CHARACTERIZATION OF ANTIOXIDANT ACTIVITY AND PROTEIN FUNCTIONALITY IN SOME LEGUME CULTIVARS GROWN IN TURKEY

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ABSTRACT

CHARACTERIZATION OF ANTIOXIDANT ACTIVITY AND PROTEIN FUNCTIONALITY IN SOME LEGUME CULTIVARS GROWN IN TURKEY

Turkish chickpeas (4 cultivars) and lentils (6 cultivars) show similar total phenolic contents and free radical scavenging capacities in aqueous extracts ranging between 2869 and 4312 mg gallic acid equivalents/kg legume and 24.42 and 38.20 mmol Trolox equivalents/kg legume, respectively. However, the free radical scavenging capacity of lentil and chickpea protein extracts, range between 110 and 185 mmol Trolox/kg protein and 58 and 144 mmol Trolox/kg protein, respectively, clearly showed the higher free radical scavenging capacity of lentil proteins than chickpea proteins. Protein extracts of chickpeas and lentils showed considerable emulsifying and foaming capacities in almost at the same range, but emulsions and foams formed by chickpea proteins are more stable than those of lentil proteins. The lentil protein extracts are highly soluble and showed poor water absorption and gelling characteristics. In contrast, chickpea protein extracts showed moderate water absorption and gelling capacity. Chickpea protein extracts are also good oil absorbers with almost 1.5 to 2 fold better oil adsorption capacity than lentil protein extracts. Thus, chickpea proteins are suggested as soy and whey protein alternatives for functional proteins used in the food, drug and cosmetics industries. Considering functional properties of proteins for different cultivars, the outstanding Turkish chickpea cultivars are Gökçe and Cevdetbey, while the outstanding Turkish lentil cultivar is Alidayı. Variations in the functional properties of protein suggest the diversity of genes in chickpeas and lentils responsible for these properties. Thus, this study showed the possibility of improving functional properties of chickpeas and lentils by breeding programs.

ÖZET

TÜRKİYE'DE YETİŞTİRİLMEKTE OLAN BAZI BAKLAGİL TÜRLERİNDE ANTİOKSİDANT AKTİVİTE VE PROTEİN FONKSİYONELLİĞİNİN KARAKTERİZASYONU

Türk nohut (4 çeşit) ve mercimeklerinde (6 çeşit) suda çözünür toplam fenolik madde miktarı 2869 ve 4312 mg gallik asit eşdeğeri/kg baklagil ile 24.42 ve 38.20 mmol Troloks eşdeğeri/kg baklagil arasında değişmekte ve benzerlik göstermektedir. Ancak, mercimek ve nohutların sırasıyla 110 ve 185 mmol Troloks eşdeğeri/kg protein, ile 58 ve 144 mmol Troloks eşdeğeri/kg protein arasında değişen serbest radikal indirgeme güçleri mercimek proteinlerinin nohut proteinlerinden daha iyi bir antioksidant potensiyeli oluşturduğunu göstermiştir. Nohut ve mercimek proteinleri kaydadeğer bir emülsiyon ve köpük oluşturma gücüne sahiptirler. Ancak, nohut proteinleri çoğunlukla mercimek proteinlerine kıyasla daha stabil emülsiyonlar ve köpükler oluşturmaktadırlar. Mercimek proteinleri nohut proteinlerine göre daha yüksek bir çözünürlüğe sahip olup oldukça zayıf bir jelleşme gücü ve su absorpsiyon kapasitesi göstermektedirler. Buna karşın nohut proteinleri kayda değer düzeyde bir jelleşme gücü ve su absorpsiyon kapasitesine sahiptirler. Ayrıca, mercimek proteinleri orta düzeyde yağ absorbsiyonu gösterirken, nohut proteinleri mercimek proteinlerine göre 1.5-2 kat daha yüksek yağ absorpsiyonu göstermektedirler. Bu özellikler dikkate alınarak nohut proteinlerinin soya ve peyniraltı suyu proteinlerine alternatif olarak gıda, ilaç ve kozmetik endüstrisinde kullanılması önerilebilir. Elde edilen sonuçlara göre proteinlerinin fonksiyonel özellikleri bakımından öne çıkan nohut çeşitleri Gökçe ve Cevdetbey'dir. En üstün protein fonksiyonel özellikleri gösteren mercimek çeşidi ise Alidayı'dır. Gerçekleştirilen bu çalışmada nohut ve mercimeklerin birçok protein fonksiyonel özelliklerinin çeşit bazında ciddi değişimler göstermesi bu ürünlerde ıslah yöntemleri kullanılarak fonksiyonel özelliklerin geliştirilebileceğini göstermektedir.

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CHAPTER 1

INTRODUCTION

With its 550.000 and 610.000 metric tons of production, Turkey is one of the biggest producers of lentils and chickpeas in world, respectively (Gül and Isık 2002). Although these legumes are good sources of proteins and phenolic compounds, in Turkey there is almost no information related to the differences of contents of these health related and industrially important compounds in different chickpea and lentil cultivars. In fact, these products are currently used solely for consumption, but not for production of value added products such as commercial protein extracts. Thus, almost all of the commercial vegetable proteins used as technological ingredients in food, cosmetics and drug industry of our country are imported as soy products (Anaç and Ertürk 2003). The legume proteins has many different technological properties including emulsion, film, gel, foam formation, increase of consistency and water/oil holding capacity, retention of aroma compounds and antioxidant activity (Sikorski 1997, Arcan and Yemenicioğlu 2007, Pena-Ramos and Xiong 2002, Hu et al. 2003, Horax et al. 2004b). Thus, they are extensively used in many food products such as meat and bakery products, and beverages to improve the technological properties and nutritional values of these products. The legume proteins are also used in many different cosmetic products to obtain different functions. Moreover, after the recent findings which showed the abundance of phenolic compounds having preventive/protective effects on cardiovascular disease and cancer in legumes, there is a great interest to produce health related products from these crops (Obama, et al. 2006, Nurmi, et al. 2002, Yu, et al. 2006). Currently, there are many different soy phenolic enriched health products in the market and they are sold at relatively high prices.

Turkey is not only a principal producer of chickpeas and lentils, but it is also at a critical geographical location in which the agriculture of these products first initiated and spread to the other parts of the world. For this reason, our country is one of the few countries having many different cultivars of chickpeas and legumes grown on its land. In this study, we characterized antioxidant activities and contents of phenolic compounds, and protein functionality (capacity to form emulsions, gels and foams,

water/oil holding capacities and antioxidant activity) in different cultivars of chickpeas and lentils grown in Turkey. Turkey has unique advantages to determine the potential genetic diversity of these traits in chickpeas and lentils and use biotechnology as a tool to improve the current technological and nutritional status of its plant material. The main objectives of this project are (1) selection of suitable cultivars to produce the commercial protein extracts needed by our food, cosmetics and drug industry, (2) the preparation of the basis for breeding of chickpea and lentil cultivars with improved protein functionality and better antioxidant activity.

CHAPTER 2

AMINO ACIDS

2.1. General Properties of Amino Acids

Amino acids are the monomeric units of proteins, which are joined by a specific type of covalent linkage (Bhagavan 1992). The 20 common amino acids are called α-amino acids because they have an amino group (NH^{+}_{3}) and acidic carboxyl group (COOH) attached to C-2, which is also known as the α-carbon (Horton, et al. 1996). In addition, a hydrogen atom and a distinctive R group are also linked to a central carbon atom. The R group is often referred to as side chain which varies in size, charge, hydrogen-bonding capacity, hydrophobic character and chemical reactivity (Figure 2.1) (Armstrong 1989, Berg, et al. 2002).

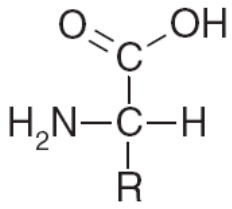


Figure 2.1 Basic structure of an amino acid
(Source: Buxbaum 2007)

Nineteen of the 20 α-amino acids are asymmetric molecules since they have four different substituents attached to their central carbon. Glycine is an exception, since it has -H as -R group and this creates two similar groups on the α-carbon. A molecule containing an asymmetric carbon atom has two stereoisomers (Mathews and Holde 1996). The streoisomers are nonsuperimposable mirror-images, and according to the position of their amino groups at α-carbon atom they are designated D (for dextro, from the Latin dexter, right) or L (for Levo, from the Latin laevus, left) (Figure 2.2) (Horton, et al. 1996). Only L amino acids are constituents of proteins (Berg, et al. 2002). Of over 100 naturally occurring amino acids, only 20 amino acids which are coded for in the genes of all organism are utilized in polypeptide biosynthesis (Armstrong 1989, Mathews and Holde 1996).

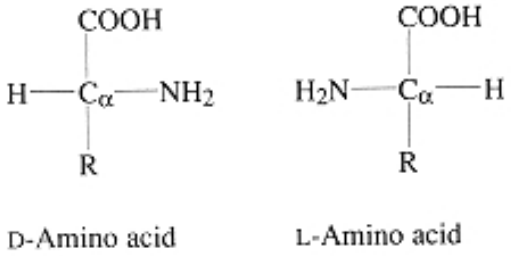


Figure 2.2 Stereoisomers of chiral amino acid

(Source: Damodaran 1996a)

At physiological pH, the amino group of an amino acid is protonated (NH_2 to NH^+_3) and the carboxyl group is ionized (COOH to COO^-). Therefore, at neutral pH, an amino acid exists in a dipolar state which is called a zwitterion (Armstrong 1989, Horton, et al. 1996). Amino acid polymerization is a condensation reaction in which the carboxyl group of one amino acid reacts with the amino group of the other to form a covalent amide bond. These amide bonds are called peptide bonds and each amino acid which contributes to the peptide bond is referred to a residue (Bhagavan 1992).

2.2. Classification of Amino Acids

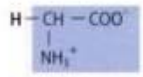
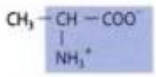
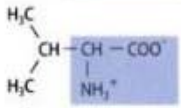
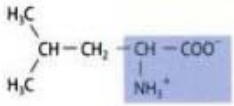
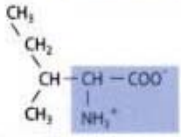
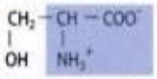
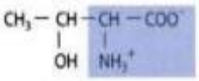
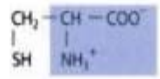
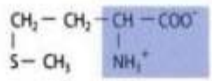
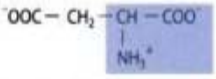
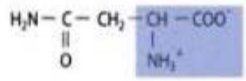
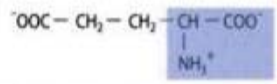
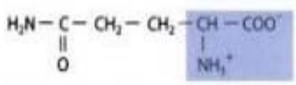
Classification of amino acids is based on the solubility and ionization properties of R groups. In every class, R groups are different in size, shape and other properties. The physical and chemical properties of the side chains greatly influence the overall three-dimensional conformation of a protein. Amino acids can be classified as aliphatic, hydroxyl, sulphur-containing, aromatic, basic and acidic. (Table 2.1) (Bhagavan 1992, Horton, et al. 1996, Mathews and Holde 1996).

2.2.1. Amino Acids with Aliphatic Side Chains

Alanine (Ala, A), valine (Val, V), leucine (Leu, L) and the structural isomer of leucine, isoleucine (Ile, I) have saturated non-cyclic aliphatic side chains (Horton, et al. 1996). The side chains of these amino acids don't have any reactive groups but as a result of the chemical and physical properties of methyl groups, they show hydrophobic

Table 2.1. L-α Amino acids present in proteins

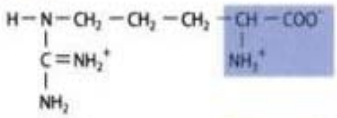
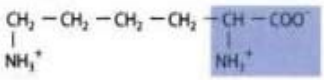
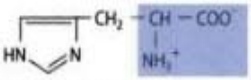
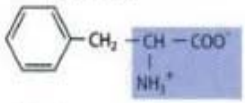
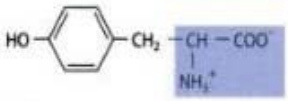
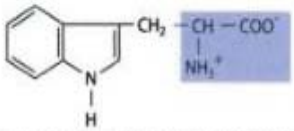
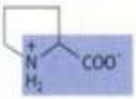
(Source: Rodwell 2003)

Name	Symbol	Structural Formula	pK_1	pK_2	pK_3
With Aliphatic Side Chains			α-COOH	α-NH_3^+	R Group
Glycine	Gly [G]		2.4	9.8	
Alanine	Ala [A]		2.4	9.9	
Valine	Val [V]		2.2	9.7	
Leucine	Leu [L]		2.3	9.7	
Isoleucine	Ile [I]		2.3	9.8	
With Side Chains Containing Hydroxylic (OH) Groups					
Serine	Ser [S]		2.2	9.2	about 13
Threonine	Thr [T]		2.1	9.1	about 13
Tyrosine	Tyr [Y]	See below.			
With Side Chains Containing Sulfur Atoms					
Cysteine	Cys [C]		1.9	10.8	8.3
Methionine	Met [M]		2.1	9.3	
With Side Chains Containing Acidic Groups or Their Amides					
Aspartic acid	Asp [D]		2.0	9.9	3.9
Asparagine	Asn [N]		2.1	8.8	
Glutamic acid	Glu [E]		2.1	9.5	4.1
Glutamine	Gln [Q]		2.2	9.1	

(cont. on the next page)

Table 2.1(cont.). L-α Amino acids present in proteins

(Source: Rodwell 2003)

Name	Symbol	Structural Formula	pK_1	pK_2	pK_3
With Side Chains Containing Basic Groups			α-COOH	α-NH_3^+	R Group
Arginine	Arg [R]		1.8	9.0	12.5
Lysine	Lys [K]		2.2	9.2	10.8
Histidine	His [H]		1.8	9.3	6.0
Containing Aromatic Rings					
Histidine	His [H]	See above.			
Phenylalanine	Phe [F]		2.2	9.2	
Tyrosine	Tyr [Y]		2.2	9.1	10.1
Tryptophan	Trp [W]		2.4	9.4	
Imino Acid					
Proline	Pro [P]		2.0	10.6	

character which has an important role in establishing and maintaining the three dimensional structure of protein (Horton, et al. 1996). Since they have a branched side chain, valine, leucine and isoleucine, may strongly participate in hydrophobic interactions (Bhagavan 1992). Another member of this class, Glycine (Gly, G) has the least complex structure since its R group is simply a single hydrogen atom. Thus, glycine gives little hydrophobic character to a protein molecule, but it may locate to small cracks and cavities within the protein structure (Horton, et al. 1996). The last member of this group is proline. Instead of a primary amino group, proline contains secondary amine group (an imine). The side chain of proline is cyclic. Thus, it mostly restricts the geometry of polypeptides (Bhagavan 1992, Horton, et al. 1996).

2.2.2. Amino Acids with Hydroxyl Group Containing Side Chains

Serine (Ser, S) and threonine (Thr, T) have aliphatic uncharged polar side chains that contain β-hydroxyl groups which impart a hydrophilic character to their structure (Berg, et al. 2002, Horton, et al. 1996). Although threonine has two asymmetric carbon atoms, only one of the four stereoisomers commonly occurs in proteins. Hydroxyl groups of these amino acids have weak ionization properties (Horton, et al. 1996). The primary alcohol groups of serine and threonine participate in esterification reactions with phosphoric acid and glycosides with sugars (Bhagavan 1992).

2.2.3. Amino Acids with Sulfur Containing Side Chains

Cysteine (Cys, C) and methionine (Met, M) are sulfur containing amino acids which have important roles for the functions of some enzymes and structural proteins (Bhagavan 1992). Methionine is a highly hydrophobic essential amino acid having a nonpolar methyl thioeter group which can serve as a donor of a methyl group in many transmethylation reactions (Bhagavan 1992, Horton, et al. 1996).

The sulfhydryl group of cystein (–SH) plays an important role in stability of folded proteins by forming covalent disulfide bonds when oxidized, particularly at alkaline pH. It also forms weak hydrogen bonds with oxygen and nitrogen. Since the sulfhydryl group is a weak acid, it loses its proton and is turned to a thiolate ion which gives a negative charge to protein surface (Horton, et al. 1996). The oxidation of two cysteines forms cystine (Figure 2.3). But this compound is not listed within the 20 amino acids since it is not coded in the DNA (Mathews and Holde 1996).

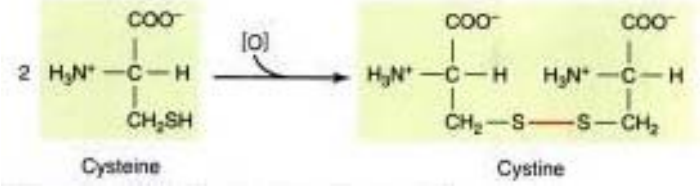


Figure 2.3. Oxidation of cysteine to form cystine
(Source: Troop 2007)

2.2.4. Aromatic Amino Acids

Phenylalanine (Phe, F), tyrosine (Tyr, Y) and tryptophan (Trp, W) carry aromatic side chains (Mathews and Holde 1996). Phenylalanine has a planar benzene ring which gives a hydrophobic character to the molecule. On the other hand, tyrosine carries a weakly acidic phenolic hydroxyl group that can participate in hydrogen bond formation with oxygen and nitrogen atoms (Bhagavan 1992). In tryptophan, there is a bicyclic indole group which is a nitrogenous aromatic ring system. As a result, tyrosine and tryptophan are less hydrophobic amino acids compared to phenylalanine (Berg, et al. 2002). The aromatic amino acids, like most compounds carrying conjugated rings, exhibit strong absorption of light in the near-ultraviolet region of the spectrum (Mathews and Holde 1996). The aromatic rings of tryptophan and tyrosine contain delocalized π electrons which strongly absorb UV light at 280 nm whereas phenylalanine absorbs light weakly at 260 nm (Berg, et al. 2002, Horton, et al. 1996, Mathews and Holde 1996).

2.2.5. Basic Amino Acids

Histidine (His, H), lysine (Lys, K) and arginine (Arg, R) have very polar hydrophilic side chains (Berg, et al. 2002). They are nitrogenous bases and positively charged at pH 7 (Horton, et al. 1996). Histidine is the least basic amino acid which has an imidazole ring capable of functioning in enzyme active sites by accepting or donating protons during reactions that occur at physiological pH range (Berg, et al. 2002). In fact, histidine is the only amino acid which may show ionization at physiological pH values. Thus, this amino acid is essential for the function of many enzymes.

Lysine is a diamino acid having a reactive amino group attached to the ε-carbon. The lysyl side chain forms ionic bonds with negatively charged groups of acidic amino acids. The ε-carbons of some lysyl residues can oxidized to reactive aldehyde groups with elimination of NH_3 then react with other ε-amino groups to form covalent cross-links between polypeptides (Bhagavan 1992). These covalent cross-links provide tensile strength and insolubility to some protein fibres.

Arginine is the most basic amino acid which has a positively charged guanidinium group attached to the δ-carbon and is stabilized by resonance between the two nitrogenous groups (Bhagavan 1992). The strongly polar (hydrophilic) basic amino acids are usually found on the exterior surfaces of folded proteins, where they can be hydrated by the surrounding aqueous environment (Mathews and Holde 1996).

2.2.6. Acidic Amino Acids and Their Amides

Aspartic acid (Asn, N) and glutamic acid (Glu, E) are dicarboxylic amino acids that carry negative charge at physiological pH (Horton, et al. 1996). Aspartic acid is a β-carboxylic acid and the anionic carboxylate group tends to occur on the surface of water-soluble proteins. The Glutamic acid is a γ-carboxylic acid and like aspartic acid, the anionic group of glutamate tends to occur on the surfaces of proteins in aqueous environments (Bhagavan 1992).

Asparagine (Asn, N) and glutamine (Gln, Q) are the uncharged amid derivatives of asparatic acid and glutamic acid. These amino acids contain a terminal carboxamide in place of a carboxylic acid (Berg, et al. 2002). Unlike to aspartic and glutamic acids, asparagine and glutamine have uncharged side chains even though these amino acids are highly polar. These hydrophilic amino acids participate in hydrogen bond formation and support protein stabilization (Bhagavan 1992, Mathews and Holde 1996).

2.3. Ionization of Amino Acids

Amino acids contain α-carboxyl and α-amino groups as ionizable groups. In addition 7 of the 20 amino acids including aspartic acid, glutamic acids lysine, arginine, histidine, tyrosine and cysteine also contain ionisable groups at their side chains. The ionic states of amino acids significantly affect their interactions (Horton, et al. 1996). The properties of proteins also change considerably when they contain heavy amino acids having ionisable side chains. In an acidic solution the amino acids bear a net positive charge due to the existence of their amino groups in protonated form ($-NH_3^+$). At neutral pH values the amino groups maintain their positive charge, but this time the carboxyl group shows ionization and forms a negative charge (COO^-). At this state the amino acids are electrically neutral (zwitterions) since they have both a positive and a

negative charge. Thus, they can not migrate when placed in an electrical field. The pH at which the dipolar ion is electrically neutral is called the isoelectrical point (pI). At alkaline pH values, the amino group is also deprotonated ($-NH_2$) and amino acid became negatively charged due to the deprotonated carboxyl group (Figure 2.4).

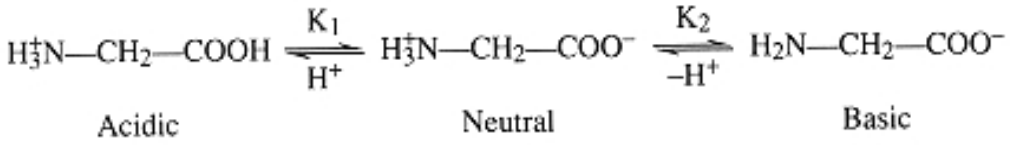


Figure 2.4. Ionic states of amino acids depending on pH value
(Source: Damodaran 1996a)

Each ionisable group in amino acids including α-amino, α-carboxyl and side chain ionisable groups has a pKa value (Table 2.1). The pKa value is a constant which corresponds to the pH value at which ionization of a weak acid occur to form equal amounts of conjugate acidic and base species. According to the pKa value, one can estimate the ionic state of an amino acid at a known pH value by using the Henderson-Hasselbalch equation given below.

The ionization of weak acidic groups, amino and carboxyl groups, of amino acids can be illustrated as:

$$[\text{Conjugate acid}] \Longleftrightarrow [\text{Conjugate base}] + H^+$$

$$RCOOH \Longleftrightarrow RCOO^- + H^+$$

$$RNH^+_3 \Longleftrightarrow RNH_2 + H^+$$

The equilibrium constant for the ionization of a weak acid can be written as;

$$Ka = [\text{Conjugate acid}]\,[H^+] / [\text{Conjugate base}]$$

$$[H^+] = Ka\,[\text{C. acid}] / [\text{C. base}]$$

$$\log [H^+] = \log Ka + \log [\text{C. acid}] / [\text{C. base}]$$

$$-\log [H^+] = -\log Ka - \log [C.\ acid] / [C.\ base]$$
$$-\log [H^+] = \log Ka + \log [C.\ base] / [C.\ acid]$$

$$-\log [H^+] = pH; \quad -\log Ka = pKa$$

pH= pKa + log [C. base] / [C. acid] Henderson-Hasselbalch equation

pKa values of different groups in amino acids are shown in Table 1.1

2.4. Hydropathy of Amino Acids

Each amino acid shows different hydrophobic or hydrophilic character according to the properties of its R group. These properties vary from highly hydrophobic to weakly polar and highly hydrophilic. The relative hydrophobicity or hydrophilicity of each amino acid is called its 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 having negative values are hydrophilic, whereas those having positive values are hydrophobic. The hydrophobicity of proteins is important since it is a driving force for their folding. On the other hand, the hydophilicity is important since it determines their reactivity in water and solubility. The hydropathy of amino acids is an important determinant about their hydrophilic or hydrophobic nature, but it doesn't give an accurate prediction whether a given residue will be found in the non-aqueous interior or on the solvent –exposed surface of a folded protein (Table 2.2) (Horton, et al. 1996).

Table 2.2. Hydrophobicity of amino acids side chains at 25°C

(Source: Damodaran 1996a)

Amino acid	ΔG_t (ethanol $\rightarrow$ water) (kJ/mol)	Amino acid	ΔG_t (ethanol $\rightarrow$ water) (kJ/mol)
Alanine	2.09	Leucine	9.61
Arginine	—	Lysine	—
Asparagine	0	Methionine	5.43
Aspartic acid	2.09	Phenylalanine	10.45
Cysteine	4.18	Proline	10.87
Glutamine	-0.42	Serine	-1.25
Glutamic acid	2.09	Threonine	1.67
Glycine	0	Tryptophan	14.21
Histidine	2.09	Tyrosine	9.61
Isoleucine	12.54	Valine	6.27

CHAPTER 3

PROTEINS

3.1. Proteins

Proteins are complex biomolecules formed by end to end covalent linking of constituent amino acids. The covalent linking of amino acids occurs with condensation between the α-carboxyl group of one amino acid and the α-amino group of another. Proteins are the most versatile macromolecules in living systems and have crucial functions as catalysts, for transportation and storage of other molecules, providing mechanical support and immune protection, transmitting nerve impulses and controlling growth and differentiation (Berg, et al. 2002). Interestingly, proteins spontaneously fold and form three-dimensional structures. The specific shape of a protein is dictated by its amino acid composition. For example, in water, proteins containing mainly hydrophobic amino acids form mostly a compact and globular shape, while proteins containing mainly hydrophilic amino acids form a rod-like expanded shape. Some proteins show different degrees of flexibility while others are quite rigid (structural proteins) (Berg, et al. 2002). All these structural characteristics of proteins are a result of amino acid composition and determine the functions of protein. To understand their complex nature one should know the peptide bond and different structural organization of proteins.

