İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

PROPERTIES AND THERMAL INACTIVATION KINETICS OF LIPASE AND PEROXIDASE FROM HAZELNUT IN RELATION TO NATURAL HAZELNUT MEAL STABILITY

Ph.D. Thesis by

Ferda GÜRTAŞ SEYHAN, M.Sc.

(506942016)

Date of submission : 16 February 2001

Date of defence examination: 27 June 2001

Supervisor (Chairman): Prof. Dr. Özgül EVRANUZ

Members of the Examining Committee Prof. Dr. Reşat APAK (İÜ.)

Prof. Dr. Dilek BOYACIOĞLU (İTÜ.)

Prof. Dr. Nuran DEVECİ (İTÜ.)

Assist. Prof. Dr. Murat ÖZDEMİR (GYTE.)

JUNE 2001

ACKNOWLEGEMENTS

Thanks are due Prof. Dr. Özgül Evranuz for her accurate supervising, valuable discussions and guidance.

I am especially grateful to my mother, my husband and my son for their spiritual support and giving courage to start and to conclude this study.

This study was conducted within the framework of the The Scientific and Technical Research Council of Turkiye (TÜBİTAK)-TOGTAG/TARP project. I thank TÜBİTAK-Marmara Research Center - World Bank Grant Program for financing the part of the study at ATO, the Netherlands. Thanks are due Prof. L.M.M. Tijskens (ATO, Netherlands) and Dr. J. C. Espin (CEBAS-CSIC, Spain) for helpful discussions and suggestions during the constructing mathematical models. Prof. H.J. Wichers (ATO, Netherlands), Dr. Türcan Gürcan (TÜBİTAK, Gebze) and Dr. Dilek Kazan (TÜBİTAK, Gebze) kindly gave permission to use their laboratories and equipment. Thanks are also due Murat Özdemir (TÜBİTAK, Gebze) for his fellowship and İbrahim Kelebek for his contribution to experimental studies.

February, 2001 Ferda Gürtaş Seyhan

CONTENTS

ABBREVIATIONS

AB	: Acetate Buffer
ABTS	: 2,2-azino-bis-(3-ethylbenz-tiazoline-6-sulphonic acid)),
App.	: Approximetely
ATO	: Instituut voor Agrotechnologish Onderzoek, Agrotechnological Research Institute
Aw	: Water Activity
BET	: Brunauer-Emmet-Teller
BSA	: Bovine Serum Albumine
cv.	: Cultivar
d.b.	: Dry Basis
d.m.	: Dry matter
DAG	: Diacylglycerol
DNPB	: Dinitrophenyl butyrate
EDTA	: Ethylenediaminetetraacetic acid
ERH	: Equilibrium Relative Humidity
FFA	: Free Fatty Acids
HCl	: Hydrochloric acid
I.U.	: International Units
LDL	: Low density lipoprotein
LIP	: Lipase
MAG	: Monoacylglycerol
MBTH	: 3-methyl-2-benzothiazolinone hydrazone
MUFA	: Monounsaturated Fatty Acids
NLR	: Non Linear Regression
N_{obs}	: Number of observetions
POD	: Peroxidase
PUFA	: Polyunsaturated Fatty Acids
PV	: Peroxide Value
RH	: Relative Humidity
R^2	: Coefficient of determination for NLR analysis
s.e	: Standart error of estimates
SPSS	: Statistical Software, SPSS Inc. Headquarters, 233 S. Wacker Drive, 11th floor, Chicago, Illinois 60606
TAG	: Triacylglycerol
TS	: Turkish Standarts
w.b.	: Wet Basis

LEGEND OF SYMBOLS

Variable	Dimension	Meaning
Act	nktal (=nmol/sec) or Abs/min	Enzyme activity
E_a	kJ/mol	Energy of activation
En	mol/L	True enzyme concentration
En_{na}	mol/L	Denatured enzyme concentration
En_0	mol/L	Enzyme concentration at t=0
$[H^+]$	mol/L	Concentration of hydrogen ions
$[OH^-]$	mol/L	Concentration of hydroxide ions
[En]	mol/L	Concentration of enzyme
$[EnH^+]$	mol/L	Concentration of enzyme-hydrogen ion complex
$[EnOH^-]$	mol/L	Concentration of enzyme-hydroxyl ion complex
$[En_{tot}]$	mol/L	Total amount of enzyme concentration
k_s	sec^{-1}	Rate constant substrate conversion
k_d	$(mol/L)^{-1}*(sec)^{-1}$	Rate constant denaturation
K_{EH}	mol/L	Acidity constant of EnH^+ species
K_{EOH}	mol/L	Basicity constant of $EnOH^-$ species
K_w	$(mol/L)^2$	Water dissociation constant
P	mol/L	Concentration of product
R	J/ mol .K (8.314)	Gas constant
S	mol/L	Concentration of substrate
t	Sec	Time
T	K (or °C)	Temperature
F	min	Thermal destruction time
Indices		
0		Initial
d		Denaturation
f		Final
i		Any
na		Not active
obs		observed
ref		Reference
s		Substrate conversion
tot		Total pool of enzymes
w		Water

CAPTION TO TABLES

CAPTION TO FIGURES

PROPERTIES AND THERMAL INACTIVATION KINETICS OF LIPASE AND PEROXIDASE FROM HAZELNUT IN RELATION TO NATURAL HAZELNUT MEAL STABILITY

SUMMARY

Turkiye is the biggest hazelnut producer of the world with the amount of about 500 000 ton per year , which is 80% of the world's production. Turkiye gets about one billion US$ annually as export (4-5% of the total export) income from hazelnut and its products. However, natural hazelnut export is only 3% and used in bakery industry. The export of processed hazelnuts is very limited and because of the quality problems the producers hesitate to process natural hazelnut meal. Especially due to the prolonged storage, during spring and summer quality problems in natural hazelnut meal create a handicap in hazelnut meal export.

Hazelnut is very nutritious and has widespread usage due to its special aroma in chocolate, confectionery, and bakery industries besides consumption as whole nut. The hazelnut is a high lipid content food and very rich in unsaturated fatty acids. Therefore hazelnut gets rancid due to lipid oxidation and hydrolysis. Lipid hydrolysis results in formation of free fatty acids causing increase of food acidity. Once the lipid hydrolysis is initiated, the quality declines rapidly since free fatty acids formed can be substrate of the oxidation reactions. Structure of the tissue of hazelnut broken down during processing of natural hazelnut meal and enzymes becomes available for oxidation and hydrolysis and becomes rancid more rapidly. Lipase, peroxidase, lipoxygenase and esterase are the enzymes found in hazelnut that catalyses hydrolysis and oxidation reactions. According to the literature, it is found that lipoxygenase shows no activity in Turkish type hazelnuts and esterase has minimal effect on quality.

Many researchers have studied use of thermal treatment for inactivation of the enzymes, which deteriorate food. In the food industry heating is the most convenient method for enzyme inactivation. To minimise heat induced quality changes in the food the heat treatment is often designed as a high temperature short time process. Therefore the conditions for minimal heat treatment to inactivate the enzymes responsible of rancidity development in hazelnuts should be determined to increase the shelf life stability of hazelnut and hazelnut products. In this respect it was aimed to determine the properties and thermal inactivation kinetics of lipase and peroxidase, which causes rancidity in natural hazelnut meal in relation to natural hazelnut meal stability. Results from our investigations on inactivation of lipolytic enzymes will be useful in shelf life and market quality improvement of hazelnuts and hazelnut products.

Activity of lipase and peroxidase in Turkish hazelnut (cv. Tombul) was studied as a function of temperature at a constant pH of 4.5, and as a function of pH at a constant temperature of 25 °C. For peroxidase the temperature for maximum activity is quite sharp at 40°C, for lipase, the temperature for maximum activity is very broad and ill defined, ranging from 40°C to 60°C. The results showed that maximum activity for both lipase and peroxidase was observed between 4.5-4.7 pH. However, lipase revealed an iso-enzyme system with a second clearly distinguishable peak around pH 7.5 in activity with pH, whereas peroxidase only showed one large peak of activity. The activity data obtained were analysed by non-linear regression using an existing model for enzyme activity as a function of pH and temperature simultaneously. The existing model was adapted to account for the iso-enzyme system found to be present in hazelnut lipase activity. The coefficients of determination accounted for (R^2) obtained for peroxidase were 0.856 and 0.962 for temperature and pH dependence respectively. For lipase the coefficients were found to be 0.721 and 0.896 respectively. These results corroborate significantly the developed fundamental models in the literature.

The thermostabilities of lipase and peroxidase was studied at 60°C and the activity measurements for both enzymes were carried out at their optimum pH and temperature conditions, determined in the first step of this study. The analysis of the data with exponential model resulted in inactivation rate constant of $3.66x10^{-3}s^{-1}$ for lipase and $5.34x10^{-3}s^{-1}$ and $0.38x10^{-3}s^{-1}$ for heat labile and heat stabile peroxidases respectively. Heating of enzyme extract for 25 min led to considerable inactivation of peroxidase and partial inactivation of lipase, reflecting the higher thermostability of lipase. Therefore for the further analysis for thermal treatments it was decided to study inactivation kinetics of lipase.

Hazelnut enzyme extract were subjected to different heating temperatures for varying periods at static conditions and the lipase activities were measured for each time-temperature treated extract. Analysing the inactivation data using exponential model for lipase obtained at higher temperatures 70, 80 and 90°C resulted in k_i values of $0.40x10^{-2}$ s^{-1} (R^2=0.9057), 5.75 $x10^{-2}$ s^{-1} (R^2=0.9894), and 15.22 $x10^{-2}s^{-1}$ (R^2=0.9685) respectively. Energy for heat inactivation were determined as 171.64 kJ/mol using the Arrhenius plot. The results were in the same range when compared with the literature values.

Whole hazelnut kernels were subjected to dynamic heat treatment at 80, 100 and 120°C for 15 min to determine the inactivation ratio of lipase. Inactivation of lipase in whole hazelnut kernel at 80°C resulted in only 26% inactivation while it was more than 80% after 1 min heating for free lipase showing that the level of heat inactivation for hazelnut lipase depended on physical environment of the enzyme (aqueous extract or kernel matrix). Heat treatment for 10 min at 100°C resulted in more than 80% inactivation of lipase in the kernel. However increasing the heating time up to 15 and 20 min or temperature to 120°C did not resulted in a further significant inactivation, while caused higher FFA values during storage.

Natural and blanched hazelnuts were stored at different temperature, relative humidity and oxygen conditions for 150 days and change in FFA were recorded for 15 day intervals. As it was expected, there was an increase in shelf life as the storage temperature (12, 22 and 35°C) and relative humidity (60 and 70%) decreased.

However, exclusion of oxygen by vacuum packaging were not found beneficial since higher FFA values were recorded for these samples. The reason may be due to higher moisture content then the untreated samples during the storage and activity of lipase independent of oxygen. It was observed that, blanched (120°C, 15 min) hazelnut meal reached 1.0% FFA value 6 times more than the natural hazelnut meal when stored at same conditions (70% RH and 22°C). As a conclusion, all these findings and the storage studies on heat treated hazelnut meal showed that the natural hazelnut meal can be stabilised as decided from the lower increase in FFA content after storage as compared to the untreated samples.

NATUREL FINDIK UNUNDAKİ ACILAŞMA İLE İLGİLİ OLARAK FINDIK LİPAZ VE PEROKSİDAZ ENZİMLERİNİN ETKİSİZLEŞTİRME KİNETİĞİ

ÖZET

Yılda 500 bin tona ulaşan fındık üretimi ile dünya üretiminin %80'ine sahip olan ülkemiz fındık ihracatından bir milyar Amerikan Doları gelir elde etmektedir. Fındık üretiminin büyük olması ile birlikte yaşanan problemler de büyük olmaktadır. Ülkemizde fındığın işlenerek satılması için teşvik verildiği halde işlenmiş fındık ihracat oranı son yıllarda ancak %25'i bulmuştur. Naturel fındık unu ihracatı sadece %3 olup genellikle pastacılıkta kullanılmaktadır. Naturel fındık ununda kalite sorunları yaşanmakta ve bu nedenle ürünün ihracatı olumsuz yönde etkilenmektedir. Firmalar ekonomik kayıplara uğradıklarından fındık unu işlemekten sakınmaktadırlar.

Fındık %55-65 arasında değişen, doymamış yağ asitleri bakımından zengin yağ içeriği nedeni ile zamanla acılaşabilmektedir. Fındıklarda acılaşma oto-oksidasyon veya hidrolitik bozulma sonucu meydana gelmektedir. Hidrolitik bozulma enzim faaliyetleri sonucu oluşmakta ve serbest yağ asitliğinin artmasına neden olmaktadır. Fındık un haline getirilirken dokunun zarar görmesi ile sıcaklık ve bağıl neme bağlı olarak enzimlerin ortamda bulunan yağ ile tepkimeye girmesi kolaylaşmaktadır. Bu nedenle naturel fındık ununun serbest yağ asitliği hızla artmakta ve ürün bir ay gibi kısa bir sürede bozulabilmektedir. Lipaz, peroksidaz, lipooksijenaz ve esteraz fındıkta acılaşmaya neden olan enzimler olarak belirlenmiştir. Yapılan araştırmalar sonucunda Türk fındıklarında lipoksijenaz enziminin bulunmadığı, esteraz enziminin ise kaliteye etkisinin çok az olduğu sonucuna varılmıştır. Bu nedenle bu çalışmada sadece lipaz ve peroksidaz enzimlerinin fındık kalitesine bağlı olarak etkisizleştirilmesi çalışılmıştır.

Gıdalarda kaliteyi etkileyen enzimlerin termal olarak etkisizleştirilmesi bir çok araştırmacı tarafından çalışılmaktadır. Gıda endüstrisinde soya fasulyesinde, balıklarda ve diğer yağlı ürünlerde acılaşmanın giderilmesi amacıyla lipoksijenaz, lipaz ve peroksidaz, portakal suyu üretiminde pektin esteraz, dondurulmuş meyve ve sebzelerde peroksidaz veya katalaz, v.b. enzimlerinin etkisizleştirilmesi kalitenin korunmasında başarılı sonuçlar vermiştir. Benzer sonuçlara yer fıstıkları, pekan, kolza tohumu, fındık gibi yağlı tohumlarda peroksidaz ve lipaz enzimlerinin ısıl etkisizleştirilmesi ile ilgili olarak yapılan çalışmalarda da rastlanılmaktadır.

Isıl işlem uygulaması sonucunda kalitenin olumsuz yönde etkilenmesini en aza indirmek amacı ile yüksek sıcaklık-kısa süre koşulları tercih edilmektedir. Bu nedenle, fındıkta acılaşmaya neden olan enzimlerin minimal ısıl işlemle etkisizleştirilmesi fındık ve fındık ile ilgili ürünlerin raf ömrünün arttırılması açısından büyük önem taşımakta ve bu koşulların belirlenmesi gerekmektedir. Bu çalışmanın amacı, naturel fındık ununda acılaşma ile ilgili olarak lipaz ve peroksidaz

etkisizleştirilmesinin naturel fındık ununun raf ömrüne etkisinin belirlenmesidir. Bunu gerçekleştirmek için, Tombul çeşidi fındıklardaki lipaz ve peroksidaz enzimlerinin özelliklerini belirlemek amacı ile sıcaklık ve pH'ya bağlı olarak ortam koşulları incelenmiş ve aktivitelerinin maksimum olduğu sıcaklık ve pH değerleri belirlenmiştir. Her iki enzimin ısıl işleme karşı dayanıklılığını tespit etmek için aynı koşullara tabi tutulan enzimlerin aktiviteleri, ilk aşamada belirlenen optimum koşullarda ölçülmüş ve sıcaklığa en dayanıklı enzim tespit edilmiştir. Tespit edilen enzimin inaktivasyon kinetiği enzim ekstraktında daha yüksek sıcaklıklarda çalışılmıştır. Bu aşamadan sonra, fındık tanesindeki enzimin etkisizleştirilmesi için gerekli olan sıcaklık-süre ilişkisi belirlenip, bunun dayanma süresine etkisi incelenmiştir. Bu çalışma sonunda elde edilen sonuçlar fındık ve fındıkla ilgili ürünlerin raf ömürlerinin uzatılması ve kalitelerinin arttırılması açısından çok büyük önem taşımaktadır. Bu çalışma kapsamında elde edilen sonuçlar aşağıda özetlenmiştir.

Birinci aşamada Tombul çeşidi Türk fındıklarında lipaz ve peroksidaz aktiviteleri 4.5 sabit pH'da sıcaklığa ve 25°C sabit sıcaklıkta pH'ya bağlı olarak tespit edilmiştir. Peroksidaz için aktivitenin en yüksek olduğu değer belirgin olarak 40°C iken, lipaz için bu değer 40-60°C arasında daha geniş bir aralığa yayılmaktadır. Sonuçlara göre lipaz ve peroksidaz enzimlerinin her ikisi için de maksimum aktivitenin gözlendiği pH aralığı 4.5-4.7 olarak belirlenmiştir. Peroksidaz enzimi için sadece geniş bir aktivite piki gözlenirken, bu çalışma ile lipaz enziminin 7.5 pH civarlarında belirgin olarak farklı bir pik göstermesi sonucu pH'ya bağlı izo-enzim varlığı ortaya çıkarılmıştır. Elde edilen aktivite verileri daha önceden fitaz enzimi için de kullanılmış olan, eşzamanlı olarak sıcaklık ve pH'ya bağlı model kullanılarak doğrusal olmayan regrasyonla analiz edilmiştir. Peroksidaz için regresyon katsayısı sıcaklık ve pH için sırasıyla 0.856 ve 0.962, lipaz için ise katsayılar 0.721 ve 0.896 olarak bulunmuştur. Sonuçlar literatürdeki geliştirilen belli başlı model değerleri ile desteklenmektedir.

Lipaz ve peroksidazın ısıya karşı dayanıklılıklarını tespit etmek amacıyla enzim ektraktı 60°C'deki su banyosunda statik koşullara tabi tutulmuştur. Etkisizleştirilme sonrası aktivite ölçümleri birinci aşamada belirlenmiş olan optimum koşullarda gerçekleştirilmiştir. Enzim ekstraktının 25 dak ısıtılması sonunda peroksidaz önemli derecede lipaz ise kısmi olarak etkisizleşmiştir. Lipaz etkisizleştirilmesi tek eksponansiyel modelle, peroksidaz etkisizleştirilmesi ise çift eksponansiyel model ile açıklanmıştır. Eksponansiyel model kullanılarak verilerin doğrusal olmayan regresyon ile analizi sonucu inaktivasyon hız sabitleri lipaz için $3.66x10^{-3}s^{-1}$ bulunmuştur. Yapılan analiz sonucu peroksidazın sıcaklığa karşı farklı dirençte olan iki farklı izo-enzime veya izo enzim gruplarına sahip olduğu sonucuna varılmış ve etkisizleştirilme hız sabitleri sıcaklığa duyarlı izo-enzim için $5.34x10^{-3}san^{-1}$ ve sıcaklığa duyarsız izo-enzim için $0.38x10^{-3}san^{-1}$ olarak hesaplanmıştır. Lipazın sıcaklığa karşı daha dirençli olduğunun belirlenmesi sonucu daha sonraki aşamalarda lipazın inaktivasyon kinetiği daha yüksek sıcaklıklarda incelenmiştir.

Fındıktan elde edilen enzim ekstraktı 70, 80 ve 90°C sıcaklıklarda ve durağan koşullarda, farklı sürelerde ısıl işleme tabi tutulduktan sonra lipaz aktivitesi ölçülmüş ve elde edilen veriler eksponansiyel model kullanılarak analiz edilmiştir. Etkisizleştirilme hız sabitleri her bir sıcaklık uygulaması sonucu elde edilen veri seti için ayrı ayrı hesaplanmış ve analiz sonunda 70, 80 ve 90°C sıcaklıklar için

etkisizleştirilme katsayısı, k_i sırası ile $0.40x10^{-2}$ san^{-1} (R^2=0.9057), $5.75x10^{-2}$ san^{-1} (R^2=0.9894) ve $15.22x10^{-2}san^{-1}$ (R^2=0.9685) olarak hesaplanmıştır. Isıl etkisizleştirilme için enerji, Arrhenius grafiği yardımı ile 171.64 kJ/mol olarak hesaplanmıştır.

Bütün fındığın dinamik koşullarda ısıl işleme tabi tutulması sonucunda lipaz aktivitesindeki azalmanın belirlenmesi amacı ile fındıklar 80°, 100° ve 120°C sıcaklıklarda hava ile dinamik koşullarda 15 dak ısıl işleme tabi tutulmuştur. Fındıkların bütün olarak 80°C'de 15 dak ısıtılması sonucu lipaz aktivitesinde sadece %26 azalma kaydedilirken, aynı sıcaklıkta enzim ekstraktının sadece 1dak bekletilmesi %80'den fazla etkisizleştirilme sonuçlanmıştır. Bu gözlem ısıl etkisizleştirilme düzeyinin enzimin içinde bulunduğu fiziksel ortamla çok ilgili olduğunu, ekstrakta olduğu gibi sulu ortamlarda sıcaklığın çok daha fazla etki ettiğini göstermiştir. Isıtma işlemi daha sonra 100°C'de farklı sürelerde gerçekleştirilmiş ve bütün fındığın 100°C'de 10 dak ısıtılmasının lipaz aktivitesinde %80 azalmaya neden olduğu gözlenmiştir. Bununla birlikte ısıtma süresinin 15 ve 20 dak'ya veya sıcaklığın 120°C'ye çıkarılması etkisizleştirilmesinde önemli bir artışa neden olmadığı gibi depolama sonunda daha fazla SYA artışına yol açtığı belirlenmiştir.

Naturel ve beyazlatılmış fındık unları sıcaklık (12, 22 ve 35°C), bağıl nem (%60 ve %70) ve oksijen koşullarının (vakumlu paket, paketsiz) farklı olduğu depolama koşullarında 150 gün boyunca depolanmış ve SYA değerleri kaydedilmiştir. Sıcaklığın ve bağıl nemin azaltılması ile SYA'ne bağlı olarak fındık unlarının raf ömründe artış gözlenmiştir. Bununla birlikte, vakumlu paketleyerek oksijensiz ortamda depolanan örneklerde daha yüksek SYA değerleri kaydedilmiştir. Vakumla paketlenmiş örneklerde ambalaj malzemesi nedeni ile örneklerin denge bağıl nem değerine ancak depolama sonunda ulaşmış ve depolama süresi boyunca vakumlu paketlenmiş örneklerin nem içerikleri diğer örneklere kıyasla daha yüksek olmuştur. Enzim aktivitesi ve bozulma reaksiyonlarının yüksek nem içeriğinde daha hızlı olması ve vakuma rağmen lipazın oksijensiz ortamlarda da tepkimeye girebilmesi vakumlu örneklerde daha fazla SYA artışına neden olmuştur. Naturel ve 120°C de 15 dak işleme tabi tutularak beyazlatılmış fındık unlarının %70 bağıl nem ve 22°C ortam koşullarında depolanması sonunda beyazlatılmış fındık ununun %1.0 SYA değerine ulaşma süresinin naturel fındıktan 6 kat daha fazla olduğu sonucuna varılmıştır. Bu gözlemler sonucunda ve elde edilen veriler ışığında ısıl işlemle lipaz aktivitesinin azaltılması ile naturel fındık ununun dayanıklılığının arttırılabileceği sonucuna varılmıştır.

1. INTRODUCTION

Turkiye is the leading country providing hazelnut to the world market with the yearly production figure of 500 000 tons for the last six years average (Anon, 2000). However, the problems associated with the hazelnut production and marketing are as big and as many as the relative importance of production. During harvesting, contact with the soil that causes contamination can not be prevented, during drying sudden rain showers that results in re-wetting or insufficient/prolonged drying are not uncommon and generally humid weather conditions provide poor storage stability. So the main problem is the retention of quality for both natural or processed hazelnut products. The hazelnut producers are incited to export added value products by the government. However, the level of processed hazelnuts has recently reached about 25% of the total due to these quality problems.

Although shells which comprises about 50% of the whole kernel, provide natural protection, hazelnuts are mostly marketed as shelled both to decrease the transportation and storage costs and to provide ease of handling to the hazelnut purchasers. The shelled kernels are processed as natural, blanched, roasted, sliced, chopped, diced, meal and paste and add unique flavour and texture to many food products, including chocolate, candies, cookies, bread, ice cream, breakfast cereals and sauce formulations. For that reason, poor quality not only decrease the market potential but also influence the products containing hazelnuts as ingredient.

Shelling and further processing of hazelnut need careful control of temperature, moisture, light and oxygen during processing and storage. For the shelled nuts, in the most cases decreasing the storage temperature, packaging under an inert gas atmosphere, blanching methods, heat treatment to inactivate enzymes are found beneficial. On the other hand, in the production of value added products, any mechanical operation like crushing, grinding, pressing, slicing causes cell damage and increased exposed surface area that results in rapid decline of quality. Lipid globules in the cell are destroyed and oil is squeezed out with these operations which can then contacts easily with the enzyme or the oxygen.

The quality deterioration due to enzymatic and oxidative reactions in hazelnuts has been the subject of various studies (Grosh et al., 1983; Hadorn et al., 1977 and 1978; Keme et al., 1983, Lopez et al., 1997a and b; Metwalli et al., 1983; Pershern et al., 1995 and Zürcher and Hadorn, 1976). In those studies it was shown that the presence of lipolytic enzymes which depend on the maturity, variety, location etc., affect the stability of hazelnuts unless their activity is not controlled.

Understanding the action and the mechanism of the enzymes responsible for the rancidity is essential for designing and developing means to control the activity of these enzymes to retain as much of the flavour and storage characteristics of natural hazelnut meal. Enzyme inactivation by heating is the most convenient method in the food industry. Effect of heat treatment for inactivation of enzymes on quality of foods has been studied by many researchers. It was reported by many researchers that lipase and peroxidase are found to be the main catalysts responsible for the rancidity development in hazelnuts (Hadorn et al., 1977; Keme and Meserli, 1976; Lopez, 1997b and Metwalli et al., 1983). Inactivation of these enzymes, by heating for example has been shown to increase the shelf life stability of natural and roasted nuts and oil seeds (Metwalli et al., 1983; Mitchell and Malphrus, 1977; Ponne et al., 1996; Sanders et al., 1993; Senter et al., 1984a and b; Svensson, 1977 and Vetrimani et al., 1992). However optimum conditions for lipase and peroxidase in hazelnuts have not been determined yet and the inactivation kinetics has not been studied for Turkish hazelnuts.

Specifically it was aimed to determine the properties and thermal inactivation kinetics of lipase and peroxidase in relation to natural hazelnut meal stability. To achieve this the study was conducted with Turkish hazelnuts, in four steps including:

- Determination of the optimum conditions for these enzymes by partially purifying the lipase and peroxidase and measuring the activities at 4.5pH as a function of temperature (5-60°C) and at 25°C as a function of pH (3-9),
- Comparison of the thermostabilities of lipase and peroxidase at 60°C to determine the most thermostabile enzyme,
- Inactivation kinetics of the free enzyme at higher temperatures (70, 80, 90°C)
- Determination of the effect of thermal inactivation of the enzyme on shelf life stability of natural hazelnut meal.

2. HAZELNUT VARIETIES, COMPOSITION, AND IMPORTANCE

Hazelnuts, or filberts (*Corylus*) have 400 distinct, named cultivars, where 20 of these are planted for commercial production. Majority of hazelnuts are produced in limited areas near large bodies of water characterised by mild, humid winters and cool summers. European hazelnuts *C. avellana L.* are produced in larger amounts than American hazelnut *C. americana*. Plants of *C. avellana* are multi-stemmed scrubs which grow 3-10m, occasionally 15m high. The major producing region in Turkiye is adjacent to the Black Sea. The cultivars and native hazelnuts of Turkiye are frequently classified as *C. pontica Koch*. The cultivars have been selected for their long, clasping, constricted husks and several small nuts per cluster. The Turkish cultivar Tombul is the most popular cultivar with 35% production ratio of the total. Tombul is the round type and mostly produced in Giresun. It is considered one of the best currently available for the market due to its easily removed pellicle (Mechlenbacher, 1990).

Hazelnut is an important nutritional food because of its potential value as a source of protein and oil. There are many literature on composition of hazelnuts (Açkurt et al., 1999; Baş et al., 1986; Beringer and Dompert, 1976; Bonvehi and Coll, 1993a,b and 1997a,b; Botta et al., 1996; Ebrahem and Richardson, 1994; Garcia et al., 1994; Hadorn et al., 1977; Özdemir, et al., 1998a; Pala et al. 1996; Parcerisa et al., 1993a,b; 1995a,b; 1997; 1998; Ruggeri et al., 1998; Savage et al., 1997; Şimşek and Aslantaş, 1999 and Villarroel et al., 1987). Data on the composition of different varieties of hazelnuts grown in Turkiye and the ranges from other literatures listed above are given in Table 2.1.

Table 2.1. The composition and fatty acids profile of Turkish Hazelnuts (Baş et al., 1986) and range of the values given in other literatures[a].

Veriaty	Energy (Kcal/ 100 g)	Fat (% dm)	Carbohydrate[b] (% dm[c])	Protein[d] (%dm)	Mineral (%dm)	Fatty Acids (% Fat)					
						Palmitic (16:0)	Palmitoleic (16:1)	Steraic (18:0)	Oleic (18:1)	Linoleic (18:2)	Unsaturated/ Saturated
Tombul	627	64.77	15.72	13.78	2.07	4.50±0.23	0.22±0.04	1.02±0.24	82.61±0.51	11.65±0.70	17.12
Palaz	633	63.25	14.21	12.43	2.05	6.71±0.01	0.83±0.09	1.670.15	79.84±0.26	10.96±0.02	10.93
Mincane	639	63.64	12.22	13.29	1.90	7.88±0.16	0.69±0.07	2.32±0.35	76.87±0.35	12.49±0.10	8.83
Foşa	651	57.70	15.35	13.95	2.16	6.74±0.02	0.72±0.08	2.15±0.08	73.10±0.65	17.30±0.68	10.25
Çakıldak	623	55.07	22.32	14.91	2.55	5.62±0.02	0.49±0.03	1.12±0.13	71.27±0.01	21.36±0.06	13.82
Kalınkara	632	64.65	16.61	11.71	1.95	5.23±0.02	0.24±0.02	1.15±0.03	78.73±0.01	14.66±0.04	14.68
Cavcava	-	62.89	15.52	11.54	1.97	9.27±0.25	0.87±0.09	2.03±0.05	71.75±0.17	16.02±0.25	7.84
Uzunmusa	-	66.40	12.77	12.38	2.12	ND[e]	ND	ND	ND	ND	ND
Sivri	644	66.28	10.88	13.55	2.05	6.61±0.06	0.34±0.01	1.71±0.07	76.19±0.01	15.14±0.00	11.02
Others	587-634	46-76.8	13.7-16.7	12.6-16	2.3-2.5	4-6.7	0.15-0.3	1.2-3.25	70.5-85.3	5.7-22.2	13.2-14.8

[a] (Açkurt et al., 1999; Beringer and Dompert, 1976; Bonvehi and Coll, 1993a,b and 1997a,b; Botta et al., 1996; Ebrahem and Richardson, 1994; Garcia et al., 1994; Hadorn et al., 1977; Özdemir, et al., 1998a; Pala et al. 1996; Parcerisa et al., 1993a,b; 1995a,b; 1997; 1998; Ruggeri et al., 1998; Savage et al., 1997; Şimşek and Aslantaş, 1999 and Villarroel et al., 1987).

[b] Total Carbohydrate

[c] dm: Dry matter.

[d] Nitrogen x 5.3.

[e] ND: No Data.

Apart from the basic food source of protein and oil, hazelnuts are high in Vitamin E folic acid and Vitamin B_6. One hundred grams of hazelnuts is enough to supply 100% of vitamin E and 25% of vitamin B_6 (Richardson, 1996). One hundred grams of hazelnut provides up to 28% of the 400μg of folate recommended for adults and 19% of the 600μg recommended for pregnant women. Hazelnut is also valuable for its rich mineral content; iron, calcium, potassium, magnesium and zinc with the ranges of 21.1-65.4mg, 0.18-0.59mg, 1-4.5 mg, 141-250 mg, 465-750 mg, 146.5-184 mg 1.96-2.44 mg per 100g edible portion (Beringer and Dompert, 1976; Beuchat and Worthington, 1978; Bonvehi and Coll, 1997b; Botta, 1996; Garcia et al., 1994; Pala et al., 1996; Parcerisa et al., 1995b and Savage et al., 1997).

2.1. Nutritional Benefits and Health Claims of Hazelnuts

Hazelnuts are not only a source of energy but they also provide certain compounds such as monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), Vitamin E, fibre, potassium, calcium, folic acid and natural sterols that enhance the nutritional value of human diet (Parcerisa et al., 1998 and Stone, 2000). Vitamin E (α-tocopherol) behave as natural antioxidant and protect the unsaturated compounds in oil by slowing down the rancidity. Vitamin E is essential for the normal functioning of muscle tissue and the reproduction system. It protects the body against cancer and also anaemia. Alpha tocopherol is the only type of Vitamin E that is active biologically (Lee, 1983). Hazelnut has 98% of the tocopherol as alpha type. Diets should contain 1 I.U. Vitamin E/ g PUFA and hazelnut is one of the oilseeds which have the high ratio of tocopherol : PUFA. The Vitamin E/PUFA was determined as 9.7 I.U./g for hazelnut which was the highest among many oil seeds (Beringer and Dompert, 1976). According to Parcerisa et al. (1998), PUFA content of Tombul is 17.89% of fatty acids and 303.8 mg/kg of lipid fractions extracted from hazelnut samples.

Hazelnuts are an excellent source of folic acid or folate which is one of the B vitamins that is often below recommended quantities in diets. Folate is an important factor in new cell production and the growth and repair of cells and is involved in the formation of DNA. Therefore it is very important for pregnant women. Hazelnuts which contain more folic acid than the other nuts are a convenient source of folate.

Folate also helps reduce the risk of neural tube defects when consumed early pregnancy. In addition folate may also play a part in reducing the cardiovascular disease (Stone, 2000).

It has been suggested by many researchers that nuts protect against ischaemic cardiovascular diseases, also due to their high MUFAs and PUFAs (Elvevol et al., 1990; Mattson, 1989; Nicolasi et al., 1990; Richardson, 1996 and Sabate et al., 1993). MUFAs plays an important role in decreasing the serum cholesterol levels (Elvevol et al., 1990 and Nicolasi et al., 1990). Hazelnuts has 75-85% oleic acid ($C_{18:1}$) of total lipid in hazelnuts (Baş et al., 1986; Beringer and Dompert, 1976 and Pala et al., 1996). Beuchat and Worthington (1978) have determined fatty acid composition from different 13 tree nuts and hazelnut has the highest oleic acid content with 76.4% of fatty acids and 14.5 unsaturated: saturated ratio. Hazelnuts also modify the lipoprotein profile in normal persons when consumed frequently. They are low in sodium and sugar. It has been observed that monounsaturated fat diet enriched with hazelnut did not influence the carbohydrate metabolism and lowered LDL and cholesterol so that can be used in diets of patients with non-insulin-dependent diabetes mellitus (Alphan et al., 1996 a and b).

