PRODUCTION OF HIGH QUALITY FUNCTIONAL PROTEINS FROM LEGUMES AND PLANT BASED AGROINDUSTRIAL WASTES AND BYPRODUCTS

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Levent Yurdaer AYDEMİR

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İZMİR

We approve the thesis of **Levent Yurdaer AYDEMİR**

Examining Committee Members:

Prof. Dr. Ahmet YEMENİCİOĞLU
Department of Food Engineering, İzmir Institute of Technology

Prof. Dr. Taner BAYSAL
Department of Food Engineering, Ege University

Prof. Dr. Figen TOKATLI
Department of Food Engineering, İzmir Institute of Technology

Assoc. Prof. Dr. Yusuf BARAN
Department of Molecular Biology and Genetics, İzmir Institute of Technology

Assoc. Prof. Dr. Figen KOREL
Department of Food Engineering, İzmir Institute of Technology

9 July 2014

Prof. Dr. Ahmet YEMENİCİOĞLU
Supervisor, Department of Food Engineering
İzmir Institute of Technology

Prof. Dr. Ahmet YEMENİCİOĞLU
Head of the Department of
Food Engineering

Prof. Dr. R. Tuğrul SENGER
Dean of the Graduate School of
Engineering and Sciences

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ABSTRACT

PRODUCTION OF HIGH QUALITY FUNCTIONAL PROTEINS FROM LEGUMES AND PLANT BASED AGROINDUSTRIAL WASTES AND BYPRODUCTS

In this thesis, technological and bioactive properties of protein concentrates and isolates form Turkish lentil and chickpea cultivars and hazelnut meal has been characterized. This study aimed to increase industrially suitable alternatives to soy and animal origin proteins. The functional properties (solubility, gelation, water and oil absorption, emulsion and foaming capacity and stability) of globulin proteins from chickpeas are comparable or superior than those of soy proteins and most animal proteins (fish and bovine gelatin, egg white and whey proteins) with the exception of gelation capacity of bovine gelatin. The chickpea proteins got extremely high water (4.9-7.9 g/g) and oil (10.9-14.6 g/g) absorption capacity. The lentil globulins showed high protein solubility (0.56-0.69 g/g) and oil absorption capacities (6.9-10.4 g/g), but missed water absorption and gelation properties. The lentil proteins showed 2-3 fold higher antioxidant capacity than the chickpea proteins, and antioxidant lentil protein fractions could be separated or concentrated by isoelectric precipitation, ion exchange chromatography and ultrafiltration. The hazelnut meal globulins showed satisfactory oil absorption (7.4-9.4 g/g) and good foaming (7.1 to 18.9 ml foam with 10 mg/ml), but poor water absorption, gelation and emulsion properties. The hazelnut proteins also form water soluble yellowish to brownish and reddish colored transparent edible films with 10-12.5 % (w/w) solutions. However, the most outstanding properties of hazelnut meal proteins is their high bioactivity including free radical scavenging (158-461 µmol Trolox/g), iron chelation (60.7-126.7 µmol EDTA/g) and angiotensin-converting enzyme inhibition (IC_{50}: 0.57-1.0 mg/mL) capacities. Thus, a functional beverage, hazelnut milk enriched with trypsin and pepsin hydrolyzed hazelnut globulins, was developed as an example functional food product. This work showed the good potential of selected Turkish plant resources as animal and soy protein alternatives.

ÖZET

BAKLAGİLLER VE BİTKİSEL KAYNAKLI TARIMSAL ATIKLAR VE YAN ÜRÜNLERİNDEN YÜKSEK KALİTEDE FONKSİYONEL PROTEİNLER ÜRETİLMESİ

Bu tezde Türk mercimek ve nohutlarıyla, fındık küspesi protein konsantreleri ve izolatlarının teknolojik ve biyoaktif özellikleri karakterize edilmiştir. Çalışmayla soya proteini ve hayvansal proteinlere alternatif olarak endüstriyel amaçlı kullanılabilecek farklı bitkisel proteinler ortaya konmuştur. Nohut proteinlerinin fonksiyonel özellikleri (çözünürlük, jelleşme, su ve yağ absorpsiyon, emülsiyon ve köpük oluşturma kapasiteleri ve stabiliteleri) soya ve pek çok hayvansal proteinlerinkiyle (balık ve sığır jelatini, yumurta akı ve peyniraltı suyu proteinleri) kıyaslanabilir veya daha üstün nitelikte bulunmuştur. Nohut proteinlerininkinden belirgin şekilde yüksek olan tek özellik sığır jelatininin jelleşme özelliğidir. Nohut proteinlerinin su (4.9-7.9 g/g) ve yağ (10.9-14.6 g/g) absorplama kapasiteleri sıradışı şekilde yüksektir. Mercimek proteinleri yüksek protein çözünürlüğü (0.56-0.69 g/g) ve yağ absorpsiyon kapasitesi (6.9-10.4 g/g), göstermekte, ancak kayda değer bir su bağlama kapasitesi ve jelleşme kapasitesi gösterememektedir. Buna karşın mercimek proteinleri nohut proteinlerine göre 2-3 kat fazla antioksidant aktivite göstermekte ve bu antioksidant proteinler izoelektrik çöktürme, iyon değiştirici kromatografi ve ultrafiltrasyon gibi tekniklerle ayrılıp yoğunlaştırılabilmektedirler. Fındık proteinleri kabul edilebilir düzeyde bir yağ absorpsiyon (7.4-9.4 g/g) ve iyi köpük oluşturma (7.1 to 18.9 ml köpük) kapasitesi göstermekte, ancak su absorpsiyon, jelleşme ve emülsiyon kapasiteleri düşük düzeyde kalmaktadır. Fındık proteinleri % 10-12.5 (w/w) konsantrasyonda kullanıldıkları zaman suda çözünürlüğü yüksek, sarı-esmer renkten kırmızımsı renge kadar değişen renkte ve şeffaf yenilebilir filmler oluşturabilmektedir. Ancak en sıradışı özellikleri yüksek serbest radikal yakalama (158-461 µmol Trolox/g), demir bağlama (60.7-126.7 µmol EDTA/g) ve angiotensin-dönüştürücü enzim inhibe etme (IC_{50}: 0.57-1.0 mg/mL) kapasitesi yani yüksek biyoaktivite göstermeleridir. Bundan dolayı bu proteinlerin fonksiyonel gıda üretiminde kullanımına örnek teşkil etmesi amacıyla pepsin ve tripsin hidrolizine uğratılmış fındık globülinleriyle zenginleştirilmiş fındık sütü üretilmiştir. Bu tez Türk bitkisel kaynaklarının soya ve hayvansal proteinlere alternatif protein üretiminde kullanılabileceğini göstermiştir.

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LIST OF SYMBOLS AND ABBREVIATIONS

°C: Degree Celsius

µg: microgram

µmol: micromole

1 D: 1 dimension

2 D: 2 dimensions

*a**: redness-greenness

AAPH: 2,2'-Azobis[2-methyl-propionamidine

ABTS: 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid

ACE: Angiotensin converting enzyme

AGE: Albumin globulin extract

ANOVA: Analysis of variances

AOAC: Association of Analytical Communities

AP: Acetone powder

ASTM: American Society for Testing and Materials

AUC: Area under curve

*b**: yellowness-blueness

BGEL: Bovine gelatine

BSA: Bovine serum albumin

CAGE: Chickpea albumin globulin extract

CGE: Chickpea globulin extract

CGE-1: Chickpea globulin extract from Canıtez cultivar

CGE-2: Chickpea globulin extract from Cevdetbey cultivar

CGE-3: Chickpea globulin extract from Gökçe cultivar

CGE-4: Chickpea globulin extract from Sarı cultivar

CIE: International Commission on Illumination

DEAE: Diethylaminoethyl

E: Elongation at break

EA: Emulsifying activity

EC: Epicatechin

EDTA: Ethylenediaminetetraacetic acid

ES: Emulsion stability

EWP: Egg white protein

FA: Foaming activity

FAPGG: N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly

FGEL: Fish gelatine

FRS: Free radical scavenging

FS: Foam stability

g: gram

GA: Gallic acid

GE: Globulin extract

GMO: Genetically modified organism

HM: Hazelnut meal

HPC: Hazelnut protein concentrate

HPC-AW-H: Acetone washed and heat applied hazelnut protein isolate

HPI: Hazelnut protein isolate

HPI-AW: Acetone washed hazelnut protein isolate

HPI-H: Heat applied hazelnut protein isolate

IC_{50}: Inhibition concentration for 50 %

ICC: Iron chelating capacity

IEF: Isoelectric focusing

IEP: Isoelectric precipitation

ISO: International Organization for Standardization

kDa: kilo Dalton

*L**: lightness value

L: litre

LAE: Lentil albumin fraction of Fırat cultivar

LAGE: Lentil albumin globulin extract of Fırat cultivar

LAGE-C1: Neutral and basic protein fraction of LAGE obtained by IEC

LAGE-C2: Acidic protein fraction of LAGE obtained by IEC

LAGE-P1: Proteins fraction ≤ 30 kDa of LAGE obtained by UF

LAGE-P2: Proteins fraction ≤ 10 kDa of LAGE obtained by UF

LAGE-P3: Proteins fraction ≤ 2 kDa of LAGE obtained by UF

LAGE-R1: Proteins fraction > 30 kDa of LAGE obtained by UF

LAGE-R2: Proteins fraction 10 - 30 kDa of LAGE obtained by UF

LAGE-R3: Proteins fraction 2- 10 kDa of LAGE obtained by UF

LGC: Least gelation concentration

LGE: Lentil globulin fraction of Fırat cultivar

LGE-1: Lentil globulin extract from Alidayı cultivar

LGE-2: Lentil globulin extract from Çiftçi cultivar

LGE-3: Lentil globulin extract from Fırat cultivar

LGE-4: Lentil globulin extract from Kafkas cultivar

LGE-5: Lentil globulin extract from Meyveci cultivar

LGE-6: Lentil globulin extract from Pul-II cultivar

LSD: Least significant differences

M: Molar

mA: milliampere

mg: milligram

mL: millilitre

mmol: millimole

mol: mole

MW: Molecular weight

nm: nanometer

NTU: Nephelometric turbidity unit

OAC: Oil absorption capacity

ORAC: Oxygen radical absorption capacity

PBS: Phosphate buffer saline

pHPI: Pepsin hydrolzed hazelnut protein isolate

RH: Relative humidity

rpm: Rotation per minute

SDS-PAGE: Sodium dodecyl sulphate – polyacrylamide gel electrophoresis

SEM: Scanning electron microscopy

SPC: Soy protein concentrate

SPE: Soy protein extract

SPI: Soy protein isolate

TCA: Trichloroacetic acid

T_d: Denaturation temperature

TEAC: Trolox equivalent absorption capacity

TFC: Total flavonoid content

tHPI: Trypsin hydrolysed hazelnut protein isolate

TPC: Total phenolic content

TPrC: Total protein content

TS: Tensile strength

UF: Ultrafiltration

UV: Ultraviolet

v/v: volume per volume

V: Volt

w/v: weight per volume

w/w: weight per weight

WAC: Water absorption capacity

WPI: Whey protein isolate

WSPC: Water soluble protein content

CHAPTER 1

INTRODUCTION

Proteins are one of the main constitutes of foods which are made up 20 different amino acids stabilized by many covalent and non- covalent interactions. They are highly complex polymers and they play several important roles such as biocatalysts (enzymes), contractile proteins (actin, myosin), structural proteins (collage, keratin), transport proteins (serum albumin, hemoglobin), metal chelation (phosvitin), antibodies (immunoglobulins), protective proteins (toxins, allergens), and storage proteins (seed proteins, casein micelles, egg albumen) in food systems (Damodaran, 1996). In an ideal meal, the food proteins are in good balance of amino acids that are easily digested and absorbed into the body with good taste, aroma, texture and colour without toxic effects. The food quality is one of the major contributors to the long and healthy life (Nakai, 1996).

Animal and plant origin proteins are used as ingredients in food systems due to their nutritive value, and different bioactive and functional properties. In industry they are benefited from their emulsifying activity, film, foam and gel formation property, oil, water and flavour binding property and thickening agent (Arcan & Yemenicioglu, 2010; Damodaran, 1996). They also contribute beneficial effect to food products and human health with their antioxidant, antihypertensive, antimicrobial, anticancerogenic, and antidiabetic activities. Moreover, they may bind some minerals to carry through the biological systems and they can act as immunomodulatory molecules. Milk proteins, muscle proteins and egg proteins are the most extensively used commercial animal proteins while soybean, legumes, oilseeds, and cereal proteins are the most studied and used plant sourced proteins (Asgar, Fazilah, Huda, Bhat, & Karim, 2010).

Recently, the economic reasons and the consumer demands originated from health concerns, religious limitations and rising trend of vegetarianism have increased the interest of food industry to use of functional and bioactive plant proteins as alternative to animal proteins. The prions associated with mammalian proteins and the safety discussions of bovine origin proteins also entails the developing those plant sourced protein products for human consumption (Alvaro, Juan Jose, Maira, Raquel, &

Miguel Ángel, 2006; Dormont, 2002; Jenkins et al., 2002; Karim & Bhat, 2009). In addition to these suspicious, it was declared by the European Food Safety Authority (EFSA) that the risk of gelatine was very low, but this did not slow down extensive studies to find functional proteins alternative to mammalian gelatine (Karim & Bhat, 2009). The plant sourced protein demand of industry is majorly being satisfied by the soybean protein isolates, concentrates and hydrolyzates. They are extensively used in foods such as meat and dairy products, infant formulas, functional foods and nutraceuticals. Although, the major source of plant origin proteins used in industry is still soybeans, different studies have also been conducted related to soy alternative legume proteins from chickpeas, common beans, lentils, cowpeas, lupins, peas and broad beans or oilseed proteins from sunflower, canola and rapeseed seeds (Bamdad, Goli, & Kadivar, 2006; Horax, Hettiarachchy, Chen, & Jalaluddin, 2004; Makri, Papalamprou, & Doxastakis, 2005; Manamperi, Wiesenborn, Chang, & Pryor, 2011; Megías et al., 2008; Morales-de León, Vázquez-Mata, Torres, Gil-Zenteno, & Bressani, 2007; Salgado, Ortiz, Petruccelli, & Mauri, 2012; Sánchez-Vioque, Clemente, Vioque, Bautista, & Millán, 1999; Wu, Aluko, & Muir, 2009; Yoshie-Stark, Wada, Schott, & Wäsche, 2006). Furthermore, due to the rapidly growing plant protein market there is a great competition to evaluate fruits, vegetables, and cereals and their processing wastes as source of commercial proteins (Boye, Zare, & Pletch, 2010a; Moure, Sineiro, Domínguez, & Parajó, 2006). There are also some studies to use suitable agroindustrial wastes as source of functional plant proteins. For example, several studies have been initiated to extract and use tomato seed waste proteins as functional ingredients (Sogi, Bhatia, Garg, & Bawa, 2005). An early study was conducted by El-Aal, Hamza, and Rahma (1986) to evaluate physico-chemical and functional properties of apricot kernel protein. Defatted meals of oilseeds are also potential sources of functional proteins (Moure et al., 2006). Salgado, Molina Ortiz, Petruccelli, and Mauri (2010) and Orliac, Rouilly, Silvestre, and Rigal (2002) investigated the potential of edible film making by using sunflower proteins while Jang, Lim, and Bin Song (2011) studied with edible films from rapeseed proteins. Besides oilseed protein, peanut meal and palm fruit meal proteins have also been evaluated for their bioactive, edible film making or functional properties to understand their value as alternative sources of functional proteins (Chee, Ling, & Ayob, 2012; Jamdar et al., 2010; Ng, Ayob, Said, Osman, & Ismail, 2013; Reddy, Jiang, & Yang, 2012).

Among main legumes, chickpeas and lentils are grown in different parts of world including America, Mediterranean Basin, China, Middle East, India and Australia. The major chickpea producers in the world are India, Turkey and Pakistan, while lentils are produced mainly by India, Turkey and Canada (Roy, Boye, & Simpson, 2010). In India and Pakistan, the grown chickpeas are Desi type, while Kabuli type chickpeas are grown mainly in Turkey. The major types of lentils produced in different parts of world are red and green lentils, but red lentils comprise 2/3 of the world production (Roy et al., 2010). Due to their high protein quality, nutritive value and antioxidant phenolic content chickpeas and lentils have a very important role for the diet in Mediterranean, Middle East, Pakistan and India (Diane, Frank, Terryl, & Julianne, 2009; Friedman, 1996; Han & Baik, 2008; Hernández-Infante, Sousa, Montalvo, & Tena, 1998; Xu & Chang, 2007). Recently, these pulses and some other legumes have also been strongly suggested by American Dietetic Association to improve diet quality of US population (Diane et al., 2009). Unfortunately, chickpeas and lentils are not extensively grown and used systematically for production of value added products suitable for industrial food applications. Therefore, different studies have been conducted on characterization of functional properties for major chickpea and lentil resources in the world at the cultivar level. For example, Kaur and Singh (2007) characterized functional properties of protein from 6 Indian Desi chickpea cultivars. Boye et al. (2010a) characterized functional properties of protein in some Canadian lentil (1 green and 1 red) and chickpea (1 Desi and 1 Kabuli) cultivars, while Lee, Htoon, Uthayakumaran, and Paterson (2007) studied functional properties of protein in 2 Australian lentil cultivars (1 green and 1 red). However, no studies have been conducted to characterize the functional properties of proteins form chickpea and lentil resources in Turkey, a major centre in the world for production of these pulses. In the first part of thesis study, functional properties of protein extracted from different Kabuli type chickpea cultivars (4 cultivars), and different red (4 cultivars) and green (2 cultivars) lentil cultivars have been characterized and compared with those of different commercially important proteins for the first time. In addition to characterization of functional properties of legume proteins, further purification of antioxidant protein fractions of one lentil protein extract (obtained from Fırat cultivar) with high antioxidant activity were carried out and the antioxidant proteins were determined based on their molecular weight, charge and structure. This study is also the first study

conducting 2-D electrophoresis of chickpea and lentil proteins obtained by the classical isoelectric precipitation method.

Turkey is the largest producer of hazelnuts (*Corylus avellana* L.) in the world and provides almost 85 % of world hazelnut requirement with its almost 650.000 metric tons of production (Demiryürek, Ceyhan, & Bozoğlu, 2012). In world food industry, most of the hazelnuts are used as ingredients for confectionary and for production of hazelnut cream. Whole roasted hazelnuts are also popular as snack food. Recently, the edible oil production from hazelnuts has also become increasingly important since hazelnut oil has similar fatty acid profile with olive oil (Yalcin, Toker, & Dogan, 2012). Due to its nutritive value and high protein content, the hazelnut meal obtained from oil extraction is mostly employed as animal feed (Xu & Hanna, 2011). In fact, recent studies showed that the Turkish hazelnut meal can successfully be used in feeding juvenile rainbow trout (Doğan & Erdem, 2010) and European sea bass (Emre, Sevgili, & Şanlı, 2008) as alternative to fish meal and soybean meal. There are also studies to use Turkish hazelnut meal in feeding of broiler chickens (Erener, Ak, & Ocak, 2009). Recently, Sharma, Su, Joshi, Roux, and Sathe (2010) studied the molecular and functional properties of different defatted nut flour proteins including hazelnut proteins. However, there are no attempts to evaluate the hazelnut meal obtained from commercial oil extraction for production of value added products including protein concentrates and isolates, and protein based edible films suitable for the food industry. This study is significant in that it is the first study to characterize the bioactive, functional and edible film making properties of different protein isolates and concentrates obtained from hazelnut meal by the commercially suitable purification procedures. In the second part of the study, four different hazelnut protein extracts were produced with different treatments such as acetone washing and heating. Then the effects of these treatments on the bioactivity and functionality of proteins were evaluated. The molecular and isoelectric properties of hazelnut proteins have also been characterized with 1D and 2D electrophoresis to prepare basis for advanced identification, characterization and purification studies. This work contributes to develop alternative commercial proteins for use of food, feed, drug and cosmetic industry. At the end of study, a new idea appeared to develop a novel food product using these functional proteins, so that bioactive hazelnut milk enriched with hydrolysed hazelnut proteins was produced. Hazelnut milk was simply obtained like home-made style and it was enriched with hazelnut protein isolates at varying concentrations. Bioactivity of hazelnut milk was

expressed in terms of its antioxidant activity. Enzymatic hydrolysis of hazelnut protein isolates was carried out by using pepsin or trypsin enzyme and protein solubility and antioxidant activity of the product was determined for 3 days storage in the refrigerator. The high amount of hydrolysed hazelnut protein enrichment yielded more protein solubility and antioxidant activity of hazelnut milk samples. In order to increase the shelf life stability of hazelnut milk, the thermal stability of antioxidant hazelnut milk proteins was determined at different temperatures and heating time. During heat application, enzymatic hydrolysis of hazelnut proteins provided better protein solubility and bioactive stability to the hazelnut milk even at high temperatures. Especially when hazelnut milk was enriched with pepsin hydrolysed hazelnut protein isolates, the antioxidant activity of hazelnut milk was generally doubled after heat treatment at all temperatures. All results indicated that pasteurization is a potential method to obtain shelf life stable bioactive hazelnut milk enriched with hydrolysed hazelnut proteins.

CHAPTER 2

PROTEINS

2.1. Proteins

Proteins are polymers which are composed of 20 different L-α-amino acids linked by peptide bonds. Peptide bond is an amide bond that is covalently formed between α-amino group of one amino acid and the α-carboxyl group of another with elimination of water molecule (Mathews & Van Holde, 1996). Amino acids can be classified depending on their solubility and ionization properties of side chain group at the α-carbon atom. They are grouped as aliphatic amino acids (Gly, Ala, Val, Leu, Ile), aromatic amino acids (His, Phe, Tyr, Tpr), hydroxyl group containing amino acids (Ser, Tyr, Thr), sulfur containing amino acids (Cys, Met), basic amino acids (Arg, Lys, His), acidic amino acids and their amides (Asp, Glu), and imino acid (Pro). Proteins play several important roles in biological and food systems such as biocatalysts, structural components, contractile proteins, hormones, transport proteins, metal chelation, antibodies, protective proteins, and storage proteins (Damodaran, 1997). They spontaneously fold and form three dimensional structures which are mainly driven by their amino acid composition. Each amino acid residues shows different hydrophobic and hydrophilic character and this situation determines protein hydropathy. Hydropathy values show the free-energy change for transfer of an amino acid residue from the interior of a lipid bilayer to water. Amino acids which have negative values are hydrophilic, whereas those which have positive values are hydrophobic. Hydropathy of amino acids and amino acid sequence in polypeptide chain are important because hydropathy is a driving force for protein folding (Moran, Horton, Scrimgeour, & Perry, 2012). For example proteins which are mainly composed of hydrophobic amino acids form mostly compact and globular shape while proteins which are mainly composed of hydrophilic amino acids form mostly rod-like expanded shape (Berg, Tymoczko, Stryer, & Clarke, 2002). Amino acid sequence and composition determine the structural characteristic and functions of a protein. It is assumed that under specific pH and temperature conditions, each polypeptide chain has one specific conformation which

corresponds to a thermodynamically stable and organized system. This form of a polypeptide chain is called its native form and this native form is related to the polarity, hydrophobicity and the steric hindrance of the side chain.

2.1.1. Protein Structures

In order to better understand the proteins, the concept of a hierarchy of levels of protein structures was created.

Primary structure of a protein defines a specific linear sequence in which the consequent amino acids are linked to end to end through peptide bonds (Voet, Voet, & Pratt, 1999). The chain length and amino acid sequence of polypeptide determine the structural configuration of the protein and do not include any information about conformation.

Secondary structure of a protein defines the local, regular conformation of the polypeptide backbone in terms of different structures such as helical forms, β-structures and random coil. It is influenced by specific primary structure of polypeptide and is determined by noncovalent interactions especially involving hydrogen bonds between C=O and N-H groups (Armstrong, 1989; Berg et al., 2002; Voet et al., 1999).

Tertiary structure of a protein defines its three dimensional conformation with secondary structure elements containing entire polypeptide chain. This spatial arrangement is formed mainly by hydrophobic interactions which are resulted of thermodynamic requirements in order to minimize the free energy of the molecule. In the final form of protein most of the hydrophobic and hydrophilic amino acid residues are located at the interior and exterior of the protein molecule, respectively. The distribution of hydrophobicity of residues determines the shape, surface activity, and solubility of proteins. Proteins consisting of large amount of hydrophilic amino acid residues tend to form a rod like shape due to the increased surface area to volume ratio whereas proteins mostly consisted of hydrophobic groups form globular shapes which decrease the surface area to volume ratio. Depending on the molecular weight of proteins, they can have domains which are the regions of the polypeptide chain fold independently into a tertiary form. At the surface of the protein molecule, some loop regions can be found which are hydrophilic groups forming hydrogen bonds to water molecules in the environment. The major loop regions of proteins are; (1) Hairpin loop;

(2) Alpha-alpha motif; (3) Beta-alpha-beta motif; (4) Greek key motif (Berg et al., 2002; Damodaran, 1997; Ludescher, 1996).

Quaternary Structure of a protein is an oligomeric structure which defines the specific conformational arrangements between polypeptide chains in multi subunit protein complex. When a protein contains more than 28 % hydrophobic amino acid residue, it is physically not possible bury all hydrophobic residues into the protein interior. Therefore, hydrophobic regions tend to interact with hydrophobic regions of adjacent polypeptide chain in aqueous solution. At the end of the process, a quaternary structure is formed and mainly stabilized by hydrophobic interactions, in addition electrostatic interactions and hydrogen bonding contributes the stability (Belitz, W, & Schieberle, 2004; Damodaran, 1997; Ludescher, 1996).

2.2. Functional Properties of Proteins

Functional properties of proteins are physicochemical properties that effect their behaviour in food systems during preparation, processing, storage and consumption, and they contribute to the quality and sensory features of food systems (Zayas, 1997). These properties are resulted from protein structure and protein environment. The interactions between protein and its surrounding solvent, other proteins, lipids, carbohydrates, ions and a wide range of other components determine the protein functionality. In addition to these factors, the functional properties are highly related to molecular weight and shape of the protein molecules, structure and conformation, charge distribution, amino acid sequence in polypeptide chain(s), degree of hydrophobicity, and protein source. The conformation of protein is highly effective on determining its functions. The globular structured proteins are very dynamic and can easily make a transition between many different conformations. Amino acid residues with polar charged groups which are located on the surface of globular protein structures increase the solubility, swelling, and hydration of proteins (Alashi, Blanchard, Mailer, & Agboola, 2013; McClements, 2002; Sikorski, 2006; Zayas, 1997).

Proteins have a major contribution on food products by influencing their appearance, colour, juiciness, mouth feel and texture (Sikorski, 2006). In food systems, the functionality of a protein is defined with complex interactions between protein and protein or protein and non-protein compounds. In order to use proteins in industry, the

protein structures and its modifications are needed to be known as well as their functional properties. These properties might be altered by processing conditions, defatting type, solvent, and temperature of extraction, drying, freezing, ultrafiltration, homogenization, and other treatments (Zayas, 1997). However, at the molecular level, the functional properties are determined by the ability of proteins to bind other molecules, to undergo conformational changes, to self-associate, and to adsorb to interfaces (McClements, 2002).

During food processing and food preparation, proteins are generally modified or denaturated which cause changing the protein functionality (Damodaran, 1997). This functionality is highly depended on protein conformation. Proteins can be found in native form, molten globule state and denaturated form. In some applications, the functionality of the protein such as emulsification is expressed when the protein is in its native state, whereas gelation property is expressed when the protein is in a denatured state (McClements, 2002). In order to understand how functionality of proteins was altered by different factors, some model systems were constructed by changing conditions such as pH, temperature, ionic strength, and interactions between protein with other components. In addition to model construction, analysis on protein solubility and hydrophobicity may also provide a prediction of protein functionality. Nevertheless, due to the complexity of foods, to develop a model system, and to predict a protein functionality are difficult. The only reliable way of determining functionality of proteins is to incorporate the protein ingredient into the formulation and to test the end-product for desired functionality (Zayas, 1997).

Protein functionality is a beneficial tool for industrial purposes and human health. Especially in food, drug and cosmetic industries emulsification, foaming, water or oil binding, gelling and bioactive properties of proteins are often utilized. Although, the protein functionality can be improved by modifications of proteins with enzymatic and chemical treatments, using a good natural source is more practical and economic way to obtain a high functional protein (Sikorski, 2006) (Table 2.1).

Table 2.1. Functional roles of food proteins in food systems
(Source: Damodaran 1997)

Function	Mechanism	Food	Protein type
Solubility	Hydrophilicity	Beverages	Whey proteins
Viscosity	Water binding, hydrodynamic size and shape	Soups, gravies, salad dressings, desserts	Gelatine
Water binding	Hydrogen bonding, ionic hydration	Meat sausages, cakes, breads	Muscle and egg proteins
Gelation	Water entrapment and immobilisation, network formation	Meats, gels, cakes, bakeries, cheese	Muscle, egg, and meat proteins
Cohesion – adhesion	Hydrophobic, ionic, hydrogen bonding	Meats, sausages, pasta, baked goods	Muscle, egg, and whey proteins
Elasticity	Hydrophobic bonding, disulfide cross-links	Meats, bakery	Muscle and cereal proteins
Emulsification	Adsorption and film formation at interfaces	Sausages, bologna, soup, cakes, dressing	Muscle, egg, and milk proteins
Foaming	Interfacial adsorption and film formation	Whipped toppings, ice cream, cakes, desserts	Egg and milk proteins
Fat and flavour binding	Hydrophobic bonding	Low-fat bakery products, doughnut	Milk, egg, and cereal proteins

2.2.1 Protein Hydration

In biological systems, water is the main solvent which is essential molecule for nearly all biological functions. The interactions between water and proteins determine the protein native form that describes its functional properties (McClements, 2002). Thermodynamic interactions between protein and water effect the protein dispersibility, solubility, swelling, viscosity, gelation, water holding capacity, coagulation, emulsification and foaming property (Alashi et al., 2013; Mattos, 2002). Distribution of hydrophilic and hydrophobic groups in proteins determines the hydration capacity of protein which increases with the greater number of hydrophilic groups located on the surface. Water binds to polar and nonpolar groups of protein molecule through dipole-dipole, charge-dipole and dipole-induced dipole interactions (Damodaran, 1997). Hydration capacity of monomeric protein can be calculated by some mathematical equations however the same equation can not be used for oligomeric proteins when subunit surfaces are partially buried as a result of protein conformation. In some cases,

the hydration capacity of proteins is a negative value because of the enormous amount of void space within the micelle structure. This molecular formation absorbs water through capillary action and physical entrapment (Damodaran, 1996).

Water binding to a protein has many steps starting with solvation of ionic groups on the protein surface and formation an unfreezable monolayer of water on protein surface. This immobilized bound water layer binds more water to protein with polar and nonpolar groups. After completion of hydration at the polar surface, hydrophobic hydration starts and monolayer coverage is actualized. Finally, hydrodynamic water which moves with protein fills into the cracks and cavities of protein molecule (Damodaran, 1997; Damodaran, 1996) (Figure 2.1).

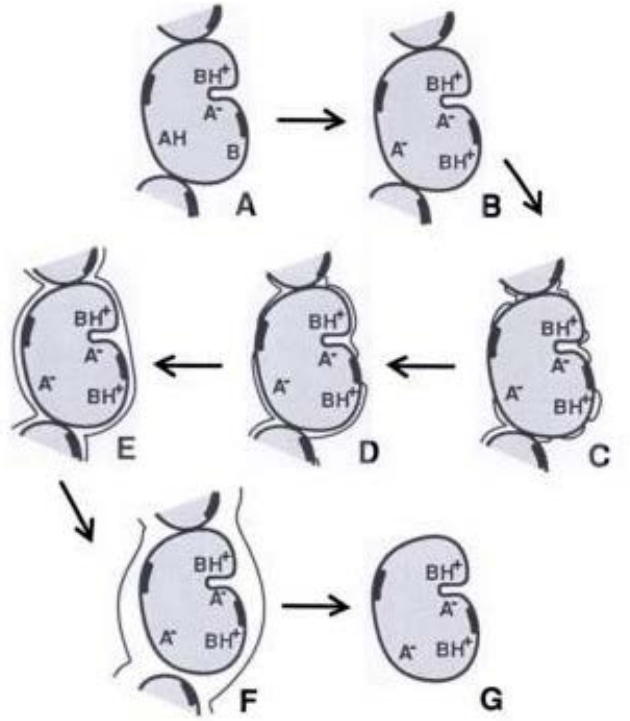

Figure 2.1. Sequence of steps involved in hydration of protein (A) Unhydrated protein. (B) Initial hydration of charged groups. (C) Water cluster formation near polar and charged sites. (D) Completion of hydration at the polar surface. (E) Hydrophobic hydration of non-polar patches; completion of monolayer coverage. (F) Bridging between protein-associated water and bulk water. (G) Completion of hydrodynamic hydration (Source: Damodaran 1996).

Protein conformation and environmental factors are the main determinants of the water-binding capacity of proteins. The interactions between protein and water are highly dependent on net charge of protein and repulsive forces between molecules. At

isoelectric point of protein, net charge is zero and water binding capacity is too low due to increased protein-protein interactions. On the other hand, with increasing the net charge of protein and repulsive forces, the water binding capacity of protein increases because protein-water interactions increase. Heat and salt ion concentration in the environment also influence the water binding capacity of proteins. Low and high salt ion concentration incompatibly change the water binding capacity. At low concentration, hydrated salt ions weakly bind to charged groups of proteins with increasing protein-water interactions (salting-in) whereas at high concentration salt ions bind large quantity of water causing protein dehydration (salting-out) (Damodaran, 1997; Damodaran, 1996; Sikorski, 2006). Heating induces low hydrogen bonding and hydration of ionic groups resulting in low water binding capacity whereas denaturation by temperature increases water binding of proteins because buried hydrophobic groups are exposed to surface and surface area to mass ratio is increased (Damodaran, 1997).

Water binding and water holding capacity are different concepts. The water binding capacity of a protein is defined as grams of water bound per gram of protein when a dry protein powder is equilibrated with water vapour at 90-95 % relative humidity (Table 2.2). On the other hand, water holding capacity is the ability of the protein to absorb water and sustain it against gravitational force within the protein matrix. Physically entrapped water, bound water and hydrodynamic water are the components of absorbed water (Damodaran, 1996). There is a positive correlation between water-holding capacity and water-binding capacity.

Due to fibrous nature and compartmentalization of the muscle, water is kept by both protein-water interactions and physical entrapment in meat and fish tissues. Water-holding capacity is the main factor affecting texture and juiciness of meat and fish products (Sikorski, 2006).

2.2.1.1. Solubility of Proteins

Protein solubility is defined as the concentration of protein in a solution which is in equilibrium with protein-protein and protein-solvent interactions (Damodaran, 1997; McClements, 2002). The ability of showing the functionality of proteins is highly related to solubility situation of proteins. Many of the functional properties are represented in high capacity when the protein is fully dissolved. Proteins are classified

into four categories according to their solubility abilities: (1) albumins are highly soluble in water at pH 6.6; (2) globulin which is soluble in dilute salt solution at pH 7.0; (3) glutelin is only soluble in acid (pH 2.0) and alkaline (pH 12.0) solution; (4) prolamin is soluble in 70 % ethanol. In food chemistry, the solubility of proteins is often defined as the percentage of the total quantity of protein contained in the food material which can be extracted by water or a suitable solvent in specific conditions (Sikorski, 2006). For utilization of proteins in beverages and liquid foods, solubility is the main characteristic to obtain dispersed colloidal systems (Zayas, 1997).

$$P_{\text{solution}} \leftrightarrow P_{\text{precipitate}}$$

Table 2.2. Hydration capacities of various proteins
(Source: Damodaran 1996)

Protein	**Hydration capacity (g water/g protein)**
Pure proteins[a]	
Ribonuclease	0.53
Lysozyme	0.34
Myoglobin	0.44
B-Lactoglobulin	0.54
Chymotrypsinogen	0.23
Serum albumin	0.33
Hemoglobin	0.62
Collagen	0.45
Casein	0.40
Ovalbumin	0.30
Commercial protein preparations[b]	
Whey protein concentrates	0.45-0.52
Sodium caseinate	0.38-0.92
Soy protein	0.33

[a]At 90 % relative humidity
[b]At 95 % relative humidity

Average hydrophobicity of amino acid residues, molecular weight and conformation of protein, pH, concentration and charge of other ions in the medium, ratio and solvent volume, particle size of the sample, duration of extraction, and temperature are the determinants for protein solubility (Alashi et al., 2013; Damodaran, 1997; Moure et al., 2006; Sikorski, 2006; Zayas, 1997). In polar solvent (water, glycerol, formamide, etc.) proteins are more soluble due to increased electrostatic repulsion and hydration of charged residues whereas in less polar solvents (ethanol,

etc.) proteins are less soluble (Belitz et al., 2004; Damodaran, 1996). Proteins with high quantity of hydrophobic groups easily dissolve in organic solvent because organic solvents cause unfolding of the protein molecules and exposure of the hydrophobic residues with a low dielectric constant (Belitz et al., 2004; Sikorski, 2006). The hydrophilicity of amino acid residues on protein surface is important for protein solubility since the solvent firstly interacts to protein surface. Hydrophilic interactions promote protein-water interactions therefore solubility increases whereas hydrophobic interactions decrease the solubility promoting protein-protein interactions (Damodaran, 1997). The pH is another important factor that determines the protein solubility because solubility is highly dependent on the repulsive and attractive intermolecular forces in the medium which are altered by pH (Zayas, 1997). At isoelectric pH value, most proteins have total zero charge which increases the hydrophobic interactions on the protein surface resulting to minimum protein solubility. When the pH is at acidic or alkali region, the interactions between protein and water increase due to formation of positive or negative charges of proteins. Especially, at alkaline pH values, proteins have net negative charge which enhances the protein solubility. Therefore, alkali region is extensively used for protein extraction although it causes dissociation and disaggregation of proteins (Zayas, 1997). Ions are also effective on protein solubility and protein precipitation. The high salt ion concentrations cause protein precipitation due to salting-out effect which can be used for extraction and fractionation of proteins. In this process multivalent anions or monovalent cations are more effective than monovalent anions or divalent cations, respectively (Belitz et al., 2004). In food industry, thermal treatments are the most important processing operations which significantly effect the protein solubility. Heat or cold denaturation of proteins is occurred by hydrophobic-hydrophobic interactions, electrostatic interactions, hydrogen bonds, van der Waals forces and steric interactions (McClements, 2002). Thermal denaturation is caused by increased interactions between hydrophobic groups or reactive groups which are exposed to surface during process (Sikorski, 2006). When the temperature is above 50 °C, irreversible denaturation starts and lowers the protein solubility by shifting the surface from more hydrophilic to more hydrophobic resulting to protein-protein interactions (Damodaran, 1997; Damodaran, 1996; Zayas, 1997). Cold denaturation of proteins is dependent on many factors such as freezing rate, freezing and storage temperature, storage time, stability of storage conditions, and thawing methods and conditions (Zayas, 1997).

2.2.1.2. Gelation

Gelation is defined as the transformation of a protein in solid-state into a three dimensional lattice to gel-like structure by heating or other agents. A gel is a dispersed system of at least two components and is an intermediate phase between solid and liquid. A gel is characterized by the lack of its fluidity and elastic deformability. Protein gel network is developed by intermolecular cross-links covalently or non-covalently by entrapping water and other-water soluble molecules with capillary forces. This network is responsible for elasticity and textural strength (Figure 2.2) (Alashi et al., 2013; Belitz et al., 2004; Damodaran, 1997; McClements, 2002; Oakenfull, Pearce, & Burley, 1997; Sikorski, 2006).

Gelation process starts with dissociation of the quaternary structure and unfolding of a protein. In this process, mostly heating is applied and proteins expose non-polar and sulfhydryl amino acid side groups, which are normally located in the protein interior, to the surface. In the second step, these reactive groups of unfolded protein interact with each other and rearrange; so that the protein aggregates and forms a pro-gel. The gelation process is completed by cooling of the pro-gel form of the viscous solution and the structure is stabilized (Damodaran, 1997; McClements, 2002; Sikorski, 2006).

According to the formation process and stabilization factors, food proteins may form two types of gels; translucent and coagulum type gel. If the protein contains high level of hydrophilic residues, it forms a soluble aggregate occurring thermo reversible translucent gel. When the gel is heated, the intermolecular hydrogen bonds can be easily broken and it melts. On the other hand, if the hydrophobic residue level is high in the protein, hydrophobic aggregation occurs and these aggregates form thermo-plastic (thermo-irreversible) coagulum type gel network. Unlikely translucent type gel, coagulum type gel does not liquefy when it is heated (Belitz et al., 2004; Damodaran, 1997; Sikorski, 2006).

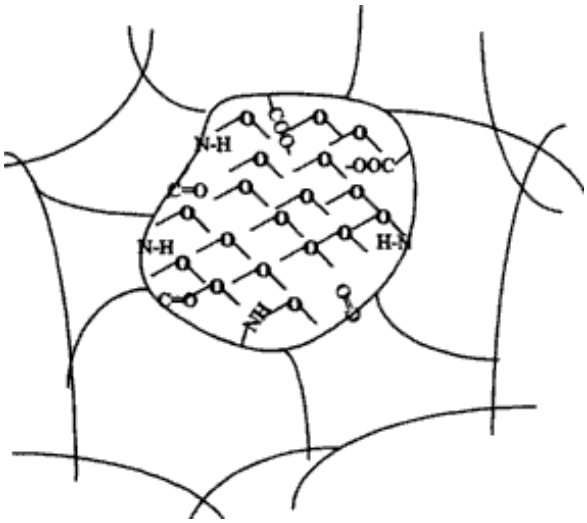

Figure 2.2. Hypothetical hydrogen-bonded state of water in a protein gel matrix (Source: Damodaran, 1996)

2.2.1.3. Flavour Binding

Proteins can bind small molecular weight substances with hydrophobic pockets crevices on the protein surface. These bound flavour compounds influence the sensory attributes of foods which are determined by nose and tongue. According to the purpose, flavour binding is preferred such as to protect the flavour molecule against the process conditions. On the other hand, binding the unsaturated fatty acid oxidation products negatively effects the proteins which will be used in industry (Damodaran, 1997; Damodaran, 1996; McClements, 2002; Walsh, 2002). In food applications, proteins are sometimes used as flavour carriers. Such proteins should bind flavours reasonably tightly and retain them during processing. The flavour compounds may bind to protein with various affinities and in the final food product the obtained flavour may be different than the expected one. The bound flavour should release during chewing in the mouth. Thus, the binding should be non-covalent to be able to contribute to aroma and taste of the protein-based products (Damodaran, 1997; Damodaran, 1996).