3.1.1. Peptide Bonds

A peptide bond is an amide bond that is covalently formed between the α-amino group of one amino acid and the α-carboxyl group of another with elimination of a water molecule (Mathews and Holde 1996). The linked amino acid moieties are called amino acid residues (Figure 3.1) (Horton, et al. 1996).

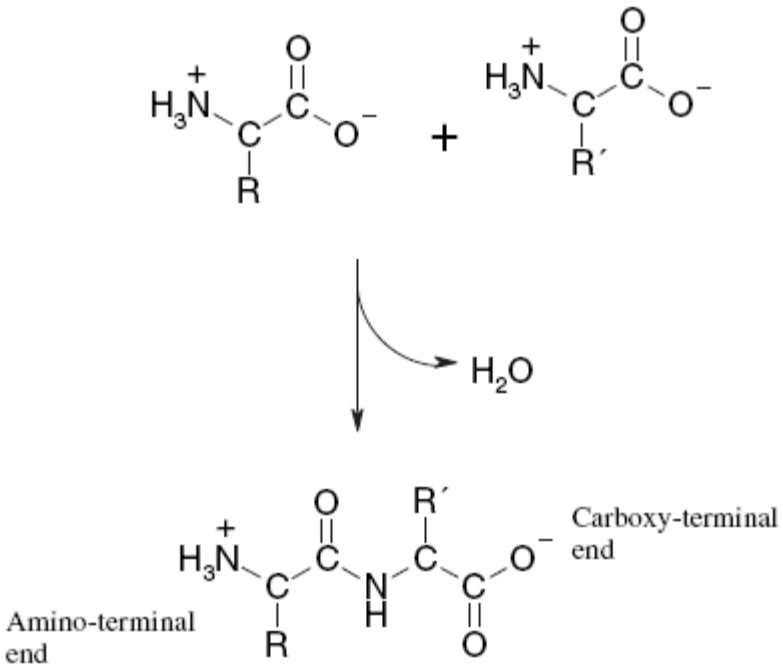


Figure 3.1 Peptide bond formation

(Source: Buxbaum 2007)

The equilibrium of this reaction lies on the side of hydrolysis rather than synthesis. However the synthesis reaction is not thermodynamically favoured and requires an input of free energy. Peptide bonds are kinetically quite stable. Therefore, every amino acid must be activated by an ATP-driven reaction before it can be incorporated into proteins (Mathews and Holde 1996). When a peptide bond is formed, there is still an amino group (NH+3) on one end of the peptide and an unreacted carboxyl group (COO-) on the other, so that at neutral pH the polypeptide has polarity (Berg, et al. 2002, Horton, et al. 1996, Mathews and Holde 1996). In fact, polypeptides are good examples of polyampholytes (Berg, et al. 2002, Mathews and Holde 1996). However, effects of changing pH are very important in the chemistry and biochemistry of proteins. A small shift in pH may alter the charges of the protein and this may affect its interactions. For example,increase of the negative charge intensity of a protein can prevent its interaction with a negatively charged molecule due to the repulsion of like charges.

A polypeptide chain consists of two parts: a main chain or backbone which is the repeating part and side chains which are the variable part. The backbone has good hydrogen bonding potential since carbonyl groups of amino acid residues are good hydrogen acceptors whereas amino groups are good hydrogen-bond donors. Thus, the H

bond plays an important role in formation and stability of protein structure (Berg, et al. 2002).

3.1.2. Primary Structure

The primary structure of a protein refers to the linear sequence in which the consequent amino acids are linked end to end through peptide bonds. The sequence of amino acids is not random and it is encoded in the genome of the cell (Voet, et al. 1999). At this level of structure, the only type of bonding is covalent peptide linkage (Armstrong 1989). It is important to know the amino acid sequence of proteins for several reasons. Firstly, the sequence of a protein is essential to understand its functions and mechanism of action. Secondly, amino acid sequence gives information about the genetic message in the DNA and the three-dimensional structure of the protein. Finally, the sequence of protein reveals much about its evolutionary history (Berg, et al. 2002).

A peptide bond forms a plane. The atoms involved in this plane are the Cα and C′ atoms (C of carboxyl group) of one amino acid and the N (of NH group) and Cα atoms of the other amino acid (Berg, et al. 2002). For a planar peptide bond, the possible configurations are trans or cis configurations. In trans configuration, the two α-carbon atoms are on the opposite sides of the peptide bond whereas in cis configuration, these groups are on the same side of the peptide bond (Figure 3.2). Nearly all peptide bonds exist in trans form since steric clashes between groups attached to the Cα atoms hinder formation of the cis form. However, when proline participates in peptide bonds, cis peptide bonds are formed because the nitrogen of proline is bonded to two tetrahedral carbon atoms by limiting the steric differences between trans and cis configuration (Berg, et al. 2002).

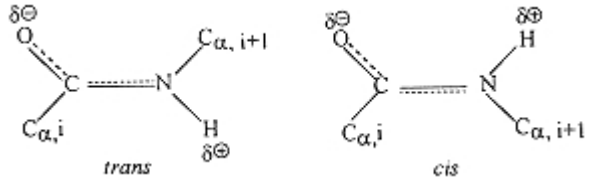


Figure 3.2 Trans and cis peptide bonds

(Source: Damodaran 1996)

The covalent peptide bond is quite rigid, but the plane formed by the bond may rotate about the α-carbon atoms. The angles of rotation around the N-Cα and the Cα-C′ atoms are called the phi (ϕ) and the psi (ψ) angles, respectively. If a protein has the same angles of rotation about the Cα-N and Cα-C ‘ bonds repeated down the length of its polypeptide chain, this will obviously rule out any sort of compact, globular folding arrangement. Instead we can expect extended highly ordered molecules (Figure 3.3).

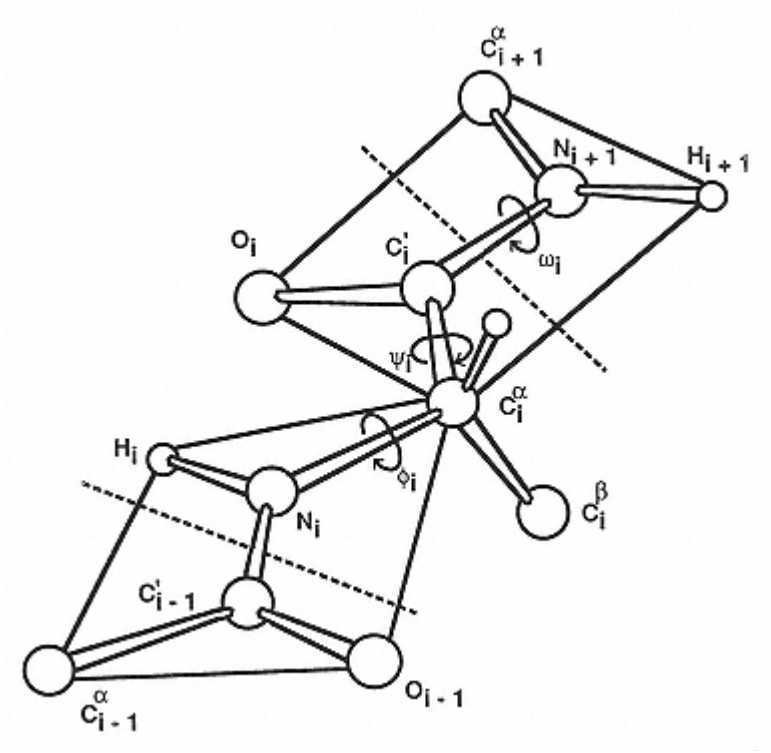


Figure 3.3 Planar configuration of the atoms of the peptide units
(Source: Damodaran 1996)

3.1.3. Secondary Structure

The secondary structure of a protein defines the regular arrangements of the polypeptide backbone in terms of different structures such as helical forms, β- structures and random coil. Secondary structure is the local spatial arrangement of a polypeptide’s backbone atoms as a result of hydrogen bonding between peptide bonds without regard to the conformations of its side chains (Armstrong 1989, Voet, et al. 1999). Different secondary structures of proteins are discussed below.

3.1.3.1. Helical Structures

Helical structures of proteins form when the ϕ and ψ angles of consecutive amino acid residues in the polypeptide backbone are twisted to a same set of values. Different combinations of ϕ and ψ angles, create several types of helical structures such as α-helix, 3_{10}-helix and π-helix (Figure 3.4). However, the α-helix with ϕ of -58^{o} and $+58^{o}$ and ψ of -47^{o} and $+47^{o}$ is the most frequently observed helical form in proteins (Damodaran 1996a). The α-helix is characterized by a pitch of 5.4 Angstrom involving 3.6 amino acid residues (Damodaran and Paraf, 1996b). Helical structures are stabilized by intramolecular hydrogen bonds formed between the –CO of each peptide bond and the -NH of the peptide bond four amino acid residues away (Armstrong 1989, Berg, et al. 2002).

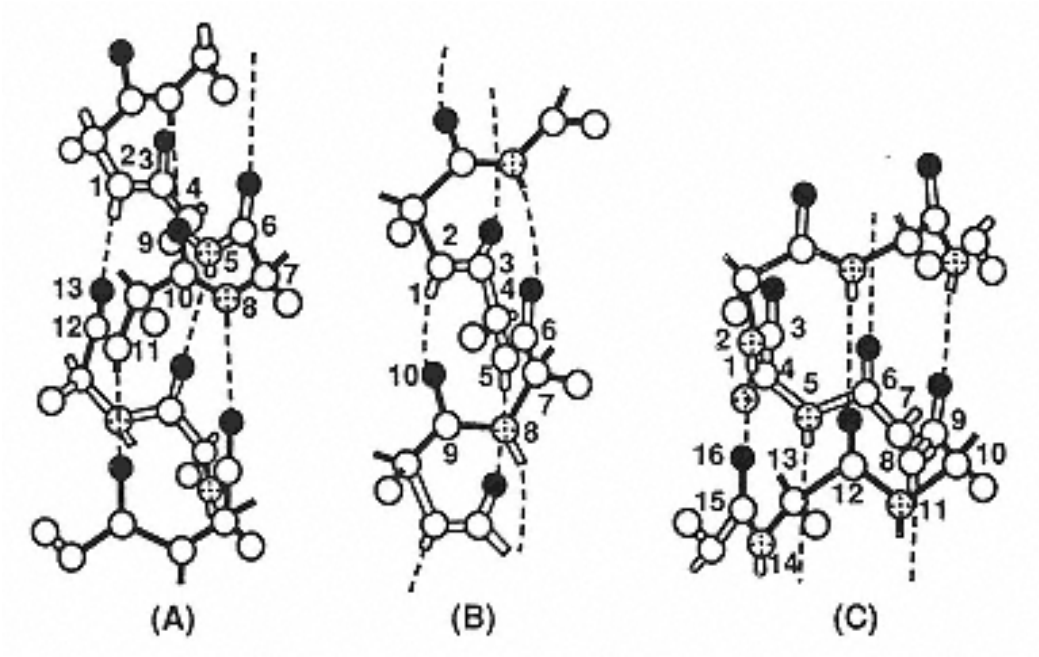


Figure 3.4. Structures of (A) alpha helix, (B) 3_{10}-helix, (C) π-helix
(Source: Damodaran 1996a)

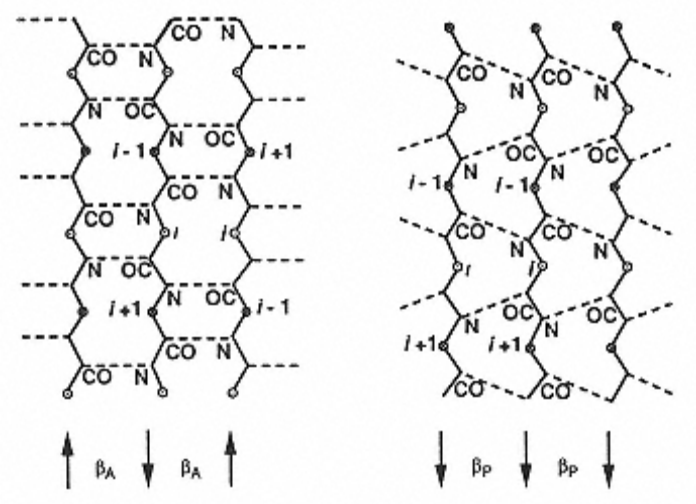
Even though the screwed structure of a helix can be right-handed or left-handed, right handed helices are energetically more favourable because there is less steric clash between the side chain and the backbone (Berg, et al. 2002). Due to van der Waals contacts, the core of the helix is tightly packed (Voet, et al. 1999). The structure is predominantly amphiphilic, that is, one half of the helical surface is hydrophilic and the

other half is hydrophobic. The hydrophobic surface participates in hydrophobic interactions with other nonpolar groups in the interior of the protein therefore such interactions contribute to the stability of the folded form of the protein (Damodaran and Paraf, 1996b).

The side chains of the amino acids influence the structure and formation of helical structures. Some amino acids such as proline are not compatible with helical structures. Proline can not participate in forming an α-helix since the nitrogen atom of this amino acid is in a rigid ring. Also, there are no hydrogen atoms on the nitrogen of a proline to form an intrachain hydrogen bond. Consecutive glutamyl and aspartyl residues with negative charges also form repulsive forces and destabilize the α-helical structure. Moreover, isoleucyl residues also prevent helical structures by causing steric hindrance with their bulky side chains (Armstrong 1989).

3.1.3.2. β-structures

The β-structures are zig zag structures which are more stretched than the helical forms. These structures can be formed from helical structures by destruction of the intrasegment H bonds by physical effects such as heat. The β-structures are fully extended rather than being tightly coiled as in the α-helix. Each β-structure contains 5 to 15 amino acid residue called the β-strand. The C=O and N-H groups of β-strands are oriented perpendicularly to the direction of the backbone (Damodaran, et al. 1996b). This structure causes formation of intersegment H bonding between the β-strands and results in the formation of β-sheets. Depending on the direction of its strands, β-sheets can be parallel or anti parallel (Figure 3.5). In parallel β-sheets, the β-strands are aligned in the same biochemical direction, amino terminal to carboxy terminal; whereas in antiparallel β-sheets, the β-strands are aligned in altering directions, amino terminal to carboxy terminal followed by carboxy terminal to amino terminal. In anti-parallel β-sheets, the individual polypeptide chains are maximally bonded to the neighbouring polypeptides, since H bond pairs in these β-structures are narrowly spaced and H atoms lie with zero angle (Armstrong 1989). In parallel β-sheets, however, the H bond pars are not narrowly spaced and H atoms lie with an angle between H bond pairs. These structural differences make anti-parallel β-sheets more stable than parallel β-sheets (Voet, et al. 1999). Both types of β-sheets are much more stable than the helical forms.



A B

Figure 3.5. Structures of (A) anti-parallel and (B) parallel β-sheets

(Source: Damodaran 1996a)

3.1.4. Tertiary Structure

The tertiary structure of a protein refers to its three-dimensional organization with secondary structure elements such as helical forms, β-structures and random coil. Formation of the three-dimensional structure of a folded protein from a linear primary configuration has thermodynamic requirements. The driving force for folding of proteins is hydrophobic interactions (Berg, et al. 2002). The folding occurs to minimize the free energy of the protein molecule. As a result, the non-polar residues locate at the protein interior, while hydrophilic residues locate at the protein surface. However, since it is not physically possible to bury all hydrophobic groups in the interior part of the protein some hydrophobic groups may also be located at the surface. When surface area is limited, it is also possible that a considerable amount of hydrophilic groups are also buried in the interior parts of protein. Generally hydrophilic groups are hydrogen bonded to each other and therefore their free energy is minimized in the apolar interior environment of the protein. The distribution of hydrophilic and hydrophobic residues determines the shape, surface topography and solubility of the protein (Damodaran, et al. 1996b). In an aqueous environment, protein folding is driven by the strong tendency of hydrophobic residues to be excluded from water (Berg, et al. 2002). Proteins containing a large number of hydrophilic residues tend to be elongated rod like shape.

In contrast; proteins containing a large number of hydrophobic residues tend to assume a globular shape (Figure 3.6) (Damodaran, et al. 1996b).

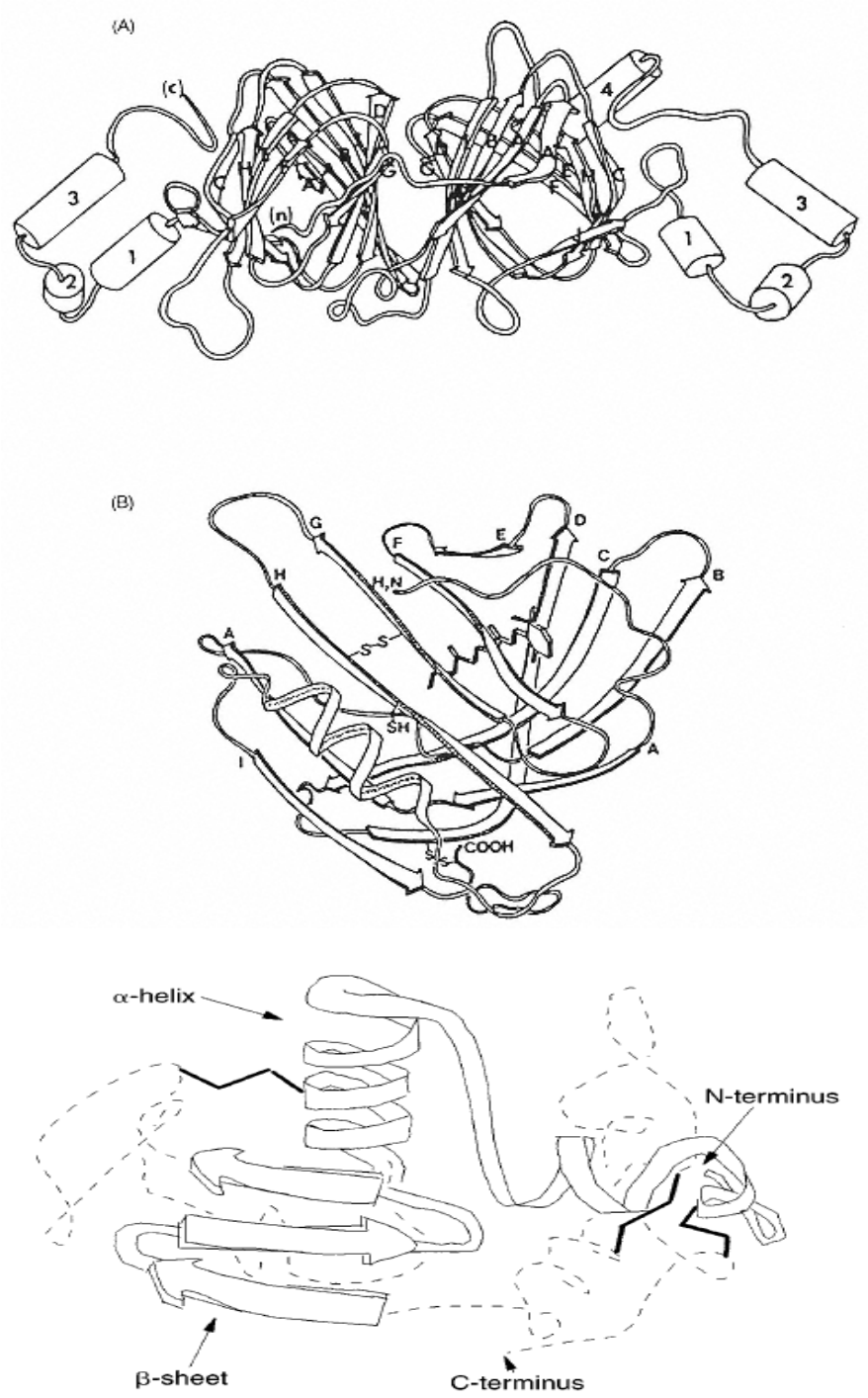


Figure 3.6. Tertiary structures of (A) phaseolin subunit, (B) lactoglobulin and (C) egg lysozyme (Source: Damodaran 1996a, Barrett and Elmore 2004)

Most protein structures are built up from combinations of secondary structure elements, α helices and β strands, which are connected by loop regions of various

lengths and irregular shape (Voet, et al. 1999). A combination of the secondary structure elements forms the stable core of the protein molecule. The loop regions are at the surface of the molecule. This is because loop regions exposed to water are rich in charged and polar hydrophilic residues. The main chain C=O and NH groups of these loop regions general do not form hydrogen bonds to each other. Instead, they are exposed to the solvent and can form hydrogen bonds to water molecules. The major loop regions of proteins are; (1) Hairpin loop (connects two adjacent antiparallel β-strands); (2) alpha-alpha motif (connects two α-helix); (3) Beta-alpha-beta motif (connects two parallel β strands linked to an intervening α-helix by two loops); (4) Greek key motif (connects four or more anti-parallel β strands with multiple loops) (Figure 3.7).

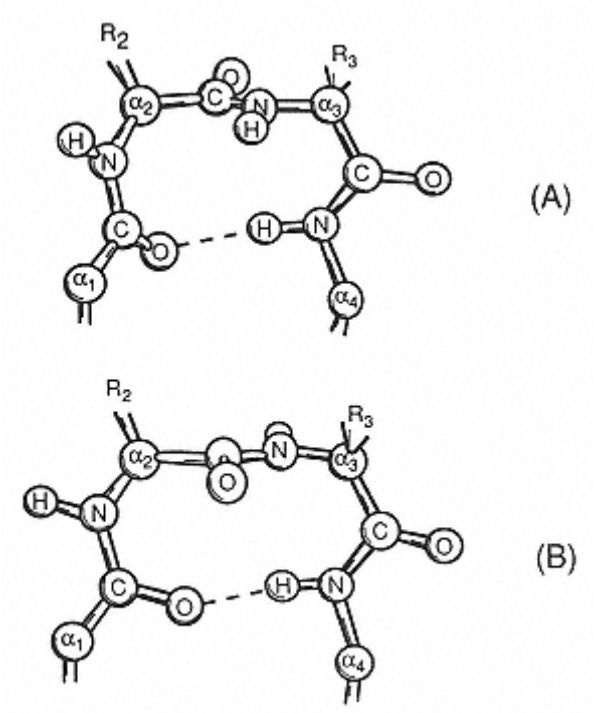


Figure 3.7. Conformations of (A) type I and (B) type II β turns
(Source: Damodaran 1996a)

In the tertiary structure, some polypeptide chains fold into two or more compact regions that may be connected by a flexible segment of polypeptide chain that is called a domain (Berg, et al. 2002). The structural stability of each domain is usually independent of the others (Damodaran, et al. 1996b). Domains of the protein may make extensive contacts with each other such that the protein appears to be a single globular

entity. Domains often have a specific function such as the binding of a small molecule (Voet, et al. 1999).

The tertiary structures of proteins are stabilized by different types of bonding and interactions. The intramolecular interactions originated from forces intrinsic to the protein molecule are Van der Waals forces and steric interactions. On the other hand, intramolecular interactions affected by surrounding solvent are hydrogen bonding, electrostatic and hydrophobic interactions. The H bonds and electrostatic interactions can contribute to protein stability in low dielectric environments such as the interior part of a protein (Damodaran, et al. 1996b). Van der Waals interactions may contribute to stability when they form at tightly packed hydrocarbon side chains (Berg, et al. 2002). The only covalent linkage involved in tertiary structure is the disulfide bond which is formed by oxidation of the sulfhydryl groups of cysteine residues (Figure 2.3) (Armstrong 1989).

3.1.5. Quaternary Structure

Quaternary structure refers to the spatial arrangement of proteins that contain more than one polypeptide chain. The subunits of such proteins contain non-polar patches on the surface. The hydrophobic interactions of different subunits in aqueous solution lead to formation of quaternary structures (oligomeric structure). The hydrophobic interactions are the main driving force in the formation of quaternary structures. However, electrostatic interactions and hydrogen bonding at the interface of the subunits may also contribute to the stability of the quaternary structure (Damodaran, et al. 1996b).

The simplest type of quaternary structure is the dimer that consists of two subunits (Berg, et al. 2002). Homogeneous proteins contain identical subunits whereas heterogeneous proteins consist of different subunits (Figure 3.8) (Armstrong 1989).