2.2. Hazelnut Production

Turkey is the largest hazelnut producer with average annual production of about 500 000 tons for the last six years and shares 80% of the world total export and production (Anon, 2000). Italy, USA and Spain follow Turkiye with total production of 100 000 , 18 150 and 16 500 tons/year (Table 2.2). However, Turkiye has 210 000 tons of ending-stocks for 2000 crop (Sabır et al., 2000). Whilst, the ending-stocks could be used in production of added-value products, 140 000 tons of the stock were allocated to oil crushing and sold with a price much lower than its economical value.

The hazelnut production is wide spread to 500 000 hectares area mainly in steep East Black Sea Region and beside its economical value the hazelnut trees protects the earth from erosion (Özdemir et al., 1998b). Eight billion people earn income from production and processing of hazelnut and Turkiye recieves one billion USD as

export income (Anon., 1995, Akdağ and Öztürk, 1993; Külünkoğlu, 1996).

Turkiye exports 76.5% of total hazelnut production and 73.5% of the export is as shelled hazelnut (Sabır et al., 2000). The high amount of shelled hazelnut export is an evidence of lack of processing ability of added value hazelnut products in Turkiye. The amounts of exported hazelnut and hazelnut products are given in Table 2.3 and the various forms along with descriptions, functional qualities and suggested applications of hazelnut products is listed in Table 2.5.

The hazelnut exportation of Turkiye to different countries are given in Table 2.4 where Germany is the first with 38% of the total export. The domestic consumption of hazelnut in Germany, Switzerland and Austria is quite high with the amounts of over 1500 g where it is only 350 g per person for Turkiye (Özdemir and Devres, 1999). Turkiye can reduce its ending stocks either cutting the hazelnut trees or by increasing the added value and the quality of hazelnut and its products which will also provide an advantage for exportation incomes.

Table 2.2. Major hazelnut producing countries, their production, supply and distribution (Anon, 2000).

Origin	Carry out	Production	Imports	Total Supply	Export	Domestic consumption	Ending Stocks
			(Tons, in-shell basis)				
Turkiye							
1996/97	40 000	500 000	0	540 000	383 000	52 000	105 000
1997/98	105 000	515 000	0	620 000	440 000	50 000	130 000
1998/99	130 000	625 000	0	755 000	420 000	180 000	155 000
1999/00	285 000	550 000	-	835 000	440 000	140 000	255 000
2000/01	150 000	550 000	-	700 000	420 000	70 000	210 000
Italy							
1996/97	50 000	95 000	41 847	186 847	76 649	109 198	1 000
1997/98	1 000	87 000	45 000	133 000	27 000	103 000	3 000
1998/99	3 000	130 000	35 000	168 000	53 000	113 000	2 000
1999/00	4 000	100 000	50 000	154 000	38 000	114 000	2 000
2000/01	3 000	100 000	50 000	153 000	45 000	106 000	2 000
US							
1996/97	4.788	16.783	9.772	31.343	16.612	14.264	467
1997/98	467	42 640	10 765	53 872	16 084	26 788	1 000
1998/99	1 000	13 154	16 000	30 154	8 000	22 154	0
1999/00	109	34 000	12 000	46 309	23 000	23 000	309
2000/01	8 000	18 150	12 600	38 750	11 600	24 200	2 950
Spain							
1996/97	5 100	6 500	12 300	23 900	7 900	15 000	1 000
1997/98	1 000	18 900	7 000	26 900	10 400	16 000	500
1998/99	500	8 000	12 500	21 000	6 000	15 000	0
1999/00	-	16 000	6 000	22 000	7 000	14 000	1 000
2000/01	10 000	16 500	10 000	36 500	14 000	16 000	6 500

Table 2.3.The ratio of exported hazelnut and products in Turkiye (Anon, 1995).

Hazelnut Products	Export Ratio (%)
Shelled	73.5
Roasted	18.1
Meal	3.2
Puree	2.5
Paste	0.5
Hazelnut oil	1.9
Unshelled	0.3

Table 2.4. Hazelnut Exportation of Turkiye to different countries (Amount:Ton, Value: 1000 US$) (Anon., 2000).

Country	1996		1997		1998	
	Amount	Value	Amount	Value	Amount	Value
Germany	248 033	85 110	372 831	77 369	324 516	81 526
Italy	21 188	64 927	20 380	88 878	21 346	86 494
France	15 812	49 378	15 199	68 665	20 274	87 103
England	9 304	27 782	8 718	40 502	8 910	39 553
The Netherlands	11 530	33 915	8 377	38 852	9 688	42 552
Bel - Luxembourg	9 933	27 666	7 474	32 632	11 108	45 817
Switzerland	9 266	26 938	9 717	42 628	9 207	38 728
Austria	4 532	12 981	3 344	14 338	4 459	18 309
USA	4 166	13 823	5 643	19 933	5 549	21 762
Spain	2 756	8 498	3 990	12 751	5 654	19 342
Greece	1 992	6 162	4 930	13 100	4 153	11 761
Sweden	2 372	6 956	2 216	10 943	2 115	9 948
Russia	2 380	6 930	2 196	10 379	2 050	9 779
Australia	1 982	4 178	1 489	5 989	1 484	6 101
TOTAL	193 533	608 096	198 980	855 105	205 137	847 073

Table 2.5. Forms of hazelnut along with descriptions, functional qualities and suggested applications (Woodroof, 1975).

	Available Forms & Description		Function	Category Applications
NATURAL Shell removed, brown skin intact	Whole	Whole nut with skin	Garnishing premium foods	Bakery, Candy, Snacks
	Diced	Three Sizes:	Good distribution of nut for uniform flavour texture and appearance	Bakery, Cereals, Candy, Breads, Snacks
	Sliced	Whole nut thinly sliced lengthwise	Nut recognition, good contrast for flavour and texture	Garnish, Low-Calorie Entrees or Toppings, Breads, Cereals, Snacks
	Meal	Finely ground; free flowing	Flour replacer, binding agent, flavouring agent	Bakery, Breads, Fillings, Snacks, Health-oriented Foods
ROASTED Brown skin is removed	Whole	Dry roasted	Intensifies flavour and crisp texture	Candy, Bakery, Snacks
	Diced	Same as Natural	Stronger flavour, darker colour and crisper texture	Dairy, Snacks, Sauces, Bakery
	Meal	Finely ground; free flowing	Flour replacer, binding agent, flavouring agent	Bakery, Breads, Fillings, Snacks, Health-oriented Foods
HAZELNUT PASTE	Sweetened mixture of ground hazelnuts and sugar; spreadable but grainy		Adds body, sweetness, flavor and moisture	Bakery fillings & Toppings, Candy
HAZELNUT BUTTER Smooth or Chunky	Finely ground nuts with consistency similar to natural peanut butter		Adds flavor, richness and increases protein content, replaces animal fat	Candy, Bakery, Entrees, Sauces, Icings, Fillings

3. STORAGE STABILITY OF HAZELNUTS

3.1. Effect of processing conditions on storage stability of hazelnuts

The hazelnut is harvested with a moisture content which can reach 25% and dried in order to reduce its moisture to a safe storage level (Hadorn et al., 1977; Lopez et al., 1997a) and keep it during the storage until processed. The influence of the drying conditions on the oxidation state of the lipid fraction in hazelnut was studied by Lopez et al. (1997a, b). Different hazelnut quality parameters were determined in unshelled and shelled hazelnuts dried under 30, 40, 50, 60, 70 and 80°C. It was observed that hydrolytic reactions affect dried hazelnut, increasing with drying temperature. Also, drying temperature higher than 50°C favour an increase in the rate of lipid oxidation in hazelnut, especially in shelled hazelnuts, with a decrease in the oxidation stability. Because of this, it was suggested to dry hazelnuts lower than 50°C (Lopez, 1997a).

Hadorn et al. (1977), suggested to dry the hazelnut kernel even lower than 5% moisture level to prevent the microbial growth and biochemical spoilage reactions and it is pointed out that lower levels may impart additional protection. It was suggested that ERH should not exceed 65% during storage of natural hazelnuts (Hadorn et al., 1977). Hence, above 70% equilibrium relative humidity (ERH) moulds grow and enzymes can become active between 60 and 70% ERH. After drying it has been suggested that the hazelnuts should be stored whole in the country of origin because shells offer a certain amount of protection (Hadorn and Zürcher, 1977). Hazelnuts are crushed and the shells are removed after a demand and exported as shelled. Because, first of all the transportation costs would be considerably high since the weight of the shell is about equal to that of the kernel and the second; natural hazelnut is more economical than the hazelnut products with added value. The shelled hazelnuts are processed (sliced, ground, chopped etc.) or roasted prior to purchase and transported as rapid as possible since the shelf life of

the processed hazelnuts are lower than the natural whole hazelnuts (Woodroof, 1975). The FFA of fresh hazelnuts is usually less than 0.3%, values above 0.7% indicate the start of the deterioration (Keme et al., 1983). Hadorn et al. (1977) reported that values of FFA more than 1% indicated an old or already deteriorated produce. It has been determined by Hadorn et al. (1977) that shelled hazelnuts keeps their quality at least one year at cool and dry conditions (8-10°C and 50-60%RH) having the same FFA value (0.4-0.7%) with whole kernels. However, at humid and warm conditions (20-22°C and 70-80%RH) shelled hazelnuts found to be deteriorated having a FFA value 1.4-16.3 while the whole kernels kept much better with FFA value of 0.4-1.8. The similar results were obtained for the other quality parameters such as oxidation value, induction time, flavour index and diene extinction value. The adverse effect of increasing moisture content and temperature was reported by Evranuz (2001) for unblanched salted roasted peanuts. It has been also observed for storage of raw peanuts that at 4°C might be safe for 5-6 months before quality loss begins, however did not inhibit its progress once the oxidative mechanism is initiated (Angelo and Ory, 1975).

If the hazelnuts contact with soil during harvest, dry insufficiently and keep at humid conditions, aflatoxins are formed due to mould growth. Aflatoxin is one of the major problem in hazelnut and hazelnut products, mainly in natural hazelnut meal. Aflatoxin is a kind of mycotoxin produced by mould at high relative humidity conditions greater than 85% (Denizel et al., 1976). It is the most potent naturally occurring carcinogen known and subjected to many studies in nuts (Sanchis et al., 1988; King et al.,, 1983; Yanniotis and Zarmboutis, 1996; Heperkan et al., 1994; Eke and Göktan, 1987 and Topal and Aran, 1987). Mehlenbacher et al. (1993) has reported that mould growth is the most serious problem that greatly shortens the shelf life of hazelnuts. Mould contamination and growth starts on the tree and increase during harvesting, processing and storage (Bullerman et al., 1984). Sun-drying which is a generally used method practised by the smallholders. This method, however is not safe because of the uncertain weather conditions (Özdemir et al., 1998b). Uncontrolled environmental conditions and the length of time involved during the drying leads to microbiological contamination and growth. Rainy weather conditions during the harvest and post-harvest treatments enhances the microbial growth (King Jr., 1983). Besides aflatoxin formation, mould activity also changes nutritional

value, texture, colour, flavour and increases the free fatty acids (Topal and Aran, 1987). It is reported that the Turkish hazelnut varieties are more susceptible to moulds than the other major hazelnut varieties in the world (Mehlenbacher et al.,, 1993). *Penicillium, Aspergillus, Mucor, Fusarium* and *Rhizospus* species are isolated from natural and roasted hazelnuts (Sanchis et al., 1988; Eke and Göktan, 1987). It has been reported by Pluyer et al. (1987) that the aflatoxin B1 in peanuts could be destroyed 30 to 45% by oven-roasting at 150°C for 30 min or microwave-roasting for 8.5 min at 0.7 kW. Since the mould growth and consequently formation of aflatoxin and also the amount of produced aflatoxins are reduced by heat treatment, the problem gains more importance for natural hazelnut meal. Although it is reported that peanut meal, containing aflatoxin B1, was detoxified 97% using horseradish peroxidase with hydrogen peroxide and then microwaves (Chitrangada and Mishra, 2000), the world export market has been aimed aflatoxin-free commodities by the end of 2000 and later annihilate the foods when aflatoxin is detected (Paster et al. 1992). The total aflatoxin (B1, B2, G1 and G2) limit decreased from 10 ppb to 4 ppb and from 4 to 2 ppb for B1 aflatoxin (Majerus et al., 1989).

The effect of roasting on hazelnut quality was studied by (Grosch et al., 1983; Metwalli et al., 1983; Lopez, 1997a and Saklar, 1999). The peroxide value for unroasted hazelnut and peanut kernels was found to be lower than roasted kernels and did not changed during a certain period of storage (Angelo and Ory, 1975; Grosch et al., 1983). Roasted nuts which are consumed as an appetiser and as raw materials in chocolate, confectionery, bakery, etc. are more susceptible to lipid oxidation than raw nuts (Sanders et al., 1993). The hydroperoxide formation in roasted hazelnuts increased during storage while the hydroperoxide formation of unroasted kernels remained unchanged during 16 weeks storage (Grosch et al., 1983). The shelf-lives of unblanced roasted peanuts have been found shorter than those roasted after blanching (Shewfelt and Young, 1977). It has been suggested by Angelo and Ory (1975) and Evranuz (1993) that roasted peanuts should be stored under nitrogen or vacuum, since refrigeration would not retard oxidation in the presence of air.

3.1.1. Hazelnut Meal Production

Hazelnut meal is finely ground; free flowing meal and functions as flour replacer,

binding and flavouring agent which can be used mainly in bakery, breads, fillings, snacks and health oriented foods. Hazelnut meal can be produced from natural or roasted hazelnuts. According to Hazelnut Meal Turkish Standards (TS-10937, 1993) hazelnut meal is defined as the product ground from shelled hazelnut (TS-3075, 1978) or hazelnuts which are processed according to techniques indicated in TS-1917 (1993). Hazelnut meal is marketed as 3 types; natural, blanched and roasted and classified as I. and II. class according to damaged and undamaged hazelnuts. The appearance, taste and flavour of the hazelnut meal should be unique and rancidity or unpleasant flavour should not be detected. There should be no living or dead insect and any other foreign matter. The particle size of the hazelnut meal should be reduced such that the percentage of the particles should not exceed 5% when the meal is sieved through 3.15mm mesh size. The aflatoxin levels should be below 5 ppb for aflatoxin B_1 and 10 ppb for the total (B_1+B_2+G_1+G_2). The total quantity of coliform bacteria is allowed max. 10 cfu/g and it is not allowed to contain Fecal coli, Salmonella or *S. Aureus*. The chemical and typical properties of the hazelnut meal are given in Table 3.1and Table 3.2 (TS-10937, 1993).

Table 3.1. The chemical properties of hazelnut meal according to classes (TS-10937, 1993).

Properties	Limits			
	I. Class		II.Class	
	Fresh	Old	Fresh	Old
Free fatty acids (as oleic acid in extracted fat)	1.0	1.4	1.3	1.5
Peroxide Value (meq g/kg)	7	9	8	10

The main problem in hazelnut meal is increase in free fatty acidity in natural type and FFA is the most common indicator of product stability used by the hazelnut industry. The quality decline in natural hazelnut meal is more rapid than blanched type. Any damage or pressure done on the surface of the kernel during shelling or transport causes oil to be squeezed out of the kernel and provides the substrate for the enzyme (Keme et al., 1983). It also happens to whole kernel during meal production. The cell structure is damaged during grinding process so that the lipid fraction

contacts easily with the enzyme (Keme et al., 1983). Beside this, raw ground hazelnut meal is more susceptible to aflatoxin contamination, increase in FFA than roasted hazelnuts (Sanchis et al., 1988).

Table 3.2. The typical properties of hazelnut meal (TS-10937, 1993).

Properties	Limits		
	Natural Hazelnut Meal	Blanched Hazelnut Meal	Roasted Hazelnut Meal
Sensory			
• Colour	Pale Brown (the same colour as the inside part of natural hazelnut)	Pale Yellow	Dark Yellow
• Flavour	Unique	Unique	Unique, free from burned smell and taste
Moisture	Max. 6% (w/w)	Max. 5% (w/w)	Max. 3% (w/w)
Microbiological			
• Total mesophylic aerobic bacteria	Max. 5000 cfu/g	Max. 2000 cfu/g	Max. 2000 cfu/g
• Total Mould and Yeast	Max. 100 cfu/g	Max. 50 cfu/g	Max. 50 cfu/g

3.2. Factors affecting storage stability of hazelnuts

Like any food, the hazelnuts are susceptible to quality loss during processing and storage. If high quality in the final products is to be retained, special attention has to be devoted to optimal processing and storage conditions (Angelo et al., 1979). Storage stability can be improved either using processing techniques or using appropriate storage conditions. The main problem affecting the quality of nuts and their products is rancidity. Hazelnut, like any oil rich food, gets rancid due to lipid hydrolysis and subsequent lipid oxidation. The spoilage mechanism of hazelnut is given in Figure 3.1. The spoilage of hazelnut kernels proceeds parallel with an increase in free fatty acids in lipids with a conjugated diene-system in hydroperoxides (Grosch et al., 1983). Once the lipid hydrolysis is initiated, quality declines rapidly: the liberated free fatty acids form substrate for the oxidation reaction. The hydroperoxides formed either by oxidation or hydrolysis is than undergo reaction by the effect of peroxidase, relative humidity and temperature

which results in formation of rancid and bitter products such as saturated, unsaturated di- and epoxy aldehydes, ketons, lactons, furans, alcohols, monobasics, dibasics, oxo & hydroxy acids, saturated, unsaturated hydrocardons (Ragnarsson and Labuza, 1977; Hadorn and Zürcher, 1977 and Sanders et al., 1993).

It is known that the process is facilitated or catalyzed by many factors, such as light, oxygen, heat, water activity, micro-organisms, metal ions, free fatty acids and enzymes (Angelo et al., 1979). The chemical as well as enzymatic reactions participate in the spoilage of hazelnuts are shown in Figure 3.1. The hydroperoxides formed during lipid oxidation are themselves starting products of autoxidation and are tasteless. Therefore the rancid taste may not be detected although hydroperoxides are in detectable quantity. The decomposition products of the fat hydroperoxides account for the rancid taste (Sturm, 1979). The main actors are, however, enzymes and temperature. On the other hand, shelf life of hazelnuts vary with respect to cultivars and affected by several factors such as unsaturated fatty acids (oleic, linoleic and linolenic acids), minerals, the quantities of natural antioxidants in the kernel (Vitamin E) or pellicle (phenolics) (Mehlenbacher, 1993).

3.2.1. Minerals

The transition metals such as cobalt, copper, iron and manganese are major proxidants in lipid systems. Copper and iron catalyse the oxidation of unsaturated lipids, increasing the rate of oxidation . They readily transfer electrons making them useful cofactors for reactive oxygen species which do not effectively initiate lipid oxidation (Labuza, 1971 and Sanders et al., 1993).

3.2.2. Fatty Acid Composition

The unsaturated fatty acid content of the hazelnut makes it a nutritional product but also makes it more susceptible to oxidation. Fatty acid content in oilseeds vary according to species, cultivars, geographical origin, climate and maturity. The rates of oxidation of fatty acids are approximately 1:10:100:200 for stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids. The relative concentrations of these fatty acids greatly influences the oxidation rates, flavour deterioration and shelf life

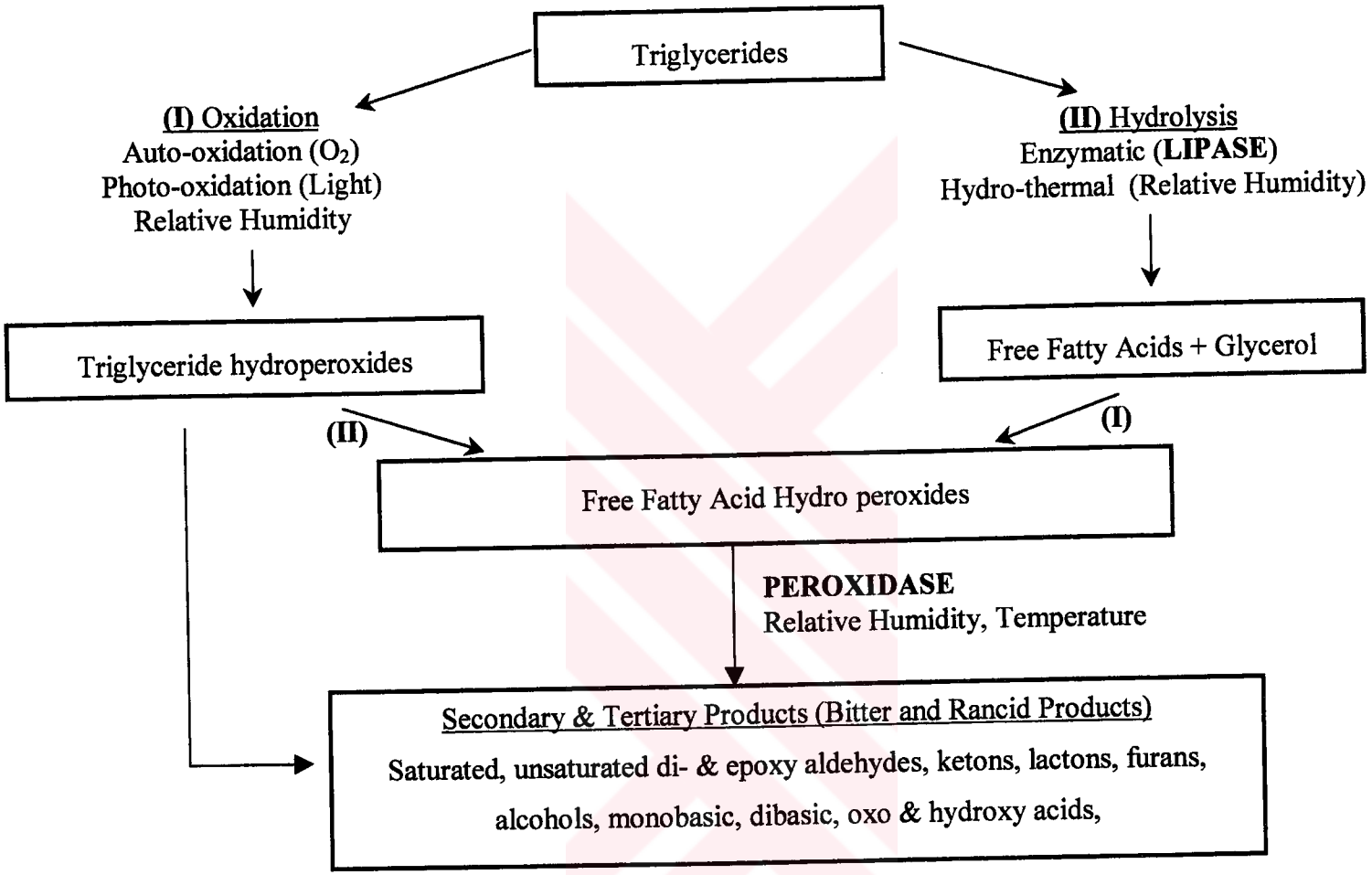


Figure 3.1. Rancidity mechanism in hazelnuts (Modified according to Ragnarsson and Labuza, 1977; Zürcher & Hadorn, 1976 and Sanders et al., 1993).

of nuts. O'Keefe et al. (1993) have compared the oxidative stability of high and normal oleic peanut oils. It was reported that the highest unsaturation exhibited the least peroxidation. Similarly almond oil has the highest stability probably due to the higher linoleic acid/oleic acid ratio and tocopherols (0.14 and 20 mg/100 g) which is similar to hazelnut oil (0.14 and 21 mg/ 100 g)) (Beringer and Dompert, 1976 and Sattar et al., 1989).

3.2.3. Tocopherols

Tocopherols occur naturally in nuts and retard off-flavour development by neutralising free radicals produced by oxidation of unsaturated lipids. Greater the stability of the hazelnut oil was explained by the higher levels of tocopherol. It has been reported by Beringer and Dompert (1976) that the tocopherol is α-tocopherol in hazelnut and almond. They have determined that Vitamin E activity of α-tocopherol was 7 times higher than γ-tocopherol and hazelnut and almond oil had the highest Vitamin E. However, the γ-tocopherol has been found the possible factor contributing to the higher oxidative stability since it has been shown to have the higher antioxidant capacity than α-tocopherol (Dougherty, 1988). Pershern et al. (1995) has reported that γ-tocopherol was in trace amounts in domestic cultivars whereas the wild type *C.cornuta* had 8.55 mg/100 g. A significant relation has been found between γ-tocopherol and oxidative stability of oils in hazelnuts grown in New Zealand (Savage et al., 1997).

3.2.4. Oxygen

Oxygen concentration is one of the most important environmental factors affecting lipid oxidation. The rate of oxidation is independent of oxygen concentration at very high oxygen partial pressure while it is proportional to oxygen concentration at low levels. (Labuza, 1971). Low-oxygen packaging is used to increase the shelf life of nuts (Sanders et al., 1975).

Fatty acid oxidation of pistachio nuts were examined under various atmospheric conditions (air and CO_2) and different temperatures (10, 20 and 30°C) by Maskan and Karataş (1998). High stability was determined during storage of pistachio nuts at 98% CO_2 concentration. Mate et al. (1996) has studied the effect of relative

humidity and oxygen concentration on peanut and walnut rancidity. It was concluded that oxidative rancidity could be controlled by decreasing the oxygen concentration surrounding the roasted peanuts and walnuts. However, the effect of exclusion of oxygen was found to be minimal for blanched unroasted peanuts. Nitrogen flushing or oxygen barrier packaging to increase the shelf life was suggested for roasted peanuts and walnuts (Mate et al., 1996).

The storage of hazelnuts under nitrogen has been studied by Keme et al. (1983) and they have reported that autoxidation and hydrolysis of fats due to the enzymes occur much more slowly under the exclusion of oxygen. They have compared the shelf life of hazelnuts stored under normal, cool storage conditions (3-6°C, 50-60% RH) and warm nitrogen atmosphere (18-25°C, 99.5% N_2) and assessed that hazelnuts can be stored during prolonged periods of time without loss of quality in both cases. It has been found interesting by Keme et al. (1983) that the higher FFA values were determined for the storage under a nitrogen atmosphere. They have proven as Hadorn et al. (1977 and 1978) that the splitting of fatty acids from lipids were enzyme-based. As it is known lipid splitting enzymes; lipases and esterases are oxygen-independent and influenced by the moisture content. The enzymatic splitting of free fatty acids by lipoxygenase and peroxidase is oxygen dependent. That means that when storing under oxygen the fatty acids are oxidised to hydroperoxidiene acids which break into short chain carbonyl compounds (Ory et al., 1984). This second stage cannot take place under nitrogen. It was also found to be interesting that storage in nitrogen under pressure produces the highest FFA-values. It was explained by Keme et al. (1983) that the cell structure was damaged by the high pressure to such an extent that the enzyme/substrate contact was increased.

3.2.5. Light

Light has been known to play an important role in the oxidative stability of edible oils and milk fat (Sattar et al., 1976). It has been observed by Sattar et al. (1976) and Sattar et al. (1989) that light damages the quality and stability of nut oils by inducing the oxidation. A decrease in oxidation rate with increasing wavelength was observed in the oils of rape seed, corn, soybean, coconut and it was pointed out that susceptibility of these oils to photo-oxidation did not depend on the degree of unsaturation alone. It was also reported that natural antioxidants such as tocopherol

has no effect against photochemical oxidation of different oils and fats. Peroxidation was reduced 6.5-12.75 times by the elimination of light when the nut oils from almond, peanut, pinenut and walnut were stored for more than 6 weeks.

3.2.6. Temperature

Although it was observed by many researchers that increase in temperature cause an increase in lipid oxidation in foods (Labuza, 1971) it was concluded that the control of relative humidity during storage was found to be more effective than the control of temperature (Hadorn et al., 1977). It was reported by Labuza (1982) that 20°C drop in temperature increased the shelf life of fried nuts two or threefold. The effect of temperature and moisture on lipid oxidation of unblanched salted roasted peanuts were examined by Evranuz (1993) and it has been determined that shelf life is doubled when the storage temperature was reduced by 15°C. Evranuz (1993) has concluded that although the lower temperatures were beneficial, control of storage temperature to increase shelf life was not practical and economic.

On the other hand, it is found to be that oxidative stability was more temperature dependent than oxygen (Maskan and Karataş, 1998). Fatty acid oxidation of pistachio nuts were examined at different temperatures (10, 20 and 30°C) by Maskan and Karataş (1998) and high stability was determined storage at 10°C at monolayer value.

As mentioned before, shelled hazelnuts keeps their quality for one tear or more at cool conditions (5-10°C) while deterioration begins after 32 weeks at warm (20-22°C) conditions (Hadorn et al., 1977). It has been observed that storage of raw peanuts at 4°C might be safe for 5-6 months before quality loss begins, however did not inhibit its progress once the oxidative mechanism is initiated (Angelo and Ory, 1975).

Beside this, the influence of temperature on an enzyme catalysed reaction vary according to foods and the enzyme itself. As the temperature increase the enzyme activity increase in such a way that the reaction rate is approximately double for every 10°C rise in temperature and on reaching a certain critical temperature the enzyme molecule begins to denature corresponding an activation energy of 48 kJ/mol approximately. The activation and denaturation parts of the activity vs. temperature

curve can be expressed as a straight line in Arrhenius plot (Svensson, 1977). During the storage of hazelnuts the activation of the enzymes are considered while denaturation part is take into account for the inactivation of the enzymes.

3.2.7. Moisture Content & Water Activity

It has been reported by many researchers that moisture content and water activity (a_w) are very important parameters during processing and storage of the nuts (Hadorn et al., 1977; Sanders et al., 1993; Ali et al., 1996; Lopez et al., 1997a,b). Thus, water affects reaction rates of oxidative and hydrolytic rancidity. At low water activity values which is in the region of adsorption of water on specific sites in the food (below the BET monolayer value) enzymatic reactions occur extremely slowly or cease entirely. This lack of enzyme activity is due to the lack of mobility for the substrate to diffuse to the active sites of the enzymes or lack of water as a reactant. However, the mobilising activity of water rather than its availability as a reactant is the critical factor in limiting enzyme action as in the case of lipases. It is reported ther liquid triglyserides undergo enzymatic hydrolysis at a much higher rate than solid triglyserides. It is known that lipid oxidation occurs rapidly at low a_w levels as low as 0-0.1. As a_w increased up to 0.5 the rate decreases and further increase in a_w beyond 0.5 results an increase in lipid oxidation rate. High lipid oxidation rate at low a_w levels is attributed to hydration of metal catalysts decreasing their effectiveness and hydrogen bonding of peroxides, thus slowing the chain reaction of autoxidation. Cause of increased oxidation at high a_w levels is due to catalyst mobility becoming better as the liquid volume increased and exposing new catalyst sites because of extensive swelling (Labuza et al., 1971).

Evranuz (1993) has showed that the shelf life decreases near or above 2.13% moisture content (BET monolayer value) that corresponds to a water activity of 0.3 (30%ERH) and increases with further increase in moisture content for roasted peanuts. Mate et al. (1996) observed the same effect by observing the rancidity in peanuts and walnuts at 21 and 53%RH. They have reported that lowering the water content of below the monolayer value (from 30% to 21% ERH) affected lipid oxidation much more than increasing the water content above the monolayer value (from 30% to 53% ERH). The contrast results were obtained for walnuts and it was explained that, an enzymatic reaction has took place in the lipid oxidation. The

acceleration of chemical and enzymatic reactions at water levels above monolayer level was found to be more important than the reduction due to the protection provided by the monolayer (Mate et al., 1996).

Maskan and Karataş (1998) has observed that there is not a significant difference between storage temperatures (10, 20 and 30°C) both for air and CO_2 storage conditions which substantiates the theory proved by Evranuz (1993) and Mate et al. (1996). Lipid oxidation and hydrolysis reactions are minimum at or near to the monolayer of water and maximum quality retention will be achieved at the monolayer (Labuza, 1971, Evranuz, 1993, Mate et al., 1996, Maskan and Karataş, 1998).

Effect of moisture content on storage stability of ground nut seed was studied by Ali et al. (1996) and the highest free fatty acid content was determined in groundnut seed with the highest moisture content (10.4%). It was explained that lipase became active at this moisture level. It was also confirmed by Lopez et al. (1997b) that lipase activity was higher in dried shelled hazelnuts than in dried unshelled hazelnuts due to the higher water activity of shelled hazelnuts. It is concluded that to reduce lipase activity during storage, moisture content of stored oilseeds should be as low as possible (Banu & Serban, 1970).

Lipid oxidation in flours of Brazil-nut and macadamia nut in relation to a_w was studied by Prado-Filho (1994). The enzymes of the nut flours were inactivated at 110°C for 2 hours and the peroxide values were determined as well as in flour without inactivation at different water activity. It was observed that at low a_w values between 0.51-0.75 the oxidation by oxygen is the effective mechanism for lipid deterioration where as at higher a_w values (0.79 and 0.81) the enzymatic activity of lipoxygenase is responsible for the lipid deterioration due to the greater mobility of the reagents (Prado-Filho, 1994).

3.2.8. Enzymes

The reaction products of enzyme-catalysed lipid oxidation are the major contributors to the production of off-flavours in raw and roasted nuts. These reaction products have a major effect on shelf life and quality of raw nuts. Understanding the action and the mechanism of enzymes responsible for the rancidity in nuts is therefore essential for designing and developing means to control the activity of these enzymes

to retain as much as possible the flavour and storage characteristics of nuts and nut products (Sanders et al., 1993).

Purr (1971), Zürcher and Hadorm (1976), Keme and Messerli (1976),. Hadorn et al., 1977, Grosh et al. (1983), Metwalli et al. (1983), Pershern et al. (1995), Bonvehi and Raosua (1996) and Lopez et al., 1997b have been studied on enzymatic activity related with rancidity in hazelnuts. The existence of lipase (EC 3.1.1.3), lipoxygenase (EC 1.13.11.12), polyphenol oxydase (EC 1.14.18.1) and peroxidase (EC 1.11.1.6) were detected in hazelnuts according to these studies. It is reported that lipase, lipoxygenase and peroxidase take part in spoilage process of cereal fats and Zürcher & Hadorn (1976) have found parallels between reaction mechanism suggested for cereal fats and reaction taking place in hazelnuts in the course of storage. The existence of lipoxygenase system is proven in cereals, soybean and peanut by many researchers (Pattee et al., 1971 and Vetrimani et al., 1992). Lipoxygenase catalyses the hydroperoxidation of free or esterified polyunsaturated fatty acids containing cis,cis-1,4-pentadiene system utilising molecular oxygen and producing 9- or 13-cis, trans-hydroperoxides, which generally decompose into acids, ketones and aldehydes with smaller carbon chains (Pattee et al., 1985). These compounds are responsible for the characteristic odour of the oxidised fatty products (Wong, 1995 and Whitaker, 1994) and increase during storage according to the storage conditions (Pattee et al., 1971).

Zürcher and Hadorn (1976) has observed lipoxygenase activity in hazelnuts which no longer had a satisfactory flavour. Pershern et al. (1995) has studied the factors influencing lipid oxidation in three cultivars of *Corylus avellana* (cv. Barcelona, Ennis, Tonda Gentile Delle Longe) and wild hazelnut *Corylus cornuta* which were held five months in common storage until shipment and 4°C until analysis. They have found that lipoxygenase activity levels varied inversely with shelf life. However, Keme and Messerli (1976) and Bonvehi and Rosua (1996) could not able to detect lipoxygenase activity in hazelnuts from Tarragona, Italy and Turkiye (cv. Tombul).