The flavour mechanism mainly depends on the moisture content of protein, even though the interactions are generally non-covalent. Dry protein powders bind flavours through van der Waals interactions, hydrogen bonding, electrostatic interactions and physical entrapment within capillaries and crevices. The flavour binding also depends on denaturation of the protein. In liquid or high moisture foods, flavours bind to protein

by hydrophobic interactions. Diffusion of flavours to the interior part of the protein may disrupt the hydrophobic interactions among protein segments, and covalent bonding of aldehydes may change the net charge of proteins. Such modifications may lead to unfolding of protein and exposure of new hydrophobic sites for more ligand binding. Denatured proteins have more ligand binding sites with weak association constants (Burova et al., 1999; Damodaran, 1997; Damodaran, 1996).

2.2.1.4. Dough Formation

In bread and bakery products, proteins play a critical role for dough formation step which determines the quality of product. Wheat flour is extensively used for manufacturing of those products. Gluten is a main structure-forming protein and responsible for rheological characteristics of dough (Lorenzo, Zaritzky, & Califano, 2008; Peressini, Peighambardoust, Hamer, Sensidoni, & van der Goot, 2008). Gluten is a mixture of gliadin and glutenin proteins which effect the gluten functionality in dough. Dough structure is based on extensive three-dimensional network of gluten protein sub-units joined together by disulfide cross-links (Davidek, Velisek, & Pokorny, 1990). The glutenin protein subunits are tyrosine rich and these amino acids can also participate in the formation of covalent structures and stabilization of crosslinks provided by disulfide bonds (Peña, Bernardo, Soler, & Jouve, 2006).

2.2.2. Surface Activity of Proteins

Proteins are good emulsifiers or foaming agents since they are amphiphilic molecules therefore they can adsorb to boundaries separating into two phases (Damodaran, 1996; McClements, 2002). They may form a viscoelastic film at the interfaces of different phases. Surface active proteins adsorb to an interface (air-water or water-oil) following by unfolding and reorientation. Then they interact with the neighbouring molecules to form a strong cohesive, viscoelastic film. Protein conformation determines the flexibility, stability, adaptability and surface hydrophobicity/hydrophilicity and these properties determine the surface activity.

Due to lower free energy of interface, proteins tend to migrate from the bulk phase to the interface (Damodaran, 1996). Hydrophobic groups provide faster migration

to the interface while hydrophilic groups prefer contact aqueous medium therefore more slowly migration occurs. After absorbtion of proteins to interface, many proteins undergo conformational changes which may promote interactions such as attractive electrostatic interactions, hydrogen bonding, hydrophobic interactions, and disulfide bonds between neighbouring proteins. Therefore better viscoelastic film formation at interfacial region is seen and this property provides better stability to foam or emulsion against to mechanical shocks which appear during processing, storage and handling. Compared to low molecular weight chemical surfactants, proteins are superior surface active agents because they have better conformational orientation at the surface (Figure 2.3) (Damodaran, 1996; McClements, 2002).

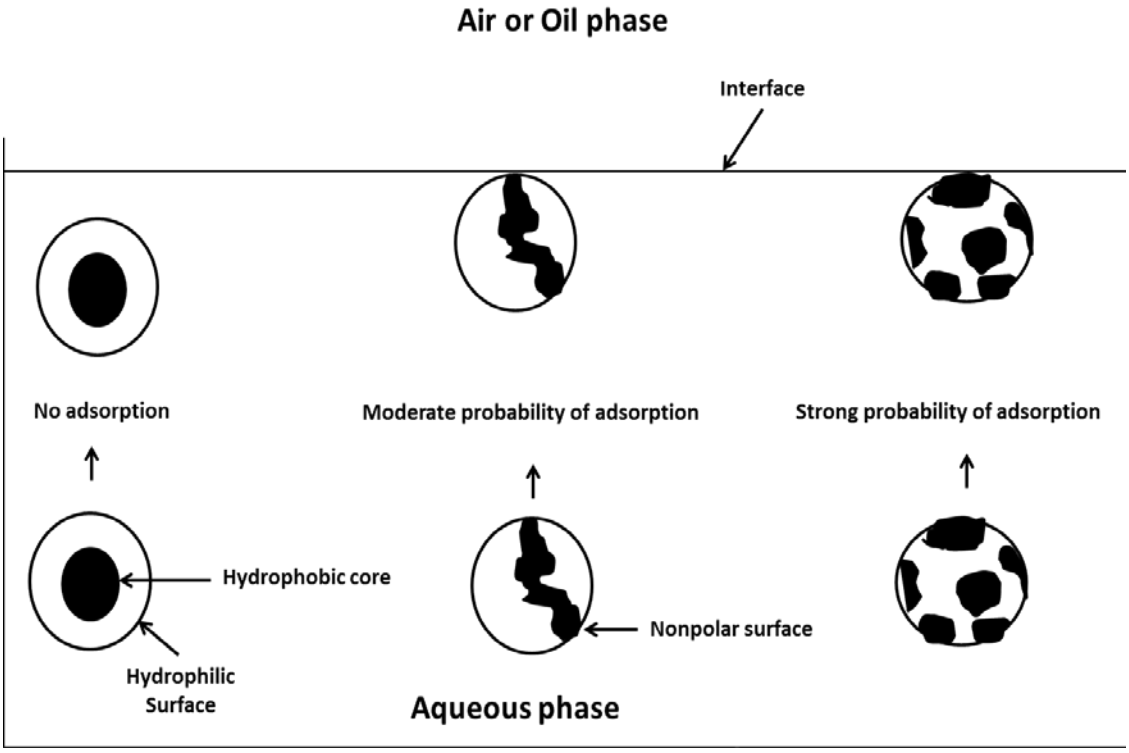

Figure 2.3. Schematic representation of adsorption of proteins at the air-water interface (Source: Damodaran 1997)

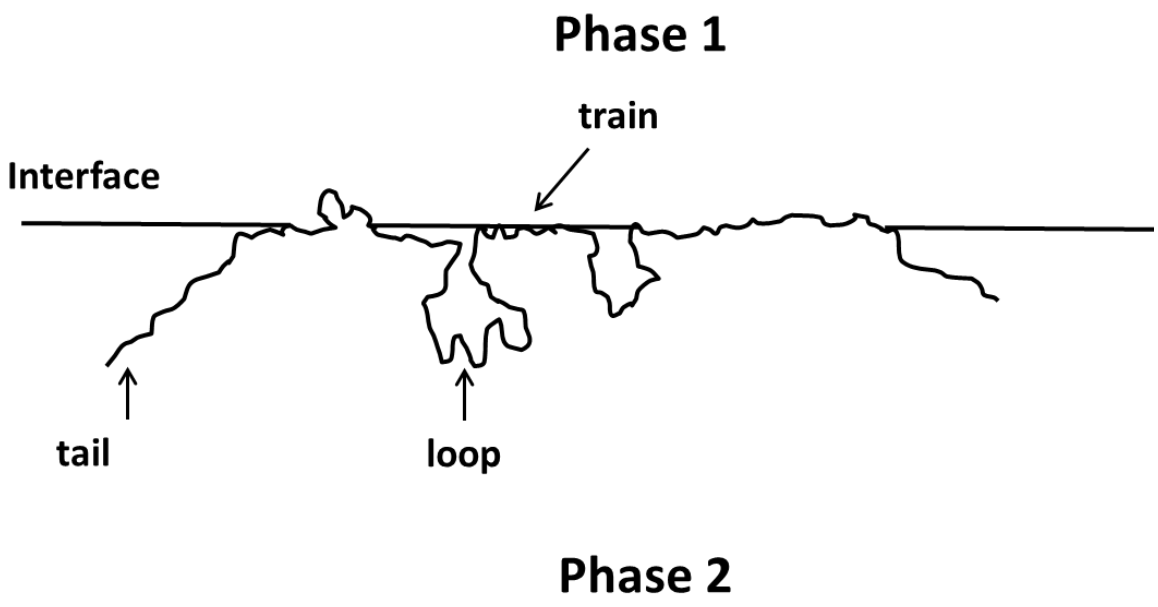

Figure 2.4. Various configurations of flexible polypeptides at an interface
(Source: Damodaran 1996)

At the interface polypeptide chains may form (1) train configurations which are the proportions directly contacting with the interface; (2) loops which suspend in the aqueous phase; (3) tails which are N- and C- terminal sites located in the aqueous phase. Train configuration contributes to stronger binding and lower interfacial tension (Figure 2.4) (Damodaran, 1996).

2.2.2.1. Emulsifying Property of Proteins

Emulsions are dispersed systems of small liquid droplets in the continuous phase of an immiscible liquid. Proteins behave as an emulsifier when they are in oil in water systems due to their amphipathic character. They easily adsorb to oil-water interface and form film structure around oil droplets. Additionally, they protect oil droplets against aggregation (Alashi et al., 2013; Belitz et al., 2004; Sikorski, 2006). Oil droplets are formed by homogenization and proteins rapidly adsorb to the newly created their surfaces by lowering the interfacial tension and leading to further droplet disruption. This situation also decreases the energy necessity to generate the small droplets and provide the protective effect to droplet aggregation (Belitz et al., 2004; McClements, 2002). During protein adsorption to oil phase through hydrophobic residues, water

molecules are displaced from this region. This mechanism is thermodynamically favourable and the diffusion rate of proteins depends on temperature, molecular weight, pH, and ionic strength. Exposure of hydrophilic and hydrophobic groups determines the adsorbability while amino acid composition, molecular weight and intramolecular disulphide bonds determines the conformative stability (Belitz et al., 2004). There are also some other intrinsic factors such as presence of low-molecular-weight surfactants, sugars, oil-phase volume, type of protein, and the melting point of the oil used and extrinsic factors such as type of equipment, rate of energy input, and rate of shear that influence the emulsion property of proteins (Damodaran, 1996). Proteins are very suitable for oil-in-water emulsions and the ideal emulsifier protein would have a relatively low molecular weight, a balanced amino acid composition in terms of charged, polar and non-polar residues, good water solubility, well-developed surface hydrophobicity, and a relatively stable conformation (Belitz et al., 2004). In water-in-oil emulsions, they have limited solubility in oil phase therefore they are not preferred as an effective emulsifier.

The protein film, which forms around the oil droplet, prevents the droplets from coalescing and flocculation with each other by using repulsion forces provided by its electrostatic charges and steric hindrance. The emulsion stability depends on van der Waals interactions, steric hindrance and electrostatic interactions (repulsion or attraction) between the droplets. Flocculation is a formation of clusters of globules producing creamy layer. In dilute emulsions, flocculation increases the product viscosity and decreases the creaming stability whereas in concentrated emulsions, it leads the formation of a three-dimensional particle network that gives the product gel-like qualities. In addition to flocculation, droplet coalescence also decreases the stability by increasing the mean droplet size and causes the changing emulsion appearance and oiling off (McClements, 2002; Sikorski, 2006).

Emulsions are highly effected by protein solubility, pH of the environment, surface hydrophobicity and denaturation ratio of proteins. Proteins with high solubility at the isoelectric point show high emulsifying activity and capacity whereas proteins with minimum solubility and lacking electrostatic repulsive forces show poor activity and capacity (Damodaran, 1996). Non-polar parts on the protein surface contacting to bulk water increase the emulsifying activity by decreasing the surface tension. Proteins often partially unfold and protein-protein interactions occur. If the proteins containing these interactions adsorb to the same droplet, polymerization through disulphide-

sulfhydryl interchange reaction occurs so that the viscoelasticity of the interfacial membrane increases. In contrast, if they are adsorbed onto different droplets, droplet flocculation is seen (McClements, 2002).

2.2.2.2. Foaming Property of Proteins

Food foams are dispersions of gas bubbles in a condensed continuous phase, which may be either predominantly liquid or solid depending on the nature of the food. They are generally formed by bubbling, whipping or shaking a protein solution (McClements, 2002; Sikorski, 2006). Proteins adsorb at the surface of gas bubbles through hydrophobic areas and lower the gas-liquid interfacial tension. After conformational changing of proteins, they form a rupture resistant, flexible and cohesive firm film surrounding the bubbles at gas-liquid interfaces (Alashi et al., 2013; Moure et al., 2006). The protein-protein interactions make the gas bubbles highly viscoelastic and provide a strong resistance to deformation. The foam stability depends on the resistance of the foams to gravitational and mechanic stress such as drainage of the liquid from the inter sheet space due to gravity, pressure or evaporation; diffusion of gas from smaller to larger bubbles; and coalescence of bubbles by rupturing of the protein film (Belitz et al., 2004; McClements, 2002; Sikorski, 2006). Similar to emulsifying property of proteins, proteins form more stable foams if they are soluble at their isoelectric pH, but they are generally less soluble; therefore the soluble protein fraction would form little of the volume of the foam while the insoluble fraction would contribute to the foam stability by increasing cohesive forces in the protein film (Damodaran, 1996). Salt ions such as Ca^{2+} and Mg^{2+} provide a contribution to the foam stability by increasing the crosslinks between the protein molecules by neutralisation of charges. Therefore salting out effect and better viscoelastic properties are appeared because the amount of the protein absorbed to the interface is increased. On the other hand, if the protein solution is incubated with salt ions for a long time, the ions cause aggregation and micellisation of the proteins resulting to low foaming property (Sikorski, 2006). Sugars also increase foam stability by increasing the viscosity of the bulk-phase and reducing the drainage of the lamella fluid. Heating has both positive and negative effect on foam formation and stability. In some cases, it causes the gelation of the protein at the interface and provides the sufficient mechanical strength to the foam. On the other hand, due to expansion of

air and decreasing the viscosity may result in bubble rupture and foam collapse. Generally, the foam formation capacity of proteins negatively correlates to their foam stability. The contradicting factors effect the foaming property of proteins. The foam forming capacity is generally related to adsorption, flexibility and hydrophobicity of proteins however foam stability is influenced by rheological properties of the protein film. Non-covalent interactions and disulphide bonds between the loops make a contribution to form a gel network with a particular viscoelastic and mechanical properties (Damodaran, 1996). Partial enzymatic hydrolyses of proteins increases the foaming capacity of the film by producing smaller and faster diffusing protein molecules. The foam stability also can be improved by embedding charged or neutral groups to proteins or thermal denaturation.

2.2.3. Bioactive Property of Proteins

In addition to their nutritional value, proteins also have positive impact on body functions or conditions which ultimately may influence health. Especially small peptides containing 3-20 amino acids unit per molecule are more bioactive in comparison with longer peptides and they are inactive form in parent protein sequence. To be able to show their bioactivity they are generally needed to be exerted from main molecule. Because of their small size, short peptides are not digested rapidly by proteolytic enzymes and can easily pass through the digestive system. Therefore they also can easily reach the blood system and are transported to target organs. Short peptides have relatively long half-life and economically cheap and easy to produce in large amounts.

Bioactive peptides can show their bioactivity as antimicrobial, antioxidant, antihypertensive, anticancerogenic, antiplatelet, antidiabetic, antiobesity, prebiotic, mineral binding or immunomodulatory molecules. Some peptides are multifunctional so that they can exhibit more than one bioactive property (Fuentes & Palomo, 2014; Ngo, Vo, Ngo, Wijesekara, & Kim, 2012; Singh, Vij, & Hati, 2014).

2.2.3.1. Antioxidant Activity of Proteins

Lipid oxidation and free radical formation are major deteriorative effects for food quality and human health. In industry, many artificial antioxidants such as BHA and BHT are extensively used, but recently they have been restricted or prohibited due to potential risks for human health such as showing suspicious cancerogenic effect (Madhavi, Deshpande, & Salunkhe, 1996; Saiga, Tanabe, & Nishimura, 2003). Therefore the demand of industry for natural sourced antioxidants such as polyphenolic compounds, carotenoids and proteins is increasing and in these area proteins are one of the suitable antioxidants due to their free radical scavenging activity and ability of chelating prooxidative transition metals (Elias, McClements, & Decker, 2005). The type of amino acids, protein sequence, distribution of hydrophobic residues, structure and length of polypeptide, and position of amino acids in the chains are the factors which determine their antioxidant activity in proteins (Chen, Muramoto, Yamauchi, & Nokihara, 1996; Rajapakse, Mendis, Jung, Je, & Kim, 2005; Saiga et al., 2003). Especially, aromatic amino acids and sulphur-containing amino acids exhibit higher antioxidant activity compared to other amino acids (Elias et al., 2005). Tryptophan, tyrosine and phenylalanine show their antioxidant activity by donating hydrogen atoms to free radicals (Elias et al., 2005; Rajapakse et al., 2005). Cysteine is a good peroxynitrite scavenger (Chen et al., 1996). Moreover, carboxyl and amino groups of acidic and basic amino acids show their antioxidant activity by chelating Fe^{2+} and Cu^{2+} ions which are prooxidants for free radical formation. Histidine exhibits bifunctional antioxidant activity with its imidazole ring by serving as both free radical scavenger and metal chelators. The hydrophobicity of proteins has an important influence on antioxidant activity because it promotes protein-lipid interaction (Chen et al., 1996; Rajapakse et al., 2005; Saiga et al., 2003). Surface exposure of antioxidant residues greatly affects their oxidation kinetics that varies from one amino acid to another (Elias et al., 2005). Moreover, (Hu, McClements, & Decker, 2003) reported that the cationic characteristics of a protein inhibit lipid oxidation due to the electrostatic repulsion of transition metals away from the lipid droplets. Enzymatic or acidic hydrolysis of proteins can increase the antioxidant activity by exposing their functional antioxidant groups (Elias et al., 2005; Saiga et al., 2003).

2.2.3.2. Antihypertensive Activity of Proteins

The most studied bioactive peptides from food sources are the antihypertensive peptides which show the activity by inhibiting the action of angiotensin-1-converting enzyme (ACE) (Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011). ACE (E.C. 3.4.15.2) is a monomeric glycoprotein which is distributed in many tissues and biological fluids. In humans there are two isoforms of ACE; somatic ACE and germinal ACE. Somatic ACE is found in many endothelial and epithelial cells and it has two homologous active sites. The structure of enzyme allows only small peptide substrates access to the active site. The enzyme plays an important role in regulating human blood pressure and fluid homeostasis. In resin angiotensin system it provides the vasoconstriction which increases the blood pressure. Therefore the ACE inhibitors such as captopril, enalapril, and lisinopril have been developed in clinical treatments. Unfortunately these drugs have side effects like dry cough and angioedema. They are also fetotoxic and cause intrauterine growth retardation, anuria, hypocalvaria, renal dysplasia, renal failure, congenital malformations, and death (Guang & Phillips, 2009).

Therefore, the peptides obtained from natural sources with antihypertensive activity have been discovered. There are many peptides produced from milk, egg, and muscle protein show in vitro antihypertensive activity furthermore some of them also display a significant antihypertensive activity in rats and human studies (Hong et al., 2008; Li, Le, Shi, & Shrestha, 2004; Martinez-Maqueda, Miralles, Recio, & Hernandez-Ledesma, 2012; Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008; Ryan et al., 2011). Among plant sources, ACE inhibitory peptides have been identified from soybean, sunflower, rice, corn, wheat, buckwheat, broccoli, mushroom, garlic, and spinach. These ACE inhibitory peptides are safer and more natural for human consumption and they could be applied as a part of daily diet in order to prevent of hypertension and they can be used as initial treatment in mildly hypertensive cases.

2.3. Modification of Proteins

Since 5000 B.C. food proteins are modified empirically to both enhance the palatability and stability of products and to improve the nutritive and organoleptic properties. Modification of proteins is extensively used in daily life and industry. For

examples; meat is ripened by modification of proteins with proteases; cheese and yoghurt are produced by enzymatically modified milk proteins. In order to increase the digestibility, egg proteins are boiled. Furthermore, the modification of proteins is also used to study the structure-function relationship of food proteins. In some situation these modifications may cause the undesirable changes or deterioration of its nutritional and functional qualities. In protein modification, the goal is to manipulate protein structure by using safe chemical and enzymatic methods or by genetic engineering (Alashi et al., 2013; Howell, 1996; Schwanke, 1997; Sun-Waterhouse, Zhao, & Waterhouse, 2014; Tavano, 2013).

2.3.1. Chemical Modification

Chemical modification of proteins includes the derivatization of the amino acid side chains and has been used to represent protein conformation and enzymatic mechanism (Howell, 1996). In Table 2.3, some amino acid side chains and type of subjected chemical modification are listed.

Acylation: Acetic (acetylation), succinic (succinylation), maleic, or citratonic anhydride is the most convenient and most frequently used methods for modification the functional properties of proteins. These agents react with all nucleophilic groups including amino groups (N-terminal and ε-amino groups of lysine), phenolic groups (tyrosine), aliphatic (serine and threonine) hydroxyl groups, sulfhydryl groups (cysteine), and imidazole (histidine groups) (Schwanke, 1997). The acylation process causes the dissociation of oligomeric proteins into their subunits (Narayana & Rao, 1991). With the blockage of amino groups with acyl residues, the isoelectric point of the proteins shifts to lower pH region and protein solubility increases in weak acidic, neutral and even alkaline solutions. However increase in water absorption capacity of proteins is observed for succinylated wheat gluten, pea, and peanut proteins, a contradictory effect is seen for sunflower and oat proteins. After acylation of soybean, faba bean, oat, pea, and sunflower proteins, an increase in the emulsion properties (emulsion capacity and stability) and foaming capacity have been reported; in contrast foaming stability of proteins was decreased due to preventing protein-protein interactions to form a continuous film around bubbles. Gelling properties are also altered by succinylation with maximizing gel strength (Schwanke, 1997). From the view

of nutritional aspects, acylation gives both positive and negative results. While the initial rate of proteolysis was increased by succinylation, the utilization of the modified amino acid residues and net protein utilization were decreased (Goulet, Ponnampalam, Amiot, Roy, & Brisson, 1987; Siu & Thompson, 1982). On the other hand, the digestibility differences were also seen between the modified and unmodified casein (Schwanke, 1997).

Table 2.3. Amino acid side chains involved in chemical modification
(Source: Howell, 1996)

Side Chain	**Chemical Modification**
Amino	Acylation, alkylation
Carboxyl	Esterification, amidation
Disulfide	Oxidation, reduction
Sulfhydryl	Oxidation, alkylation
Thioether	Oxidation, alkylation
Phenolic	Acylation, electrophilic substitution
Imidazole	Oxidation, alkylation
Indole	Oxidation, alkylation

Alkylation: Generally modification reaction is seen on sulfhydryl groups with the alkylation of amino groups with haloacetates, acrylonitrile, ethylenimine and aryls. This method can be used for protein determination of food products including milk as amino groups can react with formaldehyde (Howell, 1996).

Esterification: This process provides increased isoelectric point or net positive charge of proteins by blocking negatively charged carboxyl groups with different alcohols (methanol, ethanol, n-propanol, isopropanol) under acidic conditions (Howell, 1996; Schwanke, 1997; Sitohy, Chobert, & Haertle, 2000). This modification causes conformational changes which affect the functional properties of proteins. Due to changes in folding and peptic hydrolysis of esterified β-lactoglobulin, it becomes very susceptible to pepsin hydrolysis in aqueous medium (Sitohy et al., 2000). Moreover, esterified β-lactoglobulin shows better emulsion stability and oil adsorption properties at the oil-water interface. Esterification also effects the rheological properties of wheat gluten (Howell, 1996).

Amidation: This process includes the conversion of aspartate and glutamate to asparagine and glutamine by reacting amine group and water-soluble carbodiimide, respectively. Amidation provides covalent attachment of essential amino acids to proteins with enhancing the foaming properties (Howell, 1996).

Deamidation: It is the reverse reaction of amidation and it produces increased negative charges and hydration properties of proteins. This reaction is especially important for plant storage proteins as they are rich in amidated aspartic and glutamic acid residues. In order to minimize the peptide bond hydrolysis of vegetable proteins, deamidation process has been performed. Moreover, deamidation improves the emulsifying and foaming capacities and stabilities of glutens and provides more water soluble proteins (Schwanke, 1997).

Glycosylation: It is the attachment of sugar residues to proteins through the Maillard reaction. These protein-polysaccharide complexes show superior emulsifying properties. Furthermore, glycolysation contributes to the antimicrobial activity of lysozyme so as lysozyme-dextran complex shows antimicrobial activity against both gram negative and gram positive bacteria (native lysozyme cannot lyse gram negative bacteria) (Nakamura, Kato, & Kobayashi, 1991, 1992). This modification also increases the viscosity of protein solutions (Schwanke, 1997). It was also reported that, glycolysation of beta-lactoglobulin with Maillard reactions gave better emulsifying and foaming properties (Howell, 1996; Schwanke, 1997).

Phosphorylation: Phosphate groups can be covalently attached to proteins to increase the negative charge and the most extensive used method among phosphorylation processes is the reaction with phosphorus oxychloride (Howell, 1996). In human diet some important proteins are phosphoproteins such as milk casein, egg white albumin, and egg yolk phosvitin (Schwanke, 1997). Phosphorylation enhances the viscosity, water absorption capacity, gelation, and emulsification of proteins and it also provides better digestibility (Howell, 1996). While modified soybean and yeast protein isolates show better foaming capacity and stability, modified casein and modified gluten proteins exhibit better gelation property (Giec, Stasinska, & Skupin, 1989; Matheis, Penner, Feeney, & Whitaker, 1983; Matheis & Whitaker, 1984; Schwanke, 1997).

Acid and alkali treatments: Acid and alkali have been extensively used for protein extraction to increase solubility, to lower viscosity, and fibre formation (Howell, 1996). Mild acid hydrolysis causes improved protein solubility and emulsification and foaming properties for wheat gluten (Wu, Nakai, & Powrie, 1976). Alkali treatment

modifies proteins faster than acid treatment. It is extensively employed for the production of texturized soy proteins to be used as meat analogues and in the manufacture of gelatine from collagen (Howell, 1996).

2.3.3. Enzymatic Modification

Enzymes are mainly used to modify proteins through hydrolysis of peptide bonds to produce amino acids and small peptides. In vivo, enzymes are employed for posttranslation modifications, in addition in food processing they have been extensively used for cross-linking of polypeptide chains, phosphorylation, glycosylation, hydroxylation, and methylation. Enzymatic modification alters the functional properties of proteins. Generally proteinases, transglutaminases, protein kinases, peptidoglutaminases, peroxidases and polyphenol oxidases among enzymes are industrially used for various modifications of proteins (Howell, 1996; Schwanke, 1997; Sun-Waterhouse et al., 2014; Tavano, 2013).

Proteinases (neutral and acidic proteinases) are generally used to produce flavours and clinical nutrition products in food and pharmaceutical industry, respectively. The desired flavours are small, soluble and non-bitter peptides. In cheese production rennin is used to hydrolyse kappa-casein to initiate to coagulation. For tenderization and recovery of meat, plant enzymes such as ficin, papain, and alcalase applications are carried out. In order to decrease the strength the dough which is made from hard wheat or strong flour with a high gluten content, proteinases from *Aspergillus oryzae* are added into the dough mixture (Howell, 1996). Proteinases increase the solubility of proteins which are low water soluble in native form such as cereal proteins. Proteolytic enzymes may also improve the water absorption capacity and emulsifying capacity of several proteins (Mohri & Matsushita, 1984; Ponnampalam, Goulet, Amiot, & Brisson, 1987).

Transglutaminase has the capability to catalyse the attachment of glycosyl residue to glutamine residues of protein and this enzyme is used for glycosylation of beta-casein, pea legumin and wheat beta-gliadins. It catalyzes the incorporation of primary amines with proteins and polypeptides. It is also used in cross-linking, deamidation and phosphorylation of enzymes. It was observed that the enzyme polymerisation also enhanced the emulsifying, water binding, and gelling properties of

casein and soybean globulins. Moreover, enzymatically phosphorylated soybean proteins by protein kinases show improved emulsifying activity and emulsion stability (Howell, 1996; Schwanke, 1997).

Another method to improve the functional properties of food proteins is to subject artificial cross linking. For this purpose transglutaminase, peroxidase and polyphenol oxidase are suitable. Depending on the nature of protein cross-linking, it may shows both positive and negative effect on the gel formation process. Additions of peroxidase or lipoxygenase improve the dough-forming properties and baking performance of wheat flours while polyphenol oxidase causes strengthening of wheat gluten dough.

CHAPTER 3

MATERIALS AND METHODS

3.1. Functional Properties and Antioxidant Activity of Protein from Major Turkish Chickpea and Lentil Cultivars

3.1.1. Functional Properties of Chickpea and Lentil Proteins

3.1.1.1. Materials

The dry seeds of chickpea cultivars, Cevdetbey and Sarı, were obtained from Aegean Agricultural Research Institute in Menemen (Turkey). All other dry chickpeas and lentils were obtained from General Directorate of Agricultural Research in Ankara (Turkey). The seeds were grown in the experimental fields of research institutes for research purposes. Kabuli type chickpea cultivars were Canıtez, Cevdetbey, Gökçe, Sarı; red lentil cultivars were Alidayı, Çiftçi, Fırat, Kafkas; and green lentil cultivars were Meyveci, Pul II. The dry soybeans (non-GMO) used in soy protein extract (SPE) production (see method given in section 3.1.1.2) were purchased from a supermarket in Izmir (Turkey). Commercial whey protein isolate (WPI) obtained from sweet whey (Product name: BiPRO, Not denatured, Spray dried, Total protein content: 0.98 g/g) was kindly donated by Davisco Foods International, INC (MN, USA). Commercial soy protein isolate (SPI) obtained from non-GMO defatted and dehulled soybeans (Product name: Dunasoy 90, Total protein content: min 0.90 g/g) and soy protein concentrate (SPC) obtained from non-GMO defatted and dehulled soybeans (Product name: Dunasoy 70, Total protein content: 0.68-0.70 g/g) were from Euroduna Rohstoffe GmbH (Germany). Fish gelatine (FGEL,) from cold water fish skin and bovine skin gelatine (BGEL) (Type B, Bloom: 225) were obtained from Sigma Chem. Co. (St. Louis, MO, USA). The egg white protein (EWP) was produced from standard fresh broiler eggs obtained from a supermarket in Izmir (Turkey) by lyophilization of egg whites separated in the laboratory.

3.1.1.2 Globulin Extraction from Chickpea and Lentil Cultivars

3.1.1.2.1. Preparation of Acetone Powder from Raw Material

To remove lipids and free phenolic compounds, chickpeas, lentils and soybeans were processed to acetone powder (AP) according to the method given by Arcan and Yemenicioglu (2007). For the preparation of acetone powders, 50 g of raw materials were rehydrated with 200 mL of deionized water overnight. Then rehydrated materials were homogenized in a Waring blender for 3 minutes with 200 mL of cold acetone. The obtained slurry was filtered under vacuum through a Buchner funnel containing a Whatman No:1 filter paper and the residue remaining on the filter paper was collected. The collected residue was homogenized with 200 mL cold acetone and filtrated again for two times. The collected residue was left overnight to evaporate the acetone and stored −18 °C until it was used in the protein extraction.

3.1.1.2.2. Globulin Extraction from Acetone Powder

To obtain protein extracts, 20 g of AP was suspended in 250 mL deionized water by stirring with a glass rod 100 times. The pH of the mixture was then adjusted to 9.5 with 1 mol/L NaOH. In chickpeas, for inactivation of highly active oxidative enzyme lipoxygenase, the extracts were heated to 85 °C and maintained at this temperature for 30 minutes under continuous magnetic stirring (Arcan & Yemenicioglu, 2007). After that, the extracts were cooled to room temperature in ice water bath by stirring for 15 minutes. The lentil extracts could not be heated since they showed extreme browning by heating. Thus, they were extracted at room temperature for 45 minutes under continuous stirring. The soybean extracts were also treated similar to lentil extracts. All extracts were clarified by centrifugation for 30 minutes at 15000 × g (at 4 °C). Part of each extract was separated, lyophilized and kept as albumin-globulin extract for SDS-PAGE analysis, while the remaining extracts were further purified with the classical isoelectric precipitation (IEP) method. The IEP was applied by adjusting the pH of extracts to 4.5 with 1 mol/L acetic acid. The precipitated proteins were collected with centrifugation and resuspended in distilled water. The pH of the suspensions was once more adjusted

to 4.5 and proteins were once more precipitated and collected with centrifugation for 15 minutes at 15000 × g (at 4 °C). Finally, the obtained proteins were suspended in distilled water and lyophilized (Labconco, FreeZone, 6 liter, Kansas City, MO, USA), after adjusting their pH to 7.0. The legume protein extracts obtained by the classical isoelectric precipitation method (IEP method) contain mainly globulins. The lyophilized chickpea, lentil and soybean protein extracts stored at -18 °C for several months until they were used for characterization of their functional properties. The chickpea protein extract and lentil protein extract were also characterized for their molecular properties by 2-D electrophoresis.

3.1.1.3. Determination of Total and Water Soluble Protein Content of Protein Extracts

The total nitrogenous compounds in protein extracts were determined by the Kjeldahl method using an automated testing machine (Gerhard Vapodest 50 s and Kjeldahl Therm, Germany). 50 mg of lyophilized protein extract was heated to 420 °C with 15 mL of 96 % (v/v) H_2SO_4, catalyzer and antifoaming agent and maintained for 4 hours. After the burning step, the samples were cooled to room temperature and the titration process was carried out with 0.1M HCl. The total protein contents (TPrC) of lyophilized protein extracts were calculated by using different conversion factors (5.4 for FGEL, 6 for EWP, and 6.25 for chickpea protein extracts, lentil protein extracts and SPE). The average of three replicates was used to calculate TPrC and the results were expressed as g protein/g lyophilized protein extract.

The water soluble protein content (WSPC) of lyophilized protein extracts was determined by the Bradford method using bovine serum albumin (BSA) as standard (Bradford, 1976) (Appendix A). For Bradford solution preparation 100 mg of Coomassie Brilliant Blue, 50 mL of ethanol, and 10o mL of phosphoric acid (85 %) were dissolved in deionised water (total volume 1 L) and were filtered through filter paper. 20 mg of lyophilized protein extracts were dissolved in 10 mL of deionized water and the pH of the solution was adjusted to 9.5 with 0.1 mol/L NaOH. The solutions were magnetically stirred for 30 minutes at room temperature and they were centrifuged at 4500 × g (at 4 °C) for 20 minutes to remove insoluble residues. 50 µl of protein solution was added into 2.5 mL of Bradford solution and incubated for 5 minutes. The

absorbance measurements of solutions were conducted at 595 nm and were expressed as g soluble protein/g of protein extract. WSPC of lyophilized protein extracts was calculated as the average of three repetitions and five replications.

3.1.1.4. Determination of Gel Forming Capacity of Protein Extracts

The gel formation capacity of protein extracts was determined by finding the least gelling concentration (LGC) (Aydemir & Yemenicioglu, 2013). For this purpose, a series of protein solutions were prepared in distilled water (concentrations between 1 to 14 g/100 g or between 1 to 20 g/100 mL). All protein solutions were prepared at room temperature, except BGEL which dissolves at 50 °C. The solutions were then placed into test tubes (1.46 cm in diameter) and they were heated in a water bath at 90 °C for 1 hour. The tubes were then cooled immediately to room temperature and incubated for 2 hours at 4 °C for gel formation. The gel formation was detected by observing the flow characteristics of tube contents when tubes were turned upside down. The LGC corresponds to the lowest protein concentration (g/100 g or g/100 mL) that gives hard gel with no falling or slipping by gravity when tubes are turned upside down.

3.1.1.5. Determination of Water and Oil Absorption Capacity of Protein Extracts

To determine the water (WAC) and oil absorption capacities (OAC), 50 mg of protein extract and 1.5 mL of distilled water or commercial sunflower oil were mixed at room temperature for 20 seconds by vortex in a 2 mL centrifuge tube (Aydemir & Yemenicioglu, 2013). After mixing, the lids of tubes were closed and the tubes were incubated at 30 °C for 30 minutes. The tubes were then centrifuged at 15000 × g (25 °C) for 20 minutes and the separated free water or oil in their supernatants was removed carefully. The absorbed water or oil content was determined by weighing of the tubes. WAC and OAC were expressed as g of water or oil absorbed per g of protein extract, respectively. The tests were repeated for three times for each of the protein samples.

3.1.1.6. Determination of Emulsifying Activity and Emulsion Stability of Protein Extracts

Emulsifying activity (EA) of lyophilized protein extracts was determined by modification of the method described in Pearce and Kinsella (1978). Briefly, 20 mL of protein solution (10 mg/mL) was prepared in distilled water and its pH was adjusted to 7.0. The solution was stirred for 30 minutes at 30 °C (at 50 °C to dissolve BGEL) and 6.5 mL of commercial corn oil was added onto it at room temperature. After that, the mixture was emulsified by homogenization at 22000 rpm for 2 minutes in a homogenizer-disperser (Yellow Line, DI 18 Basic, Brazil). A 200 µL sample of the emulsion was then taken and mixed with 25 mL of SDS solution (10 mg/mL). The emulsifying activity was determined by measuring the turbidity of sample as nephelometric turbidity units (NTU) by a turbidity meter (HACH, 2100 AN, U.S.A.). The absorbance of the samples was also determined at 500 nm. The emulsion stability (ES) of samples was determined by measuring their turbidities (also absorbances) at 30^{th} and 180^{th} minutes of emulsification. All measurements were repeated for three times for each of the protein sample and results were expressed as NTU and absorbance.

3.1.1.7. Determination of Foaming Activity and Foam Stability of Protein Extracts

To determine the foaming activity (FA), a 25 mL of protein solution (10 mg/mL) was prepared from each protein extract and its pH was set to 7.0 (Aydemir & Yemenicioglu, 2013). The protein solutions were prepared at room temperature, except BGEL which dissolves at 50 °C. The solutions were then homogenized in the disperser-homogenizer at 22000 rpm for 1 minute to induce foaming. The foaming activity was determined by measuring volume of the formed foam as mL. The foam stability (FS) was determined by measuring foam volume at 30^{th} and 180^{th} minutes of foam formation. All measurements were repeated for three times for each of the protein sample.

3.1.1.8. SDS-PAGE and 2-D Electrophoresis of Chickpea and Lentil Protein Extracts

The classical SDS–PAGE was conducted for different protein extracts. In order to determine the SDS-PAGE protein patterns albumin-globulin extract of chickpea and lentil cultivars were produced. For this purpose 20 g of AP of chickpea or lentil cultivar was suspended in 250 mL deionized water by stirring with a glass rod 100 times. The pH of the mixture was then adjusted to 9.5 with 1 mol/L NaOH to solubilize proteins and extracted at room temperature for 45 minutes under continuous magnetic stirring. The extract was then clarified by centrifugation for 30 minutes at 15000 × g (at 4 °C) and its supernatant containing albumin-globulin proteins was lyophilized (Labconco, FreeZone, 6 liter, Kansas City, MO, USA) after adjusting its pH to 7.0. The extracts were lyophilized and stored at -20 °C until their SDS-PAGE was conducted on a discontinuous buffered system according to Laemmli method using separating gel (150 mg/mL) and stacking gel (50 mg/mL) (Dunn, 1989). Lyophilized samples were directly solubilized in sample buffer and centrifuged at 15000 × g for 30 minutes. The samples were then heated for 5 minutes in boiling water before electrophoresis. The electrophoresis was performed at a constant current of 12 mA. Protein fixation was performed with TCA (200 mg/mL). The gel was stained with methanol (12.3 mol/L), acetic acid (1.75 mol/L), Coomassie brilliant blue (R-250) (0.5 mg/mL) solution. The gel destaining was accomplished by using methanol (1.23 mol/L) and acetic acid (2.18 mol/L) solution. Wide molecular weight range molecular marker was used to characterize the obtained protein bands (Sigma Marker™, Sigma-Aldrich).

The two dimensional (2-D) electrophoresis (isoelectric focusing and SDS-PAGE) was applied to protein extracts with the ReadyPrep™ 2-D Starter Kit (BioRad Laboratories Inc.) according to the method given by (Arcan & Yemenicioglu, 2010). The sample was prepared in deionized water and then diluted with sample buffer. IPG Strips (ReadyStrip™, BioRad), 17 cm and pH 3-10, were used for isoelectric focusing (IEF). IEF was applied using the Protean IEF cell (BioRad) with 3 step voltage protocol: 250 V for 20 minutes (linear ramp), 10,000 V for 2.5 hours (linear ramp), and total 40,000 V × h rapid ramp step. SDS-PAGE was performed at a two steps constant current protocol: 16 mA/gel for 30 minutes, then 24 mA/gel for 5 hours in SDS (120 mg/mL) gel with PROTEAN II XL (BioRad). The protein fixation, staining and

destaining were performed as given in the classical SDS-PAGE method above. For Protein Ladder, 10-200 kDa (Fermentas International, Inc.) or and 2-D SDS-PAGE standard, pI range 4.5 – 8.5, (BioRad) were used as marker.

3.1.1.9. Statistical Analysis

Statistical analysis was carried out by using one-way analysis of variance (ANOVA) with a significance threshold of $P<0.05$ as determined by Fisher's least significant difference (LSD) method.

3.1.2. Antioxidant Properties and Characterization of Antioxidant Fractions of Lentil Protein Extracts

3.1.2.1. Antioxidant Properties of Lentil Protein Extracts

3.1.2.1.1. Materials

The dry seeds of red and green lentil cultivars were obtained from General Directorate of Agricultural Research in Ankara (Turkey). For the other protein sources, please see the section 3.1.1.1. ABTS (2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) was purchased from Sigma Chem. Co. (St. Louis, MO, USA). AAPH (2,2'-Azobis[2-methyl-propionamidine] dihydrochloride was purchased from Sigma-Aldrich Chemie GmbH (Germany). Ferrozine (3- (2-Pyridyl)-5,6-diphenil-1,2,4-triazine-4'4''-disulfonic acid monosodium salt), fluorescein sodium salt, and Trolox ((±)-6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid) were purchased from Fluka (St. Louis, MO, USA).