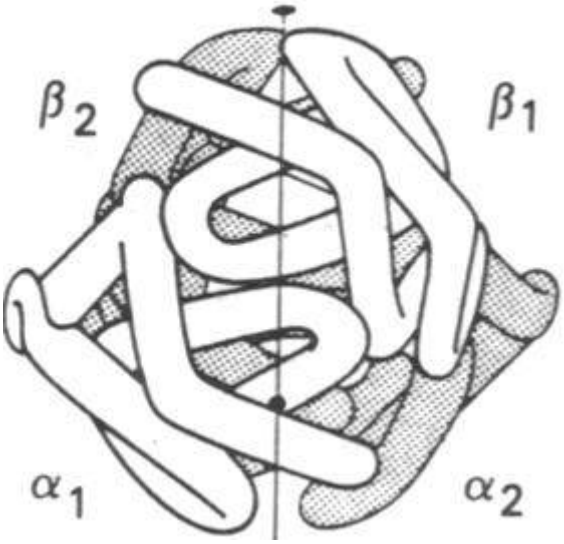


Figure 3.8. Quaternary structure of hemoglobin

(Source: Eaton, et al. 1991)

CHAPTER 4

FUNCTIONAL PROPERTIES OF PROTEINS

4.1. Functional Properties of Proteins

Functional properties of proteins are physicochemical properties that affect their behaviour in food systems during preparation, processing, storage and consumption, and contribute to the quality and sensory features of food systems (Zayas 1997). These properties of proteins result from interactions with the surrounding solvent, ions, other proteins, carbohydrates, lipids, flavours and numerous other components (Sikorski 2006, Zayas 1997). On the other hand, the functional properties of proteins are affected by the molecular weight and shape of protein molecules, structural diversity, structure and conformation, charge distribution, the primary structure, degree of hydrophobicity, and protein source (Zayas 1997). Proteins have a major contribution on food products by influencing their appearance, colour, juiciness, mouth feel and texture (Sikorski 2006).

The hydrophobicity of a protein is governed by the number of apolar amino acids in its structure. The degree of hydrophobicity affects conformation of proteins, hydration, solubility and other functional properties. The number of charged amino acids is also very important for the structure, stability and functions of protein. However, the compact structure of a protein molecule and extent of its bonding and interactions are main determinants of its functional properties (Zayas 1997). The structures of globular proteins are highly dynamic. Thus, globular proteins can rapidly fluctuate between many different conformations (McClements 2002). The polar charged amino acids on the surface of globular proteins accelerate solubility, swelling and hydration. A protein may have high water retention if it has a large proportion of hydrophilic residues (Zayas 1997).

The functionality of a protein is determined by several complex interactions among this protein and different proteins and non-protein ingredients of a food system. For utilization of protein sources for industrial purposes, it is important to improve knowledge of protein structure and its modification, and to optimize the functional properties of proteins in foods. Processing conditions, methods of defatting, type of

solvent, temperature of extraction and drying, freezing, ultrafiltration, homogenization, and other treatments are the main factors that influence the functional properties of proteins (Zayas 1997). However, at the molecular level, the functional properties are determined by the ability of proteins to bind other molecules, undergo conformational changes, self-associate, and adsorb to interfaces (McClements 2002).

The exact behaviour of proteins in real food systems is not easily understood because of the modification or denaturation of proteins during food processing and preparation (Damodaran and Paraf 1996b). This denaturated state of protein is the conformation that the protein molecule adoptes when it completely unfolds and turns to a highly flexible random coil. The structural intermediates that exist between the native and denaturated forms of globular proteins are often referred to as molten globule states. 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).

Changes in environmental conditions, interactions of proteins and the methods used for protein isolation can cause variations in the initial conformations of proteins (Damodaran and Paraf 1996b). Frequently, to understand how functionality of proteins are affected by different factors, researchers worked in model systems by changing conditions such as pH, temperature and ionic strength, and investigating interactions of the protein with other proteins and non-proteins components. The use of more than one model system is required when properties of the protein are affected by one of these conditions. In some cases, protein functionality might be predicted by analysing solubility and hydrophobicity data with multiple regression analysis. However, at present, the evaluation of the functionality of food proteins is strictly empirical and the development of a standard methodology for predicting the functional properties of a protein in various foods is necessary. Because of the complexity of foods and variation in the protein content and its physical properties, it is hard to develop models to estimate the functionality of protein in food. 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).

The functionality of proteins can yield a benefit for industrial purposes and human health. In the food, drug and cosmetics industries the functional properties of proteins such as emulsification, foaming, water or oil binding are very frequently employed. The essential amino acids of proteins provide the basic nutritional

requirements while some other functional properties such as antioxidant activity provide health benefits against diseases such as cancer and cardiovascular diseases.

The functionalities of proteins may be modified by using enzymatic and chemical treatments in order to change their structure to give better functional properties. Also by using genetic engineering it is possible to modify the functionality of proteins (Sikorski 2006). However, it is also very practical and economical to find a good natural source of a functional protein. Consequently, research to find new sources of food proteins with novel or superior functions is a growing field of interest (Table 4.1).

Table 4.1. Functional Roles of Food Proteins in Food Systems

(Source: Damodaran and Paraf 1996)

Function	Mechanism	Food	Protein type
Solubility	Hydrophilicity	Beverages	Whey proteins
Viscosity	Water binding, hydrodynamic size and shape	Soups, gravies, salad dressings, desserts	Gelatin
Water binding	Hydrogen bonding, ionic hydration	Meat sausages, cakes, breads	Muscle and egg proeins
Gelation	Water entrapment and immobilisation, network fomation	Meats, gels, cakes, bakeries, chese	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 proeins
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

(cont. on next page)

Table 4.1.(cont.) Functional Roles of Food Proteins in Food Systems
(Source: Damodaran and Paraf 1996)

Fat and flavor binding	Hydrophobic bonding	Low-fat bakery products, doughnut	Milk, egg, and cereal proteins

4.1.1. Protein Hydration

In living organisms, water is an essential molecule for nearly all biological functions because it interacts with other biological molecules such as proteins, carbohydrates, and vitamins. Water is the main solvent in the in vivo environment and the native structure of a protein is a consequence of its interaction with water, so that protein functionality mainly depends on protein water interaction (McClements 2002). The thermodynamics of protein-water interactions dominantly influence dispersibility, wettability, swelling, solubility, thickening/viscosity, water-holding capacity, gelation, coagulation, emulsification, and foaming of proteins. Hydrodynamic properties of proteins are results of molecular size, shape, and flexibility and their interactions with water. In addition, surface-active properties are consequences of the thermodynamically unfavourable interaction of exposed non-polar patches of proteins with solvent water (Damodaran and Paraf 1996b, McClements 2002).

Water molecules bind to both polar and non-polar groups in proteins through dipole-dipole, charge-dipole, and dipole-induced dipole interactions. The hydration capacity depends on the proportion of hydrophilic and hydrophobic amino acids. Thus, the greater number of hydrophilic residues on the protein surface, the greater the hydration capacity (Damodaran and Paraf 1996b). For several monomeric globular proteins, the experimental hydration capacities may be calculated by using some mathematical equations. However, the same equations can not be used for oligomeric proteins if subunit surfaces are partially buried due to protein-protein interactions. 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 (Table 4.2) (Damodaran 1996a).

Table 4.2. Hydration capacities of amino acid residues

(Source: Damodaran 1996a)

Amino acid residue	Hydration (moles H_2O/mol residue)
Polar	
Asn	2
Gln	2
Pro	3
Ser, The	2
Trp	2
Asp (unionized)	2
Glu (unionized)	2
Tyr	3
Arg (unionized)	3
Lys (unionized)	4
Ionic	
Asp^-	6
Glu^-	7
Tyr^-	7
Arg^+	3
His^+	4
Lys^+	4
Nonpolar	
Ala	1
Gly	1
Phe	0
Val, Ile, Leu, Met	1

Water binding to a protein is a stepwise process. Primarily, ionic groups on the protein surface are solvated and form an unfreezable monomolecular layer of water on the protein surface. This immobile water is called bound water (Damodaran 1996a, Damodaran and Paraf 1996b). After formation of the bound water layer, water continues to bind protein by polar and non-polar groups and water activity increases. When hydration is complete at the polar surface, hydrophobic hydration of non-polar residues starts. In other steps, bulk water condenses into the cracks and cavities of protein molecules. This water moves with the protein molecule and is known as hydrodynamic water (Figure 4.1) (Damodaran 1996a).

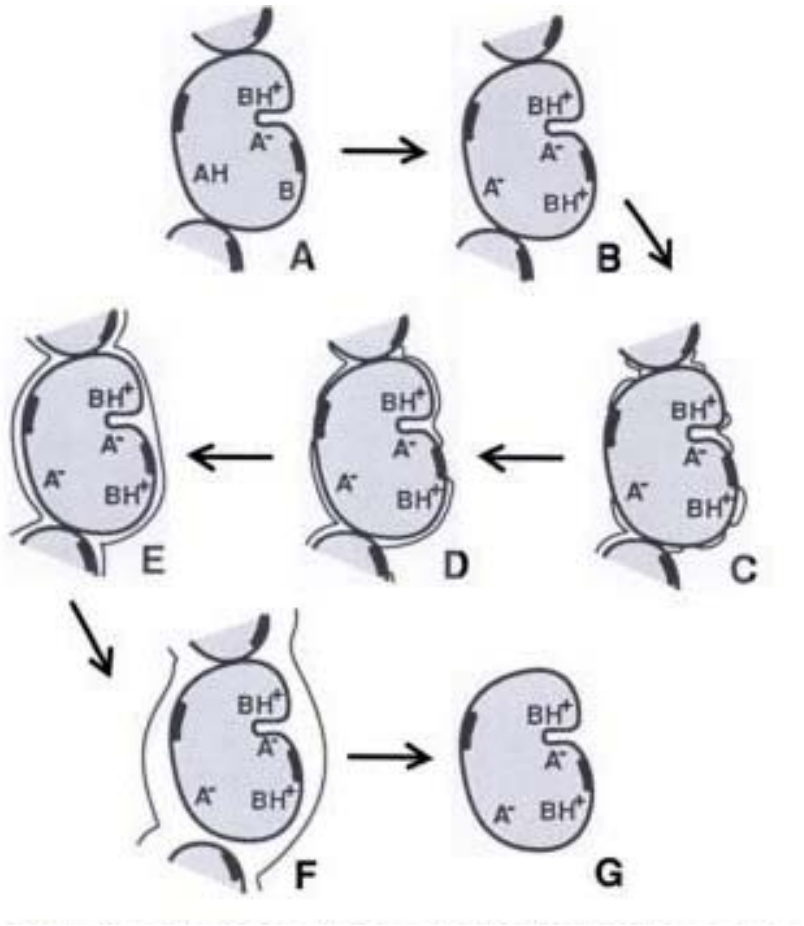


Figure 4.1. Sequence of Steps Involved in Hydration of Protein (A) Unhydrated protein. (B) Initial hydration of charged groups. (C) Water cluster formation near polar and charged sites. (D) Completion of hydration at the polar surface. (E) Hydrophobic hydration of non-polar patches; completion of monolayer coverage. (F) Bridging between protein-associated water and bulk water. (G) Completion of hydrodynamic hydration (Source:Damodaran 1996).

The water-binding capacity of proteins depends on environmental factors and protein conformation. At the isoelectric point of a protein, water binding capacity is very low due to maximal protein-protein interactions but minimal protein-water interactions. With increasing net charge and repulsive forces, protein-water interaction increases. A low concentration of salt also increases protein-water interactions due to weak binding of hydrated salt ions to charged groups on proteins (Damodaran 1996a). These hydrated salt ions increase water binding since they associate with water. On the other hand, at high salt concentrations water is bound to the salt ions and it causes dehydration of the protein.

Temperature decreases the water binding capacity by decreasing hydrogen bonding and hydration of ionic groups. However, denaturation by temperature helps binding of water by increasing the surface area to mass ratio and exposure of buried hydrophobic groups. In contrast, if denaturation causes aggregation of the proteins, water-binding capacity decreases because of increasing protein-protein interactions (Damodaran and Paraf 1996b).

Water-binding 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 4.3) (Damodaran 1996a). On the other hand, water-holding capacity refers to the ability of the protein to imbibe water and retain it against gravitational force within the protein matrix. Physically entrapped water, bound water and hydrodynamic water are the components of imbibed water (Damodaran 1996a). There is a positive correlation between water-holding capacity and water-binding capacity.

Table 4.3. Hydration Capacities of Various Proteins
(Source: Damodaran 1996a)

Protein	Hydration capacity (g water/g protein)
Pure proteins[a]	
Ribonuclease	0.53
Lysozyme	0.34
Myoglobin	0.44
β-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.

In meat and fish tissues having fibrous nature and compartmentalization of the muscle, water is held by both protein-water interactions and physical entrapment. Water-holding capacity is the main factor affecting texture and juiciness of meat and fish products. Thus, decrease of water-holding capacity brings about excessive cooking loss and thawing drip loss (Sikorski 2006).

4.1.1.1. Solubility

According to their solubility, proteins are classified into four categories: (1) albumin; soluble in water at pH 6,6 (e.g. serum albumin, ovalbumin); (2) Globulin; soluble in dilute salt solution at pH 7,0 (e.g. glycinin, phaseolin); (3) Glutelin; soluble only in acid (pH 2,0) and alkaline (pH 12,0) solutions (e.g. wheat glutelins); (4) Prolamin; soluble in 70% ethanol (e.g. zein, gliadins). Many of the functional properties of proteins show their effect when the protein is fully dissolved in water. Water solubility is defined as the concentration of protein present in an aqueous solution that is in equilibrium with protein-protein and protein-solvent interactions (Damodaran and Paraf 1996b , McClements 2002).

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

In food chemistry, the solubility of proteins is often defined as the percentage of the total quantity of protein contained in the food material that can be extracted by water or a suitable solvent in specific conditions (Sikorski 2006). For development and testing of a new protein ingredient, the solubility of the protein is one of the first tests to understand its functionality since other functional properties such as foaming, gelling, emulsion property, and thickening are generally related to solubility (Damodaran 1996a, Zayas 1997). For utilization of proteins in beverages and liquid foods, solubility is the main characteristic to obtain dispersed colloidal systems (Zayas 1997).

Average hydrophobicity of amino acid residues and charge frequency are two very important factors influencing the solubility of proteins in aqueous solution (Damodaran and Paraf 1996b). Moreover 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 other

effectors that determine protein solubility (Sikorski 2006, Zayas 1997). In highly polar solvents such as water, glycerol, formamide, dimethylformamide or formic acid, proteins favour solubility because of increasing electrostatic repulsion and hydration of charged residues (Belitz, et al. 2004, Damodaran 1996a). In a less polar solvent such as ethanol, proteins are rarely soluble because of decreasing electrostatic repulsion. On the other hand, proteins having abundant hydrophobic groups easily dissolve in organic solvents (Belitz, et al. 2004, Sikorski 2006). Organic solvents cause unfolding of the protein molecules and exposure of the hydrophobic residues owing to a low dielectric constant (Sikorski 2006).

For protein solubility, the amino acid composition of the protein surface is considered more than overall amino acid composition of the protein since solubility occurs with the interaction of surface amino acids with the solvent (Damodaran and Paraf 1996b). Hydrophilic interactions promote protein-water interactions therefore solubility increase. On the other hand, hydrophobic interactions lead to decrease of solubility (Damodaran and Paraf 1996b).

The pH of the medium is the determining factor of protein solubility. The degree of solubility in an aqueous medium is governed by repulsive and attractive intermolecular forces (Zayas 1997). For most proteins, the isoelectric pH is the pH value at which solubility is the minimum, and hydrophobic interactions on the protein surface are maximum. The exception is whey proteins. Whey proteins have a neutral charge at their isoelectric point. They also have many charged and uncharged hydrophilic residues on their surfaces and hydration of these residues prevents aggregation that would occur by hydrophobic interactions (Belitz, et al. 2004, Damodaran and Paraf 1996b). At lower and higher pH values, protein-water interactions increase due to formation of positive or negative charges of proteins, respectively. Solubility is enhanced at alkaline pH values increasing the net negative charge of proteins. Thus, alkali treatment is generally used for increasing soy and other protein solubility by causing dissociation and disaggregation of proteins (Zayas 1997).

Ions influence protein solubility according to their ionic strength and their effect on the surface tension of the solvent. Adding low concentrations of neutral salts into the protein solution increases the solubility because they interacts with surrounding water molecules and contribute to the formation of the hydration layer on the protein surface. This effect is called salting-in. In contrast, higher concentrations of salts dehydrate proteins and cause their precipitation. This process is called salting-out and it is used for

extraction and fractionation of proteins (Damodaran 1996a, Damodaran and Paraf 1996b, Sikorski 2006). For precipitation of proteins, multivalent anions are more effective than monovalent anions, while divalent cations are less effective than monovalent cations (Belitz, et al. 2004).

Thermal treatments such as pasteurization, sterilization, cooking, freezing, and chilling are of the most important processing operations that used in the food industry. Heat denaturation and cold denaturation occur by the effect of hydrophobic interactions, electrostatic interactions, hydrogen bonds, van der Waals forces and steric interactions (McClements 2002). Thermal denaturation generally influences the stability of proteins negatively owing to interactions of the surface exposed hydrophobic or reactive groups (Sikorski 2006). Between 0 and 40-50°C, the solubility of protein increases except highly hydrophobic proteins. When the temperature is above 50°C, irreversible denaturation occurs and leads to decrease of solubility (Damodaran 1996a, Zayas 1997). Heat denaturation alters the surface hydrophobicity-hydrophilicity of proteins and favours protein-protein interactions (Damodaran and Paraf 1996b). In some proteins such as collagen, the conformational changes may increase solubility (Sikorski 2006). Cold denaturation during freezing or frozen storage depends on effects of different factors. These factors are freezing rate, freezing and storage temperature, storage time, stability of storage conditions (especially temperature), and thawing methods and conditions (Zayas 1997).

4.1.1.2. 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 (Damodaran and Paraf 1996b - Sikorski 2006). 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 (Belitz, et al. 2004, Damodaran and Paraf 1996b). Proteins in foods have the ability to entrap water and other water-soluble components through capillary forces in the three-dimensional network. These networks formed by intermolecular cross-links show the characteristics of both elastic solids and viscous liquids and are responsible for elasticity and textural strength (McClements

2002, Sikorski 2006). Food gels formed by proteins may participate in gel formation in the form of solutions, dispersions, and micelles (Sikorski 2006).

Gelation starts by dissociation of the quaternary structure and unfolding of a protein. Mostly heating is applied and proteins expose non-polar and sulfhydryl amino acid side groups that are normally located in the protein interior (McClements 2002, Sikorski 2006). In the second step, these reactive side groups of unfolded molecules interact with each other and rearrange so that the proteins aggregate and form a pro-gel (Damodaran and Paraf 1996b, McClements 2002, Sikorski 2006). The gelation is completed by cooling of the pro-gel form of the viscous solution and the structure is stabilized (Damodaran and Paraf 1996b - Sikorski 2006).

Proteins in foods may form two types of gels according to the formation process and stabilization factors. If a protein contains mainly hydrophilic amino acids and hydrogen bonding is the driving force to form gel structure, the gel is a thermo-reversible translucent type gel. On the other hand, when the protein has a high frequency of hydrophobic non-polar amino acids and hydrophobic interactions are the main driving force for gelling, the gel is a thermo-plastic (thermo-irreversible) coagulum type gel (Belitz, et al. 2004, Damodaran and Paraf 1996b, Sikorski 2006). In translucent type gel, intermolecular hydrogen bonds easily break when heated. The gel forms when a solution cools and melts again when it is heated. In contrast, thermo-plastic coagulum type gel does not liquify when heated. Translucent type gels contain more water compared to a coagulum type gel (Damodaran and Paraf 1996b). Hydrogen bonding, electrostatic interactions depending on pH, charge of molecules, ionic strength and divalent ions, hydrophobic interactions, and intermolecular disulfide bonds are the stabilization factors of gel structure (Figure 4.2) (Belitz, et al. 2004, Damodaran and Paraf 1996b, Sikorski 2006).

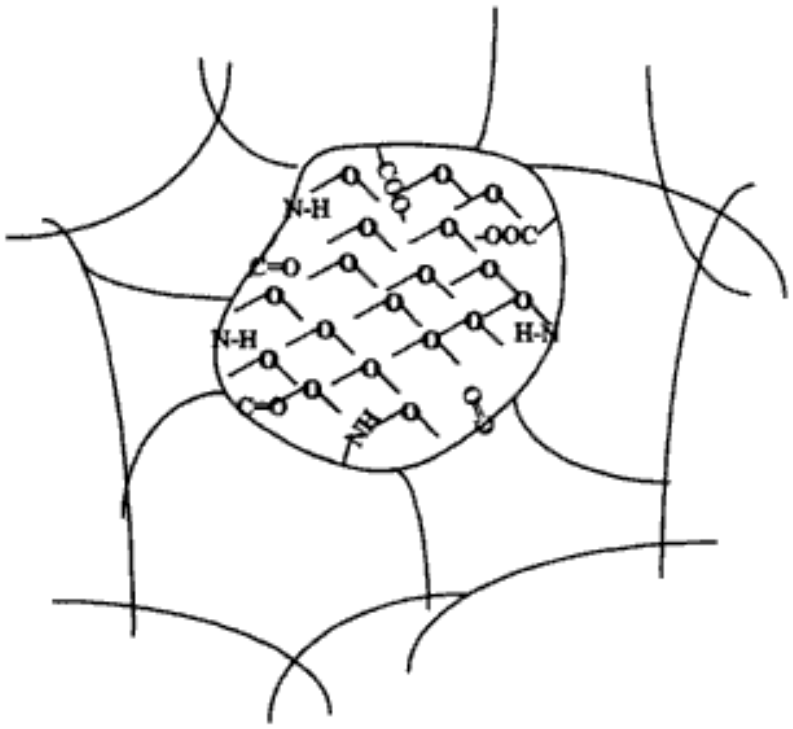


Figure 4.2. Hypothetical hydrogen-bonded state of water in a protein gel matrix (Source: Damodaran and Paraf 1996)

4.1.1.3. Flavor Binding

Proteins are odourless and they can bind small molecular weight flavorants with hydrophobic pockets or crevices on the protein's surface (Damodaran and Paraf 1996b Walsh 2002). These bound flavor compounds affect the sensory attributes of proteins and the flavor of a food product which is determined by specific receptors in the nose (aroma) or in the tongue and mouth (taste) (Damodaran 1996a - McClements 2002). The flavor binding ability of proteins is sometimes desirable, particularly if a desired flavour needs protection form harsh processing conditions. In contrast, during production of protein extracts intended for use in the food, drug and cosmetics industries, flavour binding is undesirable. Aldehydes, ketones and alcohols generated by oxidation of unsaturated fatty acids are also undesirable flavors because they can covalently bind to amino groups of lysyl side chains located in the hydrophobic parts of proteins (Damodaran 1996a).

In food applications, proteins are sometimes used as flavour carriers. Such proteins should bind flavors 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 flavor may be different than the expected one. The bound flavour should release during chewing in the mouth. Thus, the binding should be non-covalent be able to contribute to aroma and taste of the protein-based product (Damodaran 1996a, Damodaran and Paraf 1996b)

The flavor mechanism mainly depends on the moisture content of protein, even though the interactions are generally non-covalent. Dry protein powders bind flavors 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 (Burova, et al. 1999). In liquid or high moisture foods, flavors bind to protein by hydrophobic interactions (Damodaran 1996a, Damodaran and Paraf 1996b). 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 (Damodaran 1996a). 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 (Damodaran and Paraf 1996b).

4.1.1.4. Antioxidant Properties of Proteins

For human health, lipid oxidation and free radical formation are major concerns causing many diseases. The same factors are also the major causes of loss of food quality. Although, synthetic antioxidants such as BHA and BHT show strong antioxidant activity against oxidation, the use of these artificial antioxidants in foodstuffs is restricted or prohibited in some countries due to potential risks for human health (Saiga, et al. 2003). BHA and BHT show suspicious carcinogenic effects on laboratory animals (Madhavi et al. 1996). Thus, it is necessary to develop natural antioxidants such as polyphenolic compounds, caretonoids and proteins. Proteins show antioxidant activity by free radical scavenging and chelation of prooxidative transition metals (Elias, et al. 2005). In proteins, the type of amino acids, sequence, distribution of hydrophobic residues, structure and length of polypeptide, and position of amino acids in the chains are determining factors of antioxidant potential (Saiga, et al. 2003, Chen, et al. 1996, Rajapakse, et al. 2005). Aromatic amino acids and sulphur-containing amino

acids especially exhibit stronger 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 (Rajapakse, et al. 2005, Elias, 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 that are prooxidants for free radical formation (Saiga, et al. 2003). Histidine exhibits bifunctional antioxidant activity with its imidazole ring by serving as both a free radical scavenger and metal chelator (Saiga, et al. 2003, Chen, et al. 1996). The hydrophobicity of proteins has an important influence on antioxidant activity since this 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 et al. (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 hydrolysis of proteins by proteases and acidic hydrolysis can increase the antioxidant activity proteins by exposing their functional antioxidant groups (Saiga, et al. 2003, Elias, et al. 2005).

4.1.1.5. Dough Formation of Proteins

Dough formation is one of the functional properties of proteins important for production of bread and bakery products. To produce bakery products, wheat flour is predominantly used. Thus, characteristics of wheat proteins are the main factor that determines the quality of dough (Peresini 2008). Processing conditions and covalent and non-covalent interactions formed in the proteins are the components of dough formation. In wheat flour, gluten is the main structure-forming protein and it is responsible for the rheological characteristics of dough (Lorenzo 2008). Depending on process conditions, viscoelasticity, extensibility, resistance to stretching, mixing tolerance, and gas holding capacity may vary strongly (Lorenzo 2008, Peresini 2008). Gluten is a mixture of gliadin and glutenin proteins and its amino acid composition affects the functionality of gluten in the dough. Dough structure is based on an extensive three-dimensional network of gluten protein sub-units joined together by disulfide cross-links (Davidek et al. 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 (Pena 2006). Different glutenins may combine into oligomers and this highly aggregated glutenin fraction is related to dough properties (Peresini 2008). The high glutamine and hydroxyl amino acid residues give gluten water binding properties whereas cysteine and cystine residues contribute to polymerization of gluten proteins due to sulfhydryl-disulfide interchange reactions.

