzymes for improving bread quality is favoured due to some reasons which are their characteristics as biodegradable, eco-friendly, high specificity, efficient molecules (47), and lacking detrimental health effects in contrast to chemical counterpart (43).

Generally, starch-degrading enzymes are the most commonly used enzymes for retarding the firmness of bread by changing starch and soluble carbohydrate content structure (27). The enzymes have three sources which present 1) as a part of flour composition (indigenous) 2) from microorganisms, which either naturally present in the flour or added as microbial culture (endogenous) 3) or added to the flour (exogenous) (7).

1.9 Dough Quality Measurements:

Many properties of dough can be measured by different methods. Some of these methods are explained in this thesis which are:

Rheological properties (handling properties): is the measurement of the dough state

(solid, semi-solid, and liquid) and their flow changes under stressful condition (29).

Extensibility: is one of the rheological properties of dough which appears during kneading stage. It is described as the ability of the dough to stretch as much as possible without breaking. Therefore, more extensible dough means better dough, and resulting bread, quality. This property is determined by the type and amount of gluten in the wheat flour and the amount of added water to the dough (48).

Elasticity: this property can be described as dough strength. It has effects on handling quality of the dough during processing. In another word, this property provide a springback ability to the dough after sheeting it to different shapes (49).
Resistance to deformation: deformation can be defined as shearing and/or extension that may occur on dough during mechanical development (50).

1.10 Bread Quality Measurements:

There are many properties of bread that can be considered as the measurement of bread quality, as well as BS. In this thesis, some of them have been chosen for studying BS which are hardness, springiness, cohesiveness, gumminess, chewiness, and resilience (42).

1.11 Data Analysis

Various programs are available for statistical analysis for example Excel, SPSS, and Prism. However, we have chosen the R programming language for certain purposes. The reasons behind choosing such a language are its robustness, powerfulness, free-source, flexibility, and availability for all operating systems. Moreover, it provides an interactive interface for writing line codes in a way that enables us to tailor any programs or data analysis based on our research questions (51). Thus, we use R language for statistical analysis.

2. AIM AND SCOPE OF THE STUDY

The study aims to investigate not only whether 2% and/or 4% PP have positive effects on yeast growth or not but also to determine yeast enzymatic activity of certain enzymes involved in BS influenced by the addition of PP into the yeast growth medium.

There are many goals behind this project, which are:

1. Solving the problem of PP waste.
2. Trying to increase yeast growth naturally.
3. Increasing the expression of yeasts' certain enzyme.
4. Improving bread quality.
5. Retarding bread staling.

Many articles are available on isolation and production of microbial enzymes for improving baking quality, but reports on microbial strains, with multi-enzyme production quality is rare (43). Yeast has GRAS status as well among the microorganisms. So we have chosen yeast for studying their enzymes and their effects on many characteristic features of bread over ten days' of shelf life.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Microorganisms

Active dry yeast (*Saccharomyces cerevisiae*, Piyale, Turkey) was purchased from a local supermarket (Şok, Bolu, Turkey).

3.1.2 Potato Peels (PP)

PP were obtained from potatoes purchased from a local supermarket (Bolu, Turkey).

3.1.3 The Flour

The flour (Hekimoglu, Konya, Turkey) was purchased from a local supermarket (Avantaj, Bolu, Turkey).

3.2 METHOD

The study is composed of three main steps. In the first step, PP is incorporated into yeast extract-peptone-dextrose (YPD) medium alongside yeast. In the second step, the EA of amylase, invertase, cellulase, and lipase from different flasks are tested. In the final step, the enzyme crude solutions are used in the breadmaking process to investigate the effect of the enzymes on BS.

3.2.1 Experimental Groups

Yeast extract-peptone-dextrose (YPD) media

Yeast extract-peptone-dextrose (YPD) media preparation was carried out according to the formula: 10 g. of yeast extract (LAB M, UK) and 20 g of peptone from casein (Sigma-Aldrich, Merck, Germany) was mixed in 1000 mL, autoclaved and subsequently, 20 g glucose (BioChemika, UK) was added to this mixture through syringe filter (0.45 µm) (Millipore, USA).

The experimental groups were defined under three categories: in the first group 1.5×10^8 CFU/mL (yeast) were introduced to YPD media, in the second and third groups, the same amount of yeast cells were added to the media in addition to 2% and 4% PP, respectively. The treatments and their controls are shown in the below table.

Table 1 Flasks that are prepared for the whole experiments, including treatments and controls.

Flasks (Treatments)	$1.5*10^8$ CFU/mL	Amount of added PP
1st treatment	Added	No PP
Control 1	Not added	No PP
2nd treatment	Added	2%
Control 2	Not added	2%
3rd treatment	Added	4%
Control 3	Not added	4%

3.2.2 The Microbiological Assays(MAs)

MAs were done in two stages of the experiment. In the first stage, yeast culturing was carried out after incubating the previously mentioned three flask sets

at 30 °C for 48 hours. The yeast samples were cultured in yeast extract agar plates. The enumerations of the yeast were done by serial dilutions, then 1 mL from the fourth and fifth dilutions was spread onto yeast extract agar containing Petri plates, then the plates were incubated at 30 °C for 48 hours and enumerated by a colony counter (Stuart Scientific, USA). In this stage, the effect of PP on the growth of baker's yeast was investigated.

In the second stage of MAs, we did microbial analysis during studying bread quality. Sampling was done from each treated bread, alongside to their controls, in the 0 and 10th day of shelf life to investigate the effect of enzyme solutions on microbial content of them. The samples are cultured on nutrient agar (NA) (OXOID, England) and yeast extract agar (YEA) (OXOID, England) for detecting total mesophilic aerobic bacteria (TMAB) and yeast and mold content of the bread, respectively.

3.2.3 Preparation Of Enzyme Crude Solutions

After 48h of incubation, the cultures and controls were further processed to obtain enzyme crude solutions. For this purpose, samples from each flasks were taken and have been centrifuged at 5000 rpm for 10 minutes. Later on, the supernatant have been undergone to microfiltration with a pore size of 0.1 µm (Sigma-Aldrich, Merck, Germany). The enzyme crude solutions were used for subsequent EA assays and bread making process.

3.2.4 Determination Of The EA

Before making bread, the activity of the four above mentioned enzymes in yeast are studied to investigate whether the different concentration of PP has affected the production rate of the enzymes or not. The following procedures for testing the activity of the four enzymes were used:

Invertase:

DNS method have been chosen for determining invertase activity. Firstly, stock solution of substrate was prepared by dissolving 10mg/mL sucrose (SigmaAldrich, Merck, Germany) in 0.1M Na-acetate buffer (BIOCHEM CHEMOPHARMA, France). Then, the stock solutions and enzyme solutions were preheated for 5 min at 50°C. Later on, 0.3 mL of the stock solution and 0.3 mL of enzyme solutions were mixed and incubated at 50°C for 10 min. Afterwards, 0.9 mL of DNS reagent (HIMEDIA, India) was added to each tube and incubated in boiling water bath for 5 min. Finally, the absorbance of the solutions were measured at 540 nm after subsequent cooling of the solutions in the room temperature (52).

Amylase:

For testing the activity of this enzyme, 1.0 mL yeast cell suspension was mixed with 0.1 M sodium acetate (BIOCHEM CHEMOPHARMA, France) buffer (pH 4.8), and 1.0 mL starch (Merck, Germany) solution composed of 10 mg/mL in 0.15 M NaCl (BIOCHEM CHEMOPHARMA, France) was incubated for 30 minutes. By adding 1 mL of 6N HCL (CHEM-LABS, Kenya) the reaction was stopped. For estimating the unhydrolyzed starch, 1 mL was transferred to a 25 mL volumetric flask and 15 mL water was added, followed by 0.5 mL I-KI solution (0.2% 12 in 2% KI) (BIOCHEM CHEMOPHARMA, France). Volume was reached up to 25 mL with water and absorbance was read at 660 nm (53).

Cellulase:

For studying the activity of this enzyme, the dinitrosalicylic acid (DNS) method was used. For this purpose, 0.5 mL of the enzyme solution was placed into a test tube. Then the mixture was mixed with 1.8 mL 50 mM Citrate-Phosphate (BIOCHEM CHEMOPHARMA, France) buffer (pH=6.8), and 10 mg cellulose powder (Merck, Germany). Later, the reaction was stopped by transferring 3 mL DNS (HIMEDIA, India) to enzyme and buffer solution, then the mixture was incubated in a water bath at 50 °C for 30 minutes. The absorbance was determined by a spectrophotometer at O.D. 550 nm (30).

Lipase:

For lipase test, two different solution was prepared. The first solution (A) composed of 27 mg of p-NitroPhenyl palmitate (pNPP)/1 mL of isopropanol (Merck, Germany), and the second solution (B) was prepared by combining 9 mL of 50 mM Tris-HCl (pH 8) (Merck, Germany), 0.11% arabic gum (HIMEDIA, India) and 0.44% Triton X-100 (HIMEDIA, India). Later, test solution was obtained by mixing the two solutions (A+B). For assessing the lipase activity, 100 microliter (µL) of the enzyme solution was mixed with 900 µL of the test solution. Then, the tubes were incubated at 30 °C for 30 minutes. Finally, the absorbance of the samples were read at optical density (OD) 410nm, and standard curve of p-NitroPhenyl (pNP) was constructed (54).

After reading the absorbance of different tubes for different enzymes, the following equation was used to calculate the EA (U/L).

Activity (U/L) = ((absorbance variation/time)/molar extinction coefficient * path length) * 1 micromol * (total reaction volume/total enzyme volume) (55).

3.2.5 Bread Making

The breads were made according to the procedure reported in (30). This was carried out by mixing 280g flour, 1.7 fresh living yeast, and 170 mL water. However, salt was removed from the procedure as salt in wheat dough increase ionic and osmotic stress on the baker yeast (56).

After 30 min of dough proofing, the dough was partitioned into many pieces of 100g and each of them treated with 250 µL enzyme crude solutions, as long as their control solutions. The dough set on baker pan for 45 min at 45ºC for further proofing and providing time for enzymes to work on the dough. In the final stage, the dough was baked at 250ºC for 30 min and were placed in plastic bags after cooling and kept at +4 °C.

3.2.6 The Physical Assays

The Texture Analysis(TA)

Texture is one of the most important features of bread that consumers consider during assessing bread quality (57). Certain bread properties were tested by texture analyser (Stable Micro Systems Ltd., Godalming, UK). The properties include hardness, springiness, cohesiveness, gumminess, chewiness, and resilience.

TA.XT-Plus Texture Analyzer is equipped with a 5 Kg load cell. The probe radius is 36 mm edge in cylindiric form, force is 5 kg/f. A compression test was applied to toasted bread slices, using a 36 mm cylindrical aluminum probe. All bread slices had the same dimensions (10 mm × 6 mm × 1 mm, length × width × thickness). Three compression/slice were performed at a test speed of 0.5 mm/s and compressing up to 50% of the bread slice height. The maximum peak of the force-distance plot was interpreted as hardness. Cutting strength was measured using the 3 mm knife blade at a test speed of 2 mm/s.