3.1.2.1.2. Protein Extraction from Lentil Cultivars

3.1.2.1.2.1. Globulin Extraction from Lentil Acetone Powder

To obtain globulin extract from lentil cultivars, lentils firstly were processed to acetone powder (See section 3.1.1.2.1). Then, globulin extraction was conducted with the similar method described in 3.1.1.2.2 (Figure 3.1).

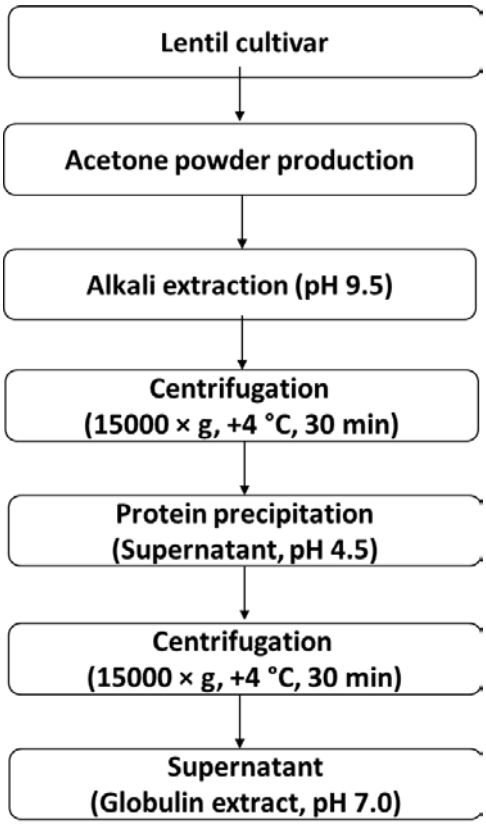

Figure 3.1. Globulin extraction from lentil cultivars

3.1.2.1.2.2. Albumin-Globulin Extraction from Lentil Acetone Powder

20 g of AP of lentil cultivar was suspended in 250 mL deionized water by stirring with a glass rod 100 times (Aydemir & Yemenicioglu, 2013) (Figure 3.2). The pH of the mixture was then adjusted to 9.5 with 1 mol/L NaOH to solubilize proteins and extracted at room temperature for 45 minutes under continuous magnetic stirring.

The extract was then clarified by centrifugation for 30 minutes at 15000 × g (at 4 °C) and its supernatant containing proteins was lyophilized (Labconco, FreeZone, 6 liter, Kansas City, MO, USA) after adjusting its pH to 7.0. Protein extract which was prepared by this method contained mainly albumins and globulins (Makri et al., 2005). The lyophilized protein extracts were stored at -18 °C for several months until they were used in tests.

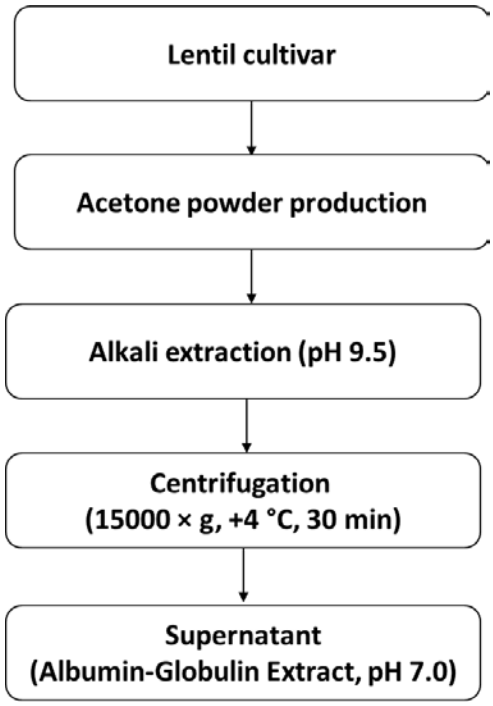

Figure 3.2. Albumin-globulin extraction from lentil cultivars

3.1.2.1.3. Determination of Total and Water Soluble Protein Content of Lentil Protein Extracts

Total and water soluble protein content of lentil protein extracts were determined according to the method described in section 3.1.1.3.

3.1.2.1.4. Determination of Total Phenolic and Flavonoid Content of Protein Extracts

The total phenolic content (TPC) of protein extracts was determined using the Folin-Ciocalteu method of Singleton and Rossi (1965). 0.25 mL of appropriately extract solution was mixed with 2.5 mL of 10 % (v/v) Folin-Ciocalteu reagent and incubated for 3 minutes at room temperature. Then 2 mL of 7.5 % (w/v) Na_2CO_3 solution was added into the mixture and further incubated for 2 hours. After incubation, the absorbance of solutions was read at 765 nm and the TPC of extracts was expressed as µg gallic acid/g extract using the calibration curve prepared by gallic acid (Appendix C). All measurements were conducted three times.

The total flavonoid content (TFC) of extracts was determined using the method described by Zhishen, Mengcheng, and Jianming (1999). Before analysis 0.25 mL of extract solution was diluted with 1 mL of deionized water. Then, 0.075 mL of 5 % (w/v) $NaNO_3$ was added into the diluted sample and mixed. After 5 minutes incubation, 0.075 mL of 10 % (w/v) $AlCl_3$ was added into the mixture and it was further incubated for 6 minutes. At the end of the incubation period, 0.5 mL of 1 mol/L NaOH solution was added into the mixture and its absorbance was determined at 510 nm following dilution with 0.6 mL of deionized water. The TFC was expressed as µg epicatechin/g of extracts using the calibration curve prepared by epicatechin (Appendix D). All measurements were conducted three times.

3.1.2.1.5. Determination of Free Radical Scavenging Activity of Protein Extracts

The antioxidant activity of protein extracts were evaluated by determination of their free radical scavenging capacity with oxygen radical absorbance capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC) tests (Appendix E, Appendix F). The samples used in the determination of antioxidant potential were prepared in distilled water. The lyophilized protein extracts were suspended in distilled water (0.01 g protein extract/mL water), dissolved by magnetic stirring for 30 minutes at room temperature and clarified by centrifugation at 15000 × g for 20 minutes (4 °C).

The ORAC method was applied by slightly modifying the procedure given by Xu and Chang (2007). Briefly, 20 µL of protein sample was pipetted into a 96 well plate (black). Then, 200 µL of 0.096 µmol/L fluorescein solution prepared in 75 mmol/L phosphate buffered saline (PBS) (contained 150 mmol/L NaCl) at pH 7.4 was added into each well and the mixtures were incubated at dark for 20 min at 37 ^{o}C. The reactions were started by adding 20 µL of 100 mmol/L AAPH (prepared in 75 mol/L phosphate buffer saline with 150 mmol/L NaCl). The fluorescence of reaction mixture was monitored periodically for 40 minutes at the excitation wavelength of 485 nm and emission wavelength of 520 nm by using a Varioskan Flash spectrophotometer (3001, Finland). The measurements were conducted as two repetitions and three replicates and calculated ORAC values were expressed as µmol Trolox equivalents per g of extract.

The TEAC method was applied against ABTS free radical by the method of Re et al. (1999). The reaction mixture was prepared by mixing 25, 50 and 75 µl of sample with 2 mL of ABTS free radical cation solution prepared in PBS at pH 7.4. The reduction in absorbance values were recorded at 734 nm with a spectrophotometer (Shimadzu Model 2450, Japan). The area under the curve (AUC) value of samples was calculated as described in the given method (for test periods of 1, 3, 6, 9, 12, and 15 minutes). All measurements were conducted for three times, and TEAC values were expressed as µmol Trolox equivalents per g of extract.

3.1.2.1.6. Determination of Iron Chelating Capacity of Protein Extracts

The iron chelating capacity (ICC) of protein extracts was determined according to the spectrophotometric method given by Arcan and Yemenicioglu (2007). 2 mL of protein extract solution was mixed with 0.1 ml of 1 mmol/L $FeCl_2.4H_2O$ solution and incubated for 30 minutes at room temperature. Then 0.1 mL of 0.5 mmol/L ferrozine was added into the solution and incubated for 10 minutes. After incubation, the absorbance of solution was read at 562 nm. The percent ICC of protein extract was determined by using deionized water in place of sample. The ICC of protein extracts was calculated by dividing the slope of the initial linear portion of their ICC (%) vs. concentration (µg/reaction mixture) curves by that slope of the same curve of the chelating agent EDTA. The average of three replicates was used for calculations and the results were expressed as µmol EDTA per g of extract.

3.1.2.2. Characterization of Antioxidant Protein Fractions in Lentil Protein Extract

3.1.2.2.1. Protein Extraction from Fırat Lentil Cultivar

Due to its high antioxidant activity, antioxidant protein fractionation studies were conducted using protein extracts obtained from Fırat lentil cultivar. Fırat lentil cultivar was firstly processed to AP by the method described in section 3.1.1.2.1. Then albumin fraction and globulin fraction of Fırat lentil cultivar were obtained by IEP described in section 3.1.2.2.2. Moreover, albumin-globulin extraction of Fırat lentil cultivar (Figure 3.3) was obtained by the method in section 3.1.2.1.2.2 in order to further fractionation with ultrafiltration and ion exchange chromatography methods.

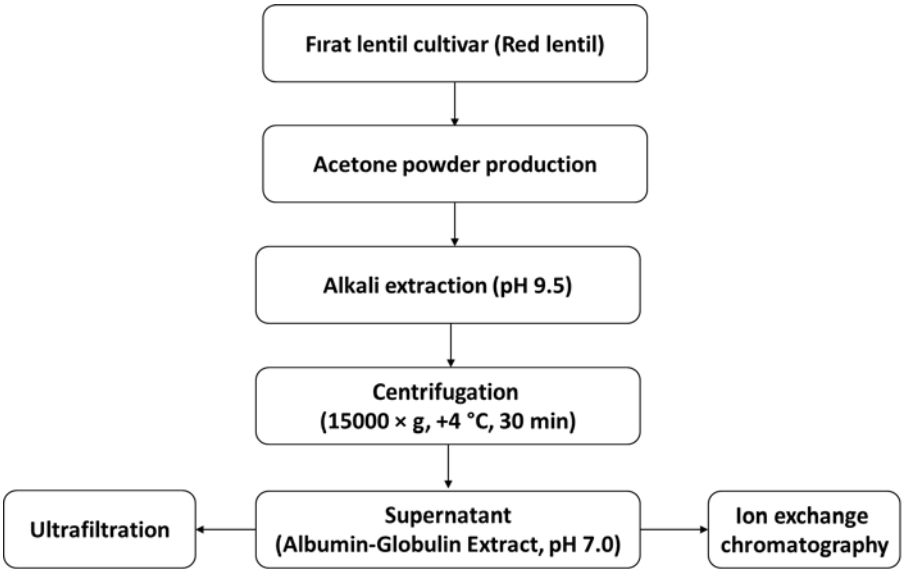

Figure 3.3. LAGE production

3.1.2.2.2. Antioxidant Protein Fractionation of Fırat Lentil Cultivar by Isoelectric Precipitation

For the preparation of lentil albumin extract (LAE) and lentil globulin extract (LGE), the classical isoelectric precipitation (IEP) method was applied which provided albumin and globulin fractions (Figure 3.4). For this purpose, 20 g of AP of Fırat

cultivar was suspended in 250 mL deionized water by stirring with a glass rod 100 times. The pH of the mixture was then adjusted to 9.5 with 1 mol/L NaOH and extracted at room temperature for 45 minutes under continuous magnetic stirring. The extract was then clarified by centrifugation for 30 minutes at 15000 × g (at 4 °C) then the pH of supernatant was adjusted to 4.5 with 1 mol/L acetic acid to precipitate the proteins. In order to separate the globulin fraction, the precipitated proteins were collected with centrifugation and washed with 150 mL of distilled then the pH of the suspension was once again adjusted to 4.5, and globulins were again collected with centrifugation for 15 minutes at 15000 × g (at 4 °C). For albumin fractionation, supernatants obtained from two centrifugation steps were gathered. The globulin precipitation was suspended in water again and then the pH of the albumin solution and globulin suspension was adjusted to 7.0. After lyophilization of the solutions, the LAE and LGE were obtained and they were used in antioxidant measurements.

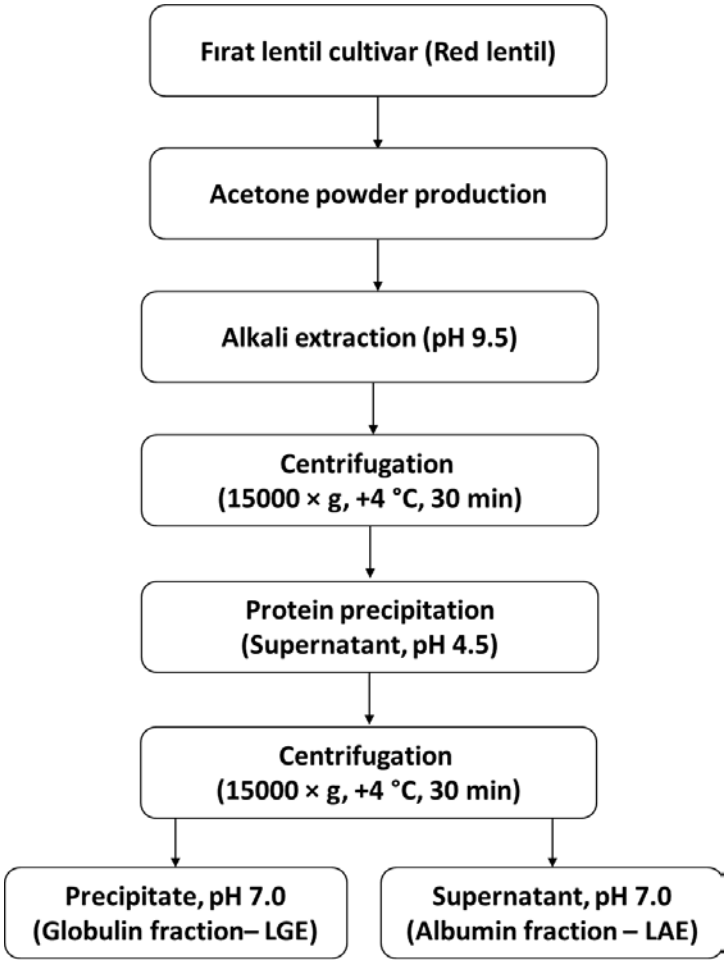

Figure 3.4. IEP fractionation of lentil proteins

3.1.2.2.3. Antioxidant Protein Fractionation of Lentil Albumin-Globulin Extract by Ion Exchange Chromatography

Ion-exchange chromatography (IEC) was applied to lentil albumin-globulin extract to obtain ionic fractions by using DEAE-cellulose anion exchange column (2.4 cm diameter, 10.0 cm height) (Arcan & Yemenicioglu, 2010). 50 mL (5 mg/mL) of LAGE solution was loaded into the column which was previously equilibrated with 0.01 mol/L Na-phosphate buffer at pH 7.0 (Figure 3.5). The flow rate of the system (Proteam-LC System 320, ISCO, the USA) was 32 drop/min. The fractions (6.5 mL) were collected in fraction collector (Foxy-200, ISCO, the USA) during 130 mL of running buffer flow and at the same time the absorbance of fractions were read at 280 nm to determine their protein content. After the DEAE-unbound protein fractions (neutral + basic) left the column, the column was eluted with a continuous linear gradient of 0-1 mol/L NaCl prepared in 0.01 mol/L Na-phosphate buffer at pH 7.0 and it was allowed the DEAE-bound protein fractions to leave the column. The water soluble protein contents of fractions were determined by Bradford method and inhibition period of antioxidant potential determinations based on TEAC were shortened to 5 minutes to complete the measurements of all samples as quick as possible to avoid the changes in antioxidant properties of protein fractions (See sections 3.1.1.3 and 3.1.2.1.5). The fractions with high protein content and antioxidant activity were gathered and named as LAGE-C1 and LAGE-C2 for DEAE-unbound fractions and DEAE-bound fractions, respectively. These samples were tested again to determine their antioxidant potential where inhibition period of tests was 15 minutes and were expressed as Trolox equivalents (μmol Trolox). The specific antioxidant potential parameters were expressed as a ratio of antioxidant capacity divided by water soluble protein content. Then the protein fractions were lyophilized and determined their antioxidant potential and water soluble protein content (See section 3.1.1.3 and 3.1.2.1.5).

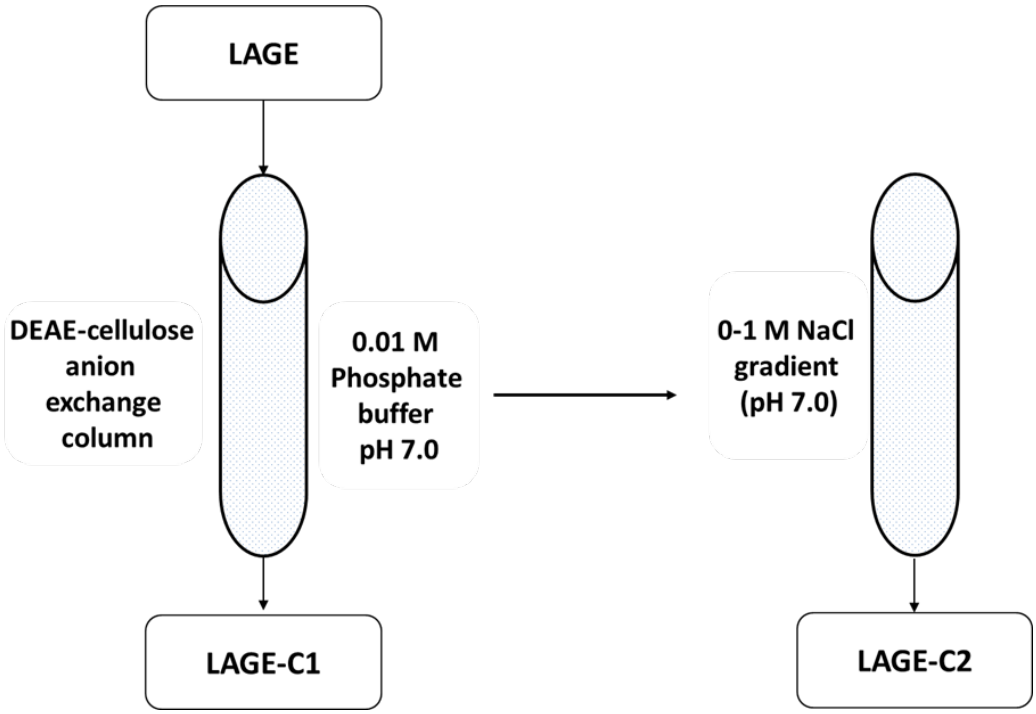

Figure 3.5. IEC fractionation of LAGE

3.1.2.2.4. Antioxidant Protein Fractionation of Lentil Albumin-Globulin Extract by Ultrafiltration

The ultrafiltration (UF) was applied to lentil albumin-globulin extract to determine the molecular weight distribution of antioxidant protein fractions (Arcan & Yemenicioglu, 2010). The UF was applied by using a laboratory scale cross-flow system (Sartorius Model Slice 200, Goettingen, Germany) equipped with Hydrosart® membranes having cut-off values of 30, 10, and 2 kDa. 250 mL, 1 % solution of lentil albumin-globulin extract was clarified by centrifugation at 15000 × g (4 °C) for 20 minutes and then it was firstly UF treated through the 30 kDa cut-off membrane (Figure 3.6). The UF process was conducted at ambient temperature and the pressure of the flow varied between 1-2 bars. The retentate (LAGE-R1) and permeate fractions (LAGE-P1) were collected separately. The Permeate-1 was diluted to 200 mL and further UF was applied to this fraction through 10 kDa cut-off membrane. The retentate (LAGE-R2) and permeate (LAGE-P2) were collected separately. The LAGE-P2 was diluted to 200 ml and once more UF treated through 2 kDa cut-off membrane. The retentate (LAGE-R3) and permeate (LAGE-P3) were collected separately. LAGE-R1, LAGE-P1, and

LAGE-R2 were assayed for their free radical scavenging activity, iron chelating capacity, and protein content by the Bradford method (for other fractions LAGE-P2, LAGE-P3 and LAGE-R3, antioxidant activities could not be determined properly). (For methods used see section 3.1.2.1.5). In addition to these test, LAGE-R1 and LAGE-R2 were analyzed for their again antioxidant potentials after they were lyophilized.

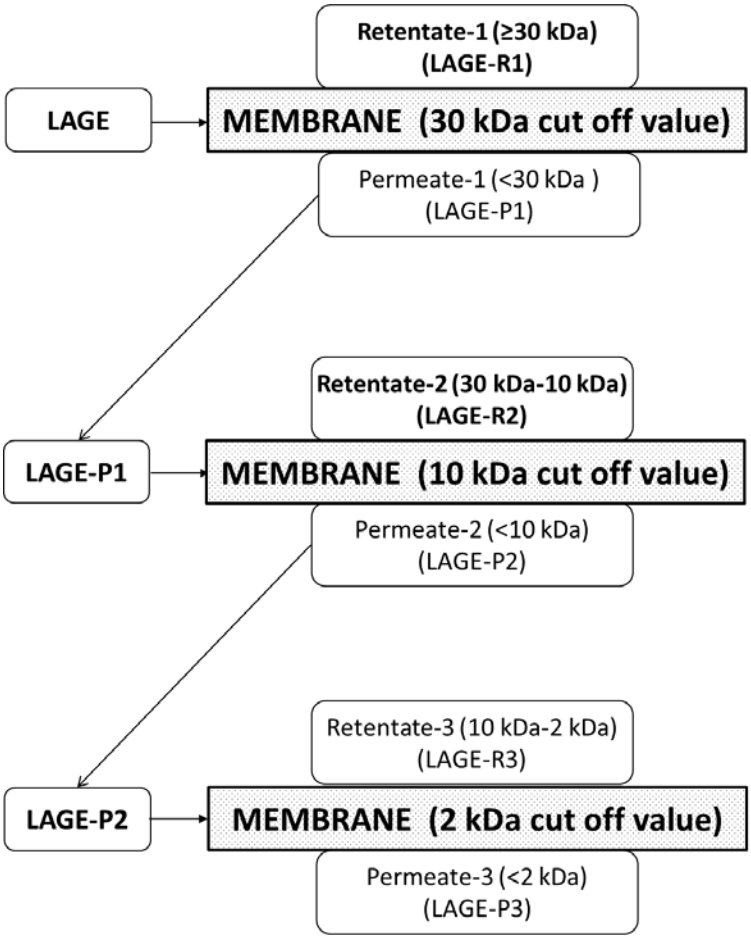

Figure 3.6. UF fractionation of LAGE

3.1.2.2.5. Determination of Total and Water Soluble Protein Content and Free Radical Scavenging Activity of Antioxidant Protein Fractions of Lentil Protein Extract

The antioxidant activity of Fırat lentil albumin-globulin extract and its fractions before and after lyophilization were evaluated by determination of their free radical scavenging capacity with oxygen radical absorbance capacity (ORAC) and Trolox

equivalent antioxidant capacity (TEAC) methods, and iron chelating capacities (ICC) (For method details, see sections 3.1.2.1.5 and 3.1.2.1.6). The ORAC measurements were conducted as three replicates and ORAC values were expressed as μmol Trolox equivalents per g of lyophilized protein extract/fraction or total μmol Trolox equivalents in the protein solution. The TEAC measurements were conducted as three replicates and TEAC values were expressed as μmol Trolox equivalents per g of lyophilized protein extract/fraction or total μmol Trolox equivalents in the protein solution. The TEAC of protein solutions (LAGE-R1, LAGE-P1 and LAGE-R2) obtained during UF was not determined by the time consuming AUC method since concentrated fractions (LAGE-R1 and LAGE-R2) tended to form haze within several hours. Instead, these protein solutions were tested at a single concentration to determine their Trolox equivalent of free radical inhibition within a fixed time period. For this purpose, 50 μl protein solutions was mixed with 2 ml of ABTS free radical cation solution and the Trolox equivalent of free radical inhibition was determined at the end of 15 minutes. All measurements were conducted for three times and results were expressed as total μmol Trolox equivalents in the protein solution (LAGE-R1, LAGE-P1, and LAGE-R2). The ICC results were expressed as μmol EDTA per g of lyophilized protein extract or total μmol EDTA equivalents in the protein solution. The average of three replicates was used to calculate ICC.

3.1.2.2.6. SDS-PAGE of Lentil Protein Extracts

The classical one dimensional SDS–PAGE of lyophilized protein extracts and fractions were conducted on a discontinuous buffered system according to Laemmli method using 15 % (w/v) separating gel and 5 % (w/v) stacking gel (Dunn, 1989) (see section 3.1.1.8). Lyophilized samples were directly solubilized in sample buffer and centrifuged at 15000 x g for 30 minutes. The samples were then heated for 5 minutes in the boiling water before electrophoresis applied as described in Arcan and Yemenicioglu (2010). The protein bands were stained by the silver staining method described by Blum, Beier, and Gross (1987). A wide molecular weight range molecular marker (Thermo PageRuler Prestained Protein Ladder 10-170 kDa) was used to characterize the obtained protein bands (Thermo Scientific). In order to make a comparison between lentil protein extracts and lentil extract, lentil water extract was

obtained. 50 g of lentils were rehydrated in 200 mL of deionised water overnight and homogenized in Waring blender for 2 minutes at high speed. Then the homogenized lentils were filtrated through 3 layers of cheesecloth and the liquid part was collected and lyophilized.

3.2. Functional, Bioactive (Antioxidant and Antihypertensive Activity), and Edible Film Forming Properties of Protein from Turkish Hazelnut Meal

3.2.1. Functional Properties of Protein from Turkish Hazelnut Meal

3.2.1.1. Materials

The hazelnut meal was kindly provided by Altaş Gıda, Ordu (Turkey). ABTS (2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid), Angiotensin converting enzyme from rabbit lung, FAPGG (N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly), and Captopril were purchased from Sigma Chem. Co. (St. Louis, MO, USA). AAPH (2,2'-Azobis[2-methyl-propionamidine] dihydrochloride was purchased from Sigma-Aldrich Chemie GmbH (Germany). Ferrozine (3- (2-Pyridyl)-5,6-diphenil-1,2,4-triazine-4'4''-disulfonic acid monosodium salt) and fluorescein sodium salt were purchased from Fluka (St. Louis, MO, USA).

3.2.1.2. Determination of Hazelnut Meal Composition

The total protein content of hazelnut meal was determined by using the classical Kjeldahl method conducted at an automated testing machine (Gerhard Vapodest 50s and Kjeldahl Therm, Germany). The total protein contents (TPrC) were calculated by using conversion factor of 6.25. Total lipid content and ash contents of hazelnut meal were determined according to AOAC 948.22 and AOAC 950.49 standard methods (AOAC, 1998), respectively. Moisture content of hazelnut meal was determined according to the ISO method number 665 by drying of the meal samples for 3 h at 103 °C (ISO, 2000). The samples were then weighted after cooling down in a desiccator and the drying was

continued with 1 hour drying periods until difference between two successive weightings was equal to or less than 0.005 g. The average of three measurements was used for all compositional analysis tests. All results were given as percent in dry weight basis. The total carbohydrate content was calculated by subtracting percentages of total protein, lipid and ash contents from hundred.

3.2.1.3. Protein Extraction from Hazelnut Meal

3.2.1.3.1. Globulin Extraction from Hazelnut Meal

For this purpose 50 g of hazelnut meal was rehydrated overnight in 500 mL of deionized water at 4 °C (Figure 3.7). The mixture was then homogenized in a Waring blender for 2 minutes. The rough particles of insoluble debris were then removed by filtering through cheesecloth and the thinned slurry was used in protein isolation. For globulin isolation the pH of the slurry was adjusted to 9.5 with 1 mol/L NaOH. To obtain the hazelnut meal protein isolate (HPI) this slurry was magnetically stirred for 45 minutes at room temperature. To obtain the hot extracted hazelnut meal protein isolate (HPI-H) the slurry at pH 9.5 was heated to 85 °C and maintained at this temperature for 30 minutes under stirring. The heated extract was then cooled to room temperature under continuous stirring for 15 minutes. After that the extract of HPI or HPI-H was clarified by centrifugation for 30 minutes at 15000 × g (at 4 °C). The protein in the obtained supernatants was then precipitated by the IEP method by adjusting the pH of extracts to 4.5 with 1 mol/L acetic acid. The precipitated proteins were collected with centrifugation and resuspended in distilled water. The pH of the suspensions was again adjusted to 4.5 and proteins were once more precipitated and collected with centrifugation. Finally, the obtained proteins were suspended in distilled water by adjusting their pH to 7.0 and lyophilized (Labconco, FreeZone, 6 liter, Kansas City, MO, USA). The unheated and heated isolates obtained were named as HPI and HPI-H, respectively and stored at -18 °C until they were analysed.

3.2.1.3.2. Globulin Extraction from Acetone Washed Hazelnut Meal

For this purpose 50 g of hazelnut meal was first rehydrated overnight in 500 mL of deionized water at 4 °C. The mixture was filtered from the cheesecloth and the hydrated softened meal pellets retained on the cheesecloth were collected. Production of AP from hazelnut meal was conducted (see section 3.1.1.2.1) For globulin extraction, 20 g of acetone washed meal powder was suspended in 250 mL deionized water and the pH of the slurry was adjusted to 9.5 with 1 mol/L NaOH. To obtain the acetone washed hazelnut meal protein isolate (HPI-AW) this slurry was mixed with magnetic stirring for 45 minutes at room temperature. The heated and acetone washed hazelnut meal protein concentrate (HPC-AW-H) was obtained by heating the slurry at pH 9.5 to 85 °C and maintaining it at this temperature for 30 minutes under continuous stirring. The heated extract was cooled at room temperature under continuous stirring for 15 minutes. The obtained HPI-AW or HPC-AW-H was then clarified by centrifugation for 30 minutes at 15000 × g (at 4 °C). The protein in the obtained supernatants was then precipitated by the classical IEP method and lyophilized. The unheated and heated isolates and concentrates were named as HPI-AW and HPC-AW-H, respectively, and stored at -18 °C until they were analyzed.

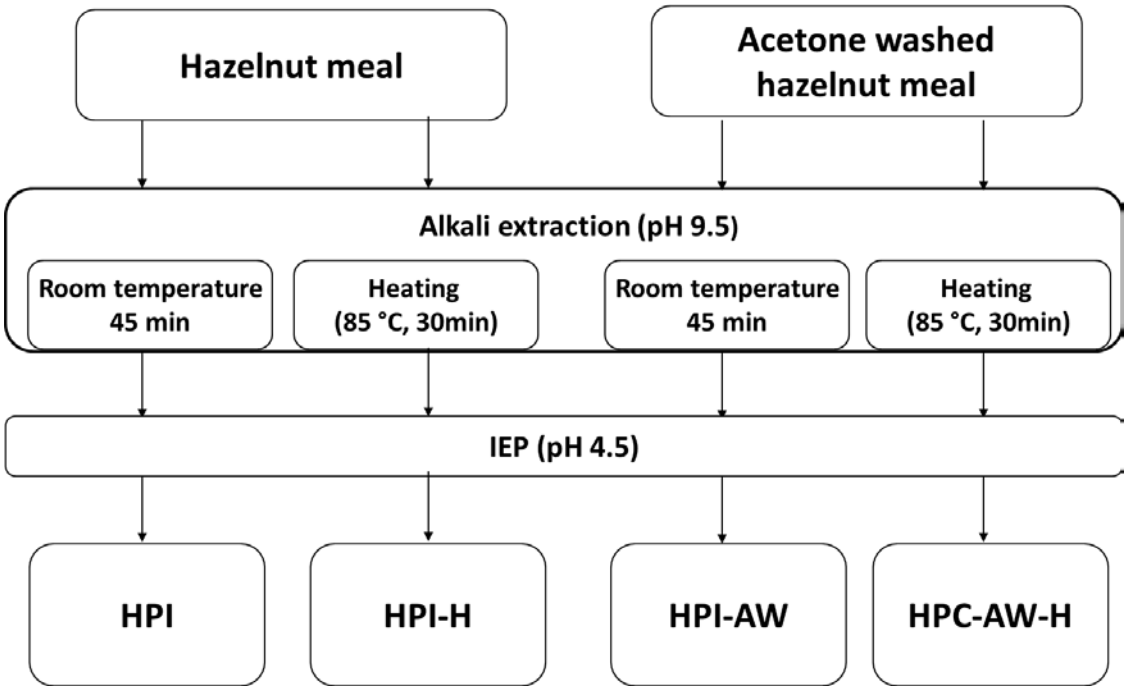

Figure 3.7. Production of different hazelnut meal protein extracts

3.2.1.4. Determination of Total and Water Soluble Protein Content of Hazelnut Meal Protein Extracts

The total protein contents of isolated protein were determined by the Kjeldahl method (see section 3.1.1.3). The extracts with total protein contents higher than 90 % were designated as hazelnut protein isolate (HPI), while extracts having lower total protein content than 90 % were designated as hazelnut protein concentrate (HPC). The solubilities of isolated proteins were determined at different pH values between 2.0 and 11.0. 20 mg portions of lyophilized protein were suspended in 10 mL of deionized water and the pH of the suspension was adjusted to different pH values between 2.0 and 11.0 by using 0.01 mol/L NaOH or 0.01 mol/L acetic acid solutions. The suspensions were then clarified by centrifugation at 4500 × g for 20 minutes at 4 °C. The water soluble protein content (WSPC) of the supernatants was determined by the Bradford method using bovine serum albumin (BSA) as standard (see section 3.1.1.3).

3.2.1.5. Determination of Water and Oil Absorption Capacity, Emulsifying and Foaming Activity and Stability, and Gel Formation Capacity of Hazelnut Meal Protein Extracts

Determination of water absorption capacity, oil absorption capacity, emulsifying activity and stability, foaming activity and stability, and gel formation capacity of hazelnut meal protein extracts were carried out according to the methods described in sections 3.1.1.4, 3.1.1.5, 3.1.1.6, and 3.1.1.7. Additionally, the slight modification was applied for the procedure of determination of gel formation capacity of hazelnut meal protein extracts. The concentrations of hazelnut meal protein extracts were between 1 and 20 % (w/v).

3.2.2. Bioactive (Antioxidant and Antihypertensive Activity) Properties of Protein from Turkish Hazelnut Meal

3.2.2.1. Determination of Free Radical Scavenging Activity and Iron Chelating Capacity of Hazelnut Meal Protein Extracts

The antioxidant activities of hazelnut meal protein extracts were evaluated by determination of their free radical scavenging capacity with oxygen radical absorbance capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC) tests and iron chelating capacities (ICC) (see section 3.1.2.1.5 and 3.1.2.1.6).

3.2.2.2. Determination of Antihypertensive Activity of Hazelnut Meal Protein Extracts

The antihypertensive activity of hazelnut meal protein extracts was determined by measuring their inhibitory effects on Angiotensin-converting enzyme (ACE) according to the method described by Shalaby, Zakora, and Otte (2006) with minor modifications. Briefly, 10 μL of 0.25 units/mL ACE prepared in 0.01 mol/L phosphate buffer saline (PBS) (NaCl concentration: 0.5 mol/L) at pH 7.0 was mixed with 10 μL of hazelnut meal protein extract solution at different concentrations (1, 2, 3, 4, 6, 8, 12, 16 mg/mL). The enzyme-protein mixture was incubated for 15 minutes at 37 °C and the enzymatic reaction was initiated by adding 150 μL 1.75 mmol/L FAPGG substrate solution (at 37 °C) prepared in PBS into this mixture. The assay was performed in 96 well microtiter plates (UV flat bottom, 8404, Thermo Fisher Scientific, Waltham, MA, USA) using the Varioskan Flash spectrophotometer. The absorbance of the reaction mixture was monitored at 340 nm for 30 minutes at 37 °C and the ACE activity was determined from the slope of the initial linear portion of absorbance-time curve. The ACE inhibition (%) was calculated according to the following formula of % ACE inhibition = [1-(activity in presence of protein extract/activity of control)] × 100. The IC_{50} values were determined as mg protein extract per mL from plots of % ACE inhibition versus protein extract concentration. The captopril was used as positive control.

3.2.3. Edible Film Forming Properties of Protein from Turkish Hazelnut Meal

3.2.3.1. Edible Film Making Method from Hazelnut Meal Protein Extracts

The edible film making properties of protein extracts were compared at optimal protein and plasticizer concentrations determined during preliminaries. Briefly, solution of hazelnut meal protein extracts at the concentration of 10 % or 12.5 % (w/w) was prepared with deionized water by continuous stirring for 30 minutes at room temperature. Then, the glycerine used as a plasticizer was added into protein solution until its concentration reached 30 % of hazelnut meal protein extract in the film making solution. The pH of the solution was adjusted to 11.0 with 1 mol/L NaOH and it was heated at 90 °C for 30 or 60 minutes. The film making solution was cooled to room temperature and it was homogenized (Heidolph, Germany, rotor Φ=6.6 mm tip) at 10000 rpm for 4 minutes with a homogenizer-disperser. Then, 5 g portions of film making solution was poured into plastic petri dishes (diameter: 8.8 cm) and dried in a controlled test cabinet (TK 120, Nüve, Turkey) at 25 °C and 50 % relative humidity (RH) for 24 hours. The thicknesses of the films were determined by scanning electron microscopy (SEM) (Philips XL 30S FEG, FEI Company, Netherlands). Films were prepared for SEM by crashing, following freezing in liquid nitrogen. The thickness of the films was measured by using Scandium software from SEM cross-sectional views of films (Olympus Soft Imaging Solutions).

3.2.3.2. Determination of Mechanical Properties of Edible Films Obtained from Hazelnut Meal Protein Extracts

Tensile strength at break and elongation at break were determined by a Texture Analyser TA-XT2 (Stable Microsystems, Godalming, UK) according to ASTM Standard Method D 882-02 (ASTM, 2002). The dried films were tested for their mechanical properties after an additional conditioning conducted at 25 °C and 50 % RH for 48 hours in the controlled test cabinet. During tests, the films were cut into 5 mm

wide and 70 mm length strips. The initial grip distance was 50 mm and crosshead speed was 25 mm/min. At least five replicates of each film were tested.

3.2.3.3. Determination of Water Solubility of Edible Films Obtained from Hazelnut Meal Protein Extracts

To determine the solubility properties of dried edible films, the films were cut into 2 × 2.5 cm pieces and they were placed into Petri dishes (diameter 8 cm) containing 10 mL deionized water at 25 °C. The Petri dishes were sealed and then shaken at 160 rpm for 3 hours using an orbital shaker (IKA, OS 5 basic, Germany) which was placed in an incubator working at 25 °C and 50 % RH. 50 μL portions of samples were taken from the test mediums at different time intervals and their soluble protein content was determined by the Bradford Method (see section 3.1.1.3). The results were expressed as amounts of solubilized protein as % of total film protein at different time intervals.

3.2.3.4. Determination of Colour of Edible Films Obtained from Hazelnut Meal Protein Extracts

The colours of the edible films were determined using a colorimeter (chromometer type CR-400, Minolta Sensing, Osaka, Japan). The films cut into 6×6 cm pieces and their colour coordinates were determined after the white calibration for illuminant D_{65}. A white background was placed on the film, and the colour measurements were performed at five different positions. Average CIE L^*, a^* and b^* values were determined to investigate the changes in lightness, redness-greenness and yellowness-blueness of films, respectively.

3.2.4. Determination of Molecular Properties of Turkish Hazelnut Meal Protein Extracts

3.2.4.1. Classical SDS-PAGE Patterns and 2-D Electrophoresis Patterns of Hazelnut Meal Protein Extracts

The classical one dimensional (1-D) SDS–PAGE of different isolated proteins were conducted on a discontinuous buffered system according to Laemmli method using 15 % (w/v) separating gel and 5 % (w/v) stacking gel (Dunn, 1989). Lyophilized samples were directly solubilized in sample buffer and centrifuged at 15000 × g for 30 minutes. The samples were then heated for 5 minutes in the boiling water before electrophoresis applied as described in Arcan and Yemenicioglu (2010). The protein bands were stained by the silver staining method described by Blum et al. (1987). A wide molecular weight range molecular marker (Thermo PageRuler Prestained Protein Ladder 10-170 kDa) was used to characterize the obtained protein bands (Thermo Scientific).

The two dimensional (2-D) electrophoresis (isoelectric focusing and SDS-PAGE) was applied to a selected isolate (HPI-AW) by using the ReadyPrep™ 2-D Starter Kit (BioRad Laboratories Inc.) according to product instructions manual. The details of this method were described previously by Arcan, et al. (2010). Unstained Protein Molecular Weight Marker, 14.4-116 kDa (Bio Basic Inc.) and 2-D SDS-PAGE standard, pI range 4.5 – 8.5, (BioRad) were used as marker.

3.3. An Example Novel Food Application with the Extracted and Characterized Protein: Bioactive Hazelnut Milk Enriched with Hydrolyzed Hazelnut Proteins

3.3.1. Production of Hazelnut Milk

Hazelnut milk production was an easy and home-made type procedure. 50 g of unroasted hazelnut was rehydrated in 200 mL of deionised water overnight in the refrigerator. Then the rehydrated hazelnuts were homogenised in Waring blender for 2

minutes at high speed and filtered the liquid through 4 layers cheesecloth. The liquid part was called as hazelnut milk.

3.3.2. Production of Bioactive Hazelnut Milk Enriched with Hazelnut Proteins

For protein enrichment of hazelnut milk samples, different concentrations of hazelnut proteins were prepared; 1 %, 2 %, 3 %, and 4 % (w/v). They were stirred for 30 minutes at room temperature at 200 rpm to maximise the protein solubility and added into the hazelnut milk samples (Figure 3.8).

3.3.3. Enzymatic Hydrolysis of Hazelnut Proteins

In order to obtain bioactive peptides of hazelnut proteins, pepsin and trypsin enzymes were used to hydrolyse the hazelnut protein. For enzymatic hydrolysis, firstly the suitable amount of hazelnut protein solution was prepared (See section 3.3.2) and the pH of the solution was adjusted to 2.5 or 7.5 for pepsin or trypsin hydrolysis, respectively. 1 % (weight of enzyme/weight of protein) of enzyme solution was added into the hazelnut protein solution and the hydrolysis of hazelnut proteins was started. The enzymatic hydrolysis continued for 3 hours at 37 °C by stirring the solution at 100 rpm. In order to stop the enzymatic hydrolysis the pH of the solution was increased to 9.5 or decreased to 2.0 for pepsin or trypsin enzymes, respectively. After this step, the pH of the hydrolyzed protein solution was adjusted to 6.25 which was the pH of hazelnut milk and then the protein solution was added into the hazelnut milk.

