# THE EFFECT OF ENZYME USE ON THE FORMATION OF CARBONYLS AND STRUCTURAL PROPERTIES OF CAKES

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# ABSTRACT


## THE EFFECT OF ENZYME USE ON THE FORMATION OF CARBONYLS AND STRUCTURAL PROPERTIES OF CAKES

Enzymes are used as additives to improve the quality parameters of cakes. However, high temperature conditions produce carbonyl-containing compounds as precursors of toxic maillard reaction products. In this study three food grade enzymes were used as agents to decrease the formation of carbonyl-containig compounds while preserving the cake quality factors. For this purpose transglutaminase, lipase and amylase enzymes were used. All of the three enzymes lowered the amounts of carbonyls with the largest decrease by lipase of 31.83% ($p<0.05$) with respect to the control cake. Transglutaminase and lipase addition changed the carbonyl profile of cakes. Both transglutaminase and lipase caused important changes in protein secondary structures with large increases in alpha helix, turns and anti-parallel beta structures, however, amylase did not cause such large changes. The three enzymes used caused the lipid/protein ratio to decrease. The level of lipid unsaturation did not change for transglutaminase and lipase, however, the level unsaturation decreased in the case of amylase indicating the formation of dicarbonyls was via Maillard reaction not due to lipid peroxidation. However, the GC-MS analysis results indicated that there was no change in the formation of neither the Maillard reaction products nor the lipid oxidation products in the head space analysis. The amorphous structure of the starch in cake samples increased depending on the enzyme concentration used.

# ÖZET

## ENZİM KULLANIMININ KEKLERDE KARBONİL GRUBU OLUŞUMU VE KEK YAPISAL ÖZELLİKLERİ ÜZERİNE ETKİLERİ

Enzimler keklerin kalite parametrelerini geliştirmek amacıyla kullanılırlar. Ancak keklerin üretiminde kullanılan yüksek sıcaklık dereceleri keklerde Maillard Reaksiyon ürünlerinin öncülleri olan karbonil içeren bileşiklerin oluşumuna da sebep olurlar. Bu çalışmada enzimler karbonil içeren bileşiklerin oluşumunu azaltırken keklerin kalite parametrelerini arttıran ajanlar olarak kullanılmışlardır. Bu amaçla transglutaminaz, lipaz ve amilaz enzimleri kullanılmıştır. Bu üç enzim oluşan karbonil içeren bileşik oranlarını düşürmüşlerdir, en büyük düşüş kontrol kekine göre 31.83% ($p<0.05$) oranla lipazla elde edilmiştir. Transglutaminaz ve lipaz eklenmesi karbonil profilini önemli oranda değiştirirken amilaz ciddi farklara sebep olmamıştır. Transglutaminaz ve lipaz kek proteinlerinin alfa heliks ve dönme, anti paralel beta tabakaları ve random koil ile ikincil yapılarında önemli değişikliklere sebep olurken amilazın etkisi kısıtlı kalmıştır. Üç enzim de lipit/protein oranında düşüşe sebep olmuştur. Lipit doymamışlığı transglutaminaz ve lipaz'da değişmezken amilaz azalmaya sebep olmuştur, bu da keklerde oluşan karbonillerin kökenlerinin Maillard Reaksiyonu ürünü oluşumuyla meydana geldiği ama lipit peroksidasyonu ile oluşmadığını işaret etmiştir. Ancak GC-MS ile gerçekleştirilen gaz analizlerinde Maillard reaksiyonu ürünlerinin ve Lipid Oksidasyonu ürünlerinin oluşumunda bir farklılık gözlenmemiştir. Kullanılan üç enzim de nişastanın amorf yapısını kullanıldıkları konsantrasyona bağımlı olarak arttırmıştır.

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# CHAPTER 1

# INTRODUCTION

## 1.1. Cakes

Cakes are one of the most commonly produced and consumed cereal products in food industry and at home all over the world. Cakes, a kind of soft wheat product, are ready-made food products produced using different formulas among various forms of bakery products (Baltacıoğlu and Uyar 2017). Cakes are prepared from wheat flour, sugar, oil and eggs prepared from the soft dough and the chemical leavening agents. Different cake doughs were prepared which are devoid of eggs due to their high lipid and cholesterol content. Similarly, different cake recipes without sugar were designed due to adverse health effects of it.

Wheat and wheat flour contain several different enzyme activities during the growth of the plant and harvesting. A commonly known enzyme of wheat enzyme is alpha amylase. Alpha-amylases have high activities, which are prefered to be used in the bread industry (Ciacco, 1982). Baking enzymes are regulary used in baking industry.

The enzyme use in food and biotechnology industries have a significant roles expecially in baking industry on the quality product.

Flour, the unstable ingredient, is the main ingredient in the baked product. High quality or high protein flours are needed for high quality bread flour (Hamer, 1995).

A cake is a medium strength, 8-9% protein, finely ground, soft dough prepared with weak wheat flour, sugar, oil and eggs, prepared according to the procedure. Chemical embossers are used in preparation. In addition, whipped egg white addition and rapid kneading act as raising agents (Elgün and Ertugay 1995).

During the cake production there are three important steps. The first one is mixing of the ingredients. At this stage protein becomes soluble in the dough and air is incorporated in the dough matrix. The second stage is stabilization of oil. At this stage this mission is commonly established by enzymes. The latest stage is processing of starch (Karaoğul et al, 2001).

Cake products are one of the most important products of the bakery industry and can be found in a wide variety of forms. It is very difficult to define the cake due to the large number of cake types and cake formulas in the industry. However, in a very general expression, cakes can be defined as a bakery products obtained by baking soft dough prepared using flour, sugar, oil, eggs, baking powder, water (sometimes milk) and sweetener (Anony. 1966, Pyler 1988, Mercan 1998).

Fat and sugar offer the cake which gave the soft structure, and increase the flavor. Eggs are emulsifying, leavening agents. They also prevent drying of the dough components. It's the baking powder's job to magnify the bubbles that cause the cake to rise to its potential. Therefore, it is added to cakes. The cake fabrication procedure includes five steps. These; mixing, accumulating, baking, cooling and packaging. The first step is mixing, the airiness of the cakes comes from the emulsion and foam resulting from egg proteins during mixing. The next step is mixing. There is foam formation in this step. The step in which the air cells are included in the dough is the mixing sequence. An increase in volume indicates an increase in the number of air pockets.

The fact that the cake is porous and soft at the same time is due to the formation of carbon dioxide, starch gelatinization and protein denaturation. A high volume and fine homogeneous moist crumb is present in a quality cake (Al-Dmoor, 2013).

In cake baking, four fundamental ingredients of cakes are mixed in a certain order which is followed by baking in an oven at higher temperature levels then 150 °C for different process times (generally about 30 minutes) (Wilderjans et al., 2013). Different process conditions such as scrambled egg white additives and fast kneading play a puffing role in cake structure (Elgün and Ertugay 1995).

During cake baking the sensory parameters of cakes such as taste, color and odor develop and the product gains its characteristic solid-amorphous structure. As a result of the elevated temperature levels the microbial load and enzymatic activity levels of the ingredients decrease and the shelf life of the final product is increased (Fellows, P., 1988). During baking the elevated temperature levels gelatinize the starch and contribute to the final solid structure. In addition, the water is evaporated. The other important macromolecular components of cake dough, proteins, become denatured and lose their secondary structures. At the final period of the cake baking intermolecular disulfide bond form and contribute to the final solid structure even further. The unsteady-state heat transfer during the baking process form a changing temperature profile which developes at the center of the product latetest. In a study the central temperature of the cakes and

related cereal products is found to reach at 95-98 °C to have a nice product structure (Ureta et al., 2014).

### 1.2. Cake Quality Factors

Several parameters are used to describe the quality of the final product. These include: the volume and the hight of the cake, symmetry values of the cakes, porosity and color.

### 1.3. Food Additives

Food additives which are not consumed as food alone or used as a characteristic component of food, with or without nutritional value, as a result of being added to food in production, treatment, processing, preparation, packaging, transportation or storage stages, are substances that are expected to be the component of that food directly or indirectly (Turkish Food Codex, 2011). Food processing aids “are not technically inevitable, although they are not consumed as food alone, used for the processing of raw materials, food or food components for a specific technological purpose, although there are no residues of the product or its derivatives in the end product; however, its residues are substances that do not pose any health risks and do not have a technological effect on the final product” (Turkish Food Codex, 2011).

### 1.4. Biotechnology

Biotechnology is a discipline that enables using cellular and biomolecular processes to develop technology and products that help improve lives on Earth and the health of our planet. Biotechnology can be divided into different branches: medical biotech, industrial biotech, environmental biotech and marine biotechnology.
Medical biotechnology focuses on the medical profession. It helps to people and create high quality life.

Industrial biotechnology makes use of living cells to. increase energy and higher technology production. Replacement of traditional industrial processes by biocatalytic

processes to obtain valuable products like cosmetics, pharmaceuticals, food additives etc and production of industrial microorganisms or enzymes are included in this group.

Environmental biotechnology depends on agriculture such as development of new biopesticides, genetically modified plants that yield higher yields, foods with increased content, more resistant plants and herbally enhanced medicines. As a result of all this, it is aimed to reduce the cost of production to help feed the growing world population.

Marine biotechnology focuses on alternative source of energy such as biofuel and biomass production.

Modern biotechnology industry produces recombinant proteins and enzymes and genetically modified organisms for various medical and food-based uses, agricultural applications.

### 1.5. Food Biotechnology

Food biotechnology is a general term that spans a wide range of applications using living organisms such as microorganisms, tissues or organisms and their parts to develop new, better and sustainable food products. It is anticipated that food biotechnology will continue to be used in the future as it is frequently used today. The food industry is also valued day by day. Many different food industries such as dairy products (ex., improved dairy products with recombinant enzymes and fermetative bacteria), brewery (ex., converting starch to sugar and then adding special yeast), agriculture (plant modifications and artificial selection and crops that can adapt to harsh climatic conditions and hybridization) and cereal inductry (ex., the positive effects of enzymes on the product quality factors) can be considered. In food biotechnology the aim is development by new and processing conditions for the better food product. This aim makes a possible uses a technology such as using living organisims (United States Department of Agriculture, Economic Research Service. 2012. Adoption of Genetically Engineered Crops in the U.S. 2012). Food biotecnology have an important mission. The mission is create lower cost and also higher nutrients by the global.

## 1.6. Enzymes

Enzymes are industrial materials used in almost every aspect of life. For example, wine industry, food industry, paint making industry, etc. Enzymes, known as food-processing enzymes, change food properties and are therefore widely used as food additives. Food processing enzymes are used in different industries such as in the dairy industry and in the manufacture of predigested foods. The use of enzymes, developing from the past to the present, makes life easier. Food studies around the world have shown that enzymes have positive effects.

Any enzyme cannot use just only one in baking application, while always need another technical enzyme classes. In the global market, some of enzyme get a number of advantages such as, lipases, amylases, xylanases. Furthermore, this enzymes types get fit to baking industry (Heil et al., 2010). Chapman said that; ''the global enzyme market is projected to grow from $5.01 billion in 2016 to $6.32 billion in 2021'' (Chapman, 2018). Various industry areas use enzymes because of their minimal costs. These type of enzymes focus only on a set of chemical reaction types or a set of intimately reactions (Bhatia, 2018).

Enzyme effects in cereals were generally studied on bread and enzymes were determined to have a positive effect. However, cereal products are not just bread. The candid question is which enzymes will be successful in cakes? It is a transglutaminase enzyme that is tested on bread and has an effect more than expected. Transglutaminase is an enzyme that can improve the functional properties of proteins by creating cross-links between amino acids or peptides. Transglutaminase is effective in improving the textural properties of bakery products. Even a very small dose can cause modification in dough properties (Kurt and Zorba 2004).

Almost all of the studies have been examined in bread and its derivatives. However, enzyme activity on the cake, which has a simple formula, has not been discussed. In the researches, the enzyme effects that are foreseen to affect the baking industry have been investigated and an enzyme has been identified by considering the proteins provided in the egg and the proteins in the flour (transglutaminase) (Alp, Bilgicli, 2008). The popularity of microbial enzymes in the food industry is because they increase the quality and variety of foods. Therefore, food diversity is expected to increase with these factors.


Figure 1. Examples to enzymes used in food industry

(Source: Corpuz, 2019)

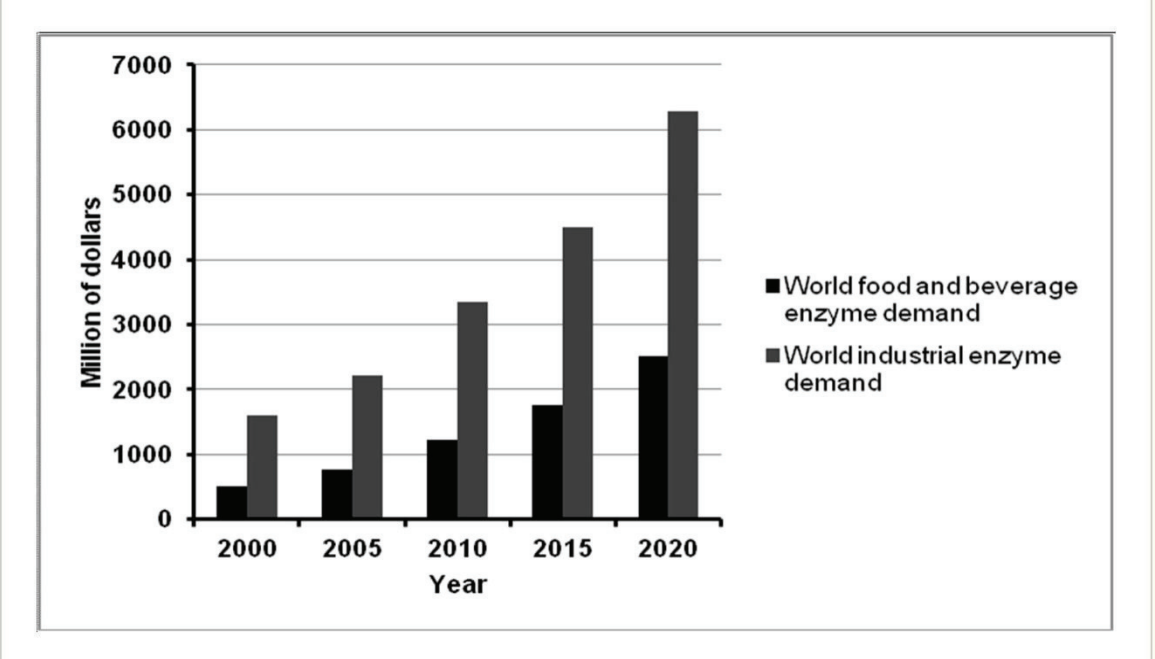



Figure 2. The enzyme market in the food industry indicates, that enzyme factors are having the main role in the world market(Source: Miguel et al, 2013)

Enzyme are used in various industries because of their contributions in the cost of final products (Bhatia, 2018).