Zürcher and Hadorn (1976) have measured the activities of peroxidase, lipase, esterase and phenolase qualitatively and indicated the amount of activity as "- : no reaction", "(+) : trace", "+: positive", "++ : highly positive" and "+++ : very highly positive". They have reported that enzyme activity differed according to province

and shelf life.

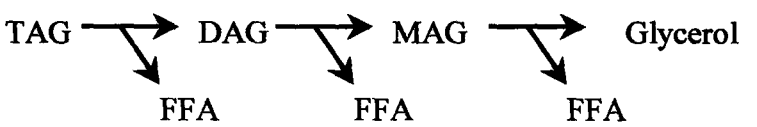
The enzymes esterase and lipase were detected in hazelnuts and found on the surface of hazelnut kernels and esterase in addition on the surface between the cotyledons. Esterase could be detected after roasting but observed to effect the quality of hazelnuts minimally. Polyphenol oxidase was found only in the hazelnuts with lignified inner walls (brown centres). It was reported that polyphenol oxidase could not be detected four months after harvesting and lipase activity was reduced after increased length of storage (Keme and Messerli, 1976). Whilst, it has been reported that peroxidase activity increased up to the 20^{th} week of storage and then remained constant. During post-harvest moist storage at 20°C for nine weeks, organellar and cytoplasmic peroxidase activity of hazelnuts increased 8 and 14 fold compared to the roughly constant level of activity at 5 °C (Gosling and Ross, 1981).

3.3. Properties and Reaction Mechanism of Lipase and Peroxidase

3.3.1. Lipase

Lipase is a single enzyme numbered as triacylglycerol acylhydrolase E.C. 3.1.1.3. Lipases catalyses the hydrolysis of triacylglycerol that gives free fatty acids which can cause soapy and rancid off-flavours. The triacylglycerol lipase generally catalyses the hydrolysis at the 1- and 3-positions of the triglyceride in the presence of C_8-C_{12} fatty acids although some higher plant and microbial lipase may release fatty acids from all three positions. It was found that the acid lipase has equal capacity to hydrolyse tri-, di- and mono-glycrides whereas the alkaline lipase can break only monoglyceride (Muto and Beevers, 1974).

The enzyme has a very wide range of properties, depending on its source, with respect to positional specificity, fatty acid specificity, pH optimum, thermostability. The general triacylglycerol hydrolysis reaction catalysed by lipases can be written as follows (Beisson et al., 2000):



Lipases are found in most organisms from the microbial, plant and animal kingdom. Commercially available lipases are usually derived from micro-organisms and an increasing number of lipases is being manufactured from recombinant bacteria and yeast. Commercial lipases are used in oleochemistry (soaps and fatty acids, fats with improved spreadability, cocoa-butter equivalents, highly digestive triglycerides, monoglycerides), detergents, paper manufacture and dairy industry (Schmid and Verger, 1998). On the other hand, especially psychrotrophic lipases often plays an important role in the spoilage of dairy products and therefore, heat is applied to inactivate these heat resistant enzymes (Owasu et al., 1992).

Since lipase catalyses the hydrolysis of triglycerides at the water/oil boundary the activity of lipase depends on moisture content and water activity (Barman, 1969, Schmid and Verger, 1998), but seems to be independent of oxygen availability (Keme et al., 1983 and Purr, 1971). Keme et al. (1983) has observed that lipase and esterase were both oxygen-independent and split off fatty acids from lipids was due to the enzymes also during the storage under nitrogen atmosphere. They have also concluded that the reaction was mainly influenced by moisture content.

Hydrolysis may occur at reduced water activity, which results in development of off-flavours associated with hydrolytic rancidity such as soapiness and sourness (Labuza, 1971). FFAs are formed as a result of lipase activity. Beside their direct action of releasing the fatty acids, lipases contribute indirectly to the oxidative rancidity: both chemical oxidation and enzymatic oxidation by lipoxygenase and peroxidase are made possible (Naesens, 1982).

Although lipases are usually not active in the resting and intact seeds, their action will pose potential problems in damaged or infected cotyledons (Sanders et al., 1993). Any damage or pressure done on the surface of the kernel during shelling, transport and processing causes oil to be squeezed out of the kernel and provides the substrate for lipase (Keme and Meserli. 1976).

Keme and Meserli (1976) mentioned that lipase content of a seed depended on the degree of maturity and the age of the seed: the higher the maturity the lower the activity. As a contrast, Henderson and Osborn (1991) has reported that in fruits of the oil palm, the lipase activity increases as ripening proceeds.

As with other enzymes lipases has its own optimal pH ranging from acid to neutral to alkaline. A lipase from *Lactobasillus plantarum* had pH optimum around 9.3 and optimum temperature at 37°C (Andersen et al., 1995). Peanuts were reported to have alkaline lipase (Sanders and Pattee, 1975). Purr (1971) has listed the optimum pH of several microbial, plant and animal lipases from different references, which lied in the area between 7.0 to 8.6 with the exception lipase from *Candida lipolytica* with the lowest optima with 5.5 and milk lipase had the highest with 9.0 (Purr, 1971). He has reported that lipase of hazelnut had 7.5 pH optimum but it was mentioned that this value was not proved. Huang and Moreau (1978) studied the two iso-lipase systems, one acidic, one alkaline, in six different oil seeds. They found that only castor bean had detectable levels both acidic and alkaline lipases. In none of the other oil seeds studied (peanut, sunflower, cucumber, cotton, corn and tomato cotyledons) the acidic lipase activity could be detected: the alkaline lipase activity dominated in the peak of gluconeogenesis during germination. The maximum lipase activity was found to be pH 9 for peanut, cucumber, sunflower, cotton and tomato cotyledons and corn scutella. Only castor bean endosperm showed maximum activity at pH 5.

Thermostability of lipase also varies considerably with their origins. Animal and plant lipases usually are less stable than microbial extracellular lipases. Purr (1971) has reported that lipases formed by micro-organisms had in general its activities optimum in temperatures between 20 and 40°C, animal lipase, like pancreas-lipase had optimum temperature between 35-37°C.

3.3.2. Peroxidase

Peroxidases are ubiquitous, iron containing enzymes which oxidise phenolic compounds and related substances using activated oxygen released from hydrogen peroxide or organic peroxides. These newly formed activated oxygen species can then react with cellular components such as proteins and lipids. In addition, the reaction of peroxidase with hydrogen peroxide leads to the formation of a porphyrin radical intermediate that can react with lipids to induce peroxidation products (Sanders et al., 1993). The reaction catalyzed by peroxidase is simply given below :

$$< \text{Donor} + H_2O_2 \leftrightarrow \text{Oxidized Donor} + 2\,H_2O >$$

Peroxidases can exist in different oxidation states and catalyse at least three different type of oxidation through a number of separate but often inter-linked chemical reactions. Peroxidase can catalyse peroxidatic, oxidatic and hydroxylation reactions. Peroxidase from horseradish has been studied extensively and the results of the studies served to get the knowledge of molecular structure, mode of action of many other peroxidases present in fruits and vegetables. It has been mentioned by Sessa and Andersson (1981) that peroxidases have been implicated in fatty acid oxidation and caused breakdown of lipid hydroperoxides. Off-flavour development due to the action of peroxidase was associated with the oxidising effect of on indigenous lipids and the phenolic constituents of the foods (Sessa and Anderson, 1981). Nevertheless, the precise role and physiological functions of peroxidases in living plants and post harvest products are still not fully understood (Robinson, 1991).

Active peroxidases are known to contribute to deteriorative changes in flavour, colour, texture and nutrition of raw and processed foods (Svenson, 1977). Another aspect to be considered is that heat denatured inactive peroxidase can either expose heme groups or mobilise them so that they are more readily available to catalyse lipid oxidation (Eriksson and Vallentine, 1973). The oxidatic reaction is much slower than the peroxidatic reaction and is a result of formation of radicals in the reaction in the presence of oxygen; therefore, peroxidase may also promote lipid oxidation with consequent off-flavour formation (Whitaker, 1994).

The observed post-harvest changes are hard to relate to specific reaction mechanisms of peroxidase activity. Much of the difficulty in understanding the effects of peroxidase activity in foods is due to the very broad type of action of this enzyme, catalysing a large number of bio-conversion. Among the deteriorative roles, it has been reported that peroxidase was involved in the regulation of plant growth and development and more specifically in metabolic activities concerned with germination metabolic activities. It has been suggested that peroxidase activity could be involved in the ripening and senescence of hazelnuts, and could play an important part in the off-flavour production (Hadorn et al. 1977; Robinson, 1991 and Rodriguez-Lopez et al., 2000,). However, peroxidase seemed to have no influence on the taste of hazelnuts as such (Hadorn et al., 1977).

Thermal inactivation of peroxidases has been studied for many years because of the

action of the enzymes causes quality loss during food preservation. The residual enzymatic activity of peroxidase is used to indicate the effectiveness of blanching treatments in fruits and vegetables since there is a high correlation between off flavour development in frozen fruits and vegetables and the residual peroxidase activity (Robinson, 1991).

It has been reported by Robinson (1991) that the rates of inactivation and reactivation of peroxidase activity to be dependent on many factors such as temperature, time of blanching, pH value and sodium chloride concentration. It is assumed in most studies on the thermal inactivation of peroxidase that the inactivation mechanism follows first order reaction kinetics. However many examples of the activity against heating time plots show non-linearity especially beyond the point where more than 50% of peroxidase activity has been destroyed (Robinson, 1991). The deviation from the linearity is explained by the presence of a mixture of heat-stable and heat-labile isoperoxidases (Ling and Lund, 1978; Naveh et al. , 1982, Guenes and Bayindirli, 1993, and Yemenicioğlu et al., 1998). A method was proposed to analyse kinetics of thermal inactivation of enzyme systems which consist of two groups differing in their thermal stability. The activation energies determined for peroxidases from different sources are given in Table 3.3.

On the other hand, lipid oxidation activities of regenerated and denatured peroxidase from horse radish was studied by Adams et al. (1996) and it was concluded that the lipid oxidation activity of horse radish peroxidase was associated with denatured forms rather than regenerated, active peroxidase. It was explained that the denatured peroxidase comprised three haemproteins where two of them possessed most of the lipid oxidation activity (Adams et al., 1996 and Eriksson and Vallentine, 1973).

3.4. Prevention of Rancidity in Hazelnuts by Enzyme Inactivation

The presence of lipolytic enzymes in hazelnut has been attributed to their poor keeping quality. Hence, inactivation of these enzymes in nuts was found necessary to increase the economic value for use in food products such as bakery, chocolate or as a source of oil (Adams, 1991; Angelo et al., 1979; Branch et al., 1987; Charalambous, 1993 and Svenson, 1977).

Table 3.3. Kinetic parameters for peroxidase based on assumption : 1st order inactivation rate.

Source-Enzyme	T, °C	Heat-labile		Heat-stabile		Reference
		k, min^{-1}	E_a, kJ/mol	k, min^{-1}	E_a, kJ/mol	
Horseradish	76.7	1.1	141.4	0.04	88.3	Ling & Lund (1978)
	82.2	2.0		0.06		
	87.2	4.6		0.08		
Corn-	100	8.9	88	0.23	75	Naveh et al. (1982)
Pinto Beans	55	19.6	195	127.7	157	Yemenicioğlu et al., (1998)
	60	67.5		137.9		
	65	103.3		839.5		
	70	281.0		2977.4		

During processing of foods, generally heat treatment, is widely used to inactivate enzymes that cause deterioration of the food during storage. Several studies have been reported on stabilisation to extend shelf-life by inactivation of enzymes through heat treatment. Samant and Rege (1990) have analysed cashew nut for lipase and other enzymes that may influence storage stability. Cheshew nut showed low activity for lipase and lipoxygenase and roasting the cheshew nut reduced lipase and other enzyme activities drastically (Samant and Rege, 1990). Inactivation of lipase, peroxidase or lipoxygenase, by heating for example has been shown to increase the shelf life stability of raw and roasted nuts (Lopez et al., 1997b; Metwalli et al., 1983; Mitchell and Malphrus, 1977; Ponne et al., 1996; Sanders et al., 1993; Senter et al., 1984a,b; Svensson, 1977 and Vetrimani et al., 1992;).

3.4.1. Inactivation of Lipase by Heating

Inactivation of lipase by heat treatment will prevent the lipase catalysed hydrolysis of glycerol esters thereby indirectly preventing the rancidity. Inactivation kinetics of lipase from different sources have been studied by many researchers and kinetic

parameters for lipase based on first order inactivation rate are given in Table 3.4. (Andersson et al., 1981; Kermasha et al., 1993; Owasu et al., 1992; Ponne et al., 1996; Swaisgood and Bozoğlu, 1984 and Vercet et al., 1997) where the thermostabilities of lipase from different sources differ considerably. Lipase from *Pseudomanas flourescens* commonly found in raw milk have been found to be partially inactivated at 100°C for 10 min and cheese made from milks containing high amounts of thease organisms developed rancidity. Lipase inactivation in milk was subjected to many researches since it was reported that although products might be commercially sterile, deterioration due to the surviving lipase activity might still occur (Andersson et al., 1981). Different denaturation states were observed for lipase from *P. flourescens* and it was contributed to the different intermediates having different degrees of molecular folding such as different degree of stabilisation of enzyme by calcium ions (Sawaisgood and Bozoğlu, 1984). No temperature inactivation was detected up to 50°C for lipase from *Candida rugosa* (Gordillo et al., 1995). However it was determined that the lipase of the palm oil could be inactivated irreversibly at 45°C for 10 h or at 55°C for 30 min in intact fruit (Henderson and Osborne, 1991).

A heat-stable and a heat-sensitive rape seed lipase species were determined where the heat-sensitive lipase was completely inactivated at 100°C and heat-stable enzyme seemed to denature only at temperatures above 100°C (Ponne et al., 1996).

Zürcher and Hadorn (1976) has studied the effect of different heat treatments on lipase, peroxidase, esterase and phenolase activity of hazelnuts. Enzyme inactivation was studied using roaster (35 min at 95-100°C and 125-128°C and 45 min at 125-128°C) and microwave heating for 1 to 4 min. It was observed that increasing the period of treatment decreased the enzyme activity. No enzymatic activity was observed after 45 min at 125-128°C and 4 min treatment using microwave. However FFA content was found to be higher than the control sample for 125-128°C treated samples and roasted flavour could be detected. Beside this, the lipase activity were decreased considerably for the hazelnut samples heated at 95-100°C and the FFA value were lower than the control sample and lighter roasted flavour. On the other hand, it was observed that after 3min roasted flavour could be noticed for microwave treatment and lipase was inactivated partially (Zürcher and Hadorn, 1976).

Table 3.4. Kinetic parameters for lipase based on first order inactivation rate.

Source of lipase	T °C	k, min^{-1}	E_a, kJ/mol	Method	Reference
P.flourescence in Nutrient Broth	100 120 140 160	0.048 0.192 0.642 1.920	83	Simple Heating	Andersson et al. (1981)
In Skim Milk	100 120 140 160	0.096 0.318 1.152 3.288	79		
P. Flourescence MC50 (extracellular)	60 70 80 90	0.612 1.105 1.393 2.524	44.77	Circulating oil bath	Swaisgood and Bozoğlu (1984)
	100 110 120 130 140 150	0.101 0.178 0.302 0.495 1.900 2.941	91.21		
P. Flourescence P38 [b]	40-60	Ns[a]	172	Low Temp. Heating	Owasu et al. (1992)
	90-140		84	Ultra High Temp.	
Wheat germ	60 70 90	0.216 0.810 3.440	87	Simple heating	Kermasha et al. (1993)
	60 70 90	0.354 3.690 10.560	106	Microwave heating	
Rape Seed [c] – extract	45-75[d]	316.2 (k_1) 27.6 (k_2)	150	Static heating	Ponne et al. (1996)
Lipase in seed	75-90[d]	5.76 (k_1) 7.44 (k_2)	49		

a : Not shown, b: partially purified, c: (k_1) and (k_2) indicates the presence of two enzymes with different thermostabilities, d: T_{ref}=90°C

The activities of the enzymes lipase, peroxidase and polyphenol oxidase were measured before and after drying under 30, 50 and 70°C and the results indicated that lipase was the most stable enzyme to heat and peroxidase was more resistant than polyphenoloxidase. High residual lipase activity was observed in hazelnuts dried up to 50°C which pointed out that lipid hydrolysis can take place in dried shelled hazelnuts. For peroxidase lower residual values were obtained and it was concluded that lipid oxidation catalysed by peroxidase were negligible (Lopez et al., 1997b). It was also observed by Grosch et al. (1983) that lipase activity in hazelnuts was considerably reduced after roasting at 119-129°C for app. 15 min while no peroxidase activity was detected after roasting.

Metwalli et al. (1983) have studied the effect of roasting on lipase activity and organoleptic quality of almonds and hazelnuts (*Corylus americana*) at 140-180°C conditions for several periods. They have found that lipase activity of hazelnuts showed a gradual decrease with increasing roasting time and temperature. The inactivation data were analysed using the thermal destruction time (TDT) concept and z value (temperature required to reduce the TDT ten-fold). Thermal destruction curves of lipase showed that z-value was 53°C and F-value was 18 min at 160°C for hazelnut. The hazelnut lipase was found to be less heat stabile than lipase from almond. It was pointed out that the time for optimum roasting was much higher than the F-value for lipase enzyme for each treatment. Thus, it was concluded that roasting these nuts could lead an increase in the shelf-life of the roasted hazelnuts and almonds (Metwalli et al., 1983).

Ekstrand et al., 1992 have studied the pH dependence and heat inactivation of lipase activity in oats. It was found that the activity was higher at neutral and alkaline pH conditions. Additionally, importance of air humidity in the inactivation of the oat lipase was emphasised and it was concluded that direct contact with water caused a much more effective heat transfer and more inactivation than dry heating. The enzyme activities after steam treatment yielded total inactivation while lipase activity were detectable for dry heat treatment. In conclusion, lipase inactivation studies in model laboratory conditions showed the importance of good heat transport into the sample, not only the use of high temperature. (Ekstrand et al., 1992).

Vetrimani and Haridas-Rao (1990) studied the inactivation of lipase to improve the

shelf-life of bran by toasting at 175°C for 40 min. Toasting decreased activity of lipase by 40%, of lipoxygenase by 100%. The fat acidity was negligible in heat stabilised bran when compared to raw bran. Although the peroxide value was higher for the toasted samples during the storage period, untoasted raw bran developed a rancid taste within 20 days, whereas a rancid flavour became perceptible in toasted bran only after 90 days (Vetrimani and Haridas-Rao, 1990).

3.4.2. Microwave Treatment

Microwave heating is used to inactivate lipase, lipoxygenase and peroxidase for stabilisation of cereal germ and bran, soybean and soy flakes, groundnut, pecan and rape seeds (Boyes et al., 1997; Jiaxun et al., 1993; Kermasha et al., 1991 and 1993; Klingler, 1994; List et al., 1990; Möller et al., 1994; Pour-el et al., 1981; Ramesh et al., 1995; Senter et al., 1984a,b and Vetrimani et al, 1992). The experimental studies showed that microwave heating was an effective method for the inactivation of lipase and the other enzymes. However Pour-el et al. (1981) concluded that peroxidase was more resistant to microwave than lipoxygenase. Lipase and lipoxygenase activities in groundnut decreased with increasing periods of microwave treatment; resulting in inactivation of 78% and 35% for 300s at 750 W microwave treated samples respectively (Ramesh et al., 1995).

Zürcher and Hadorn (1976) have studied the effect of microwave treatment on hazelnut enzymes lipase, peroxidase and polyphenol oxidase. A microwave treatment was applied to whole and ground kernels at 1400 watt with the irradiation interval of 23 cm. It was reported thet ground hazelnut required longer treatment to complete inactivation and enzymes of the whole kernels were inactivatied after 3-4 min giving a roasted taste. Lipase and esterase was found to be resistant to microwave heating.

Vetrimani et al. (1992) has studied the inactivation of lipase and lipoxygenase in cereal bran, germ and soybean by microwave. Since, lipase contributes to the quality loss in the oil extracted from these foods by increasing the FFA and making them in edible. Inactivation of lipolytic enzymes resulted in complete inactivation of lipoxygenase after 180s treatment in microwave for all samples. Whilst 50% – 75% of reduction was recorded for lipase activity in wheat bran, wheat germ, rice bran and soybean after 180s treatment. Storage of the microwave treated samples showed the effectiveness of the treatment in inactivating the enzymes and thereby increasing

the keeping quality of edible oils extracted from these foods.

Inactivation of enzymes by microwave was suggested to be more effective than conventional and mix mode (conventional and microwave together). Despite the mentioned 'non-thermal' effect of microwave, the mechanism and the effect of microwave treatment on storage stability of the food need more research to be explained (Kermasha et al., 1993).

Möller et al. (1994) studied the inactivation of rape seed lipase by microwave and steam heating and concluded that there was no significant difference between microwave and steam heating treatments. However, scanning electron microscopy results indicated that microwave heating caused explosion of oil droplets which might effect enzyme-substrate interaction and may lead the quality loss during storage. Beside this, Ponne et al. (1996) has pointed out that microwave heating caused a slightly higher FFA content in rape seed oil than did steam heating.

3.4.3. Chemical, Mechanical and Other Treatments

It is suggested that metal ions such as Fe^{3+} and Ni^{2+} could be used commercially to inhibit lipase hydrolysis of rice bran triglycerides and thus contribute to the improved quality of edible rice bran oils (Munshi et al., 1993) . The positive effect of H_2O_2 pre-treatment to increase the oxidative stability of peanuts was observed by Evranuz (2001) which was theorised that the oxidation of Fe^{2+} to Fe^{3+} in the presence of H_2O_2 made the enzymes less effective.

Lipase was completely inactivated in extracts of germinating peanuts occurred in the media containing inorganic phosphate in concentrations above 10mM. However, the phosphate inhibition was found to be ineffective for lipase from castor, cotton and pumkin seeds (Jacks and Yatsu, 1974). Lipase in oat flour was studied to inhibit lipolysis in the final product. The optimum pH for hydrolysis was approximately 7 and it was concluded that even slight pH adjustments into the alkaline range will reduce lipolysis (Liukonnen et al., 1995).

Inactivation of heat-resistant lipase from *Pseudomanas flourescens* by monothermosonication was studied by Vercet et al. (1997). It was concluded that inactivation by both heat and monothermosonication followed first order reaction kinetics and monothermosonication was more effective than heat treatment alone.

Effect of supercritical CO_2 treatment on enzyme inactivation was studied by several researchers (Dunford and Temelli, 1996; Endo et al., 1995; Ishikawa et al., 1995 and 1996). The native structure of lipase from *R.. japonicus* was lost and the enzyme in the solution was completely inactivated by treatment at 35°C and 15 MPa for 30 min (Ishikawa et al., 1995 and 1996). Effect of pulsed electric field which is a non-thermal food preservation technique have been investigated as a method for inactivation of enzymes and it is found to reduce enzyme activities of lipase by 70-85% and peroxidase activities by 30-40% (Ho et al.,1997). High pressure is an another method suggested to inactivate enzymes (peroxidase, lipase and lipoxygenase) (Hendrickx et al., 1998).

4. MATERIALS AND METHODS

4.1. Materials

Hazelnuts (cv. Tombul) were harvested in 1999 and supplied by Giresun Hazelnut Research Institute. Kernels were stored at 4 °C until crushed. After shelling, hazelnuts were sieved using a 12mm mesh sieve, vacuum packed in polyethylene films with a water vapour permeability of 5.37g/m^2.day.atm (90±2% RH, 38±1°C) and stored at -20°C until they were analysed. The component analysis was in accordance with AOAC (1990).

4.2. Reagents

ABTS (A-1888) (2,2-azino-bis-(3-ethylbenz-tiazoline-6-sulphonic acid)), H_2O_2 (30%), MBTH (M 8006) (3-methyl-2-benzothiazolinone hydrazone) and linoleic acid (L-1376) were purchased from Sigma Chemical Co. (The Netherlands). Triton X-114 was obtained from Fluka (The Netherlands). All other reagents (potassium phosphate, sodium phosphate, sodium acetate, Tris-HCl, ammonium sulphate, hydrogen peroxide, acetonitrile, butyryl chloride, 2,4-dinitrophenol, triethylamine, dichloromethane) were of analytical grade. For dialysing experiments, Spectrapor membrane (molecular weight cut-off = 3,500 daltons) was used. 2,4-Dinitrophenyl butyrate (DNPB) was synthesised according to Mosmuller et al. (1992). Reagents used for protein determination was BSA from Sigma (P-0914) and Coomassie Plus Protein Assay Reagent from Pierce.

4.3. Experimental Set-up

The experimental set up for enzyme activity and shelf life studies are given in Figure 4.1 and Figure 4.2 respectively. The hazelnut samples were separated into

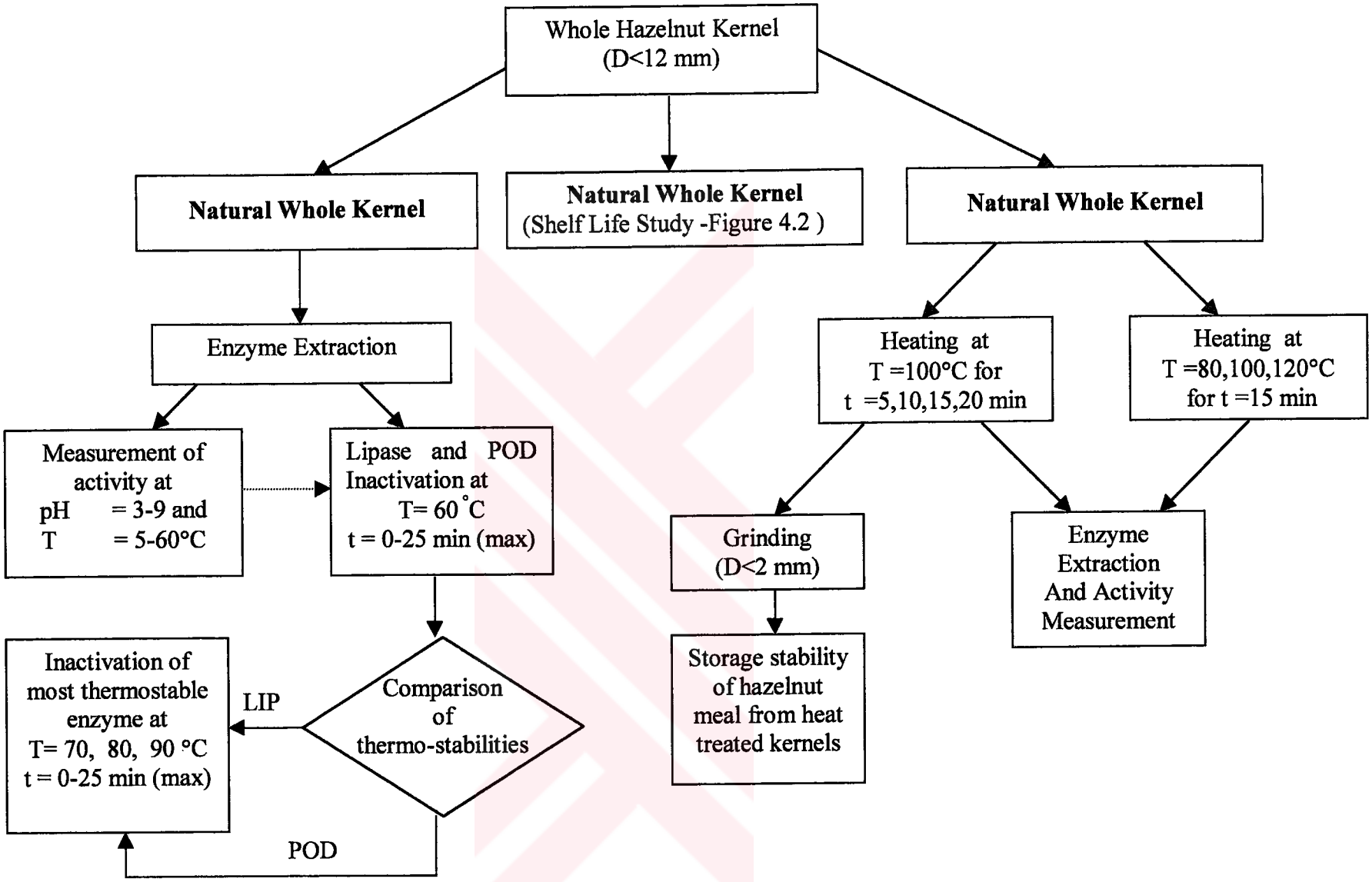


Figure 4.1. Shematic diagram of experimental set-up for enzyme activity and inactivation studies.

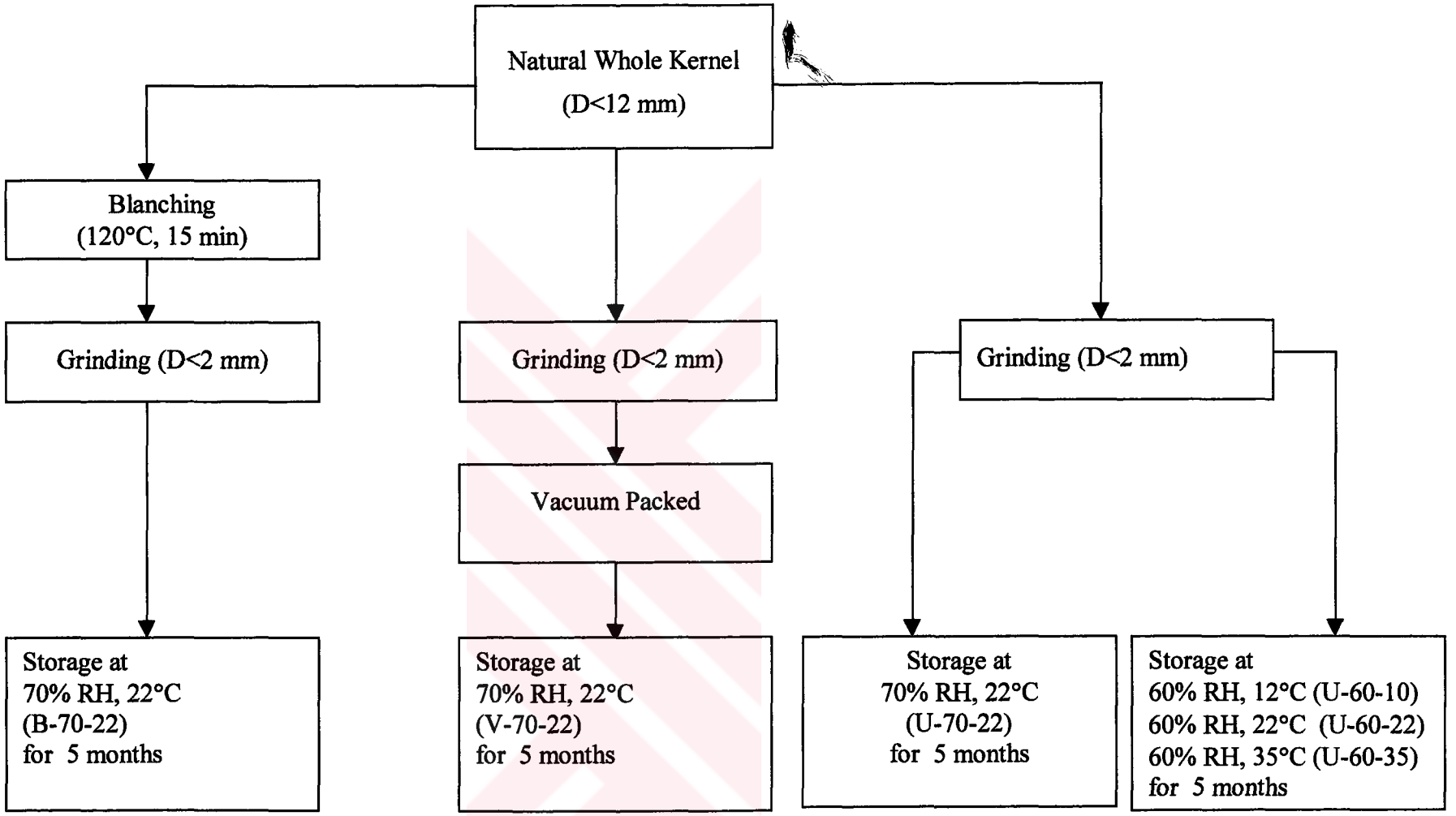


Figure 4.2. Shematic diagram of shelf life study for hazelnut meal; blanced and natural at different storage conditions.

three portions. First portion was used for the;

- Determination of the optimum conditions for those enzymes; lipase and peroxidase, and measurement of the activities at 4.5pH as a function of temperature (5-60°C) and at 25°C as a function of pH (3-9),
- Comparison of the thermostabilities of lipase and peroxidase at 60°C to determine the most thermostable enzyme,
- Inactivation kinetics of the most stable enzyme at higher temperatures (70, 80, 90°C).

The second portion was used for;

- Determination of the effect of time and temperature on the enzyme inactivation in whole hazelnut kernel.
- Determination of effect of thermal treatment on shelf life stability of hazelnut meal.

The third portion (Figure 4.2) was used for;

- Determination of effect of blanching and different storage conditions on hazelnut meal stability.

The experimental procedures are given in detail in the below sections.

4.3.1. Temperature and pH Dependence of Lipase and Peroxidase

The lipase and peroxidase, extracted using the same procedure explained in 4.4. were tested for their pH and temperature dependence. The temperature was controlled between 5-60 °C with 5°C intervals, to develop temperature profile with a circulating water bath (Julabo) with a heater/cooler and checked using a precision of ±0,1°C.

The buffer systems employed to develop pH curve were all 0.1M and consisted of acetate buffer (pH 3.0-5.5), phosphate buffer (pH 5.8-7.2) and Tris-HCl (pH 7.2-9.0). The study to determine the effect of pH on the enzymes and other activity measurents was conducted at 25 °C.

4.3.2. Comparison of Thermostabilities of Lipase and Peroxidase

The enzyme extracts of 4 ml lipase and peroxidase was deeped in 60°C water bath (Julabo, ±0,1°C) until reaching to 60°C. The 500µl of aliqouts were removed at 0, 1, 2, 3, 4 ,... 28 minutes and immediately cooled in ice bath. The aliqouts were transferred into eppendorf tubes and stored at -30°C until activity measurements according to the assay conditions mentioned above.The activity of both enzymes were measured at 37±1°C and expressed as nkatal (formation of amount of product – nmol/sec).

4.3.3. Thermal Inactivation Kinetics of Lipase in Hazelnut Enzyme Extract

Three sets of lipase extracts were separately used for inactivation kinetics for different temperatures (70, 80, and 90°C). Different initial activities were measured from each extraction process. Each lipase extract were heated in thin (5 mm diameter) test tubes in a water bath at 70, 80 and 90°C. 800 µl of samples were taken at selected times up to 20 min, immediately cooled in ice bath, transferred into eppendorf tubes and stored at -30°C until measurement of lipase activity. The lipase activity of each time-temperature treated samples were measured at room temperature (23-25°C) according to the assay conditions mentioned above and expressed as nkatal.