3.3.4. Determination of Thermal Stability of Bioactive Hazelnut Milk Enriched with Hydrolysed Hazelnut Protein

Heat treatment was applied to hazelnut milk samples enriched with hydrolyzed protein. The prepared samples were heated in water bath at different temperature (75, 85, 95 °C) and heating periods (15 and 30 minutes). After heat application the samples were immediately cooled and the protein solubility and antioxidant activity tests were

conducted for fresh prepared samples and stored samples in the refrigerator for 3 days of heat applied hazelnut milk samples which were enriched with non-hydrolysed or hydrolysed hazelnut proteins.

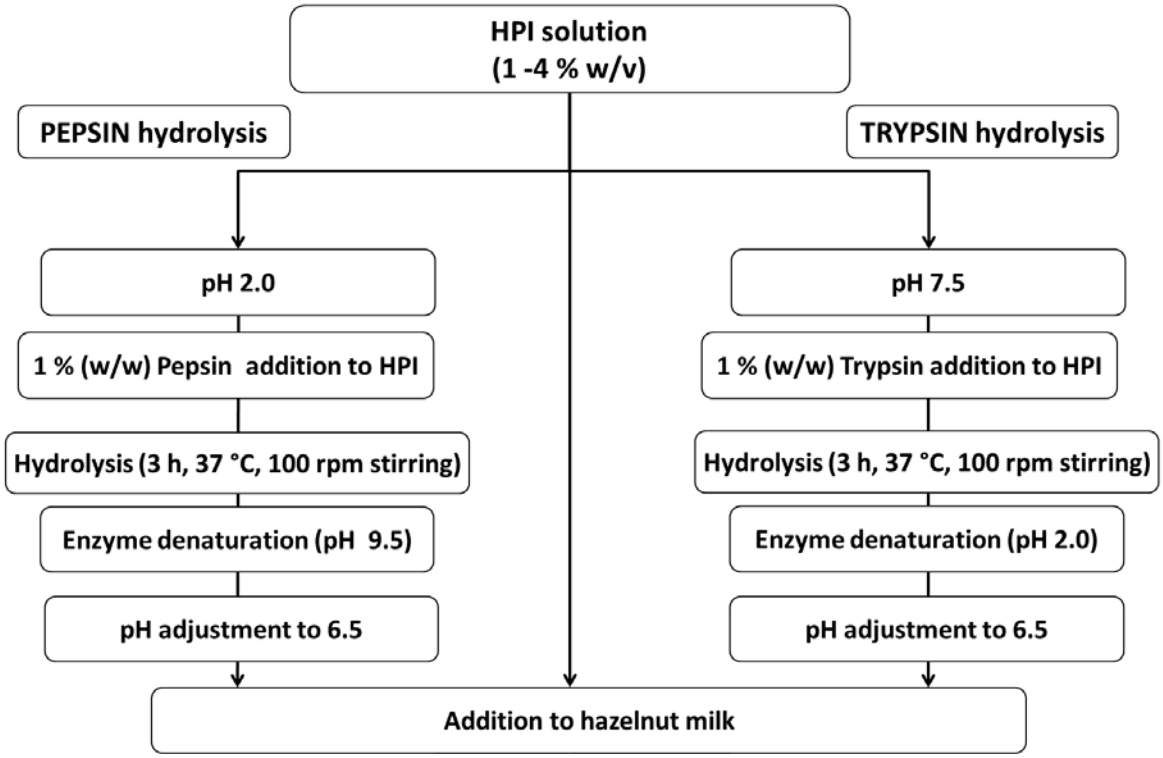

Figure 3.8. Production of hazelnut milk enriched with HPI, pHPI, and tHPI

3.3.5. Determination of Water Soluble Protein Content of Hazelnut Milk by Lowry Method

Soluble protein content of hazelnut milk samples (control samples, enriched with hydrolysed samples, and heat applied samples) were determined according to the method described by (Lowry, Rosebrough, Farr, & Randall, 1951). 0.2 mL of hazelnut milk sample were mixed with 2.1 mL Lowry reactive (245 mL of 2 % Na_2CO_3, 2.5 mL of 1 % $CuSO_4.5H_2O$, 2.5 mL 1 % Na-K tartarate in 0.1 mol/L NaOH solution) and incubated for 10 minutes at ambient temperature. After incubation 0.2 mL of Folin-Ciocalteu solution (1/10 diluted) were added into the mixture and the samples were left for 1 hour incubation at ambient temperature in dark conditions. The absorbance of the samples was read at 750 nm.

3.3.6. Determination of Antioxidant Activity of Hazelnut Milk Samples

Determination of antioxidant activity of hazelnut milk samples based on TEAC and ORAC methods was carried out according to the method described in sections 3.1.2.1.6.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Functional Properties and Antioxidant Activity of Protein from Major Turkish Chickpea and Lentil Cultivars

4.1.1. Functional Properties of Chickpea and Lentil Proteins

4.1.1.1. Total and Water Soluble Protein Content of Protein Extracts

The WSPCs of CGEs and lentil protein extracts LGEs varied between 47 and 52 g/100g and 56 and 74 g/100g, respectively (Table 4.1). The WSPCs of CGEs did not show significant differences by cultivar ($P<0.05$), but WSPCs of LGEs from different cultivars had some significant differences ($P<0.05$). The average WSPC of LGEs was almost 1.3 fold higher than that of CGEs. Except extract from one cultivar, LGEs had comparable WSPCs with WPI, the most soluble animal protein used in this study, but they showed significantly higher WSPCs than the soy proteins. The CGEs had higher WSPCs than most soy and animal proteins, but SPE and WPI showed higher WSPC than the CGEs.

The TPrC of LGEs and CGEs did not vary considerably for different chickpea and lentil cultivars, but the TPrCs determined for LGEs were significantly higher than those of CGEs ($P<0.05$). The average TPrC of LGEs was lower than that of WPI, but similar with those of SPI and SPE. The SPC and other animal proteins contained lower TPrCs than LGEs. The CGEs' average TPrC was slightly higher than those of SPC and EWP, but lower than those of the remaining proteins. Therefore, considering their total protein contents, the LGEs and CGEs obtained by the IEP procedure could be named as protein isolate and concentrate, respectively. The previous findings of Boye et al. (2010b) also showed the higher protein contents of IEP purified (at pH 4.5) lentil proteins than the chickpea proteins. The protein contents determined by these authors for IEP purified protein extracts of green lentils (0.79 g/g), red lentils (0.78 g/g) and

Kabuli chickpeas (0.64 g/g) were lower than those determined for Turkish lentil and chickpea cultivars in this study. However, due to the different processes applied to these legumes like dehulling and grinding before protein extraction, and long periods of decantation for extracts (3 to 12 hours) it is hard to attribute these differences to variations in protein contents of seed cultivars.

4.1.1.2. Gel Forming Capacity of Protein Extracts

The LGC of CGEs ranged between 5 and 7 g/100 g, while only four of LGEs formed hard fixed gels (LGC ranged between 12 and 14 g/100 g) at the studied concentration range. These results showed high variations in gelling capacities depending on pulse type and cultivar. The CGEs showed better gelling performance than lentil proteins, soy proteins and animal proteins including FGEL, WPI and EWP. However, the BGEL having LGC of 3 g/100 g showed the best gelling performance. The SPI, SPE and EWP which showed LGC of 10 g/100 g had better gelling capacity than LGEs, but WPI had comparable LGC with those of four LGEs. In the literature, very high LGCs between 11.5 to 18 g/100 mL (10.3 to 15.3 g/100 g) were reported for chickpea proteins obtained by the IEP method (Boye et al., 2010b; Kaur & Singh, 2007; Papalamprou, Doxastakis, Biliaderis, & Kiosseoglou, 2009), but LGC of 12 g/100 mL (10.7 g/100 g) reported by Boye et al. (2010b) for two lentil cultivars was only slightly lower than those obtained in this study for CGE-1 and CGE-3. These results indicated the outstanding gelling capacity of proteins from studied Turkish Kabuli type chickpea cultivars. However, it is hard to attribute the differences with the literature fully to variations in materials' protein functionality, since they used different testing methods (samples were heated in sealed or unsealed tubes, heating was applied by boiling or at 100 °C).

4.1.1.3. Water and Oil Absorption Capacity of Protein Extracts

The WACs of CGEs showed some statistically significant variations by cultivar ($P<0.05$), while LGEs had similar WAC values with the exception of LGE-3. The average WAC of CGEs was almost 6-fold higher than those of LGEs. The highest WAC was obtained for BGEL, and this was followed by those of SPI, CGEs and SPC. Three

of the LGEs showed comparable WACs with SPE, but one of the LGEs and other animal origin proteins showed inconsiderable WACs.

The OAC values of LGEs showed some significant differences by cultivar ($P<0.05$), while CGEs showed similar OAC values except that of CGE-3. The average OAC of CGEs was 1.6 fold higher than that of the LGEs. The average OAC of CGEs was also significantly higher than those of soy and animal proteins. The LGEs showed comparable OACs with SPE and EWP, but significantly higher OACs than the remaining soy and animal proteins.

In the literature, chickpea and lentil proteins obtained by the IEP procedure were reported to have WACs between 2.3 and 5.0 g/g, and 0.2 and 4.0 g/g, respectively (Bora, 2002; Boye et al., 2010b; Kaur & Singh, 2007; Lee et al., 2007; Sánchez-Vioque et al., 1999). Different reported OAC values of chickpea proteins in the literature ranged between 1.1 and 4.1 g/g (Boye et al., 2010b; Kaur & Singh, 2007; Sánchez-Vioque et al., 1999), while the only comparable OACs were reported as 1.15 and 1.25 g/g for red and green lentil types, respectively (Boye et al., 2010b). The WAC values of lentil proteins determined in this study were in the range of those reported in the literature. However, WACs of chickpea proteins and OACs of both chickpea and lentil proteins determined in this study were considerably higher than those reported in the literature. Such great differences in functional properties might come from differences in extraction and assay conditions (protein content, rehydration period and oil type) used in WAC and OAC tests, or variations in protein composition of materials due to differences in cultivars, growth conditions and climate. However, it is worth to report that the high OAC of kabuli type chickpea proteins obtained by the IEP method was also noted by Kaur and Singh (2007). These workers investigated functionality of IEP proteins from 1 kabuli type and 5 desi type Indian chickpea cultivars attributed the high OAC of kabuli type chickpea proteins to high non polar amino acid content of these proteins.

4.1.1.4.Emulsifying Activity and Emulsion Stability of Protein Extracts

The EAs of different proteins based on turbidities and absorbance of emulsions were given in Table 4.2. The EAs of CGEs and LGEs showed some limited variations by cultivar. The highest EAs were obtained for WPI, CGE-1 and LGE-6, while most

other proteins showed comparable EAs (except those of LGE-4, BGEL and SPE). The EAs determined by measuring NTU values of protein emulsions gave highly parallel results with the absorbance measurements.

Table 4.1. Protein content, gelling property, and water and oil absorption capacities of different protein extracts

Protein extract	WSPC (g/g)	TPrC (g/g)	LGC (g/100g)	WAC (g/g)	OAC (g/g)
CGE-1	0.49±0.02 d[a]	0.73±0.06 de	6	6.37±0.44 c	13.65±0.77 b
CGE-2	0.50±0.01 cd	0.71±0.02 de	5	6.64±0.71 c	14.59±0.08 a
CGE-3	0.49±0.02 d	0.77±0.01 d	7	4.90±0.26 d	10.93±0.52 c
CGE-4	0.45±0.02 d	0.73±0.02 de	5	7.94±0.52 b	14.31±0.45 ab
Average	0.48±0.02	0.73±0.03	5.75±2.6	6.46±1.24	13.37±1.68
LGE-1	0.59±0.05 b	0.90±0.01 abc	12	1.22±0.49 ef	6.90±0.10 gh
LGE-2	0.54±0.06 c	0.95±0.02 a	-[c]	1.34±0.09 ef	7.57±0.33 fg
LGE-3	0.71±0.03 a	0.88±0.02 bc	12	-[d]	9.67±1.34 d
LGE-4	0.60±0.04 b	0.93±0.01 ab	-[c]	1.47±0.22 ef	8.55±0.50 e
LGE-5	0.67±0.06 ab	0.90±0.02 abc	14	1.10±0.14 f	8.57±1.20 e
LGE-6	0.67±0.02 a	0.87±0.05 bc	14	1.08±0.51 f	10.44±0.24 cd
Average	0.63± 0.06	0.91±0.03	13±1.2	1.24±0.16	8.62±1.30
BGEL	0.12±0.01 f	0.82[b]	3	8.84±0.36 a	1.12±0.07 i
EWP	0.21±0.01 e	0.69±0.02 e	10	0.14±0.09 g	6.37±0.37 h
FGEL	0.09±0.03 f	0.85±0.01 c	-[c]	0±0 h	1.04±0.07 i
SPC	0.11±0.01 f	0.70[b]	-[c]	4.52±0.19 d	1.73±0.36 i
SPE	0.57±0.02 c	0.92±0.02 ab	10	1.69±0.62 e	8.23±1.02 ef
SPI	0.21±0.01 e	0.90[b]	10	7.94±0.44 b	1.16±0.05 i
WPI	0.67±0.06 a	0.98[b]	14	0±0 h	1.59±0.15 i

[a] Different letters in each column show significant differences at $P<0.05$,
[b] Product's manual data,
[c] No hard gel formation between 1 to 14 (g/100g),
[d] No water absorption at the test conditions

The results of ES tests were given for 30 and 180 minutes incubation periods. The ESs of both CGEs and LGEs showed statistically significant variations by cultivar. The rapid destabilization of emulsions formed by LGE-1 and LGE-4 at the end of 30 minutes clearly showed the lower ES of these proteins than the other lentil proteins. The BGEL and EWP also lost a significant portion of their ESs within 30 minutes, but the remaining proteins showed quite similar ESs at the end of this short incubation period. The differences among the ESs of proteins were observed more clearly by incubation of emulsions for 180 minutes. The highest ES values were observed for CGEs and LGEs. However, the average stabilities (%) observed in 180 min for CGEs (50.3 ± 17.1) was almost 1.6 fold higher than that of LGEs (34.1 ± 9.1). The CGE-2 and CGE-4 showed

the highest ES of CGEs, while ES of LGE-5 was the highest of LGEs ($P<0.05$). Three of the CGEs and one LGE showed significantly higher ESs than all soy and animal proteins ($P<0.05$). Due to different units and indices used in determination of protein emulsification properties, it is hard to compare our results with those in the literature. However, results of Boye et al. (2010b) who determined greater emulsion stability of Canadian kabuli and desi type chickpea proteins than green and red lentil proteins showed parallelism with results obtained in this study.

4.1.1.5. Foaming Activity and Foam Stability of Protein Extracts

For both CGEs and LGEs the FAs showed some statistically significant variations by cultivar ($P<0.05$). The average foam volume showing FA of CGEs (11.0 ± 2.6 mL) was slightly higher than that of LGEs (8.7 ± 2.7 mL). The highest FA was observed for BGEL. This was followed by FAs of FGEL, LGE-3 and CGE-4 which were quite similar with each other, but significantly higher than those of other proteins, except WPI and CGE-2. Three of the CGEs showed similar FA with WPI, but five of the LGEs had statistically significantly lower FAs than the WPI ($P<0.05$). Most of the LGEs showed similar FA with SPI and SPC. The lowest FAs were obtained for three of the LGEs, CGE-3, SPE and EWP.

The FS ranking contradicted with FA ranking since foams of most LGEs and CGEs were more stable than those of gelatins. The foams of FGEL and SPC showed the least stability and rapidly destabilized by 30 minutes incubation. With the exception of CGE-1 which showed very low FS, the CGEs and LGEs showed similar FSs and maintained a considerable portion of their stability after 180 minutes. Although the most stable foams were obtained for CGEs and LGEs, BGEL, SPI, and EWP showed comparable FSs with some of the LGEs and CGEs. In contrast, the remaining soy and animal proteins had inconsiderable foaming stabilities. The FS results obtained in this study for chickpea and lentil proteins contradicted with those of Boye et al. (2010b) who reported significantly higher foam stability for Canadian kabuli and desi chickpea proteins than the green and red lentil proteins. Thus, it is clear that further studies are needed to determine importance of different factors such as cultivar, growth conditions and climate on foaming properties and other functional properties of pulse proteins.

Table 4.2. Emulsifying and foaming activities and stabilities of different protein extracts

Protein extract	EA (NTU)	ES $_{30\ min}$ (%)	ES $_{180\ min}$ (%)	FA (mL)	FS $_{30\ min}$ (%)	FS $_{180\ min}$ (%)
CGE-1	339±3ab[a]	97.3±4.8ab	41.7±11.1cd	11.7±0.9d	82.6±15.5de	29.4±9.2g
CGE-2	316±3bc	97.3±1.8ab	73.1±4.9a	12.0±1.0cd	98.8±1.4a	57.2±1.8ef
CGE-3	304±10c	92.2±3.1abc	33.5±4.0def	7.2±0.4fg	95.2±3.0abc	67.7±12.6bcde
CGE-4	316±8bc	93.0±5.8abc	52.8±6.8b	13.1±0.5bc	95.7±1.5ab	73.2±5.2abc
Average	319±15	95.0±2.7	50.3±17.1	11.0±2.6	93.1±7.2	56.9±19.5
LGE-1	291±10c	65.2±8.5d	29.6±9.0efgh	9.1±0.2e	100.0±0.0a	77.7±0.2ab
LGE-2	312±7bc	92.3±6.4abc	24.4±1.2ghi	9.1±0.6e	91.2±7.7abcd	73.9±9.2abc
LGE-3	295±7c	96.3±7.3abc	29.4±4.8fgh	13.6±1.4b	96.2±2.3ab	71.5±13.0abcd
LGE-4	242±10d	42.4±17.7e	32.5±7.0defg	6.0±1.0g	90.2±5.8abcd	69.2±8.3bcde
LGE-5	309±21bc	93.4±2.6abc	49.9±4.5bc	7.6±0.2efg	99.2±1.4a	59.8±3.2def
LGE-6	330±21b	100.0±2.7a	38.8±8.3def	6.9±0.8fg	99.0±1.7a	84.7±9.9a
Average	297±30	81.6±22.9	34.1±9.1	8.7±2.7	96.0±4.3	72.8±8.4
BGEL	246±16d	54.5±2.1d	17.8±2.9i	14.9±0.3a	88.3±2.9bcd	62.1±10.7cdef
EWP	294±32c	13.8±8.7f	0.0±0.0j	6.3±0.2g	83.3±7.8d	54.4±4.9f
FGEL	321±24bc	98.4±4.6ab	22.3±4.6ghi	13.7±1.2b	25.8±3.0h	0.0±0.0h
SPC	314±17bc	92.9±6.9abc	23.9±3.2ghi	8.1±1.7ef	45.7±4.8g	6.8±1.7h
SPE	227±2d	90.3±5.1abc	22.2±6.1ghi	6.5±1.0g	67.8±2.2f	25.2±3.9g
SPI	312±12bc	87.5±7.0bc	19.7±5.5hi	9.1±0.7e	86.6±4.3cd	61.5±5.9def
WPI	365±15a	86.1±0.4c	28.7±3.0fgh	11.6±0.7cd	74.5±7.5ef	2.5±1.8h

[a] Different letters in each column show significant differences at $P<0.05$

4.1.1.6. Molecular Properties of Chickpea and Lentil Proteins

To determine the molecular weight distribution of proteins in chickpeas and lentils and in their different cultivars, classical SDS-PAGE was conducted in albumin-globulin extracts collected before isoelectric precipitation (Figure 4.1). The chickpea and lentil albumin-globulins showed different band patterns. Thus, it is possible to differentiate chickpea and lentil proteins from each other with their SDS-PAGE profiles. In contrast, the band patterns of different cultivars of the same pulse type were quite similar. For chickpea albumin-globulins, the main protein bands were obtained around 20 kDa and between 30 and 40 kDa, while lentil albumin-globulins gave dense bands between 15 and 20 kDa, 30 and 40 kDa, and 40 and 70 kDa.

The detailed molecular and isoelectric properties of selected CGE (CGE-3) and LGE (LGE-3, a red lentil) obtained by isoelectric precipitation of albumin-globulin extracts were also determined by 2-D electrophoresis (Figure 4.2 and Figure 4.3). This was done to provide basis for optimal isoelectric precipitation and ultrafiltration used in purification of these proteins. The CGE proteins were distributed mainly between pI 4.5 and 5.9 at MW between 15.0 and 76.0 kDa, while LGE proteins distributed mainly

between pI 4.8 and 5.9 at MW below 66.2 kDa. These profiles clearly showed lower pI and MW range of lentil proteins than the chickpea proteins. The most intensive protein spots of CGE concentrated at MW between 24 and 27 kDa, while the most intensive spots of LGE concentrated between 21 and 23 kDa, and around 26 kDa. Both CGE and LGE showed less intense spots above 30 kDa and this suggested low IEP recovery of these proteins appeared as dense bands in classical SDS-PAGE of albumin-globulin extracts.

4.1.1.7. Identification of Legume Cultivars with Multiple Protein Functionalities

From chickpea proteins, the CGE-2 from Cevdetbey cultivar and CGE-4 from Sarı cultivar had the lowest LGC, highest WAC and OAC, and highest ES values. The CGE-4 also had the highest FA of chickpea proteins. In contrast, CGE-3 showed the lowest WAC, OAC and FA, and highest LGC of chickpea proteins.

In lentils, the proteins did not show multiple very high functionalities as chickpea proteins. All LGEs showed considerably low WACs due to their high solubility in water. The LGE-3 showed the highest solubility in water, thus, lacked any measurable WAC. However, LGE-3 had one of the lowest LGCs and one of the highest OACs of lentil proteins. LGE-3 also showed the best performance in FA and FS tests of LGEs. The LGE-6 had the highest OAC and showed a good performance in EA and ES tests of LGEs. In contrast, the LGE-2 and LGE-4 did not show gelation at the test conditions. LGE-1 and LGE-4 had the worst performances in EA and ES tests of LGEs. The LGE-4 also showed one of the worst performances in FA and FS tests of lentil proteins.

4.1.1.8. Correlation of Functional Properties of Chickpea and Lentil Proteins

The statistical analysis did not indicate any significant positive correlations between WSPC or TPrC and any of the measured parameters of LGEs and CGEs. For both LGEs and CGEs, there were also no positive significant correlations among EA, ES, FA, FS, but a significant negative correlation existed between FS and EA (r^2=-

0.776) of CGEs. Some significant negative correlations also existed between TPrC and WSPC (r^2=-0.611), and WSPC and WAC (r^2=-0.612) of LGEs, and between TPrC and OAC (r^2=-0.739) of CGEs. For CGEs, there were also positive correlations between WAC and OAC (r^2=0.816), and WAC and FA (r^2=0.932). In contrast, a significant negative correlation was determined between WAC and FA (r^2=-0.687) of LGEs. These results showed the complexity of correlations among different functional properties of LGEs and CGEs. However, it seemed that the determination of TPrC might be used to obtain some information about OAC, WAC and FC of chickpea proteins and WSPC, WAC and FC of lentil proteins. The remaining functional properties of chickpea and lentil proteins are not related mainly to protein content or solubility. In the literature, data related to correlations among different functional properties of chickpea and lentil proteins scarce. It was only Kaur and Singh (2007) who reported strong negative correlation of peak denaturation temperature (T_d) determined by differential scanning calorimetry with TPrC and OAC of protein extracts obtained from 5 desi and 1 kabuli chickpea cultivars. Further studies are needed to investigate effect of protein composition, conformation and interactions on protein functionality and understand variations in functional properties of proteins from different cultivars of pulses.

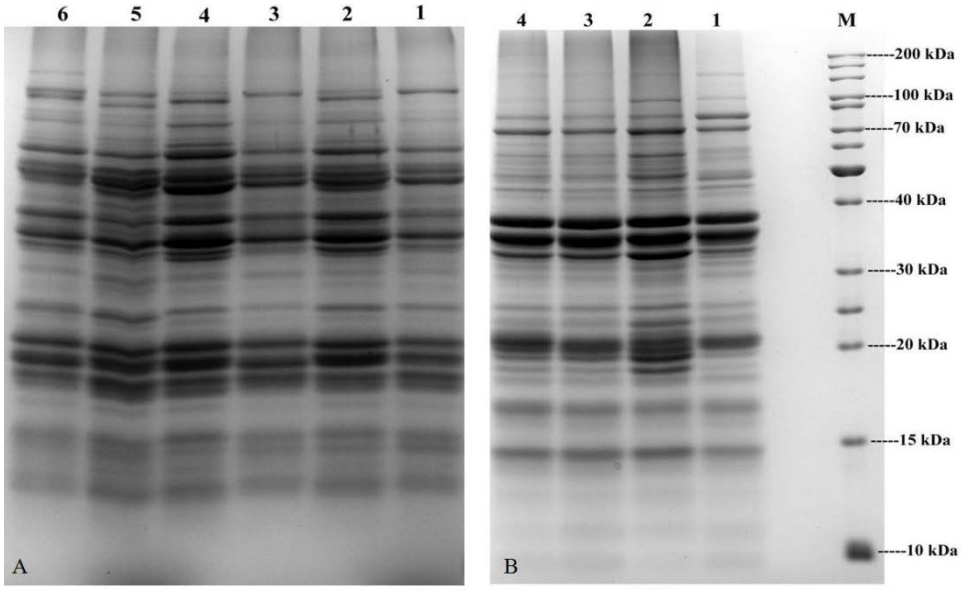

Figure 4.1. SDS-PAGE profiles of albumin-globulin extracts before isoelectric precipitation (Albumin-globulin extracts in A: 1: Alidayı, 2: Çiftçi, 3: Fırat, 4: Kafkas, 5: Meyveci, 6: Pul-II; in B: 1: Canıtez, 2: Cevdetbey, 3: Gökçe, 4: Sarı)

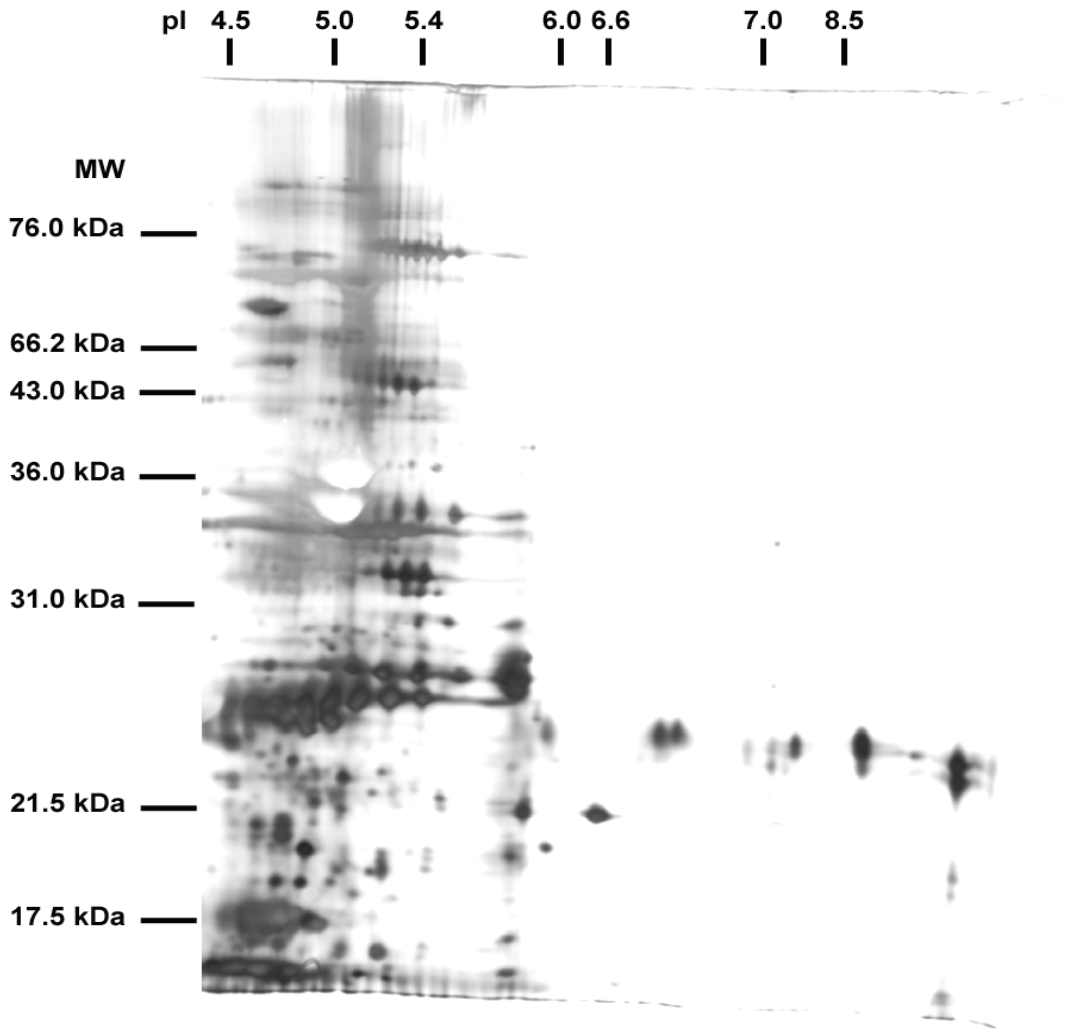

Figure 4.2. 2-D electrophoresis of chickpea proteins extract after IEP (CGE-3)

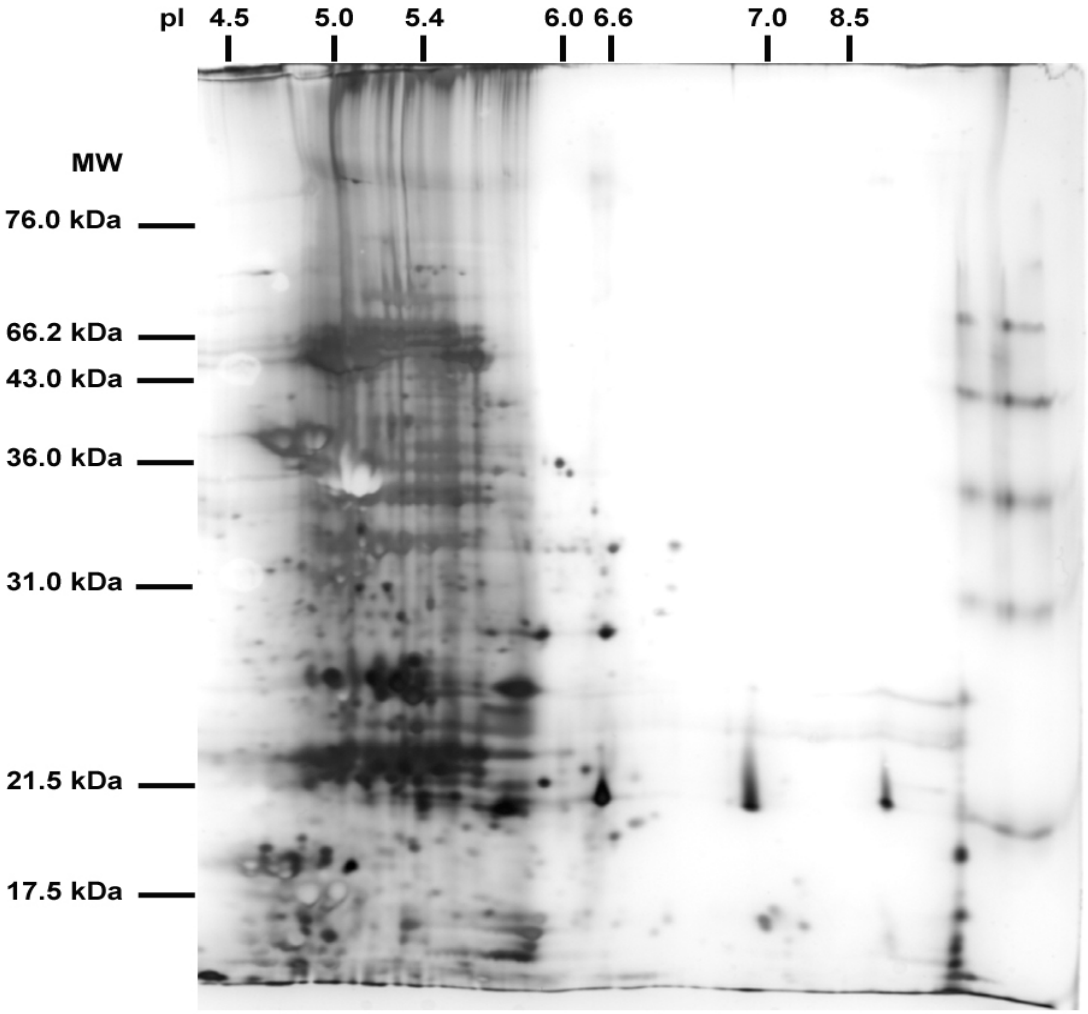

Figure 4.3. 2-D electrophoresis of lentil proteins extract after IEP (LGE-3)

The results of this study clearly showed the potential of chickpea and lentil proteins as commercial functional protein alternatives. Most of the functional properties of chickpea proteins are comparable with or superior than those of soy and animal origin proteins tested in this study. In fact, there was only gelling capacity of bovine gelatin which considerably surpassed a functional property of chickpea proteins. The most outstanding functional properties of studied pulse proteins were extremely high oil and water absorption capacity for chickpea proteins and high oil absorption capacity and solubility of lentil proteins. Both types of pulse proteins were also capable to form highly stable emulsions and foams. In contrast, water absorption capacity and gelling capacity are critical missing functional properties of lentil proteins. Thus, blending of lentil and chickpea proteins could be suggested when high protein solubility is the

primary desired functionality, but some water absorption and gelling capacity is also needed. The outstanding chickpea and lentil cultivars with superior protein functionality and cultivars with inferior protein functionality were identified. However, further studies are needed to monitor and characterize the functional properties of protein form different chickpea and lentil cultivars obtained at different growing conditions, harvesting periods and climates and determine magnitude of possible variations in protein functionality at standard extraction and assay conditions.

4.1.2. Antioxidant Properties and Characterization of Antioxidant Fractions of Lentil Protein Extracts

4.1.2.1. Antioxidant Properties of Lentil Protein Extracts

The preliminary studies conducted by comparing ORAC and TEAC based free radical scavenging capacities of globulin extracts from chickpeas and lentils clearly showed the higher antioxidant potential of lentil proteins than the chickpea proteins. The average ORAC and TEAC based antioxidant capacities of lentil globulin extracts (for 6 cultivars) was almost 2 fold higher than those for chickpea globulin extracts (see Appendix B). Thus, this section is focussed on characterizing antioxidant potentials of different lentil protein extracts and fractions obtained by different methods.

4.1.2.1.1. Total Phenolic and Flavonoid Content, Free Radical Scavenging Activity, and Iron Chelating Capacity of Lentil Protein Extracts

Although the lentil protein extracts were obtained from acetone powders to remove free soluble phenolic compounds, both the lentil albumin-globulin extracts (LAGE) and the lentil globulin extracts (LGE) obtained from different cultivars had considerably high phenolic and flavonoid contents (Table 4.3). The high phenolic and flavonoid contents of globulin extracts were particularly important since these extracts obtained also from acetone powders and were prepared by two times isoelectric precipitation and washing with water. These results clearly indicated the presence of

high amounts of bound phenolic compounds at the surfaces of lentil proteins. This finding compares well with previous report of (Han & Baik, 2008) who found that the bound phytochemicals contributed to 82-85 % of total antioxidant activity in lentils. Most of the globulin extracts contained significantly lower TEAC, ORAC and ICC than those of corresponding albumin-globulin extracts because IEP precipitated mainly globulins not albumins and other possible non-protein antioxidants in lentil albumin-globulin extracts. In the literature, antioxidant capacity of legume albumins including those from chickpeas, broad beans, white and brown beans and light peas were well studied and characterized by different workers (Arcan & Yemenicioglu, 2007; Okada & Okada, 1998; Wolosiak & Klepacka, 2002). However, there were no reports about the globulin fraction of lentil proteins.

Lentil albumin-globulin extracts and lentil globulin extracts of Alidayı and Fırat cultivars showed significantly higher TPC and TFC values than other lentil albumin-globulin and globulin extracts ($P<0.05$). Among albumin-globulin extracts, Alidayı and Fırat cultivars also showed the highest TEAC values. In contrast, there were no significant differences among ORAC values of lentil albumin-globulin and globulin extracts ($P<0.05$). There were also no considerable differences among ICC of lentil albumin-globulin extracts, but globulin extract of Fırat and Meyveci showed significantly higher ICC than those of lentil globulin extracts. These results clearly showed the presence of some variations in phenolic contents and TEAC values of lentil albumin-globulin extracts, while lentil globulin extracts showed variations mainly in their phenolic contents and ICC values.

In order to better evaluate the antioxidant potential of lentil proteins, the average antioxidant parameters determined for lentil albumin-globulin and globulin extracts from different lentil cultivars were also compared with those of commercial SPI, SPC, and with those of SPE produced from soybeans by the same method used for lentil globulin extract. The average TPC and TFC values of lentil albumin-globulin and globulin extracts were statistically significantly higher than those of soy proteins ($P<0.05$). In the literature, Pinto, Lajolo, and Genovese (2005) reported the total isoflavones content of different types of soy protein isolates (SPI) as 1540 µg/g for multipurpose SPI, 850 µg/g for low hydrolysis SPI and 1070 µg/g for the nutritional beverage SPI, while DiSilvestro, Mattern, Wood, and Devor (2006) reported 3950 µg isoflavone per g of high isoflavone SPI. Thus, it appeared that the TFC values of lentil albumin-globulin and globulin extracts were comparable with those of high

isoflavonoid soy protein extracts. On the other hand, the lentil albumin-globulin extracts also had higher average TEAC and ORAC than those of SPI, SPC and SPE, while lentil globulin extracts had comparable ORAC, but higher TEAC than those of the soy proteins. The average TEAC values determined in this study for lentil albumin-globulin and globulin extracts were also comparable with that of chickpea albumin-globulin extract (156 µmol Trolox/g), but lower than that of chickpea albumin-globulin pepsin hydrolyzate (277 µmol Trolox/g) (Arcan & Yemenicioglu, 2010). Moreover, the average ORAC determined for lentil crude protein extracts was significantly higher than those reported for 12 different soy protein hydrolyzate fractions, while average ORAC of lentil globulin extracts was within the range of those for soy fractions reported to have ORAC values between 23.8 and 83.8 µmol Trolox/g (Zhang, Li, & Zhou, 2010). On the other hand, the average ICC value of lentil globulin extracts was comparable with those of SPI, SPC and SPE, while lentil albumin-globulin extracts showed 7 to 18 fold higher averages ICC than those of soy proteins. The average ICC of lentil albumin-globulin extracts was also higher than that of chickpea albumin extract (50 µmol EDTA/g) (Arcan & Yemenicioglu, 2007), but lower than that of chickpea albumin-globulin extract (156 µmol EDTA/g) prepared by a quite similar extraction procedure with lentil albumin-globulin extract used in this thesis (Arcan & Yemenicioglu, 2010). Previous findings showed that the lentil proteins formed mainly by globulins had 5.6 fold higher iron dialysability than raw lentils (Lombardi-Boccia, Ruggeri, Aguzzi, & Cappelloni, 2003). These results suggested that lentil proteins could bind iron without considerable reduction in its bioavailability. Such proteins could be used in iron fortified food produced for people suffering from iron-deficiency anaemia to minimize undesirable colour and oxidative changes in food catalysed by the iron added for fortification. Further studies are needed to develop specific food applications for lentil proteins.

Table 4.3. Total and water soluble protein contents and different antioxidant parameters of lentil protein extracts

Cultivars	TPrC (g/g)	WSPC (g/g)	TPC (µg GA/g)	TFC (µg EC/g)	TEAC (µmol Trolox/g)	ORAC (µmol Trolox/g)	ICC (µmol EDTA/g)
Lentil Albumin-Globulin (AG) Extracts from Different Cultivars							
Alidayı (R)[a]	0.51±0.02h[b]	0.45±0.03f	23129±124bc	3515±373b	242±6a	165±53ab	126±2a
Çiftçi (R)	0.58±0.01g	0.45±0.03f	21023±620cd	2466±75cd	158±0d	153±17ab	126±2a
Fırat (R)	0.57±0.01g	0.47±0.01f	24313±227ab	3609±36b	218±4b	192±21a	126±2a
Kafkas (R)	0.59±0.01g	0.49±0.02f	20453±351cd	2669±39cd	181±5c	169±19ab	125±1a
Meyveci (G)	0.53±0.02h	0.46±0.02f	20599±269cd	2364±60cd	168±1cd	162±4ab	129±0a
Pul-II (G)	0.58±0.02g	0.44±0.01f	19985±62d	2147±91de	185±1c	142±30b	116±3b
Lentil Globulin (G) Extracts from Different Cultivars							
Alidayı (R)	0.90±0.01bc	0.61±0.5de	26376±607a	4121±273ab	133±0e	91±9c	16±2d
Çiftçi (R)	0.95±0.02a	0.56±0.06e	20300±594d	2582±361cd	131±0e	72±18cde	17±0d
Fırat (R)	0.88±0.02cd	0.74±0.03a	26108±1411a	4290±360a	162±0d	93±11c	22±2c
Kafkas (R)	0.93±0.01ab	0.62±0.04cd	15696±385e	1724±278e	113±0e	64±1cdef	12±1de
Meyveci (G)	0.90±0.02bc	0.67±0.06bc	22153±83bcd	2806±124c	122±0e	77±8cd	26±4c
Pul-II (G)	0.87±0.05cde	0.69±0.02ab	19742±1628d	2513±132cd	127±0e	49±4cdef	10±1ef
Lentil-AG (Av)[c]	0.56±0.03 A	0.46±0.02 A	21584±1729 A	2794±618 A	192±32 A	164±17 A	124±5 A
Lentil-G (Av)	0.91±0.03 B	0.65±0.06 B	21729±4083 A	3006±1000 A	129±16 B	74±16 B	17.4±6.3 B
Different Soybean Protein Extracts							
SPC	0.70	0.11±0.01h	3630±237h	231±10f	47±0fgh	82±13ef	7±1fg
SPE	0.84±0.02e	0.57±0.02de	11388±1052f	162±36f	37±0hg	90±8def	17±0d
SPI	0.90	0.21±0.01g	6055±153g	308±16f	59±0f	143±14cdef	16±0d

[a]R: red lentil; G: green lentil; [b]data with different small or capital letters in each column evaluated separately to show significant difference at $P<0.05$; [c]average parameters for lentil albumin-globulin extracts or lentil globulin extracts

4.1.2.1.2. Correlation of Antioxidant Parameters of Lentil Protein Extracts

The analyses of data were carried out for antioxidant parameters of lentil albumin-globulin and globulin extracts separately (Table 4.4 and 4.5). In both protein extracts, there was a strong correlation between TPC or TFC and TEAC values. On the other hand, it was clearly seen that there were not any correlations between the protein contents or soluble protein contents and antioxidant properties of lentil protein extracts. Thus, the bound phenolics on proteins should be the main contributor to the antioxidant capacities of lentil albumin-globulin and globulin extracts.