### 1.6.1. Transglutaminase

Transglutaminase is an enzyme that can change the protein structure in foods. This enzyme, which enables the formation of high molecular weight polymers, creates covalent cross-links between the molecules in the structure of proteins and glucose. Due to these features, it has a wide range of uses, including meat, milk and cereal products. The transglutaminase enzyme can improve the nutritional properties of processed foods, it provides positive effects in various quality features, especially texture, and shows effects such as gel structure, increasing mechanical strength and reducing textural deformation. This enzyme is also provided to prevent food additives and excessive salt use, which are known to cause various harm in terms of human health. It contributes to increasing the economic value of many foods with low economic value and also has an important effect on the development of new products.

The cereal-based food industry are commonly based on product by cakes and breads.The enzyme of lipases has a multiple function in the industry (Pareyt and others 2011). The most several instances are using to the enzyme in egg yolk, thus in the baked products. So, the researchers generally focus are on endogenesis lipids (which are phospholipids are affect on to the monoacylglycerols) while find in wheat flour and also egg.

Transglutaminase enzyme is also effective in correcting the texture defects that occur in various grain products made with low quality flours and increasing the bread volume. It causes modification on dough properties even when used at a very small dose. It has been observed that with the addition of transglutaminase enzyme, the volume of breads increased and strengthened (Motoki and Seguro 1998).

### 1.6.2. Lipase

Baked food industry have the four common type of enzymes are used: lipases, proteases, amylases and cellulases (Singh and Sachan, 2019). The lipase enzyme has lots of effect for the cake. Mostly, lipase enzymes used in the bread manufacturing, shown

that positive effect. Lipase enzymes multi usable, while prefer many of industry like in the food etc. (Gerits et al, 2014).

However, in the bakery industry have many reasons for select to enzymes. The bread making formula depend on added to high level by lipase. Because, the lipase enzyme decrease the cost. In addition it provides an easy storage conditions. Significant thing is, lipases are simply fabricated at basic conditions (Casas-Godoy et al., 2012) (Gerits et al., 2014). Lipases only focus on lipids or fat portions of the dough which increase the tolerance and, improving cakes volume (Berry, 2010).

The flavor production and fragrance manufacturing industry generally prefer to lipase enzymes. Lipases are have role of the key in transformation acyl groups from ester to others (SA et al, 2017).

The enzyme of lipases have multiple functions in the industry (Pareyt and others, 2011). The most several instances are using to the enzyme in egg yolk, thus in the baked products. So, the researchers generally focus on lipids (which are phospholipids have effects on the monoacylglycerols) while find in wheat flour and also egg.

### 1.6.3. Amylase

The alpha-amylases have found wide utilization in food industry. Almost all the part of baking, brewing etc these enzymes are takes in charge (Rveendran et al, 2018). The two types of amylases generally take in breadmaking. And, two of them obtain the positive effect in process (Miguel et al, 2013). The α-amylase hydrolyzes damaged or pasted starch; forms short, straight-chain dextrins. The α-amylase enables the formation of fermentable sugars required for ethyl alcohol fermentation in dough. Indirectly, it provides bread with swelling, porous structure, desired taste and color (Erem and Certel, 2006). Alpha amylase enzyme is widely used in bakery to improve product quality and improve dough properties (Newberry et al. 2018, He and Hoseney, 1991).

## 1.7. Carbonylation in Foods

In addition to the effects of elevated temperature levels on the starch and protein structure during cake baking, the high temperature levels also cause some addition reactions to take place one of which is the formation of carbonyl compounds. Carbonyl group is a functional group formed by the double covalent bond between carbon and

oxygen atoms as seen in figure 3. Many differnet chemical componds contain the carbonyl group including aldehydes, ketones, carboxylic acids, esters and amides (Solomons, T. W. G., 1996).

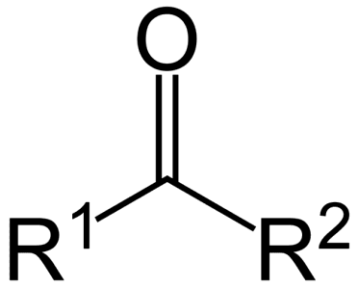



Figure 3. The carbonyl group ($R^1$ and $R^2$ indicate different functional groups)

The reactive carbonyl groups formed during the heat processing were found to have detrimental effects on the health because of their elevated reactivity potentials. As the figure 3 indicated the carbonyl groups had more then one functional groups with different chemical activities in living tissues and organisms (Semchyshyn, H. M., 2014). In addition to their adverse effect on food quality these reactive carbonyl chemicals were found to play important roles in diffent disease pathologies (Bastos, D H. M B., Gugliucci, A., 2015; Laurianne P., L., Peterson, D. G. 2018).

The health effects of the reactive carbonyls: Reactive carbonyl compounds were found to react covalently with the cellular DNA, glutathione and proteins. The products of these reactions contribute to the formation of Advanced Glycolization End Products (AGE) which were associated with the pathology of some metabolic diseases (Bastos & Gugliucci, 2015). In addition these compounds were found to have an antioxidant activity and to kill tumor cells (Somoza, V., 2005). However, the effects of these compounds were not characterized thoroughly.

The Presence of the Reactive Carbonyl Compounds in Foods: The presence of the reactive carbonyl componds have been known for a long time (Fujioka, K, Shibamoto, T. 2004; O'brien, P. J. Et al, 2005; Stevens, J. F. and Maier, C. S., 2008). One of the food types that contain the reactive carbonyls were of animal origin (O'brien, P. J. et al., 2005).

A second example of the reactive carbonyl compound formation is the high temperature fruit juice processing as a consequence of the browning reactions (Paravisini, L., Peterson, D. G., 2018). We believe that the formation of carbonyl compounds should be studied as a function of temperature and the proportions of several food ingredients. Such a study should include the contribution of these compounds to the food sensory properties in addition to the deleterious effects of them on the human health. This subject seems to be a trade-off between the positive and the negative effects of these compounds (Paravisini, L., Peterson, D. G., 2019).

Formation of many carbonyl compounds such as dicarbonyls is based on Maillard Reactions. Maillard Reactions are known to include the reactions between sugars with a reducing carbonyl and proteins and amino acids (Pripis-Nicolau, L. Et al. 2000, Chen, X. ve Kitts, D. D., 2011, Kroh, L. W. Et al,, 2008). Another mechanism of the formation of these carbonyl include lipid peroxidation reactions as the final products of the heating processes in food processing (Wang, Y ve Cui, P, 2015). These heating processes induce the formation of carbonyls and the presence of molecular oxygen facilitates these reactions (Trammell, D. L. Et al, 1986).

Carbonyl-containing compounds also form as basic reaction of Maillard Reaction or lipid peroxidation as seen in Figure 18.

In his experiments with cakes Ceylan, C (Unpublished results) found an increase in the ratio of 1740 $cm^{-1}$/Amid I band at the center of the cakes depending on the temperature level (35, 85 and 112 °C). This increase was found to be enormous at 112 °C in his model cake studies. This ratio indicates the ratio of carbonyl containing compounds and proteins. This ratio is used as the neutral lipid/protein ratio in cell and tissue based cancer and cell culture studies (Ceylan, C et al., 2012). In this study, the cake baking process was found to have three consecutive phases with inter-phase parts. The rheological study showed parallels with the temperature increase behavior at the centre of the baking cake. His results indicated that the rheological and temperature increse profiles were parallel to the changes in the molecular changes indicated by the FTIR analysis. As a consequence of these, prevention of the formation of these compounds in cake baking by using food enzymes was ascertained to have important consequences for human health and nutrition while preserving the quality parameters of the final product.

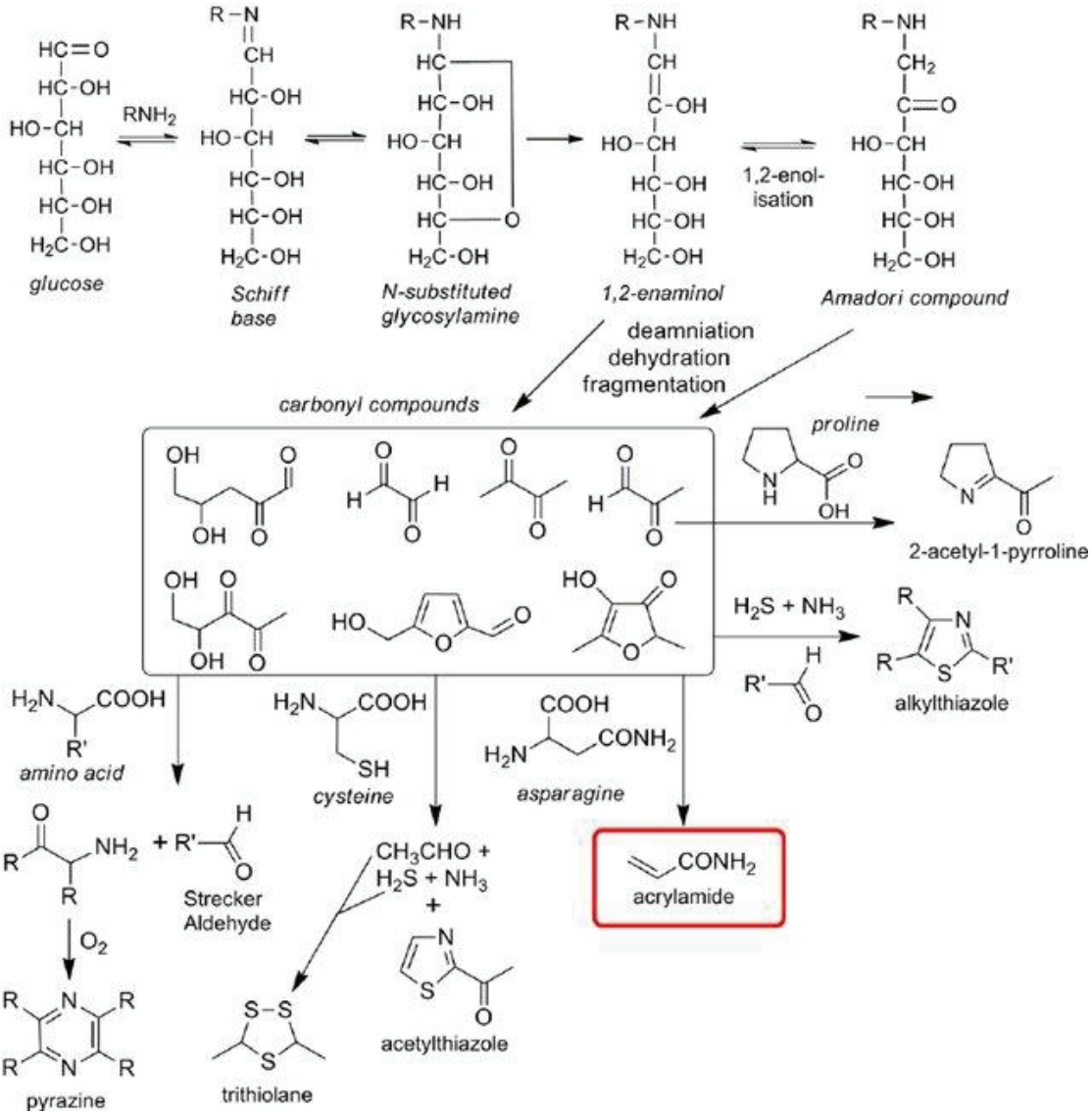



Figure 4. Formation of dicarbonyls in Maillard Reaction

(Source: Halford et al., 2012)

## 1.8. Hypothesis of This Study

The formation of carbonyl groups can be prevented or at least minimized by using different functional ingredients such as enzymes, antioxidants, hydrocolloids and surface active food additives. The basic hypothesis of this study is that the enzymes added as the food additives can achieve this goal while preserving the other cake quality parameters. The enzymes were selected according to their effects on different macromolecular ingredients of cakes namely, proteins (transglutaminase), lipids (lipase) and carbohydrates (amylase).

For this purpose the carbonyl amounts formed in cakes were determined by FTIR experiments in terms of the carbonyl group intensity at around 1740 $cm^{-1}$/ the protein band intensity in terms of Amide I band intensity between 1700 $cm^{-1}$ and 1600 $cm^{-1}$. Following this, cake quality parameters were studied for different enzyme levels used in the study.

# CHAPTER 2

# METHODOLOGY

## 2.1. Enzymes Used in the Study

Fungal alpha amylase with 52164 - 63756 units/g activity (Smart Kimya Tic. ve Dan. Ltd. Şti), Lipase with 2575 - 3150 units/g activity (Smart Kimya Tic. ve Dan. Ltd. Şti) and Transglutaminase 1576-3620units/g activity (Smart Kimya Tic. ve Dan. Ltd. Şti) were used in the study.

## 2.2. Preparing Cake Dough and Baking Cakes

All cake ingredients (wheat flour, sugar, fresh eggs and baking powder) were purchased from a local supermarket. The cake formulation was similar to that of low-rate sponge cakes, but was adjusted so that it does not contain milk or added fat. The amount of ingredients were as follows: 48.6 grams (g) of eggs, 60 g of wheat flour, 60 g of tea sugar, 2.4 g of baking powder, and (0.2-0.050) g of enzyme. Cake dough was prepared in two stages. First, eggs and sugar was weighed in a laboratory beaker (1 lt) and mixed in the mixer for 5 minutes at 50 rpm (DLH Stirrer, VELP Scientifica). Then dough and baking powder were added in order. 150.0 g of dough was will be weighed into another beaker and placed in a convection oven heated to 180 ° C. The cake product to be taken from the oven at the specified temperature was removed from the beaker and immediately cooled and subjected to the planned tests. There are two types of cake preparation. One of them control cake (no added enzyme) the other one is an enzyme added in dough cake.

## 2.3. Measurement of Cake Symmetry Values

Cake symmetry values are among the important quality parameters showing the morphological-structural formation and quality of the cakes produced. The first tests to be performed after cooling are determination of cake symmetry values (Chesterton, A. K. S. et al., 2015). Cake symmetry values will be obtained as follows: the cake base will be measured by measuring with a ruler and the cake heights will be taken with four equal

points to go up the cake surface. Parameters such as Volume index, Symmetry Index and Uniformity Index will be calculated from the cake heights at two points in the center and surrounding areas.

### 2.4. Volume Measurement

Cakes were weighed within one hour after baking, volumes of cakes were measured using colza grains andspecific volumes were calculated (Lee, 1985).

### 2.5. Cake Height

Cake height will be performed precisely using a Verbier caliper. For this purpose, the distance between the highest point of the cake and its base will be measured at room temperature 1 hour after the cake cools. Multiple measurements (n >= 3) will be made and averaged.

### 2.6. Gas Chromatography Mass Spectrometry

The cake dough samples were prepared and placed in the glass sample holders. The sampels were then placed in the GC-MS instrument (Agilent 5973Network Mass Selective Detector).

### 2.7. FTIR Analysis

The baked cake samples were lyophilized in a freeze drier (Labconco, FreeZone 18 liter freeze dry system) overnight to remove water. The samples were homogenized to in an agate mortar. The sample powder spectral analysis was carried out using a Perkin-Elmer spectrometer equipped with MIR TGS detector (Spectrum 100 Instrument, Perkin Elmer Inc., Norwalk, CT, USA). FTIR spectra of the samples were recorded between 4000 and 450 $cm^{-1}$. Interferograms were averaged for 20 scans at 4 $cm^{-1}$ resolutions. The background spectrum was automatically subtracted from the spectra of the samples. Spectrum 100 software (Perkin Elmer) was used for all data manipulations. From each sample, at least three different scans, which gave identical spectra, were performed. These replicates (n=3) were averaged and the averaged spectra for each sample were then used for further data manipulation and statistical analysis. Then, the spectra were interactively

baselined from two arbitrarily selected points. Finally, the spectra were normalized in specific regions for visual comparison of the control and baked cake samples.