4.3.4. Effect of Temperature on Lipase in Hazelnut Kernel

200g of raw hazelnut with 6.36% moisture content (wet basis) and 0.77 water activity were heated in forced air pilot scale roaster (73 cm x 205 cm x 161 cm, Pasilac, APV, UK) at 80°, 100° and 120°C for 10 minutes. Hazelnut samples were held in mono-layer in a rectangular (20 cm x 15 cm) wire mesh tray. After heating the kernels were immediately cooled to room temperature and peeling ratio was determined prior to -30°C storage until enzyme extraction. The activity of lipase were measured at room temperature (23-25°C) immediately after enzyme extraction. Activity measurements of the lipase extract were carried out in triplicate. Protein content in the extracts were determined according to 3.4. and the activities are expressed as nkat/mg protein.

4.3.5. Effect of Lipase Inactivation on Stability of Natural Hazelnut Meal

200g of natural hazelnut with 6.36% moisture content (w.b.) were heated at 100°C for 5,10, 15 and 20 minutes. The lipase was extracted from heat treated samples and the activity was measured as mentioned in 2.9.2.1. Heat treated hazelnuts were ground according to Hazelnut Meal Turkish Standards (TS 10937, 1993) and stored in environmental test chambers (Sanyo, MLR-3500HT, ±1°C, Japan) at 35°C and 60% relative humidity conditions for 1 month. Free fatty acids, peroxide value, moisture content and peeling ratio values were measured before and after 1 month of storage.

4.3.6. Effect of Processing and Storage Conditions on Stability of Hazelnut Meal.

Change in free fatty acids and moisture contents of hazelnut meals from natural and blanched kernels were determined at different storage conditions. Schematic diagram of the experimental set-up is given in Figure 4.2. Hazelnut meals from blanched and un-blanched hazelnuts were stored at different conditions in environmental test chambers (Sanyo, MLR-3500HT, Japan) for 5 months. Blanching were conducted at 120°C for 15 minutes. Blanched, un-blanched and vacuum packed, un-blanched samples were stored at 70%RH, 22°C. In addition, un-blanched hazelnut meal were stored at 60% relative humidity at 12 and 35°C. The summary of the storage conditions for different hazelnut samples are given in Table 4.1. Free fatty acids, peroxide values and moisture contents were measured with 15 days intervals during 6 months of storage.

4.4. Enzyme Extraction

The enzyme extract was obtained according to the method of Espín et al. (1997) with some modifications. Kernel homogenisation, centrifugation and dialysis were carried out at 4 °C. 60g of hazelnut kernel kept at -20 °C were homogenised with 200 ml of cold 0.1M phosphate buffer pH 7.25 containing 20 mM EDTA and 2% TX-114, for 2 min in a blender at low speed (Waring). The homogenate was kept at 4 °C for 60 min before being centrifuged at 28,500xg for 30min. The supernatant was collected and used as crude enzyme extract. It was then subjected to temperature-induced phase

partitioning by warming to 35°C for 15min. This solution was centrifuged at 7,500xg for 15min at 25 °C. The detergent rich phase was discarded and the supernatant was acidified to pH 4.5 with acetic acid. The solution was centrifuged at 28500 g for 20 min. The supernatant brought to 25% saturation with solid ammonium sulphate under continuous stirring at 4°C. After 20min solution was centrifuged at 28,500xg for 20min and the pellet discarded. Additional ammonium sulphate was added to the clear supernatant to give 80% saturation, and the resultant mixture stirred for 20 min at 4 °C. Enzymes from hazelnut were extracted by ammonium sulphate precipitation and then dialysis overnight. Ammonium sulphate precipitation offered advantage of preventing turbidity in the reaction mixture during the spectrophotometric measurements. The solution was then centrifuged at 28500 g for 20min and the precipitate dissolved in a minimal volume of 0.1M sodium phosphate buffer pH 7.25. The enzyme solution was dialysed overnight vs. 4 L of distilled water. The enzyme extract was stored at -30 °C without loss of the original activity after 1 month. The protein content of the enzyme extracts were determined after each extraction except inactivation studies conducted at 60, 70, 80 and 90°C. The inactivation kinetic studies did not necessitate to determine protein contents for each data point since the data sets were analyzed separately.

Table 4.1. Hazelnut Samples and conditions for shelf life study

Hazelnut Sample	T, °C	%Relative Humidity	Initial Moisture Content	Symbol
Blanched	22	70	3.40	B-70-22
Unblanched-vacuum packed	22	70	6.36	V-70-22
Unblanched	22	70	6.36	U-70-22
Unblanched	12	60	6.36	U-60-12
Unblanched	22	60	6.36	U-60-22
Unblanched	35	60	6.36	U-60-35

The purification yield of the enzymes could not be estimated because of the turbidity of the assay mixture during the activity measurements and protein determination at each purification step.

4.5. Protein Determination in the Enzyme Extracts

Protein content was determined by using the method of Bradford (1976) using BSA as standard. BSA solution (0.1μg/1μl solution) was mixed with water in 1 ml plastic spectrophotometer quvettes as indicated in Table 4.2 and 500 μl Pierce Reagent was added to make volume 1 ml. The solution was mixed using a Vortex and let stand for 10 min. The absorbance of the mixture was read at 595 nm. The calibration curve were drawn using the measured absorbance data. The enzyme extract was diluted to be able to read the absorbance within the range for calibration curve using 10 μl diluted enzyme extract and 490 μl of water and 500μl Pierce Reagent. The absorbance was read as indicated above and the mg protein in the enzyme extract were calculated using the value read from calibration curve. During the inactivation kinetic studies it is not necessary to determine protein contents for each data point since the data sets were analysed separately. The protein content of the extracts used for the pH and temperature dependence and inactivation studies and the details of the protein determination are given in Appendix A.

Table 4.2. The amounts of the reagents and mixtures used for protein determination in enzyme extracts.

BSA (μl)	0	10	20	30	40	50	60	70	80	90	100
Distilled Water (μl)	500	490	480	470	460	450	440	430	420	410	400
Pierce Reagent (μl)	500	500	500	500	500	500	500	500	500	500	500

4.6. Enzyme assays

The spectrophotometric assays were recorded in an ultraviolet-visible Perkin-Elmer Lambda-2 spectrophotometer (Überlingen, Germany), on-line interfaced to a Pentium-100 microcomputer (Ede, Netherlands) or by UV-1601, Shimadzu Spectrophotometer (Kyoto, Japan). Experiments were performed at least in triplicate.

4.6.1. Peroxidase Activity

Peroxidase activity was assayed spectrophotometrically at 414 nm using ABTS as described by Arnao et al. (1996). The assay medium was 50mM buffer, 10mM ABTS and 2mM H_2O_2 solutions in 1 ml of total volume. ABTS and H_2O_2 solutions were prepared daily with oxygen free distilled water, kept under N_2 gas and covered with aluminium foil to avoid light effect during analysis.

The activity was calculated using the equation as described by Beer's law :

$Abs = \varepsilon.c.l$

where Abs is the absorbance of the sample at the wavelength, c is the concentration in the molarity units and l is the path length (1 cm) of sample the light beam traverses and ε is an intrinsic constant of the molecule, known as the extinction coefficient or molar absorbtivity (Copeland, 1996). The oxidation of ABTS was followed by observing the increase in absorbance at 414nm ($\varepsilon_{414}=3.11x10^4\ M^{-1}cm^{-1}$). One unit of the enzyme is defined as katal (consumption of mol of substrate/sec) and the activity is given as nkat/mg protein.

4.6.2. Lipase Activity

Lipase activity was measured according to a colorimetric method by recording the change in absorbance at 350nm with DNPB as substrate (Mosmuller et al., 1992). Assay mixtures of DNPB were prepared by adding 50µl of a 50mM stock solution of DNPB in acetonitrile (1mM final concentration) into 2.45 ml of 50 mM buffer, pH 4.5 kept at room temperature. To ensure complete emulsification of the substrate the mixture was sonified for 30 sec with a Sonics VC-300 sonifier (set at level 6 and pulser on 50%), using a microprobe. 2.5 ml of the sonified substrate mixture were mixed with 0.1 ml of enzyme extract.

The activity was calculated using the same procedure as followed for peroxidase. The hydrolysis of DNPB was followed by observing the increase in absorbance at 360nm ($\varepsilon_{360}=1.48x10^4M^{-1}cm^{-1}$). One unit of the enzyme activity is defined as nkatal (consumption of nmol of substrate/sec) and the activity is given as nkat/mg protein for pH and temperature dependence of lipase and the inactivation studies of lipase in

hazelnut kernel. Activity of lipase was expressed in nkatal for inactivation kinetic studies.

4.7. Chemical and Physical Analysis

Moisture content and peeling ratio of the samples were obtained just after the treatment. Moisture content of the samples were determined by drying in an oven at 103°C for 4 h according to AOAC (1990). Peeling ratio was determined by counting visually the 95% peeled kernels out of 100 kernels. Samples of hazelnut from heat treated and storage experiments were kept at -20°C until analysis. Oil was obtained from hazelnut samples by pressing with a lab-scale press prior to analysis. After pressing the oil, samples were analysed immediately for free fatty acids (Ab 5-49) and peroxide value (Cd 8-53) according to AOAC (1990). The analysis to determine the composition of the hazelnut at initial conditions are carried out according to AOAC (1990). All the analysis were carried out in triplicate.

4.8. Model Development

4.8.1. Effect of Temperature

Use of temperature to control rates of enzyme-catalyzed reactions is quite important. Storage at low temperatures decrease the effect of enzyme, while increase in temperature till the so-called "optimum temperature" causes an increase in reaction rate but at higher temperatures there is a decrease in rate. Effect of temperature on stability of an enzyme may be determined by incubation the enzyme solution at various temperatures (at both activating as well as denaturating temperatures) while the other conditions are kept constant (Whitaker, 1994).

Enzyme activity is generally measured as the amount of some specific substrate converted per unit time. This activity, as observed by activity measurements, is a combination of a true concentration of the enzyme, multiplied by its specific reaction rate constant. Assuming that a first order rate process is characterised by a rate of disappearance of reactant and specific rate constant increases with increasing temperature according to Arrhenius law the Equation 4.1 can be solved as explained

below. At higher temperatures the enzyme starts to denaturate (second reaction Equaition 4.1), thereby effectively decreasing the amount or concentration of the active enzyme configuration. Eventually, the enzyme loses its activity entirely. The rate constant of that part of the enzyme, that is still active, however, continues to increase with temperature. This principle has been used, verified and calibrated for a number of enzymes in a variety of products (Ponne et al., 1996; Tijskens et al. 1997a, Tijskens et al., 1997b, Tijskens et al., 1997c, Tijskens et al., 1998, Tijskens et al., 1999a and Tijskens et al., 1999b).

The fundamental assumptions in the development of a model on enzyme activity as a function of temperature are:

$$S + En \xrightarrow{k_s} P + En$$
$$En \xrightarrow{k_d} En_{na} \qquad \text{(Equation 4.1)}$$

where ;

[S] (mol/L) : Concentration of substrate
[En] (mol/L) : True enzyme concentration
[P] (mol/L) : Concentration of product
$[En_{na}]$ (mol/L) : Denatured enzyme concentration
$k_s(sec^{-1})$: Rate constant substrate conversion
k_d $(mol/L)^{-1}*(sec)^{-1}$: Rate constant denaturation.

This system is worked out and applied to phytase by Tijskens et al. (2001).

From this simplified mechanism, a set of differential equations can be derived based on the rules of chemical kinetics where t(sec) is time:

$$\frac{\delta[En]}{\delta t} = -k_d[En]$$

$$\frac{\delta[S]}{\delta t} = -k_s[En].[S]$$

$$\frac{\delta[P]}{\delta t} = k_s[En]\cdot[S]$$

$$\frac{\delta[En_{na}]}{\delta t} = k_d[En] \qquad \text{(Equation 4.2)}$$

The general solution for this set of differential equations at constant conditions of temperature is, for the active enzyme configuration, En :

$$[En] = [En_0] \cdot e^{-k_d t} \qquad \text{(Equation 4.3)}$$

The apparent activity Act (nkat, as measured in experiments) is, as already mentioned, represented by the product of actual enzyme concentration; [En] and the specific activity;k_s (Godfrey and West, 1996). This results in an expression for the activity of any enzyme at any constant temperature:

$$Act = k_s \cdot [En] = k_s \cdot [En_0] \cdot e^{-k_d t} \qquad \text{(Equation 4.4)}$$

In this equation k_s and En_0(enzyme concentration in mol/L at t=0) only appear in combination with each other. It is therefore impossible to estimate both variables at the same time. Both parameters are therefore combined in a new parameter called Act_0. This results in the final equation:

$$Act = Act_0 \, e^{-k_d t} \qquad \text{(Equation 4.5)}$$

In most instances, the variation of inactivation rate at higher temperatures higher than the temperature for maximum enzyme activity follows the emprical law of Arrhenius which allows the determination of an activation energy (Adams, 1991). It is assumed in this study that, in all these above equations, the rate constants k_d and k_s, and hence also enzyme activities; Act and Act_0, depend on temperature according to Arrhenius law:

$$k_i = k_{i,ref} \, e^{\left(\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right)} \qquad \text{(Equation 4.6)}$$

where index i refers to any of the processes and ref refers to a chosen reference temperature T_{ref}.With the combined equations 4.5 and 4.6, the activity of an enzyme can be described and predicted by Equation 4.7; as already reported (Ponne et al., 1996; Tijskens et al. 1997a, Tijskens et al., 1997b, Tijskens et al., 1997c, Tijskens et al., 1998, Tijskens et al., 1999a and Tijskens et al., 1999b).

$$Act = Act_0 \, e^{-k_{i,ref} \exp\left(\frac{Ea}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \cdot t} \qquad \text{(Equation 4.7)}$$

4.8.2. Effect of pH

The effect of acid [H^+] ions or basic [OH^-] ions on the activity of an enzyme is probably caused by a change in stereo configuration at or in the neighbourhood of the active sites (Fersht, 1984; Whitaker, 1994). As in almost all protonation reactions, these reactions will occur very fast. The different configurations are instantaneously in equilibrium. The protonated and hydroxylated enzymes are assumed to be completely inactive or at least less active. This can be represented by the following mechanisms:

$$EnH^+ \underset{K_{EH}}{\rightleftharpoons} En + H^+$$

$$EnOH^- \underset{K_{EOH}}{\rightleftharpoons} En + OH^- \qquad \text{(Equation 4.8)}$$

where;

K_{EH} (mol/L) and K_{EOH} (mol/L) are the dissociation constants of EnH^+ and $EnOH^-$ respectively .

The water dissociation (K_w; $(mol/L)^2$) is defined as:

$K_w = [H^+].[OH^-] \cong 10^{-14}$ at 25°C and

$$[OH^-] = \frac{K_w}{[H^+]} \qquad \text{(Equation 4.9)}$$

where;
[H^+] (mol/L) : Concentration of hydrogen ions
[OH^-](mol/L) : Concentration of hydroxide ions

The amount of [EnH^+] and [$EnOH^-$] can now be expressed in terms of actual amount of active enzyme and pH by :

$$[EnOH^-] = \frac{K_w.[En]}{K_{EOH}.[H^+]}$$

$$[EnH^+] = \frac{[En].[H^+]}{K_{EH}} \qquad \text{(Equation 4.10)}$$

where;

[EnH^+] (mol/L) : Concentration of enzyme-hydrogen ion complex

[$EnOH^-$] (mol/L) : Concentration of enzyme-hydroxyl ion complex

The amount of enzyme in any configuration has to remain constant :

$$[En_{tot}] = [EnH^+] + [EnOH^-] + [En] \qquad \text{(Equation 4.11)}$$

[En] (mol/L) : Concentration of enzyme
[En_{tot}] (mol/L) : Total amount of enzyme concentration

Combining equations 4.10 and 4.11 and solving for En, an expression (Equation 4.12) is obtained for the active enzyme at any [H^+] concentration (or pH):

$$[En] = \frac{[En_{tot}]}{1 + \frac{[H^+]}{K_{EH}} + \frac{K_w}{K_{EOH}} \cdot \frac{1}{[H^+]}} \qquad \text{(Equation 4.12)}$$

This model, for amount of available active enzyme configuration, can be converted into an apparent activity (Act; nkat), as for the temperature model. This result in:

$$Act = \frac{k_s[En_{tot}]}{1 + \frac{[H^+]}{K_{EH}} + \frac{K_w}{K_{EOH}} \cdot \frac{1}{[H^+]}} \qquad \text{(Equation 4.13)}$$

Again, in this equation k_s and [En_{tot}] only appear in combination with each other. It is therefore impossible to estimate both variables at the same time. Both parameters are therefore combined in a new parameter called Act_0. This results in the final equation:

$$Act = \frac{Act_0}{1 + \frac{[H^+]}{K_{EH}} + \frac{K_w}{K_{EOH}} \cdot \frac{1}{[H^+]}} \qquad \text{(Equation 4.14)}$$

The term of formulation in Equation 4.14 is separated into componenets to allow for both iso-enzymes at the same time.

$$Act = \frac{Act_{01}}{1 + \frac{[H^+]}{K_{EH1}} + \frac{K_w}{K_{EOH1}} \cdot \frac{1}{[H^+]}} + \frac{Act_{02}}{1 + \frac{[H^+]}{K_{EH2}} + \frac{K_w}{K_{EOH2}} \cdot \frac{1}{[H^+]}} \qquad \text{(Equation 4.15)}$$

Equation 4.14 and 4.15 describes how the activity of the total pool of enzyme changes with H^+ concentration, over the complete range from pH 0 up to pH 14. Whitaker (1994) and Fersht (1984) deduced this equation in a somewhat different form.

4.8.3. Inactivation Kinetics

4.8.3.1. Thermal Inactivation of Lipase

The transformation of an enzyme into an inactive species may vary from a simple, unimolecular process to a complex one involving a number of enzyme molecules. The order of the reaction may therefore be unity or may be greater or less than unity. It is important to distinguish between the order with respect to concentration and the order with respect to time. Out of four main classes of denaturation behaviour as proposed by Laidler and Bunting (1973) (in Adams, 1991) the second type is assumed to be applicable to this study. The order with recpect to concentration, as it is determined at the begining of the reaction is assumed unity and the order with respect to time is assumed to be greater than unity (Adams, 1991).

It is assumed that the native enzyme may consist of a number of iso-enzymes and the inactivation process may involve a number of simultaneous, unimolecular processes. The inactivation data sets for lipase are analyses assuming the existance of at least two iso-enzymes. However according to Ponne et al., 1996, two different inactivation mechanisms found in the lipase extract, a fair amount of lipase activity remained after heating. For this reason, three different inactivation steps were distinguished in their model :

$$Act_{lipase-1} \xrightarrow{k_1} Act_{lipase-inact}$$

$$Act_{lipase-2} \xrightarrow{k_2} Act_{lipase-inact} \qquad \text{(Equation 4.16)}$$

$$Act_{lipase-3} \xrightarrow{k_3} Act_{lipase-inact}$$

The method applied in measuring lipase activity does not discriminate between the lipases: the sum of activities can be measured :

$$Act_{tot} = Act_{lipase-1} + Act_{lipase-2} + Act_{lipase-3} \qquad \text{(Equation 4.17)}$$

Heat induced inactivation of enzymes can usually be described by a first order reaction kinetics, which results in the analytical solution shown in Equation 4.5.

Act (nkat) and t(sec) is the activity of the enzyme and inactivation time respectively. The inactivation rate k_i (sec^{-1}) is a function of temperature most probably according to Arrhenius's law (Equation 4.6), where T_{ref} is the reference temperature chosen within the range of measurements.

Based on the behaviour of the activity at test conditions only one iso-enzyme system is estimated to be active, including a fixed invariable part of the activity; Act_f . Analyses were made assuming k_2 and k_3 equal zero leading to the following model (Equation 4.18):

$$Act = Act_1 e^{-k_1 t} + Act_f \qquad \text{(Equation 4.18)}$$

In this equation Act_1 is the variable activity and Act_f is an invariable part for each data series. The initial activity is then Act_1+Act_f. Because different series had different initial activities since for each inactivation procedure at a certain temperature, new lipase extract was extracted from the hazelnuts.

4.8.3.2. Thermal Inactivation of Peroxidase

Enzyme extract with POD activity was incubated at 60 °C. Aliquots were removed after different incubation times and POD activity was assayed in the above conditions. The experimental data were fitted by non-linear regression (NLR) to the following double-exponential equation as similarly described for the lipase inactivation:

$$Act = Act_1 e^{-k_1 t} + Act_2 e^{-k_2 t} + Act_f \qquad \text{(Equation 4.19)}$$

where Act is the residual POD activity at any incubation time, the parameters Act_1 and Act_2 are the POD activities (two groups of POD iso-enzymes with different thermal stability) at time zero for the simple uniexponential equations that form the double exponential (one uniexponential equation for each POD iso-enzyme group). k_1 and k_2 are the apparent first-order rate constants, which describe the rate of inactivation of the two groups of POD iso-enzymes and t is the incubation time at 60°C.

Commonly accepted criteria for NLR, such as the value of the norm (the lower the

norm, the better the fit), was applied in the fits of experimental data to different possible equations. Moreover, the best kinetic model function that fits the experimental data will lead to the most even distribution of residuals, i.e., the differences between observed and predicted responses (residual values scattered close to 0 value) (Espín et al., 2000).

5. RESULTS AND DISCUSSION

5.1. Composition of shelled hazelnuts

In this study Turkish hazelnut (cv. Tombul) from 1999 harvest were used where the initial properties are given in Table 5.1. Hazelnuts were shelled, sieved and the ones below 12 mm mesh size were vacuum packed and stored at -20 °C until analysed.

Table 5.1. Composition of shelled hazelnuts at the beginning of the experiments.

Composition (w.b.)	Amount
Moisture, %	6.36
Protein, %	14.26
Ash, %	2.09
Total carbohydrate, %	14.29
Total fat, %	63.00
Fatty acid distribution, (% of fat)	
• Palmitic acid ($C_{16:0}$)	5.91
• Stearic acid ($C_{18:0}$)	2.44
• Oleic acid ($C_{18:1}$)	83.14
• Linoleic acid ($C_{18:2}$)	8.5
Oleic acid :linoleic acid ratio	9.78
Sucrose, g/ 100 g	2.5
Invert sugar, g/100 g	Not detected
Vitamin E, mg/100g	18.71
Peroxide value, meq/kg	0
Free fatty acid, as % oleic acid	0.17

5.2. Temperature and pH Dependence of Lipase and Peroxidase from Hazelnut

Before studying temperature and pH dependence of lipase and peroxidase extraction and assay conditions needed to be determined. For this purpose, the same enzyme extract was used for both lipase and peroxidase as was done by Bonvehi and Rosuo, 1996 and Lopez et al., 1997b. Then, enzyme activities were measured by the spectrophotometric method as suggested by Arnao et al. (1996) for peroxidase and that by Mosmuller et al. (1992) for lipase. In these methods it is necessary to work with clear enzyme extract solutions. Turbidity was reported a problem especially of oily foods such as hazelnut (Pershern et al., 1995). In this study, in order to obtain a clear extract, enzyme extraction method (Arnoa et al., 1996) was slightly modified. pH adjustment of crude extract to 4.5 prior to ammonium sulphate precipitation yielded a clearer extract. After partial purification, qualitative tests for lipase and peroxidase were conducted for confirmation of the presence of these enzymes.

5.2.1. Effect of Temperature

Temperature dependence of the enzymes were determined at 4.5pH within the range of 5-60°C. The activity of peroxidase and lipase increased with increasing temperatures up to about 40 °C, as expected. Above that temperature, peroxidase started to denature rapidly. The peroxidase and lipase activity of hazelnut (cv. Tombul) at various temperatures are shown in Figure 5.1 and Figure 5.2. For peroxidase the temperature for maximum activity is quite sharp at 40°C, for lipase, the temperature for maximum activity is very broad and ill defined, ranging from 40°C to 60°C.

There were studies in literature reporting that drying temperatures up to 70°C cause a partial denaturation in lipase activity (Lopez et al. 1997b) which is in consistent with this study. Our observations together with the literature findings indicated that the lipase type in hazelnut was quite resistant to heat. Thus, lipid hydrolysis could take place in dried hazelnuts even for the hazelnuts dried at 70°C. On the other hand, considerable decrease in peroxidase activity at the same low temperatures corresponds to negligible lipid oxidation catalysed by peroxidase (Lopez et al., 1997b). All these findings corroborate the behaviour as shown in Figure 5.2.

5.2.2. Effect of pH

Effect of pH on peroxidase and lipase activities of hazelnut (cv. Tombul) were determined at 25°C within the pH range of 3-9 and the results are shown in Figure 5.3 and Figure 5.4. The results showed that maximum activity for peroxidase and lipase was observed between 4.5-4.7 pH. The occurrence of a second relatively small peak for lipase at around 7.5pH indicated the presence of an iso-enzyme and this was roven by the model studies explained in the next section. The pH optimum found in this study was in acid range as reported for peroxidase in peanuts (Huystee et al., 1990) and lipase in castor endosperm (Altaf et al., 1997). An overview of pH for maximum activity of peroxidase and lipase for selected agricultural products is given in Table 5.2. Since, the temperature and pH dependence has not been studied for lipase and peroxidase in hazelnuts before, a direct comparison could not be made. The only reported value for optimum pH of hazelnut lipase was given as 7.5 by Purr (1971) without being proven.

Table 5.2. Overview of reported pH for maximum activity in peroxidase from agricultural products, mainly nuts.

Product	**Opt. pH**	**Reference**
		Peroxidase
Peanut	4.3	van Huystee et al. (1990)
Soybean	5.0-6.0	Chuang and Chen (1988)
Tomato	4.6-6.0	Rodríguez-López et al. (2000)
Walnut	5.5	Piffaut and Metche (1991)
Hazelnut	4.5-4.7	This study
		Lipase
Castor endosperm	4.5	Altaf et al. (1997)
Lupin seed	5.0	Sanz and Olias (1990)
Pinus edulis	4.5	Hammer and Murphy (1993)
Cereal bran, germ & soybean	7.8*	Vetrimani et al. (1992)
Oats	7.0	Ekstrand et al. (1992)
Hazelnut	7.5[a]	Purr, (1971)
	4.5-4.7	This study

[a] : Not proven datum

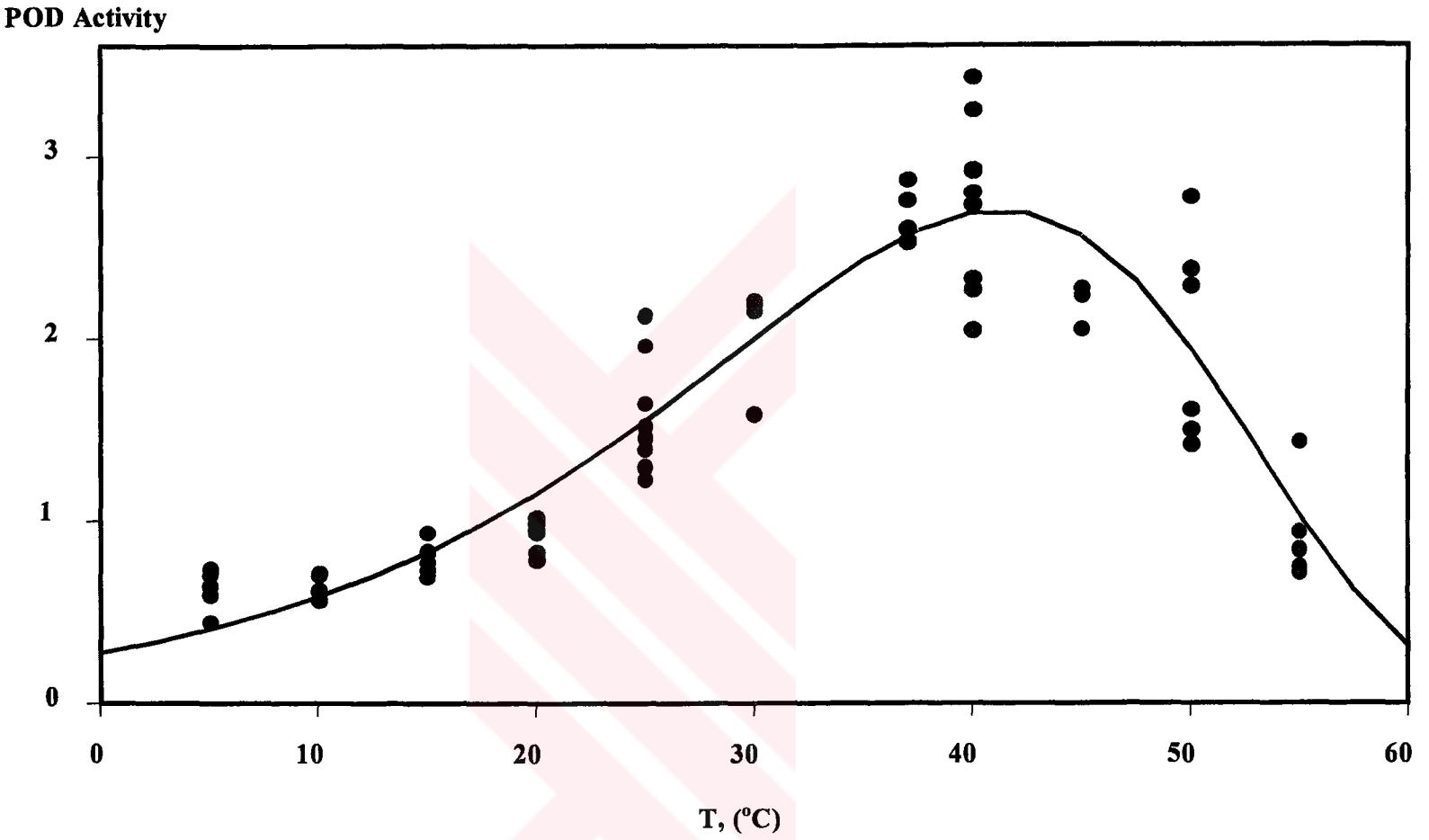


Figure 5.1. Effect of temperature on hazelnut (cv. Tombul) peroxidase activity. Conditions were: 50 mM buffer pH 4.5, 10 mM ABTS and 2 mM H_2O_2 and 0.36 mg/ml protein of enzyme extract. (POD activity=nkat/mg protein)

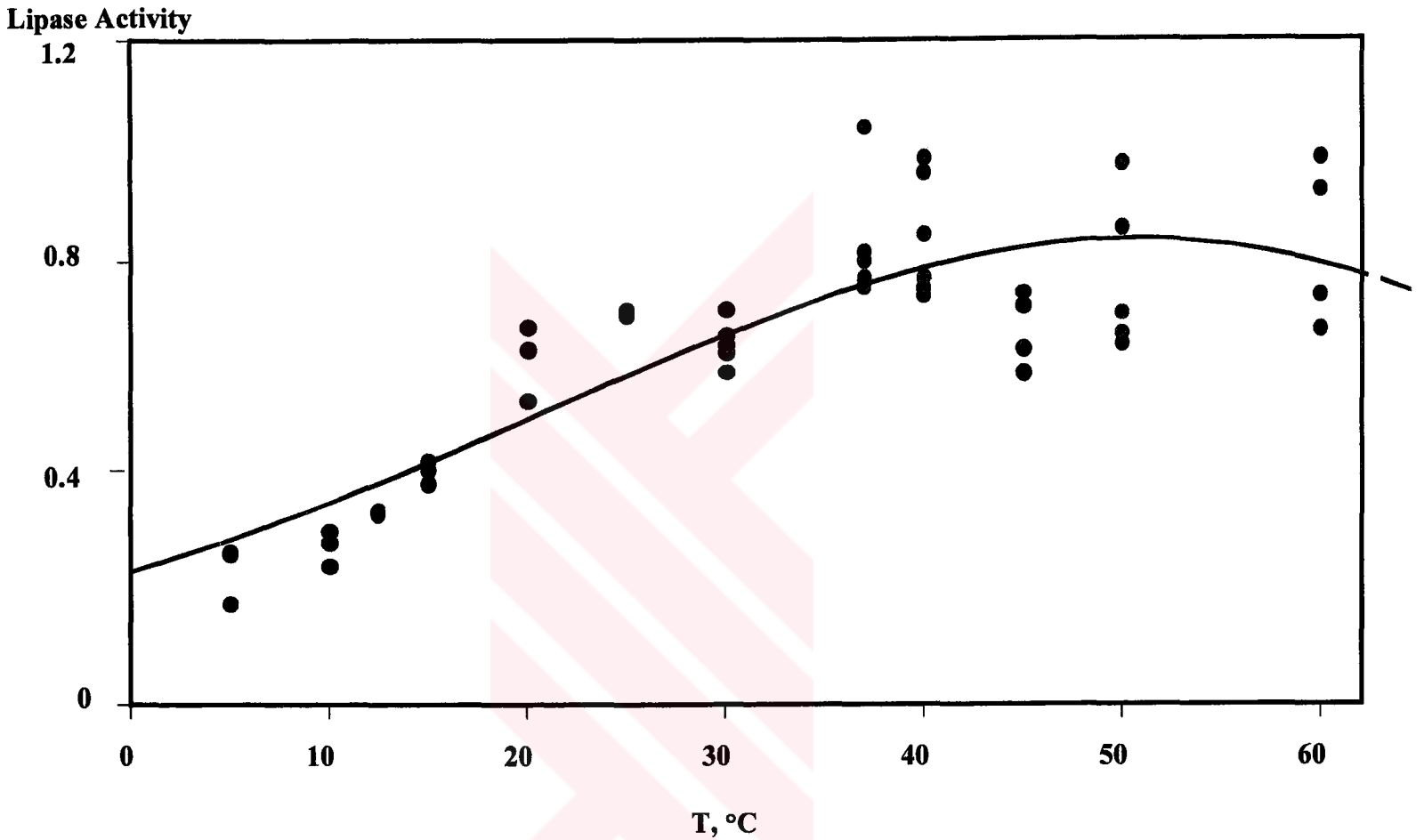


Figure 5.2. Effect of temperature on hazelnut (cv. Tombul) lipase activity. Conditions were: 50 mM buffer pH 4.5, 1 mM DNPB and 0.36 mg/ml protein of enzyme extract. (Lipase activity=nkat/mg protein)