The Determination Of The Water Holding Capacity (WHC) Of The Bread

The WHC was determined according to the method of Gould and others (58). Based on this method, 1 g of the sample was added to a centrifugal tube, alongside 10 mL of distilled water and mixed by vortex (Nüve, NM 110, Turkey) for 30 seconds. the samples were left behind at ambient temperature for 2 h to be hydrated. Later on, a bench top centrifuge was used for centrifugation of the samples at 2800 rpm for 10 min. Finally, the pellets were weighed and incorporated to the following equation for finding WHC.

$$\text{Water holding capacity (g/g)} = \frac{\text{weight of hydrated sample (g)} - \text{weight of dry sample (g)}}{\text{weight of dry sample (g)}}$$

The Determination Of The Oil Holding Capacity (OHC) Of The Bread

The OHC was determined according to the method of Caprez and others (59). Based on this method, 1 g of the sample was added to a centrifugal tube, alongside 10 mL of corn oil and mixed by vortex (Nüve, NM 110, Turkey) for 30 seconds. the samples were left behind at ambient temperature for 1 h to be hydrated. Later on, bench top centrifuge was used for centrifugation of the samples at 2800 rpm for 10 min. Finally, the pellets were weighed and incorporated to the following equation for finding OHC.

$$\text{Oil holding} = \frac{\text{weight of pellet (g)} - \text{weight of dry sample (g)}}{\text{capacity (g/g) weight of dry sample (g)}}$$

3.2.7 Sensory Evaluation

Sensory evaluation was done for the breads by constructing a taste panel composed of 30 persons, ranging between 21 to 60 years old. They consist of 13 females and 17 males. Within these people, 19 of them were smokers and 11 of them were non-smoker. They have rated the breads from 0 (as worst) to 10 (as best).

3.2.8 Statistical Analysis Tool

All statistical analysis workflows were performed using open-source R Statistical Software (version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria). Plots were generated using *ggplot2* and *ggpattern* packages. t-tests, ANOVA, Tukey-HSD post-hoc tests were performed in *stats* package.

4. RESULTS AND DISCUSSION

4.1 Microbiological Tests

MAs were carried out in two main steps of the project for two different reasons. Firstly, viable count (colony count) of the yeast were carried out on YPD media. This step was done in early stages of the experiments to investigate which treatment promotes yeast growth the most. Secondly, enumerating total mesophilic aerobic bacteria (TMAB) on nutrient agar were investigated. This step was carried out in the last step of the experiment to assess the microbiological quality of the breads from each treatment and their controls.

4.1.1 Yeast Growth

The effect of PP on yeast growth was investigated. The untreated showed the least number of colony, while treatment with 2% peel showed the most (p-value 0.04). Colonies with 4% peel was more than the untreated. However, the differences of colonies of 4% treated was not significant, neither with untreated nor 2% treated. Thus, 2% is the optimum concentration of PP for enhancing yeast growth, while yeast growth is obviously decreased by adding 4% PP (Figure 2).

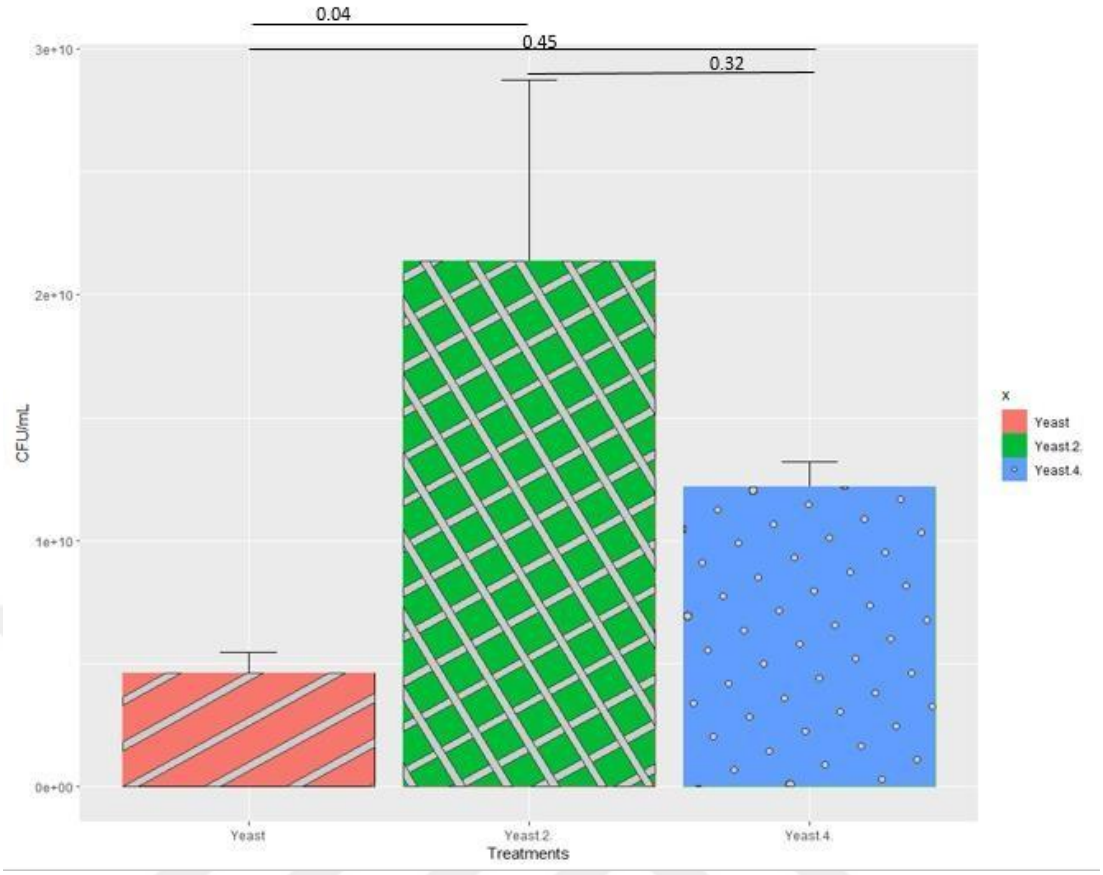

Figure 2 Colony Forming Units: greatest colony number was observed in treatments with 2% PP.

Moreover, 1% of potato wastes was used in other studies for increasing growth of many *Bacillus sp.*. As a result, the growth of *B. subtilis* JS-2004 was in the highest rate at 48h of incubation, while the growth of *Bacillus flavothermus*, *Bacillus amyloliquefaciens*, and *Bacillus sp. ANT-6* were in the maximum rate after 24h of incubation, using the same amount of potato wastes (60).

Many other studies have been conducted for assessing the effects of different food wastes on the growth of different microorganisms, including yeasts. For example, food waste compost was used for enhancing bacterial and fungal growth in the rhizosphere of lettuce. At the same time, commercial compost and mineral fertilizer were used alongside control group. As a result, the maximum growth of fungi and bacteria were observed in the treatments with food waste composite. Fungal growth were 30-500 times higher than those of the other treatments throughout 2,4,6 growing weeks period (61).

4.1.2 Total Mesophilic Aerobic Bacteria(TMAB)

Microbiological content of the breads was one of our main focus in this study to test the staling. The total mesophilic aerobic bacteria content of the breads was analyzed by the assays in 0, 5, and 10th days. As a result, no growth was detected (except a few replicates which are contaminated in each run of experiment). These results were obtained while the breads were kept in closed nylon bags at +4 °C.

4.2 Enzyme Activity

Yeast, yeast+2% PP, and yeast+4% PP were the three treatment groups investigated for their effects on invertase, cellulase, and amylase. One way ANOVA and Tukey HSD post-hoc test was used to find p-values. Generally, an increasing trend of EA was observed as more PP is added to the media. In another words, the treatments without PP showed the least enzymatic activity, while the treatments with 4% PP showed the highest enzymatic activity.

In some other studies, different food wastes were used in different ways for increasing EA. For example, acid phosphatase, alkaline phosphatase, dehydrogenase activity were significantly higher in the soil treated with food waste compost, in comparison to commercial compost, mineral fertilizer, and control (61).

4.2.1 Invertase

From Figure 3a, it can be seen that adding PP to the media will increase the expression of invertase. In another words, the amount of invertase increased significantly when 2% PP was added to the media (p-value 0.045). However, a nonsignificant p-value (0.3) was recorded between the activity of the enzyme in the flasks with 2% PP and flasks with 4%PP. Moreover, there was a huge significant difference between the amount of invertase which are produced in the flasks containing no peel and 4% PP (p-value 0.001).

Other attempts were done for increasing invertase activity, using food wastes. For instance, invertase activity in the fruit body of edible fungi *Pleurotus*

ostreatus NRRL-0366 was nearly doubled when lemon pulp was used in solid state fermentation process. At the same system, no changes in the activity of the enzyme was recorded when different types of papaya substrates were added (62). So, choosing the right food waste is important for increasing the activity of the enzyme.

4.2.2 Cellulase

Expression of cellulase was increased by adding PP to the growth media. Also, 4% PP have greater impact in elevating cellulase amount in comparison to 2% counterpart. Totally, treatment with 4% peel showed the best result for increased cellulase amount, showing a p-value which is highly less than 0.05 (p-value 3e05)(Figure 3b).

Other food wastes, rather than PP, were used for enhancing cellulase expression. Cellulase expression in *Saccharomyces cerevisiae* SCPW 17 was reported to be elevated by using corn cob. Sugar cane bagasse is the second best substrate for enhancing activity of the enzyme. These substrate was chosen as the two best substrate for cellulase expression when compared to opete bagasse, ugu pod, and rice husk (63).

4.2.3 Amylase

Amylase activity was increased by adding PP to the yeast growth medium, and significant change was recorded in the second treatment (yeast+2% PP). According to this result, the optimum concentration of PP for increasing amylase activity is 2% (Figure3c).

There are other ways for increasing amylase activity. For example, optimising pH and temperature are among the effective methods of improving amylase activity. For example, amylase, from *B. licheniformis* HULUB1 and *B. subtilis* SUNGB2, production was at peak in the growth temperature of 45˚ C and pH 6 (64).

Additionally, using industrial food wastes is another way for increasing amylase expressions in microorganisms. For example, 1% potato waste was used

for investigating α-amylase expression rates throughout time intervals in many *Bacillus spp.* The maximum rate of α-amylase expression was observed at 48h *B. subtilis* JS2004. Also, the highest α-amylase activity in *Bacillus flavothermus*, *Bacillus amyloliquefaciens*, and *Bacillus spp. ANT-6* was observed after 24 h, using the same amount of the potato waste (60).

So, amylase activity can be increased by considering some variables such as the type and concentration of the substrate, pH, and temperature. Through optimization of these factors, maximum yield of amylase can be achieved.

4.2.4 Lipase

Lipase activity was also measured. The result of the lipase activity was different from all the other tested enzymes. The maximum expression rate of lipase was occurred in the first treatment and the minimum expression rate observed in the second treatment. However, none of the changes between the treatments were significant. This result was obtained because of two main reasons. Firstly, PP contains a small portion of lipid, nearly 1% (13). Secondly, patatin is the major protein type in potato which inhibit lipase activity (65). So, PP cannot be recommended for increasing lipase activity (Figure 3d).