For the determination of protein secondary structural changes, the second derivative spectra were obtained by applying a Savitzky-Golay algorithm with thirteen points. The second derivatives were normalized between 1700 and 1600 $cm^{-1}$. The peak minima of the second derivative signals were considered because they correspond to the peak maxima of the original absorption spectra (Ceylan et al, 2012).

### 2.8. Statistical Analysis

The differences between the batter and baked cake sample groups were compared using the Mann-Whitney U Test. The statistical results are expressed as means ± standard deviation. $p<0.05$ was considered statistically significant.

# CHAPTER 3

# RESULTS

## 3.1. FTIR Study on the Effect of Transglutaminase on Cakes

FTIR spectroscopy was used to analyze the effect of enzyme addition on the macromolecular structure of cakes. For this purpose cakes were added with a certain amounts of enzymes prior to baking process and then baked.

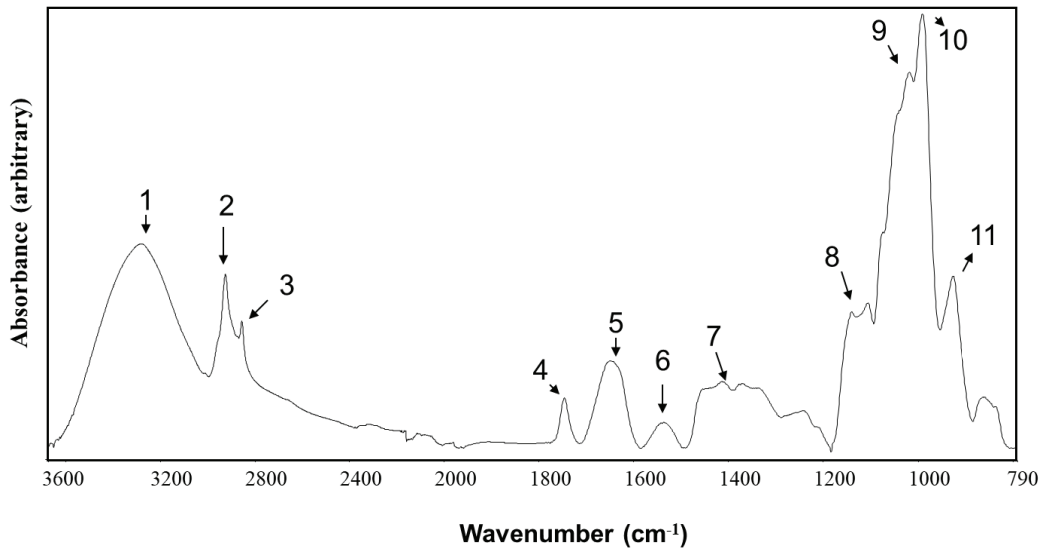


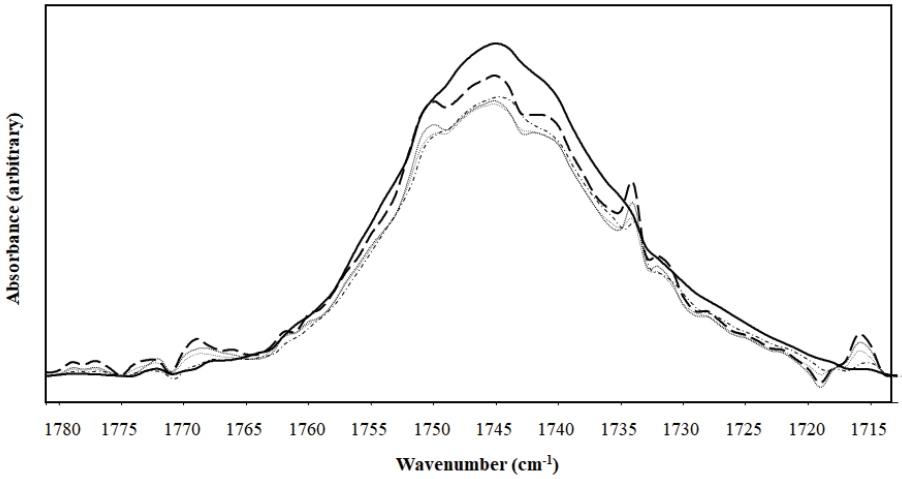

Figure 5. A general FTIR automatically smoothed and baselined spectrum of baked cake sample

Table 1. The major peaks in FTIR spectrum of cakes

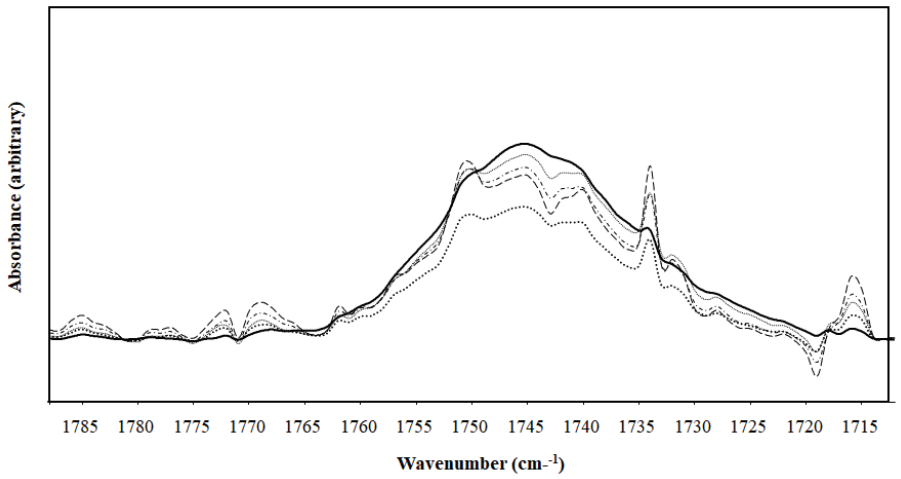
| Band Number | Wavenumbers ($cm^{-1}$) | Definition of the spectral assignment |
|---|---|---|
| 1 | 3292 | Amide A: Mainly N-H stretching of proteins with the little contribution from O-H stretching of polysaccharides and intermolecular bonding. |
| 2 | 2924 | $CH_2$ asymmetric stretch: mainly lipids, and the little contribution from proteins and carbohydrates. |
| 3 | 2854 | $CH_2$ symmetric stretch: mainly lipids, with the little contribution from proteins, nucleic acids and carbohydrates. |
| 4 | 1745 | Ester C=O stretch: carbonyl containing compounds. |
| 5 | 1652 | Amide I: proteins, mainly C=O stretch. |
| 6 | 1543 | Amide II: proteins, mainly N-H bend and C-N stretch. |
| 7 | 1416 | $CH_2$ bending: mainly lipids, with the little contribution from proteins, $CH_3$ asymmetric bending: methyl groups of proteins. |
| 8 | 1105 | Carbohydrates |
| 9 | 1043 | C-O stretch: carbohydrates. |
| 10 | 987 | $OCH_3$: polysaccharides. |
| 11 | 923 | C-C or C-O stretch: from the skeletal phospholipids head. |

### 3.2. The Effect of Enzymes on the Carbonyl Formation in Cakes

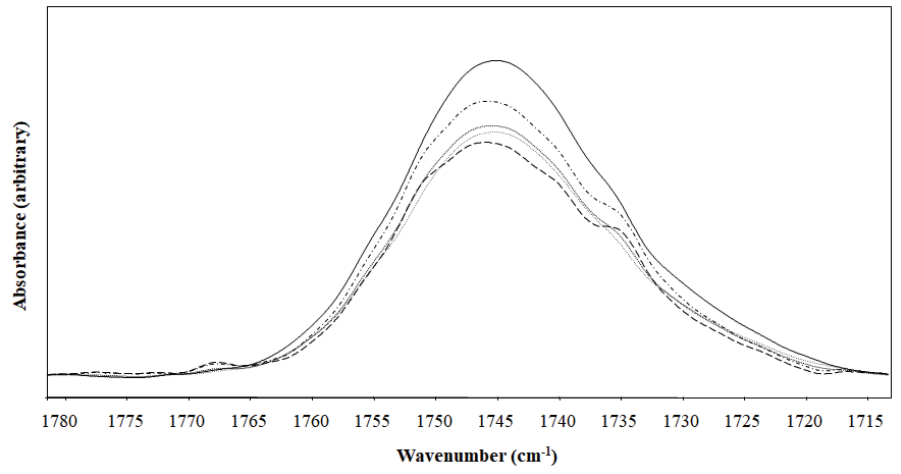
Formation of carbonyl-containing chemicals such as aldehydes, ketones and carboxylic acids were studied using FTIR spectroscopy. The peak aroud 1740 $cm^{-1}$ is due to the absorption of the caobonyl bond. Similarly, the band between 170 $cm^{-1}$ and 1600 $cm^{-1}$ is called the Amide-I band and due to the absorption of carbonyl bond stretching (80%), N-H and N-C stretching in the structure of proteins. Therefore, the intensity ratio of the band around 1740 $cm^{-1}$ and the Amide-I band can be used as a measure of the ontent of the carbonyl-containing compounds. Experiments were designed to figure out the effect of enzyme use on the carbonyl content of the cake samples by addition of the transglutaminase, lipase and amylase at designated concentrations. The results can be seen in Figure 6 below. The results indicated that enzyme added cake samples had lower carbonyl contents with 8.6, 17.07, 16.40 and 17.05% decreases with respect to the control cake samples for the transglutaminase amounts of 0.05, 0.1, 0.15 and 0.2 g respectively when the spectra baselined between 1775 $cm^{-1}$ and 1586 $cm^{-1}$ and then normalized in the same region. The results indicated that the amount of carbonyl-containing compounds decreased with respect to the control cake samples in a parallel fashion with increased transglutaminase amounts as seen in Figure 5 a. For the lipase addition case, the results indicated that enzyme added cake samples had lower carbonyl contents with 15.46, 11.57, 5.18 and 31.83% decreases with respect to the control cake samples for the lipase amounts of 0.05, 0.1, 0.15 and 0.2 g respectively when the spectra baselined between 1781 $cm^{-1}$ and 1586 $cm^{-1}$ and then normalized in the same region. The results indicated that the amount of carbonyl-containing compounds decreased with respect to the control cake samples in an almost parallel fashion with increased lipase amounts as seen in Figure 5 b. For the amylase addition case, the results indicated that enzyme added cake samples had lower carbonyl contents with 26.31, 12.99, 20.65 and 22.61% decreases with respect to the control cake samples for the amylase amounts of 0.05, 0.1, 0.15 and 0.2 g respectively when the spectra baselined between 1782 $cm^{-1}$ and 1586 $cm^{-1}$ and then normalized in the same region. The results indicated that the amount of carbonyl-containing compounds decreased with respect to the control cake samples not in a parallel fashion with increased amylase amounts as seen in Figure 5 c.




a




b




c

Figure 6. Carbonyl and amide I band of a) transglutaminase, b) lipase and c) amylase treatment in cakes

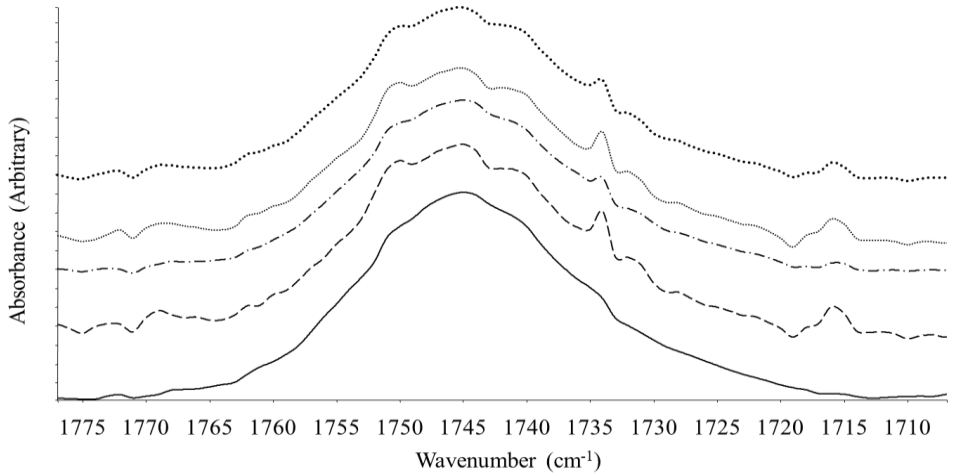
### 3.3. Effect of Enzyme Use on the Carbonyl Band Profile

The carbonyl-containing compounds in the cake samples within the 1781 $cm^{-1}$ and 1713 $cm^{-1}$ range. The peaks were identified as lipase, transglutaminase and amylase with 1745 $cm^{-1}$ .

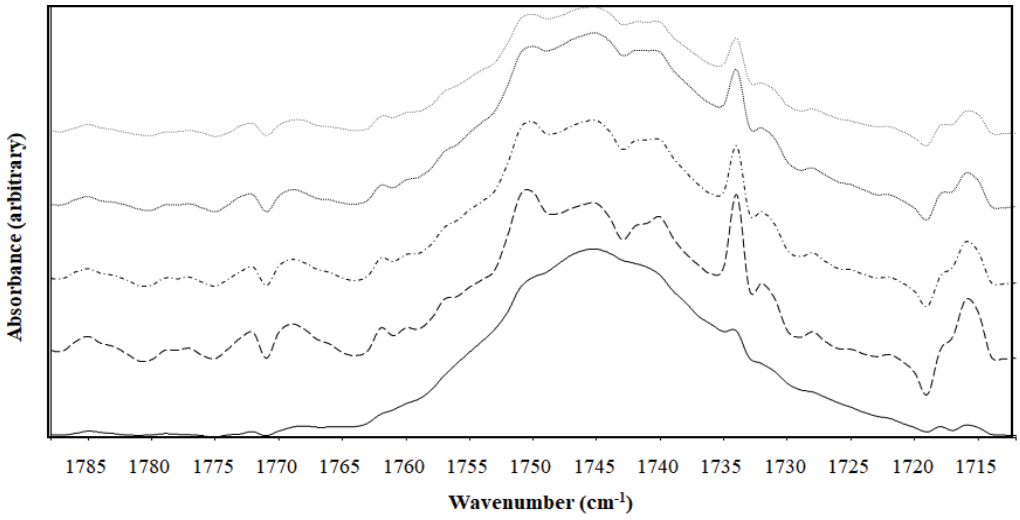
In the transglutaminase addition case, we saw level of significance should be given changes in the absorbances of 1734 $cm^{-1}$ (aldehydes), 1732 $cm^{-1}$ (possibly aldehydes), 1740 $cm^{-1}$ (esters), 1749 $cm^{-1}$ (peracids), 1769 $cm^{-1}$ (carboxylic acids) and 1715 $cm^{-1}$ (carboxylic acids). We saw level of significance should be given changes in the overall band shapes of the transglutaminase added cake samples as indicated in Figure 7 a. The major band in this region was at around 1745 $cm^{-1}$. This band possibly is indicative of aldehydes.