4.1.2. Surface Activity of Proteins

Proteins are amphiphilic molecules that have the ability to adsorb to boundaries separating two phases (air-water or water-oil). This property of proteins has a great influence on their effectiveness as emulsifier and foaming agents (Damodaran 1996a, McClements 2002). Depending on their surface activity and concentration, proteins may form a viscoelastic film at the interfaces of different phases. A good surface active protein should rapidly adsorb to an interface, unfold and reorient at an interface, and interact with the neighbouring molecules to form a strong cohesive, viscoelastic film (Damodaran 1996a). The main determinant of the surface activity of proteins is the protein conformation. Stability/flexibility ratio of its polypeptide chain(s), ease of adaptability to changes in the environment, and distribution of hydrophilic and hydrophobic groups in the protein surface have a large influence on their surface activity.

Proteins migrate spontaneously from the bulk phase to the interface because the free energy of the interface is lower than the free energy of the bulk phase (Damodaran 1996a). The rapidity of protein adsorption to the interface is related to the distribution of hydrophobic and hydrophilic residues on the protein surface. If the surface has a large number of hydrophobic groups migration to the interface is favourable, whereas proteins having a large number of hydrophilic residues cannot migrate rapidly to the interface because its free energy will be lower in the aqueous phase compared to the interface (Damodaran 1996a). Many proteins undergo conformational changes after adsorption to the interface and this unfolding property of proteins may promote interactions such as attractive electrostatic interactions, hydrogen bonding, hydrophobic interactions and disulfide bonds between neighbouring proteins. This tendency may

result in formation of a viscoelastic interfacial region (McClements 2002). The viscoelastic-film formation property of proteins is essential to prevent destabilization of the foam or emulsion by mechanical shocks that occur during processing, storage and handling (Damodaran 1996a). This property of proteins makes them superior surface active agents than low molecular weight chemical surfactants. Low molecular weight surfactants have hydrophobic and hydrophilic groups only at the ends of the molecule. Thus, they have a conformational limitation to show orientation at the surface (Figure 4.3) (Damodaran 1996a).

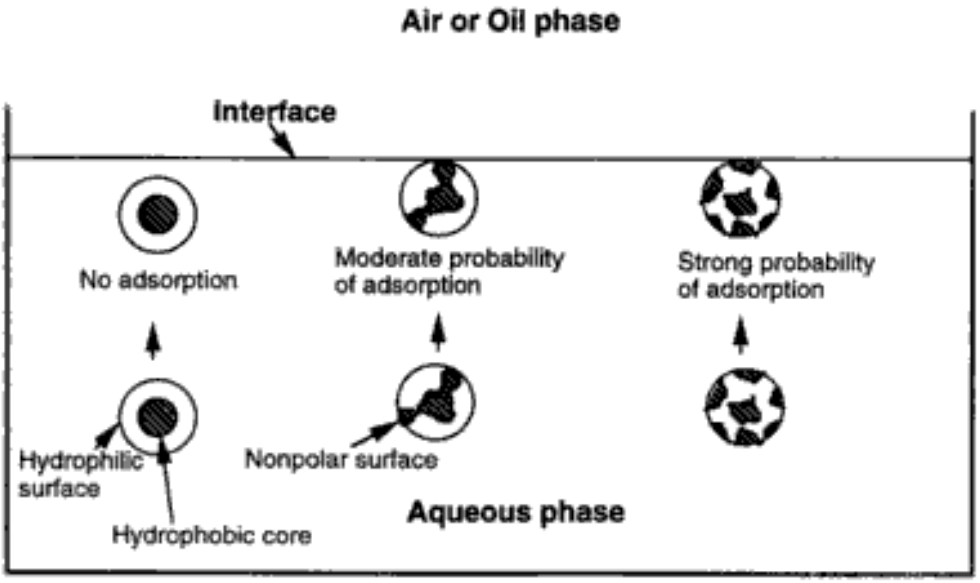


Figure 4.3. Schematic representation of adsorption of proteins at the air-water interface (Source: Damodaran 1996b).

At the interface polypeptide chains may form train, loop, and tail configurations. Depending on the conformational characteristic of proteins, trains are the proportions that directly contact with the interface, while loops suspend in the aqueous phase. The tails are N- and C- terminal sites and usually they are located in the aqueous phase. Train configuration contributes to stronger binding and lower interfacial tension (Figure 4.4) (Damodaran 1996a).

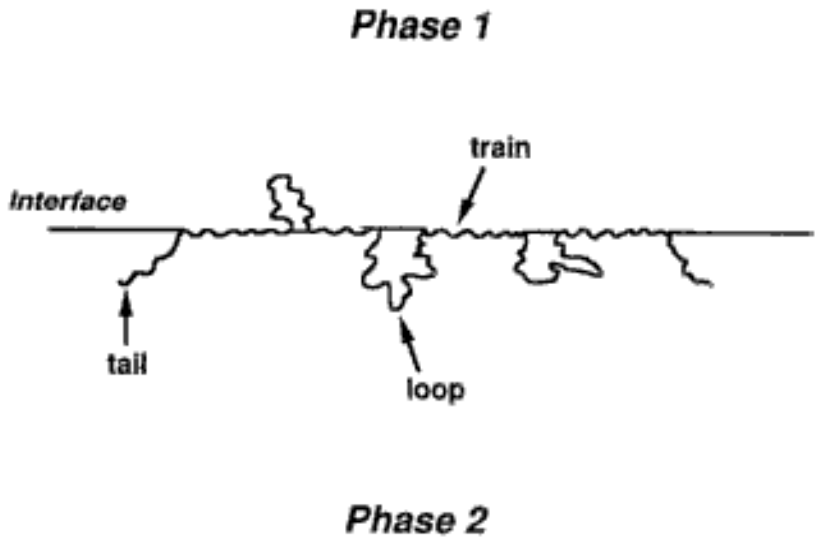


Figure 4.4. Various configurations of a flexible polypeptides at an interface (Source: Damodaran 1996a).

4.1.2.1. Emulsifying Properties of Proteins

Emulsions are dispersed systems of small liquid droplets in the continuous phase of an immiscible liquid (Belitz, et al. 2004, Sikorski 2006). In oil-in-water interfaces, proteins having amphipathic character can adsorb to oil-water interfaces and protect oil droplets against aggregation so they behave as an emulsifier (Belitz, et al. 2004, McClements 2002). For emulsifying activity, proteins must rapidly adsorb to surfaces of newly created oil droplets formed during homogenization. By adsorption of proteins, interfacial tension decreases and this leads to further droplet disruption. Also, this reduces the amount of energy to generate small droplets, and form a protective membrane to prevent droplet aggregation (McClements 2002). Adsorption is thermodynamically favourable because hydrophobic amino acid residues can be away from the hydrogen bonding network of the surrounding water in the bulk phase. When the protein contacts with the oil phase, water molecules are displaced from the hydrophobic regions of the oil-water boundary layer. In this mechanism, the diffusion rate depends on temperature, molecular weight, pH, and ionic strength; adsorbability depends on exposure of hydrophilic and hydrophobic groups; and conformative stability depends on amino acid composition, molecular weight, and intramolecular disulfide bonds (Belitz, et al. 2004). In addition, presence of low-molecular-weight surfactants, sugars, oil-phase volume, type of protein, and the melting point of the oil used are the

other intrinsic factors. Moreover, type of equipment, rate of energy input, and rate of shear are the extrinsic factors that influence the emulsion property of proteins (Damodaran 1996a). As a result, 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). Proteins are very suitable for oil-in-water food emulsions. However, their limited solubility in oil prevents their use in water-in-oil emulsions.

The protein film, which forms around the oil droplet, prevents the droplets from coalescing and flocculation with each other by using repulsion provided by its electrostatic charges and steric hindrance (McClements 2002, Sikorski 2006). The coating of lipid droplets by protein provides an energy barrier to coalescence. The net positive charge or negative charge formed by protein at the film surface creates a repulsion among different oil droplets and prevents their floculation. Flocculation is formation of clusters of globules and thus rapid creaming due to the action of gravitational force (Sikorski 2006). The stability of emulsions depends on van der Waals interactions, steric hindrance and electrostatic interactions (repulsion or attraction), among the droplets. Droplet coalescence may result in an increase in mean droplet size that decreases the creaming stability, causes changing of emulsion appearance, and oiling-off (McClements 2002). In dilute emulsions, flocculation causes the increased product viscosity and the decreased creaming stability. In concentrated emulsions, flocculation leads to the formation of a three-dimensional particle network that gives the product gel-like qualities. (McClements 2002, Sikorski 2006).

Solubility, pH, surface hydrophobicity, and partial denaturation affect the emulsion property of proteins. Proteins capable of creating electrostatic repulsive interactions at isoelectric point are loaded at the interface and promote formation of a highly viscoelastic film contributing to emulsion stability. Besides that, proteins having high solubility at the isoelectric point show maximum emulsifying activity and emulsifying capacity. In contrast, proteins insoluble at the isoelectric point or poorly hydrated and lacking electrostatic repulsive forces are known as the poor emulsifiers (Damodaran 1996a).

Surface hydrophobicity defined by the portion of non-polar surface of the protein that is in contact with the surrounding bulk water increases the emulsifying activity by decreasing surface tension (Damodaran 1996a). Proteins often partially

unfold after they adsorb to the surface of emulsion droplets. Protein-protein interactions between proteins adsorbed to the same droplet cause increase of the viscoelasticity of the interfacial membrane by polymerization through disulfide-sulfhydryl interchange reaction, whereas interaction of proteins adsorbed onto different droplets lead to droplet flocculation (McClements 2002).

4.1.2.2. Foaming Property

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 (McClements 2002, Sikorski 2006). In foam formation, proteins are the main surface-active agents that help lower the gas-liquid interfacial tension and the formation of a rupture resistant, flexible, cohesive film surrounding the bubbles (Belitz, et al. 2004, Damodaran 1996a, Sikorski 2006). Generally, foams are produced by bubbling, whipping, or shaking a protein solution (Damodaran 1996a, McClements 2002). Proteins adsorb at the surface of gas bubbles through hydrophobic areas and undergo conformational changes to produce a thin, firm film at gas-liquid interfaces (McClements 2002, Sikorski 2006). Protein-protein interactions during unfolding make the gas bubbles highly viscoelastic and resistant to deformation (McClements 2002).

Foam stability is the ability of a foam to resist gravitational and mechanic stress (Damodaran 1996a, McClements 2002). The foams may be disproportionate by destabilization factors such as drainage of the liquid from the intersheet 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, Sikorski 2006). When the proteins are soluble at their isoelectric pH, foamability and foaming stability are higher because protein-protein interactions and formation of a viscoelastic film at the interface are promoted. In general, proteins are less soluble at their isoelectric pH. Thus, the soluble protein fraction would form little of the volume of foam, but the insoluble fraction would contribute to the foam stability by increasing cohesive forces in the protein film (Damodaran 1996a). By adding salt ions such as divalent Ca^{2+} and Mg^{2+}, foam stability may be increased owing to cross-linking of protein molecules and creation of better viscoelastic properties. Salt ions contribute to the neutralization of charges resulting in salting out of the protein (Damodaran 1996a).

The lack of repulsion of charges increases the amount of protein absorbed to the interface and foam stability increases. On the other hand, if the solution is incubated with the salts for a long time, aggregation and micellization of proteins may be promoted and this situation results in reduction of film formation ability (Sikorski 2006). Sugars also increase foam stability by increasing the viscosity of the bulk-phase and reducing the drainage of the lamella fluid.

In some cases, heating has a positive impact on foam formation and foam stability depending on gelation of the protein at the interface. Although sufficient mechanical strength developed during heating stabilizes the foam, expansion of air and decreasing viscosity may cause bubble rupture and collapse of the foam (Damodaran 1996a). Generally, proteins having high foaming capacity show less foam stability, while proteins having good foam stability have poor foaming capacity (Damodaran 1996a). This occurs due to contradicting factors affecting foam forming capacity and foam stability. Adsorption, flexibility, and hydrophobicity mainly affect foam forming capacity, while foam stability is influenced by rheological properties of the protein film. On the other hand, non-covalent interactions and disulfide bonds between the loops help the production of a gel network having certain viscoelastic and mechanical properties (Damodaran 1996a). Foam stability may be improved by chemical and physical modifications. Partial enzymatic hydrolysis produces smaller and more quickly diffusing protein molecules having better solubility. Foam stability may also be improved by introducing charged or neutral groups to proteins and by partial thermal denaturation.

CHAPTER 5

MATERIALS AND METHODS

5.1. Materials

The cultivars were provided by the General Directorate of Agricultural Research in Ankara (Turkey) and the Aegean Agricultural Research Institute in Menemen (Turkey) (Table 5.1). Acetone, ethanol, hydrochloric acid (32%), orto- phosphoric acid, acetic acid (96%), di-sodium hydrogen phosphate, sodium carbonate, sodium hydroxide pellets, sodium chloride, sodium dihydrogen phosphate monohydrate, dodecyl sulphate sodium salt, potassium peroxodisulfate, potassium sodium tartrate tetrahydrate and Coomassie Brilliant blue G-250 were purchased from Merck KGaA (Darmstadt). ABTS (2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid), sodium nitrite and bovine serum albumin were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Ferrozine (3- (2-Pyridyl)-5,6-diphenil-1,2,4-triazine-4'4''-disulfonic acid Monosodiun salt, ferrous chloride tetrahydrate, aluminum chloride phosphate and Folin-Ciocalteu's phenol reagent were purchased from Fluka (Switzerland).

Table 5.1 Cultivars

CULTIVARS	**SOURCE**
Chickpea	
Canıtez	General Directorate of Agricultural Research
Cevdetbey 98	Aegean Agricultural Research Institute
Gökçe	General Directorate of Agricultural Research
Sarı 98	Aegean Agricultural Research Institute
Lentil	
Ali dayı (red)	General Directorate of Agricultural Research
Çiftçi (red)	General Directorate of Agricultural Research
Fırat (red)	General Directorate of Agricultural Research
Kafkas (red)	General Directorate of Agricultural Research
Meyveci (green)	General Directorate of Agricultural Research
Pul II (green)	General Directorate of Agricultural Research

5.2. Methods

5.2.1. Determination of Total Phenolic and Flavonoids Content and Antioxidant Capacity of Different Turkish Chickpea and Lentil Cultivars

5.2.1.1.Preparation of Legume Water Extracts

To prepare legume water extracts, 10 g chickpeas or lentils were first rehydrated in 50 ml distilled water for 16-18h at room temperature. A 10 g rehydrated sample was then crushed in a ceramic mortar. The obtained paste was further homogenized in a Waring blender in 90 ml distilled water for 3 minutes and the homogenate was filtered through 3-layers of cheesecloth to collect the filtrate (the cake was collected for ethanolic extraction). The 30 ml of the obtained filtrate was centrifuged for 30 min at 15000 g (+4°C) for clarification and assayed for its total phenolic and flavonoids content and antioxidant capacity. This extract is designated aqueous extract. The precipitate obtained during centrifugation was combined with the precipitate obtained from cheesecloth filtration. The total precipitate was then suspended in 30 ml ethanol and homogenized with an IKA homogenizer-disperser at 14000 rpm for 2 min. The homogenate was clarified by centrifugation at 15000 g (+4°C) for 30 min and then assayed for its total phenolic and flavonoids content, and antioxidant capacity. This extract was designated ethanolic extract.

5.2.1.2. Determination of Total Phenolic Content of Legumes

The total phenolic content of crude legume water extracts was determined using the Folin-Ciocalteu method of Singleton and Rossi (1965). A 0.5 ml sample of appropriately diluted aqueous or ethanolic extract was mixed with 5 ml of 1/10 diluted Folin-Ciocalteu reagent. After 3 minutes incubation, 2 ml of a 7.5 % Na_2CO_3 solution was added to the mixture and shaken. The mixture was further incubated for 2 hours, and its absorbance at 765nm was measured with a spectrophotometer. Total phenolic contents of legumes were expressed as milligrams of gallic acid equivalents per kg of dry legumes. All measurements were conducted five times.

5.2.1.3. Determination of Total Flavonoids Content of Legumes

The total flavonoids content of legumes was determined using the method described by Zhishen (1999). Before analysis 1 ml of legume water extract was diluted with 4 ml of distilled water. Then, 0.3 ml of 5 % $NaNO_3$ was added into the diluted sample and mixed. After 5 min incubation, 0.3 ml of 10 % $AlCl_3$ was added into the mixture and it was further incubated for 6 min. At the end of the incubation period, 2 ml of 1 M NaOH solution was added into the mixture and its absorbance was determined at 510nm following dilution with 2.4 ml distilled water. The total flavonoids content was expressed as milligrams of epicatechin equivalents per kg of dry legumes. All measurements were conducted five times.

5.2.1.4.Determination of Free Radical Scavenging Capacity of Legumes

The antioxidant activity of legumes used in this work has been mainly based on free radical scavenging capacity. The tests were conducted using the ABTS radical by the method given in Re et al (1999). The ABTS free radical cation was obtained by treating 7 mM ABTS solution with 2.45 mM potassium persulfate. The ABTS radical solution was diluted with 5 mM pH 7.4 phosphate buffer containing 150 mM NaCl (PBS) until its absorbance reached 0.70 units at 734 nm. The reaction mixture was prepared by mixing 5, 10 or 15 µl of legume water extract with 2 ml of ABTS radical cation solution. The absorbance of each reaction mixture was then monitored and recorded after 1, 3, 6, 9, 12, and 15 min. To calculate the AUC, the percent inhibition / concentration values for the extracts and trolox were plotted separately against test periods. The division of the areas of curves for each legume water extract to that of trolox was used to calculate the AUC value. All measurements were conducted three times and antioxidant activity was expressed as trolox equivalents (mmol) per kg of dry legumes.

5.2.1.5. Determination of Iron Chelating Capacity of Legumes

For determination of iron chelating capacities of legumes, the method given by Rajapekse et al. (2005) was used with small modifications. In this method, 2 ml of

legume water extract was mixed with 0.1 ml, 1 mM $FeCl_2.4H_2O$ solution and the mixture was incubated for 30 min at 30°C. Following incubation, 0.1 ml 5mM ferrozine was added into the mixture and the mixture was incubated at 30°C for 10 min. The absorbance of the mixtures were then determined at 562nm. The intensity of the developed blue colour indicates weak iron chelating capacity. For each sample, the percentage of chelation of iron was determined from the equation given below and the results were then expressed as millimoles of EDTA (a standard metal chelating agent) per kg of dry legumes. All measurements were conducted three times.

% iron chelating = [1- (absorbance of the sample at 562 nm) / (absorbance of control at 562 nm)] × 100.

5.2.2. Determination of Functional Properties of Proteins for Different Turkish Chickpea and Lentil Cultivars

5.2.2.1. Preparation of Acetone Powders Used for Protein Extraction

To remove phenolic compounds and lipids, legumes were processed to acetone powder according to the method given by Arcan and Yemenicioğlu (2006). For this purpose, 70 g legume samples were rehydrated in 200 ml distilled water for 16-18h. 100g rehydrated legume was then homogenized in a Waring blender for 2 min with 200 ml cold acetone. The obtained slurry was filtered using a Buncher funnel under vacuum with Whatman No:1 filter paper and the solid residue on the filter paper was collected. This residue was extracted two more times at the same conditions by using fresh cold acetone and the final residue was left overnight to evaporate the acetone. The dry acetone powder obtained was stored at -18°C until used for protein extraction.

5.2.2.2. Preparation of Chickpea or Lentil Protein Extract

Chickpea and lentil proteins were extracted by the alkaline extraction method given in Kaur (2006). This method extracts albumin and globulin fractions which form most of the legume proteins (Wang, et al. 2003). To obtain the total protein extract (TPE) of chickpeas or lentils, 20 g acetone powder was suspended in 250 ml distilled

water under continuous magnetic stirring. The pH of the suspension was then adjusted to 9.5 by 1N NaOH solution to maximize protein solubility. For preparation of chickpea protein extract (CPE), the temperature of the obtained extract was brought to 85^{o}C to inactivate the highly active lipoxygenase enzyme. The chickpea extract was then continuously stirred at this temperature for 30 min for extraction and then it was cooled down to room temperature. For preparation of lentil protein extract (LPE), the extract was not heated to 85^{o}C, due to the low amounts of lipids in these legumes and extreme browning that occurred during heating at this temperature. Instead the extraction of lentil TPE was conducted at room temperature for 30 min under continuous magnetic stirring. At the end of the extraction period the pH of both chickpea and lentil extracts was adjusted to 7.0 with 1N acetic acid solution. The extracts containing solubilized albumin and globulins were clarified by centrifugation at 15000 x g (+4°C) for 30min. The clarified supernatant was then lyophilized for determination of functional properties and soluble protein content.

5.2.2.3. Determination of Soluble Protein Content of Protein Extracts

The soluble protein content of protein extracts were determined by the Bradford method using bovine serum albumin (BSA) as standard (Bradford 1976). The lyophilized protein extracts were prepared for analysis by dissolving in deionized water at pH 9.5. The solutions were magnetically stirred for 30 min at room temperature and centrifuged at 3500 g for 20 min to remove insoluble fractions. The sample preparation was repeated three times and the protein assay was conducted five times for each preparation.

5.2.2.4. Determination of Free Radical Scavenging Activity of Protein Extracts

The free radical scavenging activity of protein extracts were tested against ABTS free radical by the method of Re et al (1999) given in section 5.2.1.4. The lyophilized protein extract was prepared for analysis by dissolving in distilled water. This was conducted by suspending 0.1 g lyophilized protein in 9.9 ml distilled water by stirring with a magnetic stirrer for 30 min at 30°C. The solution was then centrifuged at

15000 g (+4°C) for 30 min for clarification. The reaction mixture was prepared by mixing 25, 50 or 75 µl of legume protein extract with 2 ml of ABTS radical cation solution. The absorbance of the reaction mixture was then monitored for 15 min. To calculate the AUC, the percent inhibition / concentration values for the extracts and trolox were plotted separately against test periods. The division of the areas of curves for each legume water extract to that of trolox was used to calculate the AUC value. All measurements were conducted three times and antioxidant activity was expressed as trolox equivalents (millimol) per kg of lyophilized protein.

5.2.2.4. Determination of Iron Chelating Capacity of Protein Extracts

The iron chelating capacity of lyophilized protein extracts were determined according to the method of Rajapekse et al. (2005) given in section 5.2.1.5. The lyophilized protein extract was prepared for analysis by dissolving in distilled water. This was conducted by suspending 0.1 g lyophilized protein in 9.9 ml distilled water by stirring with a magnetic stirrer for 30 min at 30°C. The solution was then centrifuged at 15000 g (+4°C) for 30 min for clarification.

5.2.2.5. Determination of Emulsifying Capacity and Emulsion Stability of Protein Extracts

Emulsifying capacities of lyophilized proteins were determined by the method described in Pearce and Kinsella (1978). In this method, 20 ml of 1% protein solution was suspended in distilled water. The pH of the protein solution was then adjusted to 7.0 and the solution was stirred for 30 min at 30°C. Then 6.5 ml of oil was added into the protein solution and emulsified by homogenization at 22000rpm for 2 min in a homogenizer-disperser (Yellowline, DI 18 Basic, Brazil). A 200µl sample of the emulsion was then taken and mixed with 5 ml of SDS (1%) solution. The emulsifying capacity was determined by reading absorbance of the sample at 500 nm. The turbidity of the sample was also determined as NTU units by using a HACH turbidimeter (2100 AN, the U.S.A.). The emulsion stability of proteins was determined by monitoring of absorbance value at 500nm and turbidity after the 30^{th} and 180^{th} min of emulsification

and calculation of percent retention of the emulsifying activity. All measurements were conducted three times.

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

To determine the water and oil absorption capacities of protein, the method given by Bora (2002) was used with minor modifications. A 50 mg sample of lyophilized protein and 1,5 ml of water or commercial sunflower oil were mixed for 2 min in a 2 ml centrifuge tube. After mixing, the lids of tubes were closed, the tubes were incubated at 30°C for 30min and centrifuged at 15000 g (+25°C) for 20min. The separated free water or oil in the supernatant was removed carefully and the absorbed water and oil content was determined by weighing the tubes. Water or oil absorptions were expressed as g of liquid retained per g of protein.

5.2.2.7. Determination of Foaming Capacity and Foam Stability of Protein Extracts

To determine the foaming capacity, 25 ml of 1% protein solution was prepared and its pH was set to 7.0. The solution was then homogenized in a disperser-homogenizer (Yellowline, DI 18 Basic, Brazil) at 22000rpm for 1 min to induce foaming. The foaming capacity was determined by measuring the volume of the formed foam immediately. The foam stability was determined by monitoring foam volume at the 30^{th}, 60^{th}, 180^{th} and 360^{th} min of foam formation.