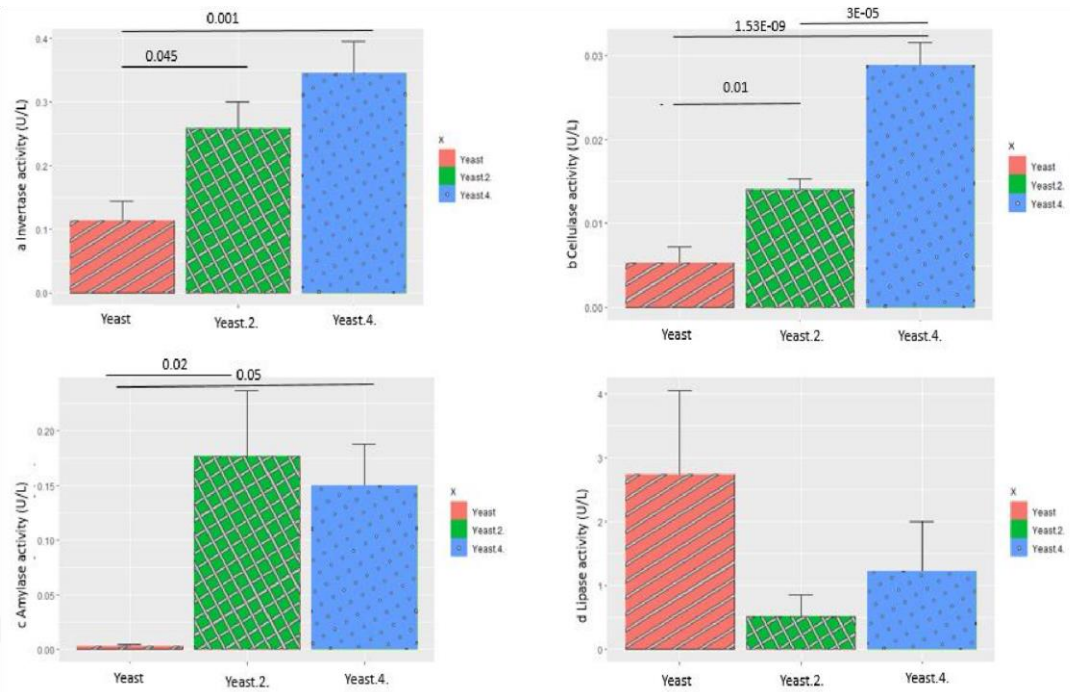

Figure 3 Effects of PP on enzyme activity. a, invertase activity is increased by adding and increasing PP to the culture media of the yeast. b, cellulase activity have been increased as increased concentration of PP was added. c, amylase activity was increased by adding PP, especially in the second treatment. d, lipase activity was decreased by adding PP.

4.3 The TA

Several physical properties have been measured, in the 0th and 10th day of the bread shelf-life, to find out the effects that the peel have on both bread quality and staling rate.

4.3.1 Hardness

Hardness of the breads with 4% peel was less than the hardness of the breads which treated with enzymes from no PP treatment. Also, the hardness was decreased in the treatment with 2% peel in the 0th day. However, in the 10th day, no significant differences can be observed among the treatments and their controls (Figure 4).

Also, the hardness of treated bread with enzymes from yeast+4% peel showed the smallest p-value in comparisons to other groups. So, enzymes from

treatment (yeast+4% peel) have the best effect, in terms of hardness changes, on staling rate in comparison to the ones from only yeast, and yeast+2% PP (Figure 5).

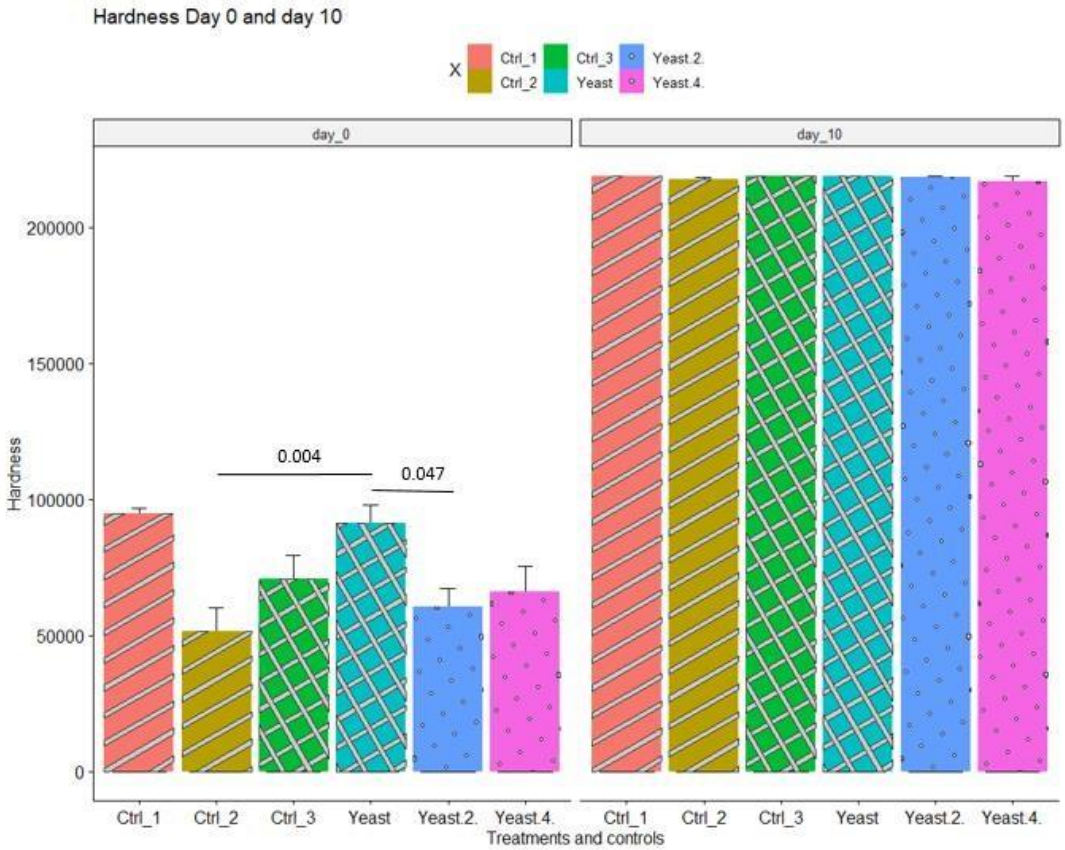

Figure 4 Second treatment (in day 0) provides the most positive effect on bread hardness. However, the hardness (in day 10) of all treatments show nearly the same value.

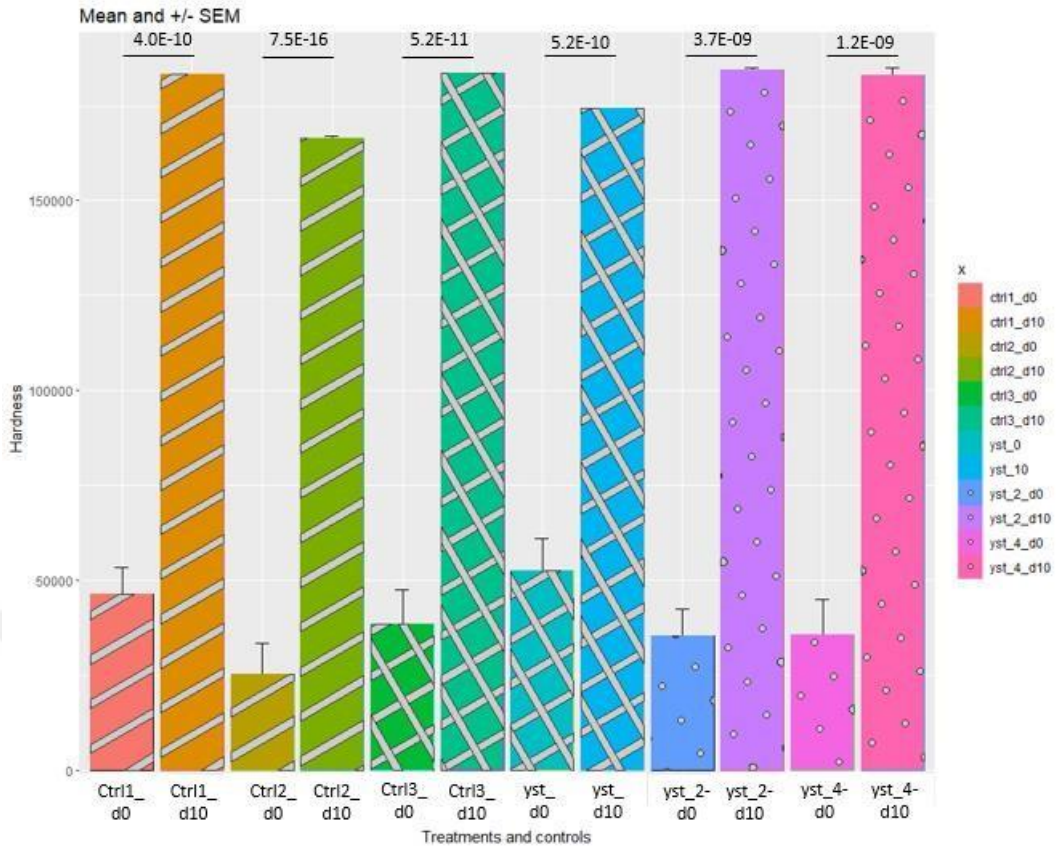

Figure 5 t-test for hardness of breads after 10 days. There are significant differences by means of hardness of breads over 10 days. However, treatments with 2% and 4% show smaller p-value in comparison to other groups.

One of the enzymes that have a big role in decreasing bread hardness and staling rate is α-amylase (66,67). This capacity of the enzyme occurs due to the production of fermentable sugars and gas and elevation in low molecular weight saccharides content. As a result, the gas retention capacity and volume of the bread will be increased and softer bread will be produced with longer shelf life (68).

Xylanase have been tested for assessing its effects on bread hardness. It is considered as one of the enzymes that reduce the hardness of panettones bread (66) and brown pan bread (69). Also, lysophospholipase, a type of lipase, was concluded for its significant improving effects on the bread hardness (66). Thus, many enzymes have significant effects on improving bread hardness in different ways.

Additionally, hardness of breads, which were made by germinated brown rice, were reduced by 34%-90% in comparison to control breads. This result was

explained not only by an increase in starch and protein degradation, but also in specific volume. These chemical reactions were occurred as the result of amylase and protease activation during germination of brown rice (70).

Moreover, addition of 0.1% laccase, and 0.001% and 0.01% protease significantly decreased crumb hardness in oat bread. However, 0.1% glucose oxidase significantly increased the bread hardness (71). So, choosing the right enzymes for enhancing bread quality is crucial.

Previously, other studies have been conducted for assessing the effects of enzymes on staling rates. For example, enzyme crude solution from rice wine, which was produced through glutinous rice fermentation by *Rhizopus oryzae*), was used in bread making. TA have been studied during 7 storage days. Generally, the hardness of treated breads and controls were increased as storage time increased. However, hardness of the treated breads with the 5%, 10%, and 15% enzyme cocktail, from rice wine, remained lower than the hardness of the control bread at any storage time. At the same time, the breads which are treated with 20% enzyme solution had harder texture of bread due to excessive deterioration of proteins by protease activity (34). A similar study showed the same result in yeast grown in YPD medium with increased protease activity, supplemented by Guatemala coffee grains (72).