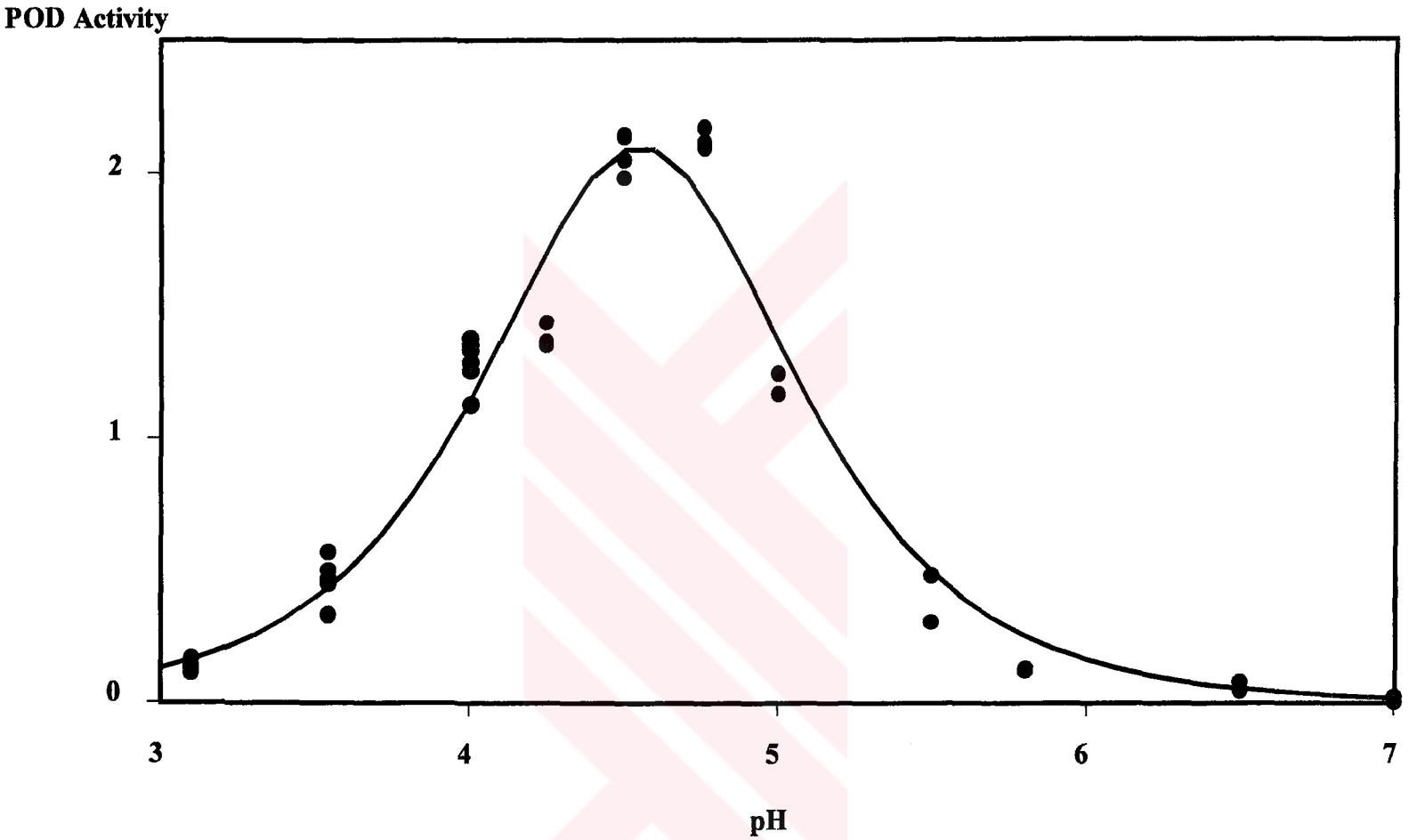


Figure 5.3. Effect of pH on hazelnut (cv. Tombul) peroxidase activity at 25 °C. Conditions were: 50 mM buffer (pH 3-7), 10mM ABTS and 2mM H_2O_2 and 0.44 mg/ml protein of enzyme extract. (POD activity=nkat/mg protein)

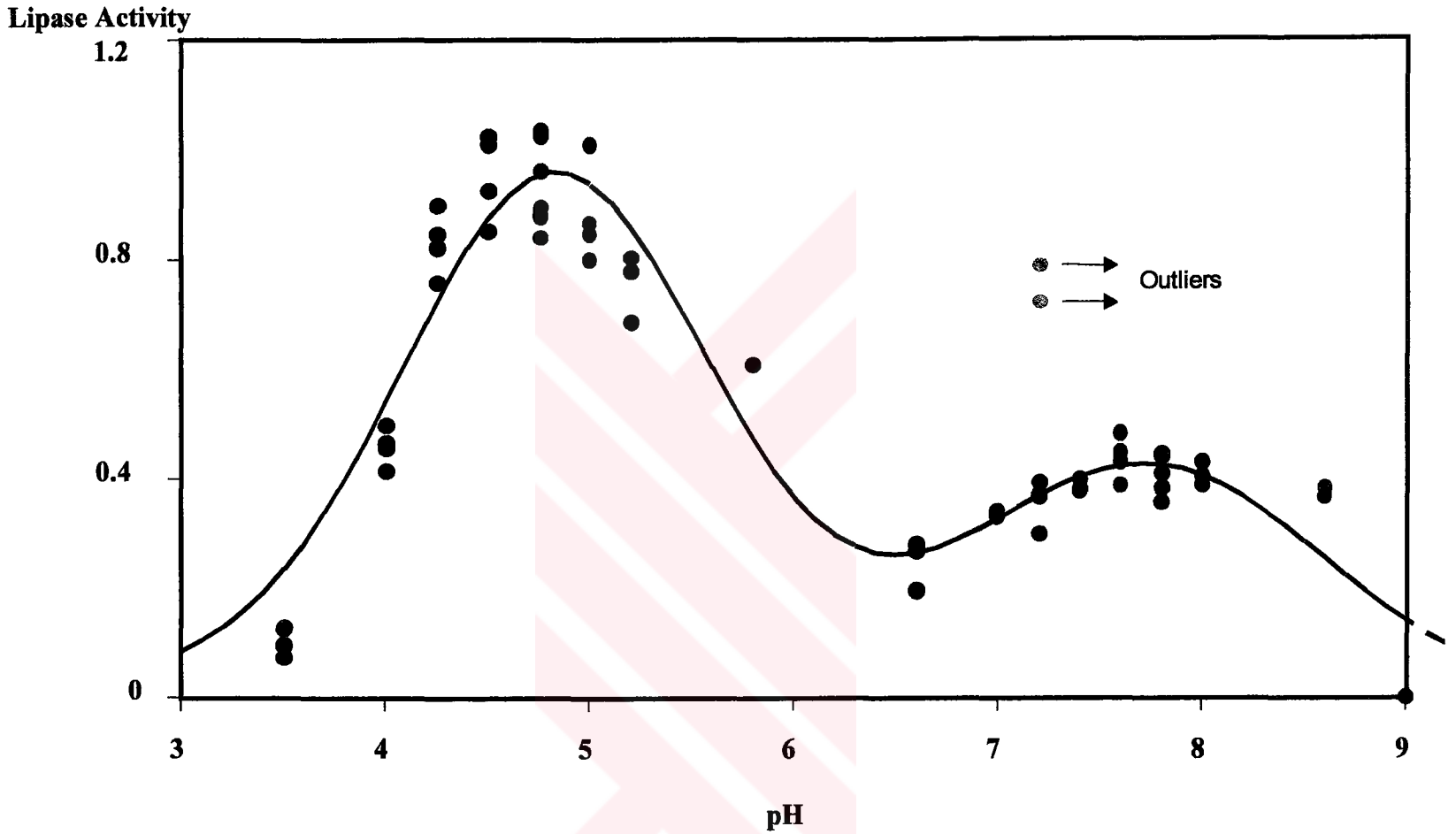


Figure 5.4. Effect of pH on hazelnut (cv Tombul) lipase activity at 25 °C. Conditions were: 50 mM buffer pH (3.5-9), 1 mM DNPB and 0.44 mg/ml protein of enzyme extract. (Lipase activity=nkat/mg protein)

5.2.3. Duplicate Reliability

Several methods were essayed for the measurement of lipase and peroxidase activity. Especially, spectrophotometric methods were preferred for the enzymes because of the limited laboratory facilities. Spectrophotometric methods for assaying peroxidase are popular (Chuang and Chen, 1988; Gosling and Ross, 1981; Huystee et al., 1990; Rodriguez-Lopez et al., 2000 and Yemenicioğlu et al., 1998). However, maximum activity was determined by ABTS assay mixture among guaiacol and TBC (4-*tert*-butylcatechol) and gave good duplicate reliability as also mentioned by Arnao et al., 1996.

As it is reviewed extensively by Thomson et al. (1999) and Beisson et al. (2000) that numerous methods are available for measuring lipase. Titrimetric methods for assaying lipase are appear to offer adequate sensitivity in many applications and the performance is enhanced by the pH-stat method. However, titrimetric methods tend to be time consuming, which may be unsuitable for large scale screening of lipolytic activity (Thomson et al., 1999). The need to increase the speed and sensitivity of assays for lipase led to the development of colorimetric methods. Synthetic substrates such as p-nitrophenyl derivatives of fatty acids allowed spectrophotometric methods. Hydrolysis of the substrate by lipase releases p-nitrophenol. Because the substrate is synthetic, results should be confirmed. The method described by Mosmuller et al. (1992) offered a rapid and sensitive assay and used for activity measurement of lipase from rapeseed successfully by Ponne et al., 1996. However, the measuring error between duplicates (see Figure 5.1 to Figure 5.4) for lipase is considerably large. Especially at higher temperatures above 30°C the error is more evident. Mosmuller et al. (1992) applied the procedure only at 25°C and did not mention the effect of higher temperatures on DNPB. Beside this, it is suggested that measurement of lipase activity requires stable emulsions. Emulsification of the substrate was performed by 10 fold of the suggested volume (24.5 ml instead of 2.45 ml) because of the lack of sonifier microprobe and impossibility of sonication of such a small amount of emulsion volume. This large standard error which may arise due to the explained reasons will have a considerable effect on the reliability of the parameters estimated by statistical analysis.

5.2.4. Statistical Analysis

5.2.4.1. Effect of Temperature

Based on the combination of Equations 4.5 and 4.6, the individual data (no means taken) on the activity of peroxidase and lipase at constant pH, were analysed using non-linear regression analysis (Genstat, Rothamsted, UK) as a function of treatment temperature, at the constant pH of 4.5 and a constant denaturation time of 25 s. The results of the analyses are estimated using equation 4.7 and shown in Table 5.3.

Table 5.3. Statistical analysis of temperature dependence of peroxidase and lipase activity.

		Peroxidase		Lipase	
	Unit	Estimated	s.e.	Estimated	s.e.
$Act_{0,ref}$	(nkat/mg protein)	5.87	1.70	2.56	na
E_s	kJ/mol	48.94	7.96	34.45	na
$k_{d,ref}$	$(mol/L)^{-1} x(sec)^{-1}$	$3.32x10^{-2}$	$0.87x10^{-2}$	$4.55x10^{-2}$	na
$E_{d,}$	kJ/mol	88.55	$1.04 x10^{-4}$	25.65	na
R^2		0.856		0.721	
N_{obs}		62		48	
t	sec	25		25	
T_{ref}	°C	45		45	

na: not available

For peroxidase the obtained explained part (R^2) is slightly high, and the standard errors of estimates (s.e.) are within the normal range. The reason for slightly high R^2 might be the presence of iso-enzymes with different thermal stability, which will be confirmed later in this study. The analysis for lipase is much less reliable. The explained part (R^2) is too low for the statistical system to estimate the standard errors of estimates (s.e.). The reason for this low reliability has to be found in the large deviation between the duplicates.This indicates probably that the measuring method used for this enzyme is not quite as good as for the POD enzyme. Another reason for this low reliability can be found in the not yet complete denaturation of lipase at the temperatures studied (Figure 5.2). At the temperature range studied (5°C-60°C) for a reaction time of 25 sec. it was seen that considerable inactivation of lipase could not be achieved. As also determined by Ory, (1969) (in Reed, 1975) that lipase from

castor bean was quite heat stable at 60°C. Therefore E_d was found rather low when compared with the activation energy values listed Table 3.4.

Combined effect of time and temperature on enzyme activation and deactivation was simulated with Equation 4.7. based on the developed model and the parameters estimated for POD and shown schematically in Figure 5.5.

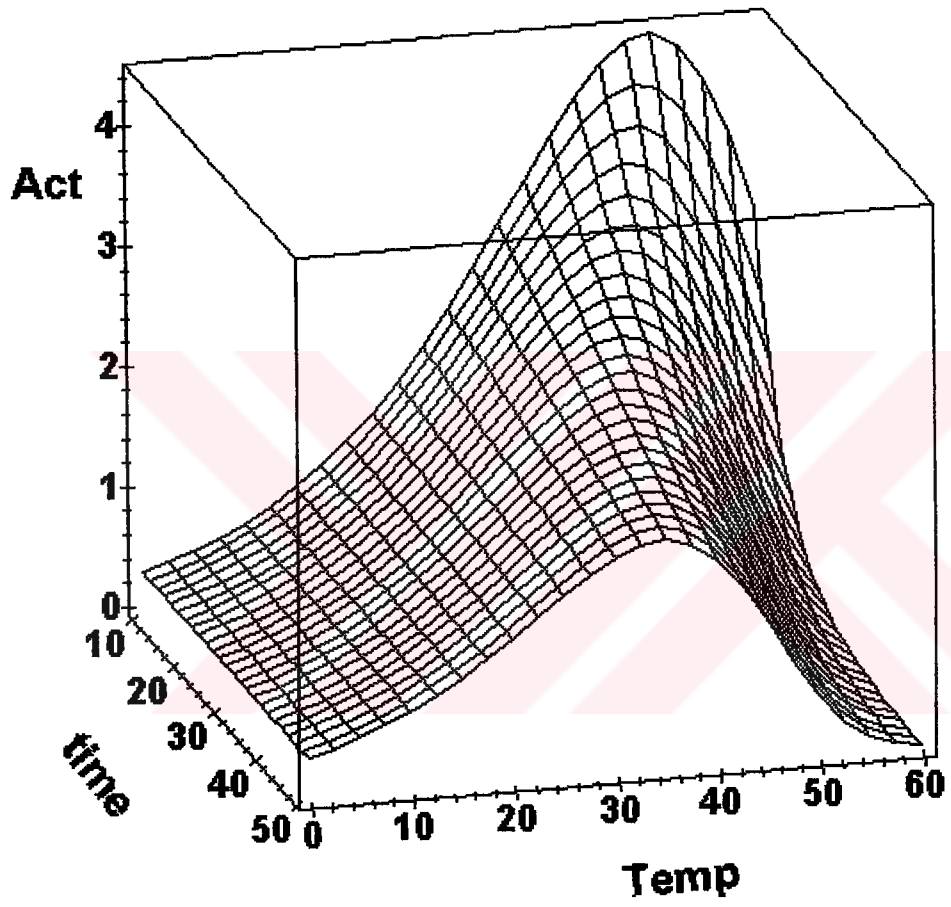


Figure 5.5. Simulated behaviour of POD as a function of time and temperature. Based on eq. 4.5 and 4.6 and the estimated parameters as shown in Table 5.3.

5.2.4.2. Effect of pH

The data on the pH dependency of enzyme activity were analysed using non-linear regression based on Equation 4.14. The data of peroxidase clearly indicate (Figure

5.3) that with respect to pH dependency, only one iso-enzyme is present. The existence of iso-enzyme systems based on pH dependence for peroxidase in nuts has not been explicitly reported in literature. The data were therefore, analysed as is based on Equation 4.15. The data on lipase, however, showed (Figure 5.4) a possible existence of two iso-enzymes with respect to pH dependency. The formulation in Equation 4.14 was for an enzyme exhibiting a single activity and Equation 4.15 a double activity. Therefore Equation 4.15 was used to allow for both iso-enzymes at the same time. Whether or not both iso-enzymes are different configurations of the same enzyme or fundamentally different enzymes, could not be concluded based on the available data. Two measured values (indicated in Figure 5.4) did clearly not belong to the observed behaviour and were discarded as outliers. The results of the statistical non-linear regression analysis are shown in Table 5.4.

The dissociation constants K_{EH} and K_{EOH}, for both enzymes and for both configurations are given in Table 5.4. As can be seen from this table, the R^2 for both enzymes are high enough. These findings not only corroborate the developed model on pH dependency, but also that this model can be used to detect and describe the existence of iso-enzymes or different enzyme configurations. The significance of the determination of pH and temperature profiles for lipase and peroxidase is that assaying conditions for quantity measurements and characterisation studies can be handled more precisely.

5.3. Comparison of Thermostabilities of Peroxidase and Lipase

The temperature dependence for lipase and peroxidase were discussed in the previous section. The temperature optimum has been shown to be time- and pH-dependent and therefore is only applicable to specific conditions. It will increase in inverse proportion to the length of the reaction time used to measure the enzyme activity. Therefore, the thermostability of an enzyme can not be correlated with the temperature optimum (Svensson, 1977). Since the temperature optima of these enzymes must always be passed in heat treatment processes intended to inactivate the enzymes, the highest temperature, 60°C, applied in this study was chosen to compare the thermostabilities of peroxidase and lipase.

Table 5.4. Results of non-linear regression analysis for pH dependence of lipase and POD.

Lipase		Estimated	s.e.	pK
$En_{tot,1}$	mol/L	1.4045	0.1235	
$En_{tot,2}$	mol/L	0.5550	0.08204	
K_{EH1}	mol/L	6.37×10^{-5}	1.30×10^{-5}	4.20
K_{EOH1}	mol/L	2.87×10^{-9}	7.60×10^{-10}	8.54
K_{EH2}	mol/L	1.11×10^{-7}	5.30×10^{-8}	6.96
K_{EOH2}	mol/L	3.41×10^{-6}	1.61×10^{-6}	5.47
R^2		0.896		
N_{obs}		65		
T_{ref}	°C	25		
POD		Estimated	s.e.	pK
En_{tot}	mol/L	26.846	35.691	
K_{EH}	mol/L	4.80×10^{-6}	6.76×10^{-6}	5.32
K_{EOH}	mol/L	6.05×10^{-11}	8.60×10^{-11}	10.22
R^2		0.962		
N_{obs}		41		
T_{ref}	°C	25		

The effect of static heat treatment in water bath at 60°C is indicated in Figure 5.6 and Figure 5.7. Activities of lipase and peroxidase decreased gradually with increasing periods of heat treatment. It was observed that continuing the heat treatment to extended periods did not inactivate the lipase further. Deviation from first order kinetics in lipase inactivation is also observed by many reseachers and explained that such inactivation courses are usually attributed to presence of iso-enzymes with different thermostabilites or one enzyme with different active conformations or formation of aggregates with other components (Bozoğlu et al., 1984; Hassanien and Mukherjee, 1986; Ponne et al., 1996; Swaisgood and Bozoğlu, 1984 and Vetrimani et al., 1992). Ponne et al. (1996) explained the inactivation of lipase in rape seed using a single-exponential model with a constant remaining activity. Therefore inactivation data for lipase were analysed using a single exponential model described in Equation 4.18. In this model it is assumed that the

level of remaining activity is constant, that is the inactivation rate (k_2 and k_3) for the heat stable lipases is zero as suggested by Ponne et al. (1996).

The explanation of peroxidase inactivation using two-exponential function was already suggested by Ling and Lund (1978), Naveh et al. (1982) and Yemenicioğlu et al. (1998) who used an un-purified extract as in this case. Therefore, the presence of at least two distinct iso-peroxidases or group of iso-peroxidases with different heat stabilities can be easily assumed. Because of the possible presence in the extract of two groups of POD iso-enzymes with different thermal stability (POD groups 1 and 2), a double-exponential (Equation 4.19) was used to explain the decrease of POD activity after incubation at 60°C.

NLR data was carried out with time as explaining variable using SPSS (SPSS Inc., Chicago, Illinois). NLR analysis of the lipase and peroxidase inactivation data for 60°C resulted in coefficient of determination that accounted for 0.9245 and 0.9826.

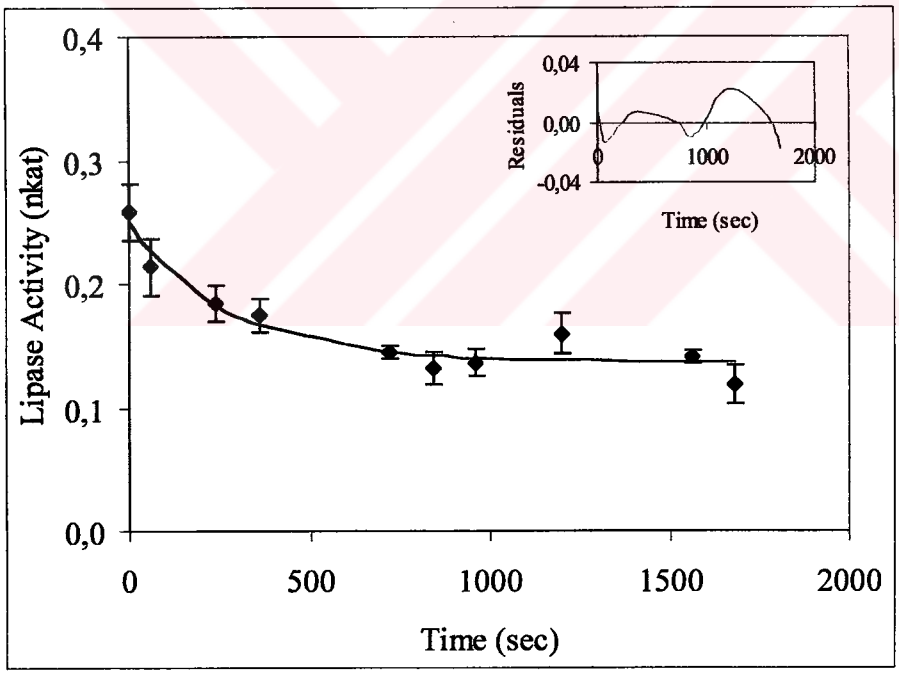


Figure 5.6. Activity of free lipase as a function of time at 60°C. Simulation results are indicated with the line $k_1=3.66x10^{-3}s^{-1}$ and $R^2=0.9245$.

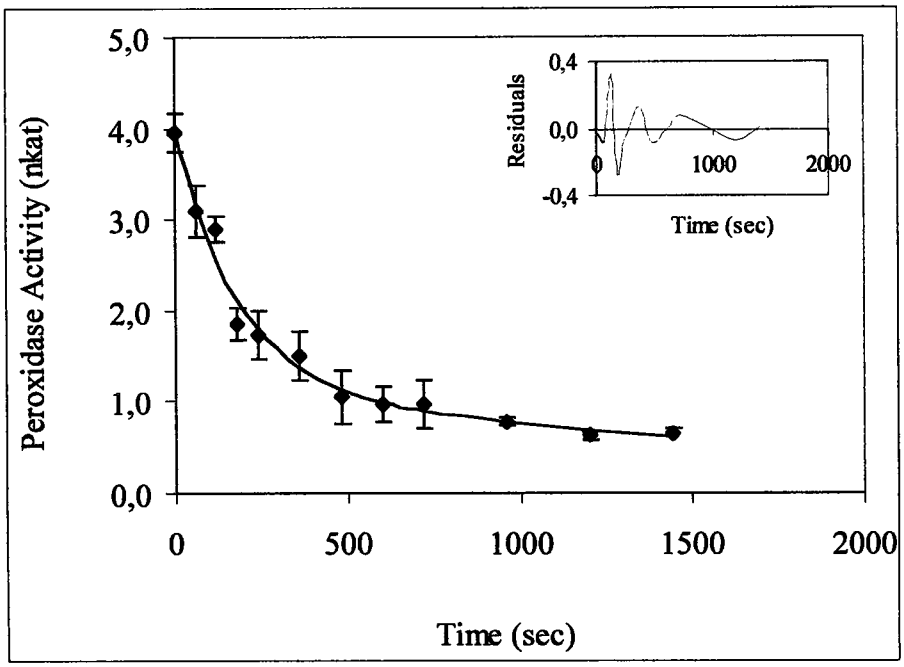


Figure 5.7. Activity of free peroxidase as a function of time at 60°C. Simulation results are indicated with the line $k_1=5.34x10^{-3}$ s^{-1} and $k_2=0.38x10^{-3}$ s^{-1} and $R^2=0.9826$.

Since it is reported that the usual F-tests for regression and lack of fit were not valid, in general, in the non-linear case (Drapper and Smith, 1966) comparission of the observed and predicted values and the residuals are made visually and given in Appendix C6 (Figure C.1 and Figure C.2) and Appendix C7 (Figure C.6 and Figure C.7) . The graphs of predicted versus observed values and residuals versus predicted values are used to help decide the used models were acceptable. The models used for lipase and peroxidase were appeared to be visually statistically sufficient to describe the inactivation kinetics at 60°C.

Analysis of the data with single exponential model described in Equation 4.18 resulted in explanation of experimental variance with a k_1 of $3.66x10^{-3}s^{-1}$ inactivation rate for lipase. The peroxidase extract apparently contained one iso-enzyme or group of iso-enzymes with relatively low thermal stability (k_1 = $5.34x10^{-3}$ s^{-1}). The second iso-enzyme or group of iso-enzymes were more resistant at 60 °C (k_2 =$0.38x10^{-3}s^{-1}$) although the initial residual activity (Act_2) was lower (Figure 5.7). Estimated values of k_i and R^2 are given in Table 5.5.

Results from this free enzyme inactivation showed that lipase is more heat stable than peroxidase. In addition, the characterisation of the thermal inactivation of POD

at 60°C encourage us to extend and to make deeper such study isolating the different POD iso-enzymes and with more incubation temperatures. However, it should be noted that regenerated and denatured peroxidase is a potential lipid oxidation catalyst because of the haem cleaved from the active site on extended heating (Adams et al., 1996 and Eriksson and Vallentine, 1973). Beside this, in some vegetables the extra time required to inactivate peroxidase permanently was found to be excessive and the loss in product quality was serious (Robinson, 1991). Moreover, to obtain a high quality product the above study is extended to lipase that catalyses the hydrolytic breakdown of the lipids in hazelnut and resulting in rapid increase of FFA.

Table 5.5. Results of statistical analysis of heat inactivation of lipase and peroxidase from hazelnut at 60°C.

Parameter	Enzymes	
	Lipase	Peroxidase
k_1 (sec^{-1})	$3.66x10^{-3}$	$5.34x10^{-3}$
k_2 (sec^{-1})	-	$0.38x10^{-3}$
k_1 (min^{-1})	0.22	0.32
k_2 (min^{-1})	-	0.023
Act_1 (nkat)	0.1129	1.0945
Act_2 (nkat)	-	2.8873
Act_f (nkat)	0.1366	-
R^2	0.9245	0.9826

5.4. Effect of Temperature on Inactivation Hazelnut Lipase

Since lipase type in Tombul hazelnut appeared to be quite resistant to heat, modelling and inactivation kinetics of lipase activity were determined to improve the quality of hazelnut and hazelnut products, so the natural hazelnut meal.

A number of experiments have been performed to show the effect of temperature and time in the inactivation of free lipase and lipase in hazelnut kernel. The experimental conditions for kinetic study were quite different than the heating conditions of hazelnut to inactivate lipase. It is known that inactivation kinetics depends very much on the physical environment. Obviously moisture content in the hazelnut lipase extract is much higher than in whole kernel. As it was also reported by Ponne et al. (1996) that in aqueous environments lipase is more easily inactivated by heating.

The inactivation kinetics of free lipase were conducted in a water bath with static

conditions and the data obtained were used to determine the inactivation parameters. On the other hand hazelnuts were subjected to dynamic heating conditions to inactivate lipase. The dynamic conditions were conducted to be able to compare the heating treatments with the industrial applications and determine the effect of heating treatment on shelf life stability of natural hazelnut meal.

In this study, although microwave heating promise potential use in enzyme inactivation, it was not found to be an alternative method for inactivation trials, since as reported by Zürcher and Hadorn (1976) and Möller et al. (1994) microwave treatment for a complete inactivation cased with a roasted taste and the microstructure is damaged during the treatment.

5.4.1. Thermal Inactivation Kinetics of Free Lipase from Hazelnut

The static heat treatment in water bath (70, 80 and 90°C) were carried out with free lipase (aqueous extract). The lipase activity gradually decreased with increasing periods of heat treatment and temperature (Figure 5.8, Figure 5.9 and Figure 5.10). The model (Equation 4.18) used for 60°C was also applicable to the 70, 80 and 90°C data set. The presence of a remaining activity was confirmed as reported before.

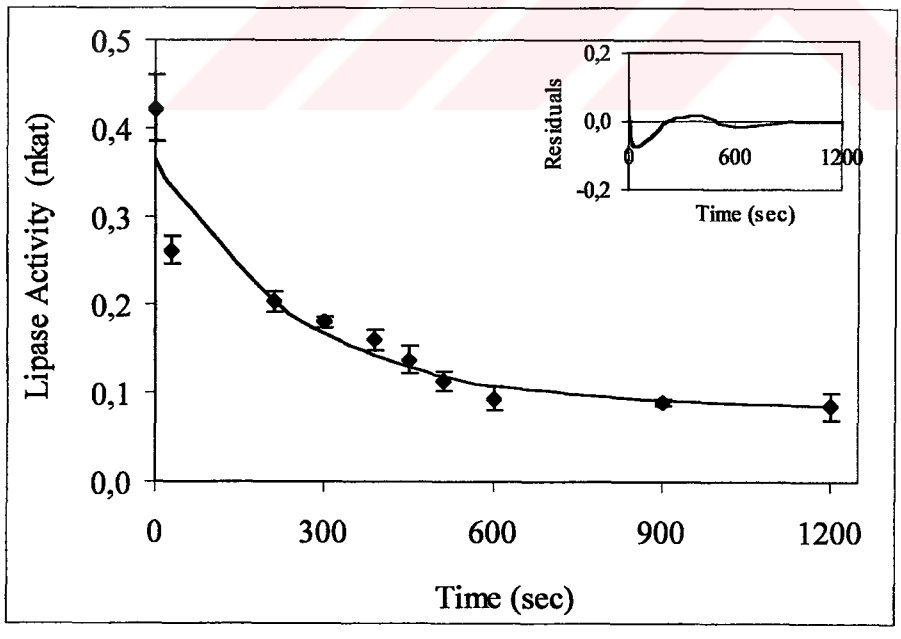


Figure 5.8. Activity of lipase as a function of time at 70°C. Simulation results are indicated with the line $k_{1,70}$=0.40 x10^{-2} s^{-1} and R^2=0.9057.

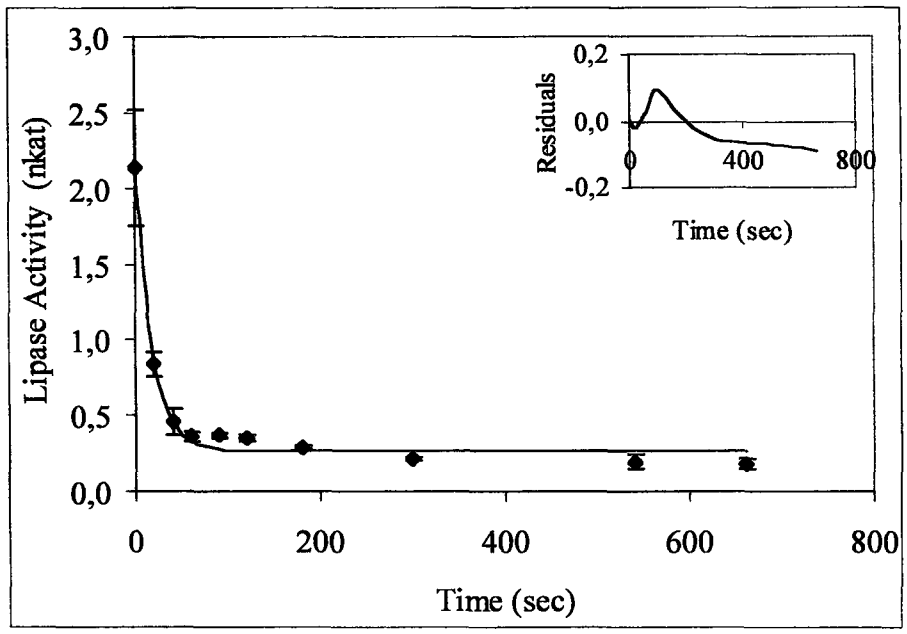


Figure 5.9. Activity of lipase as a function of time at 80°C. Simulation results are indicated with the line $k_{1,80}$=5.75 x10^{-2} s^{-1} and R^2=0.9894.

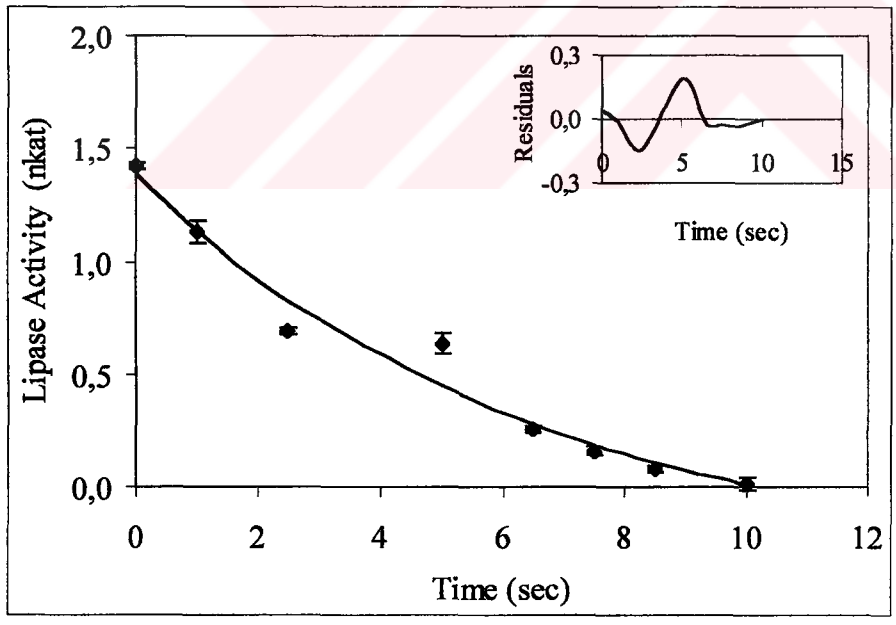


Figure 5.10. Activity of lipase as a function of time at 90°C. Simulation results are indicated with the line $k_{1,90}$=15.22x10^{-2} s^{-1} and R^2=0.9685.

In addition to the discussion about remaining activity of lipase in previous section, Cogan (1977) has suggested that first phase represents inactivation of an extracellular lipase and the second slow phase, a more heat resistant intracellular lipase. Andersson et al. (1981) has explained the two stage inactivation of lipase in skim milk as the result of the formation of an association complex of lipase with the components of skim milk such as polysaccharides and divalent cations.

The association of the enzyme was explained by the high standard deviations in the first seconds of the inactivation course and related to the dissociation of an enzyme-inhibitor complex as a result of heat treatment. It was also pointed out that lipase can be inactivated only partly and 90% inactivation of lipase was extremely long even at high temperatures. Thus, analysing the inactivation data in this study in all kinds of combinations and approaches with non-linear regression, the statistical system keeps coming back with an estimation that only a one-enzyme system is active, but includes a fixed invariable part of the activity as mentioned by Ponne et al., (1996) not an isoenzyme with higher thermostability. However, according to Tijskens, 2001 it was explained that the fixed invariable part still remained unexplained and it was pointed out that no evidence could be determined to say that it is an enzymatic configuration.

NLR analysis of the data was carried out for each temperature series separately using time and temperature as explaining variables. Analysis of data with the single exponential model described in Equation 4.18 resulted in estimated parameters (given in Table 5.6). The activity of free lipase from hazelnut at various times and different temperatures (70, 80 and 90°C) are shown in Figure 5.8, Figure 5.9 and Figure 5.10 where the lines indicate the simulated results. The inactivation rate constant k_i increases as the temperature of heat treatment increase. The determined k_i values for 70, 80 and 90°C are 0.40×10^{-2} s^{-1} (R^2=0.9057), 5.75×10^{-2} s^{-1} (R^2=0.9894), and $15.22 \times 10^{-2} s^{-1}$ (R^2=0.9685) respectively. Comparission of the observed and predicted values and residuals are made visually and given in Appendix C6 (Figure C.3, Figure C.4 and Figure C.5) and Appendix C7 (Figure C.8, Figure C.9 and Figure C.10). In almost all the cases (70, 80 and 90°C) the used model (Equation 4.18) can be regarded as statistically sufficient to predict the inactivation kinetics.