Table 4.4. Correlation of antioxidant parameters of lentil albumin-globulin extracts (*P<0.05*)

	TPC	TFC	TEAC	ORAC	ICC	TPrC
TFC	0.937					
TEAC	0.751	0.804				
ORAC	-	-	-			
ICC	-	-	-	-		
TPrC	-	-	-	-	-	
WSPC	-	-	-	-	-	-

Table 4.5. Correlation of antioxidant parameters of lentil globulin extracts (*P<0.05*)

	TPC	TFC	TEAC	ORAC	ICC	TPrC
TFC	0.928					
TEAC	0.720	0.768				
ORAC	0.712	0.721	0.616			
ICC	0.525	-	-	0.579		
TPrC	-	-	-	-	-	
WSPC	-	-	-	-	-	-0.611

4.1.2.2. Characterization of Antioxidant Protein Fractions in Lentil Protein Extract

In order to better evaluate the antioxidant capacities of lentil proteins, the lentil albumin-globulin extracts were fractionated by different methods. Among albumin-globulin extracts, the highest antioxidant activities were obtained for Alidayı and Fırat cultivars. Thus, in this study albumin-globulin extract of Fırat lentil cultivar was used for fractionation of antioxidant proteins due to its relatively high antioxidant activity. Ion exchange chromatography, ultrafiltration, and isoelectric precipitation were applied to lentil albumin-globulin extract (LAGE) of Fırat cultivar for further purification of proteins. Since lyophilization process might cause some variations on antioxidant activities of proteins, the antioxidant activity determinations were carried out both before and after the lyophilization.

4.1.2.2.1. Characterization of Antioxidant Protein Fractions of Lentil Protein Extract Obtained by Ion Exchange Chromatography

DEAE-cellulose cation exchange chromatography was conducted to determine the antioxidant activities of protein fractions based on their molecular charges. For this purpose firstly 18 fractions (each was 6.5 mL) were collected and then NaCl gradient was started. During salt gradation 16 fractions were collected and protein contents of fractions were determined by measuring their absorbance at 280 nm. Then peak points of high protein content ~~of~~ fractions were combined and named as LAGE-C1 and LAGE-C2. The LAGE-C1 and LAGE-C2 correspond to the fraction numbers from 6 to 10 and from 22 to 28, respectively (Figure 4.4). LAGE-C1 was composed of basic and neutral proteins since it was eluted before initiation of salt gradient while LAGE-C2 was composed of acidic proteins bound by the column. Although, IEC fractionated a high portion of protein during the chromatography, 60 % of TEAC and ORAC values of fractions lost and purity of free radical scavenging antioxidant fractions drop moderately (Table 4.7). In contrast, iron binding proteins retained with a yield over 100 % and purified effectively. Purification folds for ICC were 1.6 and 1.8 for LAGE-C1

and LAGE-C2 fractions, respectively. The increase of purification yield (over 150 %) for ICC following IEC suggested regeneration of iron binding capacities of protein by this chromatographic process. These results clearly showed that the basic and neutral lentil proteins had higher specific TEAC and ORAC based antioxidant capacities than acidic lentil proteins. On the other hand, the ICC based antioxidant capacities of basic and neutral and acidic proteins are not considerably different.

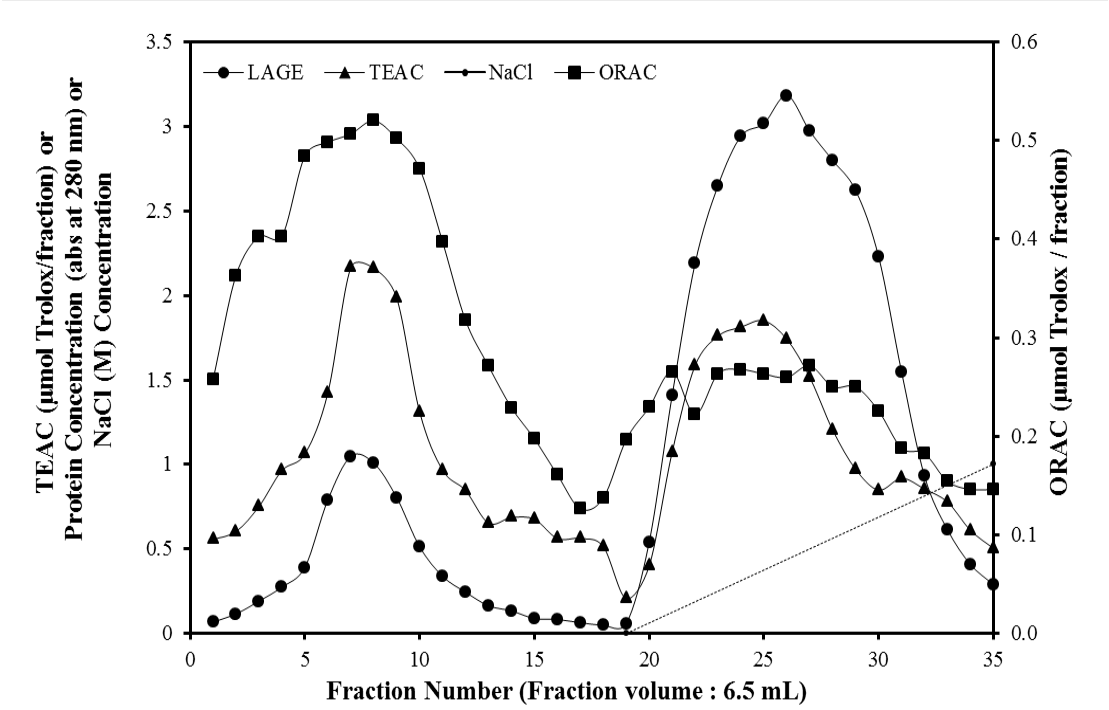

Figure 4.4. DEAE-cellulose chromatography of LAGE

4.1.2.2.2. Characterization of Antioxidant Protein Fractions of Lentil Protein Extract Obtained by Ultrafiltration

In order to determine the molecular weight distribution of antioxidant proteins, UF was applied to LAGE at different cut-off values (30, 10 and 2 kDa) and antioxidant activity measurements were conducted in separated fractions based on their TEAC, ORAC, and ICC (Table 4.7). The retentate and permeate fractions retained above and passed below 30 kDa, 10 kDa and 2 kDa cut-off value membranes were named; LAGE-R1 (> 30 kDA) and LAGE-P1 (≤ 30 kDA), LAGE-R2 (between 30-10 kDA) and LAGE-P2 (≤ 10 kDA), and LAGE-R3 (between 10-2 kDa) and LAGE-P3 (≤ 2 kDa),

respectively. A significant portion of protein (almost 37 %) insolubilized during UF due to concentration effect and it remained at the surface of 30 kDa cut-off membrane. This protein loss decreased the free radical scavenging activity based on TEAC values of fractions. However, the antioxidant activity reduction was lower in ORAC values of UF fractions (78 % of initial ORAC was remained). LAGE-R1 showed more than 50 % of antioxidant capacity based on TEAC (28 µmol Trolox) and ORAC (14 µmol Trolox) and this result showed that an important part of the antioxidant activity associate with proteins having MW ≥30 kDa. Similar findings were reported for the ultrafiltrated chickpea protein extracts by Arcan and Yemenicioglu (2010). LAGE-P1 fraction had quite low total TEAC and ORAC but the purities of these proteins were increased between 5 and 8 fold. In contrast, LAGE-P1 had a considerably high total ICC (17 µmol EDTA) which purified almost 32 fold by the UF treatment. This result clearly showed the concentration of iron binding proteins below 30 kDa. LAGE-R2 fraction composed of proteins with molecular weights between 10 and 30 kDa possessed very low total TEAC and ORAC, but the proteins having free radical scavenging based antioxidant activity increased in their purities between 3 and 3.5 fold. The LAGE-R2 also showed very high ICC which purity increased 32 fold by UF. For the UF fractions with molecular weight less than 10 kDa, TEAC, ORAC and ICC values were quite low and protein contents were below the detection limit of the assay method.

4.1.2.2.3. Characterization of Antioxidant Protein Fractions of Lentil Protein Extract Obtained by Isoelectric Precipitation

IEP was highly effective in separating albumins form globulins which precipitate easily at their pI (Tablo 4.7). There is only 5 % loss of protein during the IEP. A very large portion of LAGE was formed by globulin fraction which account for almost 90 % of total soluble protein in lentils. The LGE formed almost 75 %, 90 % and 45 % of total TEAC, ORAC and ICC, respectively. However albumin extract (LAE) was only consisted of 5 % of soluble protein in LAGE and forms almost 15 % and 40 % of total TEAC and ORAC based antioxidant capacity, respectively.

Although the LGE formed most of the soluble protein, the IEP did not increase the purity of its antioxidant activity based on TEAC, ORAC and ICC. The purity of

LAE for TEAC and ORAC based antioxidant activity increased 3 to 7 fold, but the ICC of this fraction did not purify by the IEP.

4.1.2.2.4. Characterization of Lyophilized Antioxidant Protein Fractions of Lentil Protein Extract

The total and soluble protein contents and TEAC, ORAC and ICC based antioxidant activities of fractionated lentil proteins were also tested following lyophilization. This was done to determine the possible adverse effects of lyophilization on protein solubility and bioactivity. It is well known that the lyophilization might cause aggregation and complexation of proteins by causing formation of noncovalent type bonds and interactions (ionic and hydrophobic interactions and H bonds). The aggregation and complexation of proteins is a primary factor causing loss of their solubility or blocking of their antioxidant groups. Thus, it might affect the commercialization of protein preparations.

Table 4.6. Summary of DEAE-cellulose cation exchange chromatography, isoelectric precipitation and ultrafiltration of lyophilized lentil albumin-globulin extract

Lyophilized Fraction	TPrC (g/g)	WSPC (g/g)	TEAC (µmol Trolox/g)	ORAC (µmol Trolox/g)	ICC (µmol EDTA/g)
LAGE	0.58±0.01b[a]	0.41±0.02c	217±4a	192±21b	126±2c
Fractions obtained by ion-exchange chromatography					
LAGE-C1	0.44±0.03c	0.38±0.01c	97±2c	70±1d	278±9a
LAGE-C2	0.12±0.02d	0.07±0.01e	10±1e	15±1e	65±7e
Fractions obtained by isoelectric precipitation					
LGE	0.84±0.05a	0.72±0.03a	162±1b	92±11d	22±2f
LAE	0.40±0.04c	0.11±0.01e	71±3d	142±4c	90±3d
Fractions obtained by ultrafiltration					
LAGE-R1	0.83±0.06a	0.49±0.02d	73 ± 9d	223±9a	94±3d
LAGE-R2	0.48±0.03bc	0.27±0.02e	108±3c	159±17bc	159±9b

[a] Different letters in each column show significant differences at $P<0.05$.

Table 4.7. Summary of DEAE-cellulose cation exchange chromatography, isoelectric precipitation and ultrafiltration of red lentil albumin-globulin extract

Fractions	WSPC (mg)	TEAC (µmol Trolox)	Specific TEAC (µmol Trolox/mg protein)	ORAC (µmol Trolox)	Specific ORAC (µmol Trolox/mg protein)	ICC (µmol EDTA)	Specific ICC (µmol EDTA/mg protein)
LAGE	96±8	52±7	0.5 (1.0)[a]	27±1.5	0.3 (1.0)	48±4	0.5 (1.0)
Fractions obtained by ion-exchange chromatography							
LAGE-C1	30±1	9.0±0.1	0.3 (0.6)	4.5±0.5	0.2 (0.7)	23±6	0.8 (1.6)
LAGE-C2	56±3	12±0.1	0.2 (0.4)	6.5±0.3	0.1 (0.3)	50±2	0.9 (1.8)
Fractions obtained by isoelectric precipitation							
LGE	5.4±0.4	8.0±0.0	1.5 (3.0)	11±0.0	2.1 (7.0)	1±1	0.2 (0.4)
LAE	85±2	38±0.1	0.5 (1.0)	24±0.6	0.3 (1.0)	21±1	0.3 (0.6)
Fractions obtained by ultrafiltration							
30kDa cut-off membrane							
LAGE-R1	59±2	28±0.1	0.5 (1.0)	14±0.2	0.2 (0.7)	23±1	0.4 (0.8)
LAGE-P1	1.1±0.1	3.0±0.0	2.7 (5.4)	2.5±0.0	2.3 (7.7)	17±1	16 (32)
10kDa cut-off membrane							
LAGE-R2	0.7±0.0	1.2±0.0	1.7 (3.4)	0.6±0.0	0.9 (3.0)	11±1	16 (32)
LAGE-P2	ND[b]	0.5±0.0	NC[c]	1.2±0.2	NC	2.1±0.1	NC
2kDa cut-off membrane							
LAGE-R3	ND	0.2±0.0	NC	1.5±0.0	NC	0.6±0.0	NC
LAGE-P3	ND	0.15±0.	NC	1.3±0.1	NC	0.4±0.0	NC

[a] Purification fold of parameters were given within the parenthesis.
[b] ND: The soluble protein content of fractions could not be determined.
[c] NC: The specific parameters of fractions could not be calculated.

The results given in Table 4.6 clearly showed the very low protein solubility for LAGE-C2, LAE and LAGE-R2. In contrast, the LGE and LAGE-R1 had the highest soluble protein contents. The highest ORAC based antioxidant capacity was observed for LAGE-R1 while the highest TEAC based antioxidant capacity was observed for LGE. The free radical scavenging capacities of LAGE-R1 and LGE did not purify with IEC and IEP, respectively. However, it appeared that these proteins owe their high antioxidant capacity to their high solubility following lyophilization. The highest ICC was observed for the LAGE-C1 and this showed the stability of ICC of this protein against lyophilization. In contrast, the LAGE-C2 lost majority of its ICC following lyophilization.

4.1.2.2.5. SDS-PAGE Patterns of Lyophilized Antioxidant Protein Fractions of Lentil Protein Extracts

Figure 4.5 A and B represented the SDS-PAGE patterns of LAGE and its fractions obtained in order to characterize their molecular weight distribution at 2 and 10 mg/mL (w/v) protein concentrations. The fractions which had low WSPC (LAE, LAGE-R2, and LAGE-C2) had clear protein bands only at 10 mg/mL concentration while other fractions having higher solubilities had dense bands even at 2 mg/cm^2. The similar patterns of LEGE-R1 and LGE indicated that the LAGE-R1 consisted mainly of globulin proteins. As expected, LAGE-R2 contains protein bands mainly above 30 kDa. Moreover, protein bands of LAE are characteristic and different from LGE. It is worth to note that the dense protein band around 23 kDa are distinctive for LAE and appeared only in LAGE-R2 as a dramatically less dense protein band.

This study clearly shows that red lentil proteins extract has the good potential to be used as natural antioxidant food ingredient. Different antioxidant lentil protein fractions can easily be purified by different methods and then be lyophilized successfully without a considerable loss in their antioxidant activity and protein solubility. The lyophilized lentil globulins obtained by the IEP have high protein solubility and TEAC. The lyophilized protein fractions obtained as retentate by ultrafiltration at 30 kDa could be employed successfully by exploiting their sufficient solubility and high ORAC based antioxidant capacity. The lyophilized LAGE-C1 obtained by IEC showed limited protein solubility but extraordinary high ICC.

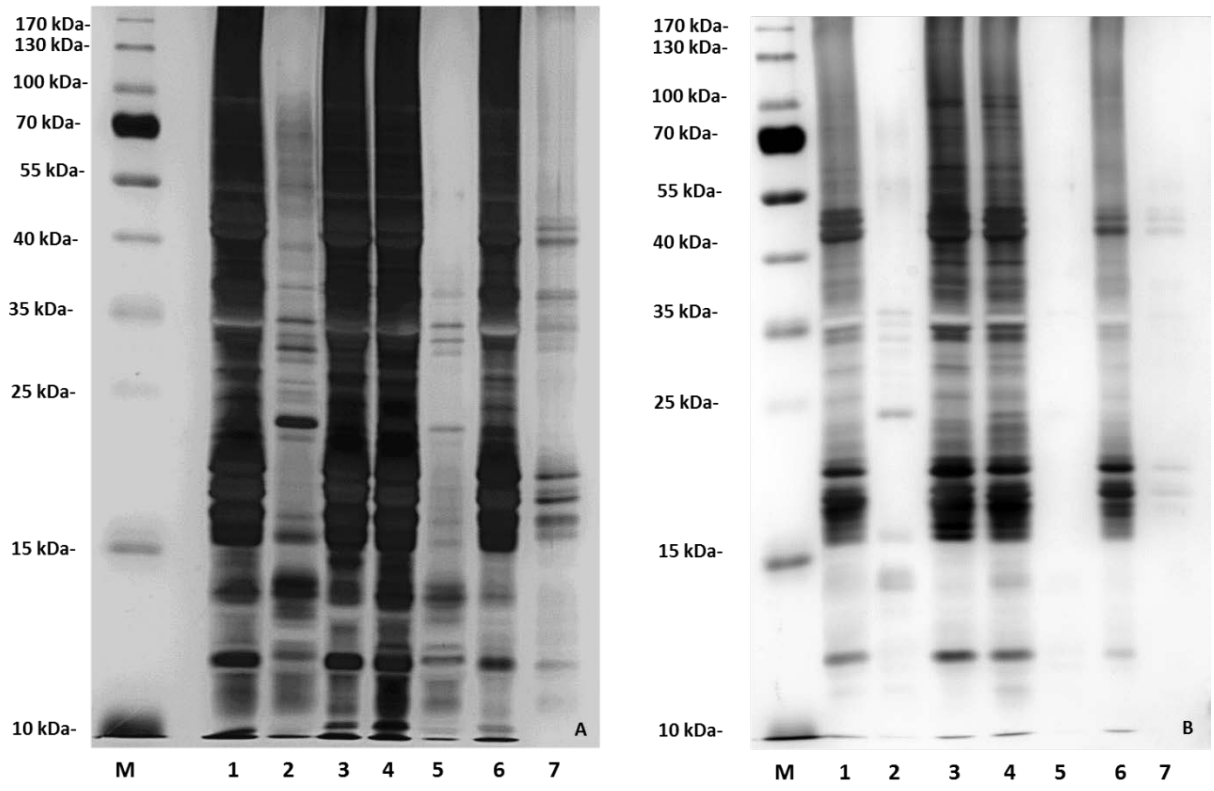

Figure 4.5. SDS-PAGE profiles of LAGE and its different fractions (protein fractions in A and B; M: Marker , 1:LAGE, 2:LAE, 3:LGE, 4:LAGE-R1, 5:LAGE-R2, 6:LAGE-C1, 7:LAGE-C2. In A and B; protein concentrations were 10 mg/mL and 2 mg/mL, respectively)

4.2. Functional, Bioactive (Antioxidant and Antihypertensive Activity), and Edible Film Forming Properties of Protein from Turkish Hazelnut Meal

4.2.1 Functional Properties of Protein from Turkish Hazelnut Meal

4.2.1.1. General Composition of Turkish Hazelnut Meal

The compositional analysis of hazelnut meal obtained from oil extraction showed the presence of 54.4 % protein, 3.1 % lipid, 7.0 % ash and 35.5 % carbohydrate in dry weight of this waste. The TPrC determined for the hazelnut meal is higher than those of 46.8 % determined by Emre et al. (2008) for Turkish hazelnut meal and those

of 44.8 % determined by Doğan and Erdem (2010) for the dry weight of similar meals. These results clearly showed that the protein content of hazelnut meal was comparable with that of soybean meal which could vary between 48.5 % and 58.1 % in dry weight basis (Thakur & Hurburgh, 2007). Moreover, it also appeared that the hazelnut meal used in this work had higher protein content than sunflower and rapeseed meals which reported to contain TPrCs of 32 and 35 % in dry weight basis, respectively (Lomascolo, Uzan-Boukhris, Sigoillot, & Fine, 2012). Thus, it can be concluded that the Turkish hazelnut meal is a good source for protein extraction.

4.2.1.2. Total Protein Content of Hazelnut Meal Protein Extracts

The protein extracts used in this study were obtained directly from meal, or from acetone washed meal to eliminate the oxidized lipid residues and brown phenolic pigments which could interact with the proteins and modify their properties (Aewsiri et al., 2009; Tsai & She, 2006). The extraction was conducted at room temperature to obtain total soluble protein fractions or at 85 °C to denature and eliminate the heat labile protein and other heat labile impurities which might form haze or precipitate during food processing and storage.

The protein yields from hazelnut meal were 0.09, 0.10, 0.17, and 0.18 g protein/g dry meal for the extracts of HPC-AW-H, HPI-AW, HPI-H, and HPI, respectively**.** On the other hand, the TPrCs of proteins isolated from hazelnut meal by different treatments varied between 86 % and 94.5 %. The TPrCs of HPI, HPI-H and HPI-AW were not significantly different ($P > 0.05$), but HPC-AW-H had significantly lower protein content than the other extracts. These findings suggested that the classical unfolding effects of acetone on proteins (Brandts & Hunt, 1967; Roe, 2001) increased the protein-protein interactions and led the precipitation of proteins. Moreover, it also appeared that the heating of acetone treated proteins caused further insolubilization of a specific heat labile HPC-AW-H fraction during extraction.

4.2.1.3. Water Soluble Protein Content of Hazelnut Meal Protein Extracts

The protein extracts gave a U shaped solubility profile between pH 2.0 and 11.0 with minimal solubility at pH 4.5 which was also employed during purification with IEP (Figure 4.6). This profile is quite similar with those of soybean meal proteins and other nut proteins from defatted cashew nut, walnut and Brazil nut (Neto, Narain, Silva, & Bora, 2001; Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009; Ramos & Bora, 2005; Swamylingappa & Srinivas, 1994; Sze-Tao & Sathe, 2000). For HPI-AW, HPC-AW-H and HPI, the maximal solubility was observed at pH 2.0 while HPI-H showed 2.5-3.0 fold lower solubility then these proteins at the indicated acidic pH value. At pH 3.0, the HPI-AW and HPC-AW-H showed 2.0-2.5 folds higher solubility than HPI and HPI-H. This result clearly showed that the acetone washing of meal contributed to increased solubility of protein at acidic pH values. The HPI and HPI-H had similar pH-solubility profiles between pH 3.0 and pH 7.0, but HPI-H had slightly higher solubility than HPI between pH 8.0 and 10. The HPC-AW-H and HPI-AW also showed similar solubility profiles at alkaline pH values, but HPC-AW-H had 1.9 and 1.4 fold higher solubility at pH 6.0 and 7.0 than HPI-AW, respectively. These results clearly indicated that the hot extraction was an effective treatment to increase solubility of hazelnut proteins. However, the solubility profile obtained for HPC-AW-H at acidic, neutral and alkaline pH values suggested that the combinational application of acetone washing and hot extraction was the most effective treatment to increase protein solubility.

4.2.1.4. Gel Formation Capacity of Hazelnut Meal Protein Extracts

The gelling properties of isolated protein between 1 and 20 % (w/v) showed the limited gel formation capacity of hazelnut proteins (Table 4.8). The highest gelling capacity was determined for HPC-AW-H which formed fixed gels at 14 %. The HPI-H and HPI-AW formed fixed gels at the protein concentration of 20 %, while HPI did not form any gel at the studied concentration range. It is well known that the unfolding and exposure of critical number of functional groups, such as hydrogen bonding and hydrophobic groups are the essential steps in the sol to gel state transformation

mechanisms of proteins (Damodaran, 1996). Thus, these results supported our previous hypothesis that the acetone washing and hot extraction had possible roles in protein unfolding and exposing reactive groups responsible for the bioactive properties of hazelnut proteins. It is also once more proved that the modifications in protein conformation pronounce by the combinational application of acetone washing and heat treatment. The LGC of HPC-AW-H obtained from hazelnut meal is comparable with that of peanut (14 w/v) protein concentrate (Sharma et al., 2010), but it is still considerably lower than those of hazelnut (12 w/v), almond (6 w/v), Brazil nut (8 w/v) and cashew nut (8 w/v) protein isolates or concentrates obtained from defatted nut flour with alkaline extraction and dialysis (Sharma et al., 2010). The LGC of HPC-AW-H is also lower than those of the commercial soy protein isolates (10 % w/v) and whey protein isolates (14 % w/v) tested in this work as reference proteins at the same conditions (Table 4.1). These results clearly showed the poor gelling properties of isolated hazelnut meal proteins.

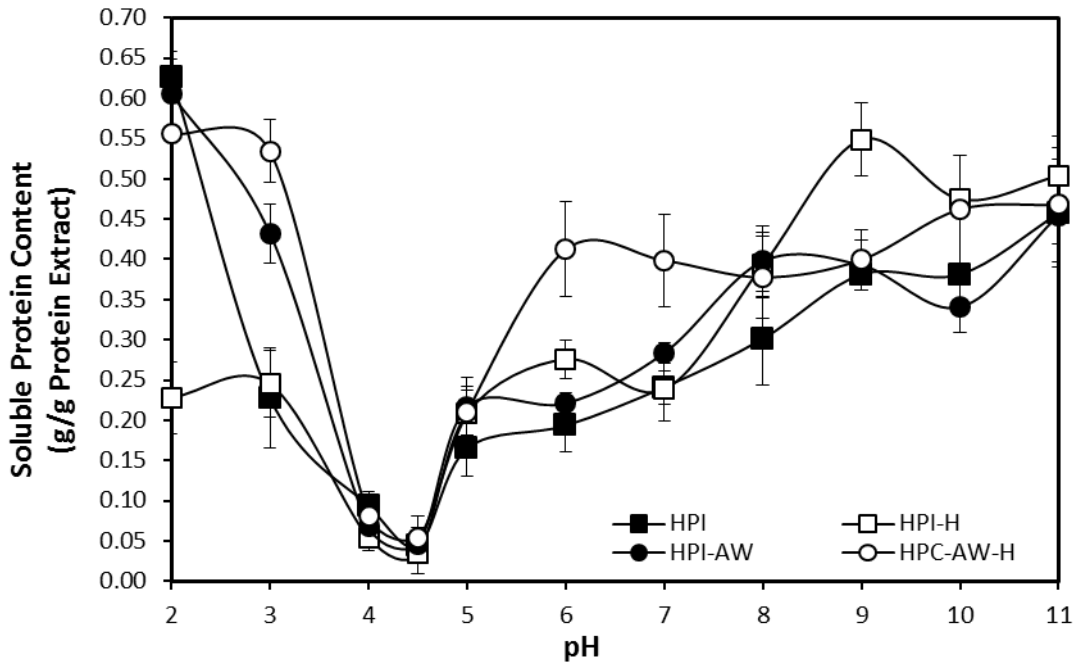

Figure 4.6. pH solubility profiles of different hazelnut meal protein extracts

4.2.1.5. Water and Oil Absorption Capacity of Hazelnut Meal Protein Extracts

Due to their low water binding capacities, the hazelnut protein extracts showed no or very little WAC values. Measurable WACs were obtained only for the heated protein extracts (HPI-H and HPC-AW-H) and this proved the modification of proteins during heating. However, no effect of acetone washing on WAC was determined. On the other hand, the OACs of protein extracts, varied between 7.4 g/g and 9.4 g/g, showed no statistically significant differences ($P > 0.05$). Thus, it was clear that the acetone washing and/or hot extraction had no effect on OAC. Although the isolated hazelnut meal proteins showed poor WACs, their OACs are 1.3 to 2.5 fold higher than those of protein extracts obtained from flours of hazelnuts (90 % protein), almonds (93 % protein), pistachios (80 % protein) and peanuts (80-82.5 % protein) obtained by alkaline extraction and dialysis (Sharma et al., 2010). The OACs of isolated hazelnut meal proteins were also comparable with those of non-GMO soy protein isolate (OAC: 8.23 g/g) and those of protein isolates from various red and green lentil cultivars (average OAC for 6 cultivars: 8.62 g/g) obtained with the same extraction and purification method (IEP) applied for HPI (Table 4.1). The IEP protein isolates from flaxseed meal (7.97 g/g) also showed similar OAC with the hazelnut meal protein isolates (Krause, Schultz, & Dudek, 2002). However, it is worth to note that the OACs of isolated hazelnut meal proteins were 4 to 8 fold higher than those of wheat protein isolate (1.73 g/g) reported by (Ahmedna, Prinyawiwatkul, & Rao, 1999), and those of commercial whey (1.59 g/g) and soy (1.16 g/g) protein isolates reported by (Table 4.1).

4.2.1.6. Emulsifying Activity and Emulsion Stability of Hazelnut Meal Protein Extracts

The highest EA was determined for HPI-AW, followed by HPI and HPC-AW-H with similar EAs, and HPI-H (Table 4.9). However, it was hard to report some dramatic differences among the EAs of protein extracts since the turbidities of their emulsions varied at a narrow range, between 302 and 340 NTU.

Table 4.8. Gel formation capacity, and water and oil absorption capacity of hazelnut meal protein extracts

Protein extract	Gelling properties of different isolate concentrations (%, w/v)[a]							WAC (g/g)	OAC (g/g)
	1-8	10	12	14	16	18	20		
HPI	-	-	-	-	+	+	+	0	9.1 ±0.97 a[b]
HPI-H	-	-	-	-	+	++	+++LGC[c]	0.32 ±0.21a	9.4 ±0.75 a
HPI-AW	-	-	-	-	+	++	+++LGC	0	8.8 ±0.15 a
HPC-AW-H	-	+	++	+++LGC	+++	+++	+++	0.21 ±0.11a	7.4 ±0.80 a
WPI	-	+	++	+++LGC	+++	+++	+++	0±0	1.59±0.15b
SPI	-	+++LGC	+++	+++	+++	+++	+++	7.94±0.44b	1.16±0.05b

[a] + : increased viscosity-no gelation; ++: slush-like unfixed gel; +++: fixed hard gel
[b] Different letters in each column show significant differences at $P<0.05$
[c] LGC: Least gelling concentration

The HPI, HPI-H and HPC-AW-H showed quite similar ESs and maintained 80 to 100 % of their turbidities in 30 minutes. In contrast, the HPI-AW showed considerably lower ES than the other protein extracts and lost almost 40 % of its turbidity within 30 minutes. The higher ES of HPC-AW-H than HPI-AW and higher ES of HPI-H than HPI within 30 minutes showed the contribution of heat treatment applied during hot extraction in emulsifying properties of proteins. However, this was a limited contribution since all protein extracts showed very low ESs and lost almost 85 % of their initial turbidities within 180 minutes. Due to differences in emulsion formation conditions (type of oil, protein concentration and speed of homogenization), and in units and indices used in expression of emulsification properties, it is hard to compare the present results with those in most of the literature. However, it is worth to report that the EA and ES determined for the isolated hazelnut protein were comparable with those of commercial soy protein isolate and commercial whey protein isolate tested at the same conditions.

4.2.1.7. Foaming Activity and Foam Stability of Hazelnut Meal Protein Extracts

The FAs of different protein extracts showed greater variation than EAs and changed between 7.1 and 18.9 mL. The HPI-AW showed a very high FA which was almost 1.5 fold higher than those of HPI-H and HPC-AW-H, and 2.7 fold higher than

that of HPI. Thus, it seemed that the modifications in surface activities of protein at air-water interface caused by acetone treatment alone were highly optimal to maximize the FA of HPI-AW. The significantly lower FA of HPC-AW-H than HPI-AW showed the adverse effect of hot extraction on foaming activity of protein obtained by acetone treated meal. However, 1.8 fold higher FA of HPI-H than HPI showed the beneficial effect of hot extraction alone in improving FA of proteins untreated with acetone. These results clearly showed the sharp differences created in surface properties of protein at air-water interface by combinational or individual application of acetone washing and hot extraction.

Table 4.9. Emulsifying and foaming activities and stabilities of hazelnut meal protein extracts

Protein extract	EA (NTU)	$ES_{30\ min}$ (NTU)	$ES_{180\ min}$ (NTU)	FA (mL)	$FS_{30\ min}$ (mL)	$FS_{180\ min}$ (mL)
HPI	326±5bc[a]	268±14b	53±5b	7.1±0.6cd	6.7±0.7d	5.5±0.6b
HPI-H	302±6d	308±14a	47±10b	12.9±1.0b	12.3±0.4b	10.9±0.3a
HPI-AW	340±3b	202±24c	42±16b	18.9±1.1a	16.9±2.0a	6.9±3.2ab
HPC-AW-H	327±3bc	285±1ab	46±1b	12.3±1.3b	11.3±0.9b	8.2±0.5ab
WPI	365±15a	314±1a	105±3a	11.6±0.7bc	10.1±1.5bc	0±0c
SPI	312±12cd	273±7b	62±6b	9.1±0.7c	7.9±0.8cd	5.6±0.1b

[a] Different letters in each column show significant differences at $P<0.05$

The FS of protein isolates at 30 minutes corresponded to almost 90 % of their initial FAs and this clearly showed the stability of obtained foams. The limited changes in FS of HPI, HPI-H, and HPC-AW-H in 180 minutes clearly proved the high resistances of these protein extracts' foams to destabilization. In contrast, the high initial FA gained by HPI-AW was not stable and it reduced almost by 63 % during 180 minutes incubation. These results clearly showed the different factors affecting the foaming activities and stabilities. The HPI-AW also formed the least stable emulsions. Thus, it was clear that the acetone induced modifications in conformation of HPI-AW did not contribute to its ES and FS which were related with increased protein-protein interaction and formation of a thick and viscoelastic film at oil-water and air-water interfaces, respectively (Damodaran, 1996). The results obtained in this work for foaming properties of hazelnut meal protein extracts contradict with those obtained for hazelnut protein extract by Sharma et al. (2010). These authors did not report a considerable FA and FS for hazelnut protein extract obtained by alkaline extraction and

dialysis from hazelnut flour. Sharma et al. (2010) attributed the poor foaming properties of hazelnut and some other nut proteins to their compact globular conformation which hided their surface active properties. Thus, it appeared that the higher foaming properties of hazelnut meal protein extracts are due to protein unfolding occurred during oil processing and heat treatment applied during extraction for protein modification. The FAs determined in this work for different isolated hazelnut meal proteins were also either comparable with or higher than those of protein isolates obtained from defatted meals of 6 lentil cultivars (average FA: 8.7 mL), 4 chickpea cultivars (average FA: 10.98 mL) and a non-GMO soybean cultivar (6.5 mL) prepared with the IEP method and those of commercial soy (9.1 mL) and whey (11.6 mL) protein isolates tested at the same conditions (concentration, pH, speed of mixing) applied in the current study. The hazelnut protein isolates (except HPI-AW) also had higher foam stabilities than 1 % (w/v) solutions of IEP pea protein isolate, broad bean protein isolate and lupin protein isolate which lost 25 to 30 % of their initial foam volume at pH 7.0 within 30 minutes (Makri et al., 2005) and 1 % solutions of commercial soy protein isolate and whey protein isolate which lost almost 38 % and 100 % of their initial foam volume at pH 7.0 within 180 minutes, respectively.

4.2.2. Bioactive (Antioxidant and Antihypertensive Activity) Properties of Protein from Turkish Hazelnut Meal

4.2.2.1. Free Radical Scavenging Activity and Iron Chelating Capacity of Hazelnut Meal Protein Extracts

The free radical scavenging (FRS) based antioxidant capacities of isolated proteins are given in Table 4.10. The TEAC and ORAC values of extracts were significantly different from each other and varied between 158 and 365 µmol Trolox/g and 219 and 461 µmol Trolox/g, respectively. The highest TEAC and ORAC values were obtained for HPC-AW-H, followed by those of HPI-AW, HPI-H and HPI. The HPI-H, HPI-AW and HPC-AW-H showed 1.2 and 1.5 fold, 1.6 and 1.8 fold, and 2.3 and 2.1 fold higher TEAC and ORAC than HPI, respectively. Thus, it is clear that the individual or combinational application of acetone washing and hot extraction has beneficial effects on antioxidant capacity based on FRS of proteins. The acetone

washing of meal was more effective on increased antioxidant capacity of extracts than the hot extraction, but the combinational application of acetone washing and hot extraction was the most effective method to improve the antioxidant capacity of proteins. The increased FRS based antioxidant activity of protein extracts by acetone and heat treatment could be related with their effects on protein unfolding and resulting increased accessibility to protein antioxidant groups such as those of histidine, tyrosine, phenylalanine, tryptophan and cysteine which were capable to donate protons to free radicals (Hu et al., 2003; Je, Park, & Kim, 2004; Rajapakse et al., 2005). It is also possible that the slight differences in solubility of extracts at the pH of antioxidant activity measurements (pH 7.4) and removal of some prooxidants in the meal by acetone washing and heat denaturation contribute to the differences in FRS based antioxidant activity of the isolated protein. Further studies are also needed to understand the contribution of non proteinous compounds including carbohydrate-phenolic complexes and polymeric phenolic compounds in antioxidant activity of extracts. HPC-AW-H needs a particular attention since it has the highest bioactivity, but lowest purity.

On the other hand, the ICC of protein extracts varied between 60.7 and 126.7 µmol EDTA/g. The highest ICC was obtained for HPI-H followed by those of HPC-AW-H, HPI-AW and HPI. Although the ICC values were significantly different from each other at $P < 0.05$, the HPI and HPI-AW had very similar ICC values. The ICC of HPC-AW-H was also only 1.3 fold lower than that of HPI-H and this revealed the limited contribution of acetone washing to improve ICC of proteins. In contrast, the HPI-H and HPC-AW-H obtained by hot extraction showed almost 2.1 and 1.6 fold higher ICCs than HPI and HPI-AW, respectively. The contribution of hot extraction in ICC of proteins should be related with the specific conformational changes of heating in areas accommodating iron binding groups. It seemed that the heating unburied and exposed the iron binding groups such as those of basic amino acids like lysine, histidine and arginine and those of acidic amino acids like aspartate and glutamate (Rajapakse et al., 2005; Saiga et al., 2003). This is an interesting finding suggesting the selective effects of acetone and heat treatment on protein conformation and resulting bioactivity.

4.2.2.2. Antihypertensive Activity of Hazelnut Meal Protein Extracts

Antihypertensive activities of isolated protein were determined based on their ACE inhibitory capacities (Figure 4.7). Protein extracts applied at increasing concentrations between 0.1 and 1 mg/mL inhibited the ACE activity at a dose dependent manner. The inhibition level in the presence of 1 mg/mL of different protein extracts changed between 50 and 70 %. The HPC-AW-H, which had 1.5-1.8 folds lower IC_{50} than the other proteins, showed the highest antihypertensive activity. The HPI-H and HPI-AW showed quite similar IC_{50} values which were almost 1.2 fold lower than that of HPI, respectively. These results clearly indicated the greater contribution of combinational application of acetone washing and hot extraction in improving antihypertensive properties of proteins than the individual application of these treatments. On the other hand, although the HPI showed the lowest inhibitory activity between concentrations of 0.1 and 1 mg/mL, the increase of protein extract concentration from 1 to 5 mg/mL increased the inhibition caused by this extract almost 25 % (data not shown). For the other protein extracts, the dose dependency of the ACE inhibition was lost or weakened by increase of extract concentration from 1 to 5 mg/mL. Thus, the ACE inhibition level of all isolated protein at 5 mg/mL became similar and changed at a narrow range between 70 and 80 %. These results clearly showed that the reactivity of protein in different extracts with ACE was different. It is also proved that the 20 to 30 % of ACE was highly resistant to inhibition caused by the protein. However, the inhibition of a significant portion of ACE activity by hazelnut proteins suggested the considerable affinity of these proteins to the hydrophobic enzyme active site or anionic enzyme inhibitor binding site that is distinct from enzyme's catalytic site (Guang & Phillips, 2009; Ryan et al., 2011). Moreover, it is also important to note that the ranking of the antihypertensive activities of extracts showed parallelism with those of FRS based antioxidant capacities. The r^2 values determined between TEAC and IC_{50} for antihypertensive activity (r^2: -0.919), and ORAC and IC_{50} for antihypertensive activity (r^2: -0,899) also supported the relation among these bioactive properties. In the literature, inconsiderable ACE inhibitory activities were reported for commercial soybean protein isolate (Lo & Li-Chan, 2005), flaxseed meal protein isolate (Marambe, Shand, & Wanasundara, 2008) and pumpkin meal protein isolate (Vaštag, Popović, Popović, Krimer, & Peričin, 2011) unless they were hydrolyzed by proteases. The ACE

inhibitory potential of hazelnut protein extracts was also higher than that of protease digested pea protein isolate (1.36 mg/mL) and comparable with that of protease digested whey protein isolate (0.90 mg/mL) (Vermeirssen, Van Camp, & Verstraete, 2002).

Table 4.10. Bioactive properties of hazelnut meal protein extracts

Protein extract	TEAC (µmol Trolox/g)	ORAC (µmol Trolox/g)	ICC (µmol EDTA/g)	ACE inh. IC_{50} (mg/mL)
HPI	158 ±0.6d[a]	219 ±8d	61 ±8.4d	1.0±0.01a
HPI-H	193 ±2.5c	337 ±7c	127 ±0.7c	0.82±0.01b
HPI-AW	254 ±4.2b	387 ±14b	64 ±2.7b	0.87±0.01c
HPC-AW-H	365 ±1.8a	461 ±29a	101 ±2.0a	0.57±0.02d

[a] Different letters in each column show significant differences at $P < 0.05$.