4.3.2 Springiness

Springiness of the treatments and their controls showed no significant differences in both time periods (Figure 6). The results of paired, one way t-test have showed significant differences between the breads' springiness in the 0 and 10th day, except the springiness of the control 1 and yeast+4% peel (Figure 7).

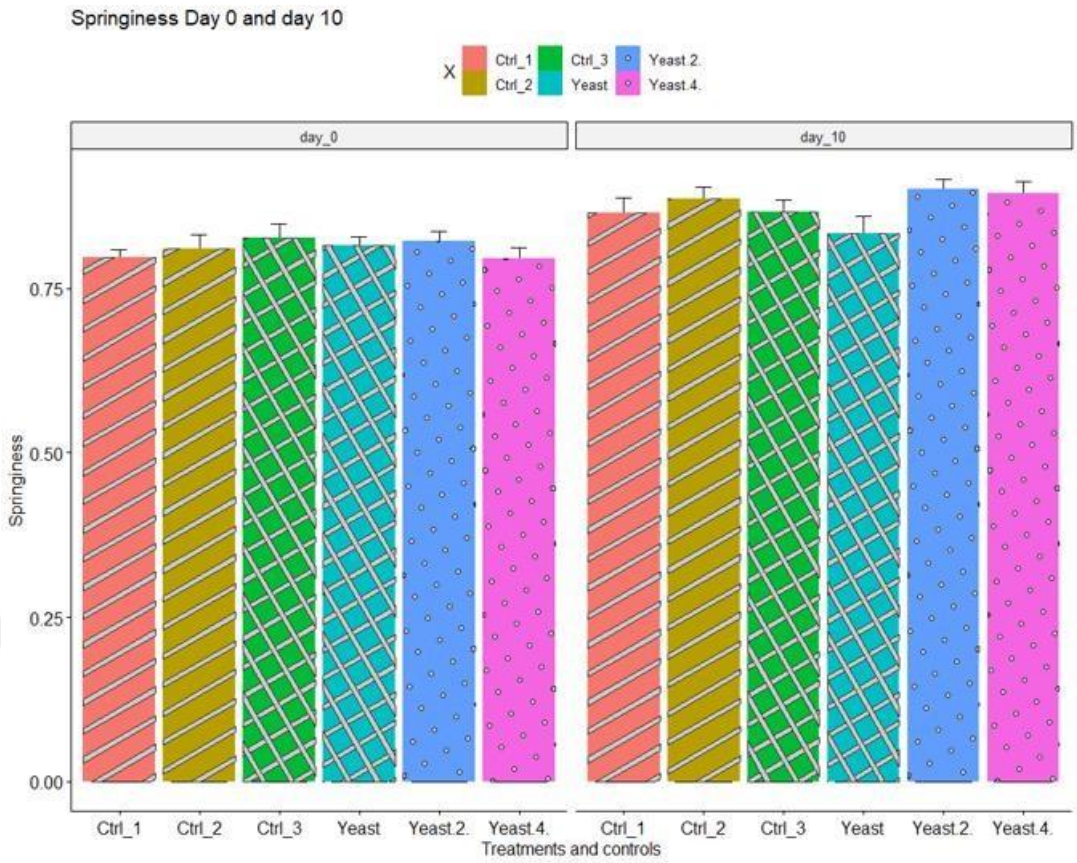

Figure 6 Springiness (in both day 0 and 10) show no significant differences.

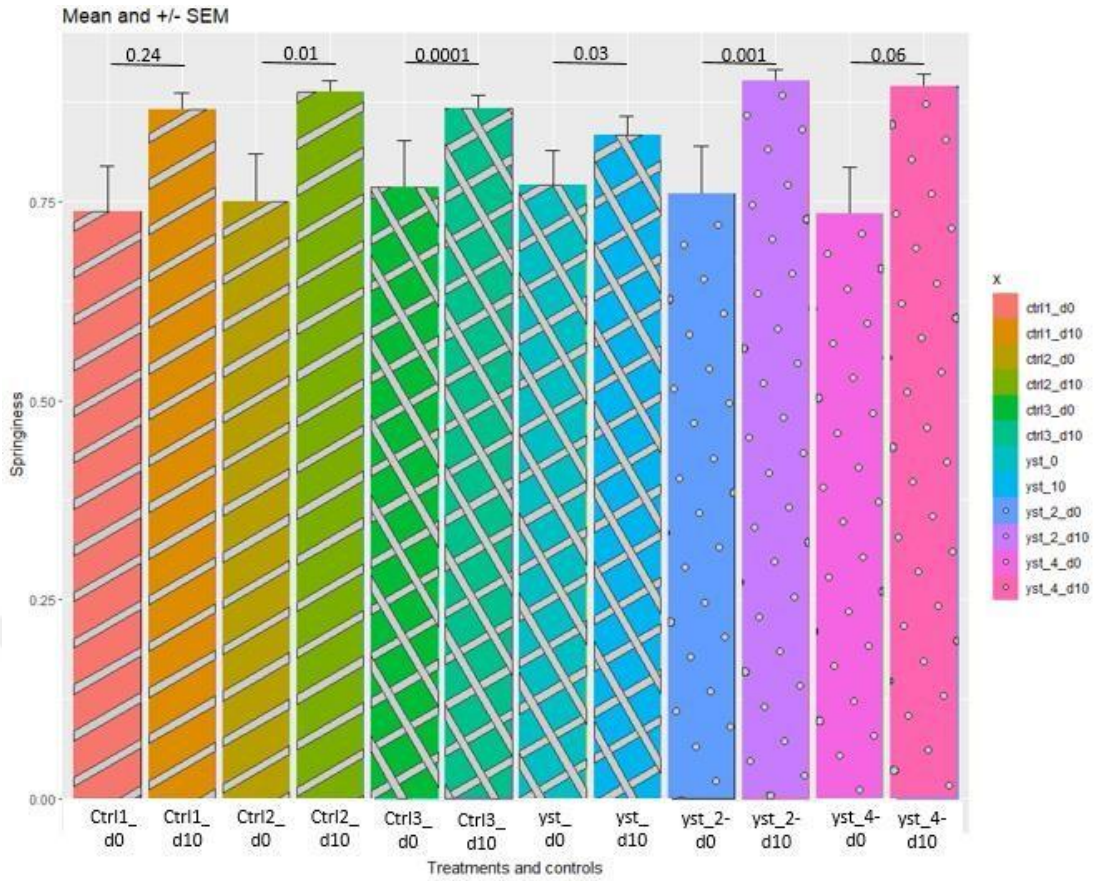

Figure 7 t-test results for the springiness of the breads after 10 days. The means of springiness of the breads have changed significantly except those of control 1 and yeast+4%.

There are other studies investigating the effect of different enzymes on bread springiness. For example, no change in springiness occurred as a result of xylanase, and xylanases/hemicellulases addition (73). Based on these results, enzymes have no effects on bread springiness.

Additionally, springiness of the breads were analyzed. No obvious differences were detected between treated breads and control in the first day. As a function of time, this property of the breads decreased in controls. Thus untreated groups were opposite to consumers' satisfaction for breads (34).

4.3.3 Gumminess

Gumminess of the breads were near to each other in the 0th day. However, adding PP, in 2%, to the yeast culture decreased the gumminess of the bread mostly in 10th day. Also, its control have least gumminess value. There is a great significant value between control 2 and treatment 1 (p-value 0.005). This may occur due to the direct effect of the added PP on bread quality, rather than on EA. It needs further investigation (Figure 8). Regarding gumminess changes over the 10 days, the changes were significant in all treatments and controls (Figure 9).

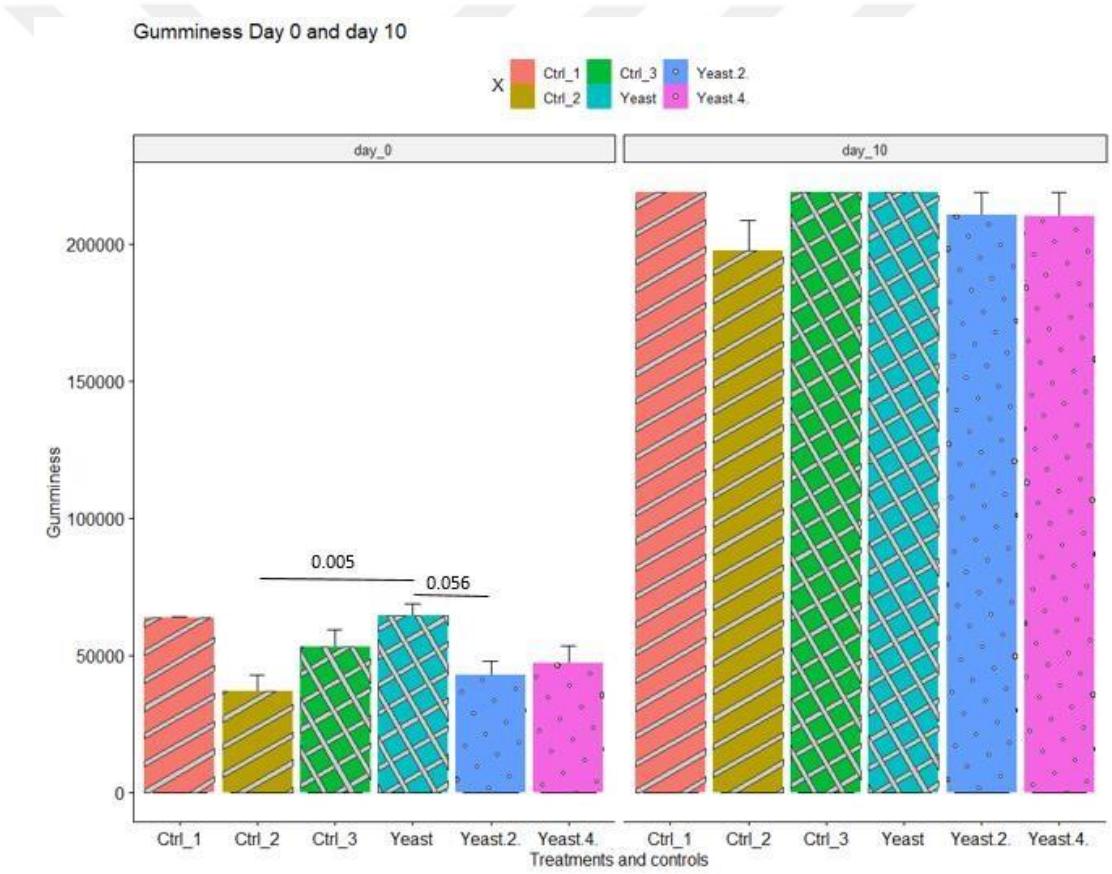

Figure 8 Gumminess (in day 0) in second treatment have the least value. The same is true for its control. However, the gumminess values (in day 10) show no significant changes among the treatments and their controls.