In the lipase addition case we saw level of significance should be given changes in the absorbances of 1734 $cm^{-1}$ (aldehydes), 1732 $cm^{-1}$ (possibly aldehydes), 1740 $cm^{-1}$ (esters), 1750 $cm^{-1}$ (peracids), 1768 $cm^{-1}$ (carboxylic acids), 1771 $cm^{-1}$ (unidentified) and 1715 $cm^{-1}$ (carboxylic acids) as seen in Figure 7 b. The major band in this region was at around 1745 $cm^{-1}$. This band possibly is indicative of aldehydes.

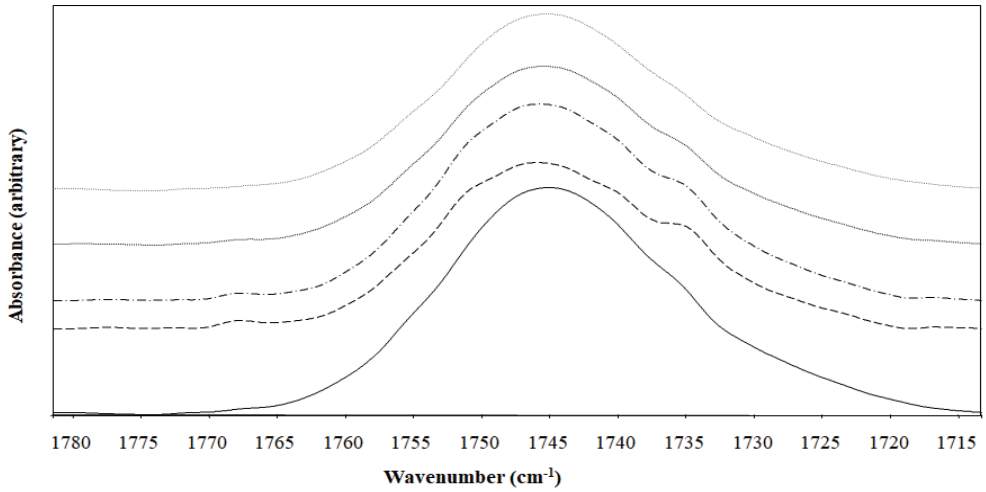
In the amylase addition case we did not see a drastic change in the carbonyl bands of the control and amylase added cake samples. However, the shoulder around 1735 $cm^{-1}$ indicated an inrease in the amount of aldehydes as seen in figure 7 c.




a




b




c

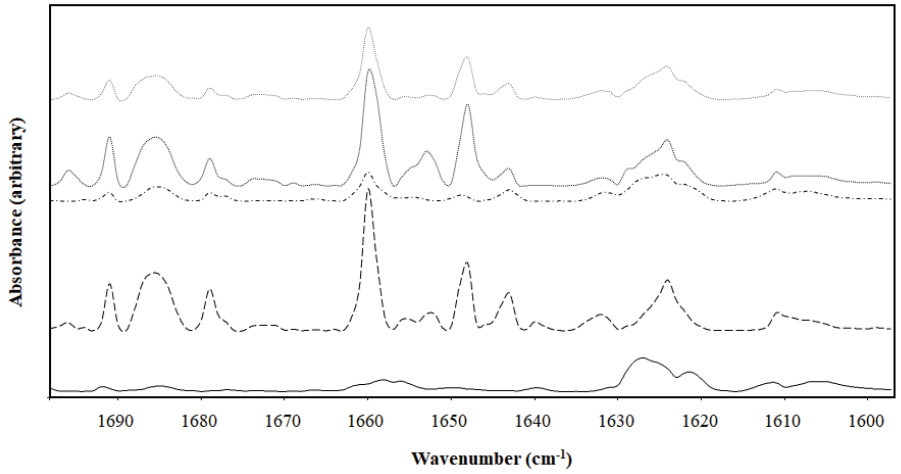
Figure 7. Carbonyl peak profile of a) transglutaminase, b) lipase and c) amylase treatment in cakes

### 3.4. The Effect of Enzyme Use on The Cake Protein Secondary Structure

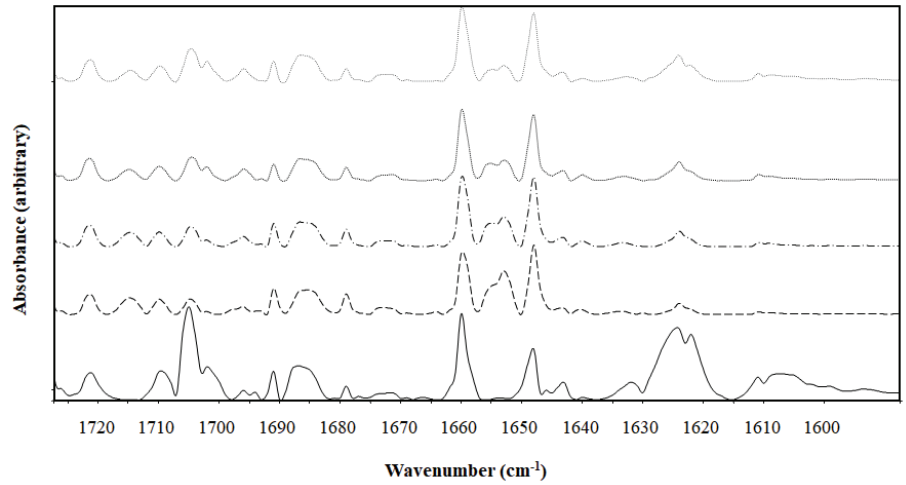
To analyze the effect of enzyme use on the protein structure the amide I peak between 1600 $cm^{-1}$ and 1700 $cm^{-1}$ was analyzed using second derivative analysis followed by vector normalization. The peaks of the minimum points of the second derivative spectra are given in Table 2. In the transglutaminase addition case the enzyme addition caused significant changes in the second structure of the cake proteins. We observed level of significance should be given increases in the alpha helix bands with respect to beta sheet structures at around 1630 $cm^{-1}$. Similarly, level of significance should be given increases were observed for the anti-parallel beta sheet and turns structures around 1685 $cm^{-1}$, 1690 $cm^{-1}$, 1679 $cm^{-1}$ and 1673 $cm^{-1}$. We also observed in the random coil structures at 1649 $cm^{-1}$ and 1643 $cm^{-1}$ position as seen in Figure 8 a. Similar results were obtained with the lipase application as well as seen in Figure 8 b. As opposed to the first two cases, the amylase application did not cause as level of significance should be given changes in the protein secondary structures as seen in Figure 8 c. However, there was a slight increase in the alpha helix band at 1660 $cm^{-1}$.

Table 2. Peak assignment for the secondary structure peaks of cake samples

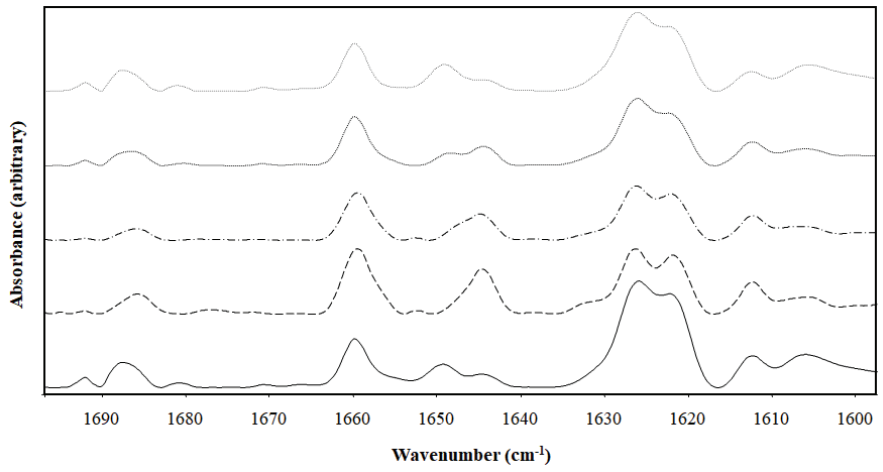
| Peak Number | Mean Frequencies ($cm^{-1}$) | Assignment |
|---|---|---|
| 1 | 1695 | Anti-Parallel Beta Sheet |
| 2 | 1691 | Anti-Parallel Beta Sheet |
| 3 | 1681 | Anti-Parallel Beta Sheet, Turns |
| 4 | 1670 | Turns |
| 5 | 1659 | Alpha Helix |
| 6 | 1649 | Random Coil |
| 7 | 1635 | Beta Sheet |
| 8 | 1624 and 1622 | Aggregated Beta Sheet |




a




b




c

Figure 8. Secondary structure profile of a) transglutaminase, b) lipase and c) amylase treatment in cake proteins

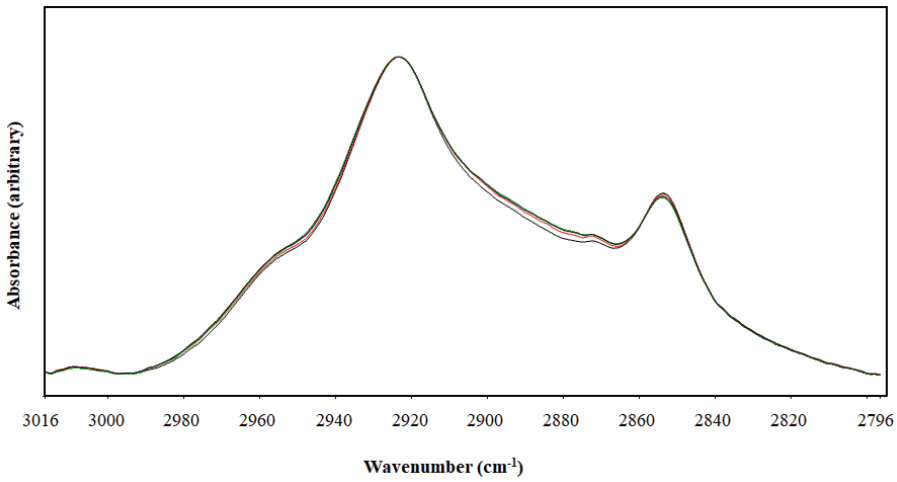
### 3.5. The Effect of Enzyme Use in the Aliphatic Chain Range of the FTIR Spectrum in Cake Baking

The bands between 2995 $cm^{-1}$ and 2800 $cm^{-1}$ are indicative of lipid aliphatic gruous with the exception of 2872 $cm^{-1}$ which is indicative of proteins basically. In addition, the peak around 3009 $cm^{-1}$ is indicative of unsaturation level of lipids. This band is used to indicate possible lipid peroxidation in many biological and biophysical studies. When the band are baselined followed by normalization the lipid-to-protein ratio was obtained from the $CH_2$ symmetric and $CH_3$ symmetric stretching band intensity ratios were considered.

The lipid/protein ratio was found to decrease by 4.13, 7.05, 6.16 and 7.13% for the 0.05, 0.1, 0.15 and 0.2 g transglutaminase addition respectively as seen in Figure 9 a. There was almost no change in the unsaturation ratio of the lipids.

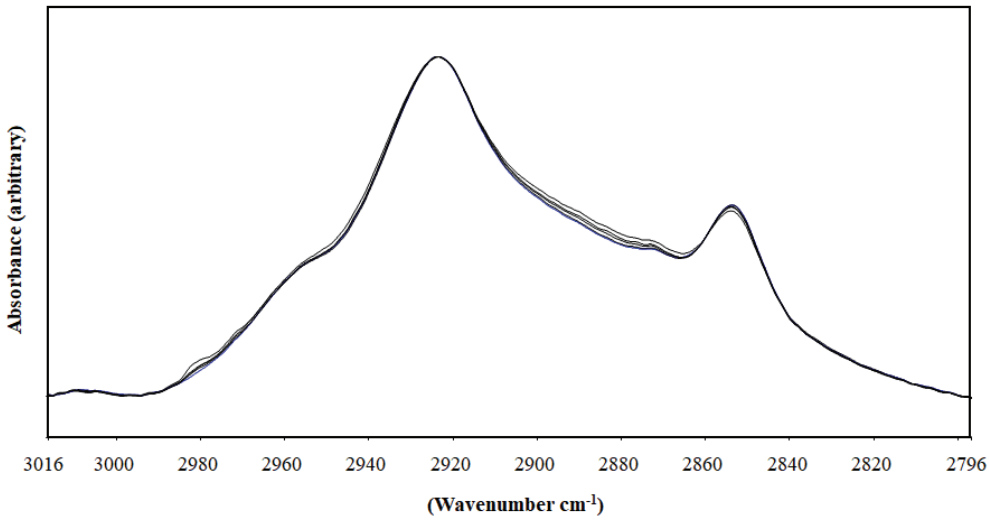
For the lipase addition case, the lipid/protein ratio also decreased by 4.23, 2.78, 0.54 and 5.8% for the 0.05, 0.1, 0.15 and 0.2 g lipase addition respectively as seen in Figure 9 b. However in the case of lipase additon there was a slight decrease in the level of unsaturation of the lipids.

For the amylase case, 11.21, 5.2, 8.24 and 10.3% for the 0.05, 0.1, 0.15 and 0.2 g amylase addition respectively as seen in Figure 9 c. In the case of amylase additon there was a decrease in the level of unsaturation of the lipids as well.