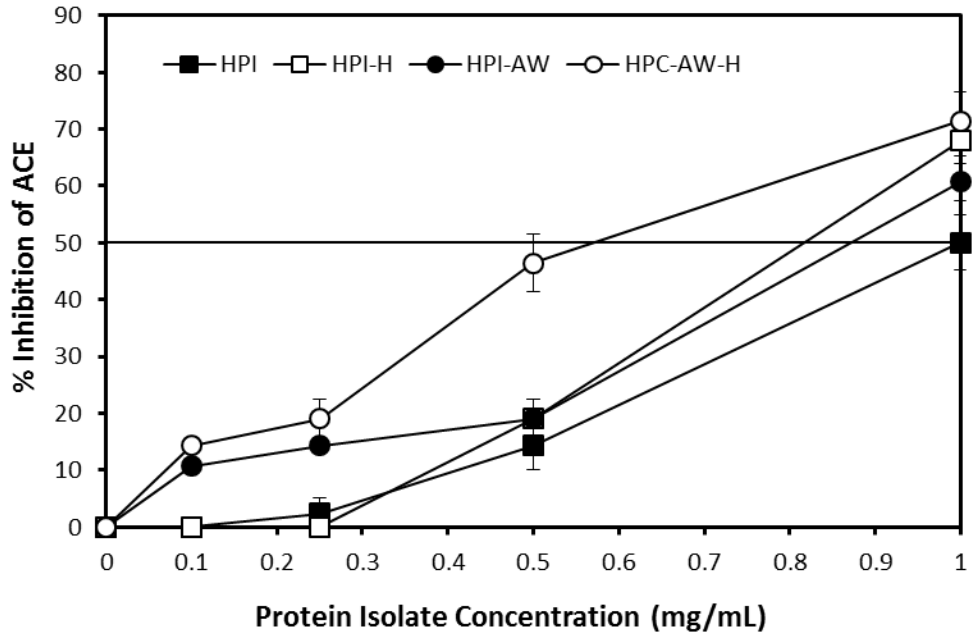

Figure 4.7. Antihypertensive activities of hazelnut meal protein extracts

4.2.3. Edible Film Forming Properties of Protein from Turkish Hazelnut Meal

4.2.3.1. Mechanical Properties of Edible Films Obtained from Hazelnut Meal Protein Extracts

The elongation at break (E) and tensile strength (TS) values of films obtained from different protein extracts were given at Figure 4.8A and 4.8B. In general, the use of different protein extracts and modifications in film making conditions caused considerable changes in mechanical properties of the films. For example, the increase of the extract concentration from 10 % to 12.5 % and the heating time from 30 minutes to 60 minutes during film making increased the E of HPI, HPI-H and HPI-AW considerably. In contrast, the increase of isolate concentration and heating time caused a sharp reduction in E of HPC-AW-H. The overall E measurements at different conditions showed that the most flexible films were formed by HPI (E between 62 % and 144 %) followed by HPI-H, HPI-AW and HPC-AW-H. On the other hand, the change of the extract concentration and the heating time during film making caused limited or inconsiderable reductions in TSs of HPI and HPI-H, while TSs of HPI-AW and HPC-AW-H increased or reduced dramatically by change of film making conditions. The HPI-H gave the highest TSs (between 5.6 and 7.9 MPa) at different film making conditions while the films of HPI-AW and HPC-AW-H obtained at 10 % extract concentration with 60 minutes heating gave the lowest TSs. These E and TS measurements conducted at different film making conditions clearly showed that the use of HPI and HPI-H in film making was more advantageous than the use of HPI-AW and HPC-AW-H in obtaining flexible films with sufficient resistances against mechanical stresses. Thus, it is evident that the use of extracts obtained by acetone washing alone or in combination with hot extraction during protein extraction is not beneficial when hazelnut proteins will be employed in edible film production.

4.2.3.2. Water Solubility of Edible Films Obtained from Hazelnut Meal Protein Extracts

The solubility profiles of different edible films obtained by changing protein extract concentration (10 % and 12.5 %) and heating period (30 minutes and 60 minutes) of film making solutions are given at Figure 4.9A to 4.9D. High protein solubility form films indicates low protein denaturation during film preparation and weak interactions among film components, while low solubility indicates high protein denaturation and some considerable interactions among film components. The results obtained by 3 hours incubation of films in distilled water showed that the films of hazelnut protein extracts were not water resistant and they disintegrated in water due to solubilization of part of their protein. However, in most of the extracts the use of suitable protein concentration and heating time could be effective to minimize the protein solubility of films and to improve their water resistances. For example, the use of 12.5 % isolate concentration with 60 minutes heating period was the most effective procedure to reduce the protein solubility of films from all extracts. In contrast, the most soluble films were obtained from HPI-AW (10%-60min), followed by HPI-H (10%-30) and HPC-AW-H (10%-60min). Thus, it was likely that the combinational application of high protein concentration and long heating period enhanced the protein-protein interactions within the film matrix while it reduced the protein-solvent interactions. Moreover, it is clear that the individual or combinational application of acetone washing and hot extraction have no beneficial effects in controlling protein solubilization of edible films.

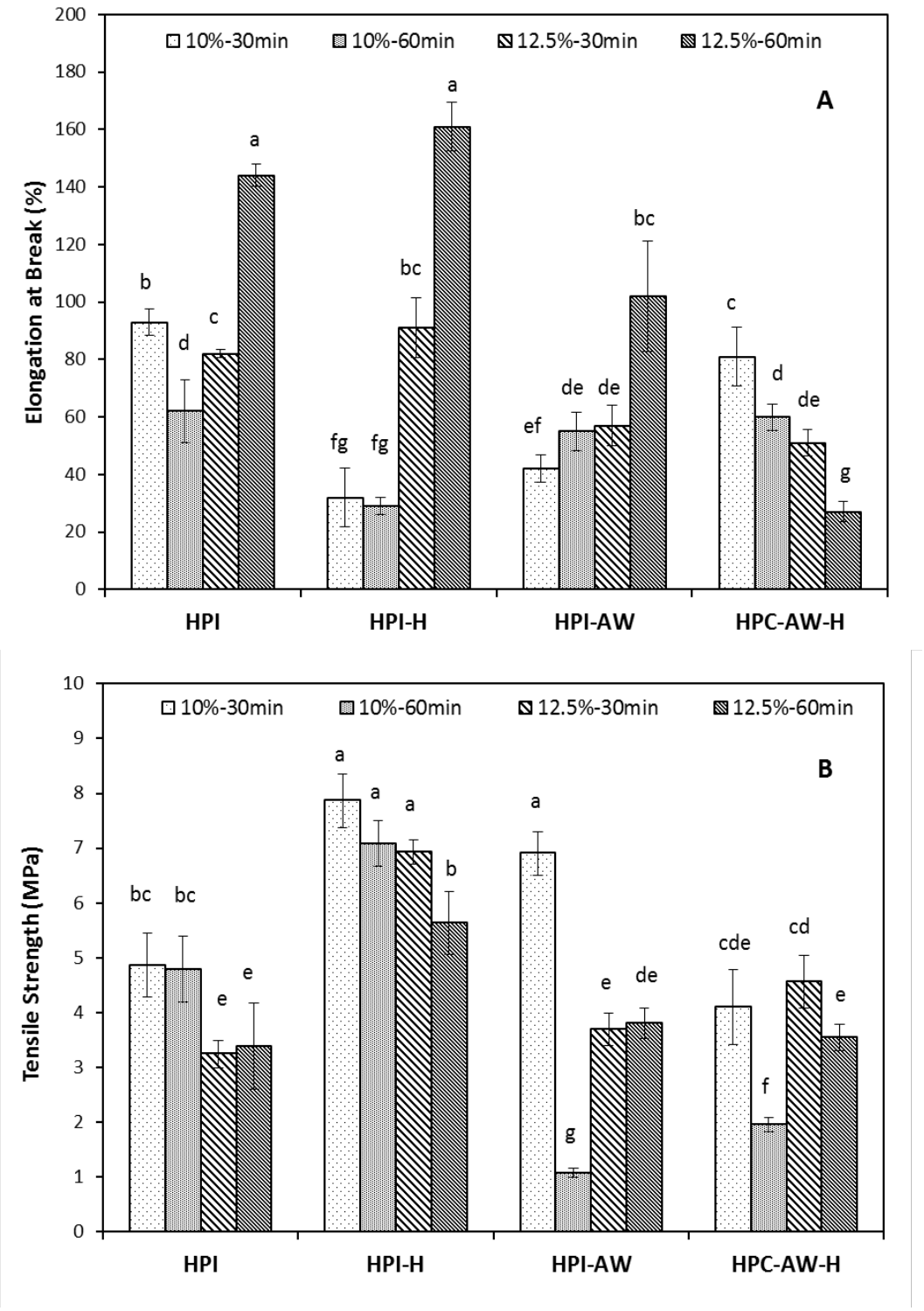

Figure 4.8. Mechanical properties of edible films obtained from hazelnut meal protein extracts (A: elongation at break; B: tensile strength)

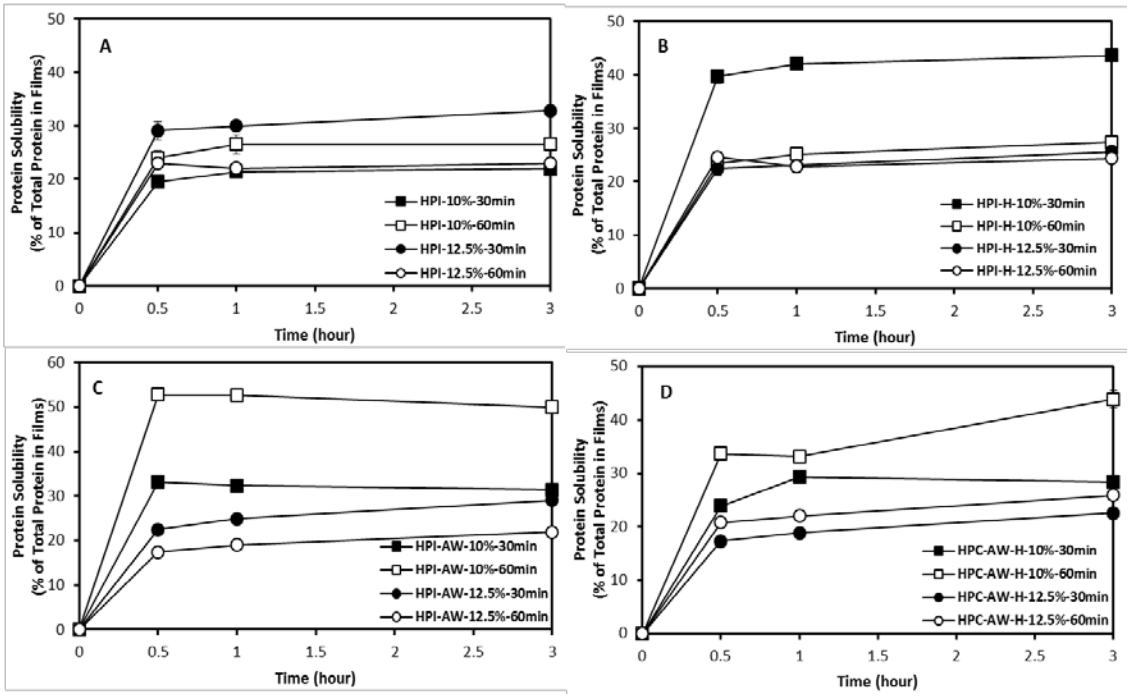

Figure 4.9. Effects of protein concentration and heating applied during film making on water solubilities of edible films from hazelnut meal protein extracts

4.2.3.3. Colour of Edible Films Obtained from Hazelnut Meal Protein Extracts

The photos of films obtained at the extract concentration of 12.5 % showed a colour change in films from yellowish to brownish and brownish to reddish depending on type of extract used in film making and change of heating time used for film making solutions (Figure 4.10).

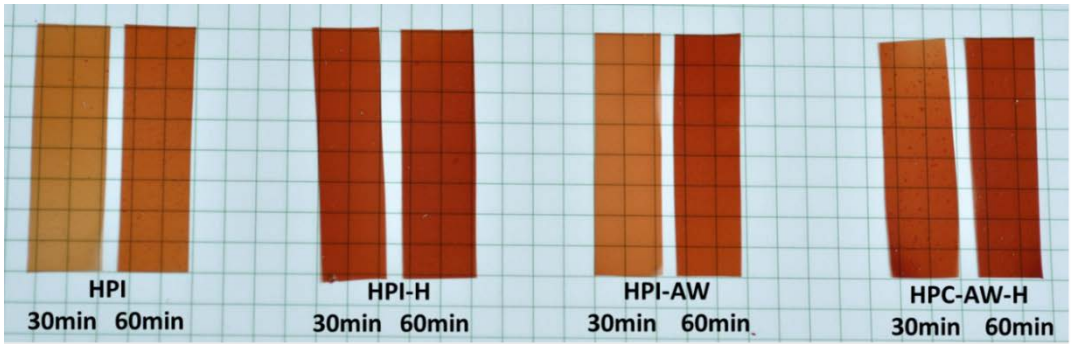

Figure 4.10. Photos of edible films obtained from hazelnut meal protein extracts (protein extract concentration: 12.5 %)

However, all films were transparent and did not contain any haze or cloud indicating the insolubilization of proteins before drying of films. As expected, the increase of heating time from 30 minutes to 60 minutes during film making caused visually detectable increases in the darkness of all films. The darkening observed by increased heating in HPI and HPI-H films were limited and did not cause any significant reductions in L^* values of the films (Table 4.11) *(*$P>0.05$*).* In contrast, increase of heating time during film making caused apparent darkening and significant reductions in L^* values of HPI-AW and HPC-AW-H films ($P<0.05$). The increased heating time was not effective on film a^* values which represented the redness-greenness of the films while it caused significant reductions in film b^* values (except HPI films) which represent yellowness-blueness. The overall results showed that the HPI gave the lightest colored films at both 30 minutes and 60 minutes heating period, followed by HPI-AW and HPC-AW-H films obtained by 30 minutes heating. In contrast, films of HPI-H were quite dark at both heating conditions.

Table 4.11. Colour of edible films obtained from hazelnut meal protein isolates

Protein extract	Heating time (min)	L^*	a^*	b^*
HPI-12.5%	30	53.3±0.5a[a]	21.3±0.6a	34.2±0.4ab
	60	53.2±4.4a	22.3±3.4a	34.5±1.5a
HPI-H-12.5%	30	40.0±1.1d	29.0±1.1b	28.7±1.3c
	60	37.7±1.0d	28.1±0.4b	25.7±1.0d
HPI-AW-12.5%	30	48.0±5.3b	29.2±3.8b	34.5±0.5a
	60	41.2±1.7cd	31.5±0.5b	32.0±1.6b
HPC-AW-H-12.5%	30	45.8±1.2bc	29.2±0.9b	33.0±0.5ab
	60	38.8±1.2d	30.5±0.5b	28.5±1.8c

[a] Different letters in each column show significant differences at $P<0.05$

4.2.4. Molecular Properties of Turkish Hazelnut Meal Protein Extracts

4.2.4.1. Classical SDS-PAGE Patterns of Hazelnut Meal Protein Extracts

The classical SDS-PAGE patterns of hazelnut meal protein extracts at different concentrations, 5 and 10 mg/mL, are given in Figure 4.11. The molecular weight (MW) of protein in different extracts changed at a narrow range between 14.4 and 48.5 kDa. In

fact, it could be reported that the majority of the hazelnut proteins were low MW ones concentrated between 20 and 25 kDa. The protein extracts gave similar protein band patterns, but different band widths and intensities depending on protein extract concentration. For example, HPI-H, HPI-AW and HPI gave almost similar band widths and intensities at the concentration of 5 mg/mL, while HPC-AW-H at the same concentration gave weaker and thinner bands than the other extracts. This result proved that the acetone treated proteins were more heat labile than the untreated proteins, and this explained the lower total protein content of HPC-AW-H than the other isolates. As expected, the protein bands become more intense at the protein extract concentration of 10 mg/mL. The increase of protein concentration caused clearly identifiable increases in the band widths of most HPI-H, HPI-AW and HPC-AW-H bands, while only slight increases were observed in the band widths of HPI fractions. These results suggested some limited modifications and a limited increased heterogeneity in acetone and heat treated protein fractions. However, it seems that the significant differences in bioactivity, functionality and film making properties of protein extracts are not due to major differences in MW of their monomeric fractions but due to differences in their conformations caused by the acetone washing and/or hot extraction. The SDS-PAGE of different nut proteins including hazelnut proteins has been previously studied by Sathe et al. (2009) with details. These authors solubilized nut proteins from defatted nut flour and then investigated their SDS-PAGE profiles without applying isoelectric precipitation of proteins. There were some differences in the band intensities and profiles obtained by these authors and those obtained by the current study for hazelnut proteins, but concentration of most intensive protein bands determined by Sathe et al. (2009) between 20 and 30 kDa (close to 20 kDa) compared well with the results of current study.

4.2.4.2. 2-D Electrophoresis Patterns of Hazelnut Meal Protein Extract

The MW patterns and isoelectric properties of HPI-AW extract were also further characterized by using 2D electrophoresis. The HPI-AW was particularly selected for this purpose since it was not heat treated but acetone washed to remove oxidized polymeric phenolic substances and lipid oxidation products which could form complexes with proteins and mask their charges. The 2D pattern of HPI-AW is given in

Figure 4.12. The results of pI profiles clearly showed the great variations in isoelectric profiles of hazelnut proteins. The majority of the high MW proteins had pI between 5.0 and 5.4, while low MW fractions concentrated between pI 7.0 and 8.5. Sathe et al. (2009) applied isoelectric focusing of hazelnut proteins extracted from defatted flour with Tris-HCl buffer at pH 8.1 found that these proteins migrated mainly around pI 5.85 (between pI > 5.20 and pI < 6.55). Moreover, these authors did not determine any minor hazelnut protein fractions between pI 7.0 and 8.5. However, it is hard to understand the reasons of differences between results of Sathe et al. (2009) and the current work which uses different material (defatted and acetone treated meal) and different methods for extraction, purification and isoelectric focusing of protein. On the other hand, the MW pattern of HPI-AW proteins obtained by the 2D electrophoresis was quite similar with that obtained with the classical SDS-PAGE. Interestingly, the 80 kDa protein band observed during 2D electrophoresis was not detected during classical SDS-PAGE of hazelnut proteins. The classical SDS-PAGE involves the treatment of sample with SDS and a heating step which is essential to effectively transform quaternary structures to their tertiary structures. Unfortunately this procedure conducted at harsh conditions might sometimes cause loss of some protein fractions due to denaturation and insolubilization. The 2D electrophoresis does not involve the heating step and it is a better procedure to monitor the full MW patterns of proteins.

In conclusion, this study clearly showed the bioactive, functional and edible film making properties of hazelnut meal proteins. The acetone washing and hot extraction of meals were quite beneficial to improve the bioactive properties of protein extracts based on antioxidant capacity and antihypertensive activity. The acetone washing and hot extraction also improved the gelling and solubility properties of hazelnut protein extracts, but got limited beneficial effects in improving edible film making properties. Due to their high bioactivity, the hazelnut protein extracts could be very suitable to develop novel functional foods and to contribute to human health. It might be quite interesting to enrich hazelnut cream and hazelnut containing confectionary with bioactive hazelnut proteins and develop novel hazelnut protein rich functional beverages such as hazelnut milk. However, it is quite important to support these studies with more solid animal or human tests and understand the real magnitudes of potential health benefits of hazelnut proteins. The low water absorption capacity, gelation capacity and emulsion stability were the limitations of the technological capacity of isolated hazelnut meal proteins. However, good foaming properties and oil absorption capacities might

still be exploited to obtain beneficial effects in many different food systems. For example, flavored hazelnut protein solutions could be used in hot and cold drink foams prepared just before serving or they could be tested to improve textural properties of foam type foods such as ice-cream. The hazelnut meal proteins showed good film forming abilities and gave flexible, water soluble edible films depending on processing conditions. These films might be suitable for bioactive packaging which involves incorporating nutrients and bioactive compounds into film forming solution and applying it onto surface of suitable food by dipping, spraying or brushing. However, the film making should be conducted at mild heating conditions to avoid darkening of the films. Further studies are continuing in our laboratories to employ hazelnut protein extracts in different food systems and to develop bioactive or active edible films from hazelnut proteins. This work clearly showed the importance of hazelnut meal as source of value added protein based products and food ingredients.

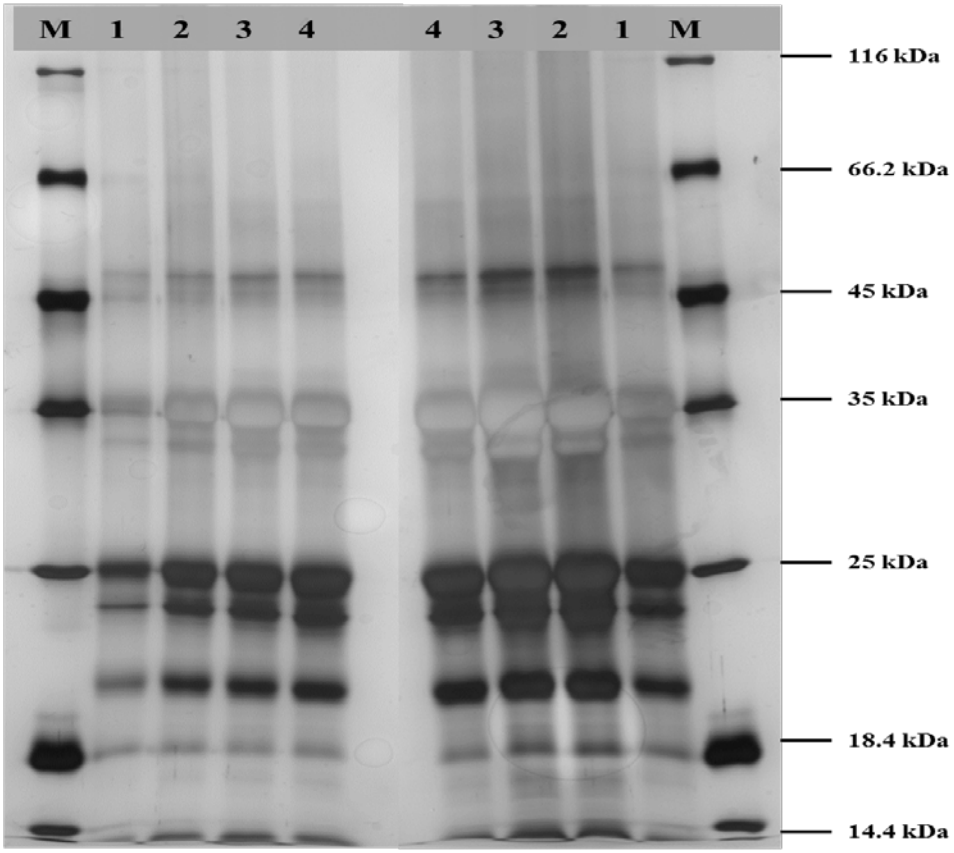

Figure 4.11. SDS-PAGE profiles of hazelnut meal protein extracts (M: Marker, 1:HPC-AW-H, 2:HPI-AW, 3:HPI-H, 4:HPI; protein concentrations of M, 1,2,3,4 (left) and 4,3,2,1,M (right) were 5 and 10 mg/mL, respectively.

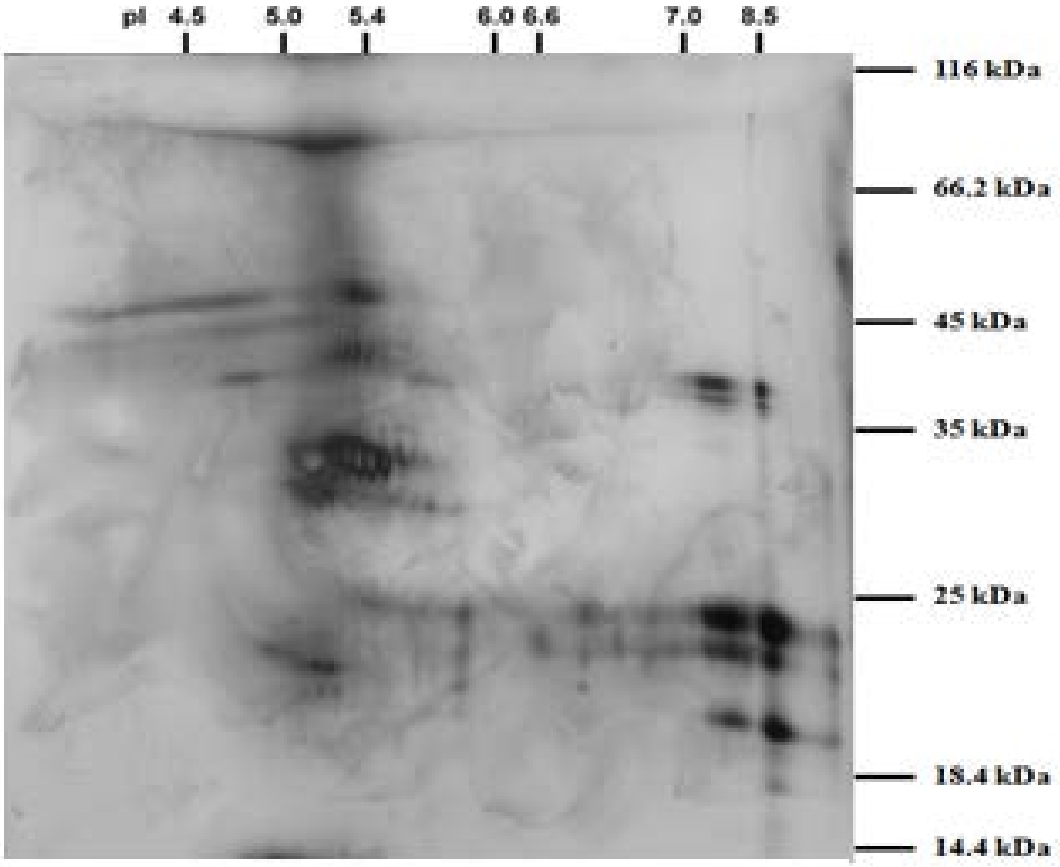

Figure 4.12. 2D electrophoresis patterns of HPI-AW: pI (migration: vertical) MW (migration: horizontal)

4.3. An Example Novel Food Application with the Extracted and Characterized Protein: Bioactive Hazelnut Milk Enriched with Hydrolyzed Hazelnut Proteins

4.3.1. Protein Solubility and Antioxidant Activity of Hazelnut Milk Enriched with Hydrolyzed Hazelnut Protein

Due to the health problems associated with mammalian origin milk, preparation of plant based milk from legumes, cereals and oil seeds have become highly popular. In the world, soy beans are the major sources for plant based milk products. In the literature, there are many studies about soy milk and soy milk based products, rice milk and almond milk based products (Bedani, Rossi, & Isay Saad, 2013; Cruz et al., 2009;

Liu & Chang, 2013; Nik, Tosh, Poysa, Woodrow, & Corredig, 2008; Nilufer-Erdil, Serventi, Boyacioglu, & Vodovotz, 2012; Osthoff, Hugo, van Wyk, de Wit, & Meyer, 2010; Park & Oh, 2005; Sivanandan, Toledo, & Singh, 2008; Telang & Thorat, 2010; Valencia-Flores, Hernandez-Herrero, Guamis, & Ferragut, 2013). Unfortunately, very little work has been conducted related to hazelnut milk (HM) based beverages. Thus, this part of the thesis aimed to produce bioactive hazelnut milk enriched with hydrolysed hazelnut proteins.

In this study, hazelnut protein isolate (HPI) at varying concentrations, from 1 % to 4 % (w/v), was added into HM prepared by the classical method and then soluble protein content and antioxidant activity of HM enriched with HPI were characterized (Figure 4.13, 4.16, 4.19). The protein contents and antioxidant parameters were tested both for freshly prepared samples and for three days cold stored samples to detect possible effects of solubility changes on antioxidant properties in the developed HM beverage. The increase of HPI in the HM up to 3 % increased soluble protein contents, and TEAC and ORAC based antioxidant potentials of HM beverage. However, the increase of HPI concentration in the HM to 4 % caused solubility problems in the HM and this caused slight to moderate reductions in soluble protein content and TEAC based antioxidant activity of HM samples. On the other hand, the use of pepsin (pHPI) or trypsin hydrolysed (tHPI) HPI instead of HPI solved this problem and enabled concentration dependant increase of soluble protein content, ORAC and TEAC up to 4 % concentration (Figure 4.14, 4.15, 4.17, 4.18, 4.20, 4.21). The use of pHPI or tHPI in enrichment caused significant increases in soluble protein contents of HM. For example, the maximum soluble protein content of 40 % reached at 3 % of HPI in the HM increased up to 50 % by use of 4 % of pHPI and up to 45 % by use of 4 % of tHPI in the HM. The maximum TEAC of 7 µmol Trolox obtained by use of 3 or 4 % HPI in HM was also increased up to 14 µmol Trolox by use of 4 % pHPI or tHPI in the HM. In contrast, the increase in the ORAC of HM was not considerably affected by use HPI, pHPI or tHPI.