5.2.2.8. Determination of Gel Formation Capacity of Protein Extracts

The gel formation capacity of protein extracts was determined by the method given in Horax et al (2004). For the tests, a series of concentrations of protein solutions in distilled water were prepared from 1% to 8% (w/w) with increments of 1%. The solutions placed in 1.46 cm diameter and 15.9 cm long test tubes were then heated in a water bath at 90°C for 1h, immediately cooled to room temperature and incubated for 2 h at 4°C for gel formation. The gel formation was detected by inverting the tubes and

observing flow characteristics of tube contents. The grading of the gel formation capacity was done as follows: (+++): very hard thick gel formation with no flow when inverted; (++): hard gel formation with no flow when inverted but with a very little slipping occurring at the surface due to gravity; (+) gel formation with slight flow occurring from the surface when inverted, some slipping also occurred in the remaining mass by gravity; (±): weak gel formation with most of the mass flowing slowly when inverted; (-) no gel formation with the mass flowed rapidly when inverted.

5.2.3. Determination of Functional Properties of Hydrocolloids Extract for Different Turkish Chickpea and Lentil Cultivars

In this study, the major legume hydrocolloids extract was obtained for comparison of its functional properties with the legume protein extracts. The hydrocolloids extract contains proteins, starch and cellulose. Potential functional properties of such a crude extract enable obtaining a very cheap functional source from chickpeas and lentils for technological purposes.

5.2.3.1. Preparation of Chickpea or Lentil Hydrocolloids Extract

For the extraction of major legume hydrocolloids, 20 g acetone powder was suspended in 250 ml distilled water under continuous magnetic stirring. The pH of the suspension was then adjusted to 9.5 by 1N NaOH solution to maximize protein solubility. For preparation of chickpea hydrocolloids extract (CHE), the temperature of the obtained extract was brought to 85^{o}C to inactivate the highly active lipoxygenase enzyme. The chickpea extract was then continuously stirred at this temperature for 30 min for extraction and then it was cooled down to room temperature. For preparation of lentil hydrocolloids extract (LHE), the obtained extract was not heated, due to the low amounts of lipids in these legumes and extreme browning that occurred during heating at this temperature. Instead, the extraction was conducted at room temperature for 30 min under continuous magnetic stirring. At the end of the extraction period, the pH of both chickpea and lentil extracts was adjusted to 4.5 with 1N acetic acid solution. The proteins and other hydrocolloids such as starch, cellulose and pectates were then precipitated by centrifugation at 15000 x g (+4°C) for 30min. The precipitate was then

collected, dissolved in deionized water and lyophilized after setting of its pH to 7.0. This extract contained only globulin proteins which contain the major protein fraction in legumes but it lacked the albumin fraction which is a minor fraction forming highly water soluble protein fractions. The functional properties and soluble protein content of CHE and LHE were determined by using the same methods used for analysis of CPE and LPE.

5.2.4. Determination of SDS-PAGE Patterns of Protein Extracts

The SDS–PAGE patterns of CPE or LPE were determined on a discontinuous buffered system according to Laemmli method (Dunn, 1989) by using 15% separating gel and 5% stacking gel. Lyophilized samples were directly solubilized in sample buffer and centrifuged at 15000 x g for 30 minutes. The samples were then heated for 5 min in boiling water before electrophoresis. The electrophoresis was performed at a constant current of 20 mA. Protein fixation was performed with TCA (20 %).The gel was stained with 50 % methanol, 10 % acetic acid, 0.05 % Coomassie brilliant blue (R-250) solution. The gel destaining was accomplished by using 5 % methanol and 12.5 % acetic acid solution.

5.2.5. Statistical Analysis

Statistical analysis of extracts were carried out by using ANOVA with a significance threshold of $P<0.05$ as determined by Fisher's protected least significant difference and correlation coefficients between traits (functional properties of extracts) were calculated by QGENE with a significance threshold of $P<0.01$.

CHAPTER 6

RESULTS AND DISCUSSIONS

6.1. The Lentil and Chickpea Cultivars Used in This Study

In this study we characterized the antioxidant potential and protein functionality of 4 different chickpea and 6 different lentil cultivars grown extensively in Turkey. The list of cultivars used and 1000 kernel weight of these cultivars are given in Table 6.1.

Table 6.1. List of lentil and chickpea cultivars.

CULTIVARS	1000 kernel weight (grams)
Chickpea	
Canıtez	52.99 ± 0.78 a
Cevdetbey 98	54.57 ± 0.65 a
Gökçe	41.84 ± 0.83 c
Sarı 98	46.25 ± 1.01 b
Lentil	
Ali dayı	4.57 ± 0.08 c
Çiftçi	3.23 ± 0.03 e
Fırat	3.75 ± 0.38 d
Kafkas	3.09 ± 0.06 e
Meyveci	7.72 ± 0.04 a
Pul II	6.70 ± 0.20 b

a-e: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

6.2.1. Determination of Total Phenolic and Flavonoids Content and Antioxidant Potential of Turkish Chickpea and Lentil Cultivars

The antioxidant potentials of chickpea and lentil cultivars were determined by assaying their total phenolic and flavonoids contents and free radical scavenging and iron binding capacities.

6.2.1.1. Total Phenolic Content of Chickpea and Lentil Cultivars

The total phenolic content of different lentil and chickpea cultivars are given in Table 6.2. The total phenolic content in chickpeas and lentils ranged between 2869 and 3588 mg gallic acid/kg and 3193 and 4312 mg gallic acid/kg, respectively. In chickpeas the average total phenolic content for the 4 cultivars was 3131 mg gallic acid/kg whereas the 6 lentil cultivars had average phenolic content of 4032 mg gallic acid/kg. This result clearly showed the slightly higher total phenolics content of lentils than that of chickpeas.

For chickpeas, the Cevdetbey cultivar showed slightly higher total phenolics content than Sarı 98, Cevdetbey and Gökçe cultivars which showed quite similar total phenolics content. For lentils, Fırat, Pul, Kafkas and Çiftçi cultivars showed similar total phenolics content which was slightly and moderately higher than those of Meyveci and Alidayı cultivars, respectively. These results clearly showed the very limited variation of total phenolics contents of Turkish chickpea and lentil cultivars

Table 6.2. Total phenolic content of Turkish chickpea and lentil cultivars

Cultivars	Total phenolic content (mg gallic acid / kg)
Chickpea	
Canıtez	2869 ± 34 b
Cevdetbey 98	3588 ± 55 a
Gökçe	3012 ± 225 b
Sarı 98	3699 ± 114 b
Lentil	
Ali Dayı	3193 ± 25 c
Çiftçi	4032 ± 27 a
Fırat	4312 ± 58 a
Kafkas	4269 ± 92 a

(cont. on next page)

Table 6.2.(cont.) Total phenolic content of Turkish chickpea and lentil cultivars

Meyveci	3884 ± 46 b
Pul II	4275 ± 90 a

a-d: Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

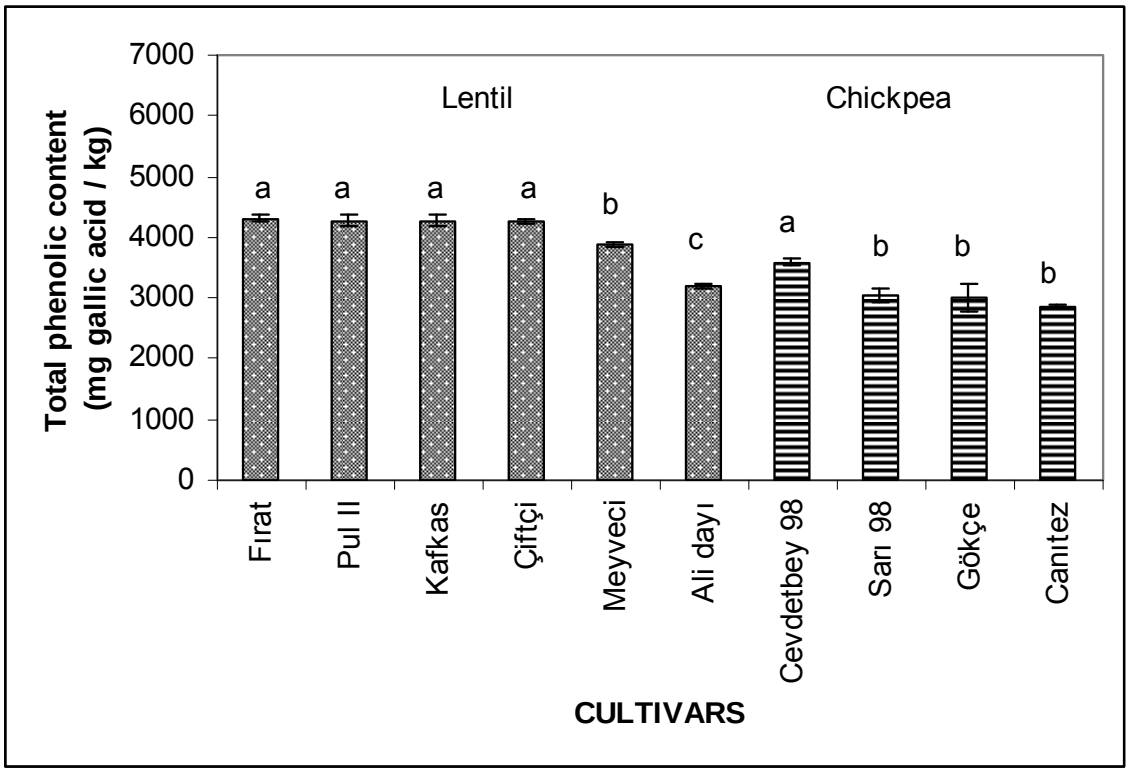


Figure 6.1. Ranking of total phenolics content of Turkish chickpea and lentil cultivars. Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

6.2.1.2. Total Flavonoids Content of Chickpea and Lentil Cultivars

The total flavonoids content of lentil cultivars is given in Table 6.3. However, the total flavonoids content could not be determined in chickpeas by the applied method. The chickpea samples gave a highly turbid sample due to the colloidal stability of proteins in the assay reaction mixture. This problem was observed also by Xu and Chang (2007) during assay of total flavonoids content in chickpeas. On the other hand, in lentils the total flavonoids content ranged between 422 and 721mg epicatechin/kg.

The average total flavonoids content of the 6 lentil cultivars was 555 mg epicatechin/kg. Thus, considering their average total phenolic content, the total flavonoids content form almost 13.8 % of total phenolics in lentils. Unlike total phenolic content, the total flavonoids content of lentils showed considerable variation. The Pul II cultivar contained the highest flavonoids content, while Çiftçi cultivar had the lowest total flavonoids content, 0.6-fold less than Pul-II.

Table 6.3. Total flavonoids content of Turkish lentil cultivars

Cultivars	**Total flavonoid content (mg epicatechin / kg)**
Chickpea	
Canıtez	-
Cevdetbey 98	-
Gökçe	-
Sarı 98	-
Lentil	
Ali Dayı	540 ± 18 bc
Çiftçi	422 ± 16 d
Fırat	621 ± 106 b
Kafkas	468 ± 15 cd
Meyveci	566 ± 46 b
Pul II	721 ± 77 a

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

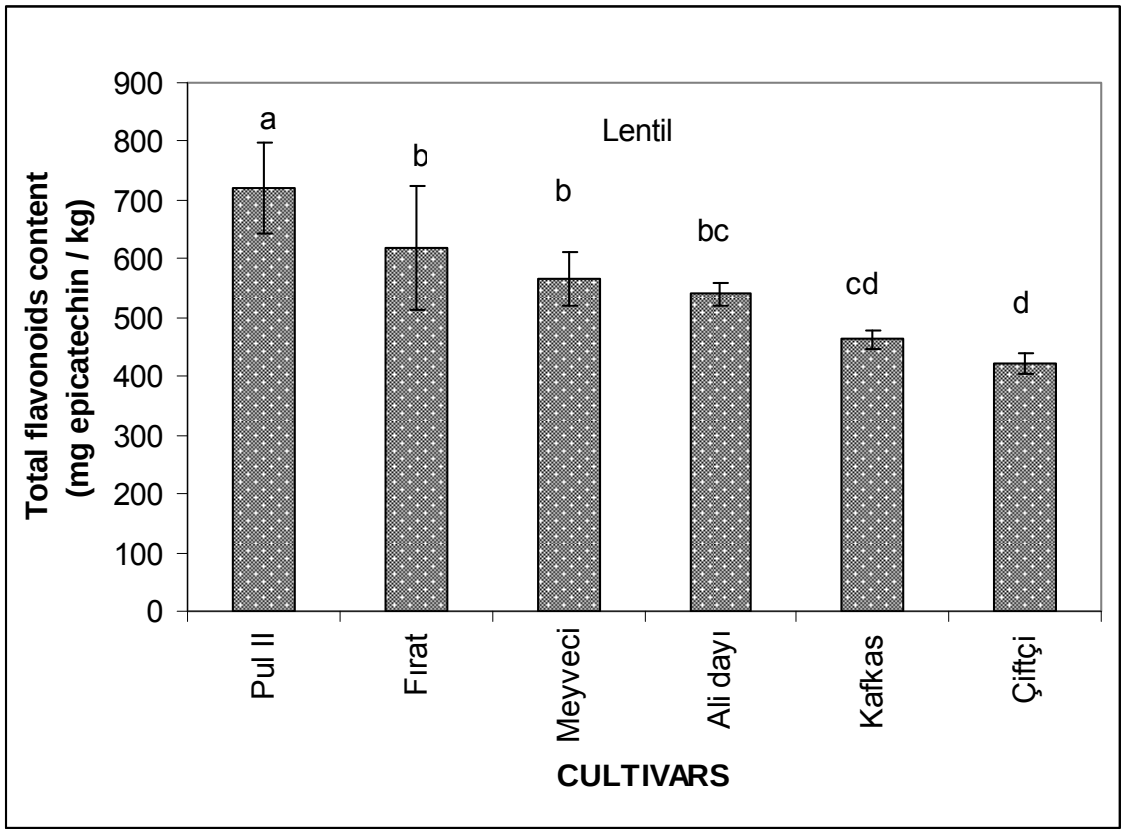


Figure 6.2. Ranking of total flavonoids content of Turkish lentil cultivars. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference

6.2.1.3. Free Radical Scavenging Capacity of Chickpea and Lentil Cultivars

The free radical scavenging capacity of Turkish chickpea and lentil cultivars was determined against ABTS free radical (Table 6.4). The results obtained for free radical scavenging capacity of different lentil cultivars showed a very narrow range of variation between 33.51 and 36.85 mmol Trolox/kg. This result was quite in line with total phenolic contents of lentils which also showed limited variation. However, it contradicts with total flavonoids content of this legume which showed considerable variation. Thus, it seems that the lentil flavonoids did not have an outstanding free radical scavenging capacity than the other phenolics in this product. On the other hand, it is interesting to observe more considerable variation in free radical scavenging capacities of chickpea cultivars. For chickpeas, the highest free radical scavenging capacity was observed for Gökçe, while Cevdetbey showed the lowest free radical scavenging capacity. Similar to

lentils, chickpeas showed very limited variation in their phenolics content. Thus, the variation in their free radical scavenging capacity may be related with the variation in their total flavonoids content which could not be assayed in this study.

Table 6.4. Free radical scavenging capacity of Turkish chickpea and lentil cultivars

Cultivars	Free radical scavenging capacity (mmol Trolox / kg)
Chickpea	
Canıtez	28.66 ± 1.73 c
Cevdetbey 98	24.41 ± 0.39 d
Gökçe	38.20 ± 0.47 a
Sarı 98	32.22 ± 0.47 b
Lentil	
Ali Dayı	35.15 ± 0.45
Çiftçi	33.51 ± 0.65
Fırat	34.00 ± 0.84
Kafkas	35.63 ± 0.19
Meyveci	36.85 ± 1.55
Pul II	35.28 ± 3.05

a-d: Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

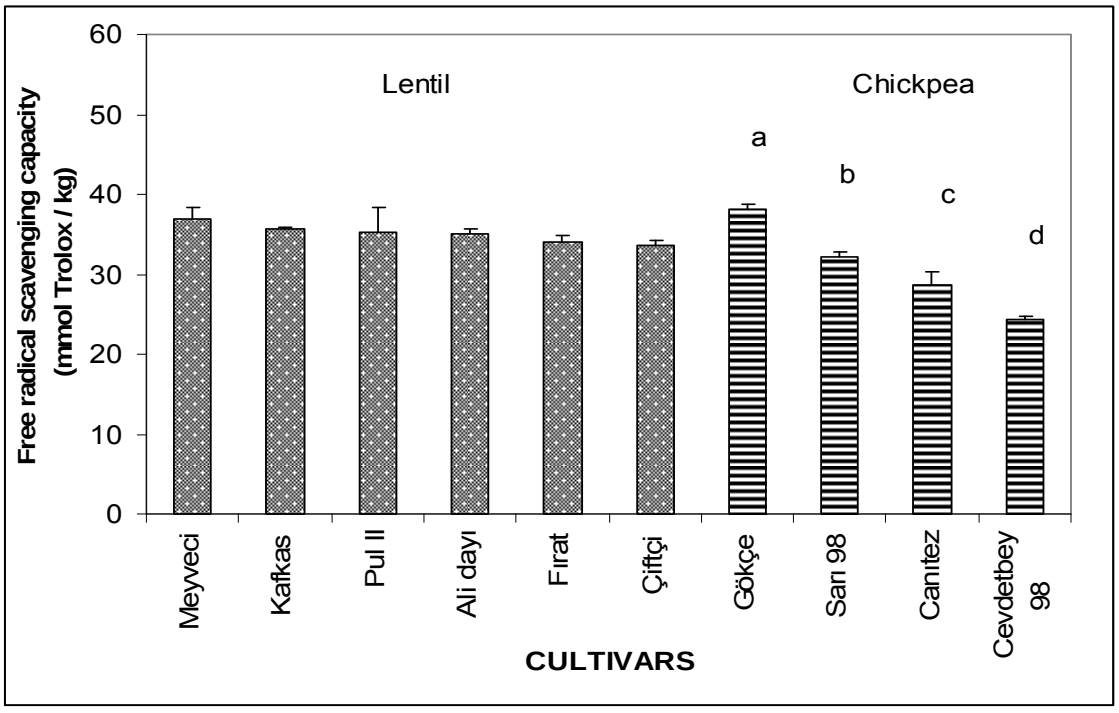


Figure 6.3. Ranking of free radical scavenging capacity of Turkish chickpea and lentil cultivars. Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

The average free radical scavenging capacities of lentils and chickpeas were quite close and determined as 35.07 and 30.87 mmol Trolox/kg, respectively.

6.2.1.4. Iron Chelating Capacity of Chickpea and Lentil Cultivars

The iron chelating capacities of chickpeas and lentils are given in Table 6.5. The iron chelating capacities of chickpea and lentils ranged between 59 and 161, and 39 and 77 mmol EDTA/kg, respectively. Thus, it is clear that the iron chelating capacities of chickpeas and lentils varied considerably and moderately, respectively. In chickpeas, Gökçe showed the highest iron chelating capacity, while Cevdetbey showed the lowest iron chelating capacity. This result clearly showed the high antioxidant potential of Gökçe which also showed the highest free radical scavenging capacity. For lentils which showed a more limited variation in their values, Kafkas and Pul-II cultivars showed the highest and lowest iron chelating capacities, respectively. The Kafkas and Pul-II cultivars showed similar total phenolics content and free radical scavenging capacities. However, interestingly, Pul-II contained significantly higher total flavonoids content than Kafkas. This result clearly showed that the lentil flavonoids are not extraordinary free radical scavengers or iron chelators.

The average iron chelating capacities of chickpeas and lentils were 98 and 61 millimol EDTA/kg, respectively, and these results clearly showed the greater average iron chelating capacity of chickpeas than the lentils.

Table 6.5. Iron chelating capacity of Turkish chickpea and lentil cultivars.

Cultivars	Iron chelating capacity (mmol EDTA / kg)
Chickpea	
Canıtez	61 ± 4.17 d
Cevdetbey 98	81 ± 5.04 c
Gökçe	161 ± 4.48 a
Sarı 98	59 ± 2.05 b
Lentil	
Ali Dayı	54 ± 2.51 c
Çiftçi	73 ± 3.68 ab
Fırat	67 ± 0.71 b
Kafkas	77 ± 1.89 a
Meyveci	57 ± 5.96 c
Pul II	39 ± 1.44 d

a-d: Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

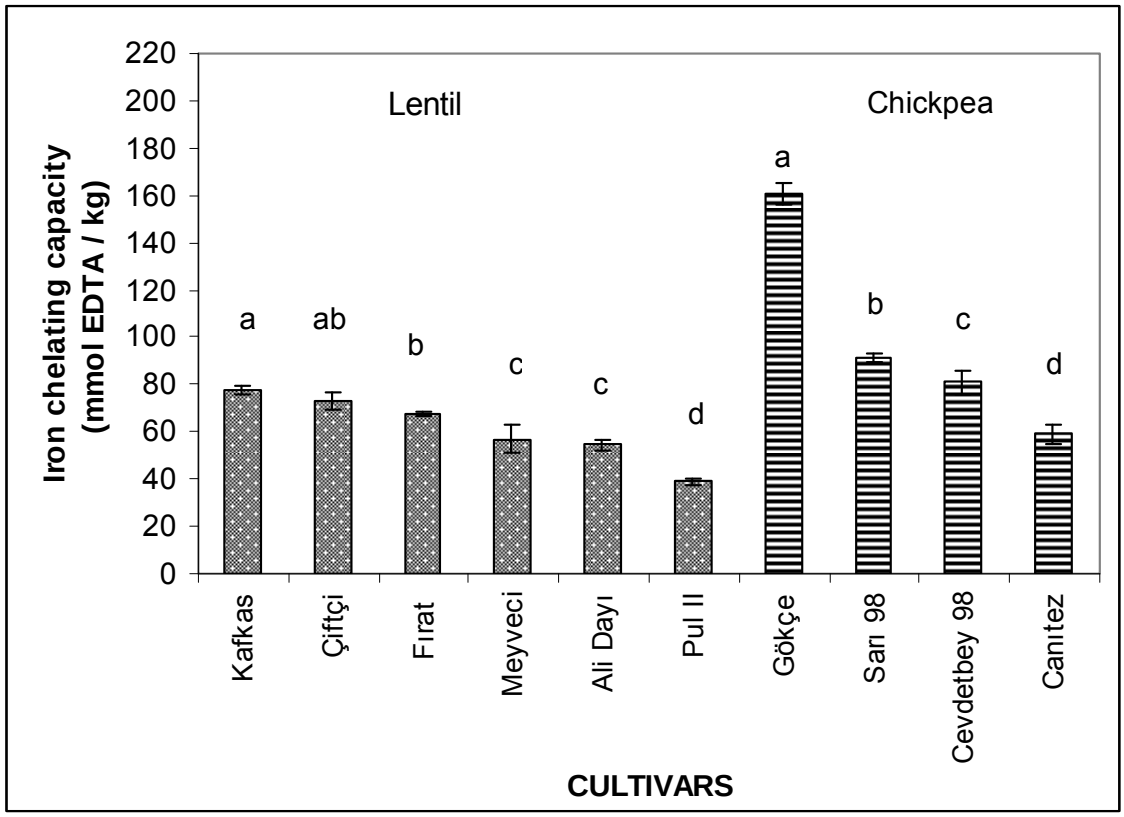


Figure 6.4. Ranking of iron chelating capacity of Turkish chickpea and lentil cultivars. Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

6.2.2. Determination of Functional Properties of Proteins for Different Turkish Chickpea and Lentil Cultivars

The functional properties of lentil and chickpea proteins were determined by obtaining extracts formed mainly by albumins and globulins. The functional properties of protein extracts were also compared with the functional properties of chickpea and lentil hydrocolloids extracts formed by globulins, cellulose, starch and pectates. The hydrocolloids extract is a very crude preparation containing both proteins and carbohydrates. Thus, comparison of the functional properties of the protein extract with the hydrocolloids extract helps understand the major functions associated specifically with proteins. Also, the potential good functional properties of the hydrocolloids extract enables use of this preparation for specific purposes as a cheap source of functional ingredients.

6.2.2.1. Soluble Protein Contents of Chickpea and Lentil Protein Extracts

The legume proteins including chickpeas and lentils showed their maximum solubility at alkaline pH values at or above pH 9.0 (Damodaran 1996a). Therefore, the soluble protein contents of protein and hydrocolloids extracts were studied by conducting solubilizations at pH 9.5. The soluble protein contents of CPE and LPE are given in Table 6.6. The soluble protein content of CPE from different cultivars did not show considerable variation and changed between 18.2 and 26.2 %. A similar limited variation in protein solubility was observed also for LPE of different cultivars (Table 6.6). For LPEs, the highest protein contents were obtained for Kafkas and Meyveci cultivars, while Pul II and Fırat showed the lowest protein solubility (Figure 6.5). For CPEs, the Gökçe and Cevdetbey cultivars had the highest soluble protein content while Canıtez showed the lowest protein solubility. The average soluble protein contents of CPE and LPE from different cultivars were 22.5 and 39.6, respectively. The soluble proteins are mainly formed by albumins. However, the globulins also show considerable solubility in the alkaline pH. The insoluble protein fractions are mostly formed by high molecular weight globulin fractions and protein-carbohydrate complexes.