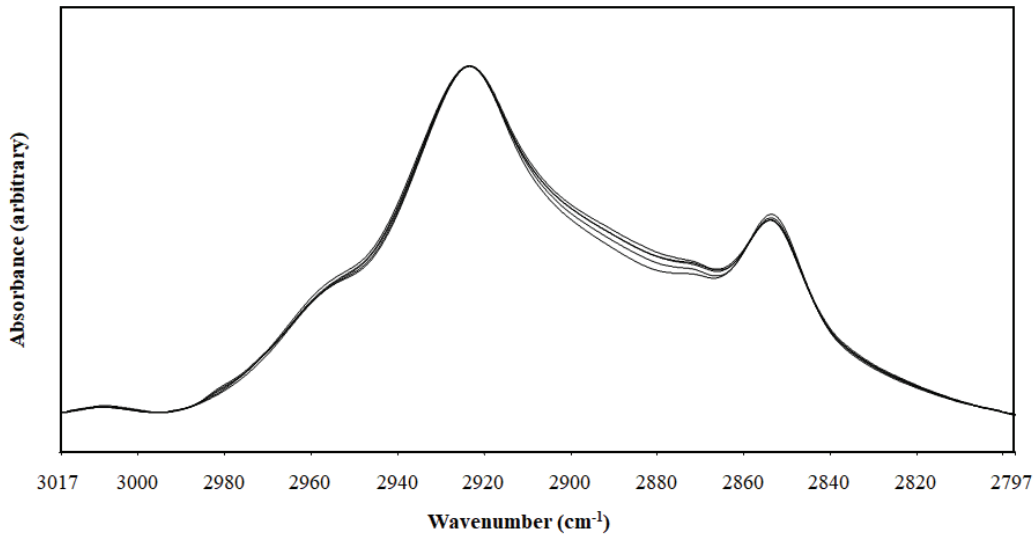



a

Figure 9. The effect of enzyme use on the aliphatic range for a) transglutaminase, b) lipase and c) amylase treatment in cake samples **(cont. on next page)**




b




c

**Figure 9. (cont.)**

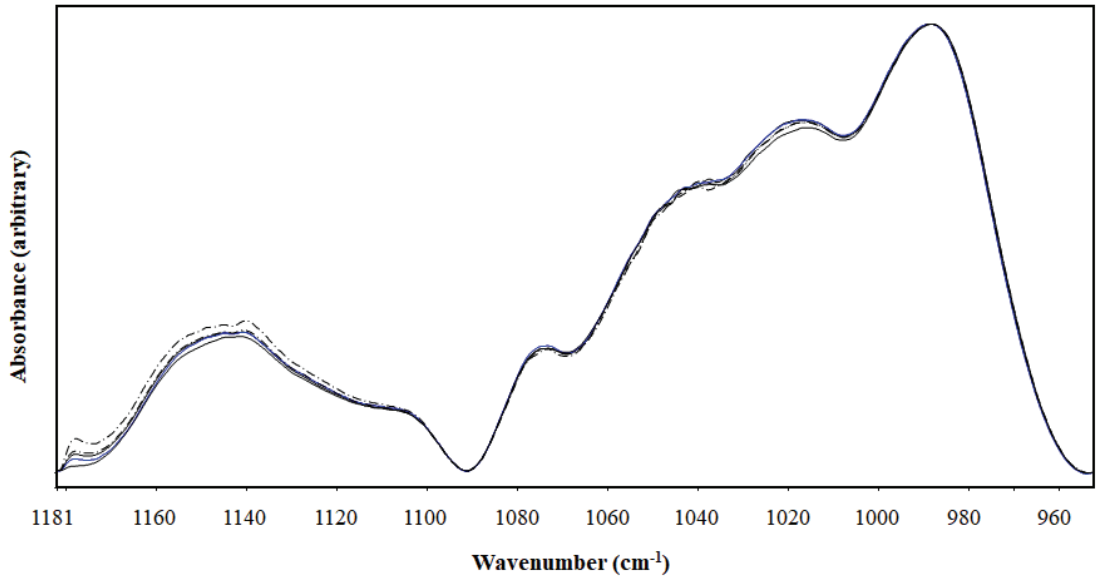
### 3.6. The effect of enzyme use on the fingerprint region

The region between 1500 $cm^{-1}$ and 800 $cm^{-1}$ is called the fingerprint region in FTIR spectrum. This region is full of band originating from lipids, nucleic acids, proteins and glycogen if any and changes from every sample studied. Therefore we investigated this region to obtain structural differences induced by the enzyme addition. The most important band within this region is at 1019 $cm^{-1}$ which is indicative of amorphous nature on starch in cake samples when the band was normalized with respect to 988 $cm^{-1}$ band. In transglutaminase added samples the intensity of this band increased depending on the amount of enzyme added. This ratio was found to increase by 3.79, 2.32, 0.61 and 1.99% for the 0.05, 0.1, 0.15 and 0.2 g transglutaminase addition respectively as seen in Figure 10 a. For the lipase addition case, the ratio was found increase by 1.93, 1.41, 2.05 and 1.92% for the 0.05, 0.1, 0.15 and 0.2 g lipase addition respectively as seen in Figure 10 b. For the amylase addition case, the ratio was found increase by 3.91, 2.67, 1.91 and 1.27% for the 0.05, 0.1, 0.15 and 0.2 g amylase addition respectively as seen in Figure 10 c.

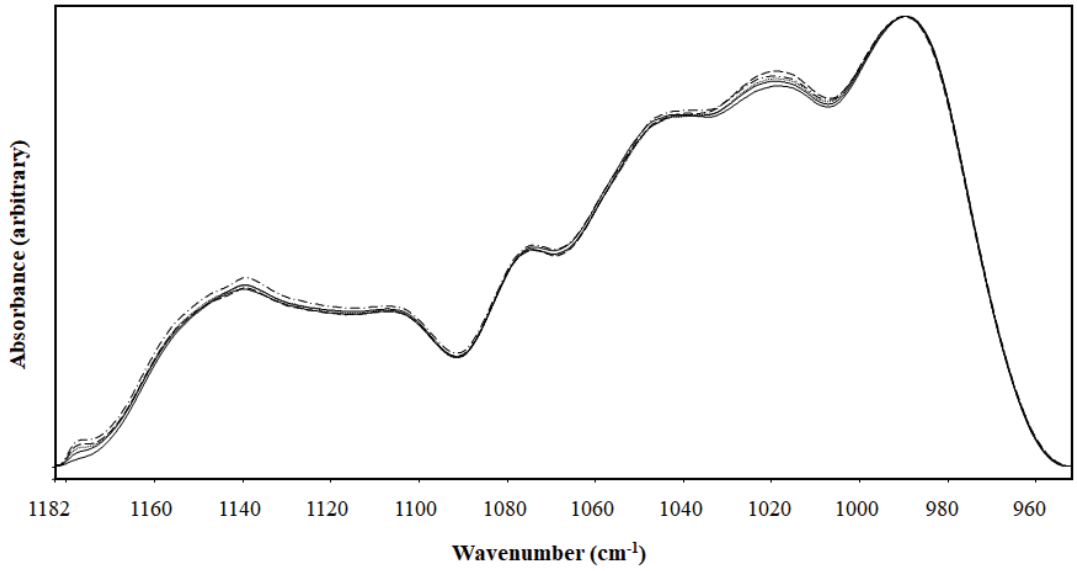

a

Figure 10. The effect of enzyme use on the fingerprint region for a) transglutaminase, b) lipase and c) amylase treatment in cake samples

**(cont. on next page)**




b




c

**Figure 10. (cont.)**

### 3.7. The Effect of Enzymes of the Quality Parameters of Cakes

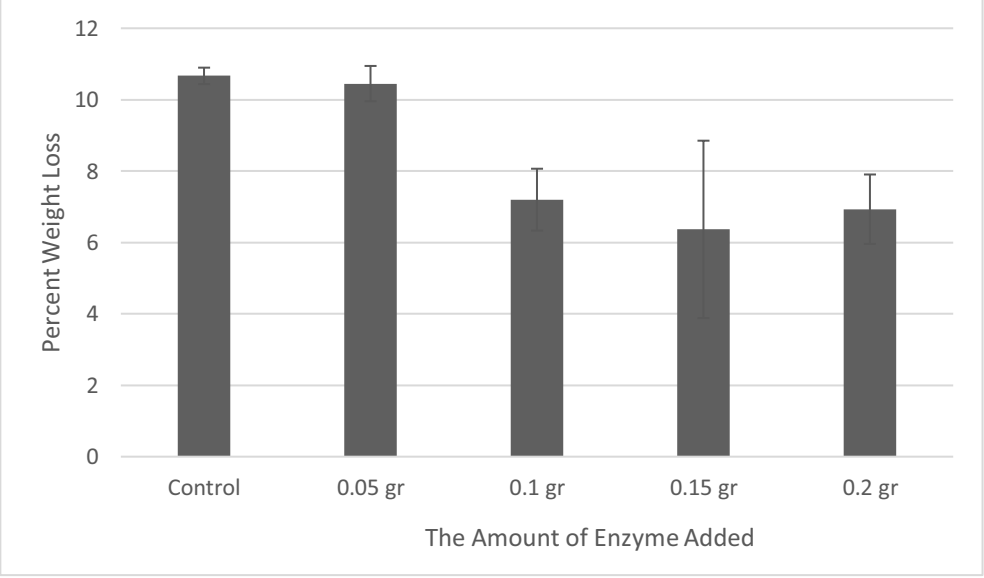
#### 3.7.1. Cake Weight Loss Results

In order to analyze the cake weight loss experiments were carried out to see the effect of cake making process. The results can be seen in Figure 11 below.

Cake baking is the mainly fluid dough is transformed to the solid product (Abdullah, 2007).

We focus on this idea, and also we saw the some produced about the texture analyses. So, we tried the different doses transglutamines enzyme into the cake experiments. Seen that, the very interesting results graph.

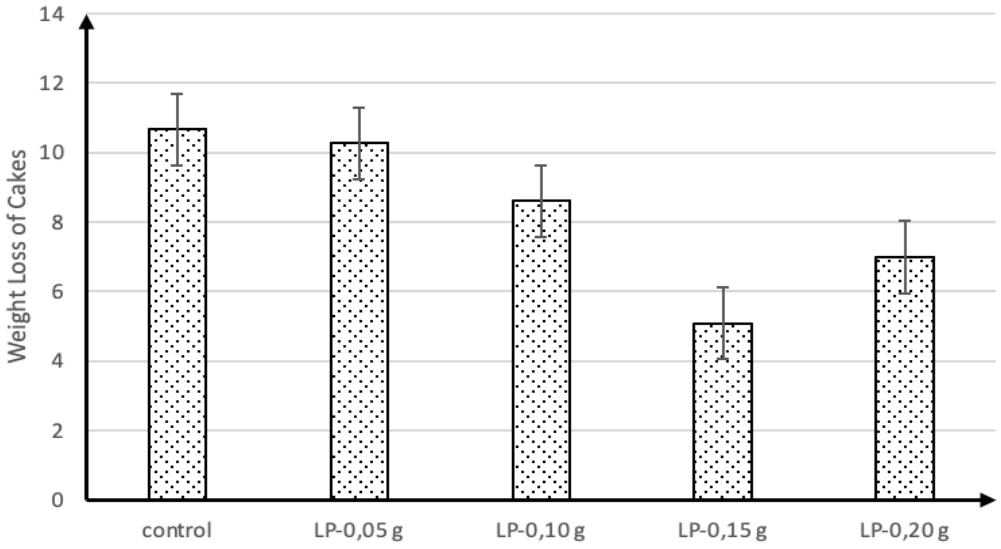
These experiment results including volume expansion, denaturation of protein and browing reactions (changing the color) to the cake (Sablani, 2002).



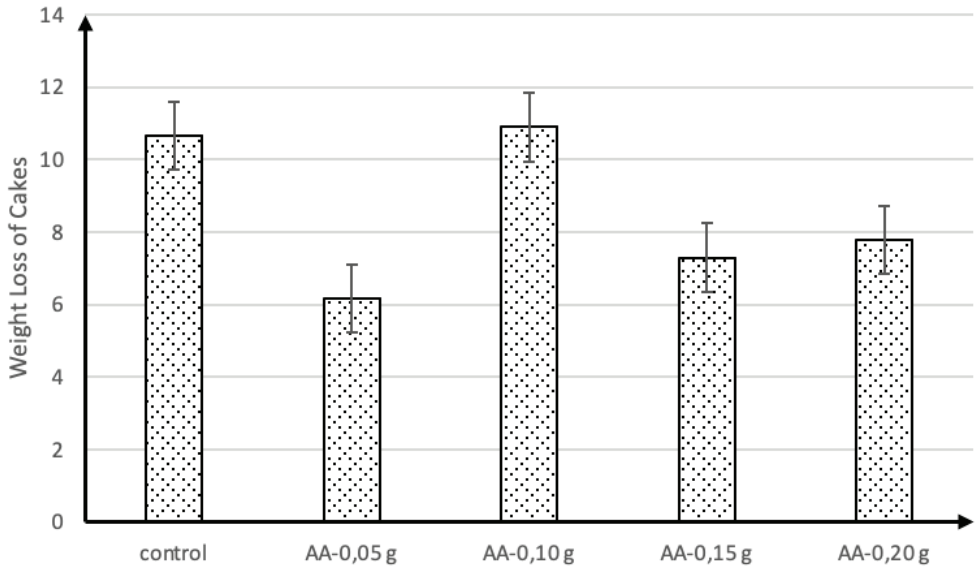

a

Figure 11. The effect of a) transglutaminase, b) lipase, and c) amylase addition on cake weight loss

**(cont. on next page)**



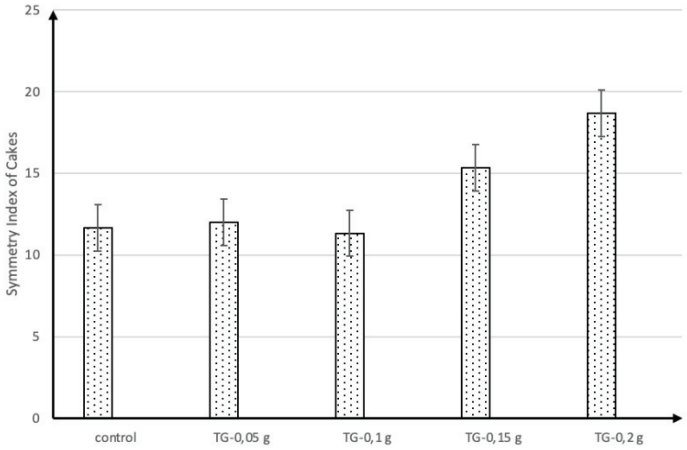

b



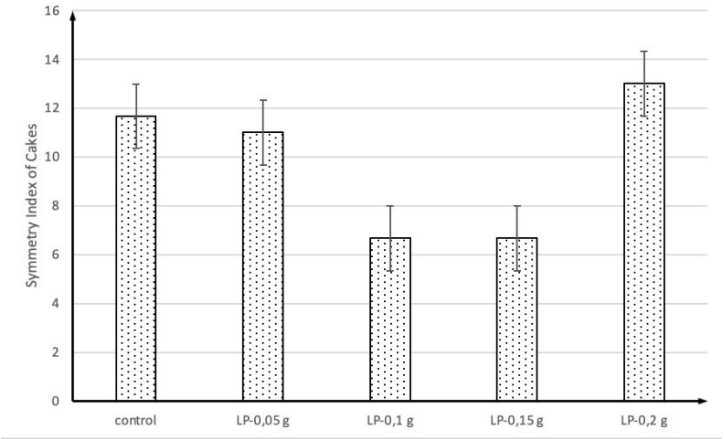

c

**Figure 11. (cont.)**

### 3.8. The Effect of Ezyme Use on the Symmetry, Uniformity and Volume Indexes of Cakes




a

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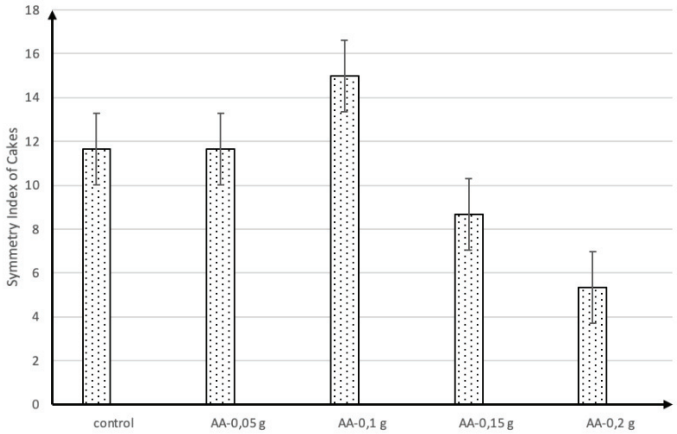
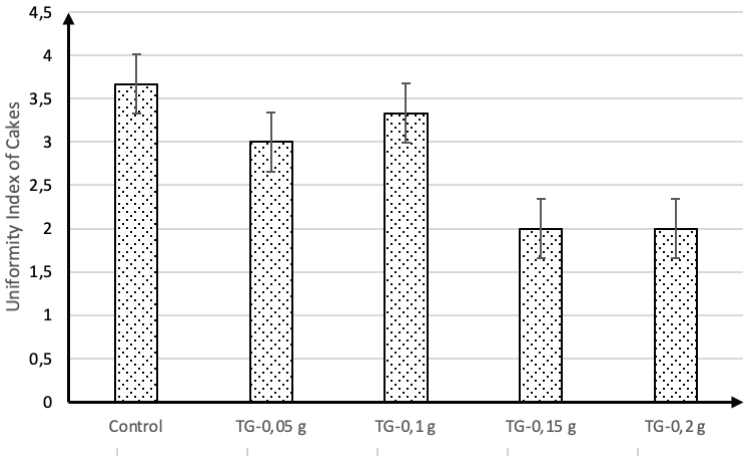
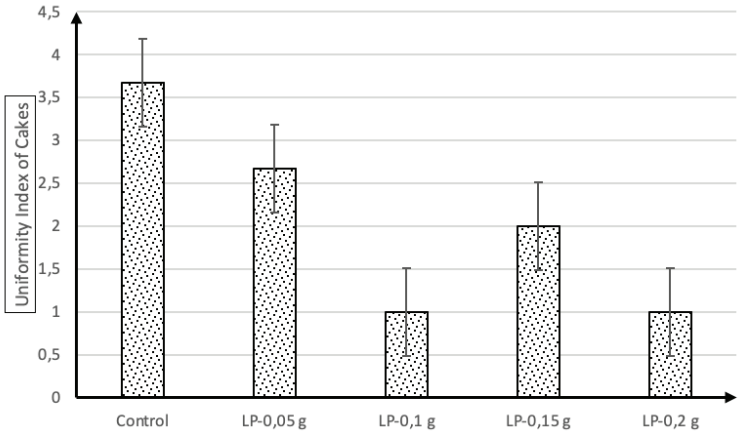