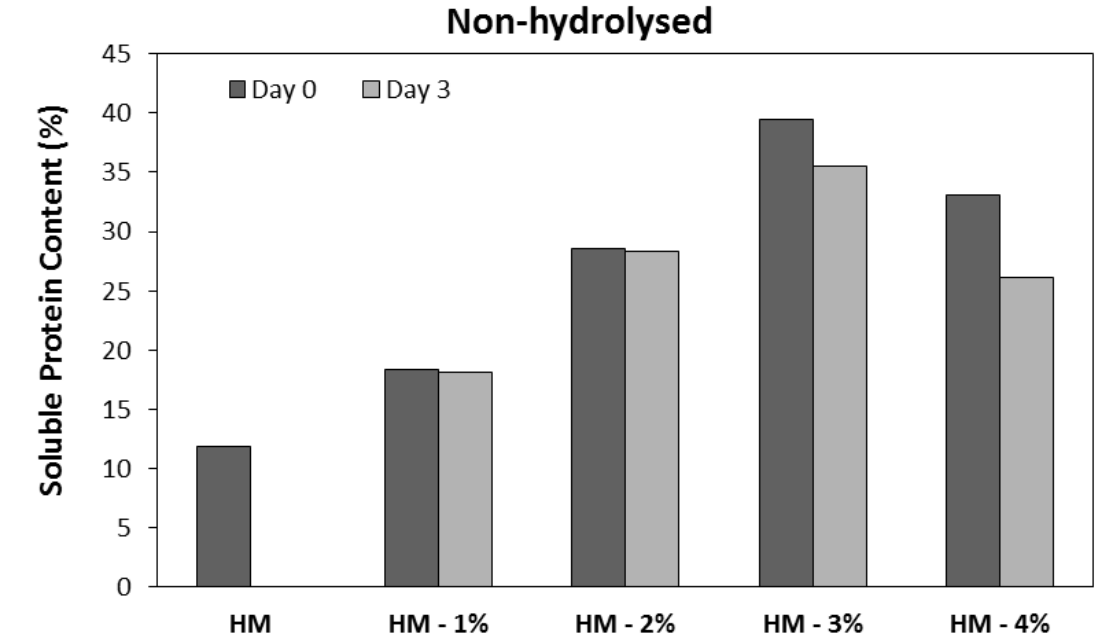

Figure 4.13. Soluble protein content of HM enriched with HPI

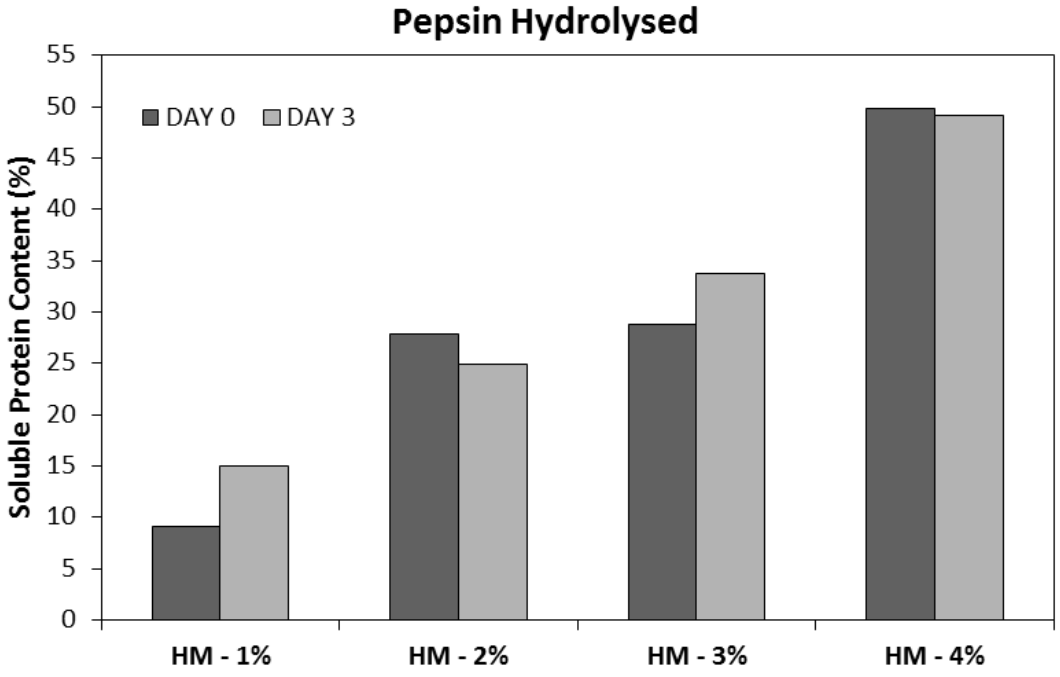

Figure 4.14. Soluble protein content of HM enriched with pHPI

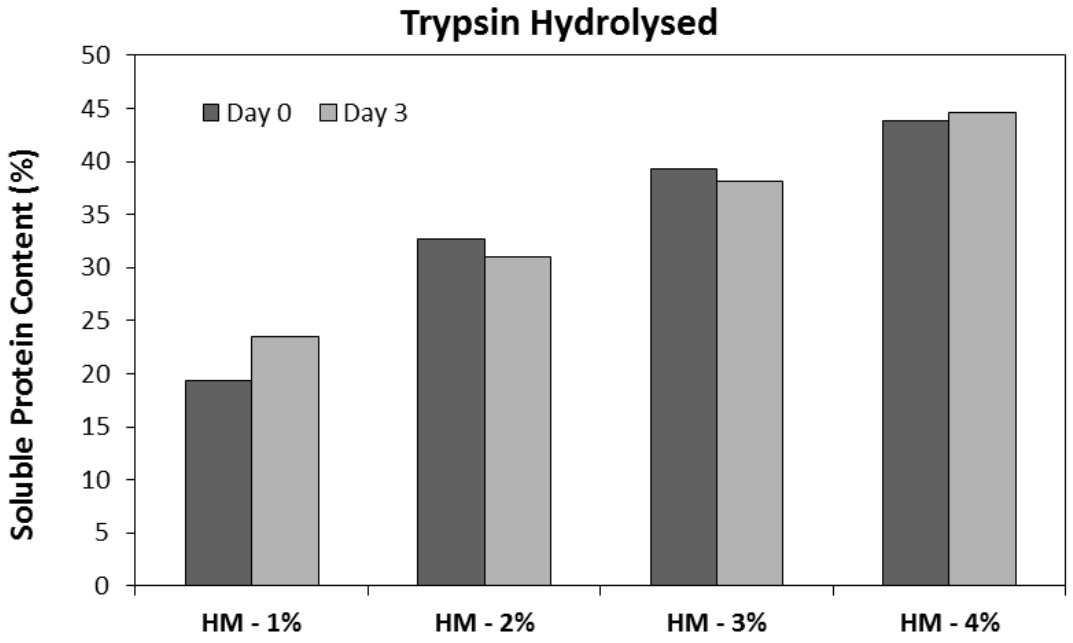

Figure 4.15. Soluble protein content of HM enriched with tHPI

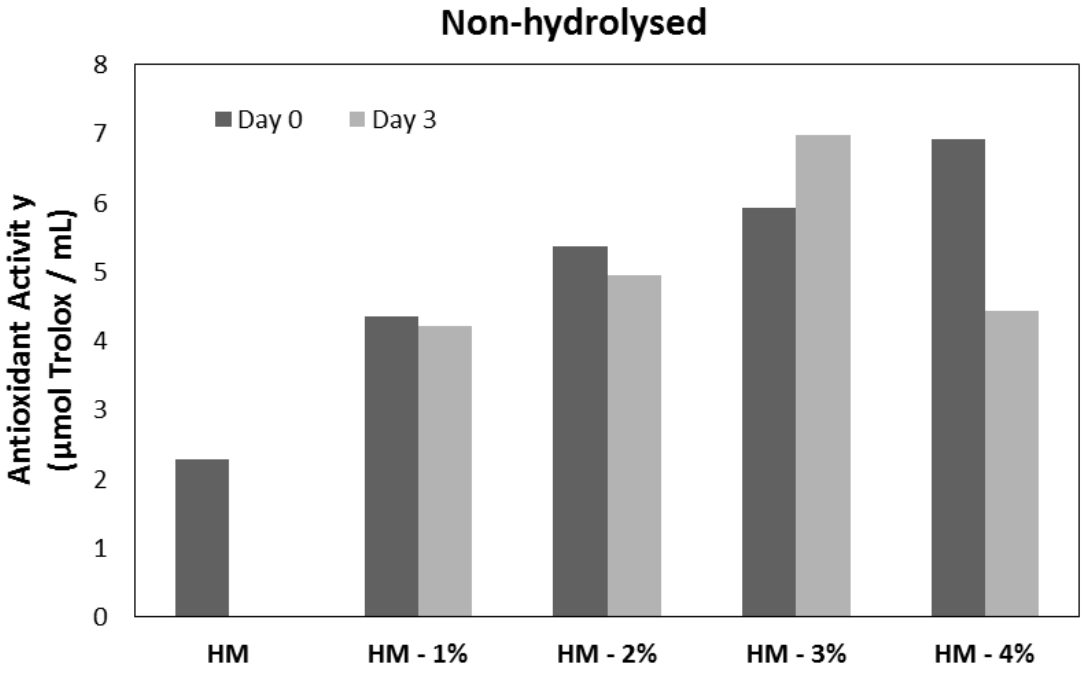

Figure 4.16. Antioxidant activity of HM enriched with HPI based on TEAC

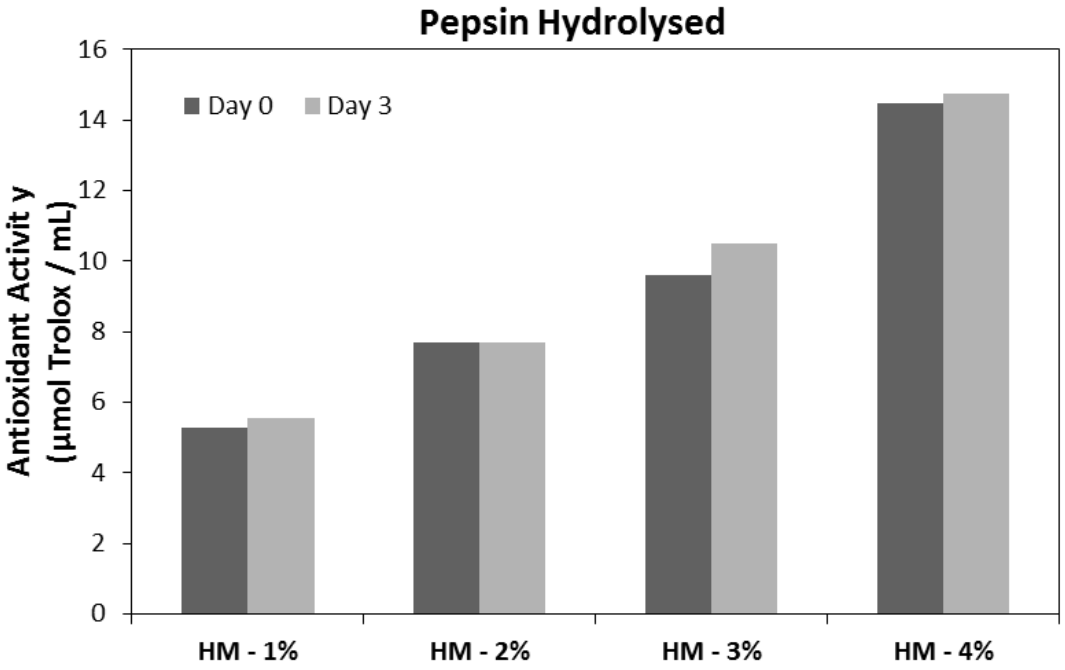

Figure 4.17. Antioxidant activity of HM enriched with pHPI based on TEAC

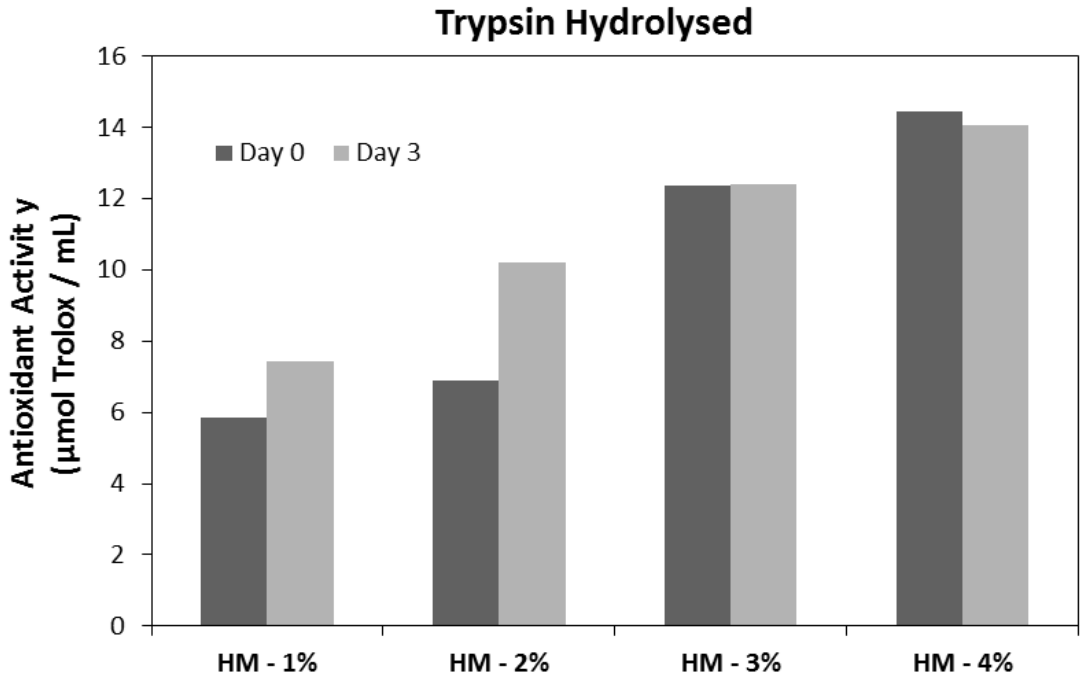

Figure 4.18. Antioxidant activity of HM enriched with tHPI based on TEAC

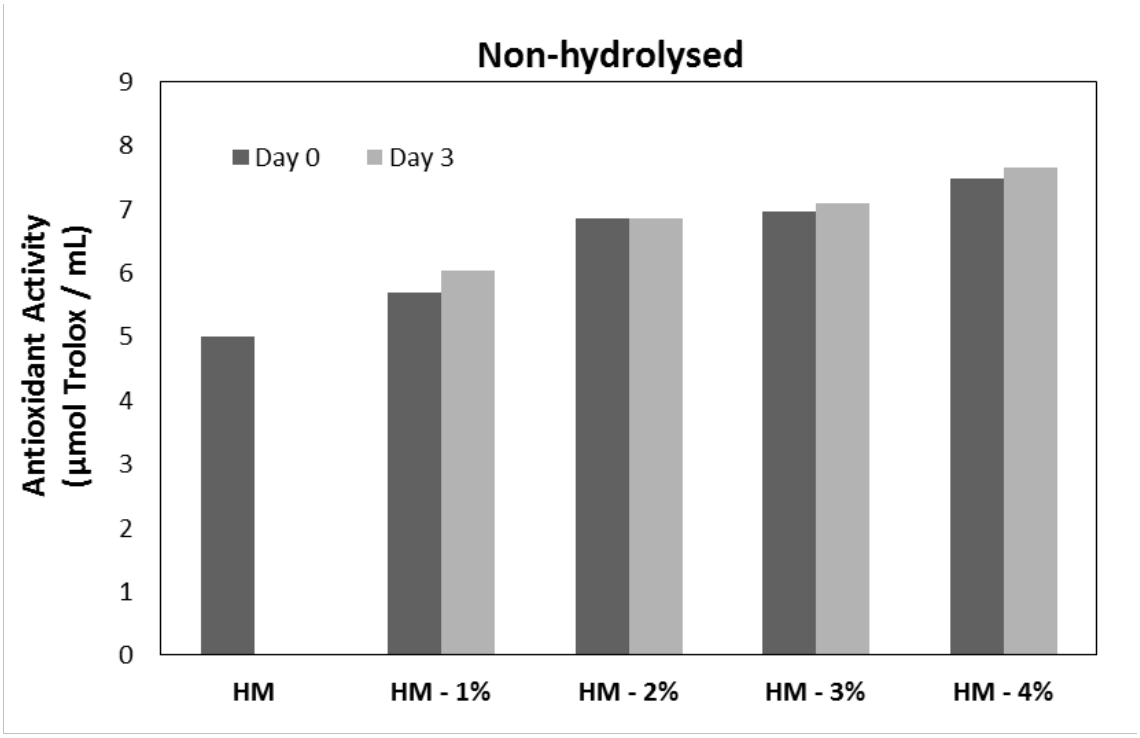

Figure 4.19. Antioxidant activity of HM enriched with HPI based on ORAC

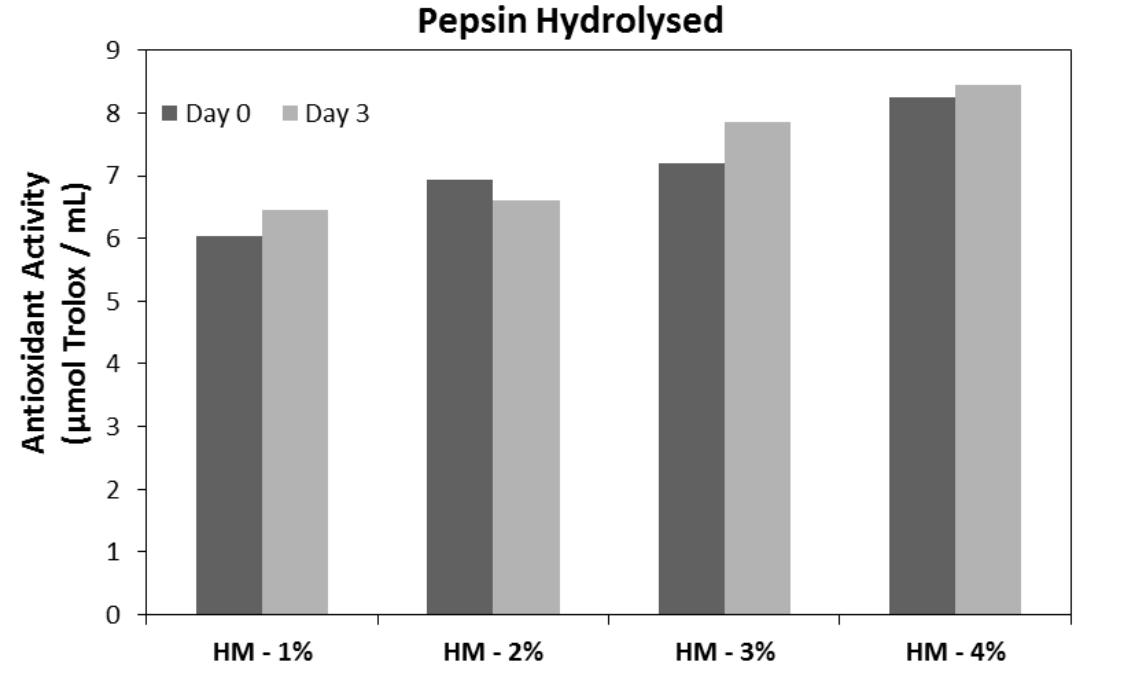

Figure 4.20. Antioxidant activity of HM enriched with pHPI based on ORAC

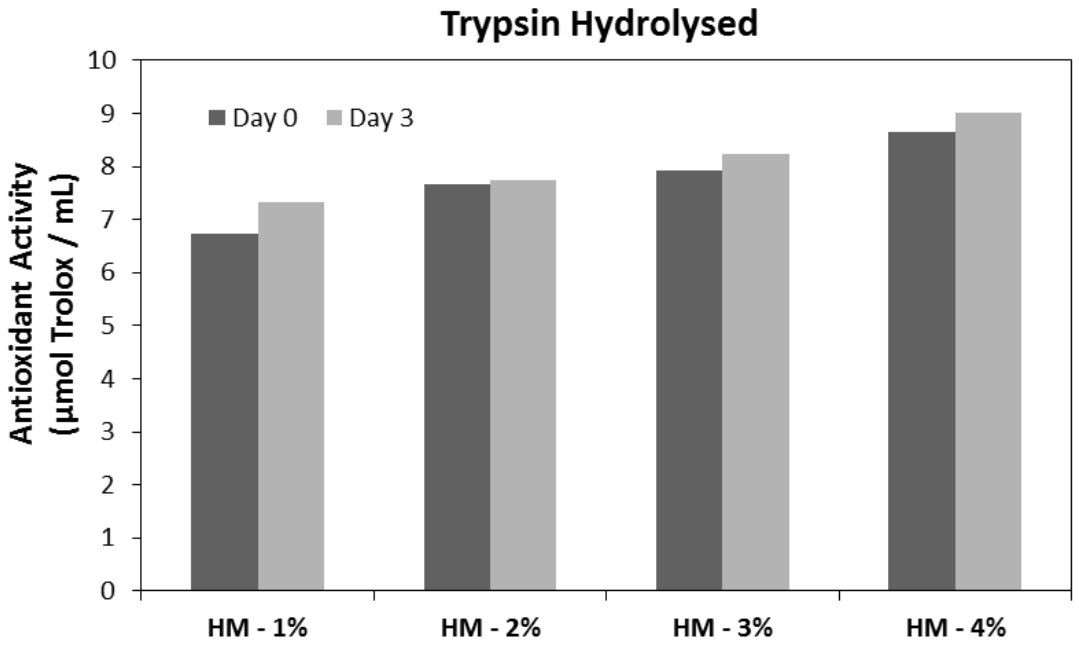

Figure 4.21. Antioxidant activity of HM enriched with tHPI based on ORAC

4.3.2. Thermal Stability of Bioactive Hazelnut Milk Enriched with Hydrolysed Hazelnut Protein

In order to determine the thermal stability of protein enriched hazelnut milk (HM) samples, heat treatment was applied at different temperatures (75, 85, and 95 °C) and different time periods (15 and 30 minutes). After heat treatment the samples with HPI, pHPI or tHPI were tested for their protein solubility and antioxidant activities based on TEAC and ORAC (Figures from 4.22 to 4.30). The results clearly showed the resistance of protein solubility, and ORAC and TEAC based antioxidant capacities of 2 % or 4 % HPI enriched HM heated at 75 °C and 85 °C for 15 or 30 minutes. In fact, a slight increase in protein solubility and antioxidant capacity of some HPI enriched samples was observed by increase of heating temperature from 75 °C to 85 °C. Heating at 95 °C for 15 minutes caused limited changes in protein concentrations of HPI enriched samples, but significant changes were observed in ORAC and TEAC of HM samples heated at 95 °C for 15 or 30 minutes. In contrast, the use of 2 % or 4 % tHPI or pHPI in enrichment of HM increased the thermal stability of proteins in HM and

maintained their protein solubilities; ORAC and TEAC based antioxidant capacities following heating. This result clearly showed the increased thermostability of proteins when their MW was reduced by proteolytic depolymerization.

Further studies are needed to clarify the molecular changes in HPI by trypsin and pepsin treatment, but it is clear that the enzyme treatment is quite beneficial to increase protein solubility, TEAC and ORAC based antioxidant capacity and thermostability of HPI added into HM samples.

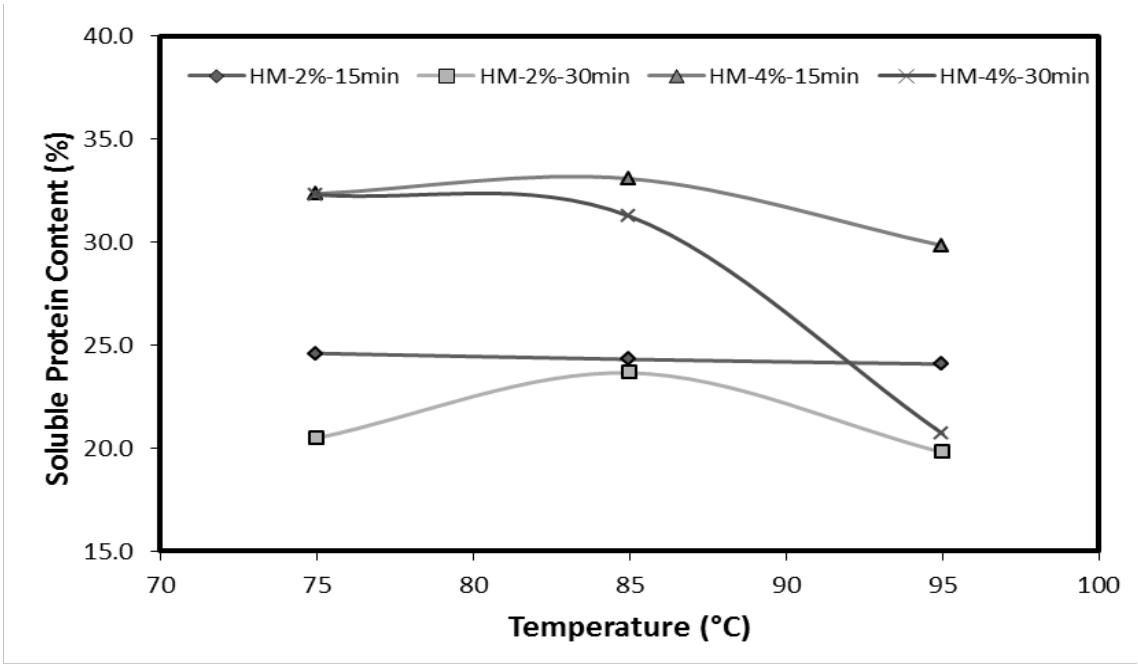

Figure 4.22. Soluble protein content of heat treated HM enriched with HPI.

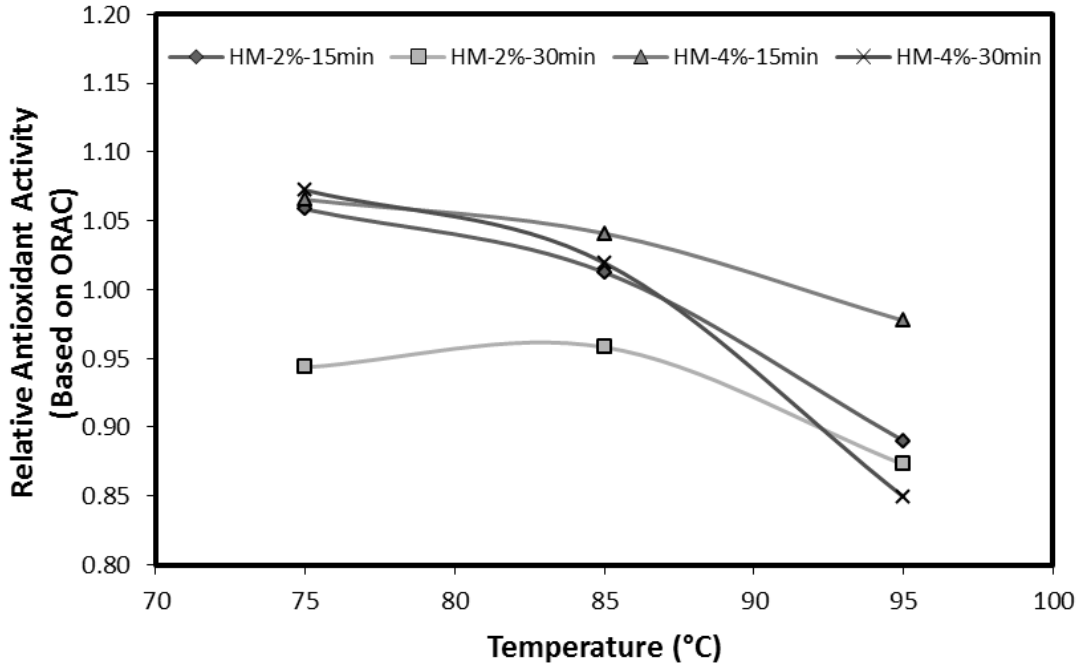

Figure 4.23. Relative antioxidant activity of heat treated HM enriched with HPI

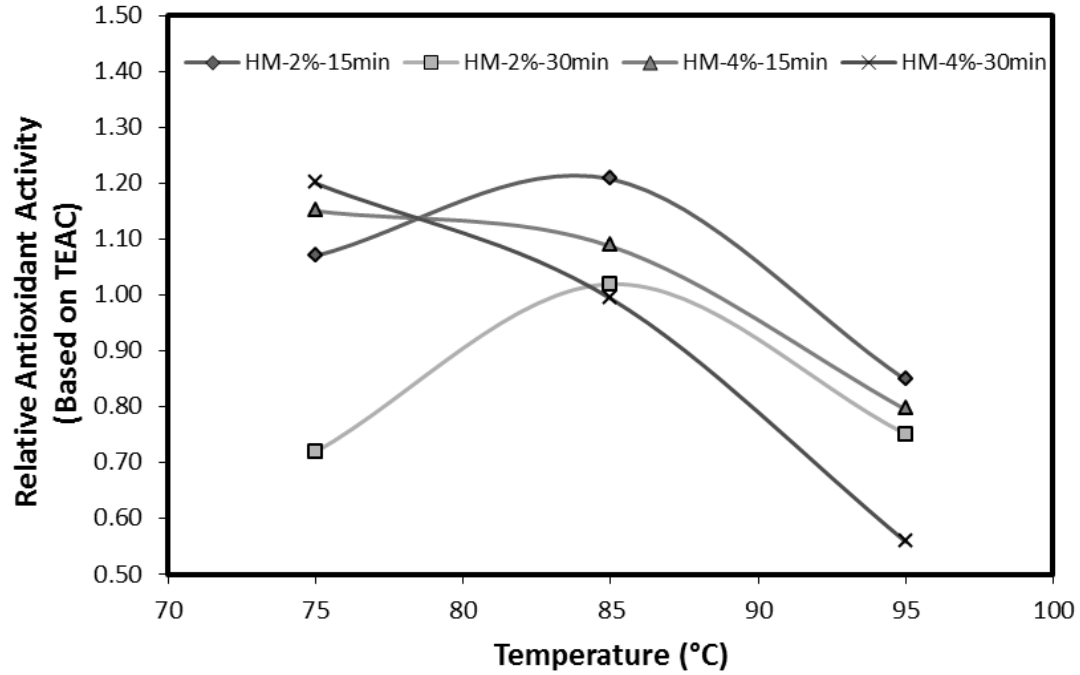

Figure 4.24. Relative antioxidant activity of heat treated HM enriched with HPI

Soluble protein content and relative antioxidant activity values of HM–2% and HM–4% samples enriched with pHPI were determined. HM–2% samples had lower but stable protein solubilities at different heat application temperatures whereas HM–4% samples lost their protein solubility at increased heat application temperature and time. In contrast to low protein solubility, heat application improved the antioxidant activity of HM–2% enriched with pHPI. They had 1.5 and 2 folds relative antioxidant activity based on ORAC and TEAC values, respectively. Heat application also slightly increased or decreased the antioxidant activities of HM–4% enriched with pHPI. During storage, enrichment with hydrolyzed proteins of HM helped for stability of antioxidant activity. In contrast to pepsin hydrolysis, heat application did not provide a considerable improvement to the relative antioxidant activity of HM samples enriched with tHPI although the similar protein solubility values were obtained with those of pepsin hydrolysed HM samples. HM-2% and HM–4% samples were highly stable against the temperature increment and they all generally preserved their antioxidant activity during heat application and storage. Only for HM–2%-15min and HM–2%-30min samples lost 1/3 of their relative antioxidant activity.

For HM samples enriched with enzyme hydrolyzed hazelnut proteins, protein solubilities and antioxidant activities were not effected by temperature or heating time. The main factor effecting the bioactivity was enriched protein concentration into the hazelnut milk. The results revealed that, the HM enriched with hydrolyzed hazelnut proteins is suitable for pasteurization in order to increase the shelf life stability of product by providing higher antioxidant activity.

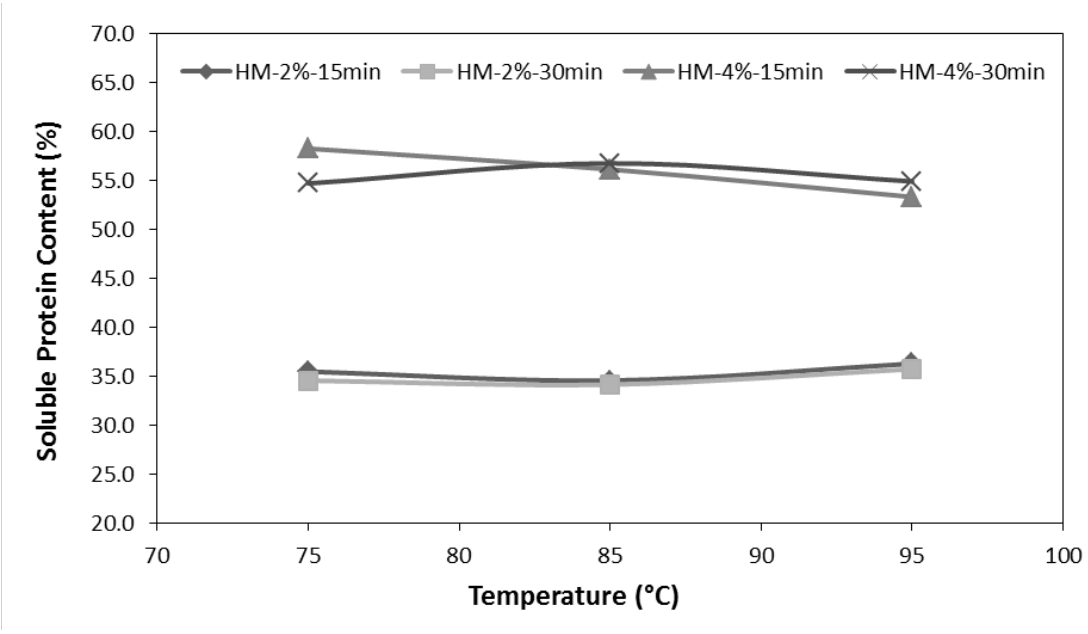

Figure 4.25. Soluble protein content of heat treated HM enriched with pHPI

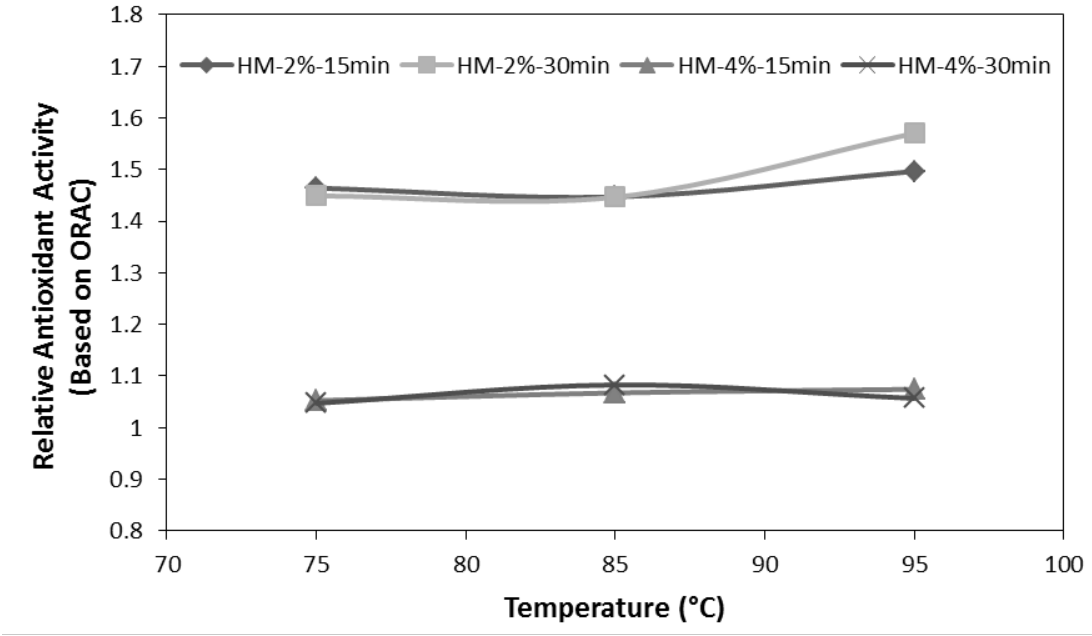

Figure 4.26. Relative antioxidant activity of heat treated HM enriched with pHPI

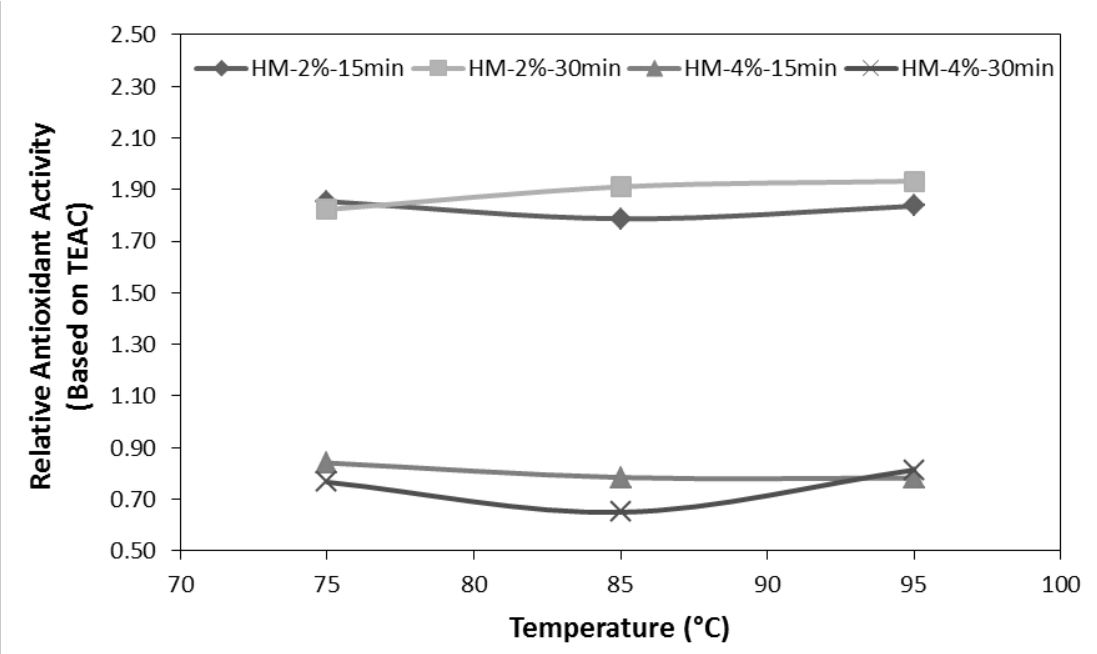

Figure 4.27. Relative antioxidant activity of heat treated HM enriched with pHPI

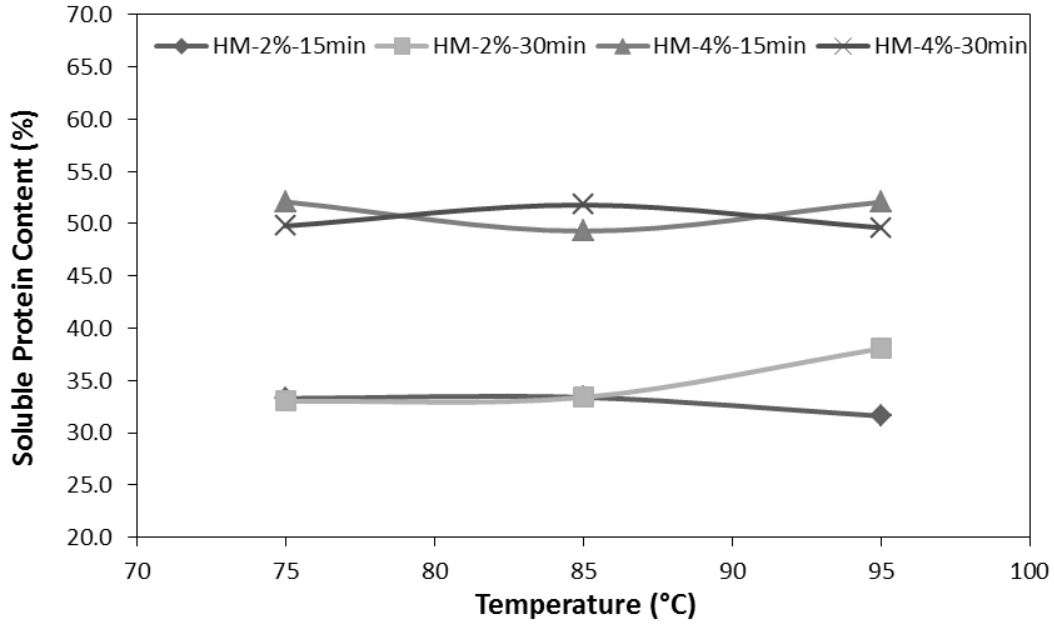

Figure 4.28. Soluble protein content of heat treated HM enriched with tHPI

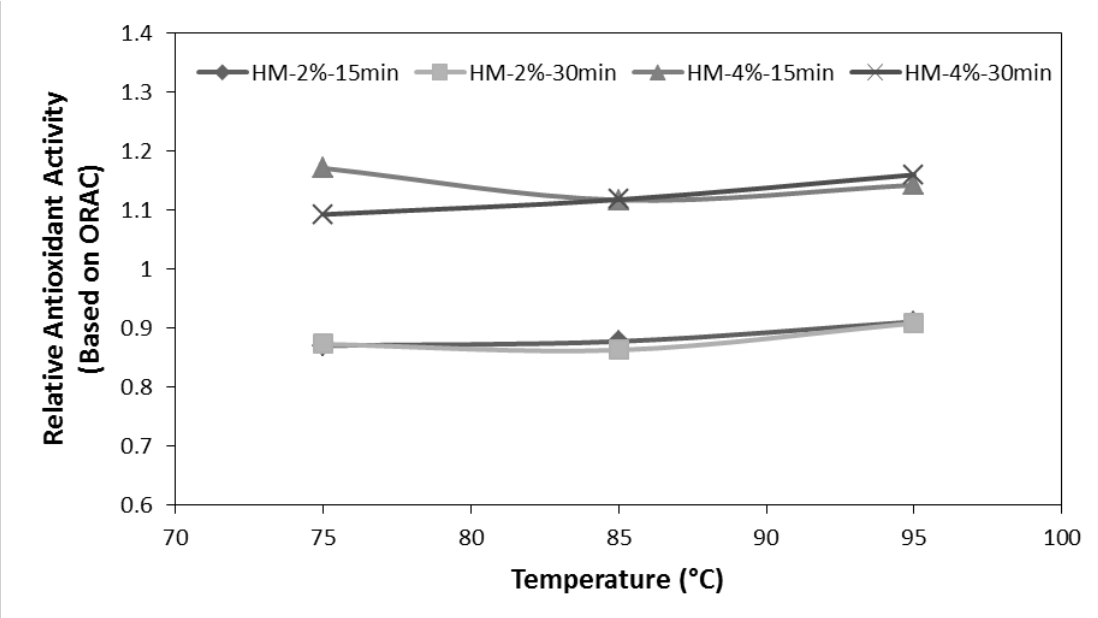

Figure 4.29. Relative antioxidant activity of heat treated HM enriched with tHPI

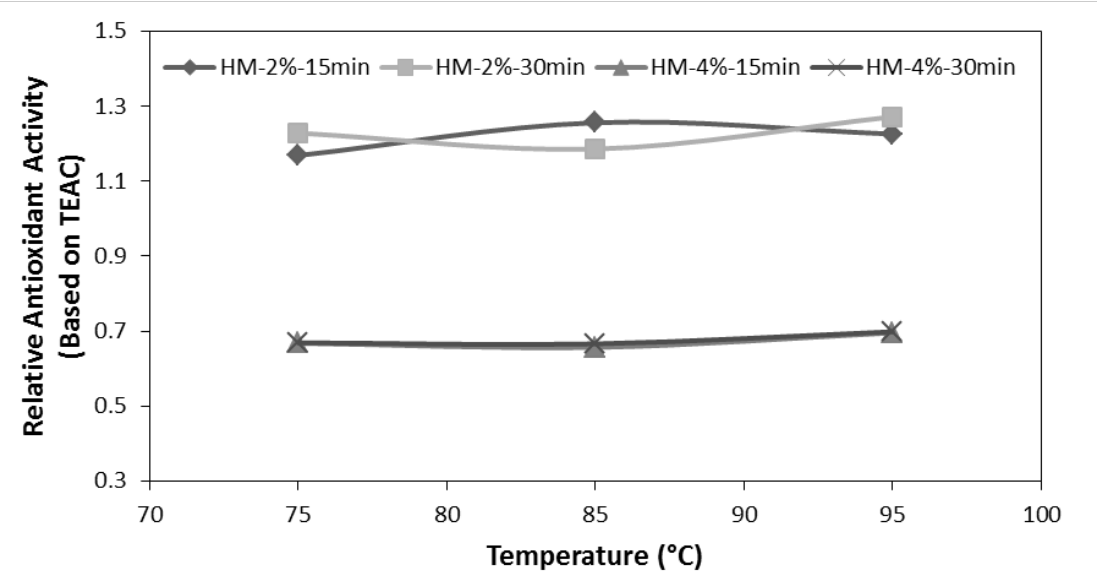

Figure 4.30. Relative antioxidant activity of heat treated HM enriched with tHPI

CHAPTER 5

CONCLUSIONS

The results of this study clearly showed the potential of chickpea and lentil proteins as commercial functional protein alternatives. Most of the functional properties of chickpea proteins are comparable with or superior than those of soy and animal origin proteins tested in this study. In fact, there was only gelling capacity of bovine gelatine which considerably surpassed a functional property of chickpea proteins. The most outstanding functional properties of studied legume proteins were extremely high oil and water absorption capacity for chickpea proteins and high oil absorption capacity and water solubility for lentil proteins. Both types of legume proteins were also capable to form highly stable emulsions and foams. In contrast, water absorption capacity and gel formation capacity are critical missing functional properties of lentil proteins. Thus, blending of lentil and chickpea proteins could be suggested when high protein solubility is the primary desired functionality, but some water absorption and gel formation capacity is also needed. The outstanding chickpea and lentil cultivars with superior protein functionality and cultivars with inferior protein functionality were identified. Further studies are needed to monitor and characterize the functional properties of protein from different chickpea and lentil cultivars obtained at different growing conditions, harvesting periods and climates and to determine magnitude of possible variations in protein functionality at standard extraction and assay conditions.

The antioxidant activity measurements conducted to understand the bioactive potential of pulse proteins showed that lentil protein extracts had better antioxidant potential than chickpea protein extracts. The specific antioxidant lentil proteins could be purified, concentrated or fractionated with different methods including ion exchange chromatography, isoelectric precipitation and ultrafiltration (cross-flow system). The antioxidant activity measurements of lentil albumin and globulin fractions separated by isoelectric precipitation showed that the lentil albumins show higher TEAC based free radical scavenging activity than the lentil globulins. In contrast, the lentil globulins had higher ORAC based free radical scavenging and iron chelating capacity than the lentil albumins. The lyophilisation could be applied to obtain antioxidant globulin fractions while antioxidant properties of albumins disappeared considerably following

lyophilisation. Ultrafiltration of proteins revealed that majority of lentil proteins and resulting total antioxidant activity based on TEAC, ORAC and ICC exist mainly above 30 kDa. However, the protein fractions with high specific antioxidant activity concentrate mainly below 30 kDa (between 10 and 30 kDa and below 10 kDa). The lyophilisation did not cause considerable changes in ORAC based antioxidant capacity of proteins having MW ≥30 kDa, and ICC of proteins between 10 and 30 kDa. On the other hand, the DEAE-cellulose chromatography gave fractions with low free radical scavenging based antioxidant capacity but high iron chelating capacity which was maintained following lyophilisation for at least one of the main fractions.

The results obtained for hazelnut meal proteins showed the importance of extraction pre-treatments (acetone washing and heating) on functional, bioactive and edible film forming properties of these proteins. The acetone washing of meal and heat application during protein extraction were quite beneficial to improve the antioxidant and antihypertensive activity based bioactive properties of hazelnut proteins. The acetone washing and hot extraction also improved the gel formation capacity and water solubility properties of hazelnut protein extracts, but it provided limited beneficial effects in improving their edible film making properties. The low water absorption capacity, gelation capacity and emulsion stability were the limitations of the technological capacity of isolated hazelnut meal proteins. However, good foaming properties and oil absorption capacities might still be exploited to obtain beneficial effects in many different food systems. The hazelnut meal proteins showed good film forming abilities and gave flexible, water soluble edible films depending on processing conditions. These films might be suitable for bioactive packaging which involves incorporating nutrients and bioactive compounds into film forming solution and applying it onto surface of suitable food by dipping, spraying or brushing. However, the film making should be conducted at mild heating conditions to avoid darkening of the films.

Due to their high bioactivity, the hazelnut protein extracts could be very suitable to develop novel functional foods and to contribute to human health. It is quite important to support the *in-vitro* bioactivity measurements in this study with more solid animal or human tests and to understand the real magnitudes of potential health benefits of hazelnut proteins. However, it might be quite interesting to enrich hazelnut cream and hazelnut containing confectionary with bioactive hazelnut proteins and develop novel hazelnut protein rich functional beverages such as hazelnut milk. As an example

novel functional food product, a bioactive beverage was produced by enriching hazelnut milk with enzymatically hydrolysed hazelnut globulin proteins. The enzymatic hydrolysis of globulins used for enrichment was conducted by trypsin and pepsin enzymes and this caused considerable increases in solubility, free radical based antioxidant capacity and thermostability of proteins added into hazelnut milk. This example study clearly showed the possibility of producing hazelnut protein enriched functional hazelnut milk and suitability of using pasteurization to increase shelf-life of this product.

REFERENCES

Aewsiri, T., Benjakul, S., Visessanguan, W., Eun, J. B., Wierenga, P. A., & Gruppen, H. (2009). Antioxidative activity and emulsifying properties of cuttlefish skin gelatin modified by oxidised phenolic compounds. *Food Chemistry, 117*(1), 160-168.

Ahmedna, M., Prinyawiwatkul, W., & Rao, R. M. (1999). Solubilized wheat protein isolate: Functional properties and potential food applications. *Journal of Agricultural and Food Chemistry, 47*(4), 1340-1345.

Alashi, A. M., Blanchard, C. L., Mailer, R. J., & Agboola, S. O. (2013). Technological and bioactive functionalities of canola meal proteins and hydrolysates. *Food Reviews International, 29*(3), 231-260.

Alvaro, A., Juan Jose, B., Maira, B.-R., Raquel, M. P., & Miguel Ángel, M.-G. (2006). Vegetable protein and fiber from cereal are inversely associated with the risk of hypertension in a Spanish cohort. *Archives of medical research, 37*(6), 778-786.

AOAC. (1998). Official Methods of Analysis. Washington, DC: Association of Official Analytical Chemists.

Arcan, I., & Yemenicioglu, A. (2007). Antioxidant activity of protein extracts from heat-treated or thermally processed chickpeas and white beans. *Food Chemistry, 103*(2), 301-312.

Arcan, I., & Yemenicioglu, A. (2010). Effects of controlled pepsin hydrolysis on antioxidant potential and fractional changes of chickpea proteins. *Food Research International, 43*(1), 140-147.

Armstrong, F. B. (1989). *Biochemistry*. New York: Oxford University Press.

Asgar, M. A., Fazilah, A., Huda, N., Bhat, R., & Karim, A. A. (2010). Nonmeat protein alternatives as meat extenders and meat analogs. *Comprehensive Reviews in Food Science and Food Safety, 9*(5), 513-529.

ASTM. (2002). Standard test method for tensile properties of thin plastic sheeting-D882-02. In ASTM (Ed.), *Annual Book of American Standard Testing Methods*. Philadelphia, PA.

Aydemir, L. Y., & Yemenicioglu, A. (2013). Potential of Turkish Kabuli type chickpea and green and red lentil cultivars as source of soy and animal origin functional protein alternatives. *Lwt-Food Science and Technology, 50*(2), 686-694.

Bamdad, F., Goli, A. H., & Kadivar, M. (2006). Preparation and characterization of proteinous film from lentil (Lens culinaris): Edible film from lentil (Lens culinaris). *Food Research International, 39*(1), 106-111.

Bedani, R., Rossi, E. A., & Isay Saad, S. M. (2013). Impact of inulin and okara on Lactobacillus acidophilus La-5 and Bifidobacterium animalis Bb-12 viability in a fermented soy product and probiotic survival under in vitro simulated gastrointestinal conditions. *Food Microbiology, 34*(2), 382-389.

Belitz, H. D., W, G., & Schieberle, P. (2004). *Food Chemistry*. New York: Springer.

Berg, J. M., Tymoczko, J. L., Stryer, L., & Clarke, N. D. (2002). *Biochemistry*. New York: W. H. Freeman Company.

Blum, H., Beier, H., & Gross, H. J. (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis, 8*(2), 93-99.

Bora, P. S. (2002). Functional properties of native and succinylated lentil (*Lens culinaris*) globulins. *Food Chemistry, 77*(2), 171-176.

Boye, J., Zare, F., & Pletch, A. (2010a). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414-431.

Boye, J. I., Aksay, S., Roufik, S., Ribereau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010b). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International, 43*(2), 537-546.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1–2), 248-254.

Brandts, J. F., & Hunt, L. (1967). Thermodynamics of protein denaturation. III. Denaturation of ribonuclease in water and in aqueous urea and aqueous ethanol mixtures. *Journal of the American Chemical Society, 89*(19), 4826-4838.

Burova, T. V., Grinberg, N. V., Golubeva, I. A., Mashkevich, A. Y., Grinberg, V. Y., & Tolstoguzov, V. B. (1999). Flavour release in model bovine serum albumin/pectin/2-octanone systems. *Food Hydrocolloids, 13*(1), 7-14.

Chee, K. L., Ling, H. K., & Ayob, M. K. (2012). Optimization of trypsin-assisted extraction, physico-chemical characterization, nutritional qualities and functionalities of palm kernel cake protein. *LWT - Food Science and Technology, 46*(2), 419-427.

Chen, H.-M., Muramoto, K., Yamauchi, F., & Nokihara, K. (1996). Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *Journal of Agricultural and Food Chemistry, 44*(9), 2619-2623.

Cruz, N. S., Capellas, M., Jaramillo, D. P., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009). Soymilk treated by ultra high-pressure homogenization: Acid coagulation properties and characteristics of a soy-yogurt product. *Food Hydrocolloids, 23*(2), 490-496.

Damodaran, S. (1997). Food Proteins: An Overview. In S. Damodaran & A. Paraf (Eds.), *Food Proteins and Their Applications* (pp. 1-24). New York: Marcel Dekker.

Damodaran, S. (Ed.). (1996). *Amino acids, peptides and proteins* (4 ed.). New York: Marcel Dekker.

Davidek, J., Velisek, J., & Pokorny, J. (1990). *Chemical changing during food processing*. Czechoslovakia: Czechoslovak Medical Press.

Demiryürek, K., Ceyhan, V., & Bozoğlu, M. (2012). Risk attitudes of organic and conventional hazelnut producers in Turkey. *Human and Ecological Risk Assessment: An International Journal, 18*(2), 471-482.