Table 6.6. The soluble protein content of CPE and LPE

Cultivar	Water soluble protein content (%)
CPE	
Canıtez	18.2 ± 1.1 b
Cevdetbey 98	24.7 ± 2.9 a
Gökçe	26.2 ± 2.5 a
Sarı 98	20.9 ± 2.2 ab
LPE	
Ali Dayı	39.7 ± 3.5 abc
Çiftçi	38.7 ± 4.6 abc
Fırat	34.7 ± 2.5 c
Kafkas	45.0 ± 2.0 a
Meyveci	42.3 ± 4.0 ab
Pul II	37.3 ± 2.5 bc

a-c: Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

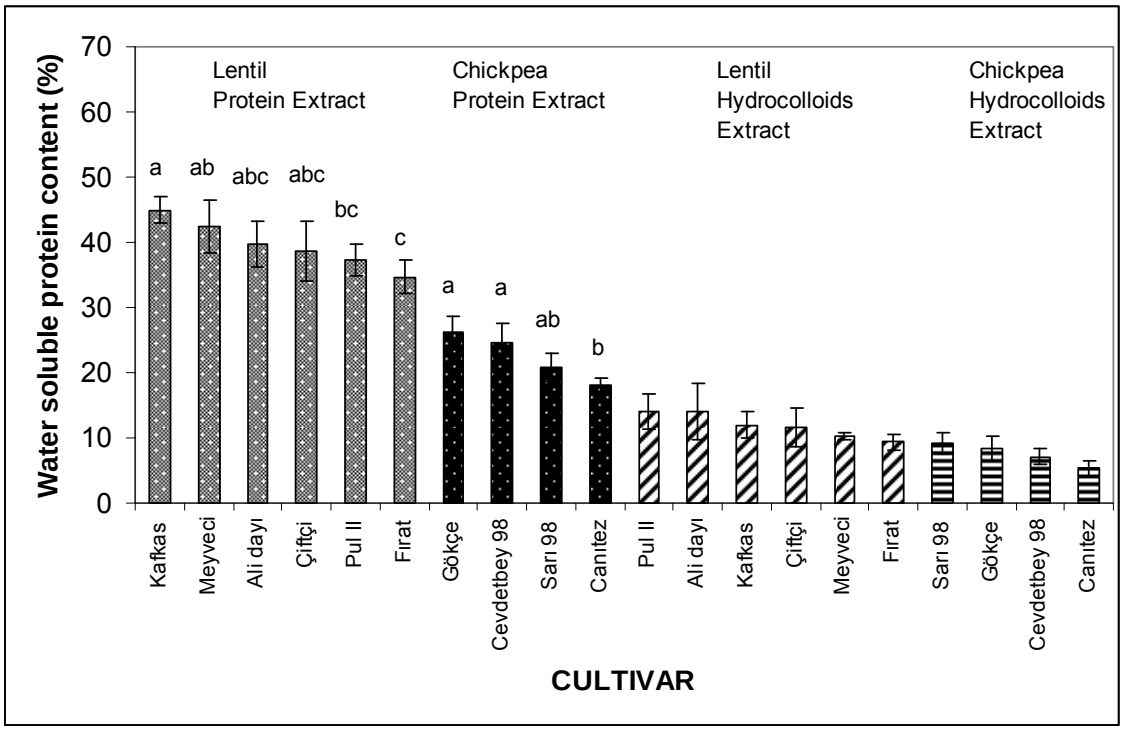


Figure 6.5. Soluble protein content of chickpea and lentil protein and hydrocolloids extracts. Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

6.2.2.2. Free Radical Scavenging Capacity of Chickpea and Lentil Protein Extracts

The free radical scavenging capacity of lentil protein extracts (LPE) and chickpea protein extracts (CPE) are given in Table 6.7. The free radical scavenging capacity of CPE showed variation and changed between 58 and 144 mmol Trolox/kg. The highest free radical scavenging activity was obtained for CPE of Cevdetbey cultivar, while that of Sarı 98 showed the lowest free radical scavenging capacity (Figure 6.6). In LPE, on the other hand, the free radical scavenging capacity varied between 110 and 185 mmol Trolox/kg. The average free radical scavenging activities of CPE from the 4 cultivars and LPE from the 6 cultivars were 95 and 138, and this result clearly showed the higher antioxidant potential of LPE than the CPE.

Both LPE and CPE showed considerably higher free radical scavenging capacity than LHE and CHE. In fact, this was expected since hydrocolloid extracts containing both proteins and carbohydrates showed lower solubility than the protein extracts.

Table 6.7. Free radical scavenging capacity of chickpea and lentil protein extracts and hydrocolloids extract.

Cultivar	**Free radical scavenging capacity (mmol Trolox / kg)**	
	Protein extract	**Hydrocolloid extract**
	Chickpea	
Canıtez	90 ± 0.01 b	20 ± 0.015 c
Cevdetbey 98	144 ± 0.02 a	18 ± 0.002 d
Gökçe	89 ± 0.02 b	39 ± 0.008 a
Sarı 98	58 ± 0.04 c	28 ± 0.007 b
	Lentil	
Ali Dayı	185 ± 0.02 a	41 ± 0.003 a
Çiftçi	148 ± 0.01 b	31 ± 0.010 c
Fırat	144 ± 0.03 b	19 ± 0.003 e
Kafkas	110 ± 0.01 d	32 ± 0.003 c
Meyveci	128 ± 0.01 c	23 ± 0.002 d
Pul II	119 ± 0.01 cd	39 ± 0.010 b

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

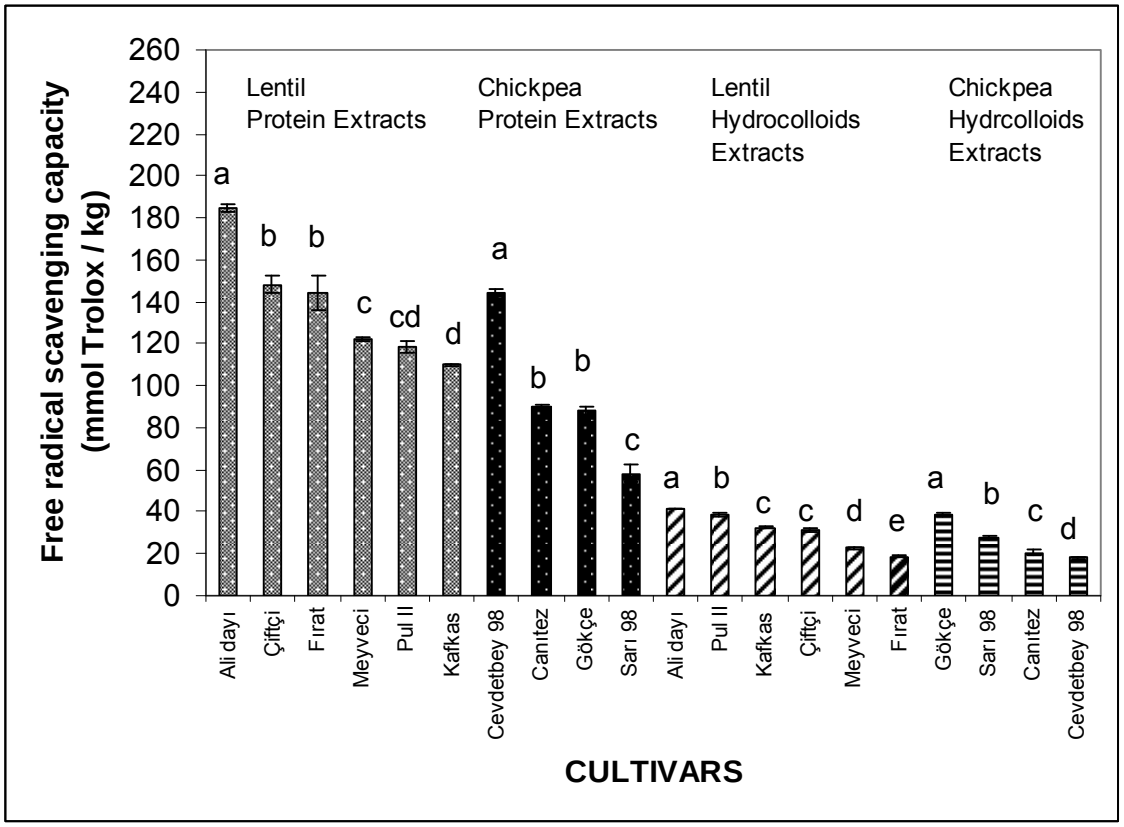


Figure 6.6. Ranking of free radical scavenging capacity of chickpea and lentil protein extracts and hydrocolloids extracts. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

6.2.2.3. Iron Chelating Capacity of Chickpea and Lentil Protein Extracts

The iron chelating capacities of CPE and LPE are given in Table 6.8. Both the LPE and CPE showed considerable variation in iron chelating capacity. The iron chelating capacity of LPE changed between 21.9 and 45.8 mmol EDTA/kg, while CPE changed between 20.7 and 48.4 mmol EDTA/kg. The average iron chelating capacities of CPE and LPE were also similar (33.9 and 32.7 mmol EDTA/kg, respectively).

The CHE and LHE from different cultivars showed lower iron chelating capacity than the CPE and LPE of corresponding cultivars, respectively. However, iron chelating capacities of CHE were considerably higher than those of LHE. This result clearly

showed the good iron chelating capacity of chickpea carbohydrates since protein extracts of chickpeas and lentils showed similar average iron chelating capacities.

Table 6.8. Iron chelating capacity of chickpea and lentil protein extracts and hydrocolloids extracts.

Cultivar	Iron chelating capacity (mmol EDTA / kg)	
	Protein extract	Hydrocolloid extract
Chickpea		
Canıtez	20.7 ± 0.3 d	18.7 ± 3.7 a
Cevdetbey 98	48.4 ± 1.5 a	12.0 ± 0.8 b
Gökçe	26.8 ± 2.7 c	11.7 ± 1.9 b
Sarı 98	41.0 ± 3.4 b	10.3 ± 3.3 b
Lentil		
Ali Dayı	38.5 ± 1.9 a	6.9 ± 0.4 a
Çiftçi	32.6 ± 4.9 c	5.2 ± 2.0 b
Fırat	21.9 ± 1.7 d	5.5 ± 1.2 ab
Kafkas	45.8 ± 2.7 a	5.5 ± 3.2 ab
Meyveci	31.7 ± 1.4 c	6.4 ± 1.1 ab
Pul II	12.7 ± 2.3 d	6.7 ± 1.3 a

a-d:Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference

6.2.2.4. Emulsifying Capacity and Emulsion Stability of Protein Extracts

In this study, the emulsifying capacity of LPE and CPE was determined by evaluating the turbidity of formed emulsions both by a turbidity meter (as NTU) and a spectrophotometer (absorbance at 500nm). The absorbance measurement by spectrophotometric method is used very frequently for determination of turbidity of emulsions since turbidity meter measuring the turbidity directly from scattered light in NTU is not available in most laboratories.

The results of emulsifying capacity of LPE and CPE are given in Table 6.9. The results of emulsifying activity measured by the absorbance method showed a high parallelism with those determined by the turbidimetric method. In both LPE and CPE, the

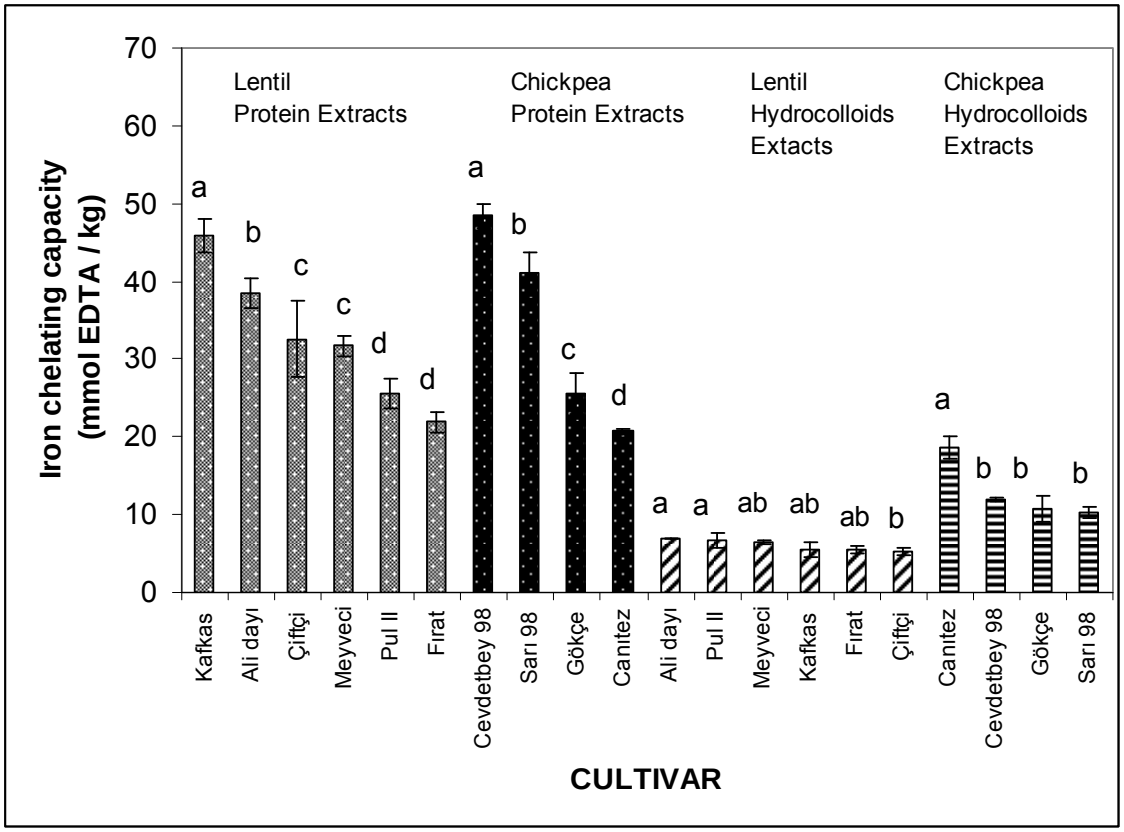


Figure 6.7. Ranking of iron chelating capacity of chickpea and lentil protein extracts and hydrocolloids extracts. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

the emulsifying capacities varied in a very narrow range (Figure. 6.8 and 6.9). It is also observed that the CPE and LPE had similar emulsifying capacities.

The CHE and LHE also showed comparable emulsifying capacities with protein extracts. This result clearly showed the similar emulsifying capacities of lentil proteins and carbohydrates.

Table 6.9. Emulsifying capacities and emulsion stabilities of chickpea and lentil protein extracts.

Cultivar	Emulsifying capacities and emulsion stabilities of protein extracts					
	0 min		30 min		180 min	
	NTU	absorbance	NTU	absorbance	NTU	absorbance
			CPE			
Canıtez	325 ±16 (100) a	0.788 ±0.04 (100) a	292 ±18 (91.5) a	0.687 ±0.12 (91.7) a	83.7 ±17 (25.7)	0.370 ±0.04 (46.9)
Cevdetbey 98	305 ±17 (100) ab	0.707 ±0.04 (100) ab	154 ±28 (50.7) bc	0.426 ±0.06 (60.3) b	66.9 ±8.5 (21.9)	0.258 ±0.02 (36.5)
Gökçe	310 ±27 (100) ab	0.729 ±0.07 (100) ab	154 ±26 (49.4) c	0.451 ±0.08 (61.6) b	66.9 ±15 (21.6)	0.260 ±0.05 (35.7)
Sarı 98	271 ±12 (100) b	0.630 ±0.05 (100) b	177 ±30 (64.9) b	0.410 ±0.06 (65.3) b	77.9 ±7.5 (28.7)	0.300 ±0.02 (47.6)
			LPE			
Ali Dayı	290 ±12 (100)	0.655 ±0.02 (100)	177 ±10 (61.0) a	0.426 ±0.01 (65.1) a	63.5 ±7.7 (21.9)	0.230 ±0.03 (35.1)
Çiftçi	302 ±13 (100)	0.723 ±0.02 (100)	165 ±12 (54.6) ab	0.430 ±0.03 (59.4) ab	59.0 ±3.4 (19.5)	0.223 ±0.01 (30.8)
Fırat	302 ±18 (100)	0.722 ±0.02 (100)	139 ±16 (46.1) bc	0.386 ±0.01 (53.5) bc	53.6 ±6.9 (17.7)	0.201 ±0.02 (27.8)
Kafkas	307 ±10 (100)	0.727 ±0.05 (100)	165 ±17 (53.5) ab	0.418 ±0.03 (57.5) b	54.6 ±6.1 (17.8)	0.194 ±0.03 (26.6)
Meyveci	311 ±5 (100)	0.720 ±0.02 (100)	122 ±6 (39.1) c	0.360 ±0.02 (49.3) cd	63.8 ±16.4 (20.5)	0.230 ±0.05 (31.9)
Pul II	312 ±10 (100)	0.728 ±0.04 (100)	119 ±11 (38.2) c	0.310 ±0.02 (42.8) d	72.9 ±11.6 (23.4)	0.232 ±0.03 (31.9)

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference

Table 6.10. Emulsifying capacities and emulsion stabilities of chickpea and lentil hydrocolloids extracts.

Cultivar	Emulsifying capacities and emulsion stabilities of hydrocolloid extracts			
	0 min		30 min	
	NTU	absorbance	NTU	absorbance
		CHE		
Canıtez	231 ± 6 (100) b	0.522 ± 0.02 (100) b	89 ± 10 (38.5) a	0.225 ± 0.02 (43.0) a
Cevdetbey 98	274 ± 11 (100) a	0.642 ± 0.05 (100) a	84 ± 5 (30.7) b	0.256 ± 0.01 (40.0 a
Gökçe	249 ± 15 (100) ab	0.577 ± 0.04 (100) ab	57 ± 3 (22.9) c	0.188 ± 0.01 (32.7) b
Sarı 98	245 ± 14 (100) b	0.590 ± 0.02 (100) ab	87 ± 2 (35.5) ab	0.250 ± 0.01 (42.3) a
		LHE		
Ali Dayı	251 ± 2 (100) bc	0.595 ± 0.01 (100) c	127 ± 15 (50.5) a	0.341 ± 0.02 (57.4) a
Çiftçi	243 ± 2 (100) c	0.599 ± 0.01 (100) c	86 ± 9 (35.2) bc	0.262 ± 0.02 (43.7) b
Fırat	273 ± 10 (100) a	0.685 ± 0.03 (100) a	114 ± 6 (41.8) ab	0.385 ± 0.01 (41.8) bc
Kafkas	261 ± 11 (100) abc	0.636 ± 0.03 (100) abc	77 ± 11 (29.6) c	0.217 ± 0.01 (34.1) c
Meyveci	262 ± 12 (100) ab	0.610 ± 0.02 (100) bc	96 ± 9 (36.5) bc	0.280 ± 0.04 (45.4) b
Pul II	269 ± 4 (100) a	0.653 ± 0.01 (100) ab	105 ± 12 (39.0) b	0.303 ± 0.02 (46.4) b

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference

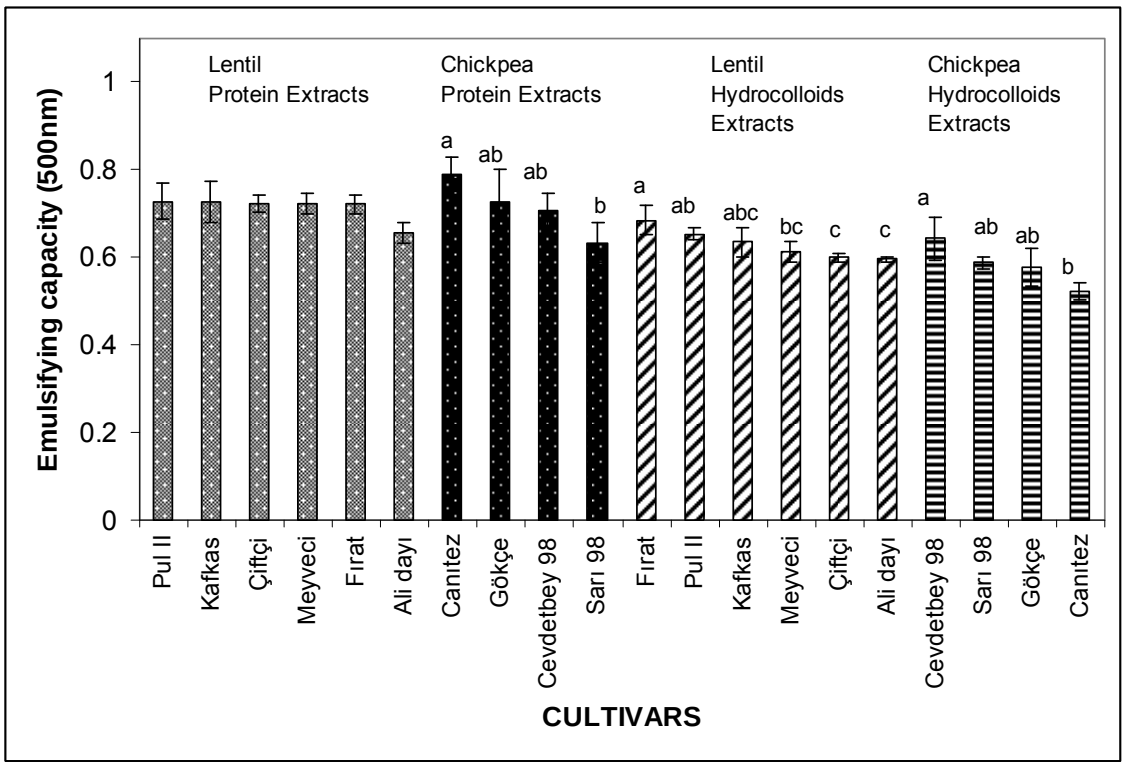


Figure 6.8. Emulsifying capacities of chickpea and lentil protein and hydrocolloids extracts based on spectrophotometric method. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

The emulsion stability of LPE and CPE was also determined by measuring turbidity or absorbance value of emulsions formed by CPE, LPE, CHE and LHE after 30 min from emulsion formation. The CPE and LPE were assayed for their turbidity and absorbance values after 180 min of emulsion formation since they showed high emulsion stability. The emulsion stabilities based on retention of formed emulsion turbidity in 30 min are given in Figure. 6.10 and 6.11. Careful analysis of these figures once more showed the parallelism between NTU and absorbance measurement methods except slight changes in ranking of similar values. For CPE, the Canitez cultivar showed the highest emulsifying stability based on measurements with both methods. The other cultivars had moderately lower emulsion stability with inconsiderable variation. Significant variation was also not observed for LPE of different cultivars. However, in measurements by both methods, Alidayı and Pul II cultivars had the highest and lowest emulsion stabilities, respectively.

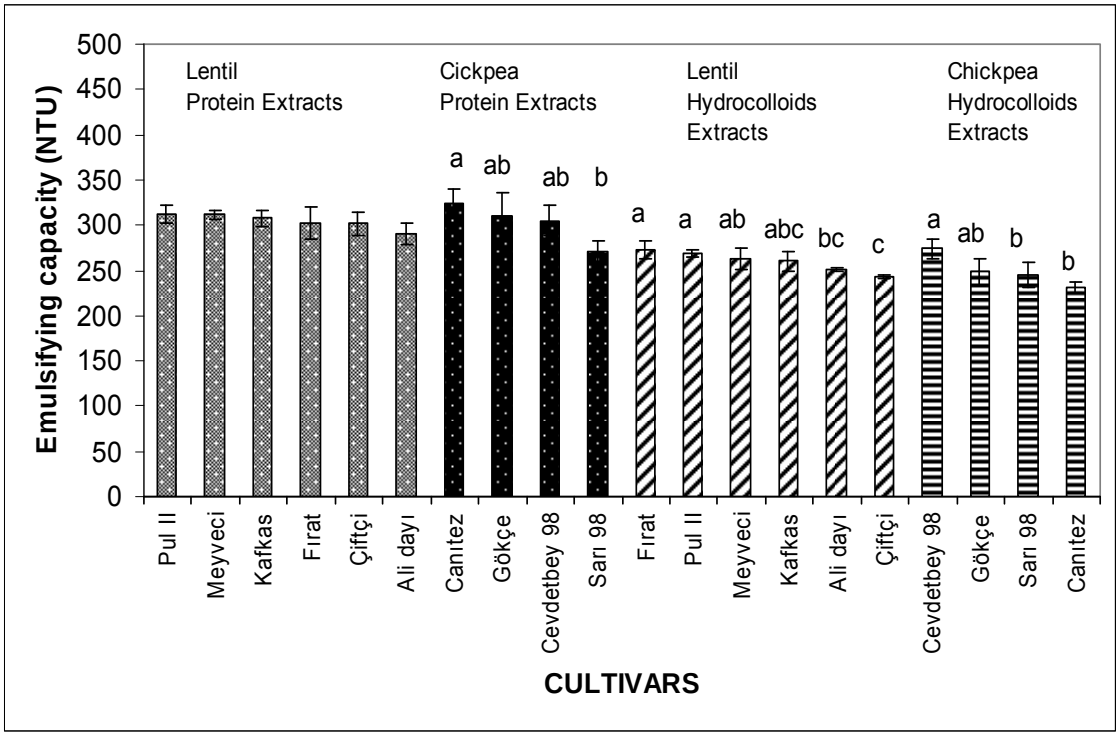


Figure 6.9. Emulsifying capacities of chickpea and lentil protein and hydrocolloids extracts based on turbidimetric method. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

The emulsion stabilities of CHE from different cultivars were considerably lower than those of CPE. In contrast, less significant reductions occurred in emulsion stabilities when LHE was used instead of LPE. Both CHE and LHE of different cultivars showed very limited variation. However, both methods showed maximum emulsion stability of hydrocolloids extract from Canıtez chickpea and Alidayı lentil cultivars. The minimum emulsion stabilities were obtained with hydrocolloids extracts of Kafkas lentil and Gökçe chickpea cultivars. The globulin proteins are the only major component which exist both in protein and hydrocolloids extracts. Thus, the parallelism between emulsion stabilities of protein and hydrocolloids extracts suggests the significant roles of these proteins in emulsion stability.