c

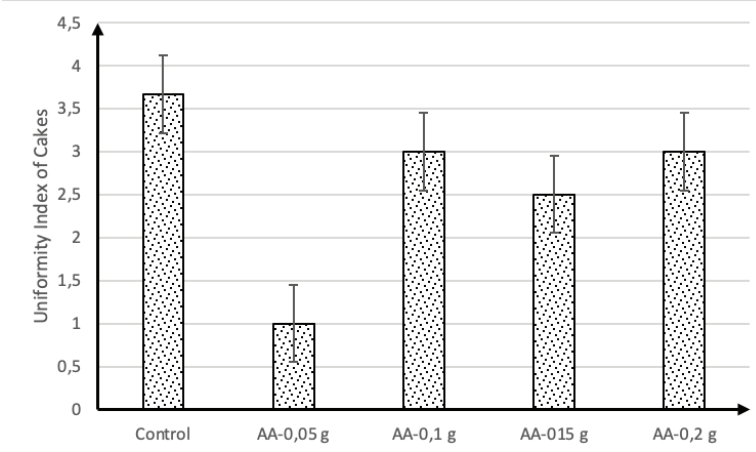
Figure 12. The effect of enzyme use on the symmetry indexes for a) transglutaminase, b) lipase and c) amylase treatment in cake samples




a

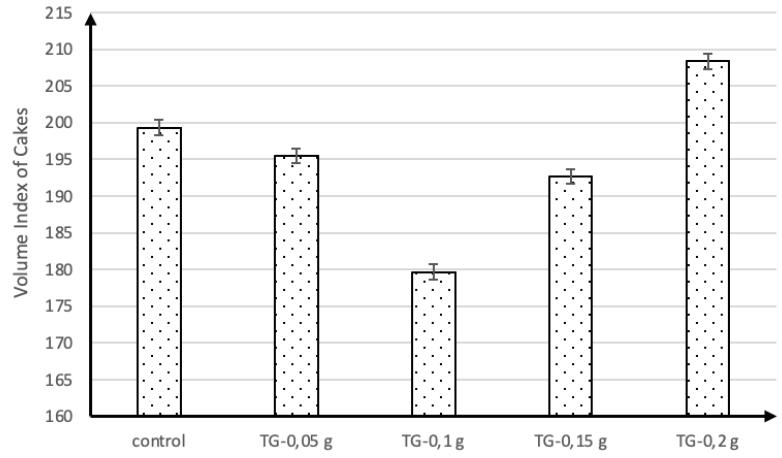



b

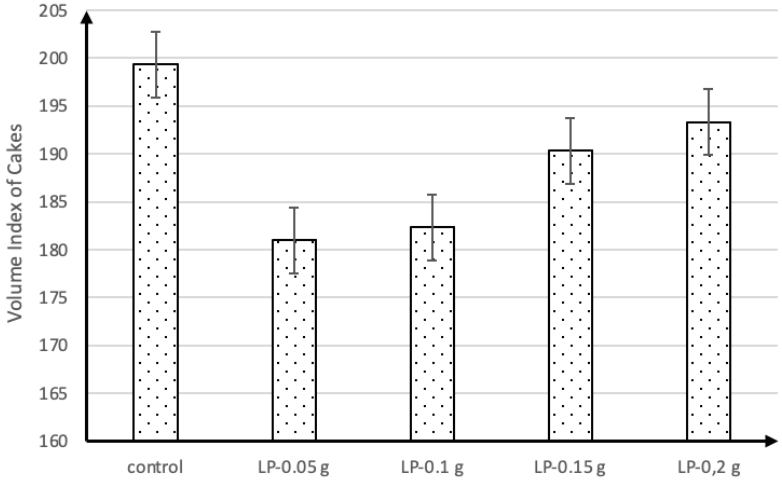



c

Figure 13. The effect of enzyme use on the symmetry indexes for a) transglutaminase, b) lipase and c) amylase treatment in cake samples




a

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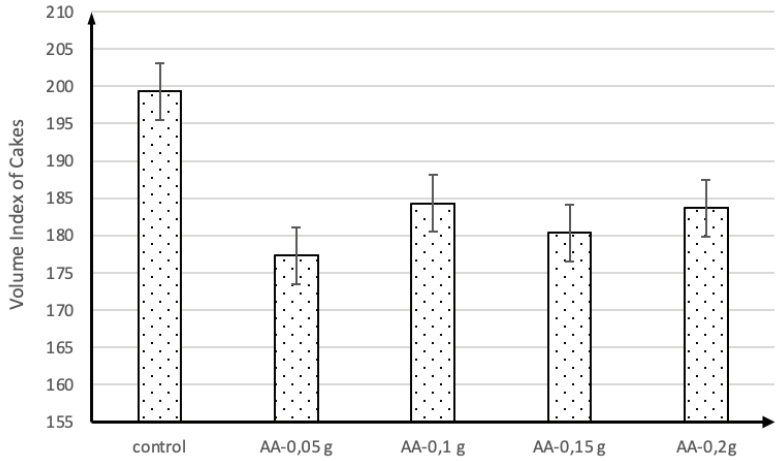
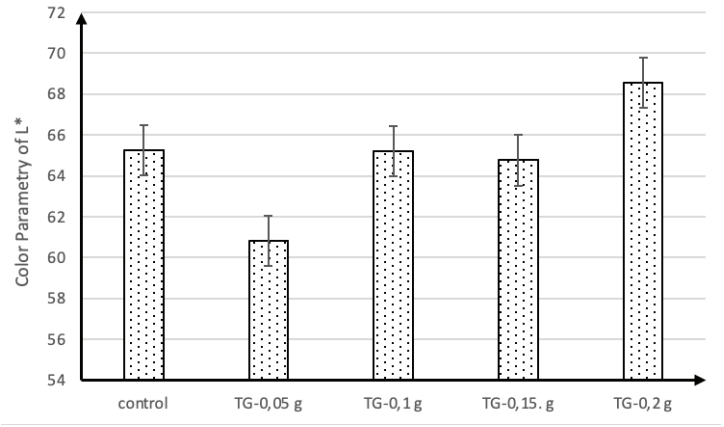


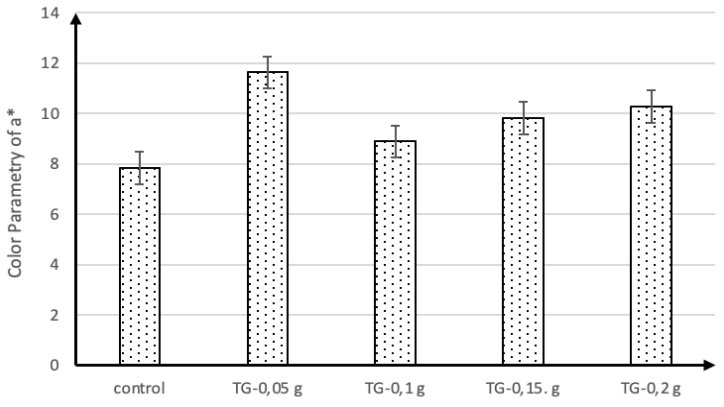

c

Figure 14. The effect of enzyme use on the symmetry indexes for a) transglutaminase, b) lipase and c) amylase treatment in cake samples

### 3.9. The Effect of Enzymes on Cake Color




a

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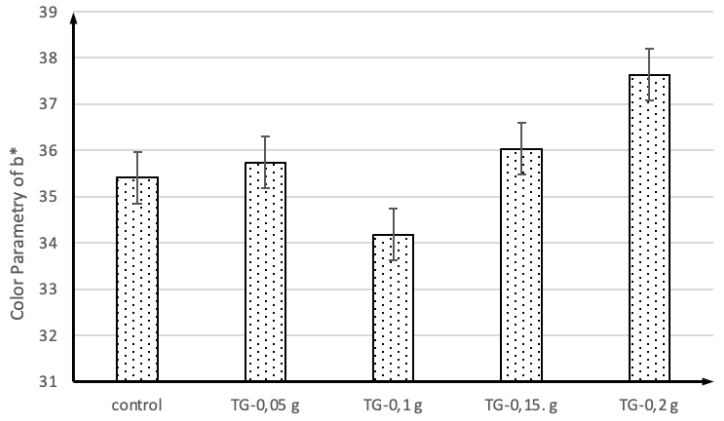
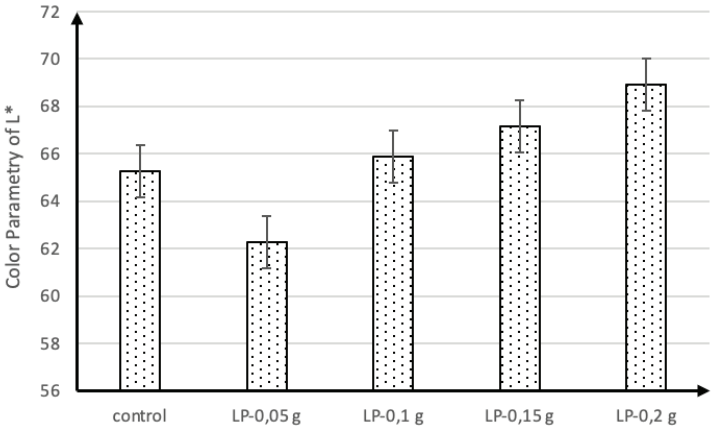
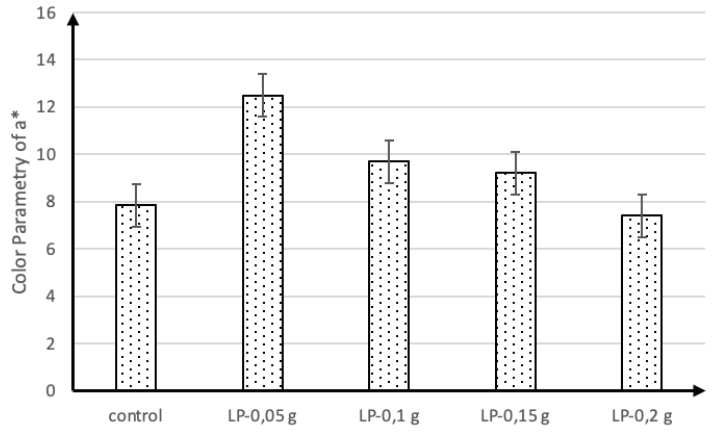



c

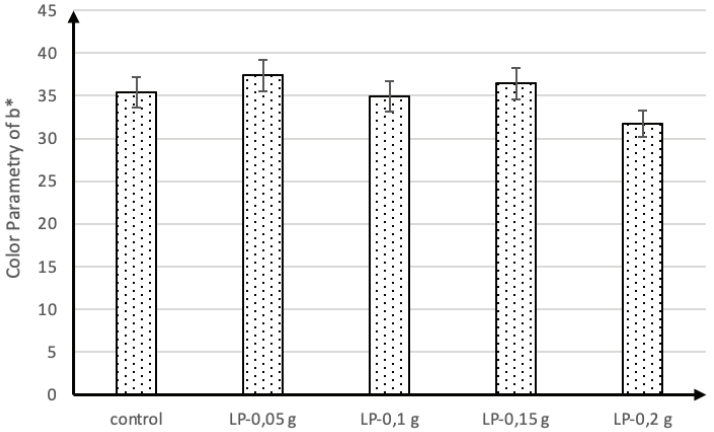
Figure 15. The change in the color parameters a) L parameter, b) a parameter, c) b parameter of cakes as a result of cake baking for different transglutaminase concentrations (Control, 0.5, 0.1, 0.15 and 0.2 g transglutaminase/150 gram initial cake weight)




a

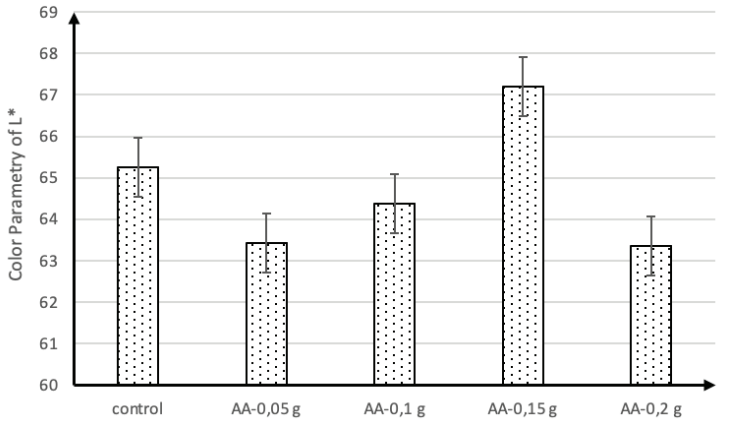



b

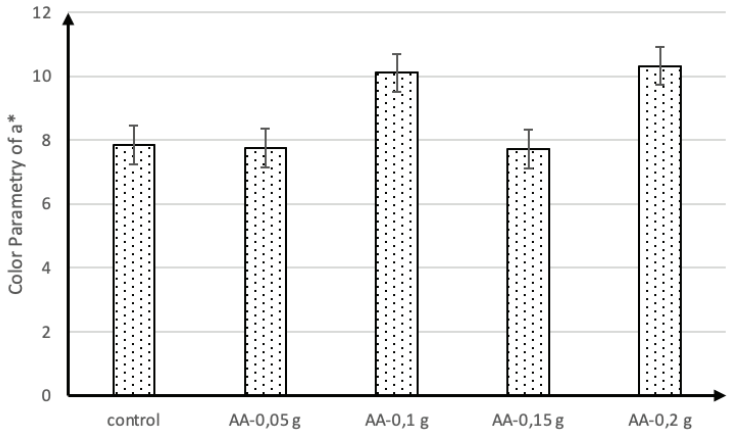



c

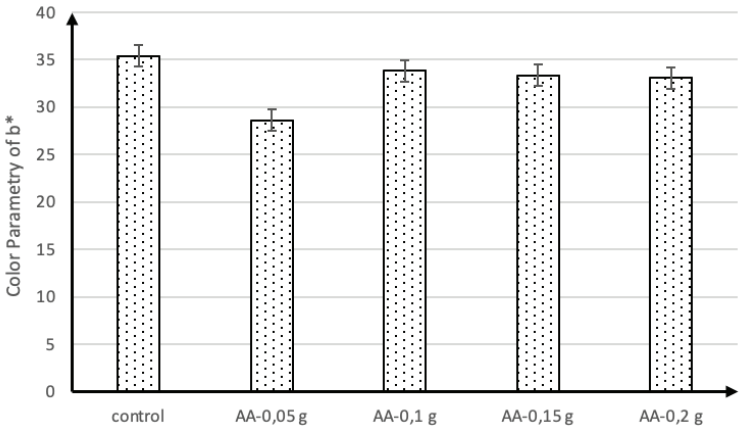
Figure 16. The change in the color parameters a) L parameter, b) a parameter, c) b parameter of cakes as a result of cake baking for different lipase concentrations (Control, 0.5, 0.1, 0.15 and 0.2 g lipase/150 gram initial cake weight)