Diane, C. M., Frank, R. L., Terryl, J. H., & Julianne, M. C. (2009). Consumption of dry beans, peas, and lentils could improve diet quality in the US population. *Journal of the American Dietetic Association, 109*(5), 909-913.

DiSilvestro, R. A., Mattern, C., Wood, N., & Devor, S. T. (2006). Soy protein intake by active young adult men raises plasma antioxidant capacity without altering plasma testosterone. *Nutrition Research, 26*(2), 92-95.

Doğan, G., & Erdem, M. (2010). Effects of hazelnut meal levels on growth performance, feed utilization and digestibility in juveline rainbow trout (*Oncorhynchus mykiss). Turkish Journal of Fisheries and Aquatic Sciences, 10*, 181-186.

Dormont, D. (2002). Prion diseases: pathogenesis and public health concerns. *FEBS Letters, 529*(1), 17-21.

Dunn, M. J. (1989). Electrophoretic analysis methods. In E. L. V. Haris & S. Angal (Eds.), *Protein purification methods: A practical approach* (pp. 18-40). New York: Oxford University Pres Inc.

El-Aal, M. H. A., Hamza, M. A., & Rahma, E. H. (1986). In vitro digestibility, physico-chemical and functional properties of apricot kernel proteins. *Food Chemistry, 19*(3), 197-211.

Elias, R. J., McClements, D. J., & Decker, E. A. (2005). Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase β-lactoglobulin in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 53*(26), 10248-10253.

Emre, Y., Sevgili, H., & Şanlı, M. (2008). A preliminary study on the utilization of hazelnut meal as a substitute for fish meal in diets of European sea bass (*Dicentrarchus labrax* L.). *Aquaculture Research, 39*(3), 324-328.

Erener, G., Ak, F. B., & Ocak, N. (2009). A study on feeding hazelnut kernel oil meal as a protein source for broiler chickens. *Animal Science Journal, 80*(3), 305-309.

Friedman, M. (1996). Nutritional value of proteins from different food sources. A review. *Journal of Agricultural and Food Chemistry, 44*(1), 6-29.

Fuentes, E., & Palomo, I. (2014). Antiplatelet effects of natural bioactive compounds by multiple targets: Food and drug interactions. *Journal of Functional Foods, 6*(0), 73-81.

Giec, A., Stasinska, B., & Skupin, J. (1989). A protein isolate for food by phosphorylation of yeast homogenate. *Food Chemistry, 31*(4), 279-288.

Goulet, G., Ponnampalam, R., Amiot, J., Roy, A., & Brisson, G. J. (1987). Nutritional value of acylated oat protein concentrates. *Journal of Agricultural and Food Chemistry, 35*(4), 589-592.

Guang, C., & Phillips, R. D. (2009). Plant food-derived angiotensin I converting enzyme inhibitory peptides. *Journal of Agricultural and Food Chemistry, 57*(12), 5113-5120.

Han, H., & Baik, B. K. (2008). Antioxidant activity and phenolic content of lentils (Lens culinaris), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. *International Journal of Food Science and Technology, 43*(11), 1971-1978.

Hernández-Infante, M., Sousa, V., Montalvo, I., & Tena, E. (1998). Impact of microwave heating on hemagglutinins, trypsin inhibitors and protein quality of selected legume seeds. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 52*(3), 199-208.

Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., & Chi, L. (2008). The antihypertensive effect of peptides: A novel alternative to drugs? *Peptides, 29*(6), 1062-1071.

Horax, R., Hettiarachchy, N. S., Chen, P., & Jalaluddin, M. (2004). Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). *Journal of Food Science, 69*(2), 114-118.

Howell, N. K. (1996). Chemical and Enzymatic Modification. In S. Nakai & H. W. Modler (Eds.), *Food Proteins Properties and Characterization*. New York: Wiley-WHC.

Hu, M., McClements, D. J., & Decker, E. A. (2003). Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. *Journal of Agricultural and Food Chemistry, 51*(6), 1696-1700.

ISO. (2000). Oilseeds-Determination of moisture and volatile matter content: ISO 665:2000(E).

Jamdar, S. N., Rajalakshmi, V., Pednekar, M. D., Juan, F., Yardi, V., & Sharma, A. (2010). Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. *Food Chemistry, 121*(1), 178-184.

Jang, S. A., Lim, G. O., & Bin Song, K. (2011). Preparation and mechanical properties of edible rapeseed protein films. *Journal of Food Science, 76*(2), 218-223.

Je, J. Y., Park, P. J., & Kim, S. K. (2004). Radical scavenging activity of hetero-chitooligosaccharides. *European Food Research and Technology, 219*(1), 60-65.

Jenkins, D. J., Kendall, C. W., Jackson, C.-J. C., Connelly, P. W., Parker, T., Faulkner, D., Vidgen, E., Cunnane, S. C., Leiter, L. A., & Josse, R. G. (2002). Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am J Clin Nutr, 76*(2), 365-372.

Karim, A. A., & Bhat, R. (2009). Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids, 23*(3), 563-576.

Kaur, M., & Singh, N. (2007). Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry, 102*(1), 366-374.

Krause, J.-P., Schultz, M., & Dudek, S. (2002). Effect of extraction conditions on composition, surface activity and rheological properties of protein isolates from flaxseed (*Linum usitativissimum* L). *Journal of the Science of Food and Agriculture, 82*(9), 970-976.

Lee, H. C., Htoon, A. K., Uthayakumaran, S., & Paterson, J. L. (2007). Chemical and functional quality of protein isolated from alkaline extraction of Australian lentil cultivars: Matilda and Digger. *Food Chemistry, 102*(4), 1199-1207.

Li, G.-H., Le, G.-W., Shi, Y.-H., & Shrestha, S. (2004). Angiotensin I–converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutrition Research, 24*(7), 469-486.

Liu, Z. S., & Chang, S. K. C. (2013). Nutritional profile and physicochemical properties of commercial soymilk. *Journal of Food Processing and Preservation, 37*(5), 651-661.

Lo, W. M. Y., & Li-Chan, E. C. Y. (2005). Angiotensin I converting enzyme inhibitory peptides from in vitro pepsin-pancreatin digestion of soy protein. *Journal of Agricultural and Food Chemistry, 53*(9), 3369-3376.

Lomascolo, A., Uzan-Boukhris, E., Sigoillot, J. C., & Fine, F. (2012). Rapeseed and sunflower meal: a review on biotechnology status and challenges. *Applied Microbiology and Biotechnology, 95*(5), 1105-1114.

Lombardi-Boccia, G., Ruggeri, S., Aguzzi, A., & Cappelloni, M. (2003). Globulins enhance in vitro iron but not zinc dialysability: a study on six legume species. *Journal of Trace Elements in Medicine and Biology, 17*(1), 1-5.

Lorenzo, G., Zaritzky, N., & Califano, A. (2008). Optimization of non-fermented gluten-free dough composition based on rheological behavior for industrial production of "empanadas" and pie-crusts. *Journal of Cereal Science, 48*(1), 224-231.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry, 193*, 265-275.

Ludescher, R. D. (1996). Physical and Chemical Properties of Amino Acids and Proteins. In S. Nakai & H. W. Modler (Eds.), *Food Proteins Properties and Characterization* (pp. 23-70). the USA: Wiley-VCH.

Madhavi, D. L., Deshpande, S. S., & Salunkhe, D. K. (1996). *Food Antioxidants*. New York: Marcel Dekker, Inc.

Makri, E., Papalamprou, E., & Doxastakis, G. (2005). Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea, broad bean) in admixture with polysaccharides. *Food Hydrocolloids, 19*(3), 583-594.

Manamperi, W. A. R., Wiesenborn, D. P., Chang, S. K. C., & Pryor, S. W. (2011). Effects of protein separation conditions on the functional and thermal properties of canola protein isolates. *Journal of Food Science, 76*(3), 266-273.

Marambe, P. W. M. L. H. K., Shand, P. J., & Wanasundara, J. P. D. (2008). An in-vitro investigation of selected biological activities of hydrolysed flaxseed (*Linum usitatissimum* L.) proteins. *Journal of the American Oil Chemists' Society, 85*(12), 1155-1164.

Martinez-Maqueda, D., Miralles, B., Recio, I., & Hernandez-Ledesma, B. (2012). Antihypertensive peptides from food proteins: a review. *Food & Function, 3*(4), 350-361.

Matheis, G., Penner, M. H., Feeney, R. E., & Whitaker, J. R. (1983). Phosphorylation of casein and lysozyme by phosphorus oxychloride. *Journal of Agricultural and Food Chemistry, 31*(2), 379-387.

Matheis, G., & Whitaker, J. R. (1984). Chemical phosphorylation of food proteins: an overview and a prospectus. *Journal of Agricultural and Food Chemistry, 32*(4), 699-705.

Mathews, C. K., & Van Holde, K. E. (1996). *Biochemistry*. New York: The Benjamin/Cummings Publishing Company, Inc.

Mattos, C. (2002). Protein–water interactions in a dynamic world. *Trends in Biochemical Sciences, 27*(4), 203-208.

McClements, D. J. (2002). Modulation of globular protein functionality by weakly interacting cosolvents. *Critical Reviews in Food Science and Nutrition, 42*(5), 417-471.

Megías, C., Pedroche, J., Yust, M. M., Girón-Calle, J., Alaiz, M., Millán, F., & Vioque, J. (2008). Production of copper-chelating peptides after hydrolysis of sunflower proteins with pepsin and pancreatin. *LWT - Food Science and Technology, 41*(10), 1973-1977.

Mohri, M., & Matsushita, S. (1984). Improvement of water absorption of soybean protein by treatment with bromelain. *Journal of Agricultural and Food Chemistry, 32*(3), 486-490.

Möller, N., Scholz-Ahrens, K., Roos, N., & Schrezenmeir, J. (2008). Bioactive peptides and proteins from foods: indication for health effects. *European Journal of Nutrition, 47*(4), 171-182.

Morales-de León, J. C., Vázquez-Mata, N., Torres, N., Gil-Zenteno, L., & Bressani, R. (2007). Preparation and characterization of protein isolate from fresh and hardened beans (*Phaseolus vulgaris* L.). *Journal of Food Science, 72*(2), 96-102.

Moran, L. A., Horton, H. R., Scrimgeour, K. G., & Perry, M. D. (2012). *Principles of Biochemistry*. the USA: Pearson Education, Inc.

Moure, A., Sineiro, J., Domínguez, H., & Parajó, J. C. (2006). Functionality of oilseed protein products: A review. *Food Research International, 39*(9), 945-963.

Nakai, S. (1996). An overview. In S. Nakai & H. W. Modler (Eds.), *Food Proteins Properties and Characterization* (pp. 1-22). the USA: Wiley-VCH.

Nakamura, S., Kato, A., & Kobayashi, K. (1991). New antimicrobial characteristics of lysozyme-dextran conjugate. *Journal of Agricultural and Food Chemistry, 39*(4), 647-650.

Nakamura, S., Kato, A., & Kobayashi, K. (1992). Bifunctional lysozyme-galactomannan conjugate having excellent emulsifying properties and bactericidal effect. *Journal of Agricultural and Food Chemistry, 40*(5), 735-739.

Narayana, K., & Rao, M. S. N. (1991). Effect of acetylation and succinylation on the physicochemical properties of winged bean (Psophocarpus tetragonolobus) proteins. *Journal of Agricultural and Food Chemistry, 39*(2), 259-261.

Neto, V. Q., Narain, N., Silva, J. B., & Bora, P. S. (2001). Functional properties of raw and heat processed cashew nut (*Anacardium occidentale*, L.) kernel protein isolates. *Food / Nahrung, 45*(4), 258-262.

Ng, K. L., Ayob, M. K., Said, M., Osman, M. A., & Ismail, A. (2013). Optimization of enzymatic hydrolysis of palm kernel cake protein (PKCP) for producing hydrolysates with antiradical capacity. *Industrial Crops and Products, 43*, 725-731.

Ngo, D.-H., Vo, T.-S., Ngo, D.-N., Wijesekara, I., & Kim, S.-K. (2012). Biological activities and potential health benefits of bioactive peptides derived from marine organisms. *International Journal of Biological Macromolecules, 51*(4), 378-383.

Nik, A. M., Tosh, S., Poysa, V., Woodrow, L., & Corredig, M. (2008). Physicochemical characterization of soymilk after step-wise centrifugation. *Food Research International, 41*(3), 286-294.

Nilufer-Erdil, D., Serventi, L., Boyacioglu, D., & Vodovotz, Y. (2012). Effect of soy milk powder addition on staling of soy bread. *Food Chemistry, 131*(4), 1132-1139.

Oakenfull, D., Pearce, J., & Burley, R. W. (1997). Protein Gelation. In S. Damodaran & A. Paraf (Eds.), *Food Proteins and Their Applications* (pp. 111-142). the USA: Mercel Decker, Inc.

Ogunwolu, S. O., Henshaw, F. O., Mock, H.-P., Santros, A., & Awonorin, S. O. (2009). Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut. *Food Chemistry, 115*(3), 852-858.

Okada, Y., & Okada, M. (1998). Scavenging effect of water soluble proteins in broad beans on free radicals and active oxygen species. *Journal of Agricultural and Food Chemistry, 46*(2), 401-406.

Orliac, O., Rouilly, A., Silvestre, F., & Rigal, L. (2002). Effects of additives on the mechanical properties, hydrophobicity and water uptake of thermo-moulded films produced from sunflower protein isolate. *Polymer, 43*(20), 5417-5425.

Osthoff, G., Hugo, A., van Wyk, P., de Wit, M., & Meyer, S. (2010). Characterization of a spray-dried soymilk powder and changes observed during storage. *Food Science and Technology International, 16*(2), 169-178.

Papalamprou, E. M., Doxastakis, G. I., Biliaderis, C. G., & Kiosseoglou, V. (2009). Influence of preparation methods on physicochemical and gelation properties of chickpea protein isolates. *Food Hydrocolloids, 23*(2), 337-343.

Park, K. B., & Oh, S. H. (2005). Production and characterization of GABA rice yogurt. *Food Science and Biotechnology, 14*(4), 518-522.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins - evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*(3), 716-723.

Peña, E., Bernardo, A., Soler, C., & Jouve, N. (2006). Do tyrosine crosslinks contribute to the formation of the gluten network in common wheat (Triticum aestivum L.) dough? *Journal of Cereal Science, 44*(2), 144-153.

Peressini, D., Peighambardoust, S. H., Hamer, R. J., Sensidoni, A., & van der Goot, A. J. (2008). Effect of shear rate on microstructure and rheological properties of sheared wheat doughs. *Journal of Cereal Science, 48*(2), 426-438.

Pinto, M. d. S., Lajolo, F. M., & Genovese, M. I. (2005). Effect of storage temperature and water activity on the content and profile of isoflavones, antioxidant activity, and in vitro protein digestibility of soy protein isolates and defatted soy flours. *Journal of Agricultural and Food Chemistry, 53*(16), 6340-6346.

Ponnampalam, R., Goulet, G., Amiot, J., & Brisson, G. J. (1987). Some functional and nutritional properties of oat flours as affected by proteolysis. *Journal of Agricultural and Food Chemistry, 35*(2), 279-285.

Rajapakse, N., Mendis, E., Jung, W.-K., Je, J.-Y., & Kim, S.-K. (2005). Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. *Food Research International, 38*(2), 175-182.

Ramos, C. M. P., & Bora, P. S. (2005). Functionality of succinylated Brazil nut (*Bertholletia excelsa HBK*) kernel globulin. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum), 60*(1), 1-6.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Reddy, N., Jiang, Q., & Yang, Y. (2012). Preparation and properties of peanut protein films crosslinked with citric acid. *Industrial Crops and Products, 39*(0), 26-30.

Roe, S. (2001). *Protein Purification Applications: A Practical Approach*: Oxford University Press.

Roy, F., Boye, J. I., & Simpson, B. K. (2010). Bioactive proteins and peptides in pulse crops: Pea, chickpea and lentil. *Food Research International, 43*(2), 432-442.

Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F., & Stanton, C. (2011). Bioactive peptides from muscle sources: Meat and fish. *Nutrients, 3*(9), 765-791.

Saiga, A., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry, 51*(12), 3661-3667.

Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S., & Mauri, A. N. (2010). Biodegradable sunflower protein films naturally activated with antioxidant compounds. *Food Hydrocolloids, 24*(5), 525-533.

Salgado, P. R., Ortiz, S. E. M., Petruccelli, S., & Mauri, A. N. (2012). Functional food ingredients based on sunflower protein concentrates naturally enriched with antioxidant phenolic compounds. *Journal of the American Oil Chemists Society, 89*(5), 825-836.

Sánchez-Vioque, R., Clemente, A., Vioque, J., Bautista, J., & Millán, F. (1999). Protein isolates from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization. *Food Chemistry, 64*(2), 237-243.

Sathe, S. K., Venkatachalam, M., Sharma, G. M., Kshirsagar, H. H., Teuber, S. S., & Roux, K. H. (2009). Solubilization and electrophoretic characterization of select edible nut seed proteins. *Journal of Agricultural and Food Chemistry, 57*(17), 7846-7856.

Schwanke, K. D. (1997). Enzyme and Chemical Modification of Proteins. In S. Damodaran & A. Paraf (Eds.), *Food Proteins and Their Applications*. New York: Marcel Dekker, Inc.

Shalaby, S. M., Zakora, M., & Otte, J. (2006). Performance of two commonly used angiotensin-converting enzyme inhibition assays using FA-PGG and HHL as substrates. *Journal of Dairy Research, 73*(2), 178-186.

Sharma, G. M., Su, M., Joshi, A. U., Roux, K. H., & Sathe, S. K. (2010). Functional properties of select edible oilseed proteins. *Journal of Agricultural and Food Chemistry, 58*(9), 5457-5464.

Sikorski, Z. E. (2006). *Chemical and Functional Properties of Food Components*. Florida: CRC Press.

Singh, B. P., Vij, S., & Hati, S. (2014). Functional significance of bioactive peptides derived from soybean. *Peptides, 54*(0), 171-179.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144-158.

Sitohy, M., Chobert, J.-M., & Haertle, T. (2000). Study of factors influencing protein esterification using β-lactoglobulin as a model. *Journal of Food Biochemistry, 24*(5), 381-398.

Siu, M., & Thompson, L. U. (1982). In vitro and in vivo digestibilities of succinylated cheese whey protein concentrates. *Journal of Agricultural and Food Chemistry, 30*(4), 743-747.

Sivanandan, L., Toledo, R. T., & Singh, R. K. (2008). Effect of continuous flow high-pressure throttling on rheological and ultrastructural properties of soymilk. *Journal of Food Science, 73*(6), 288-296.

Sogi, D. S., Bhatia, R., Garg, S. K., & Bawa, A. S. (2005). Biological evaluation of tomato waste seed meals and protein concentrate. *Food Chemistry, 89*(1), 53-56.

Sun-Waterhouse, D., Zhao, M., & Waterhouse, G. N. (2014). Protein modification during ingredient preparation and food processing: Approaches to improve food processability and nutrition. *Food and Bioprocess Technology, 7*(7), 1853-1893.

Swamylingappa, B., & Srinivas, H. (1994). Preparation and properties of protein isolate from hexane-acetic acid treated commercial soybean meal. *Journal of Agricultural and Food Chemistry, 42*(12), 2907-2911.

Sze-Tao, K. W. C., & Sathe, S. K. (2000). Walnuts (*Juglans regia* L): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. *Journal of the Science of Food and Agriculture, 80*(9), 1393-1401.

Tavano, O. L. (2013). Protein hydrolysis using proteases: An important tool for food biotechnology. *Journal of Molecular Catalysis B: Enzymatic, 90*(0), 1-11.

Telang, A. M., & Thorat, B. N. (2010). Optimization of process parameters for spray drying of fermented soy milk. *Drying Technology, 28*(12), 1445-1456.

Thakur, M., & Hurburgh, C. (2007). Quality of US soybean meal compared to the quality of soybean meal from other origins. *Journal of the American Oil Chemists' Society, 84*(9), 835-843.

Tsai, P. J., & She, C. H. (2006). Significance of phenol-protein interactions in modifying the antioxidant capacity of peas. *Journal of Agricultural and Food Chemistry, 54*(22), 8491-8494.

Valencia-Flores, D. C., Hernandez-Herrero, M., Guamis, B., & Ferragut, V. (2013). Comparing the effects of ultra-high-pressure homogenization and conventional thermal treatments on the microbiological, physical, and chemical quality of almond beverages. *Journal of Food Science*, *78*(2), 199-205.

Vaštag, Ž., Popović, L., Popović, S., Krimer, V., & Peričin, D. (2011). Production of enzymatic hydrolysates with antioxidant and angiotensin-I converting enzyme inhibitory activity from pumpkin oil cake protein isolate. *Food Chemistry, 124*(4), 1316-1321.

Vermeirssen, V., Van Camp, J., & Verstraete, W. (2002). Optimisation and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. *Journal of Biochemical and Biophysical Methods, 51*(1), 75-87.

Voet, D., Voet, J. G., & Pratt, C. W. (1999). *Fundamentals of Biochemistry*. New York: John Wiley & Sons, Inc.

Walsh, G. (2002). *Proteins Biochemistry and Biochemistry*. New York: Wiley.

Wolosiak, R., & Klepacka, M. (2002). Antioxidative properties of albumins in enzymatically catalyzed model systems. *Electronic Journal of Polish Agricultural Universities. Series Food Science and Technology, 5*(1).

Wu, C. H., Nakai, S., & Powrie, W. D. (1976). Preparation and properties of acid-solubilized gluten. *Journal of Agricultural and Food Chemistry, 24*(3), 504-510.

Wu, J. P., Aluko, R. E., & Muir, A. D. (2009). Production of angiotensin I-converting enzyme inhibitory peptides from defatted canola meal. *Bioresource Technology, 100*(21), 5283-5287.

Xu, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science, 72*(2), 159-166.

Xu, Y. X., & Hanna, M. A. (2011). Nutritional and anti-nutritional compositions of defatted Nebraska hybrid hazelnut meal. *International Journal of Food Science and Technology, 46*(10), 2022-2029.

Yalcin, H., Toker, O. S., & Dogan, M. (2012). Effect of oil type and fatty acid composition on dynamic and steady shear rheology of vegetable oils. *Journal of Oleo Science, 61*(4), 181-187.

Yoshie-Stark, Y., Wada, Y., Schott, M., & Wäsche, A. (2006). Functional and bioactive properties of rapeseed protein concentrates and sensory analysis of food application with rapeseed protein concentrates. *LWT - Food Science and Technology, 39*(5), 503-512.

Zayas, J. F. (1997). *Functionality of Proteins in Food*. Berlin: springer.

Zhang, L., Li, J. R., & Zhou, K. Q. (2010). Chelating and radical scavenging activities of soy protein hydrolysates prepared from microbial proteases and their effect on meat lipid peroxidation. *Bioresource Technology, 101*(7), 2084-2089.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555-559.

APPENDIX A

BSA STANDARD CURVE

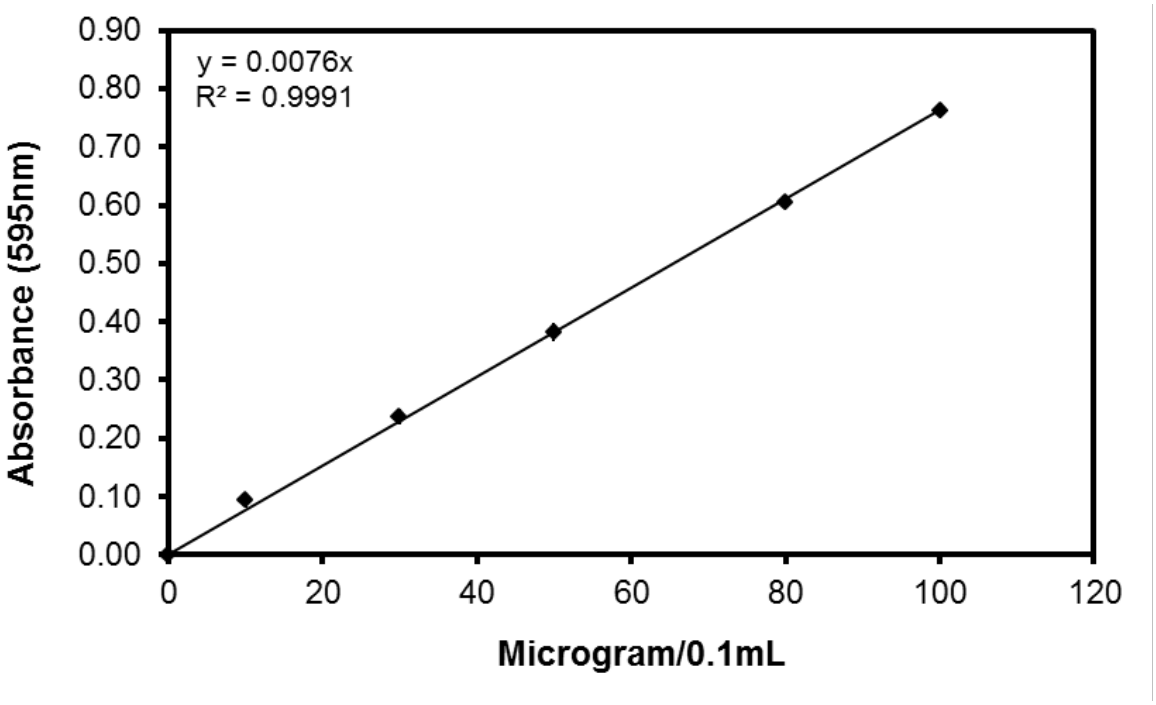

Figure A.1. Bovine serum albumin standard curve for Bradford method

APPENDIX B

ANTIOXIDANT ACTIVITIES OF CHICKPEA AND LENTIL GLOBULIN EXTRACTS

Table B.1. Antioxidant activities of chickpea and lentil globulin extracts based on TEAC and ORAC

Protein Extracts	TEAC (μmol Trolox/g)	ORAC (μmol Trolox/g)
Globulin extracts from chickpea cultivars		
CGE-1	72.31±0.0.2	33.60±3.30
CGE-2	70.83±0.03	37.41±3.82
CGE-3	67.84±0.02	39.77±3.43
CGE-4	68.10±0.01	36.89±5.77
Average of CGEs	69.77±2.2	36.9±2.5
Globulin extracts from lentil cultivars		
LGE-1	132.64±0.03	90.08±8.60
LGE-2	131.06±0.04	71.52±18.17
LGE-3	161.67±0.05	92.40±10.91
LGE-4	112.76±0.04	63.45±0.66
LGE-5	121.94±0.07	76.57±8.34
LGE-6	126.96±0.26	48.51±3.76
Average of LGEs	131.17±16.6	73.8±16.6

APPENDIX C

GALLIC ACID STANDARD CURVE

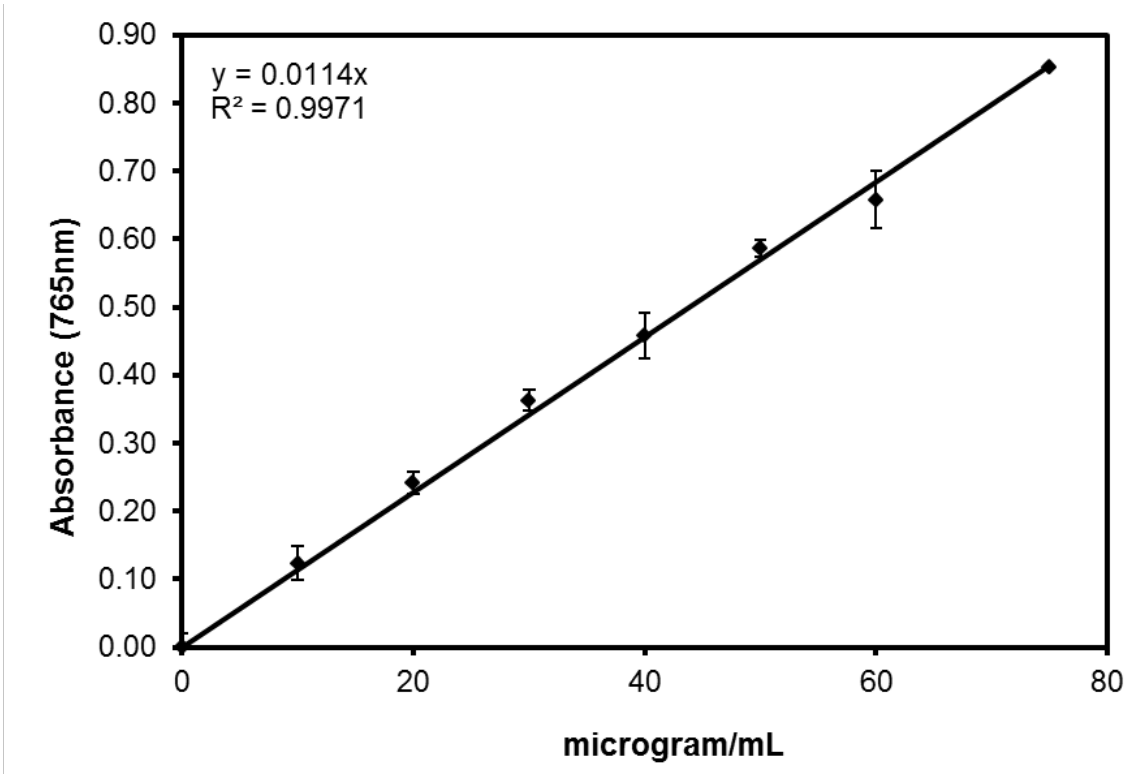

Figure C.1. Gallic acid standard curve for determination of TPC

APPENDIX D

EPICATECHIN STANDARD CURVE

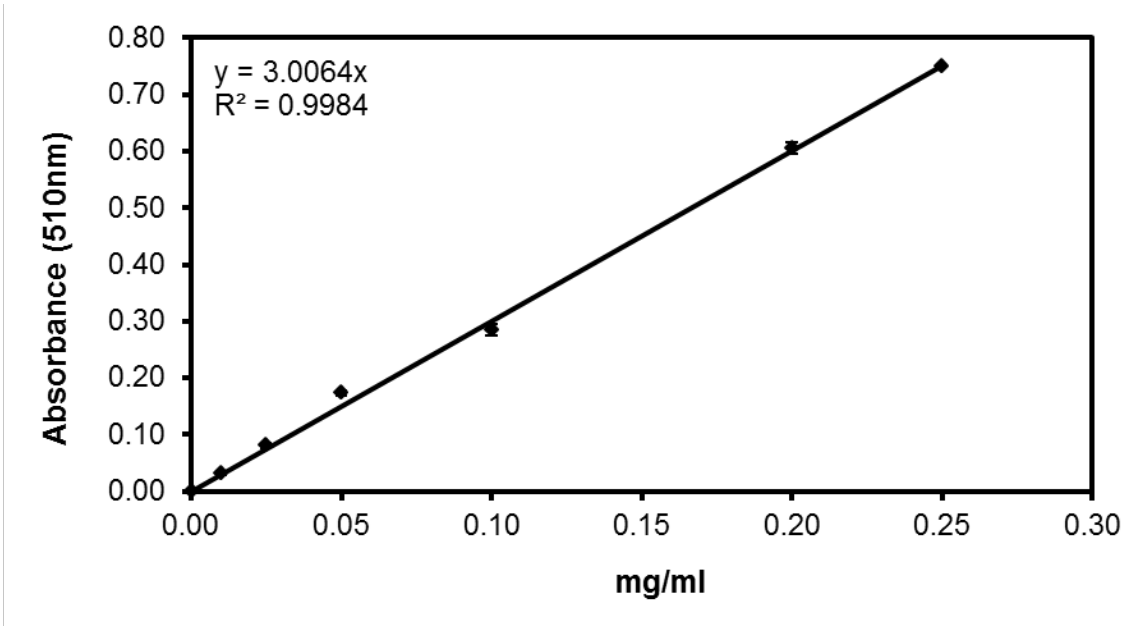

Figure D.1. Epicatechin standard curve for determination of TFC

APPENDIX E

TROLOX STANDARD FOR TEAC METHOD

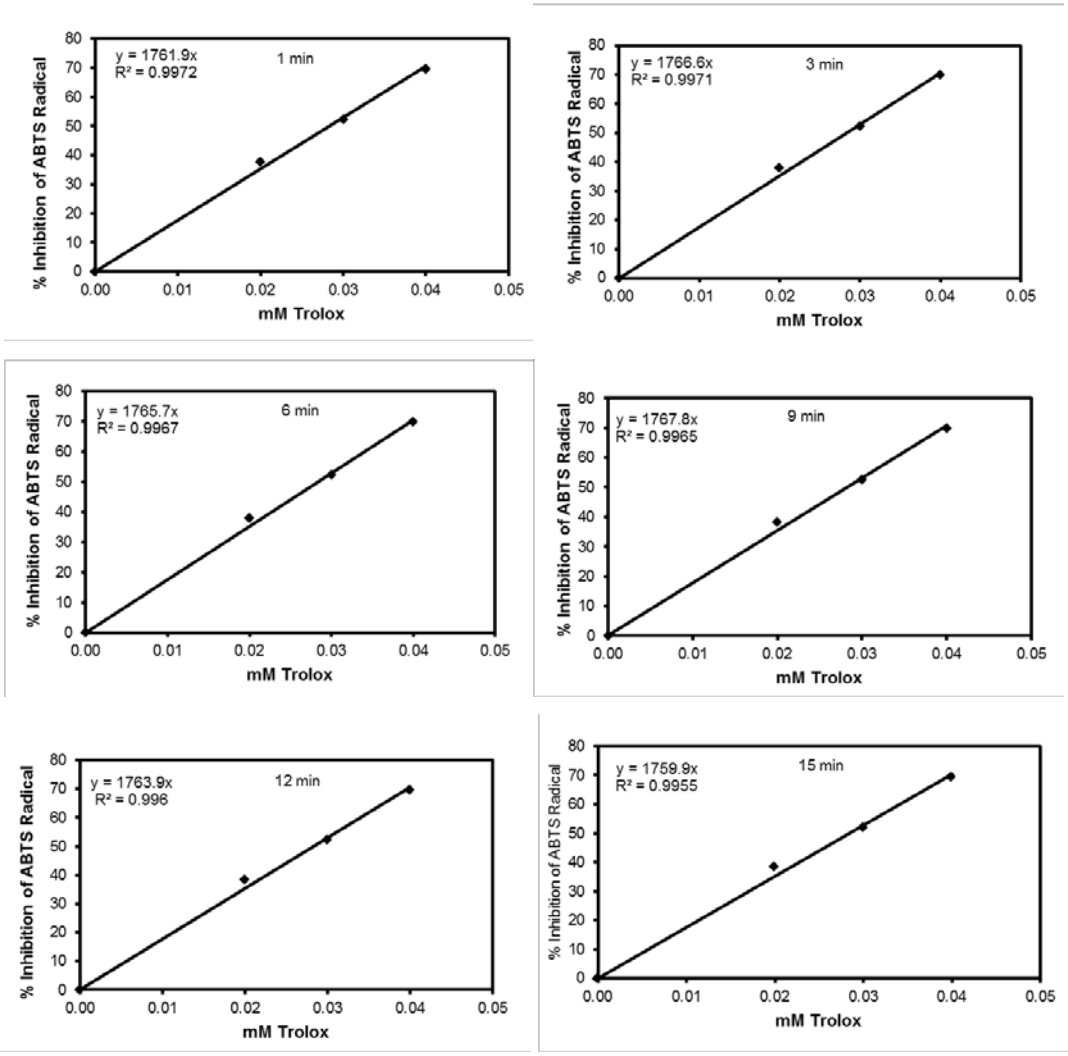

Figure E.1. Trolox standard for determination of antioxidant activity based on TEAC

APPENDIX F

TROLOX STANDARD FOR ORAC METHOD

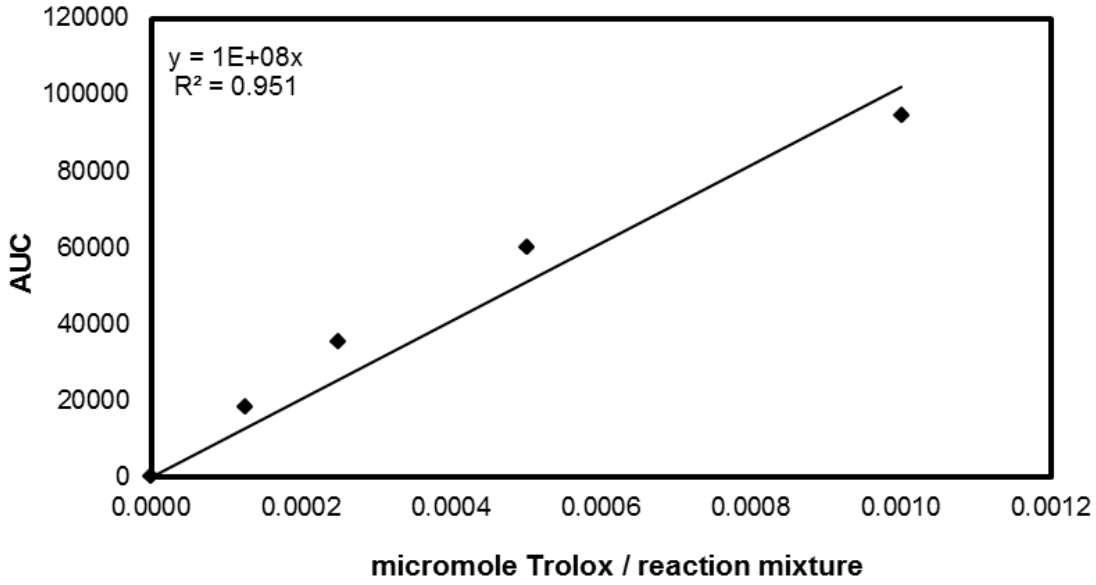

Figure F.1. Trolox standard curve for determination of antioxidant activity based on ORAC

APPENDIX G

EDTA STANDARD CURVE

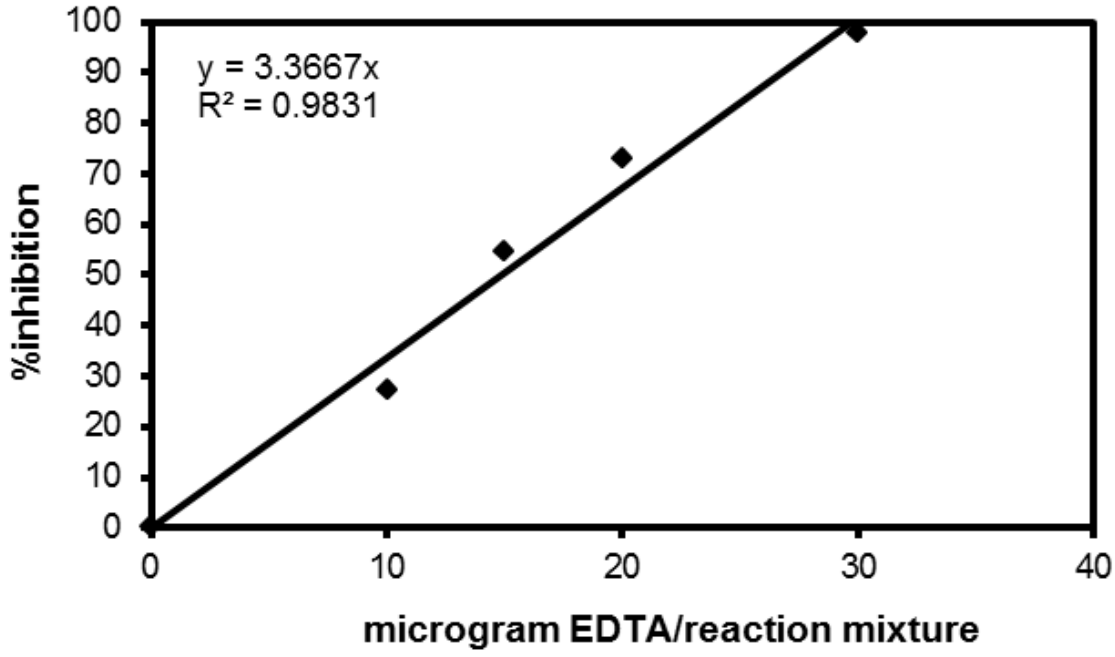

Figure G.1. EDTA standard curve for determination of ICC

VITA

Levent Yurdaer Aydemir
Place of Date and Birth: 17/11/1983 – Söke, Aydın, TURKEY
leventaydemir@iyte.edu.tr levent.aydmr@gmail.com

EDUCATION

PhD, Department of Food Engineering, Faculty of Engineering, İzmir Institute of Technology, İzmir, TURKEY (2008-2014).
MSc, Biotechnology and Bioengineering, Institute of Engineering and Science, İzmir Institute of Technology, İzmir, TURKEY (2006-2008)
BSC, Department of Biochemistry, Faculty of Science, Ege University, İzmir, TURKEY (2001-2005)

WORK EXPERIENCE

Research Assistant, İzmir Institute of Technology, Department of Food Engineering, İzmir, TURKEY, (2008-2014)

HONORS AND ACHIEVEMENTS

2. International Food R&D Brokerage Project Competition, First Prize Winner, 2013; The project titled "The Evaluation of Hazelnut Meal in Production of Value Added Protein Isolates Suitable for Food, Drug and Cosmetic Industry" was chosen as a first winning project among 176 projects.

Innovation Week of Turkey, 2013; The project titled "The Evaluation of Hazelnut Meal in Production of Value Added Protein Isolates Suitable for Food, Drug and Cosmetic Industry" was chosen by Turkish Exporters Assembly to be presented in Innovation Week of Turkey.

PUBLICATIONS

Aydemir, L. Y. , Gökbulut, A. A., Baran, Y., & Yemenicioğlu, A. (2014). *Food Hydrocolloids*, 36, 130-142.

Aydemir, L. Y. & Yemenicioğlu, A. (2013). *LWT - Food Science and Technology*, 50 (2), 686-694.

Alkan, D., **Aydemir, L. Y.**, Arcan, I., Yavuzdurmaz, H., Atabay, H. I., Ceylan, C., & Yemenicioglu, A. (2011). *Journal of Agricultural and Food Chemistry*, 59(20), 11003-11010.

Aydemir, L. Y. & Yemenicioğlu, A. (2013). *Plant Biochemistry and Physiology*,1:4 (Short communication).