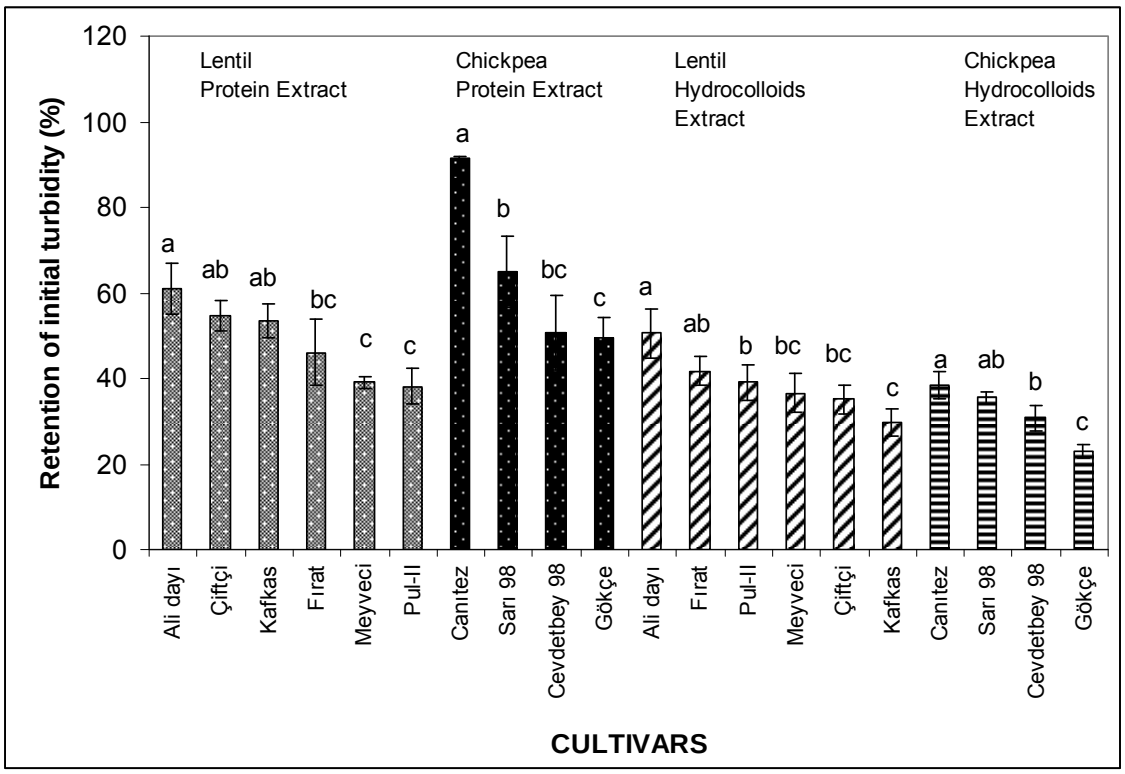


Figure 6.10. Emulsion stability of chickpea and lentil protein and hydrocolloids extracts after 30 min of emulsion formation (measurements were based on turbidimetric method). Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

6.2.2.5. Water Absorption Capacity of Chickpea and Lentil Protein Extracts

The results of water absorption capacities of CPE and LPE are given in Table 6.11. It is interesting to note that the LPE of all cultivars did not show a measurable water absorption capacity. The lyophilized LPE did not absorb water sufficiently, instead the protein extracts solubilized in the water and drained from the test tubes rapidly when they were inverted to remove unbound water. This result clearly showed the high content of water soluble albumins in lentils. In contrast, CPE did not solubilize in the added water and absorbed and fixed a considerable amount of water. The water absorption capacity of CPE from different cultivars showed significant variation (Figure 6.12). For example, the Sarı 98 and Gökçe cultivars absorbed almost 3.5 fold higher amounts of water than Canıtez and Cevdetbey cultivars.

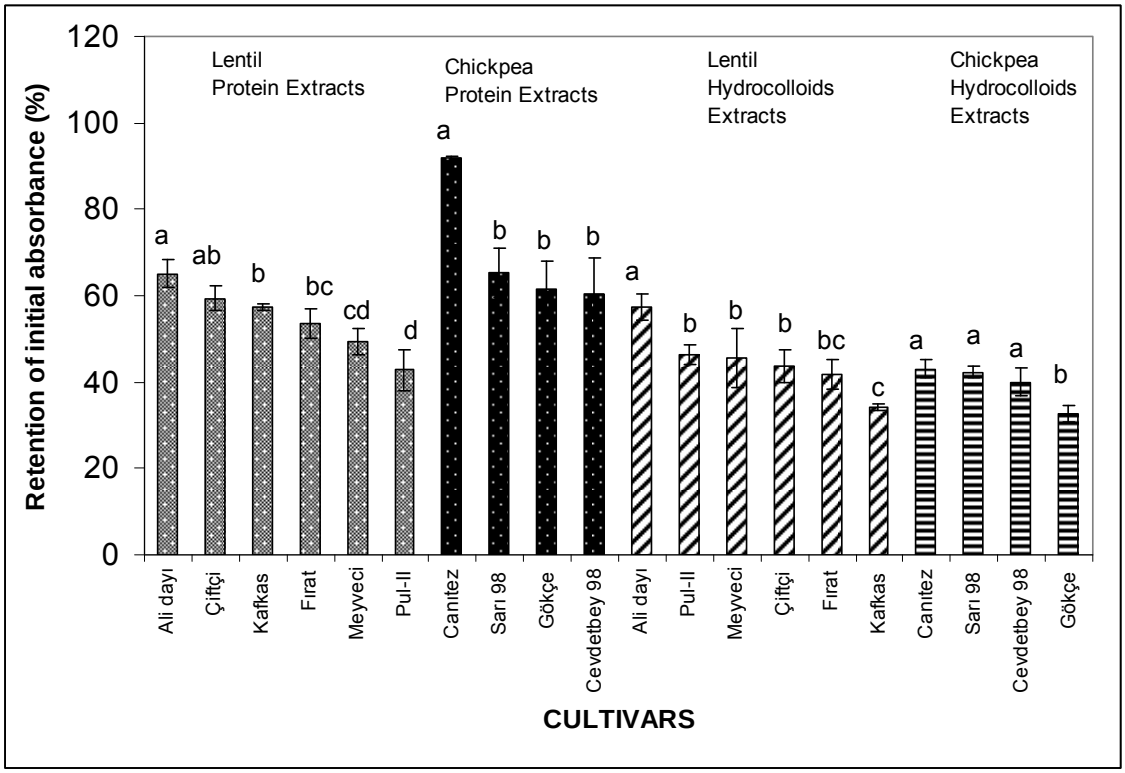


Figure 6.11. Emulsion stability of chickpea and lentil protein and hydrocolloids extracts after 30 min of emulsion formation (measurements were based on spectrophotometric method). Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

Unlike LPE, the LHE of different cultivars showed moderate water binding capacity which showed almost no variation. On the other hand, it is important to note the extremely high water binding capacity of CHE from different cultivars. In fact, the average water absorption capacity of CHE is almost 2.9 and 3.2 fold higher than those of CPE and LHE, respectively.

Table 6.11. Water absorption capacity of chickpea and lentil protein and hydrocolloids extracts.

Cultivar	Protein Extract	Hydrocolloid Extract
	Water absorbtion capacity (g / g)	
	Chickpea	
Canıtez	1.1 ± 0.1	6.8 ± 0.4 b
Cevdetbey	1.0 ± 0.6	7.3 ± 1.4 b
Gökçe	3.5 ± 0.5	5.3 ± 1.3 a
Sarı 98	3.7 ± 0.6	7.6 ± 0.3 a
	Lentil	
Ali Dayı	0	2.1 ± 0.4
Çiftçi	0	2.1 ± 0.2
Fırat	0	2.5 ± 0.2
Kafkas	0	2.0 ± 0.03
Meyveci	0	2.0 ± 0.03
Pul II	0	2.0 ± 0.2

a-b: Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

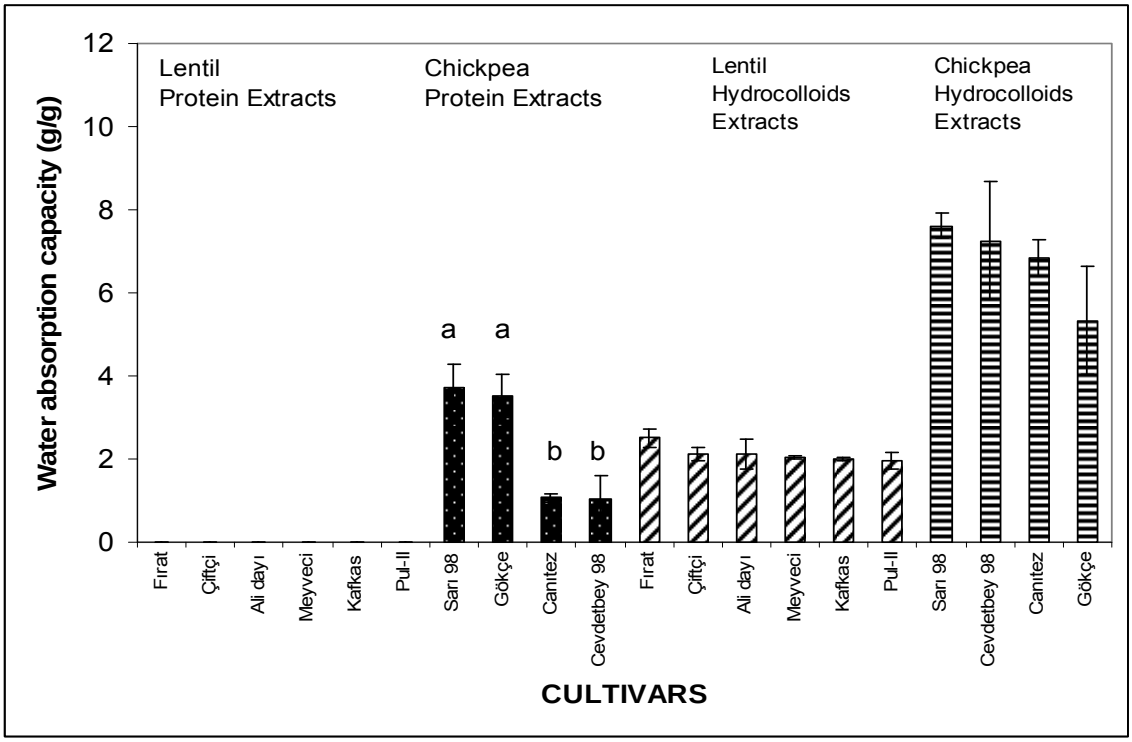


Figure 6.12. Water absorption capacity of chickpea and lentil protein and hydrocolloids extracts. Values followed by different letters are significantly different at P<0.05 as determined by Fisher's protected least significant difference.

6.2.2.6. Oil Absorption Capacity of Chickpea and Lentil Protein Extracts

The oil absorption capacities of CPE and LPE are given in Table 6.12. The oil absorption capacities of LPE varied in a narrow range between 3.2 and 4.7 g/g. The CPE showed considerably higher oil absorption capacity than LPE, but there was also no considerable variation in values of different chickpea cultivars. For chickpeas, the highest and lowest oil absorption capacities were observed for Alidayı and Fırat cultivars, while in lentils the highest and lowest oil absorption capacities were observed for Sarı 98 and Canıtez, respectively.

The average oil absorption capacities of LHE and CHE from different cultivars (3.7 g/g and 6.1 g/g) was close to those of LPE and CPE (3.9 g/g 6.9 g/g), respectively, and showed almost no variation (Figure 6.13). These results clearly showed the good potential of hydrocolloids extract as a water and oil binding agent. Particularly, the CHE can be used as a good oil and water absorbing agent.

Table 6.12. Oil absorption capacity of chickpea and lentil protein and hydrocolloids extracts.

Cultivar	Protein extract	Hydrocolloid extract
	Oil absorbtion capacity(g / g)	
	Chickpea	
Canıtez	5.4 ± 0.1 b	5.6 ± 0.3
Cevdetbey	7.1 ± 1.0 ab	6.6 ± 0.9
Gökçe	7.4 ± 0.9 a	6.6 ± 0.8
Sarı 98	7.7 ± 0.5 a	5.6 ± 0.4
	Lentil	
Ali Dayı	4.7 ± 0.2 a	4.0 ± 0.1
Çiftçi	3.6 ± 0.1 cd	3.7 ± 0.1
Fırat	3.2 ± 0.4 d	3.7 ± 0.2
Kafkas	3.8 ± 0.3 bc	3.7 ± 0.4
Meyveci	3.7 ± 0.1 bcd	3.4 ± 0.4
Pul II	4.2 ± 0.3 ab	3.7 ± 0.4

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

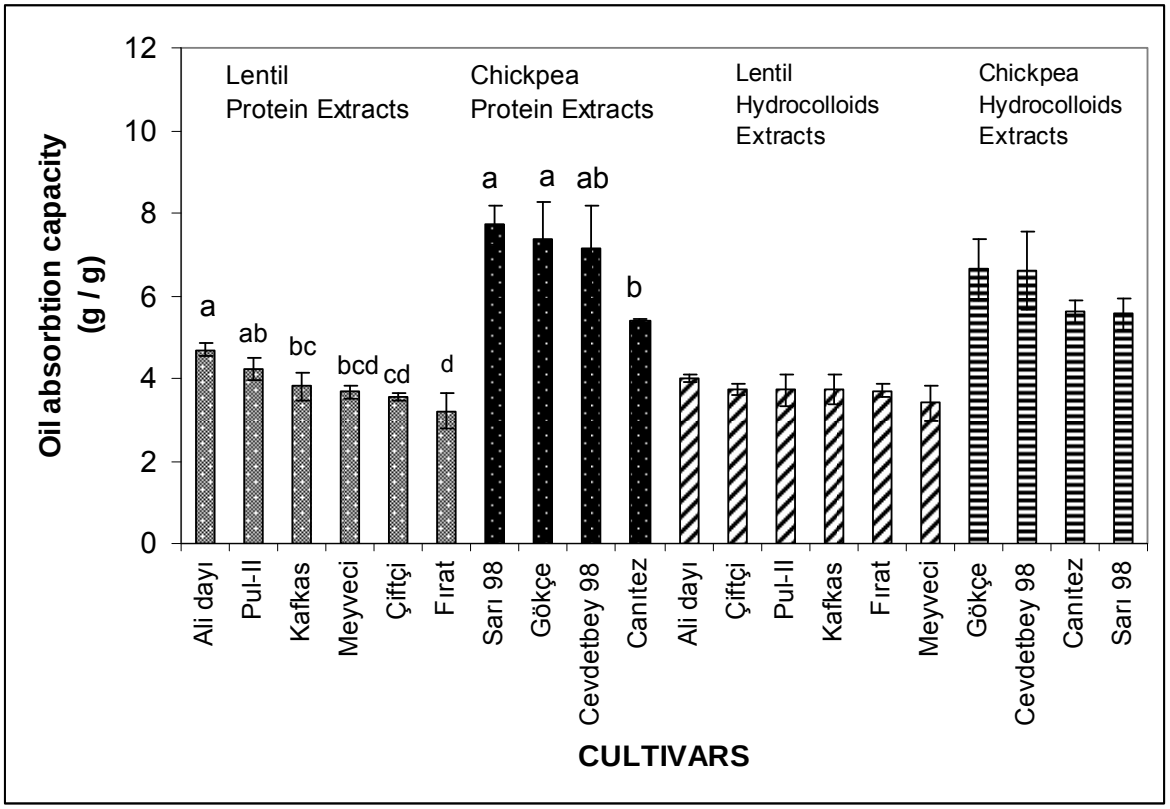


Figure 6.13. Oil absorption capacity of chickpea and lentil protein and hydrocolloids extracts. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

6.2.2.7. Foaming Capacity and Foam Stability of Protein Extracts

The foaming capacities of CPE and LPE are given in Figure. 6.14. For CPE, the foaming capacities of 3 of the 4 cultivars were similar but lower than that of Cevdetbey cultivar which showed the highest foaming capacity. In LPE, the foaming capacity changed between 8.9 and 15.3 ml. The highest foaming capacity was obtained for Alidayı cultivar, while Fırat cultivar showed the lowest foaming capacity. The average foaming capacities of CPE (13.3 ml) and LPE (12.1 ml) showed similar foaming activities of chickpea and lentil proteins.

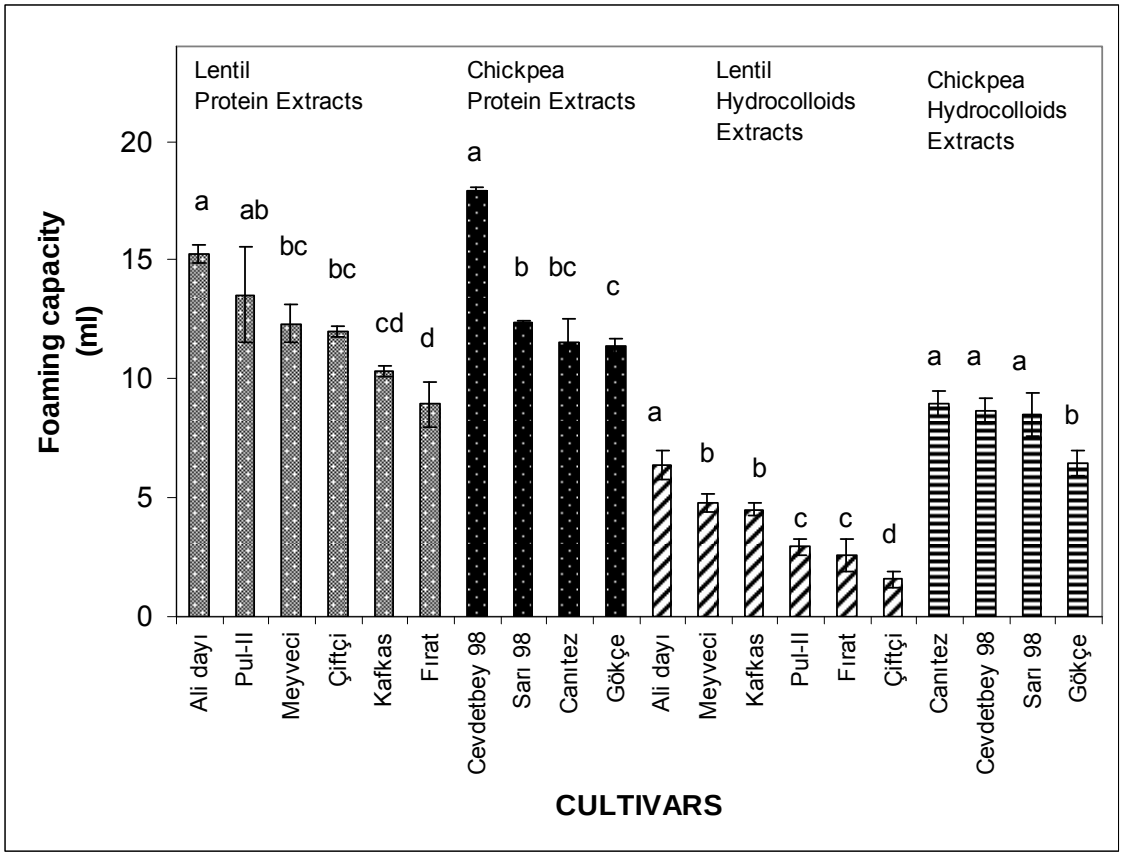


Figure 6.14. Foaming capacities of chickpea and lentil protein and hydrocolloids extracts. Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference.

The CHE of different cultivars showed considerable foaming capacity, comparable to those of CPEs. The hydrocolloids extracts do not contain albumins, proteins that solubilize easily in water and form a major part of the foam by agitation. Thus, it seems that not only albumins but also globulins or some carbohydrates also had foaming activity in chickpeas. In contrast, LHE showed considerably lower foaming capacity than LPE, except for Alidayı cultivar. This result clearly showed the importance of albumins for foaming activity of lentil hydrocolloids extracts.

The foam stability of LPE, CPE, LHE and CHE were determined by measuring foam capacity following 30, 60, 180 and 360 min of foam formation. For different CPEs, the results obtained clearly showed the high foam stability of proteins from Gökçe and Cevdetbey cultivars (Table 6.13). In these cultivars over 70 % and 35 % of foaming capacity was maintained following 180 and 360 min of foam formation, respectively. In contrast, in Sarı 98 and Canıtez cultivars the foaming capacities retained after 360 min were less than half those of Gökçe and Cevdetbey cultivars at the same

conditions. For LPE, the average foam stability observed was considerably lower than that of CPE. A great variation was also observed in foam stabilities which varied between 1% and 28% retention of foam capacity after 360 min.

The foam stabilities of CHE and LHE are also given in Table 6.14. The foam stabilities of CHE from different chickpea cultivars showed parallelism with those of CPEs. CHE of Gökçe and Cevdetbey chickpea cultivars again showed considerably higher foam stabilities than Sarı 98 and Canıtez. However, a moderate reduction also occurred in foam stabilities of Gökçe and Cevdetbey chickpea cultivars by use of CHE instead of CPE. The LHE showed very limited foaming capacity. However, it is quite interesting to observe drastic changes in foam stabilities of LHE from different lentils. The use of LHE instead of LPE caused considerable increases in foam stabilities of Alidayı, Kafkas and Meyveci cultivars, while foam stability of Fırat increased only slightly. These results clearly showed the contribution of carbohydrates to foam stability of LHE of these cultivars. It is likely that the increased viscosity by carbohydrates contributed to the increased foam stability in LHE of these cultivars. In contrast, considerable and moderate reductions in foam stability occurred in Çiftçi and Pul II cultivars by use of LHE instead of LPE. The initial foam capacities of LHE in these cultivars were also very low. Thus, it seems that these cultivars contained minimum amounts of foam-forming proteins and foam stabilizing factors (viscosity provided by carbohydrates and proteins).

Table 6.13. Foaming capacity and foam stability of lentil and chickpea protein extracts.

Cultivar	Retention of foaming capacity (%)				
	0 minute	30 minutes	60 minutes	180 minutes	360 minutes
			CPE		
Canıtez	11.6 ±0.1 (100) bc	10.6 ±1.4 (91.4) bc	9.1 ±1.4 (88.7) bc	3.5 ±0.8 (30.0) d	1.7 ±0.1 (15.0) c
Cevdetbey 98	17.9 ±0.2 (100) a	17.0 ±0.6 (94.8) a	15.5 ±0.5 (86.2) a	13.3 ±0.5 (74.0) a	6.6 ±1.3 (37.0) a
Gökçe	11.3 ±0.4 (100) c	10.0 ±0.3 (88.2) c	9.7 ±0.3 (85.9) c	8.2 ±0.6 (72.0) b	5.0 ±0.3 (44.0) b
Sarı 98	12.4 ±0.03 (100) b	11.6 ±0.3 (98.7) b	10.7 ±0.3 (86.0) b	4.9 ±0.3 (39.0) c	1.6 ±0.4 (13.0) c
			LPE		
Ali Dayı	15.3 ±0.4 (100) a	15.1 ±0.3 (99.0) a	14.9 ±0.2 (97.8) a	13.3 ±0.4 (87.0) a	4.4 ±0.3 (28.0) a
Çiftçi	12 ±0.2 (100) bc	11.7 ±0.1 (97.4) b	11.1 ±0.1 (92.6) b	10.6 ±0.3 (88.7) b	1.4 ±0.6 (12.0) b
Fırat	8.9 ±1.0 (100) d	7.9 ±1.1 (88.1) c	7.1 ±1.1 (79.8) c	2.8 ±2.5 (31.0) d	0.8 ±0.8 (9.0) bcd
Kafkas	10.3 ±0.2 (100) cd	9.5 ±0.4 (92.3) c	8.6 ±0.6 (82.9) c	1.9 ±0.5 (18.0) d	0.5 ±0.03 (5.0) c
Meyveci	12.3 ±0.9 (100) bc	11.4 ±1.0 (92.6) b	7.3 ±1.4 (39.5) c	2.3 ±1.4 (19.09) d	0.1 ±0.01 (1.0) c
Pul II	13.5±2.0 (100) ab	12.9±2.2 (95.0) ab	11.9±1.7 (87.5) b	6.9 ±1.1 (51.0) c	0.3 ±0.09 (18.0) d

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference

Table 6.14. Foaming capacity and foam stability of lentil and chickpea hydrocolloids extracts.