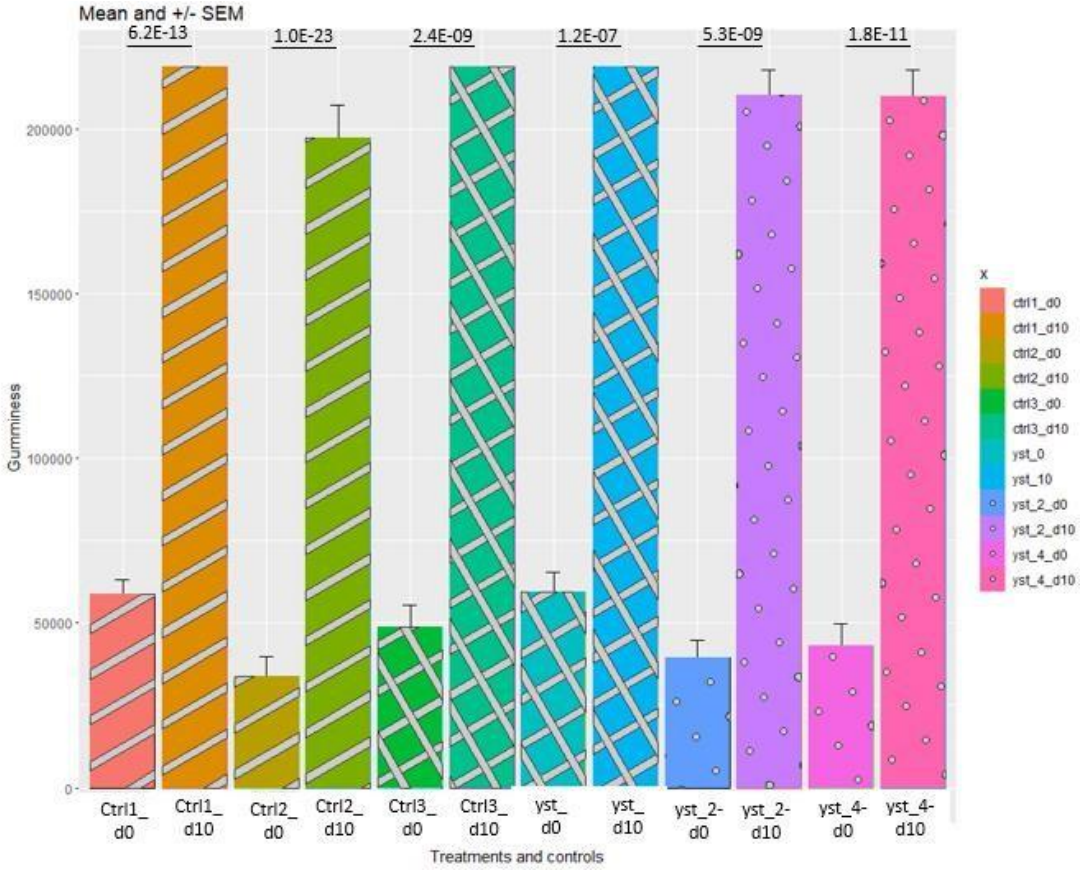

Figure 9 t-test result for gumminess of the breads after 10 days. Significant differences were observed by the means of gumminess in all groups over 10 days.

According to another study, adding some enzymes such as xylanase and cellulase will significantly decrease gumminess of breads (73). A similar study conducted by Adamcı(2019) showed the same result in yeast grown in YPD medium with increased protease activity, supplemented by Guatemala coffee grains (72). As a result, some enzymes have positive effects on decreasing bread gumminess.

4.3.4 Chewiness

The only significant differences of breads' chewiness can be found between the treatment with no PP and the control 2 (p-value 0.007) in the 0 day (Figure 10). Additionally, chewiness of all groups were significantly changed over 10 days (Figure 11).

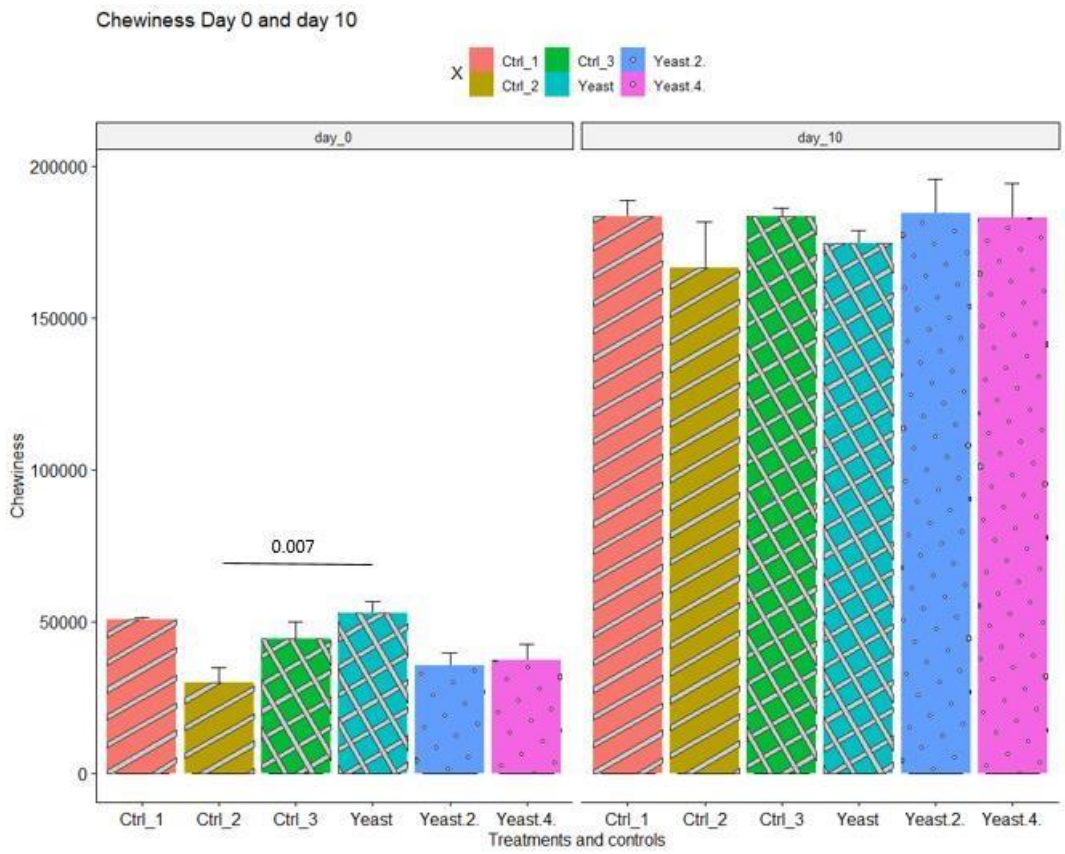

Figure 10 Chewiness (in day 0) shows significant difference between the second control and the first treatment group, while no significant changes can be seen in day 10.

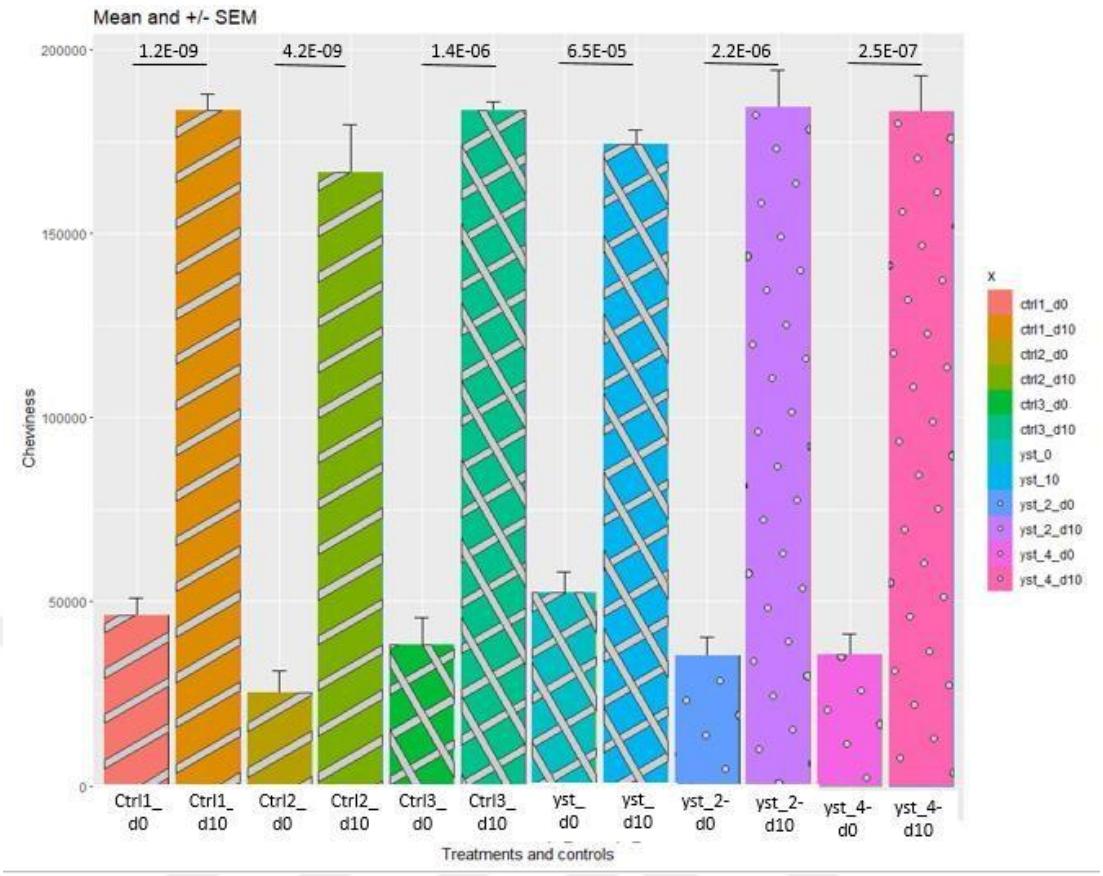

Figure 11 t-test result of chewiness of the breads. Significant difference was observed by means of chewiness of the breads over 10 days in all bread groups.

Contrary to the result that have been achieved through our work, there is a study on effects of enzymes on decreasing bread chewiness. In this study, significant decrease in the chewiness of bread was observed by the act of xylanase, amylase, cellulase (67), and glucanase (73). Thus, using appropriate type of enzymes is important for decreasing bread chewiness. A similar study conducted and showed the same result in yeast grown in YPD medium with increased protease activity, supplemented by Guatemala coffee grains (72).

4.3.5 Cohesiveness

No significant differences can be seen between treatments and their controls in both days. However, significant increase of cohesiveness was found in the control 3 in comparisons to the control 1 in the 0th day analysis (p-value 0.002). This may occurred as a result of the peel content as the cohesiveness value of the control 2

laid in between the control 1 and control 3 (Figure 12). Also, significant changes in cohesiveness were occurred in all groups over the 10 day (Figure 13).