a




b

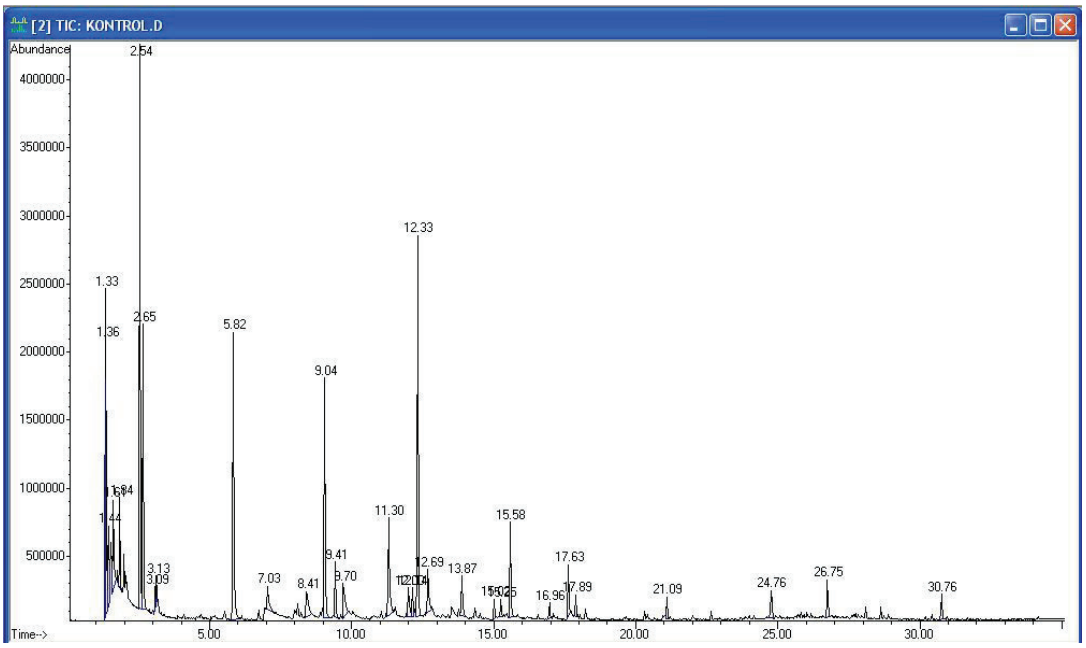



c

Figure 17. The change in the color parameters a) L parameter, b) a parameter, c) b parameter of cakes as a result of cake baking for different amylase concentrations (Control, 0.5, 0.1, 0.15 and 0.2 g alpha amylase/150 gram initial cake weight)

### 3.10. Head-space Gas Chromatography-Mass Spectrometry (GC-MS) Study

A head-space gas chromatography-mass spectrometry study was carried out to understand the differences in the production of gaseous compounds. The results can be sen in Figure 18 and the peaks and their corresponding peak areas are given in Table 3. As seen in the Figure 18 and Table 3 there is almost no change in the amount of lipid degredation products such as 2-pentylfuran, nonanal and octanal. Similarly there is almost no change in the Maillard Reaction products such as benzaldehyde, furfural, 2,5-dimethyl pyrazine and trimethyl pyrazine.




a) Control cake head space analysis

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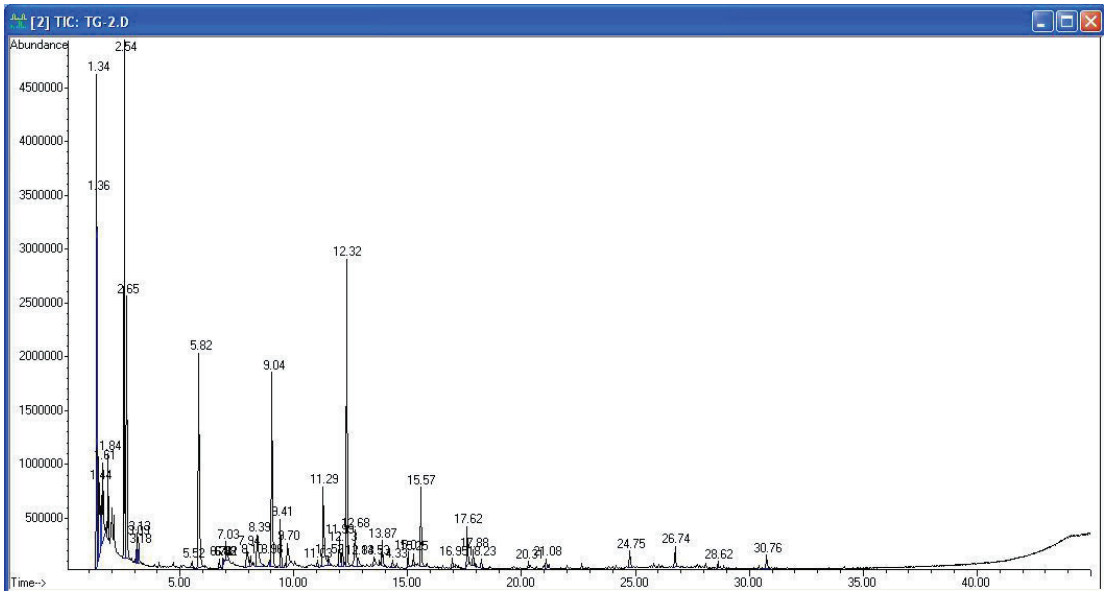
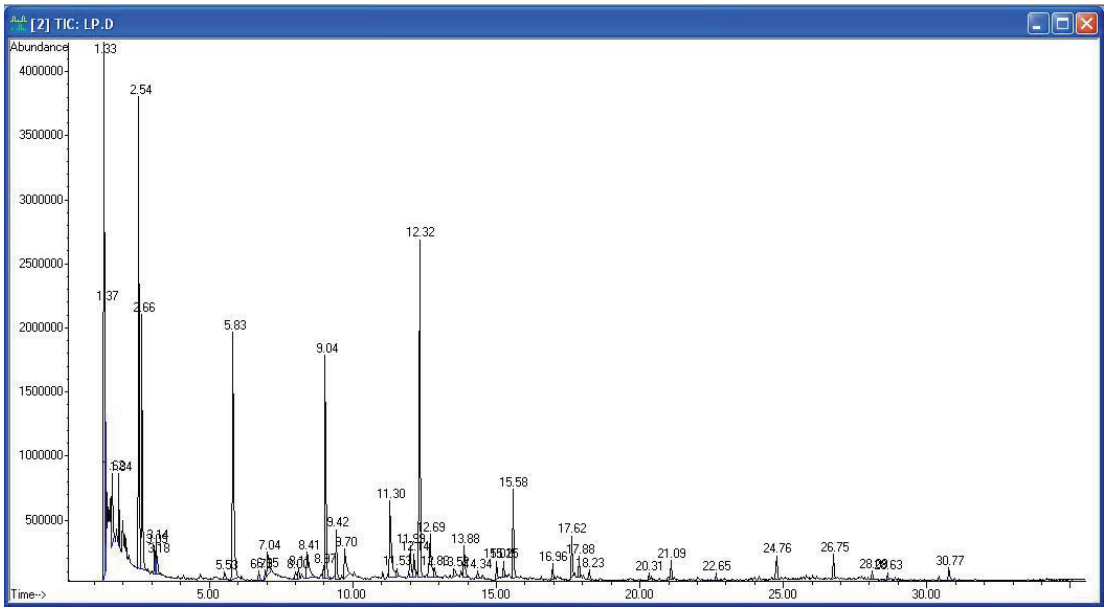
Figure 18. Gas chromatography-mass spectrometry head space analysis of control and 200 mg enzyme added cake samples

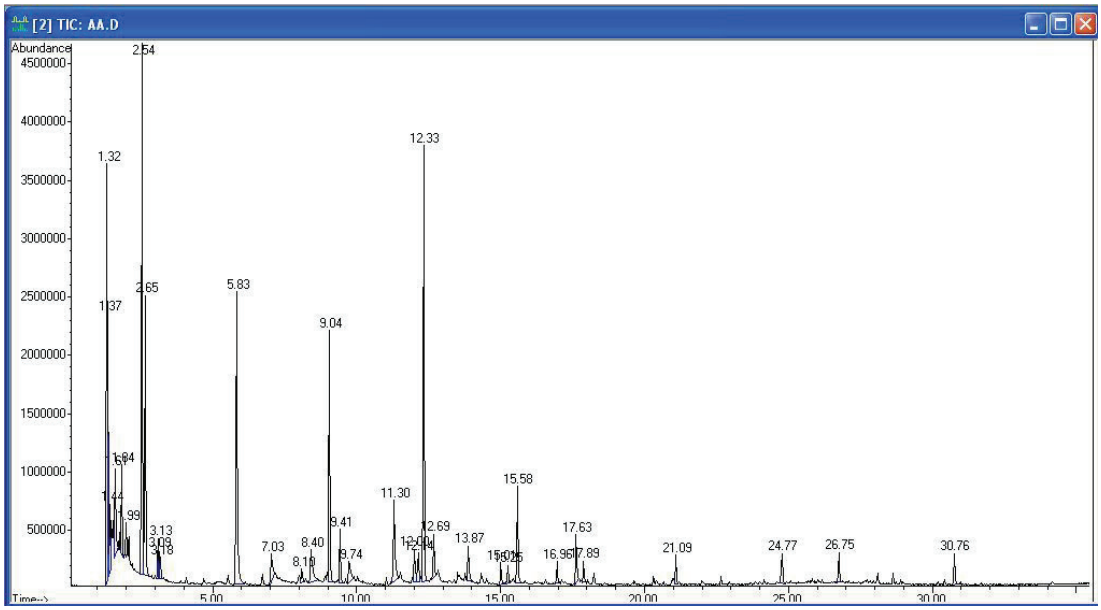



b) Transglutaminase treated cake head space analysis




c) Lipase added cake head space analysis

**(cont. on next page)**




d) Amylase cake head space analysis

**Figure 18. (cont.)**

Table 3. Gas Chromatography-mass spectrometry head space analysis peak areas for the component peaks

| | control | TG | LP | AA |
|---|---|---|---|---|
| Acetone | 1.61 | 1.61 | | 2.5 |
| Butanal | | 1.84 | | 1.23 |
| 2,3 Butanedione | | | | 0.55 |
| Butanal, 3-methyl- | 2.54 | 2.54 | 2.54 | 2.54 |
| Butanal, 2-methyl- | 2.65 | 2.65 | 2.66 | 2.65 |
| 2,3-Pentanedione | 3.09 | 3.09 | | 3.09 |
| Pentanal | 3.13 | 3.13 | 3.14 | 3.13 |
| Furan, 2-ethyl- | | 3.18 | 3.19 | 3.18 |
| Hexanal | 5.82 | 5.82 | 5.83 | 5.83 |
| 3-Furaldehyde | | | 7.04 | 7.03 |
| 2-n-Butylacrolein | | 8.1 | 8.1 | 8.1 |

**(cont. on next page)**

**Table 3. (cont.)**

| | | | | |
|---|---|---|---|---|
| 1-Hexanol | 8.41 | 8.39 | 8.41 | 8.4 |
| 2-Heptanone | | 9.04 | 9.04 | 9.04 |
| Heptanal | | 9.41 | 9.42 | 9.41 |
| Pyrazine, 2,5-dimethyl- | 9.7 | 9.7 | 9.7 | 9.74 |
| Benzaldehyde | 11.3 | 11.29 | 11.3 | 11.3 |
| 1-Octen-3-ol | 12 | 11.99 | 11.99 | 12 |
| 2,5-Octanedione | 12.13 | 12.13 | 12.13 | 12.14 |
| Furan, 2-pentyl- | 12.33 | 12.32 | 12.33 | 12.33 |
| Octanal | 12.69 | 12.68 | 12.69 | 12.69 |
| Benzeneacetaldehyde | 13.87 | 13.87 | 13.88 | 13.87 |
| 4-Pyridazinamine | | | | 15.01 |
| 2-Nonanone | 15.25 | 15.25 | 15.25 | 15.25 |
| Nonanal | 15.58 | 15.57 | 15.58 | 15.58 |
| Benzoic acid,2-[(trimethylsilyl)o | 16.96 | | | 16.96 |
| Azulene | 17.63 | 17.62 | 17.62 | 17.63 |
| 2-Decanone | 17.89 | 17.88 | 17.88 | 17.89 |
| Fluoren-9-ol, 3,6-dimethoxy-9 | | | | 21.1 |
| 5H-Naphtho[2,3-c]carbazole, 5-meth | | | | 24.77 |
| Hexadecane | 26.75 | 26.74 | 26.74 | 26.74 |
| Tetradecanal | | | | 30.76 |
| Hexadecanal | 30.76 | | | |
| Eicosane | 24.76 | 24.75 | | |
| 2H-1,4-Benzodiazepin-2-one, 7-chlo | 21-Jan | | 20.31 | |
| 1-Adamantanol | 15.02 | 15.01 | 15.01 | |
| Heptanal | 9.41 | | | |
| Furfural | 7.03 | | 7.03 | |
| propanal, 2-methyl- | 1.83 | | | |
| 16-Octadecenal | | | 30.77 | |
| Heptadecane | | 28.62 | 28.63 | |
| Tris(trimethylsilyl)borate | | | 28.09 | |

**(cont. on next page)**

**Table 3. (cont.)**

| | | | | |
|---|---|---|---|---|
| Pentadecane | | | 24.76 | |
| tetradecane | | | 22.65 | |
| 1,4-Hexadiene, 3-ethyl- | | | 20.31 | |
| Decanal | | 18.23 | 18.23 | |
| p-Trimethylsilyloxyphenyl, (trimeth | | 16.95 | 16.96 | |
| 2-Octenal | | 14.33 | 14.34 | |
| 1,3-Hexadiene, 3-ethyl-2-methyl- | | 13.53 | 13.52 | |
| Pyrazine, trimethyl- | | 12.84 | 12.84 | |
| Dimethyl trisulfide | | 11.52 | 11.53 | |
| Styrene 8,97 | | | 8.97 | |
| 2-Furanmethanol | | 7.94 | 8 | |
| Pyrazine, methyl- | | 6.88 | 6.95 | |
| Cyclotrisiloxane, hexamethyl- | | 6.73 | 6.73 | |
| 2-Hexanone | | 5.52 | 5.54 | |
| 3((2H)-Furanone, dihydro-2-methyl- | | | 3.09 | |
| Ethene, ethoxy- | | | 1.84 | |
| Cyclobutanol | | | 1.62 | |
| Bicyclo [4.2.0] octa-1,3,5-triene | | 8.97 | | |
| Pentane, 1-(ethenyloxy)- | | 11.04 | | |
| 2,7-Dioxatricyclo[4.4.0.0(3,8)] | | 20.31 | | |
| 2-Chloro-4-(4-methoxyphenyl)-6- | | 21.08 | | |
| Oxirane, heptadecyl- | | 30.76 | | |

# CHAPTER 4

## DISCUSSION

Cakes are one of the most commonly produced and consumed cereal products in food industry and at home all over the world. During cake baking the sensory parameters of cakes such as taste, color and odor develop and the product gains its characteristic solid-amorphous structure. As a result of the elevated temperature levels the microbial load and enzymatic activity levels of the ingredients decrease and the shelf life of the final product is increased (Fellows, P., 1988). During baking the elevated temperature levels gelatinize the starch and contribute to the final solid structure with several quality factors such as the volume and the hight of the cake, symmetry values of the cakes, porosity and color.