Energy for heat inactivation were determined using the Arrhenius plot (Figure 5.11)

and calculated as 171.64 kJ/mol according to Equation 4.18. The inactivation parameters are near to the values estimated in literature which are summarised in Table 3.4 (Andersson et al., 1981; Kermasha et al., 1993; Owasu et al., 1992 and Ponne et al., 1996). Heat treatment for and beyond 600 sec gradually reduced the lipase activity at 70°C and ceased with an inactivation of app. 80%. It was observed that effect of heat treatment on lipase inactivation were reduced after 80% inactivation and further heating did not resulted in an complete inactivation for 70°C and 80°C. However, a similar behaviour was not observed for heating at 90°C. Heating the enzyme extract for 10sec resulted in a nearly complete inactivation of the lipase. On the other hand, the term; Act_f used in Equation 4.18 is estimated as a negative value for 90°C which can not be explained scientifically. 80% of the free lipase were inactivated in 60 sec and 6 sec for heating at 80 and 90°C respectively. These results are similar with the findings of Ponne et al. (1996). According to Ponne et al. (1996) lipase activity decreased app. 85% after 60 sec at 75°C for the extract and 1.5 min at 130°C for whole rape seed.

Table 5.6. Results of statistical analysis of heat inactivation of lipase from hazelnut at static conditions.

Parameter	Temperature		
	70 °C	80°C	90°C
k_1 (s^{-1})	$0.40x10^{-2}$	$5.75x10^{-2}$	$15.22x10^{-2}$
k_1 (min^{-1})	0.24	3.45	9.13
Act_1	0.2818	1.8632	1.7594
Act_f	0.0837	0.2691	-0.3740
R^2	0.9056	0.9894	0.9685
E_a (kJ/mol)		171.64	

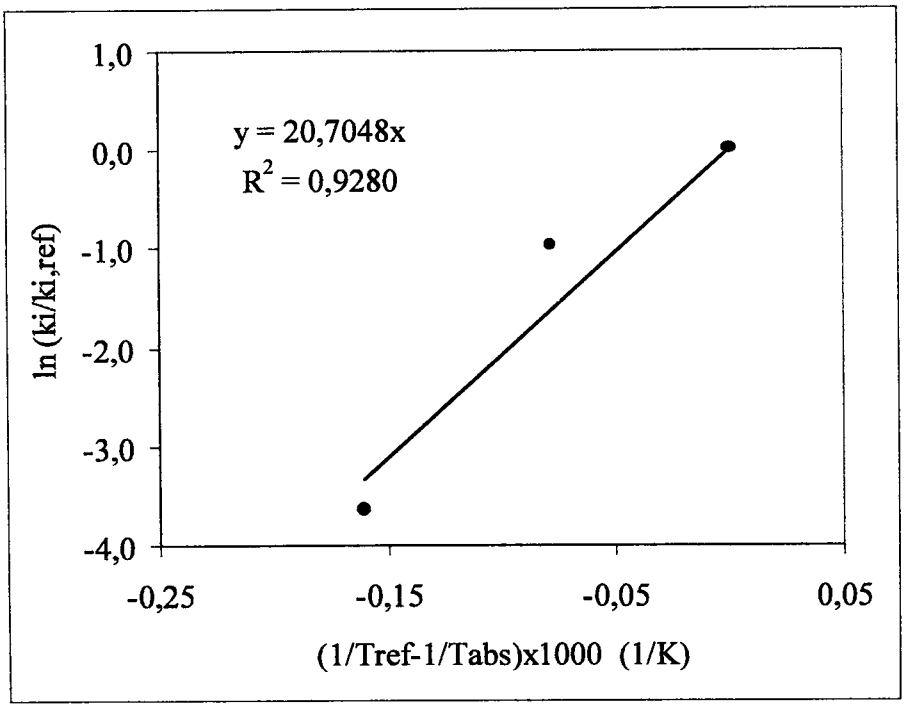


Figure 5.11. Temperature dependence of lipase inactivation by heat treatment (Arrhenius plot).

5.4.2. Effect of Temperature and Time on Lipase in Hazelnut Kernel

Whole hazelnut kernels were subjected to different temperatures in order to provide data to make a comparison between industrial applications. Temperatures below 50°C are normally used in drying of unshelled hazelnuts prior to storage and some producers treat hazelnuts with heat at 80°C for 10-20 min in order to reduce moisture content or 120°C for 10-15 min for blanching prior to natural and blanched hazelnut meal production in hazelnut industry. The temperature over 120°C was not tested since roasted flavour is not imparted by natural hazelnut users. It was reported by Saklar (1999) that hazelnuts roasted at 125°C for 15 min was slightly different from raw samples and their microstructure was similar to that of the raw hazelnuts. The blanching is done by removing the pellicle (kernel coat) because of demand for uniform white colour. Although it has been reported that pellicle and the small amount of fibre on the pellicle imparts a bitter flavour (Mehlenbacher and David, 1988 and Mehlenbacher, 1991), hazelnut meal has market for both blanched and un-blanched products.

Subjecting whole hazelnut kernels to 80, 100 and 120°C for 15 min inactivated lipase to varying degrees and resulted in different peeling ratios. The effect of temperature on hazelnut lipase inactivation and peeling ratio are given in Figure 5.12. Lipase activity in whole hazelnut kernel is markedly influenced at 100 and 120°C. Similar results were observed by the other researchers (Grosch et al., 1983 and Metwalli et al., 1983) Grosch et al. (1983) have determined that peroxidase activity could no longer be detected in Turkish origin hazelnuts after roasting (119-129°C for app. 20 min), while lipase activity was reduced but still could be detected. Metwalli et al. (1983) determined the thermal destruction time for lipase in hazelnuts (*Corylus americana*) at high temperature range between 140-180°C and reported that the evaluated optimum roasting times were much higher than the F value of the hazelnut lipase. Thermal destruction time for lipase was recorded as 42 min at 140°C which gives the roasted taste to the hazelnuts.

In this study, at 80°C the activity was reduced by only 26% after 15 min while it was 87% and 85% at 100°C and 120°C respectively. The peeling ratio which indicate the heating effect on blanching of hazelnut kernels were determined. 80% of the kernels were blanched after 120°C, 15 min heat treatment while only 50% of the kernels were blanched after 100°C, 15 min heat treatment. Heating treatments were than performed at 100°C for different periods; 5, 10, 15 and 20 min to provide transitional data between the treatments.

Heating treatments were than performed at 100°C for different periods; 5, 10, 15 and The effect of heat treatment on the inactivation of lipase in hazelnut and peeling ratio shown in Figure 5.13 indicated a decrease in the activity with increase in the duration of treatment. Heat treatment for 5 min and 10 min resulted in 69% and 82% reduction in lipase activity while after 10 min the inactivation of the enzyme were slightly influenced by treatment time and reduced by 87% and 89% for 15 and 20 min treatments respectively. The complete inactivation was not observed for all treatments. As it was also observed for inactivation studies conducted with lipase extract; although the temperatures were different, continuing the heat treatment to extended periods did not inactivate the enzyme further.

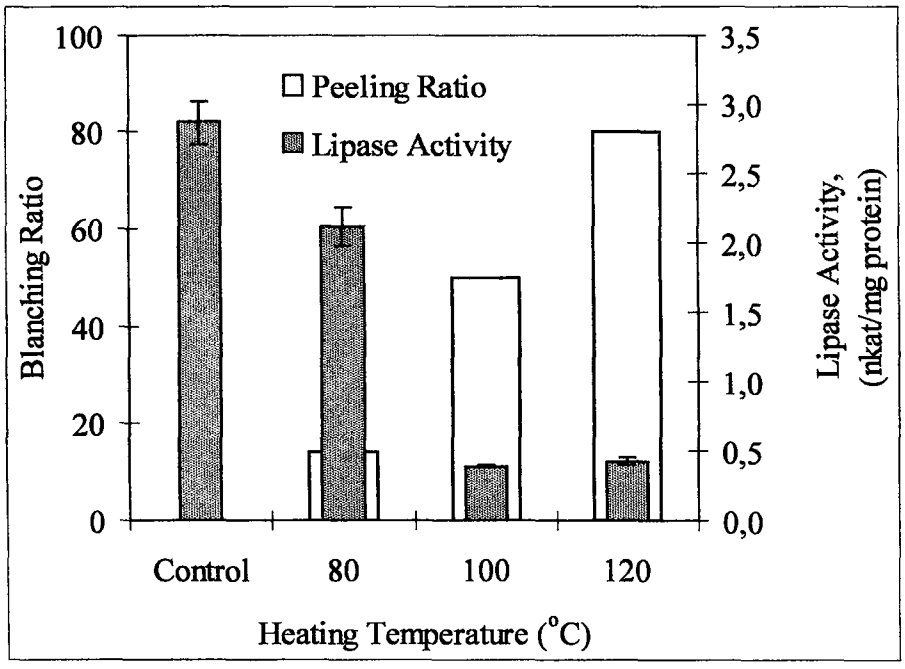


Figure 5.12. Peeling ratio and lipase activity (nkat/mg protein) of hazelnuts heated for 15 min at 80, 100,120°C having 0.376, 0.145 and 0.166 mg protein/ml enzyme extract respectively.

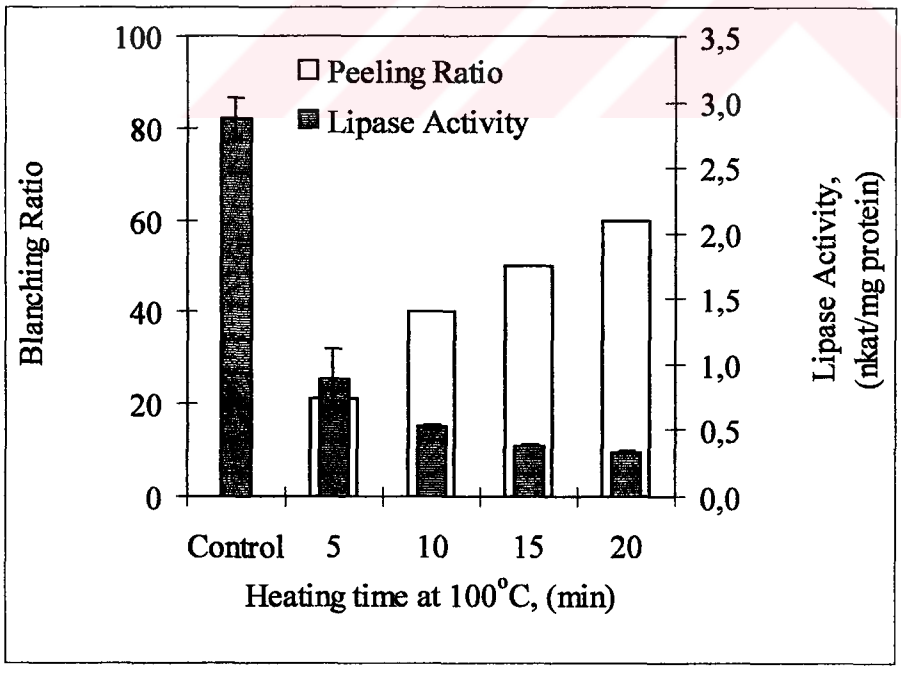


Figure 5.13. Peeling ratio and lipase activity (nkat/mg protein) of hazelnuts heated at 100°C for 5, 10,15 and 20 min having 0.166, 0.210, 0.145 and 0.172mg protein/ml enzyme extract respectively.

The heating studies indicated that inactivation of lipase was not affected after a certain time and temperature condition which corresponds to an app. higher than 80% inactivation ratio. Thus, extra heating of hazelnuts may lead slight but more inactivation, however may also cause formation of roasted flavour and decline in quality which is an undesirable situation for natural hazelnut meal customers.

5.5. Shelf Life Stability of Natural Hazelnut Meal

The stability of natural hazelnut meal were expressed as FFA content since FFA value is the most common indicator used by hazelnut industry. PV of the samples were also checked and the PV remained 0 meq/kg oil after storage. Zürcher and Hadorn (1976) has reported that lipo-hydroperoxide degradation was more rapid than the formation of hydroperoxide and concluded that hazelnuts whose taste had deteriorated never showed a greatly increased peroxide value. These results are in agreement with those of Grosch et al. (1983) and Jung et al. (1997) who have observed no change in PV of natural hazelnuts after a certain period of storage. One of the reasons for observation of no change in PV may be related with the rapid degradation of the formed hydroperoxides as mentioned by Zürcher and Hadorn (1976). On the other hand Grosch et al. (1983) have observed an increase in PV of roasted hazelnuts during storage of more than 15 weeks while increase in PV of roasted kernels with chocolate coating were lower and PV of un-roasted kernels remained unchanged. The lower rate of PV increase for coated hazelnuts may be related with exclusion of oxygen which is necessary for degradation of formed hydroperoxides (Labuza,. 1971 and Maté, et al., 1996). Another reason for the observation of no change in PV may be the less effect of thermal treatment which would result in a product with roasted properties. The studies of Zürcher and Hadorn (1976) and Saklar (1999) also confirms this observation since it was reported that roasted flavour could not be detected and the microstructure was not damaged for the hazelnuts roasted below 125°C for 15 min.

5.5.1. Effect of Lipase Inactivation on Shelf-Life Stability of Natural Hazelnut Meal

The hazelnuts subjected to heat treatments at 100°C for 5, 10, 15 and 20min were stored at 60% RH at 35°C for one month. Changes in FFA content as % oleic acid are given in Figure 5.14 in order to implicate the effect of hazelnut lipase inactivation on natural hazelnut meal stability.

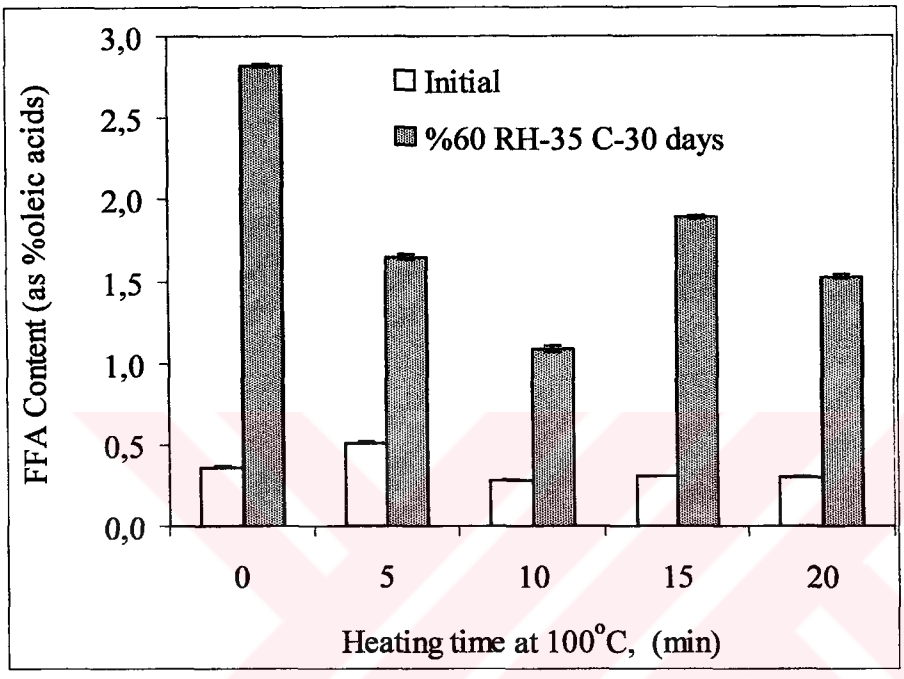


Figure 5.14. Change in FFA content (as % oleic acids) of natural hazelnut meal heated at 100°C for 5, 10,15 and 20 min before and after storage at 35°C, 60% RH for one month.

The FFA values of the hazelnut samples has changed after the heat treatments. FFA first increased at 5 min, then decreased and remained at that low level as the time of heating varied from 10 to 20 min. Change in FFA can not be explained with any known effect of time and temperature on hazelnut quality. There are several studies in the literature reporting the unexpected change in FFA just after heat treatment. Zürcher and Hadorn (1976) found that during roasting at 125-128°C FFA was increased from 0.45 to 0.52%, whereas decreased to 0.39% when roasted at 95-100°C for 35 min. For ground hazelnut they have observed that the FFA content increased after all heating treatments (63°C-0.5 hr, 80°C-1 hr and 117°C-2 hr) and

increase in FFA was the highest for heating at low temperatures (63°C-0.5 hr).

On the other hand, an increase was observed for FFA values of all samples after one month of storage. The FFA content of un-treated hazelnut meal increase 8 fold while the FFA content of the heat treated samples increased only 3-5 fold after one month of storage at 35°C, 60%RH. The critical FFA value for the industrial use of nuts is considered as 0.7% (Lopez et al., 1997a) and the limit for FFA is 1% for fresh and 1.4% for old products according to Turkish Standards (TS-10937, 1993) (Table 3.2). The hazelnut meal treated at 100°C for 5 min resulted in the lowest FFA increase and was the only application which ensures the limit although the initial FFA values were quite high (0.35%).

Beside this, unexpectedly the FFA values of the hazelnut samples treated more than 10 min were higher than the samples treated for 10 min. Whereas, higher lipase inactivation ratio was achieved for these samples. This behaviour might be explained by two reasons. Higher temperatures and long exposure time favour hydrolitic reactions and cause an increase in FFA. The studies reported in literature support this; it is reported that higher heating temperatures resulted in lipid oxidation and hydrolysis in hazelnuts. The second reason might be due to the regenerated and denatured peroxidase that acts as an oxidation catalyst. It is reported by Eriksson & Vallentin (1973) and Adams et. al (1996) that the denatured form of peroxidase enhance the lipid oxidation due to the haem cleaved from the enzyme. In this respect, during storage trial, increase in FFA in samples heated for more than 10 min might be caused by both temperature and denatured peroxidase as indicated in literature (Adams et. al., 1996; Angelo and Ory, 1975; Eriksson and Vallentin, 1973 and Lopez et. al, 1997a).

5.5.2. Effect of Processing and Storage Conditions on Free Fatty Acids of Natural Hazelnut Meal

The hazelnut meals produced from fresh hazelnuts (initial FFA=0.17%) were subjected to different processing and storage conditions and the change in FFA of oil pressed from hazelnut meals and moisture content of them are given in Figure 5.15 and Figure 5.16. The initial moisture content of the hazelnut samples were 6.36%

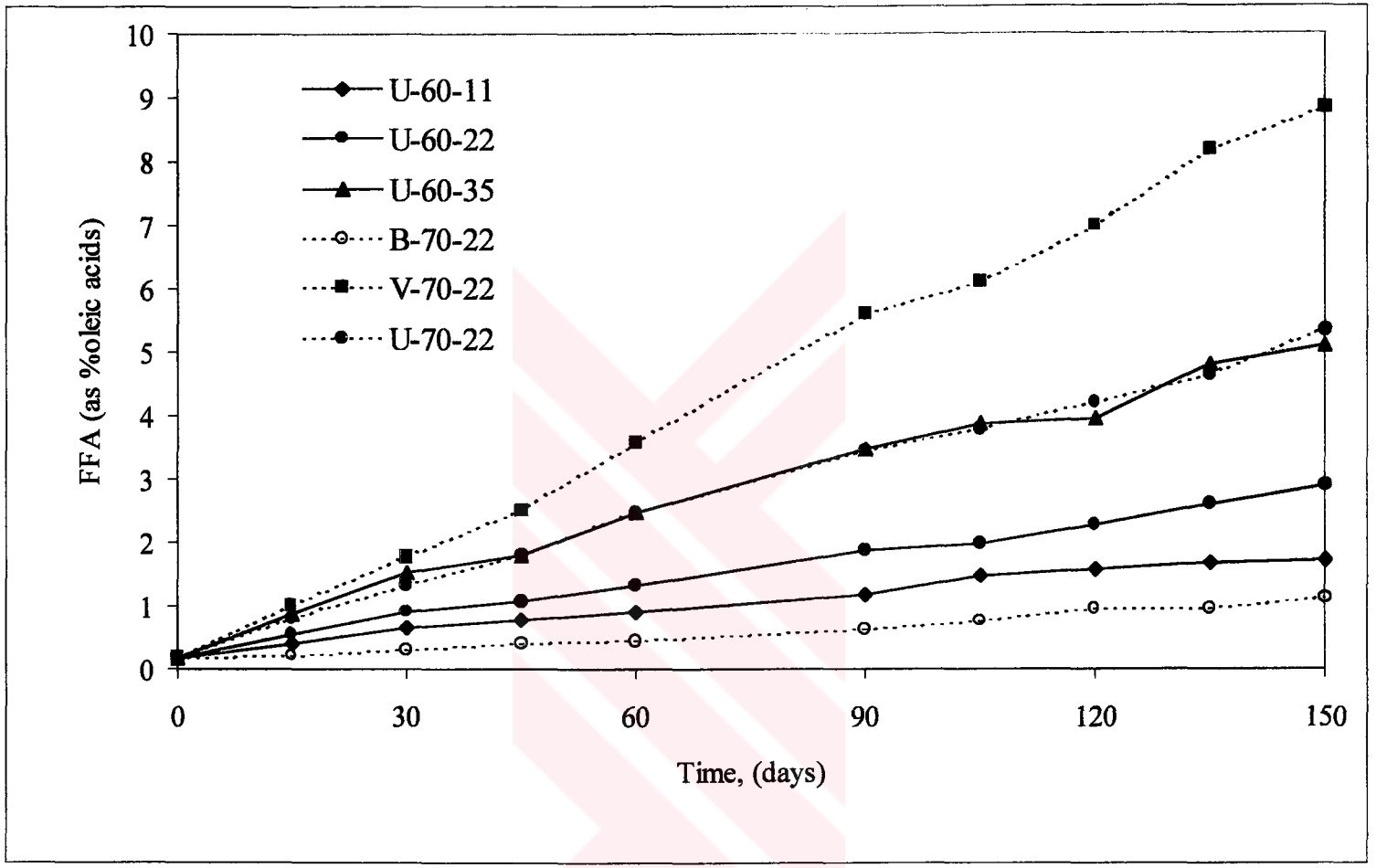


Figure 5.15. Effect of processing and storage conditions on FFA (as %oleic acid) content of hazelnut meals.

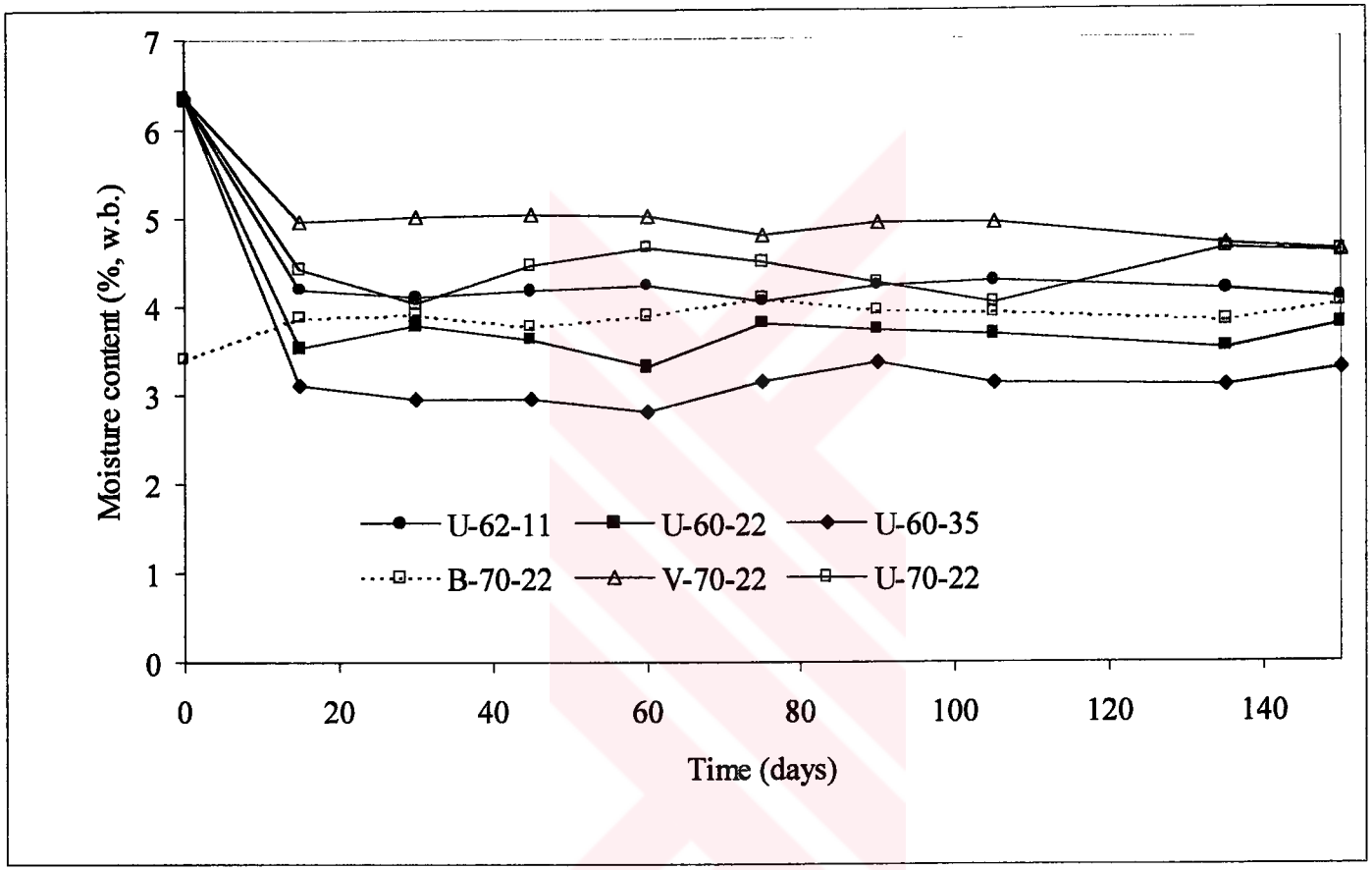


Figure 5.16. Change in moisture content (%, w.b.) of hazelnut meal samples (U: un-blanched, V: vacuum packed, B : blanched) stored at 60 and 70% RH and 12, 22 and 35oC.

and the moisture content of the blanches samples decreased to 3.40% after heat treatment. The hazelnut meal was prepared according to the method described before and placed in 11x21x4cm^3 plastic cups (200g to each cup). The blanched and un-blanched samples were exposed to storage conditions to facilitate moisture absorption or desorption. Vacuum packed samples were stored in polypropylene pouches with 5.37g/m^2.day.atm water vapour permeability (90±2% RH, 38±1°C). All the samples reached their equilibrium moisture content within 15 days or probably before.

Change in moisture content of the samples are given in Figure 5.16 . All the samples lost moisture except the blanched ones since the water activity of the blanched samples were 0.56 which is lower than 0.7 water activity (equal to 70%ERH) at the beginning. Vacuum packed samples lost moisture during storage and reached the same moisture content level with unpacked samples at the end of 150 days of storage due to the high water permeability of the packaging material.

The FFA of hazelnut meal increased during 150 days of storage. Storage at higher RH and temperature conditions caused a higher FFA content in the hazelnut samples especially for vacuum packed natural hazelnut meal. As mentioned before lipase is an oxygen independent enzyme and the activity of lipase considerably effected by the moisture content since it is an interfacial enzyme. The moisture content of the vacuum packed samples were higher than the samples stored under the same conditions. Beside this vacuum packaging enhanced the activity of lipase as described by Keme et al. (1983). It was explained that the highest FFA values were probably due to an increased availability of lipids for reaction with lipase as a result of squeezed oil (substrate) due to vacuum application and lack of oxygen for further oxidation of formed FFA.

Linear regression analysis were carried out with the time as explaining variable and the period for a start of deterioration was calculated for all samples taking the FFA value of 0.7% as limit. The results of linear regression analysis are given in Table 5.7 taking the initial FFA value; 0.17 as the intercept. The un-treated (natural) hazelnut meal stored at 22°C and 70% RH reached 1%FFA after 24days of storage which is quite short for the hazelnut meal producers. The water activity limit, allowed by

Food and Drug Administration, for raw hazelnuts during storage is 0.70. If the hazelnut meal producers do not apply any heat treatment prior to meal production and packed the meal imidiately after production, the shelf life will be 24 days, same as for the samples stored at 70%RH. The un-treated natural hazelnut meal started to deteriorate after 24days of storage at 60% RH and 35°C while the period increased approximately 2 and 3 fold for 22°C and 11°C respectively.

Table 5.7. Regression analysis for change in FFA as a function of time at different storage conditions.

Processing Condition	Storage Condition		Slope	R^2	Time (days) to reach 0.7% FFA	Time (days) to reach 1% FFA
	RH %	T °C				
Un-Treated	70	22	0.0345	0.9938	15	24
	60	35	0.0342	0.9831	15	24
	60	22	0.0181	0.9897	29	46
	60	11	0.0114	0.9746	46	73
Un-Treated & Vacuum Packed	70	22	0.0577	0.9975	9	14
Blanched	70	22	0.0059	0.9705	90	141

Coincidentally, the increase of FFA was observed to be similar for storage conditions of 60% RH & 35°C and 70% RH & 22°C. A decrease of 10% in RH resulted in 2 fold increase in shelf life of the natural hazelnut meal indicating the importance of water activity effect on lipase activity. However, the FFA content of blanched samples at 120°C for 15 min was acceptably low till 90 days, which makes heat treatment suitable for practical applications as a pre-treatment. Similarly it was also observed by Vetrimani and Haridas-Rao (1992) that free fatty acidity was negligible for heat treated wheat bran to inactivate lipolytic enzymes when compared to natural ones. Rancid taste for raw brans was detected after 20 days whereas it was 90 days for heat stabilised samples (Vetrimani and Haridas-Rao, 1992).

If we take the 1% FFA value as the acceptance limit, the shelf lives of the samples differ more evidently. It takes 51 days to reach FFA value of 1% from 0.7% for blanched sample, while it is only 9 days for untreated sample stored at same conditions.

6. CONCLUSION

The short shelf life of hazelnut meal is an obstruction for the hazelnut producers to export this product and understanding the biochemistry of such a quick loss in quality, is essential for devising means to improve the shelf life of natural hazelnut meal. In order to ensure high quality hazelnut meal, there are many factors which must be controlled carefully, i.e., enzyme activity, storage conditions, processing methods, etc. In this study, the effect of heat treatment on inactivation of enzymes and the storage conditions on stability of hazelnut meal were evaluated. Before heat treatment optimum pH and temperature conditions for lipase and peroxidase were determined since after heat treatment, it is necessary to measure the remaining activity of the enzymes at constant pH and temperature where they are stable.

Non-linear regression analysis on model formulation provided a powerful tool to estimate characteristic properties of hazelnut lipase and peroxidase activity and their inactivation kinetics. From the modelling point of view, the results of this study confirmed that models, based on generally accepted theories, and developed using the fundamental rules of chemical kinetics and equilibria, were generic in nature and applicable to many if not all situations with respect to temperature and pH dependence. Even if two iso-enzymes are present in the same sample, the behaviour can still adequately be described and predicted. On both accounts, temperature dependence and pH dependence, these results provide a further validation of the models developed for other enzymes, and for other situations.

A kinetic model were formulated (Equations 4.18 and 4.19) that can be used to predict the enzymatic activities of lipase and peroxidase during heating of enzyme extract. The model parameters all have a clear, distinct meaning, well rooted in kinetic and chemical theory and comparable with the findings in the literature. For the further studies, the developed models can be used in optimisation of storage and processing conditions to obtain stable hazelnut products of good quality.

From the product point of view, the findings in the present study indicated that the maximum activities for lipase and peroxidase are at acidic conditions of pH 4.5. Our work has demonstrated for the first time that, only the lipase activity of hazelnut among the other nuts is characterised by the presence of both acidic and alkaline iso-enzymes.

Heating treatments of enzyme extract in this study showed that moist condition during heating may be more effective in inactivating lipase as compared to heat treatment of hazelnuts to inactivate lipase in the kernel matrix. Moisture, heat and enzyme activity, in combination are important factors for lipase activity which lead to FFA formation. While sufficient moisture may be necessary to generate heat what may be more important is the content of enzyme-bound water. Therefore it seems imperative that further work needs to be carried out to find out the effect of increasing moisture content of the hazelnut samples on the inactivation of lipase.

Evidence presented in this study indicated that although heat treatment is normally used in drying and commercial processing of natural hazelnut meal, temperatures used may not be proper to inactivate lipase. Hazelnut meal is generally subjected to heat treatment either to dry the hazelnuts (at 80°C, for 15min) or to blanch them (120°C, 15-20min). Drying conditions inactivate lipase partially and shelf life improvement is insufficient. Hazelnut meal from blanched hazelnut is produced for only domestic market and the shelf life improvement is not required for this product. The hazelnut producers are faced with the shelf life problem in exported natural hazelnut meal which are not blanched. This outcome was also confirmed with the findings in this study.

Lipase inactivation ratio is between 80 and 87% for the heat treatments at 100 and 120°C, respectively. Despite the incomplete inactivation of lipase at 120°C, stability studies at prolonged storage indicated that FFA content of blanched hazelnut meal was acceptably low during 5 month of storage. However, blanching conditions is over the appropriate heating conditions determined in this study. In this study, it was observed that heating the hazelnut kernels at 100°C for 10 min led a considerable inactivation in lipase activity, less peeling ratio than the heat treated samples at 120°C and in addition to these, less FFA increase after one month of storage. On the other hand, it is determined that although lipase was not completely inactivated, it

would lead an improvement in shelf life of natural hazelnut meal. However, further heating period or higher temperature ranges may cause more inactivation but will result loss in quality.

The observations obtained from the shelf life studies at different conditions, indicated that dry (60%RH) and cold (11°C) storage conditions result in an increase in shelf life of hazelnut meal. However, heating the hazelnuts at 100°C prior to hazelnut meal production will result in a two fold increase in shelf life when compared with the untreated hazelnut meal stored at dry and cold conditions. Therefore, it would be more effective and economic to prefer heat treatment prior to hazelnut meal production instead of keeping them at cold and dry conditions. However, the conditions for the heat treatment should be determined in industrial scale in order to avoid insufficient or extra heat treatment to inactivate lipase. Vacuum application for the packaging of untreated hazelnut meal was found to be an improper method for shelf life improvement since the lipase was still active in the package. For that reason, as a recommendation for the hazelnut producers, it is advised to heat the hazelnuts at 100°C prior to hazelnut meal production and then pack the meal with a low water vapour and oxygen permeable packaging material to improve the shelf life of the product.

7. REFERENCES

Açkurt, F., Özdemir, M., Biringen, G. and Löker, M., 1999. Effects of geographical origin and variety on vitamin and mineral composition of hazelnut (Corylus avellana L.) varieties cultivated in Turkey, *Food Chemistry,* **65 (3)**, 309-313.

Adams, J.B., 1991. Review: Enzyme inactivation during heat processing of food-stuffs, *International Journal of Food Science and Technology*, **26**, 1-20.

Adams, J.B., Harvey, A. and Dempsey, C.E., 1996. Regenerated and denatured peroxidase as potential lipid oxidation catalysts, *Food Chemistry,* **57 (4)**, 505-514.

Akdağ, Z and Öztürk, İ. 1993. *Meyve ve Sebze İşleme Sanayii Özel İhtisas Komisyonu : Fındık İşleme Alt Komisyon Raporu, Yedinci Kalkınma Planı.* TC DPT, 37 pages, Ankara.