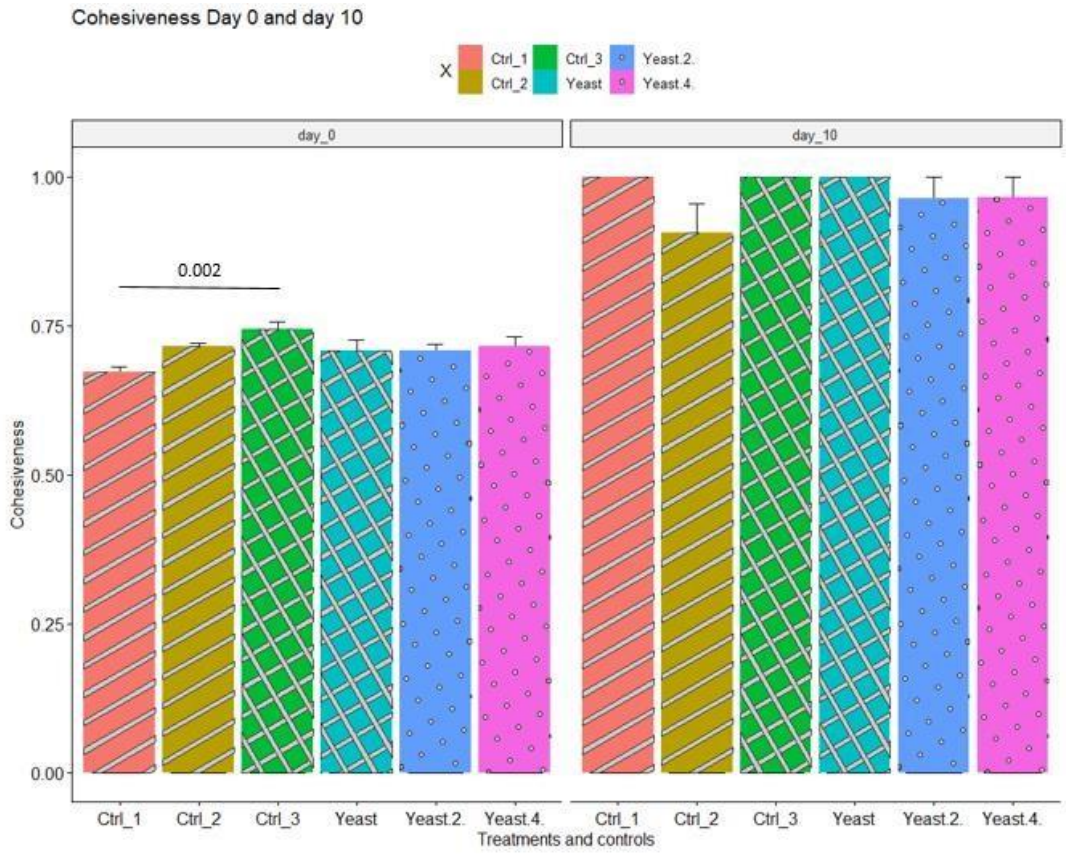

Figure 12 Cohesiveness change (in day 0) is significant between control 1 and 3 with no significant changes in day 10.

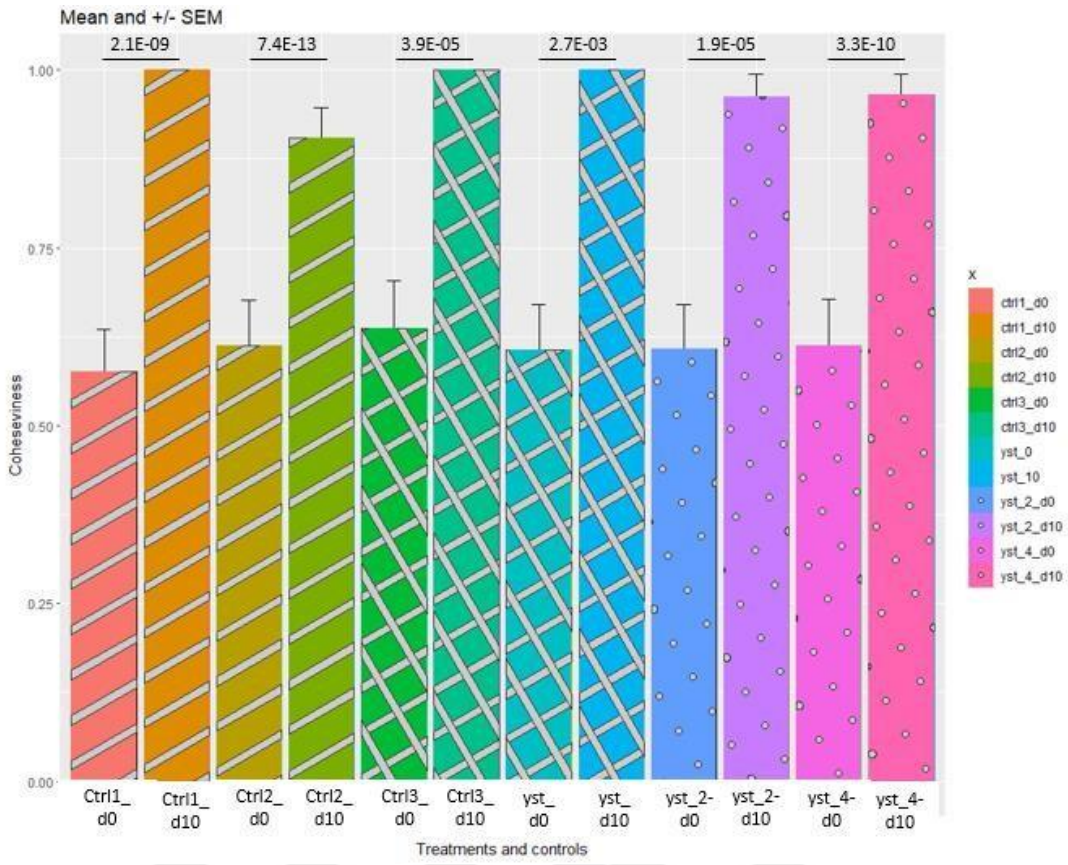

Figure 13 t-test for cohesiveness of the breads after 10 days. Significant difference was observed by means of cohesiveness of the breads over 10 days. However, the smallest p-value among all the groups is revealed in the treatment with only yeast (no peel).

Additionally, there are other studies which concluded that some enzymes, such as xylanase, cause significant increase in cohesiveness of bread while there are other enzymes, such as cellulase and glucanase, which lack significant effect on bread cohesiveness. Also, the combination of xylanases/hemicellulases showed significant increase in cohesiveness (73). So, bread cohesiveness can be significantly increased by using some effective enzymes or some combination of them.

Also, cohesiveness of the breads were analyzed in another study. No significant differences were detected between treated breads and control in the first day. However, cohesiveness of the breads decreased in controls over time. Thus untreated groups were opposite to consumers' satisfaction for breads (34).

In another study, BS is reduced by adding amylase, and amylase/xylanase enzyme solutions. Staling retardation was observed by significant increase in cohesiveness and decrease in hardness. In the same experiment, the opposite effects of xylanase enzyme was recorded on breads' properties throughout their shelf life (72).

4.3.6 Resilience

Resilience of the controls, in the 0th day, was increased as higher amount of the peel was incorporated into the media. Significant difference was achieved between the control 1 and the control 3 (p-value 0.002). This means that PP changed the resilience of the breads directly rather than changing it by affecting enzyme activities (Figure 14). Also, resilience of breads were significantly changed over the time (Figure 15).

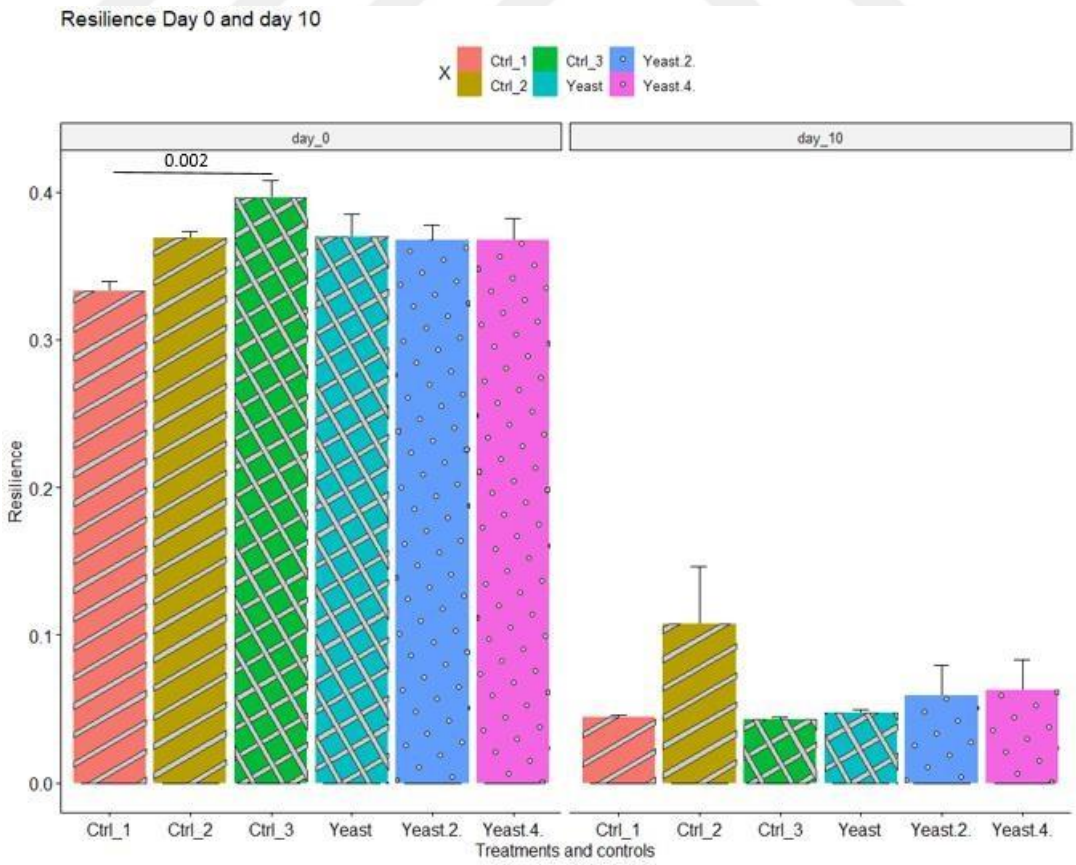

Figure 14 The significant difference between control 1 and control 3 (in day 0) in terms of resilience was shown, while No significant difference can be seen between the groups in the 10th day.