Enzymes are industrial materials used in almost every aspect of life. For example, wine industry, food industry, paint making industry, etc. Food processing enzymes are used as food additives to modify food properties. Food processing enzymes are used in starch processing, meat processing, dairy industry, wine industry and in the manufacture of predigested foods as part of the field of Food Biotechnology.

The basic hypothesis of this study is that the enzymes added as the food additives can achieve this goal while preserving the other cake quality parameters. The enzymes were selected according to their effects on different macromolecular ingredients of cakes namely, proteins (transglutaminase), lipids (lipase) and carbohydrates (amylase).

All three enzymes lowered the amounts of carbonyls significantly with the largest decrease by lipase of 31.83% with respect to the control cake. Transglutaminase and lipase addition changed the carbonyl profile of cakes significantly. Both transglutaminase and lipase caused important changes in protein secondary structures with large increases in alpha helix, turns and anti-parallel beta structures, however, amylase did not cause such large changes. The three enzymes used caused the lipid/protein ratio to decrease. The level of lipid unsaturation did not change for transglutaminase and lipase, however, the level unsaturation decreased in the case of amylase indicating the formation of dicarbonyls was via Maillard reaction not due to lipid peroxidation. In all the three enzyme cases the amorphous structure of the starch in cake samples increased depending on the enzyme concentration used.

We believe that a future work is necessary to show the effect of the three enzymes an addition to the other enzymes used in food industry on the formation of advanced Maillard Reaction compounds such as acrylamide and colored compounds such as malanoidins.

# CHAPTER 5

# CONCLUSION

Transglutaminase, lipase and amylase enzymes were used to improve the cake properties while lowering the carbonyl formation. All three enzymes used had low the amounts of carbonyls formed significantly. In addition the enzymes changed the carbonyl profiles, protein secondary structures and lipid/protein ratios. The GC-MS results indicated that there was no change in the formation of either in the Maillard Reaction Products nor the lipid oxidation products in the head space analysis.

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# APPENDIX

Table 4. Experiment results

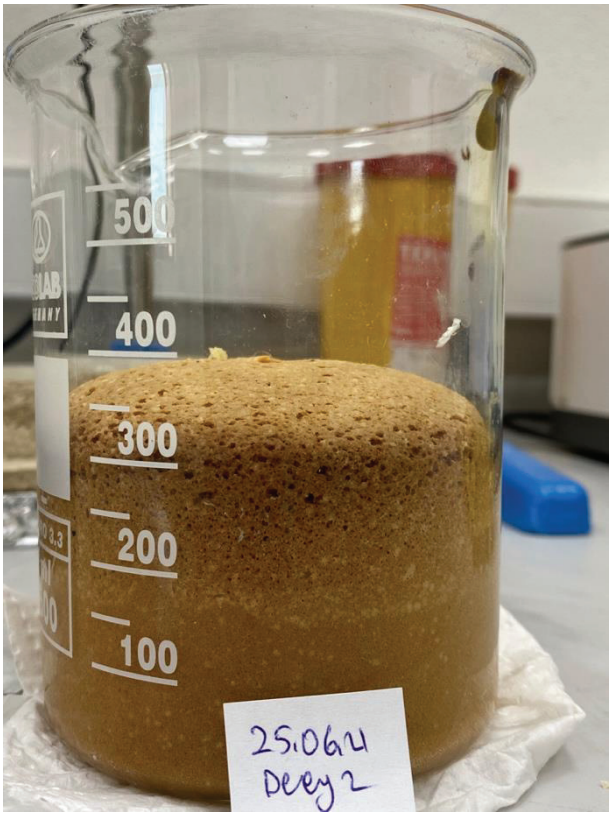
| Cake Baking Date | Volume Index (mm) | Symmetry Index (mm) | Uniformity Index (mm) | Dough Weight (g) | Baked Weight (g) | Enzyme Types | % Weight Loss | Kolza Tohumu 500ml (g) | Kolza Tohumu + Kek 500 ml (g) |
|---|---|---|---|---|---|---|---|---|---|
| 21.06.2021 | 204 | 6 | 4 | 150.0 | 133.6 | - | 10,8 | 353,2 | 251,3 |
| 21.06.2021 | 188 | 16 | 4 | 150.0 | 133.7 | - | 10,8 | 352,7 | 252,1 |
| 21.06.2021 | 206 | 13 | 3 | 150.0 | 134.4 | - | 10,4 | 355,6 | 250,2 |
| 22.06.2021 | 209 | 16 | 2 | 150.2 | 138.1 | TG-0,200 g | 8,0 | 348,1 | 249,6 |
| 22.06.2021 | 203 | 25 | 3 | 150.2 | 140.0 | TG-0,200 g | 6,7 | 350,9 | 254,8 |
| 22.06.2021 | 213 | 15 | 1 | 150.0 | 140.8 | TG-0,200 g | 6,1 | 352,1 | 248,9 |
| 23.06.2021 | 187 | 20 | 2 | 150.0 | 144.7 | TG-0,150 g | 3,5 | 354,6 | 249,1 |
| 23.06.2021 | 204 | 9 | 3 | 150.3 | 138.7 | TG-0,150 g | 7,7 | 352,7 | 248,2 |
| 23.06.2021 | 187 | 17 | 1 | 150.2 | 138.3 | TG-0,150 g | 7,9 | 354,6 | 249,6 |
| 24.06.2021 | 183 | 9 | 3 | 150.1 | 140.7 | TG-0,100 g | 6,2 | 350,6 | 250,6 |
| 24.06.2021 | 184 | 11 | 3 | 150.2 | 138.5 | TG-0,100 g | 7,7 | 351,2 | 251,2 |
| 24.06.2021 | 172 | 14 | 4 | 150.1 | 138.4 | TG-0,100 g | 7,7 | 352,3 | 249,6 |
| 25.06.2021 | 195 | 13 | 3 | 150.2 | 134.9 | TG-0,050 g | 10,1 | 352,5 | 245,7 |
| 25.06.2021 | 196 | 11 | 1 | 150.0 | 133.8 | TG-0,050 g | 10,8 | 354,7 | 247,9 |
| 01.07.2021 | 187 | 13 | 1 | 150.4 | 140.2 | LP-0,200 g | 6,7 | 355,3 | 248,9 |
| 01.07.2021 | 201 | 11 | 1 | 150.3 | 139.4 | LP-0,200 g | 7,2 | 352,7 | 238,4 |
| 01.07.2021 | 192 | 15 | 1 | 150.1 | 139.6 | LP-0,200 g | 7,1 | 352 | 247,4 |

| Cake Baking Date | Volume Index | Symmetry Index | Uniformity Index | Dough Weight (g) | Baked Weight (g) | Enzyme Types | % Weight Loss | Kolza Tohumu 500ml (g) | Kolza Tohumu + Kek 500 ml (g) |
|---|---|---|---|---|---|---|---|---|---|
| 02.07.2021 | 197 | 9 | 1 | 150.3 | 143.7 | LP-0,150 g | 4,39 | 340,8 | 155,1 |
| 02.07.2021 | 185 | 7 | 1 | 150.3 | 143.9 | LP-0,150 g | 4,25 | 343,3 | 264,6 |
| 02.07.2021 | 189 | 12 | 2 | 150.2 | 140.2 | LP-0,150 g | 6,65 | 344,2 | 240,1 |
| 05.07.2021 | 185 | 4 | 2 | 150.7 | 138.9 | LP-0,100 g | 7,83 | 343,9 | 256,2 |
| 05.07.2021 | 183 | 8 | 1 | 150.2 | 138.4 | LP-0,100 g | 7,85 | 350,6 | 249,2 |
| 05.07.2021 | 179 | 7 | 1 | 150.0 | 134.8 | LP-0,100 g | 10,13 | 345,3 | 272,8 |
| 06.07.2021 | 184 | 11 | 3 | 150.2 | 134.2 | LP-0,050 g | 10,65 | 349,3 | 257,8 |
| 06.07.2021 | 180 | 8 | 2 | 150.3 | 134.0 | LP-0,050 g | 10,84 | 340,2 | 266,3 |
| 06.07.2021 | 183 | 10 | 1 | 150.1 | 136.1 | LP-0,050 g | 9,32 | 346,7 | 264,4 |
| 13.07.2021 | 189 | 6 | 2 | 150.2 | 139.1 | AA-0,200 g | 7,39 | 348,7 | 236,4 |
| 13.07.2021 | 181 | 5 | 1 | 150.3 | 138.6 | AA-0,200 g | 7,78 | 346,3 | 264,5 |
| 13.07.2021 | 181 | 5 | 1 | 150.3 | 138.0 | AA-0,200 g | 8,18 | 340,2 | 266,3 |
| 14.07.2021 | 173 | 13 | 1 | 150.4 | 141.4 | AA-0,150 g | 5,98 | 350,2 | 257,2 |
| 14.07.2021 | 181 | 5 | 1 | 150.3 | 138.5 | AA-0,150 g | 7,85 | 347,3 | 251,4 |
| 14.07.2021 | 187 | 8 | 6 | 150.3 | 138.2 | AA-0,150 g | 8,05 | 350,5 | 233,2 |
| 27.07.2021 | 181 | 24 | 4 | 150.2 | 133.5 | AA-0,100 g | 11,11 | 341,8 | 274,8 |
| 27.07.2021 | 183 | 12 | 4 | 150.0 | 133.6 | AA-0,100 g | 10,93 | 345,9 | 256,0 |
| 27.07.2021 | 189 | 9 | 1 | 150.1 | 134.1 | AA-0,100 g | 10,65 | 349,1 | 244,2 |
| 30.07.2021 | 162 | 18 | 2 | 150.0 | 134.5 | AA-0,50 g | 10,33 | 358,4 | 276,2 |
| 30.07.2021 | 184 | 8 | 0 | 150.3 | 144.3 | AA-0,50 g | 3,99 | 350,3 | 258,4 |
| 30.07.2021 | 186 | 9 | 1 | 150.0 | 143.7 | AA-0,50 g | 4,20 | 341,7 | 270,6 |

Table 5. Experiment color results

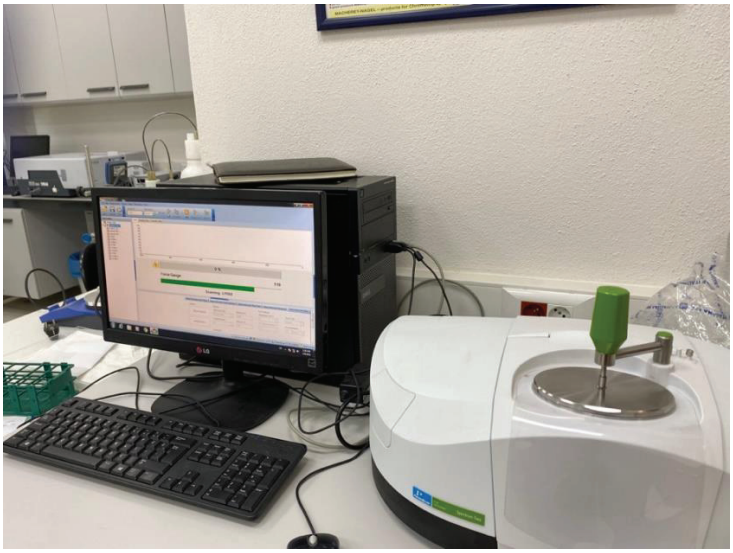
| | L* | a* | b* |
|---|---|---|---|
| LP-0,200-1 | 69.33 | 6.60 | 33.80 |
| LP-0,200-2 | 70.40 | 7.15 | 26.02 |
| LP-0,200-3 | 67.00 | 8.44 | 35.30 |
| LP-0,150-1 | 67.74 | 8.61 | 38.08 |
| LP-0,150-2 | 66.57 | 10.97 | 37.01 |
| LP-0,150-3 | 67.14 | 8.06 | 34.14 |
| LP-0,100-1 | 65.22 | 9.30 | 34.78 |
| LP-0,100-2 | 66.13 | 10.50 | 36.19 |
| LP-0,100-3 | 66.35 | 9.30 | 33.75 |
| LP-0,050-1 | 63.50 | 12.02 | 37.98 |
| LP-0,050-2 | 62.44 | 12.11 | 36.00 |
| LP-0,050-3 | 60.39 | 13.37 | 38.10 |
| TG-0200-1 | 70.73 | 10.89 | 39.65 |
| TG-0200-2 | 67.31 | 10.02 | 35.58 |
| TG-0200-3 | 67.61 | 9.87 | 37.70 |
| TG-0,150-1 | 64.89 | 10.14 | 37.17 |
| TG-0,150-2 | 65.30 | 9.49 | 36.21 |
| TG-0,150-3 | 64.09 | 9.80 | 34.70 |
| TG-0,100-1 | 65.83 | 8.85 | 32.32 |
| TG-0,100-2 | 65.17 | 8.70 | 36.21 |
| TG-0,100-3 | 64.64 | 9.10 | 34.00 |
| TG-0,050-1 | 60.22 | 12.57 | 35.64 |
| TG-0,050-2 | 61.44 | 10.67 | 35.84 |
| control-1 | 66.42 | 7.47 | 35.33 |
| control-2 | 65.48 | 7.25 | 33.84 |
| control-3 | 63.87 | 8.81 | 37.06 |


Oven





Examples of cake experiments.




The FTIR device and the mixting device.



The GC-MS device