Ali, M.H., Rahman, M.S., Ahmed, G.M., Hossain, M.A., Uddin, M.M. and Gofur, M.A., 1996. Studies on the effect of moisture during storage on major ground nut seed constituents: oil, protein, fatty acids and lipid compositions, *Bangladesh Journal of Scientific and Industrial Research,* **30 (1)**, 93-104.

Alphan, E.M., Yılmaz, T.M., Pala, M. ve Açkurt, F., 1996a. Tekli doymamış yağ asidi içeriği fındıkla zenginleştirilmiş diyetin karbonhidrat ve lipid metabolizmasına etkisi, *Gıda Teknolojisi*, **1**, 58-63.

Alphan, E., Pala, M., Açkurt, F. and Yılmaz, T., 1996b. Nutritional composition of hazelnuts and its effects on glucose and lipid metabolism, *Acta Horticulturae,* **445**, 305-310.

Altaf, A., Ankers, T.V., Kaderbhai, N., Mercer, E.I., and Kaderbhai, M.A., 1997. Acid lipase of castor bean lipid bodies: isolation and characterisation, *Journal of Plant Biochemistry and Biotechnology,* **6 (1),** 13-18.

Andersen, H.J., Ostdal, H. and Blom, H., 1995. Partial purification and characterisation of lipase from Lactobasillus plantarum MF32, *Food Chemistry,* **53 (4)**, 369-373.

Andersson, R.E.; Danielsson, G.; Hedlund, C.B. and Svensson, S.G., 1981. Effect of a heat-resistant microbial lipase on flavor of ultra-high-temperature sterilized milk. *Journal of Dairy Science*, **64 (3)** 375-379.

Angelo, A.J. St. and Ory, R.L., 1975. Effects of lipid peroxidation during storage on raw and roasted peanut proteins, *Peanut Science*, **2 (2)**, 41-44.

Angelo, A.J. St. and Ory, R.L., 1975. Effect of minor constituents and additives upon peroxidation of oil in peanut butter, *Journal of the American Oil Chemists' Society,* **52** (2) 38-40.

Angelo, A.J. St., Kuck, J.C. and Ory, R.L., 1979. Role of lipoxygenase and lipid oxidation in quality of oilseeds, *Journal of Agriculture and Food Chemistry*, **27**, 229-234.

Anonymous, 2000. Hazelnut, *The Cracker,* **1**, 38-41.

Anonymous, 1995. Fındık Ekonomik Raporu, Fiskobirlik, Giresun.

AOAC, 1990. Official methods of analysis, *Association of Official Analytical Chemists,* **15th ed.**, Washington DC.

Arnao, M.B., Cano, A., Hernández-Ruiz, J., García-Cánovas, F. and Acosta, M., 1996. Inhibition by L-ascorbic acid and other antioxidants of the 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) oxidation catalysed by peroxidase: a new approach for determining total antioxidant status of foods, *Analytical Biochemistry,* **236**, 255-261.

Banu, C. and Serban, L., 1970. Enzyme changes in dry products, Lipase activity of some oilseeds, *Industria-Alimentara,* **21 (7)**, 367-369.

Barman, T.E., 1969. Enzyme Handbook, Springer-Verlag Berlin Heidelberg. 503-504.

Baş, F., Ömeroğlu, S., Türdü, S. ve Aktaş, S., 1986. Önemli Türk fındık çeşitlerinin bileşim özelliklerin saptanması, *Gıda dergisi,* **11,** 195-203.

Beisson, F., Tiss, A., Riviere, C. and Verger, R., 2000. Methods for lipase detection and assay critical review, *Eur. J. Lipid Sci. Technol.,* 133-135.

Beringer, H. and Dompert, W.U., 1976. Fatty acid and tocopherol pattern in oil seeds. *Fette Seifen Anstrichmittel,* **78 (6)**, 228-230.

Beuchat L.R. and Worthington R.E., 1978. Fatty acid composition of tree nut oils. *Journal of Food Technology*; **13 (4),** 355-358.

Bonvehi, J.S. and Coll, F.V., 1993a. Oil content, stability and fatty acid composition of the main varieties of Catalonian hazelnuts (Corylus avellane L.), *Food Chemistry*, **48**, 237-241.

Bonvehi, J.S. and Coll, F.V., 1993b. Study of the carbohydrate fraction of the principal varieties of Tarragona hazelnuts (Corylus avellane L.), *Food Chemistry,* **46**, 285-288.

Bonvehi, J.S. and Coll, F.V., 1997a. Etude de la qualite et de la stabilite de l'huile dans l'evaluation de la noisette, *Ann. Fals. Exp. Chim.*, **90 (938)**, 21-28.

Bonvehi, J.S. and Coll, F.V., 1997b. A chemical study of the mineral fraction of Tarragona hazelnuts (Corylus avellane L.), *Acta Alimentaria,* **26 (3),** 243-253.

Bonvehi, J.S. and Rosuo, N.S., 1996. Enzymatic activities in the varieties of hazelnuts (Corylus avellane L.) grown in Tarragona, Spain, *Food Chemistry*, **56**, 39-44.

Botta, R., Gianotti, C. and Giovanni, M., 1996. Kernel quality in hazelnut cultivars and selections analysed for sugars, lipids, and fatty acid composition, *Acta Horticulturae*, **445**, 319-326.

Boyes, S., Chevis, P., Holden, J. and Perera, C., 1997. Microwave and water blanching of corn kernels: control of uniformity of heating during microwave blanching, *Journal of Food Processing and Preservation,* **21 (6)**, 461-484.

Bozoglu, F., Swaisgood, H.E. and Adams, D.M. Jr., 1984. Isolation and characterization of an extracellular heat-stable lipase produced by Pseudomonas fluorescens MC50. *Journal of Agricultural and Food Chemistry*, **32 (1)** 2-6.

Bradford, B.B., 1976. A rapid and sensetive method for the quantification of microgram quantitites of protein utilizing the principle of protein-dye binding, *Analytical Biochemistry,* **72**, 248-254.

Branch, A.L., Worthington, R.E., Roth, I.L., Chinnan, M.S. and Nakayama, T.O.M., 1987. Effect of hot water immersion on storage stability of non-

refrigerated peanuts, *Peanut Science,* **14 (1)**, 26-30.

Bullerman, L.B., Schroeder, K.Y. and Park, K.Y., 1984. Formation and control of mycotoxins in food, *J.Food Proctection,* **47**, 637-646.

Charalambous, G., 1993. Shelf Life Studies of Foods and Beverages, Chemical, Biological, Physical and Nutritional Aspects. Elsevier, Publishers B.V., Amsterdam, the Netherlands.

Chitrangada-Das and Mishra, H.N., 2000. Effect of aflatoxin B1 detoxification on the physicochemical properties and quality of ground nut meal, *Food-Chemistry,* **70 (4)**, 483-487.

Chuang, W.J. and Chen, A.O., 1988. Purification and characterization of vegetable soybean peroxidase, *Food Science,China,* **15 (1)**, 12-26.

Cogan, T.M. 1977. A review of heat resistant lipases and proteinases and the quality of dairy products. *Irish Journal of Food Science and Technology*; **1 (2)**, 95-105.

Copeland, R.A., 1996. Enzymes. A practical introduction to structure, mechanism, and data analysis. Pp. 306. VCH Publishers Inc., New York, NY 10001, USA.

Damper, N.R. and Smith, H., 1966. Applied Regression Analysis. pp. 299. John Wiley and Sons, Inc., New York, NY, USA.

Denizel, T., Rolfe, E.J. and Jarvis, B., 1976. Moisture-equilibrium relative humidity relationships in pistachio nuts with particular regard to control of aflatoxin formation. *Journal of the Science of Food and Agriculture*, **27 (11)** 1027-1034.

Dougherty, M.E., 1988. Tocopherols as Food Antioxidants, *Cereal Foods World,* **33**, 222-223.

Dunford, N.T. and Temelli, F., 1996. Effect of supercritical CO_2 on myrosinase activity and glucosinalate degradation in canola, *Journal of Agricultural and Food Chemistry*, **44 (8)**, 2372-2376.

Ebrahem K.S. and Richardson, D.G., 1994. Oil content, fatty acid composition, and vitamin E concentration of 17 hazelnut varieties, compared to other types of nuts and oils seeds, *Acta Horticulturae*, **351**, 685-692.

Eke, D. ve Göktan, D., 1987. Kabuklu fındıklarda Aspergillus flavus gelişmesi ve aflatoksin oluşumu, *Gıda Sanayi*, **4**, 36-43.

Ekstrand, B., Gangby, I. and Akesson, G., 1992. Lipase activity in oats-distribution, pH dependence, and heat inactivation, *Cereal-Chemistry,* **69 (4)**, 379-381.

Elvevol, E.O., Moen, P., Olsen, R.L. and Brox, J., 1990. Some possible effects of dietary monounsaturated fatty acids in cardiovascular disease, *Artheriosclerosis*, **81**, 71-4.

Endo, Y., Murakami, Y., Fujimoto, K., Ajiri, M. and Arai, K., 1995. Inactivation of oxidoreductase containing metals by supercritical carbon dioxide, *Journal of Japanese Society of Food Science and Technology [Nippon-Shokuhin-Kogyo-Gakkaishi],* **42 (11)**, 932-936.

Espín, J.C., Trujano, M.F., Tudela, J. and García-Cánovas, F., 1997. Monophenolase activity of polyphenol oxidase from haas avocado, *Journal of Agriculture and Food Chemistry,* **45**, 1091-1096.

Espín, J. C., Soler-Rivas, C. & Wichers, H. J., 2000. Characterization of the total free radical scavenging capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *Journal of Agriculture and Food Chemistry,* **48**, 648-656.

Eriksson, C.E. and Vallentin, K., 1973. Thermal activation of peroxidase as a lipid oxidation catalyst. *Journal of the American Oil Chemists' Society,* **50** (7), 264-268.

Evranuz, E.O., 2000. Effect of H_2O_2 dip pre-treatment on oxidative stability of unblanched salted roasted peanut, Effect of moisture content and temperature, *European Journal of Lipid Science and Technology*, **102 (3),** 189-193.

Evranuz, E.O., 1993. The effects of temperature and moisture content on lipid peroxidation during storage of unblanched salted roasted peanuts: shelf life studies for unblanched salted roasted peanuts, *International Journal of Food Science and Technology,* **28**, 193-199.

Fersht, A.W.H., 1984. Enzyme Structure and Mechanism, pp. 303-317. Freeman and Co, New York.

Garcia, J.M., Agar, I.T. and Streif, J. 1994. Lipid characteristics of kernels from different hazelnut varieties, *Tr. J. Agric. and Forestry*, **18**, 199-202.

Godfrey, T. and West, S., 1996. Industrial Enzymology, 212-242. 2nd edition, Stockton Press, NY, USA.

Gordillo, M.A., Obradors, N., Montesinos, J.L., Valero, F., Lafuente, J. and Sola, C., 1995. Stability studies and effect of initial oleic acid concentration on lipase production by Candida rugosa, *Applied Microbiology and Biotechnology*, **43 (1)**, 38-41.

Gosling, P. and Ross, J.D., 1981. Peroxidase levels in the cotyledons of hazel seed (Corylus Avellana), *Phytochemistry*, **20**, 31-33.

Grosch, W., Laskawy, G. and Senser, F., 1983. Storage stability of roasted hazelnuts *CCB Review for Chocolate, Confectionery and Bakery*, **8 (3)**, 21-23.

Guenes, B. and Bayindirli, A., 1993, Peroxidase and lipoxygenase inactivation during blanching of green beans, green peas and carrots, *Lebensmittel Wissenschaft und Technologie*, **26** (5) 406-410.

Hadorn, H., Keme, T., Kleinert, J. and Zürcher, K., 1977. The behaviour of hazelnuts under different storage conditions, *Rev. Choc., Confec., Bakery*, **2**, 25-36.

Hadorn, H., Keme, T., Kleinert, J., Messerli, M., and Zürcher, K., 1978. [Storage trials and quality tests on hazelnuts.] Lagerungsversuche und Qualitaetsprüfungen an Haselnüssen, *Gordian*, **78 (10)**, 300, 302-304, 306, 308, 310, **78 (11)**, 342-346, 348.

Hadorn, H., and Zürcher, K., 1977. Nachteilige Veraenderungen von Haselnuessen waehrend der Lagerung, [Deterioration of hazelnuts during storage.], *Gordian*, **77 (5)**, 114, 116, 118, 120.

Hassanien, F.R. and Mukherjee, K.D., 1986. Isolation of lipase from germinating oilseeds for biotechnological processes, *JAOCS*, **63 (7)**, 893-897.

Henderson, J. and Osborne, D.J., 1991. Lipase activity in ripening and mature fruit of the oil palm, Stability in vivo and in vitro, *Phytochemistry*, **30 (4)**, 1073-1078.

Hendrickx, M., Ludikhuyze, L., Broeck, I. van den and Weemaes, C., 1998. Effects of high pressure on enzymes related to food quality, *Trends in Food Science & Technology*, **9 (5)**, 197-203.

Heperkan, D., Aran, N. and Ayfer, M., 1994. Mycoflora and aflatoxin contamination in shelled pistachio nuts. *Journal of the Science of Food and Agriculture*, **66 (3)** 273-278.

Ho, S.Y., Mittal, G.S. and Cross, J.D., 1997, Effects of high field electric pulses on the activity of selected enzymes, *Journal of Food Engineering*, **31 (1)**, 69-84.

Huang, H. C. and Moreau, R. A., 1978. Lipases in the storage tissues of peanut and other oil seeds during germination, *Planta*, **141**, 111-116.

Huystee, R.van, Breda, C., Sesto, P., Beopouolos, N. and Esnault, R., 1990. Measurement and detection of peroxidase, *Plant Science*, **69**, 19-26.

Ishikawa, H., Shimoda, M., Kawano, T. and Osajima, Y., 1995. Inactivation of enzymes in an aqueous solution by micro-bubbles of supercritical carbon dioxide, *Bioscience, Biotechnology and Biochemistry*, **59 (4)**, 628-631,

Ishikawa, H., Shimoda, M., Yonekura, A. and Osajima, Y., 1996. Inactivation of enzymes and decomposition of alpha-helix structure by supercritical carbon dioxide microbubble method, *Journal of Agricultural and Food Chemistry*, **44 (9)**, 2646-2649.

Jacks, T.J., Yatsu, L.Y., 1974. Phosphate-inhibition of lipase activity in peanuts, *Journal of the American Oil Chemists' Society,* **51 (3)**, 112-113.

Jiaxun, Tao., Rao, R. and Liuzzo, J., 1993. Microwave heating for rice bran stabilization, *Journal of Microwave Power & Electromagnetic Energy,* **28 (3)**, 156-164.

Jung, M.Y., Bock, J.Y., Back, S.O., Lee, T.K. and Kim, J.H., 1997. Pyrazine contents and oxidative stability of roasted soybean oils, *Food Chemistry*, **60 (1)**, 95-102.

Keme, T. and Messerli, M., 1976. Detection of topogrphy of enzymes in hazelnuts, *CCB Review for Chocolate, Confectionery and Bakery,* **1 (4)**, 7-8.

Keme,T., Messerli, M., Shejbal, J. and Vitali,F., 1983. The storage of hazelnuts at room temperature and nitrogen (1) and (2), *CCB Review for Chocolate, Confectionery and Bakery*, **8 (1)**, 24-28 and **8 (2)**, 15-20.

Kermasha, S., Khalyfa, A., Alli, I. and Lee, B., 1991. Purification and characterisation of lipoxygenase iso-enzymes from canola (Brassica napus cv, Westar) seed, *Journal of Food Biochemistry*, **15**, 219-238.

Kermasha, S., Bisakowski, B., Ramaswamy, H. and Voort, F. van de, 1993. Comparison of microwave, conventional and combination heat treatments on wheat germ lipase activity, *International Journal of Food Science & Technology*, **28 (6)**, 617-623.

King, A.D. Jr., Halbrook, W.U., Fuller, G. and Whitehand, L.C., 1983. Almond nutmeat moisture and water activity and its influence on fungal flora and seed composition, *Journal of Food Science*; **48 (2)** 615-617.

Klingler, R.W., 1994. [Stabilization of wheat germ by microwave heating.] Stabilisierung von Weizenkeimen durch Mikrowellenerwaermung, *Getreide, Mehl und Brot*, **48 (6)**, 18-21.

Külünkoğlu, Ö., 1996. Turkish hazelnut and Fiskobirlik. *Acta Horticulturerae*. 445 pages, 347-354.

Labuza, T.P., 1971. Kinetics of lipid oxidation in foods, *CRC Critical Reviews in Food Technology*, 355-405.

Labuza, T.P., 1982. Shelf Life Dating of Foods. Food and Nutrition Press.Westport, Connecticut.

Lee, F.A., 1983. Basic Food Chemistry, pp 199-224. 2nd edition. The AVI Publishing Company, Inc. Westport, Connecticut.

Ling, A. and Lund, D.B., 1978. Determining kinetic parameters for thermal inactivation of heat-resistant and heat-labile iso-enzymes from thermal

destruction curves, *Journal of Food Science*, **43**, 1307-1310.

List, G.R., Mounts, T.L., Lanser, A.C. and Holloway, R.K., 1990. Effect of moisture, microwave heating, and live steam treatment on phospholipase D activity in soybeans and soy flakes, *Journal of the American Oil Chemists' Society*; **67 (11)**, 867-871.

Liukonnen, K., Johnson, T. and Laakso, S., 1995. Alkaline sensitivity of lipase activity in oat flour: factors contributing to inhibition, *Journal of Cereal Science*, **21 (1)**, 79-85.

Lopez, A., Pique, M.T., Boatella, J., Parcerisa, J., Romero, A., Ferran, A. and Garcia, J. 1997a. Influence of drying conditions on the hazelnut quality, I.Lipid oxidation, *Drying Technology*, **15 (3/4)**, 965-977.

Lopez, A., Pique, M.T., Ferran, A., Romero, A., Boatella, J. and Garcia, J., 1997b. Influence of drying conditions on the hazelnut quality, II.Enzymatic activity, *Drying Technology*, **15 (3/4)**, 979-988.

Majerus, P., Otteneder, H. and Hower, C., 1989. [Occurrence of ochratoxin A in porcine blood serum.] Beitrag zum Vorkommen von Ochratoxin A in Schweineblutserum, *Deutsche Lebensmittel Rundschau*, **85 (10)** 307-313.

Maskan, M, and Karatas, S., 1998. Fatty acid oxidation of pistachio nuts stored under various atmospheric conditions and different temperatures, *Journal of the Science of Food and Agriculture*, **77 (3)** 334-340.

Maté, J. I., Saltveit, M. E. and Krochta, J. M., 1996. Peanut and walnut rancidity: effect of oxygen concentration and relative humidity, *Journal of Food Science*, **61 (2)**, 465-468, 472.

Mattson, F.H., 1989. A changing role for dietary monounsaturated fatty acids, *J. of the American Dietetic Association*, **89**, 387-391.

Mehlenbacher, S.A. and David, C.S., 1988. Heritability of ease of hazelnut pellicle remonval, *Hortscience*, **23 (6)**, 1053-1054.

Mehlenbacher, S.A., 1990. Hazelnuts (Corylus) in Genetic resources of temperate fruit and nut crops. 1. Moore, J.N. and Ballington, J.R., Jr [Ed.], Wageningen, the Netherlands, *International Society for Horticultural Science.*

Mehlenbacher, S.A., 1991. Hazelnuts (Corylus), *Acta Horticulture*, **290**, 791-836.

Mehlenbacher, S.A., Smith, D.C. and Brenner, L.K., 1993, Variance components and heritability of nut and kernel defects in hazelnut. *Plant Breeding*; **110 (2)** 144-152.

Metwalli, S.M., El-Sebaiy, L.A. and Noaman, M.A., 1983. Effect of roasting on lipase activity and organoleptic quality of sweet almond and hazelnut, *J.Agric. Res. Tanta Univ.*, **9 (2)**, 1115-1125.

Mitchell, Jr. J.H. and Malphrus, R.K., 1977. Lipid oxidation in Spanish peanuts: the effect of moist heat treatment, *Journal of Food Science,* **42 (6)**, 1457-1461.

Mosmuller, E.W.J., van Heemst, J.D.H., van Delden, C.J., Franssen, M.C.R. and Engbersen, J.F.J., 1992. A new spectrophotometric method for the detection of lipase activity using 2,4-dinitrophenyl butyrate as a substrate, *Biocatalysis,* **5**, 279-287.

Möller, A.C., Pelkmans, W.C.M., Ponne, C.T. and Meijer, M.M.T., 1994. Inactivation kinetics of lipase in rapeseed, *Med. Fac. Landbouw. Univ. Gent.*, **59/4a,** 1841-1843,

Munshi, S.K., Bhatia, N., Sekhon, B.S., and Sukhija, P.S., 1993. Inactivation of rice bran lipase with metal ions, *Journal of Chemical Technology and*

Biotechnology, **57 (2)**, 169-174.

Muto, K. and Beevers, H. 1974. Lipase activities in castor bean endosperm during germination, *Plant Physiology*, **54**, 23-28.

Naesens, W., 1982. Lipase activity in a low moisture food model system, Katholieke Universiteit te Leuven, 217.

Naveh, D., Mizrahi, S. And Kopelman, I.J., 1982. Kinetics of peroxidase deactivation in blanching of corn on the cob, *Journal of Agricultural and Food Chemistry*, **30** (5), 967-970.

Nicolosi, R.J., Stucchi, A.F., Kewal, M.C., Hensesig, L.K., Hegstein, P.M. and Schefer, E.J., 1990. Effect of dietary fat saturation and cholesterol on LDL composition and metabolism, *Arterisclerosis*, **10**, 119-128.

O'Keefe, S.F., Wiley, V.A. and Knauft, D.A., 1993. Comparison of oxidative stability of high and normal oleic peanut oils, *JAOCS*, **70**, 489-92.

Ory, R.L., Angelo, A.J. St., Gwo, Y.Y., Flick, G.J., and Jr, Mod, R.R., 1984. Oxidation-induced changes in foods, *Food Technology,* **38 (3)**, 49-50.

Owasu, R.K., Makhzoum, A. and Knapp, J.S., 1992. Heat inactivation of lipase from Pseudomanas fluorescens P38: Activation parameters and enzyme stability at low or ultra-high temperatures, *Food Chemistry,* **44**, 261-268.

Özdemir, F., Topuz, A., Doğan, Ü., ve Karkacier, M., 1998a. Fındık çeşitlerinin bazı fiziksel ve kimyasal özellikleri, *Gıda,* **23(1)**, 37-41.

Özdemir, M., Seyhan, F. & Özay, G., 1998b. Hasattan Ambalaja Fındık İşlemenin Kritik Kontrol Noktalarında Tehlike Analizi . pp 39. TUBİTAK yayınları, Gebze-Kocaeli.

Özdemir, M. and Devres, O., 1999. Turkish hazelnuts: properties and effect of microbiological and chemical changes on quality, *Food Review Int.*, **15(3)**, 309-333.

Pala, M., Açkurt, F., Löker, M., Yıldız, M. ve Ömeroğlu, S., 1996. Fındık çeşitlerinin bileşimi ve beslenme fizyolojisi açısından değerlendirilmesi, *Tr. J. of Agriculture and Forestry,* **20**, 43-48.

Parcerisa, J., Boatella, J., Codony, R., Farran, A., Garcia, J., Lopez, A., Rafeces, M. and Romero, A., 1993a. Influence of variety and geographical origin on the lipid fraction of hazelnuts (Corylus avellane L.) from Spain: I. Fatty acid composition, *Food Chemistry*, **48**, 411-414.

Parcerisa, J., Rafeces, M., Castellote, A.I., Codony, R., Farran, A., Garcia, J., Lopez, A., Romero, A. and Boatella, J., 1993b. Influence of variety and geographical origin on the lipid fraction of hazelnuts (Corylus avellane L.) from Spain: II.Triglyceride composition, *Food Chemistry*, **50**, 245-249

Parcerisa, J., Boatella, J., Codony, R., Rafeces, M., Castellote, A.I., Garcia, J., Lopez, A. and Romero, A., 1995a. Comparison of fatty acid and triacylglycerol compositions of different hazelnut varieties (Corylus avellane L.) cultivated in Catalonia (Spain), *J.Agric. Food Chem.*, **43**, 13-16.

Parcerisa, J., Rafeces, M., Castellote, A.I., Codony, R., Farran, A., Garcia, J., Lopez, A., Romero, A. and Boatella, J. 1995b. Influence of variety and geographical origin on the lipid fraction of hazelnuts (Corylus avellane L.) from Spain: III.Oil stability, tocopherol content and some mineral contents, *Food Chemistry*, **53**, 71-74.

Parcerisa, J., Richardson, D.G., Rafeces, M., Codony, R., ve Boatella, J., 1997. Fatty acid distribution in polar and nonpolar lipid classes of hazelnut oil (Corylus avellane L.), *J. Agric. Food Chemistry*, **45**, 3887-3890.

Parcerisa, J., Richardson, D.G., Rafeces, M., and Jboatella, J., 1998. Fatty acids, tocopherol and sterol content of some hazelnut varieties (Corylus avellane L.) harvested in Oregon (USA), *J. Chromotography A.*, **805**, 259-268.

Paster N., Pushinsky, A., Menasherov, M. and Chet, H., 1992. Inhibitory effect of *Aspergillus niger* on the growth of *Aspergillus ochraceus* and *Aspergillus flavus*, and on aflatoxin formation, *Journal of the Science of Food and Agriculture*, **58 (4),** 589-591.

Pattee, H.E., Singleton, J.A. and Johns, E.B., 1971. Effect of storage time and conditions on peanut volatiles, *J. Agr. Food Chem.*, **19 (1)**, 134-137.

Pattee, H.E., Young, C.T. and Oupadissakoon, C., 1985. Peanut quality: effects of cultivar, growth, environment and storage in *Evaluation of Quality in Fruits and Vegetables,* H.E. Pattee (Ed.) AVI Publishing Company, Inc., Westport, Connecticut.

Pershern, A.S., Breene, W.M. and Lulai, E.C. 1995. Analysis of factors influencing lipid oxidation in hazelnuts (Corylus sp.), *J. Food Processing and Preservation*, **19**, 9-25.

Piffaut, B. and Metche, M., 1991. Proeperties of peroxidase and polyphenol oxidase in natural complexes from walnuts (Junglans regia) and in active DL-DOPA copolymers, *Journal of Food Science,* **57**, 493-506.

Pluyer, H.R., Ahmed, E.M. and Wei, C.I., 1987. Destruction of aflatoxins on peanuts by oven- and microwave-roasting, *Journal of Food Protection,* **50 (6)**, 504-508.

Ponne, C., Möller, A.C., Pelkmans, Tijkens, L.M., Bartels, P.V. and Meijer, M.M.T., 1996. Influence of microwave and steam heating on lipase activity and microstructure of rapeseed (Brassica napus), *J. Agric. Food Chem,* **44**, 2818-2824.

Pour-el, A., Nelson, S.O., Peck, E.E., Tjhio, B., and Stetson, L.E. 1981. Biological properties of VHF- and microwave-heated soybeans, *Journal of Food Science*, **46**, 880-885, 895.

Prado-Filho, L.G., 1994. Equilibrium relative humidity and lipid oxidation in Brazil-nut, macadamia nut and soybean seed flours, *Sci. Agric. Piracicaba,* **51 (2)**, 357-362.

Purr, A., 1971, Qualitatserhaltung und Verderb: Lipatische aktivitat von tierichen und pflanzlichen geweben mikroorganismen, ihr einfluss bei der herstellung und lagerung von Lebensmittel, *Gordian*, **71 (3 &4)**, 116-119, 150-153, 196-197.

Ragnarsson J.O. and Labuza, T.P., 1977. Accelerated shelf life testing for oxidative rancidity in foods - a review. *Food Chemistry,* **2 (4),** 291-308.

Ramesh, M., Haridas-Rao, P., Ramadoss, C.S., 1995. Microwave treatment of groundnut (Arachis hypogaea): extractability and quality of oil and its relation to lipase and lipoxygenase activity, *Lebensmittel, Wissenschaft und Technologie*; **28 (1)**, 96-99,

Reed, G., 1975, Enzymes in Food Processing, 2nd Ed., Academic Press. New York.

Richardson, D.G., 1996. The health benefit of eating hazelnuts: implications for blood lipid profiles, coronary heart disease, and cancer risks, *Acta Horticulturae,* **445**, 295-300.

Robinson, D.S., 1991. Peroxidase and their significance in fruits and vegetables in Food Enzymology, P.F. Fox(ed) Elsevier Applied Science, London and New York, **vol 1**, 636.

Rodríguez-López, J.N., Espín, J.C., Tuleda, J., Martínez, V., Cerda, A. and

García-Cánovas, F., 2000. Purification and kinetic characterisation of peroxidase from tomato cultivated under different salinity conditions, *Journal of Food Science,* **65 (1)**, 15-19.

Ruggeri, S., Cappelloni, M., Gambelli, L., Nicoli, S. and Carnovale, E., 1998. Chemical composition and nutritive value of nuts grown in Italy, *Ital. J. Food Sci.,* **3 (10)**, 243-252.

Sabate, J., Fraser, G.E., Burke, K., Knutsen, S.F., Bennett, H. and Lindsted, K.D., 1993. Effects of walnuts on serum lipid levels and blood pressure in normal men, *The New England J. Medicine,* **328**, 603-607.

Sabır, S., Anderson, J. , Marti, J. and Calcagni, P., 2000. Hazelnut. *The Cracker,* **3**, 38-39.

Saklar, S., 1999. Optimization of hazelnut roasting process by using response surface methodology. *PhD Thesis*, METU, Ankara, Türkiye.

Samant, S.K., Rege, D.V., 1990. Some enzymes and enzyme inhibitors from charoli and cashew nut, *Journal of Food Science and Technology, India,* **27 (4)**, 231-232.

Sanchis, V., Quilez, M.L., Viladrich, R., Vinas, I. and Canela, R., 1988. Hazelnuts as possible substrate for aflatoxin production, *J. Food Protection*, **51**, 289-292.

Sanders, T.H., Pattee, H.E. and Singleton, J.A., 1975. Lipoxygenase isozymes of peanut, *Lipids,* **10 (11)**, 681-685.

Sanders, T.H., Vercellotti, J.R. and Grimm, D.T., 1993. Shelf life of Peanuts and peanut products in *Shelf Life Studies of Foods and Beverages*, Edited by Charalambous, G., Elsevier Sc. Publishers B.V., Amsterdam, Netherlands, 289-309.

Sanders, T.H., and Pattee, H.E., 1975. Peanut alkaline lipase, *Lipids,* **10 (1)**, 50-54.

Sanz, L.C. and Olias, J.M., 1990. Characterisation of lupin seed lipase, *Food Chemistry*. **37 (3)**, 221-228.

Sattar, A., deMan, J.M. and Alexander, J.C., 1976. Effect of wavelength on light induced quality deterioration of edible oils and fats, *Can. Inst. Food Sci. Technol. J.,* **9 (3)** 108-113.

Sattar, A., Jan, M., Ahmad, A., Hussain, A. and Khan, I., 1989. Light induced oxidation of nut oils, *Die Nahrung*, **33 (2)**, 213-215.

Savage, G.P., McNEil, D.L. and Dutta, P.C., 1997. Lipid composition and oxidative stability of oils in Hazelnut (Corylus avellane L.) grown in New Zealand, *JAOCS*, **74 (6)**, 755-759.

Schmid, R.D. and Verger, R., 1998. Lipases: Interfacial enzymes with attractive applications, *Angew. Chem. Int. Ed.*, **37**, 1608-1633.

Senter, S.D., Forbus, W.R.Jr., Nelson, S.O. and Horvat, R.J., 1984a. Effect of dielectric and steam heating treatments on the pre-storage and storage characteristics of pecan kernel, *Journal of Food Science*, **49**, 1532-1534.

Senter, S.J., Forbus, W.R.Jr., Nelson, S.O., Wilson, R.L.Jr. and Horvat, R.J., 1984b. Effects of dielectric and steam heating treatments on the storage stability of pecan kernels, *Journal of Food Science,* **49 (3)**, 893-895

Sessa, D.J. and Anderson, R. L., 1981. Soybean peroxidases: purification and some properties. *Journal of Agricultural and Food Chemistry*, **29 (5)** 960-965.

Shewfelt, A.L. and Young, C.T., 1977. Storage stability of peanut-based foods: a review, *Journal of Food Science,* **42 (5)**, 1148-1152, 30 ref.

Stone, D. 2000. Health benefits of hazelnut. *Ceral Foods World.* **45 (9)**, 424-426.

Sturm, W., 1979. Praktische Qualitaetsprüfungen an Haselnüssen. I. Sichtbarmachen ranziger roher Kernel, *Deutsche-Lebensmittel-Rundschau*, **75 (3)**, 85-88.

Svenson, S. 1977. Inactivation of enzymes during thermal processing in *Physical, Chemical and Biologycal Changes in Foods Caused by Thermal Processing,* Hoyem, T and Kvale, O. pp 202-217. Applied Science Pub. Lim., London.

Swaisgood, H.E. and Bozoğlu, F., 1984. Heat inactivation of the extracellular lipase from *Pseudomonas fluorescens* MC50. *Journal of Agricultural and Food Chemistry*; **32** (1), 7-10.

Şimşek, A. and Aslantaş, 1999. Fındığın bileşimi ve insan sağlığı açısından önemi, *Gıda*, **24** (3), 209-216.

Thomson, C.A.; Delaquis, P.J. and Mazza, G., 1999., Detection and measurement of microbial lipase activity: A review, *Critical Reviews in Food Science and Nutrition,* **39**(2), 165-187.

Tijskens, L.M.M. 2001. Personal communication. ATO , P.O.Box 1700, 6700 AA Wageningen, the Netherlands.

Tijskens, L.M.M., Greiner, R., Biekman, E.S.A. and Konietzny, U., 2001. Modelling the effect of temperature and pH on the activity of enzymes: the case of phytases. *Accepted Biotechnology and Bioengineering.*

Tijskens, L.M.M., Hertog, M.L.A.T.M. and van Dijk, C., 1999a. GESSI: a Generic Enzyme System for Stimulation and Inactivation during storage and processing, *Proceedings Second International Conference* AGRI-FOOD QUALITY II (Hägg M., Ahvenainen R., Evers A.M., Tiilikkala K. eds) p. 81-86, 22-25 April 1998, Turku, Finland.

Tijskens, L.M.M., Rodis, P.S., Hertog, M.L.A.T.M., Proxenia N. and van Dijk C., 1999b. Activity of pectin methyl esterase during blanching of peaches, *J. Food Eng.*, **2**, 167-177.

Tijskens, L.M.M., Rodis, P.S., Hertog, M.L.A.T.M., Kalantzi, U. and van Dijk, C., 1998. Kinetics of polygalacturonase activity and firmness of peaches during storage, *J. Food Eng.*, **35**, 111-126.

Tijskens, L.M.M. and Rodis, P.S. 1997a. Kinetics of enzyme activity in peaches during storage and processing, *Food Technol. and Biotechnol.*, **35**, 45-50.

Tijskens, L.M.M., Rodis, P.S., Hertog, M.L.A.T.M., Waldron, K.W., Ingham, L., Proxenia, N. and van Dijk, C., 1997b. Activity of peroxidase during blanching of peaches, carrots and potatoes, *Journal of Food Engineering,* **34**, 355-370.