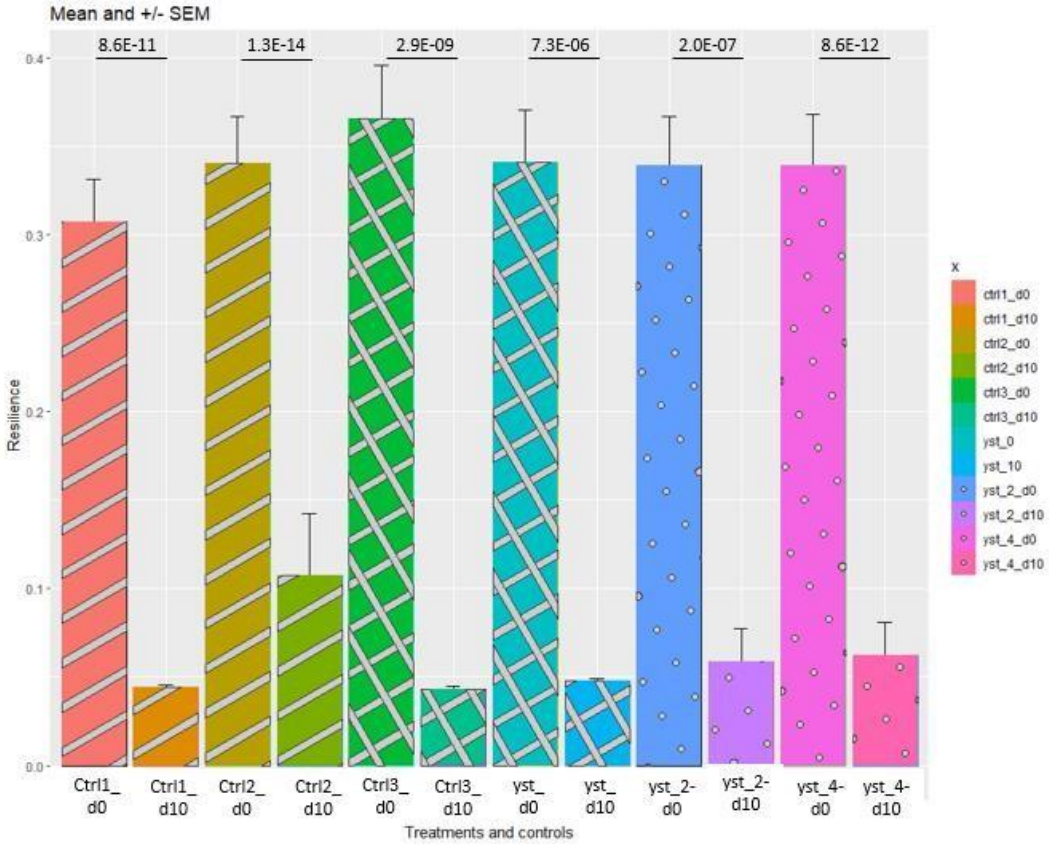

Figure 15 t-test for resilience of breads after 10 days. Significant differences were observed by means of resilience of the breads in all groups over 10 days.

However, there are other studies which concluded that some enzymes, such as xylanase, result in significant increase in bread resilience while there are other enzymes, such as cellulase and glucanase, show no significant effect on bread resilience (73). Also, the combination of xylanases/hemicellulases significantly increased resilience (73).

In a study which was conducted by (67), the decreasing effects of maltogenic α-amylase on resilience was recorded. So, changes in breads' resilience is depending the type of the enzyme as well as the some chemicals that are present in PP.

4.4 Water Holding Capacity(WHC)

WHC of the breads significantly differ between some of the treatments and controls in 0th day. WHC is reduced significantly by adding PP in the controls. Adding more PP reduced the WHC greater. In another words, there are significant differences between control1 and control3, control2 and control3, control1 and treatment3, and control3 and treatment2.

Regarding the treatments, breads which are treated with enzymes from yeast+4% peel showed the least WHC, while treatments with yeast+2% peel showed the most. On the other hand, no significant difference was recorded in WHC of breads in the 10th day (Figure 16).

In another study, WHC of steamed rice bread was increased by adding 0.5 % Flavourzyme®. The explanation for such an elevation in WHC was raising in the content of polar groups. Another explanation was exposure to hydrophilic groups by the activity of enzymes (74). The same explanations can be applied to our results too.

On the other hand, Flavourzyme® in concentration of 1.0 and 1.5% decreased WHC of the bread. This was occurred as proteins, which regulate WHC, were degraded by the act of excessive enzymatic activity (74).

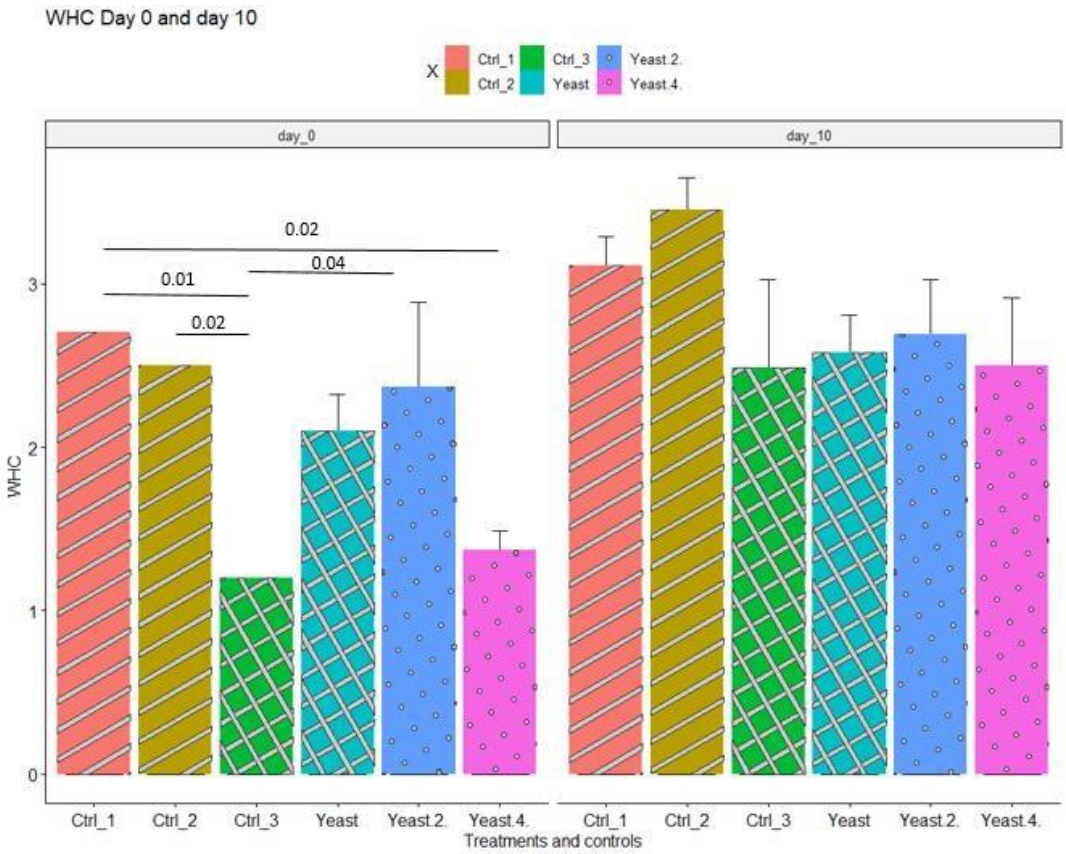

Figure 16 WHC of the breads in day 0 shows significant differences between certain treatments and controls. However, WHC of breads in 10th day, shows no significant differences.

4.5 Oil Holding Capacity(OHC)

OHC of the breads (in 0th day) were increased in controls by adding more PP, while it was decreased in the treatments as more PP is increased. Overall, the changes are not significant. In the 10th day, significant difference was recorded between control2 and control3 (p-value 0.007) (Figure 17).

Other studies explained the changes in OHC of breads by the act of enzymes. For example, increasing the OHC of bread was stated by Pourmohammadi & Abedi, 2021 as the act of *Aspergillus usamii* protease. The protease works on wheat gluten and expose its hydrophobic regions to aqueous regions. Moreover, endopeptidases from *Eurygaster spp*, *Aelia spp*, *E. integriceps* shows their increasing effects on

breads' OHC by enzymatic hydrolysis of glutenin, thus exposing hydrophobic regions to aqueous phase (75).

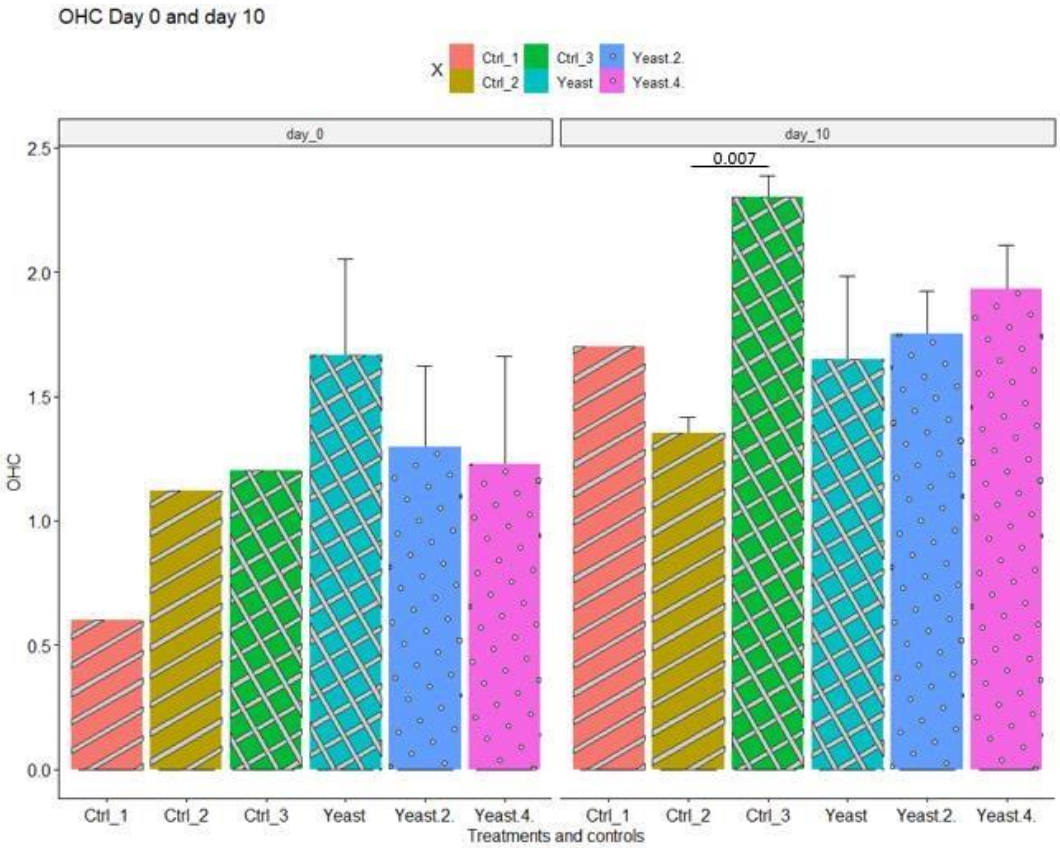

Figure 17 OHC in day 0 shows no significant difference. OHC in day 10 shows a significant difference between control2 and control3.

4.6 Sensory Evaluation

For assessing organoleptic qualities of the breads, the results of the means are shown for each criteria in the following table. Generally, breads with enzymes from yeast+4% peel was the best in the sensory evaluation; rated as the best bread in terms of chewiness, hardness, and overall taste. Moreover, other organoleptic properties have been rated in the same way for all breads (Table 2).

Table 2 Results of sensory evaluation of the breads by 30 people.

SAMPLE	SWEET-NESS	HARD-NESS	BITTER-NESS	SALTI-NESS	ODOR	APPEAR-ANCE	FLAVOR	CHEWI-NESS	OVERALL
Yeast	9.53±0.51	6.43±0.82	9.5±0.63	0.3±0.54	9.4±0.6	9±0.9	8.4±0.68	6.4±0.67	7.37±0.29
Ctrl1	9.63±0.49	6.5±0.68	9.5±0.63	0.3±0.52	9.2±0.8	9.3±0.8	8.4±0.67	6.4±0.63	7.4±0.22
Yeast+2%	9.47±0.68	7.5±0.63	9.5±0.51	0.3±0.54	9.3±0.8	8.97±0.85	8.3±0.61	7.4±0.8	7.6±0.26
Ctrl2	9.47±0.51	8.5±0.68	9.5±0.51	0.4±0.63	9.1±0.9	8.97±0.7	8±0.7	6.7±0.76	7.54±0.22
Yeast+4%	9.6±0.6	8.6±0.46	9.6±0.5	0.3±0.48	9.1±0.8	9.4±0.5	8.4±0.57	9.3±0.55	8.05±0.18
Ctrl3	9.5±0.7	8.5±0.51	9.5±0.57	0.26±0.45	9.2±0.8	9.3±0.6	8.1±0.82	8.2±0.83	7.8±0.23

5 CONCLUSIONS AND RECOMMENDATIONS

Throughout following our research questions and dedicative works, we will conclude our results in following paragraphs:

Among the treatments (yeast, yeast+2% peel, yeast+4% peel), yeast+4% peel have the greatest effects on increasing the activity of cellulase, and invertase while yeast+2% treatment have the most positive effect on amylase concentration, which are important for bread quality. Regarding lipase activity, PP inhibits its activity, especially in the second treatment (yeast+2%).

Regarding yeast growth, the maximum yeast growth rate observed in treatments with yeast+2% while yeast+4% and yeast come as second and third

order. This may occurred due to the presence and ease in availability of PP as a substrate in growth medium of yeast in yeast+4% peel, instead it may prefer expressing enzymes.

When it comes to the physical properties of bread qualities, we can see that breads which are treated with enzymes from yeast+4% peel have the best qualites in comparison to other treatments in general, either in the 0th and 10th day. On the other hand, little delaying effects on BS were recorded between 0th and 10th days. Sensory evaluation of the breads was another variable which strengthened our hypothesis, confirming that enzyme solution from yeast+4% treatment positively affected the organoleptic properties of the breads.

When microbiological status of breads is considered, no observed effects of enzymes can be seen. This may be the result of proper storage of the breads, avoiding microbial growth within these 10 days.

Based on our conclusions, we would like to recommend the following suggestions for further investigation:

Assessing the effects of the enzyme solutions on microbiological qualities of the breads, which are stored in room temperature rather than + 4 °C, would be an important research question for further investigation. Also, testing the effects of each partially purified enzymes and their combinations on bread quality is also important to know if the enzymatic effects on the breads is symbiotic or solitary.

Different concentration of PP, rather than 2% and 4%, can be investigated for assesing their effects on enzyme activity and bread quality. In this way, the optimum concentration of PP for enzyme activity and bread quality improvement would be found. Moreover, different concentration of enzyme solutions can be further investigated for their effects on bread quality and BS rate.

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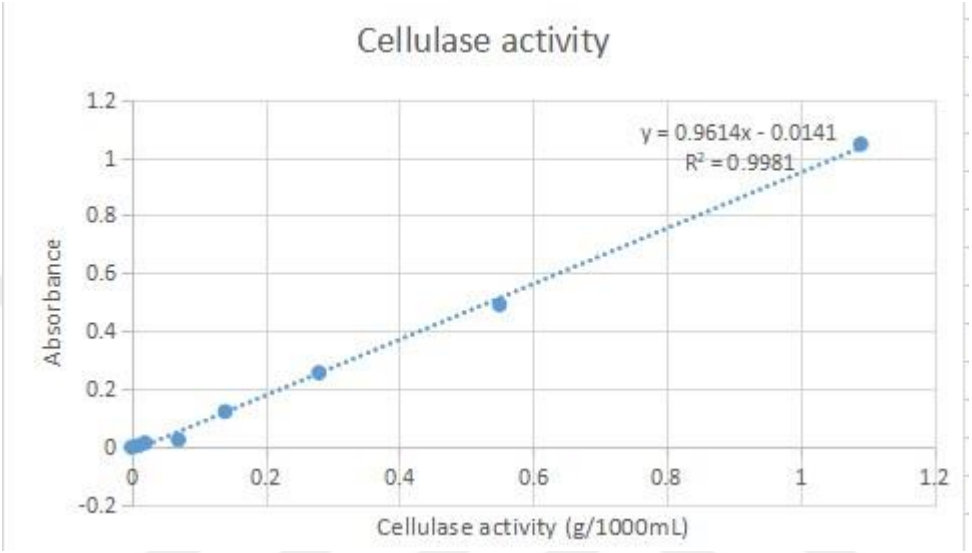
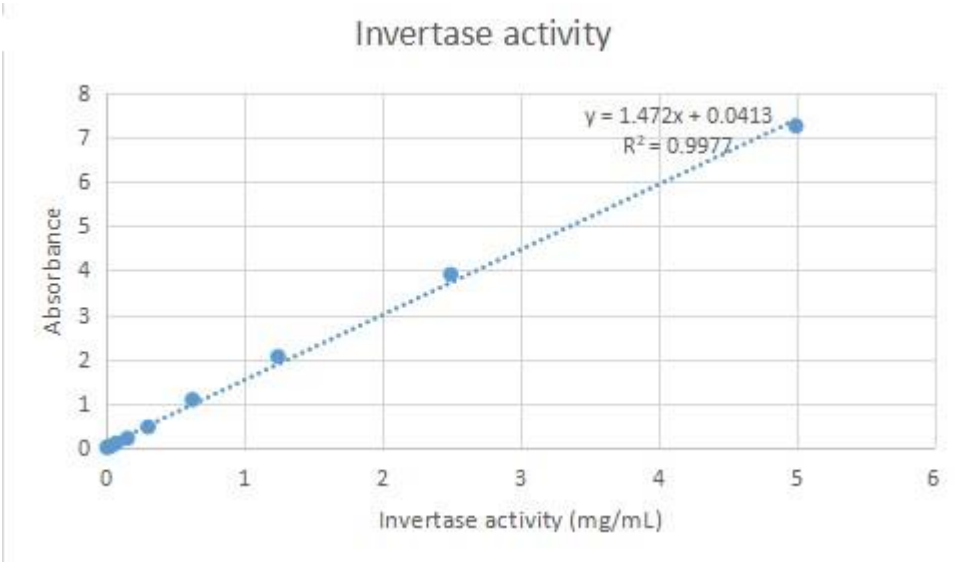
APPENDICES

7 APPENDICES

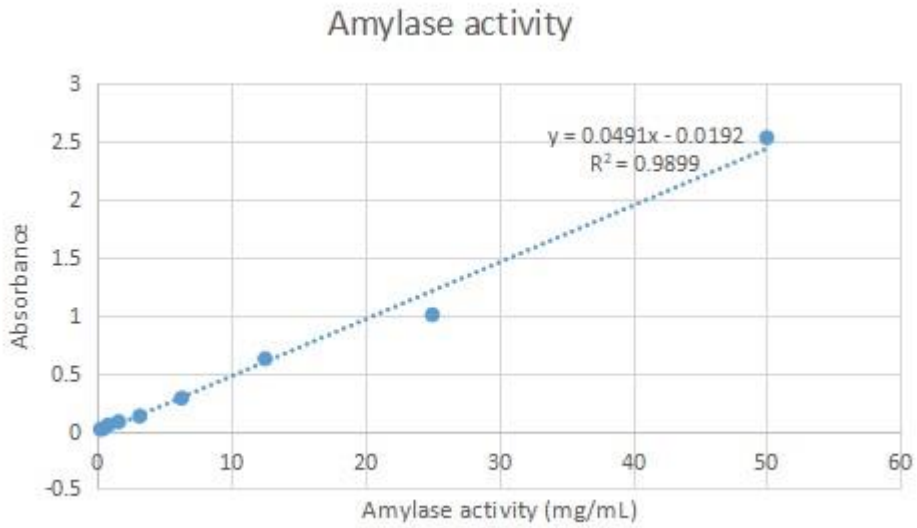
7.1 The Standard Curve Of Cellulase Activity At OD 550 nm



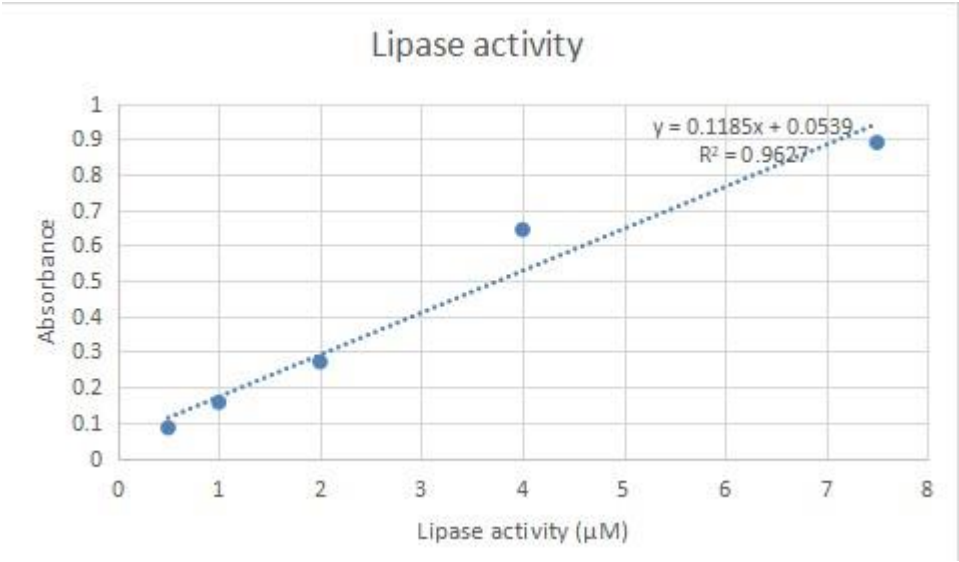
7.2 The Standard Curve Of Invertase Activity At OD 540 nm



7.3 The Standard Curve Of Amylase Activity At OD 660 nm



7.4 The Standard Curve Of Lipase Activity At OD 410 nm



8 CURRICULUM VITAE

Name SURNAME **:**Hawnaz Najmalddin

Place and Date of Birth **: Iraq- 30/10/1993**

Bachelor's Degree **: University of Sulaimani**

e-mail **: hawnaz.najmalddin@univsul.edu.iq**

List of Publications **:** Najmalddin, H. O., Taher, A. A., & Mohammed, S. A. (2015). Evolution, and phylogeny of herv-s family in some closely related primates.

Yarwais, Z. H., Najmalddin, H. O., Omar, Z. J., & Mohammed, S. A. (2020). Automated Data Collection of Drosophila Movement Behaviour Assays Using computer Vision in Python.

Yurdugul, S., & Najmalddin, H. O. (2021). Vegetable and Fruit Waste Production Related to Consumption in Turkey and Certain Middle East Countries.