Tijskens, L.M.M., Waldron, K.W., Ng A., Ingham L., and van Dijk, C., 1997c. The kinetics of pectin methyl esterase in potatoes and carrots during blanching, *J. Food Eng.*, **34**, 371-385.

Topal, Ş. ve Aran, N., 1987. Bazı yağlı tohumların küf florası ve taşıdığı riskler, *E.Ü. Mühendislik Fakültesi, B. Gıda Mühendisliği*, **5 (2)**, 47-61.

TS-1917, 1993. Prossed Hazelnut Kernels, *Türk Standartları Enstitüsü,* Ankara.

TS-3075, 1978. Decorticated Hazelnuts, *Türk Standartları Enstitüsü,* Ankara.

TS-10937, 1993. Hazelnut Meal, *Türk Standartları Enstitüsü,* Ankara.

Vercet, A., Lopez, P. and Burgos, J., 1997. Inactivation of heat-resistant lipase and protease from Pseudomonas fluorescens by manothermosonication, *Journal of Dairy Science,* **80 (1)**, 29-36.

Vetrimani, R., Haridas-Rao, P., 1990 Studies on stabilization of wheat bran, *Journal of Food Science and Technology, India,* **27 (6)**, 332-335.

Vetrimani,R., Jyothirmayi,N., Haridas-Rao, P., Ramadoss, C.S., 1992. Inactivation of lipase and lipoxygenase in cereal bran, germ and soybean by microwave treatment, *Lebensmittel, Wissenschaft und Technologie*, **25 (6)**, 532-535.

Villarroel, M., Biolley, E., Schneeberger, R., Ballester, D. and Ramirez, S., 1987. Amino acid composition of Chilean hazelnuts, *Food Chemistry*, **25**, 155.

Whitaker, R., 1994. Principles of Enzymology for the Food Sciences (2nd Ed.). Marcel Dekker, Inc. New York, USA.

Wong, D.W.S., 1995. Lipoxygenase, Chp 8. in *Food Enzymes Structure and Mechanism,* 237-270.Chapman & Hall.

Woodroof, J.G., 1975. Tree Nuts, Production, Processing, Products. 2nd ed. Avi Publishing Company Inc., Westport, Connecticut.

Yanniotis, S. and Zarmboutis, I., 1996. Water sorption isotherms of pistachio nuts. *Lebensmittel Wissenschaft und Technologie,* **29 (4)** 372-375.

Yemenicioğlu, A, Özkan, M., Velioğlu, S. and Cemeroğlu, B., 1998. Thermal inactivation kinetics of peroxidase and lipoxygenase from fresh pinto beans (Phaseolus vulgaris), *Z. Lebensm. Unters Forsch.,* **206**, 294-296.

Zürcher, K. and Hadorn, H., 1976. Qualitätsbeurteilung von Haselnusskernen *Kakao+Zucker,* **1&2**, 3-8, 26-34.

APPENDIX A. PROTEIN CONTENT DETERMINATION OF ENZYME EXTRACTS

Protein content of the enzyme extracts were determined using Pierce Coomassie Plus Protein Assay Reagent. Pierce Pierce Coomassie Plus Protein Assay Reagent is quick and ready-to-use modification of the well-known Bradford (1976) dye–binding, colorimetric method for total protein quantitation. This Bradford reagent modification greatly reduces the tendency of Comassie dye-containing reagents to give a characteristically nonlinear response curve. Pierce Coomassie Reagent results in a substantially more linear response curve in a defined range of protein concentration. This method involves the binding of Coomassie Brillant Blue G-250 to protein. The binding of the dye to the protein causes a shift in the absorption maximum of the dye from 465 to 595 nm, and it is the increase in absorption at 595 which is monitored. The assay is reproducible and rapid with the dye binding process virtually complete in approximatelly 2 min with good color stability for 1 hr. There is little or no interference from cations such as sodium or potassium nor from carbohydrates such as sucrose.

The amount of the proteins determined in enzyme extracts are calculated by using the calibration curves given in Figures A.1. and A.2. and the results are given in Table 1.

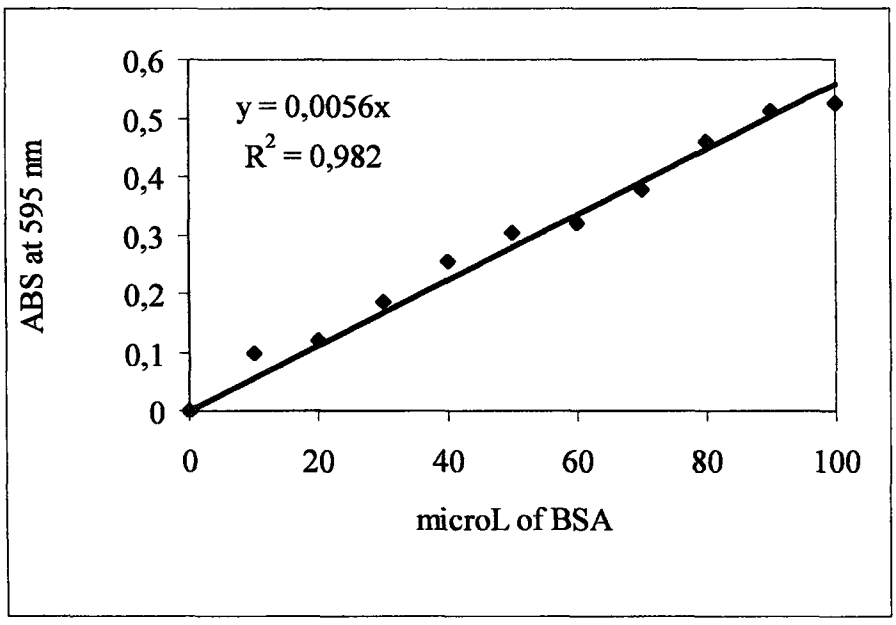


Figure A. 1. Calibration curve for protein determination of enzyme extracts used for pH and temperature dependence of lipase and peroxidase

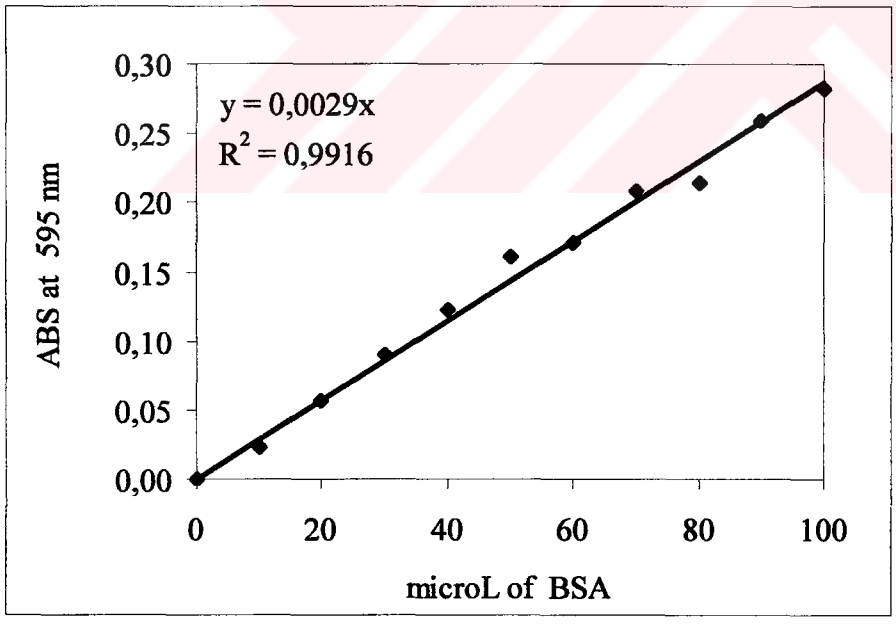


Figure A. 2. Calibration curve for protein determination of enzyme extracts used for lipase inactivation studies.

Table A. 1. Amount of proteins determined in enzyme extracts used for the activity measurements.

Enzyme Extract	Absorbance	μg protein	mg protein in 1 ml enzyme extract
pH profile[a]	0.248	44.24	0.442
Temperature profile[a]	0.204	36.34	0.363
80°C-15 min[b]	0.109	37.59	0.376
120°C-15min[b]	0.033	11.38	0.114
100°C-5min[b]	0.048	16.55	0.166
100°C-10min[b]	0.061	21.03	0.210
100°C-15min[b]	0.042	14.48	0.145
100°C-20min[b]	0.05	17.24	0.172
Control [b]	0.042	14.48	0.145

a : determined using the calibration curve given in Figure A. 1
b : determined using the calibration curve given in Figure A. 2

APPENDIX B. SYNTHESIS OF DNPB

DNPB were synthesized as explained by Mosmuller et al.(1992). It was synthesized by slowly adding 5.74 g butyryl chloride to stirred mixture of 7.1 g 2,4-dinitrophenol and 4.68 g triethylamine in 100 ml dichloromethane. The reaction mixture was stirred at room temperature for one hour, was then washed several times with sodium bicarbonate solution and dryied on sodium sulfate. After solvent removal 6.96 g 2,4-dinitrophenyl butyrate was obtained as having a bad smell, yellow liquid, bp. 162°C (0.2 mm Hg). In order to obtain a product which is completely free of dinitropenol it is essential to use excess of butyryl chloride. The product can be stored at room temperature without any detectable decomposition. It is advised caution in using the DNPB with laboratory gloves since the component is toxic.

APPENDIX C. SPSS OUT PUTS FOR INACTIVATION KINETICS

C1. SPSS OUT PUT FOR THE DATA OF INACTIVATION OF PEROXIDASE AT 60°C

NLR-60-POD

All the derivatives will be calculated numerically.

The following new variables are being created:

Name	Label
PRED_	Predicted Values
RESID	Residuals

Iteration	Residual SS	A1	A2	KD1	KD2
1	862,9724387	10,0000000	1,00000000	,000100000	,001000000
1.1	9871,514215	65,5012066	-61,648146	,002161243	,047461992
1.2	654,6487609	11,4179004	-7,7748595	,000315537	,014345468
2	654,6487609	11,4179004	-7,7748595	,000315537	,014345468
2.1	196,8501332	8,61098781	-7,5604573	,000680207	,012182065
3	196,8501332	8,61098781	-7,5604573	,000680207	,012182065
3.1	5,110336443	3,18304318	0,457490634	,000909445	,010813209
4	5,110336443	3,18304318	0,457490634	,000909445	,010813209
4.1	5,3334E+35	1,41601926	2,52101289	,000746449	-,02792193
4.2	,4063514835	2,11340595	1,95128463	,000994735	,008947878
5	,4063514835	2,11340595	1,95128463	,000994735	,008947878
5.1	2,637390836	1,17098002	2,77816254	,000552604	,002631216
5.2	,3143476603	1,91673086	2,11015300	,000986448	,007999655
6	,3143476603	1,91673086	2,11015300	,000986448	,007999655
6.1	,2848779010	1,71719813	2,28440831	,000896043	,006775585
7	,2848779010	1,71719813	2,28440831	,000896043	,006775585
7.1	,2474919453	1,42194330	2,57233433	,000684617	,006015633
8	,2474919453	1,42194330	2,57233433	,000684617	,006015633
8.1	,2316379323	1,03528223	2,94169799	,000367004	,005095395
9	,2316379323	1,03528223	2,94169799	,000367004	,005095395
9.1	,2280540940	1,10367427	2,87891905	,000391854	,005353759
10	,2280540940	1,10367427	2,87891905	,000391854	,005353759
10.1	,2280258633	1,09420392	2,88764578	,000383775	,005340776
11	,2280258633	1,09420392	2,88764578	,000383775	,005340776
11.1	,2280258418	1,09459510	2,88730907	,000384065	,005341901
12	,2280258418	1,09459510	2,88730907	,000384065	,005341901
12.1	,2280258417	1,09456894	2,88733099	,000384044	,005341824

Run stopped after 27 model evaluations and 12 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1,000E-08

Nonlinear Regression Summary Statistics
Dependent Variable: PODNKAT

Source	**DF**	**Sum of Squares**	**Mean Square**
Regression	4	46,63695	11,65924
Residual	8	0,22803	0,02850
Uncorrected Total	12	46,86498	
(Corrected Total)	11	13,11670	

R squared = 1 - Residual SS / Corrected SS = 0,98262

Parameter	**Estimate**	**Asymptotic Std. Error**	**Asymptotic 95 % Confidence Interval**	
			Lower	Upper
A1	1,094568944	0,477607638	-0,006796244	2,195934133
A2	2,887330987	0,460129771	1,826269831	3,948392142
KD1	0,000384044	0,000412000	-0,00056603	0,001334118
KD2	0,005341824	0,001323636	0,002289513	0,008394134

Asymptotic Correlation Matrix of the Parameter Estimates

	A1	A2	KD1	KD2
A1	1,0000	-0,9467	0,9662	0,9049
A2	-0,9467	1,0000	-0,9256	-0,7749
KD1	0,9662	-0,9256	1,0000	0,8298
KD2	0,9049	-0,7749	0,8298	1,0000

C2. SPSS OUT PUT FOR THE DATA OF LIPASE INACTIVATION AT 60°C

NLR-LIP-60°C

All the derivatives will be calculated numerically.

The following new variables are being created:

Name	Label
PRED	Predicted Values
RESID	Residuals
D.A1	d(Pred)/dA1
D.AF	d(Pred)/dAF
D.KD	d(Pred)/dKD

Iteration	Residual SS	A1	AF	KD
1	0,0052838499	0,100000000	,100000000	,001000000
1.1	0,0221628695	0,048874413	,192185544	,003021351
1.2	0,0024955937	,116303743	,111613566	,001357529
2	0,0024955937	,116303743	,111613566	,001357529
2.1	0,0022040884	,101969440	,137477734	,002228103
3	0,0022040884	,101969440	,137477734	,002228103
3.1	0,0013475670	,108110128	,139059518	,003345505
4	0,0013475670	,108110128	,139059518	,003345505
4.1	0,0012425853	,112757241	,136498758	,003615015
5	0,0012425853	,112757241	,136498758	,003615015
5.1	0,0012423430	,112901073	,136581204	,003650160
6	0,0012423430	,112901073	,136581204	,003650160
6.1	0,0012423389	,112907170	,136599977	,003655070
7	0,0012423389	,112907170	,136599977	,003655070
7.1	0,0012423388	,112907823	,136602723	,003655742
8	0,0012423388	,112907823	,136602723	,003655742
8.1	0,0012423388	,112907909	,136603102	,003655834

Run stopped after 17 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1,000E-08

Nonlinear Regression Summary Statistics
Dependent Variable : LIP60

Source	DF	Sum of Squares	Mean Square	
Regression	3	0,29265	0,09755	
Residual	7	1,242339E-03	1,774770E-04	
Uncorrected Total	10	0,29389		
(Corrected Total)	9	0,01647		

R squared = 1 - Residual SS / Corrected SS = 0,92455

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
A1	0,112907909	0,012232645	0,083982299	0,141833519
AF	0,136603102	0,006956348	0,120153953	0,153052250
KD	0,003655834	0,001087265	0,001084860	0,006226808

Asymptotic Correlation Matrix of the Parameter Estimates

	A1	AF	KD
A1	1,0000	-0,4101	0,0825
AF	-0,4101	1,0000	0,6458
KD	0,0825	0,6458	1,0000

C3. SPSS OUT PUT FOR THE DATA OF INACTIVATION OF LIPASE AT 70°C

NLR-LIP-70°C

All the derivatives will be calculated numerically.

Iteration	Residual SS	A1	K1	AF
1	,2564541584	,100000000	,010000000	,010000000
1.1	1,068281857	,261197497	-,00055890	,115087730
1.2	,0897414162	,132747907	,000790978	,025662928
2	,0897414162	,132747907	,000790978	,025662928
2.1	,0420945422	,182068822	,001271005	,046757610
3	,0420945422	,182068822	,001271005	,046757610
3.1	,0135792277	,240411320	,003111527	,092677120
4	,0135792277	,240411320	,003111527	,092677120
4.1	,0091781249	,279336294	,004014085	,084652144
5	,0091781249	,279336294	,004014085	,084652144
5.1	,0091643330	,281864159	,004042891	,083572051
6	,0091643330	,281864159	,004042891	,083572051
6.1	,0091643199	,281830853	,004046857	,083641129
7	,0091643199	,281830853	,004046857	,083641129
7.1	,0091643196	,281825926	,004047433	,083650939
8	,0091643196	,281825926	,004047433	,083650939
8.1	,0091643196	,281825203	,004047517	,083652374

Run stopped after 17 model evaluations and 8 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1,000E-08

Nonlinear Regression Summary Statistics
Dependent Variable LIPKAT70

Source	DF	Sum of Squares	Mean Square
Regression	3	0,39279	0,13093
Residual	7	9,164320E-03	1,309189E-03
Uncorrected Total	10	0,40196	
(Corrected Total)	9	0,09710	

R squared = 1 - Residual SS / Corrected SS = 0,90562

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
A1	0,281825203	0,036303762	0,195980447	0,367669959
K1	0,004047517	0,001343729	0,000870103	0,007224931
AF	0,083652374	0,028016130	0,017404753	0,149899994

Asymptotic Correlation Matrix of the Parameter Estimates

	A1	K1	AF
A1	1,0000	-0,3206	-0,6389
K1	-0,3206	1,0000	0,8224
AF	-0,6389	0,8224	1,0000

C4. SPSS OUT PUT FOR THE DATA of INACTIVATION of LIPASE AT 80°C

NLR-LIP-80°C
All the derivatives will be calculated numerically.
The following new variables are being created:

Name Label
PRED_ Predicted Values
RESID Residuals

Iteration	Residual SS	A1	K1	AF
1	5,272933222	,100000000	,010000000	,010000000
1.1	,4845160461	1,36854031	,180695793	,333397723
2	,4845160461	1,36854031	,180695793	,333397723
2.1	2,7227283	1,83791581	-,49349693	,298785888
2.2	,2534669339	1,67022010	,144176345	,361675411
3	,2534669339	1,67022010	,144176345	,361675411
3.1	330,9939445	1,81335885	-,00304513	,318701403
3.2	,2241685274	1,70676423	,131339330	,359090020
4	,2241685274	1,70676423	,131339330	,359090020
4.1	,1527807210	1,75279401	,103178696	,348201685
5	,1527807210	1,75279401	,103178696	,348201685
5.1	,0832210106	1,82219076	,047615490	,303708617
6	,0832210106	1,82219076	,047615490	,303708617
6.1	,0342967837	1,85849786	,055560855	,268269166
7	,0342967837	1,85849786	,055560855	,268269166
7.1	,0336343734	1,86285003	,057310558	,268746543
8	,0336343734	1,86285003	,057310558	,268746543
8.1	,0336278878	1,86314196	,057506758	,269047819
9	,0336278878	1,86314196	,057506758	,269047819
9.1	,0336278378	1,86316408	,057524298	,269085098
10	,0336278378	1,86316408	,057524298	,269085098
10.1	,0336278374	1,86316596	,057525820	,269088464
11	,0336278374	1,86316596	,057525820	,269088464
11.1	,0336278374	1,86316612	,057525952	,269088756

Run stopped after 24 model evaluations and 11 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1,000E-08

Nonlinear Regression Summary Statistics
Dependent Variable LIPKAT80

Source	DF	Sum of Squares	Mean Square
Regression	3	6,04053	2,01351
Residual	7	,03363	4,803977E-03
Uncorrected Total	10	6,07415	
(Corrected Total)	9	3,15621	

R squared = 1 - Residual SS / Corrected SS = 0,98935

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
A1	1,863166124	,073412540	1,689573052	2,036759196
K1	,057525952	,005670390	,044117611	,070934293
AF	,269088756	,027418509	,204254285	,333923227

Asymptotic Correlation Matrix of the Parameter Estimates

	A1	K1	AF
A1	1,0000	0,0952	-0,3424
K1	0,0952	1,0000	0,4589
AF	-0,3424	0,4589	1,0000

C5. SPSS OUT PUT FOR THE DATA of INACTIVATION of LIPASE AT 90°C

NLR-LIP-90°C

All the derivatives will be calculated numerically.
The following new variables are being created:

Name	Label
PRED_	Predicted Values
RESID	Residuals

Iteration	Residual SS	A1	K1	AF
1	3,427871340	,100000000	,010000000	,010000000
1.1	340,3140969	-6,1964620	2,18442764	7,47398993
1.2	,8703841549	,648416306	1,17813934	,467019082
2	,8703841549	,648416306	1,17813934	,467019082
2.1	1,7027E+16	1,17623602	-1,8522487	,235449266
2.2	,6675973589	,822874314	1,02237954	,415793625
3	,6675973589	,822874314	1,02237954	,415793625
3.1	,2995826362	1,06444316	,482024685	,329793575
4	,2995826362	1,06444316	,482024685	,329793575
4.1	3,123364877	1,34361896	,052444318	,051148717
4.2	,2303402921	1,10163866	,408574172	,268973379
5	,2303402921	1,10163866	,408574172	,268973379
5.1	,1577775374	1,20385547	,283230044	,158035974
6	,1577775374	1,20385547	,283230044	,158035974
6.1	,0927855800	1,45177385	,202519003	-,05545876
7	,0927855800	1,45177385	,202519003	-,05545876
7.1	,0711000363	1,68995924	,152900663	-,30127709
8	,0711000363	1,68995924	,152900663	-,30127709
8.1	,0592930015	1,75900168	,152210111	-,37345755
9	,0592930015	1,75900168	,152210111	-,37345755
9.1	,0592929180	1,75940732	,152158840	-,37391980
10	,0592929180	1,75940732	,152158840	-,37391980
10.1	,0592929179	1,75943637	,152153096	-,37395307

Run stopped after 23 model evaluations and 10 derivative evaluations.
Iterations have been stopped because the relative reduction between successive residual sums of squares is at most SSCON = 1,000E-08

Nonlinear Regression Summary Statistics
Dependent Variable LIPKAT90

Source	DF	Sum of Squares	Mean Square
Regression	3	4,22649	1,40883
Residual	5	,05929	,01186
Uncorrected Total	8	4,28578	
(Corrected Total)	7	1,87974	

R squared = 1 - Residual SS / Corrected SS = ,96846

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
A1	1,759436372	0,382380634	0,776495660	2,742377085
K1	0,152153096	0,070525011	-0,029137217	0,333443409
AF	-0,373953075	0,415423895	-1,441834194	0,693928044

Asymptotic Correlation Matrix of the Parameter Estimates

	A1	K1	AF
A1	1,0000	-,9285	-,9759
K1	-,9285	1,0000	,9794
AF	-,9759	,9794	1,0000

C6. COMPARISION OF OBSERVED AND PREDICTED DATA FOR THE MODELS USED FOR INACTIVATION KINETICS

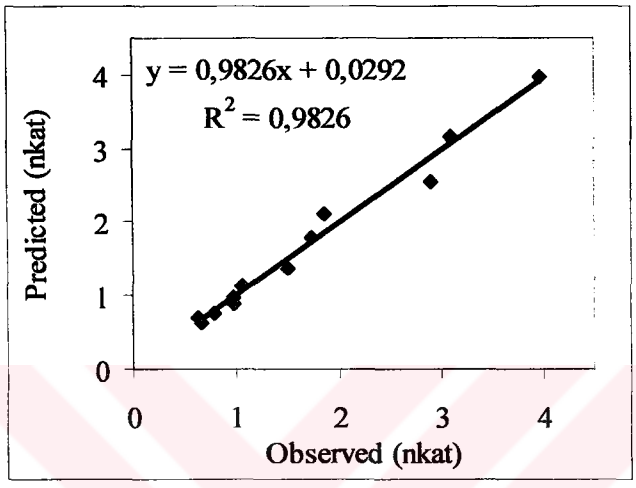


Figure C. 1. Comparision of observed and predicted data for the inactivation kinetics of POD at 60°C.

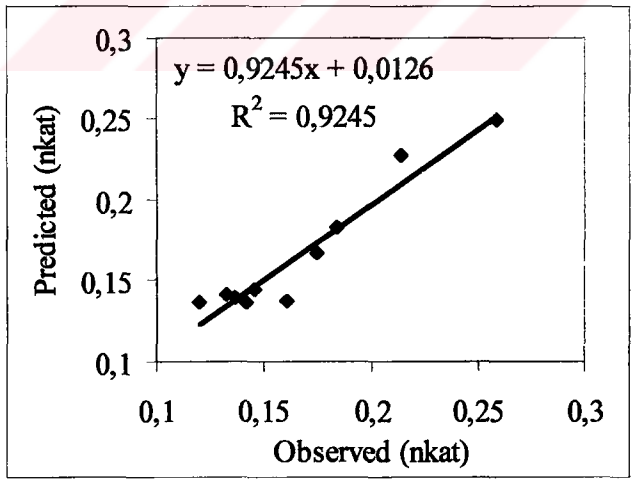


Figure C. 2. Comparision of observed and predicted data for the inactivation kinetics of lipase at 60°C.

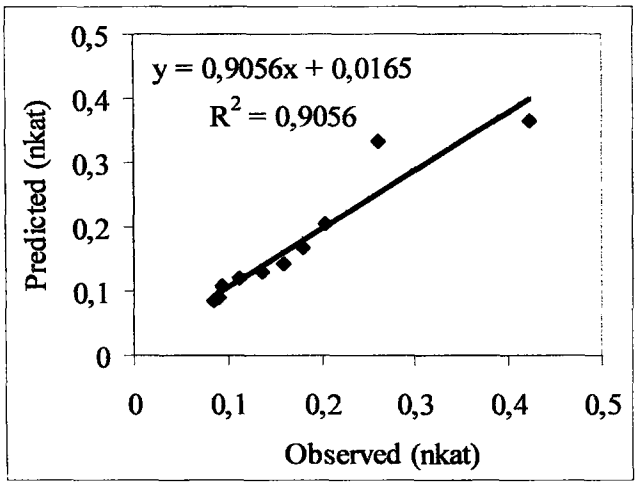


Figure C. 3. Comparision of observed and predicted data for the inactivation kinetics of lipase at 70°C.

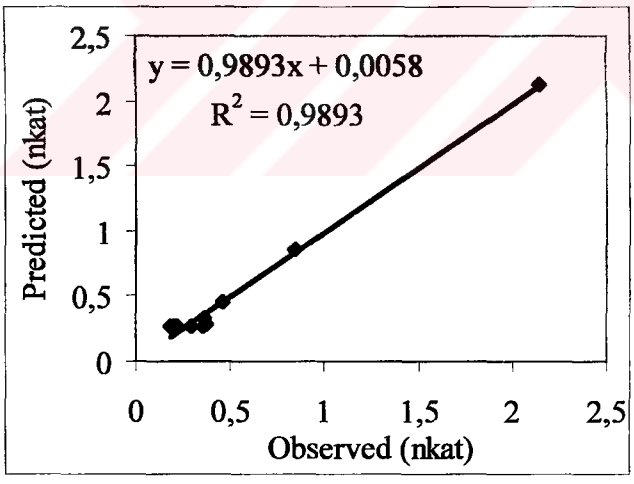


Figure C. 4 Comparision of observed and predicted data for the inactivation kinetics of lipase at 80°C.

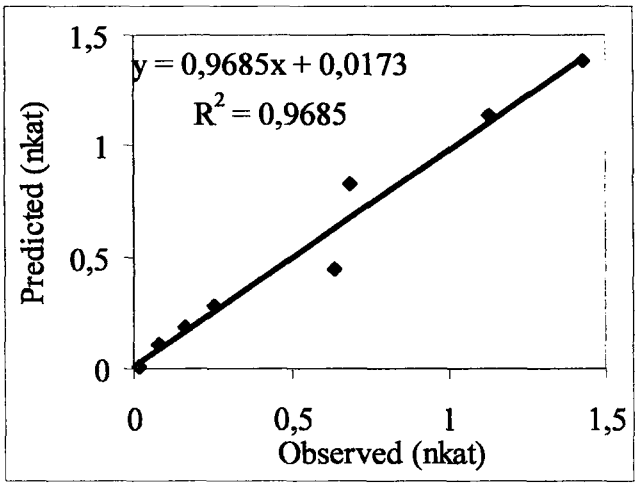


Figure C. 5 Comparision of observed and predicted data for the inactivation kinetics of lipase at 90°C.

C7. CHECKING RESIDUALS FOR RANDOM SCATTER FOR THE MODELS USED FOR INACTIVATION KINETICS

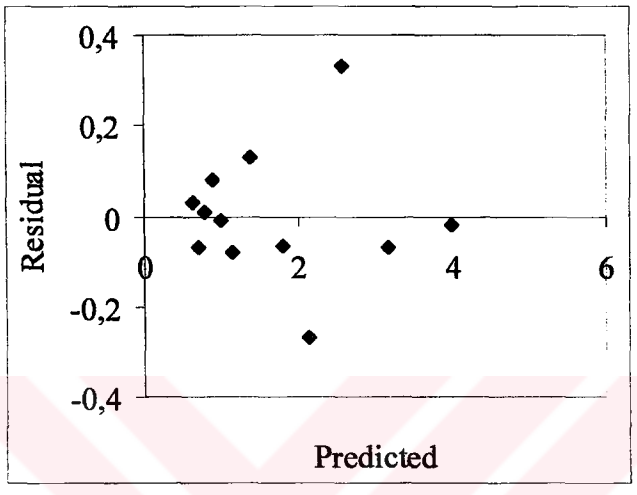


Figure C. 6. Checking residuals for inactivation of POD at 60°C.

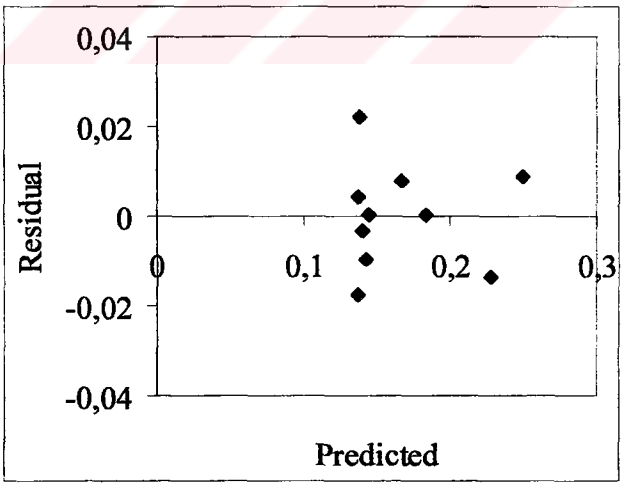


Figure C. 7. Checking residuals for inactivation of lipase at 60°C.

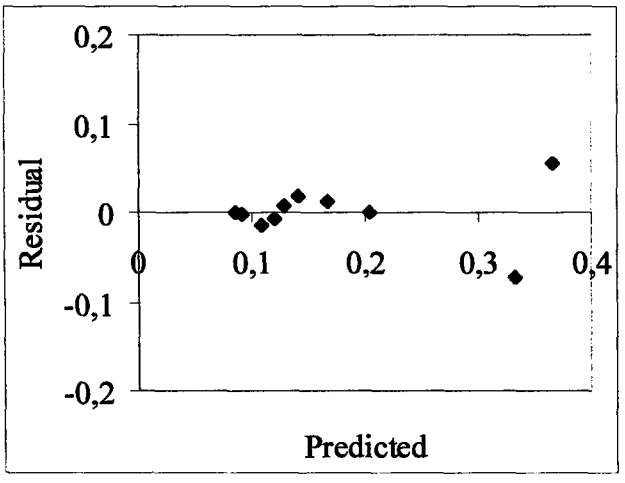


Figure C. 8. Checking residuals for inactivation of lipase at 70°C.

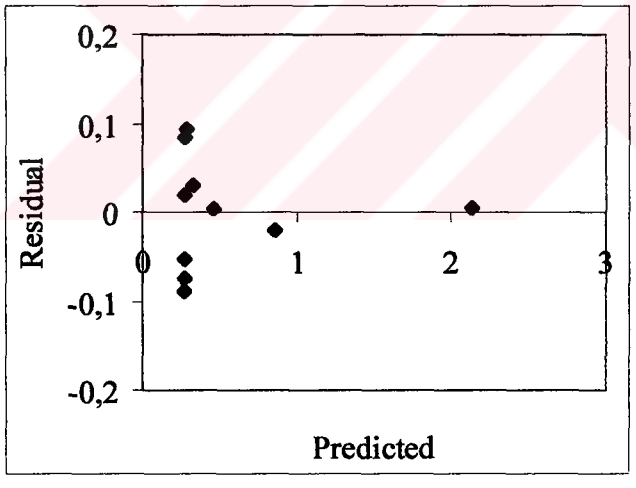


Figure C. 9. Checking residuals for inactivation of lipase at 80°C.

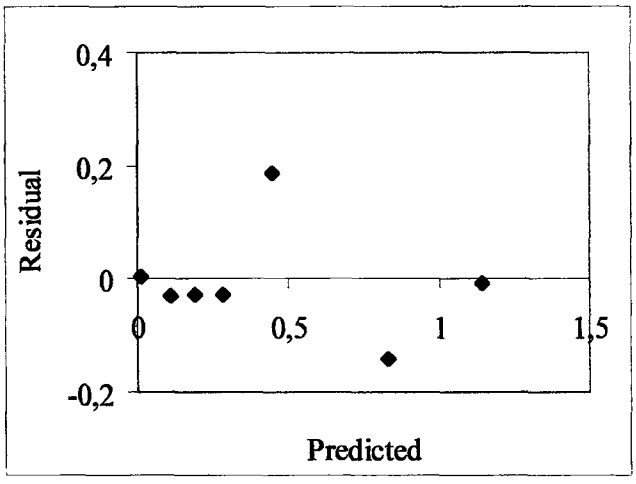


Figure C. 10. Checking residuals for inactivation of lipase at 90°C.

CIRRICULUM VITAE

Ferda Gürtaş Seyhan was born in 12.03.1969, Elazığ, Turkiye. She had B. Sc. Degree (1991) from Food Engineering Department of Middle East Technical University, (Ankara). She has worked for 6 years as an research assistant in Food Engineering Department of İstanbul Technical University (İstanbul) from 1991 to 1997 and assisted "Food Engineering Laboratory", "Food Engineering Processes I, II" and "Material and Energy Balances in Food Engineering" Lectures. She had M. Sc. Degree (1994) on "Low Temperature Drying of Cultivated Mushrooms (*A.bisporus*)" (supervised by Prof. Dr. Ö. Evranuz) from Food Engineering Program of School of Science of İstanbul Technical University (İstanbul). She has been enrolled PhD Program of Food Engineering Program of School of Science of İstanbul Technical University (İstanbul) since 1994. She has married in 1996 with Alpay Seyhan and got a son, pretty Doğaç in 1998. She has been working as senior researcher in Food Science and Technology Research Institute of TUBITAK- Marmara Research Centre (Kocaeli) since 1997. She has been studying on quality improvement of nuts and conducting a project financed by TUBITAK-TOGTAG/TARP. She got grant from TUBITAK, World Bank and studied on "Effect of lipoxygenase on quality of potato products" and "Improvement of activity measurement methods of hazelnut enzymes: lipase, peroxidase and lipoxygenase" at ATO, Wageningen, the Netherlands for 6 months (October,1999-April, 2000). She has contributed preparation of a book related with HACCP applications in hazelnut processing, seminar about "Hazelnut Quality" and has 5 international, 2 national articles and participated in 5 congress and one summer course with a poster. She is concerned with drying and processing of fruits and vegetables, nuts and enzyme activity in foods.