Cultivar	Retention of foaming capacity (%)				
	0 minute	30 minutes	60 minutes	180 minutes	360 minutes
			CHE		
Canıtez	9.0 ±0.5 (100) a	7.6 ±0.7 (86.1) a	6.7 ±0.3 (13.0) b	4.1 ±0.9 (46.0) b	1.2±0.4 (76.2) b
Cevdetbey 98	8.6 ±0.5 (100) a	8.0 ±0.6 (92.1) a	5.7 ±0.8 (66.5) b	4.0 ±0.5 (46.0) b	1.9±1.1 (22.0) b
Gökçe	6.5 ±0.5 (100) b	6.0 ±0.4 (92.8) b	9.7 ±0.3 (85.0) a	8.2 ±0.6 (68.0) a	5.0±0.3 (34.0) a
Sarı 98	8.5 ±0.9 (100) a	7.4 ±0.3 (87.0) a	6.4 ±0.5 (74.7) b	5.1 ±0.7 (59.0) b	1.3±0.9 (15.0) b
			LHE		
Ali Dayı	6.4 ±0.6 (100) a	6.1 ±0.6 (96.1) a	5.9 ±0.3 (91.8) a	4.2 ±0.5 (66.0) a	2.6 ±0.3 (41.0) a
Çiftçi	1.6 ±0.4 (100) d	1.3 ±0.3 (80.2) d	0.8±0.1 (52.0) d	0.1 ±0.03 (6.0) d	0±0 (0)
Fırat	2.6 ±0.7 (100) c	2.0 ±0.5 (87.3) cd	1.6±0.5 (62.1) c	0.9 ±0.7 (45.0) c	0.3±0.2 (11.0) b
Kafkas	4.5 ± 0.3 (100) b	4.1 ±0.3 (91.8) b	3.7±0.3 (82.4) b	2.6 ±0.3 (82.4) b	1.5±0.6 (45.0) b
Meyveci	4.8 ±0.4 (100) b	3.7 ±0.3 (76.5) b	3.3±0.2 (68.0) b	2.3 ±0.6 (68.0) b	1.4±0.3 (29.0) b
Pul II	2.9 ±0.3 (100) c	2.4 ±0.4 (81.4) c	1.0±0.1 (33.0) c	0.8 ±0.06 (33.0) c	1.4±0.09 (9.0) b

a-d: Values followed by different letters are significantly different at $P<0.05$ as determined by Fisher's protected least significant difference

6.2.2.8. Gel Formation Capacity of Chickpea and Lentil Protein Extracts

The results of gel formation capacities of CPE and LPE are given in Table 6.15. For CPE of Gökçe and Canıtez cultivars, the gel formation initiated at 4% of CPE, but 5% of CPE was needed to observe hard gel formation in protein extracts of these cultivars. In contrast, the gelation of CPE for Sarı 98 cultivar was observed at 6 % of CPE, while no gelation was observed for the CPE of Cevdetbey cultivar. The hard fixed gel was observed only for CPE of Canitez cultivar at 8% concentration. At this concentration the gel of indicated CPE was fixed and hardened, and showed no slipping when the test tube used in the gelling test was inverted. For LPE, the gelling capacity was very low. In fact, for LPE, the gelling was initiated mostly at 6 or 7 % concentration, but no LPE formed fully hardened fixed gels even at 8% concentration. The LPE of Alidayı cultivar showed initial signs of gel formation at 4% concentration, but no progress was observed in gelling of this sample by increase of protein extract concentration. The LPE of Pul II, on the other hand, showed no signs of gelation. For LPE the hardest gel formation was observed for Fırat cultivar at 8% concentration. However, the gel of this LPE was not fixed.

The CHE and LHE used in this study showed considerably higher gelling capacity than CPE and LPE (Table 6.16). In CHE and LHE the hard and fixed gels were observed in most of the cultivars at 7 % or 8% concentration. For CHE, the hard fixed gels were observed for Gökçe, Canıtez and Cevdetbey cultivars at 7% concentration. Sarı 98 is the only chickpea cultivar for which CHE showed hard but unfixed gelling. The LHE of Alidayı, Meyveci, Fırat and Kafkas cultivars showed hard and fixed gel formation at 7 %, while the same type of gel formation occurred at 8% concentration for LHE of Çiftçi. In contrast, Pul-II showed very weak gel formation which showed no progress between 5 and 8% concentrations.

Table 6.15. Gel formation capacities of chickpea and lentil protein extracts

Cultivar	Protein concentration							
	1%	**2%**	**3%**	**4%**	**5%**	**6%**	**7%**	**8%**
				Chickpea				
Canıtez	-	-	-	±	+	++	++	+++
Cevdetbey 98	-	-	-	-	-	-	-	-
Gökçe	-	-	-	±	+	+	++	++
Sarı 98	-	-	-	-	-	±	+	+
				Lentil				
Ali Dayı	-	-	-	±	±	±	±	±
Çiftçi	-	-	-	-	-	±	±	+
Fırat	-	-	-.	-	-	-	±	++
Kafkas	-	-	-	-	-	±	±	+
Meyveci	-	-	-	-	-	-	±	+
Pul-II	-	-	-	-	-	-	-	-

[a](+++): hard fixed gel formation-no flow when inverted; (++): hard gel formation-little slipping occurred when inverted; (+) gel formation-slipping occurred slowly when inverted; (-/+): weak gel formation-rapid slipping occurred when inverted; (-) no gel formation.

Table 6.16. Gel formation capacities of chickpea and lentil hydrocolloids extracts

Cultivar	Protein concentration							
	1%	2%	3%	4%	5%	6%	7%	8%
Chickpea								
Canıtez	-	-	±	+	++	++	+++	+++
Cevdetbey 98	-	-	-	±	+	++	+++	+++
Gökçe	-	-	±	±	+	++	+++	+++
Sarı 98	-	-	-	-	-	±	+	+
Lentil								
Ali Dayı	-	±	+	+	++	++	+++	+++
Çiftçi	-	-	±	±	+	++	++	+++
Fırat	-	-	±.	+	++	++	+++	+++
Kafkas	-	-	-	-	-	±	±	+
Meyveci	-	-	-	-	-	-	±	+
Pul-II	-	-	-	±	+	+	+	+

[a](+++): hard fixed gel formation-no flow when inverted; (++): hard gel formation-little slipping occurred when inverted; (+) gel formation-slipping occurred slowly when inverted; (-/+): weak gel formation-rapid slipping occurred when inverted; (-) no gel formation.

6.2.3. SDS-PAGE Profiles of Lyophilized Protein Extracts

The SDS-PAGE patterns of lyophilized CPE and LPE were given in Figure 6.15 and 6.16. In lentils there are 12 to 16 intense bands appeared in the ranges of 80-100 KDa (1-2 bands), 55-58 KDa (1 band), 43-52 KDa (3-4 bands), 32-39 KDa (3-4 bands), 24-26 KDa (1 band), and 18-21 KDa (3-4 bands). In general, different chickpea cultivars showed similar protein patterns. However, Çiftçi, Kafkas and Meyveci cultivars contained more intense bands than Alidayı, Fırat and Pul cultivars. Çiftçi, Kafkas and Meyveci cultivars also contained more protein bands than the other lentil

cultivars. On the other hand, chickpea protein extracts gave 7 to 11 intense bands in the range of 68-78 KDa (1-2 bands), 31-37 KDa (3 bands), 19-23 KDa (1-4 bands), and at almost 14 KDa and 16.5 KDa. The chickpea proteins showed quite similar patterns. However, Gökçe cultivar showed more intense bands than the other cultivars. The comparison of protein bands for chickpea and lentil proteins clearly showed the considerably different SDS-PAGE patterns of these legumes. Particularly, lack of intense protein bands in chickpeas between 40 and 60 KDa, but appearance of many intense bands for lentil proteins in this range clearly differentiates these proteins from each other. Thus, it can be concluded that the SDS-PAGE is an appropriate method to identify chickpea and lentil proteins.

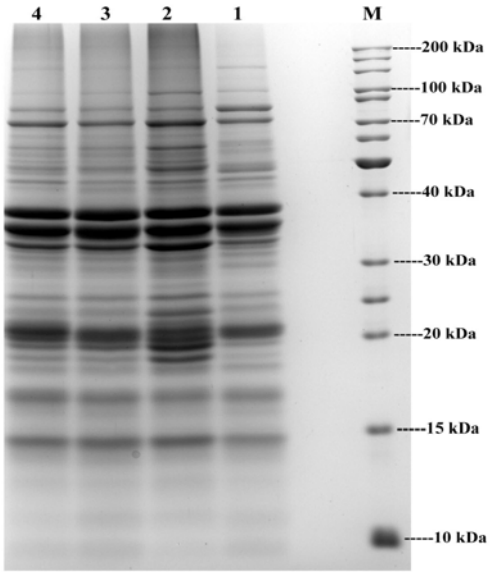


Figure 6.15. Protein profiles of lyophilized protein extracts of chickpea cultivars by SDS-Page electrophoresis. **Lines: M**: Marker. **1:** Canıtez. **2:** Gökçe. **3:** Cevdetbey 98. **4:** Sarı 98

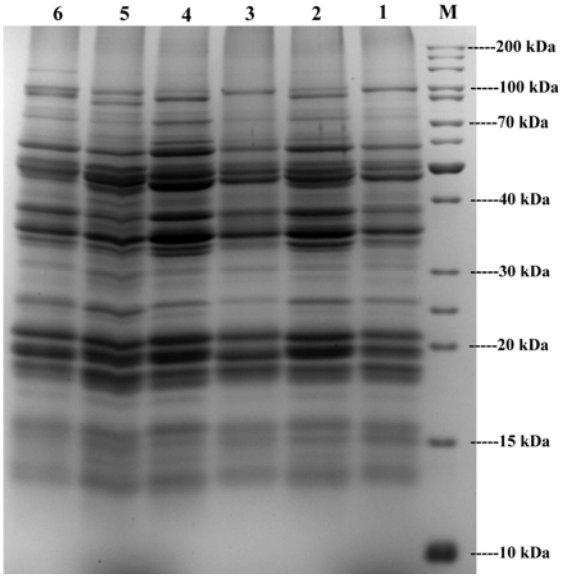


Figure 6.16. Protein profiles of lyophilized protein extracts of lentil cultivars by SDS-Page electrophoresis.**Lines: M:** Marker. **1:** Ali Dayı **2:** Çiftçi. **3:** Fırat. **4:** Kafkas. **5:** Meyveci. **6:** Pul II

6.2.4. Correlations Between Measured Parameters

The correlations between averages of different parameters of cultivars determined for legume water extracts, protein extracts (CPE or LPE) and hydrocolloids extracts (CHE or LHE) were given in Table 6.17 and 6.18 for chickpeas and lentils, respectively. In chickpea extracts, significant positive correlation ($P<0.01$) was determined between iron chelating capacity and free radical scavenging capacity. Such a correlation does not exist in lentil extracts. However, in lentil extracts there is a negative correlation between iron chelating capacity and total flavonoids content. This result suggests a possible complex formation of proteins with lentil flavonoids. Both proteins and flavonoids are iron binding compounds but it is likely that the specific complexes formed between these two compounds in lentils lack iron binding properties.

In chickpeas, a positive correlation was found between iron chelating capacity of protein extracts and total phenolic content of legume water extract. Thus, it seems that the acetone powder used in protein extraction is not completely free from phenolic residues. The lack of any correlations between free radical scavenging activity of protein extracts of chickpeas and total phenolic content of chickpea extracts suggests that the possible phenolic compounds in chickpea extracts were oxidized during acetone

powder production and they are unable to show their free radical scavenging capacity. In lentils, a negative correlation exists between lentil protein extract free radical scavenging activity and lentil extract total phenolic content. For the lentils a negative correlation also exists between iron chelating capacity of protein extracts and total flavonoids content. In contrast, there is a positive correlation between iron chelating capacity of lentil proteins and soluble protein content in lentils. The negative correlations between different types of antioxidant activity (free radical scavenging or iron binging) for lentil proteins and total phenolic or flavonoids content of lentils once more show the suppressive effect of lentil phenolic compounds on antioxidant potential of lentil proteins. It is likely that this suppressive effect is related with complex formation between proteins and flavonoids in lentils and blocking of the antioxidant groups. For chickpeas, the free radical scavenging capacity of hydrocolloids extracts correlated positively with free radical scavenging and iron chelating capacities of chickpea extracts, and water absorption capacity of chickpea protein extracts. On the other hand, the iron chelating capacity of chickpea hydrocolloids extract correlated positively with emulsion stability of chickpea protein extracts. There is also a negative correlation between iron chelating capacity of hydrocolloids extracts and oil absorption capacity of protein extracts. In lentils, the free radical scavenging capacity of hydrocolloids extracts correlated positively with foam capacity and stability of protein extracts. The iron chelating capacity of lentil hydrocolloids extracts negatively correlated with iron chelating capacity of lentil extracts.

In chickpea protein extracts there is a positive correlation between emulsion capacity and stability. In contrast, in lentil protein extracts no correlation was observed between emulsion capacity and stability. For chickpea protein extracts there are no negative correlations between any one of the parameters and emulsion capacity or stability. However, for lentil protein extracts negative correlations were observed between emulsion capacity of proteins and free radical scavenging capacity of proteins, and total flavonoids content of lentil extracts. These observations clearly showed the adverse effects of lentil falavonoids on emulsification properties of proteins. It seems that the complexation of proteins with flavonoids caused reduction of their flexibility and resulting ability to unfold and rearrange at lipid-water interface.

For the hydrocolloids extracts of chickpeas and lentils no positive correlations were observed between emulsion capacity and emulsion stability. However, emulsion capacity of chickpea hydrocolloids extract correlated positively with total phenolic

content of chickpea extracts, iron chelating capacity of protein extracts, foaming capacity and stability of protein extracts. The emulsion stability of hydrocolloids extract of chickpeas correlated positively with iron cheating capacity of chickpea protein extracts. However, there are negative correlations between emulsion stability of chickpea hydrocolloids extracts and free radical scavenging and iron chelating capacities of chickpea extracts, and free radical scavenging capacity of chickpea hydrocolloids extracts. This result suggests the positive and negative effects of phenolic compounds and other antioxidants on emulsion capacity and stability of chickpea hydrocolloids. In lentils a positive correlation was observed between emulsion capacity of hydrocolloids extracts and total flavonoids content of lentil extract. But there was a negative correlation between emulsion capacity of lentil hydrocolloids extracts and foam capacity of lentil protein extracts. The emulsion stability of lentil hydrocolloids extract correlated positively with free radical scavenging capacity and oil absorption capacity of lentil protein extracts, while there was a negative correlation between emulsion stability of lentil hydrocolloids extract and iron chelating capacity of lentil protein extract. These results suggest positive roles of lentil flavonoids on emulsion capacity of lentil hydrocolloids extracts. It is worth to note that we have determined negative effects of lentil flavonoids on emulsion capacity of lentil proteins. Thus, it seems that the flavonoids bind or crosslinked with hydrocolloids other than the proteins (carbohydrates such as cellulose, starch or pectin) make some conformational and/or molecular weight changes in these compounds and this resulted in increase of their emulsifying capacity.

Interestingly, the water absorption capacity of chickpea protein extracts correlated only with free radical scavenging capacity of chickpea extracts, while no correlations exist between any of the measured parameters and water absorption capacity of lentil protein extracts. There are no positive correlations between oil absorption capacity of chickpea protein extracts and any other measured parameter. However, a negative correlation was observed between oil absorption capacity of chickpea proteins and emulsion stability of chickpea proteins. This result showed the importance of hydrophilic groups in emulsion stability of chickpea proteins. For the lentil proteins oil absorption capacity correlated positively with free radical scavenging capacity of lentil proteins, but there is a negative correlation between oil absorption capacity of lentil proteins and iron chelating capacity of lentil extracts. For chickpeas, no correlations exist between water absorption capacity of hydrocolloids extracts and

any other measured parameters. However, in lentils water absorption capacity of hydrocolloids extracts correlated positively with iron chelating capacity of lentil extracts. There is also a negative correlation in lentils between water and oil absorption capacities of hydrocolloids extracts. For chickpeas there is a positive correlation between oil adsorption capacity of hydrocolloids extracts and foam stability of proteins. But in lentils no correlations exist between oil absorption capacity of hydrocolloids extracts and any other measured parameters.

For chickpea protein extracts the foaming capacities positively correlated with total phenolic content of chickpea extracts, free radical scavenging and iron chelating capacities of chickpea protein extracts, while there is a negative correlation between foaming capacities of chickpea protein extracts and free radical scavenging capacity of chickpea extracts. On the other hand, the foaming stability of chickpea protein extracts correlated only with free radical scavenging capacity of protein extracts. In lentils there are no significant positive correlations between foaming capacity of protein extracts and other measured parameters. But a negative correlation exists between foaming capacity of lentil protein extracts and total phenolic content of lentil extracts. For chickpeas, a positive correlation was observed for foaming capacity of hydrocolloids extracts and emulsion stability of hydrocolloids extracts. However, there are also significant negative correlations between foaming capacity of chickpea protein extracts and free radical scavenging and iron binding capacities of chickpea extracts, and free radical scavenging capacity of chickpea hydrocolloids extracts. There are no positive or negative correlations between foaming stability of chickpea hydrocolloids extracts and other investigated parameters. For lentils there is only a single negative correlation between foaming capacity of hydrocolloids extracts and total phenolic content of lentil extracts. A similar negative correlation was observed only between foam stability of lentil hydrocolloids extracts and total phenolic contents of lentil extracts. But foam stability of lentil hydrocolloids extract also correlated positively with free radical scavenging and iron chelating capacities of lentil hydrocolloids extracts, foaming capacities of lentil protein and hydrocolloids extracts.

For both chickpeas and lentils the soluble protein content of protein extracts or hydrocolloids extracts did not correlate positively with any of the investigated parameters. However, there are significant negative correlations between soluble protein contents of chickpea hydrocolloids extracts and several other parameters. For example, in chickpeas, total soluble protein content in hydrocolloids extracts correlated

negatively with emulsion capacity and stability of chickpea protein extracts. This result suggests the negative effects of hydrophilic proteins on emulsifying properties at the studied emulsification pH value.

The overall results of correlations between different parameters clearly indicated that the functional properties of lentil and chickpea proteins and hydrocolloids are affected mainly from total phenolic or flavonoids content of legumes and different types of antioxidant activity including free radical scavenging and iron chelating capacity. In contrast, the soluble protein content of extracts is not correlated significantly with different functional properties. This result clearly indicated that the technological functions of proteins in chickpeas and lentils are governed by interaction of proteins with phenolic compounds and availability of reactive groups having free radical scavenging and metal chelating activities.

Table. 6.17. Correlations between different measured parameters of chickpea water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Chickpea Water Extracts (CWE)			Chickpea Protein Extracts (CPE)			
		Total phenolic content	Free radical scavenging activity	İron chelating capacity	Water soluble protein content	Free radical scavenging activity	İron chelating capacity	Emulsifying capacity (NTU)
Free radical scavenging activity	CWE	-						
İron chelating capacity	CWE	-	0.834 (0.001)					
Water soluble protein content	CPE	-	-					
Free radical scavenging activity	CPE			-	-			
İron chelating capacity	CPE	0.814 (0.001)	-	-	-	-		
Emulsifying capacity (NTU)	CPE	-	-	-	-	-	-	
Emulsifying capacity (abs)	CPE	-	-	-	-	-		0.986 (0.000)
Emulsion stability (NTU)	CPE	-	-			-		
Emulsion stability (abs)	CPE	-	-	-		-		0.754 (0.005)
Water absorption capacity	CPE	-	0.771 (0.003)		-		-	-
Oil absorptipon capacity	CPE	-	-	-		-	-	
Foaming capacity	CPE	0.815 (0.001)	-0.729 (0.007)	-	-	0.839 (0.001)	0.803 (0.002)	-
Foam stability	CPE		-	-		0.790 (0.002)	-	-
Water soluble protein content	CHE	-	-	-	-	-	-	-0.794 (0.002)
Free radical scavenging activity	CHE	-	0.971 (0.000)	0.927 (0.000)	-	-	-	-
İron chelating capacity	CHE	-	-		-	-	-	
Emulsifying capacity (NTU)	CHE	0.861 (0.000)	-	-	-			-
Emulsifying capacity (abs)	CHE	0.800 (0.002)	-	-	-	-	0.743 (0.006)	-
Emulsion stability (NTU)	CHE	-		-0.891 (0.000)		-	-	-
Emulsion stability (abs)	CHE	-	-0.729 (0.007)		-	-	0.729 (0.007)	-
Water absorption capacity	CHE	-	-	-	-	-	-	-
Oil absorptipon capacity	CHE	-	-	-	-	-	-	-
Foaming capacity	CHE	-	-0.730 (0.007)	-0.871 (0.000)	-	-	-	-
Foam stability	CHE	-	-	-	-	-	-	-

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Table. 6.17. (cont.) Correlations between different measured parameters of chickpea water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Chickpea Protein Extracts (CPE)						
		Emulsifying capacity (abs)	Emulsion stability (NTU)	Emulsion stability (abs)	Water absorption capacity	Oil absorptipon capacity	Foaming capacity	Foam stability
Free radical scavenging activity	CWE							
İron chelating capacity	CWE							
Water soluble protein content	CPE							
Free radical scavenging activity	CPE							
İron chelating capacity	CPE							
Emulsifying capacity (NTU)	CPE							
Emulsifying capacity (abs)	CPE							
Emulsion stability (NTU)	CPE	0.705 (0.010)						
Emulsion stability (abs)	CPE	0.815 (0.001)	0.965 (0.000)					
Water absorption capacity	CPE	-	-	-				
Oil absorptipon capacity	CPE			-0.746 (0.005)				
Foaming capacity	CPE	-	-	-	-	-		
Foam stability	CPE	-		-	-	-		
Water soluble protein content	CHE	-0.805 (0.001)		-0.774 (0.003)			-	-
Free radical scavenging activity	CHE	-	-	-	0.794 (0.002)	-		-
İron chelating capacity	CHE		0.866 (0.000)	0.853 (0.000)		-0.725 (0.008)	-	-
Emulsifying capacity (NTU)	CHE	-		-	-	-	0.768 (0.004)	0.741 (0.006)
Emulsifying capacity (abs)	CHE	-			-	-	0.717 (0.009)	
Emulsion stability (NTU)	CHE	-	-	-	-	-	-	-
Emulsion stability (abs)	CHE	-	-	-	-	-		-
Water absorption capacity	CHE	-	-	-	-	-	-	-
Oil absorptipon capacity	CHE	-	-	-	-	-	-	0.734 (0.007)
Foaming capacity	CHE	-	-	-	-	-	-	-
Foam stability	CHE	-	-	-	-	-	-	-

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Table. 6.17. (cont.) Correlations between different measured parameters of chickpea water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Chickpea Hydrocolloids Extracts (CHE)						
		Water soluble protein content	Free radical scavenging activity	İron chelating capacity	Emulsifying capacity (NTU)	Emulsifying capacity (abs)	Emulsion stability (NTU)	Emulsion stability (abs)
Free radical scavenging activity	CWE							
İron chelating capacity	CWE							
Water soluble protein content	CPE							
Free radical scavenging activity	CPE							
İron chelating capacity	CPE							
Emulsifying capacity (NTU)	CPE							
Emulsifying capacity (abs)	CPE							
Emulsion stability (NTU)	CPE							
Emulsion stability (abs)	CPE							
Water absorption capacity	CPE							
Oil absorptipon capacity	CPE							
Foaming capacity	CPE							
Foam stability	CPE							
Water soluble protein content	CHE							
Free radical scavenging activity	CHE	-						
İron chelating capacity	CHE		-					
Emulsifying capacity (NTU)	CHE	-	-	-				
Emulsifying capacity (abs)	CHE	-	-		0.938 (0.000)			
Emulsion stability (NTU)	CHE	-	-0.793 (0.002)	-	-	-		
Emulsion stability (abs)	CHE	-	-0.729 (0.007)	-	-	-	0.847 (0.001)	
Water absorption capacity	CHE	-	-	-	-	-		
Oil absorptipon capacity	CHE	-	-	-	-	-	-	-
Foaming capacity	CHE	-	-0.831 (0.001)	-	-	-	0.821 (0.001)	
Foam stability	CHE	-	-	-	-	-	-	-

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Table. 6.17. (cont.) Correlations between different measured parameters of chickpea water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Chickpea Hydrocolloids Extracts (CHE)		
		Water absorption capacity	Oil absorptipon capacity	Foaming capacity
Free radical scavenging activity	CWE			
İron chelating capacity	CWE			
Water soluble protein content	CPE			
Free radical scavenging activity	CPE			
İron chelating capacity	CPE			
Emulsifying capacity (NTU)	CPE			
Emulsifying capacity (abs)	CPE			
Emulsion stability (NTU)	CPE			
Emulsion stability (abs)	CPE			
Water absorption capacity	CPE			
Oil absorptipon capacity	CPE			
Foaming capacity	CPE			
Foam stability	CPE			
Water soluble protein content	CHE			
Free radical scavenging activity	CHE			
İron chelating capacity	CHE			
Emulsifying capacity (NTU)	CHE			
Emulsifying capacity (abs)	CHE			
Emulsion stability (NTU)	CHE			
Emulsion stability (abs)	CHE			
Water absorption capacity	CHE			
Oil absorptipon capacity	CHE	-		
Foaming capacity	CHE	-	-	
Foam stability	CHE	-	-	-

Table. 6.18. Correlations between different measured parameters of lentil water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Lentil Water Extracts (LWE)				Lentil Protein Extracts (LPE)		
		Total phenolic content	Total flavonoid content	Free radical scavenging activity	İron chelating capacity	Water soluble protein content	Free radical scavenging activity	İron chelating capacity
Total flavonoid content	LWE	-						
Free radical scavenging activity	LWE	-	-					
İron chelating capacity	LWE	-	-0.718 (0.001)	-				
Water soluble protein content	LPE	-	-	-	-			
Free radical scavenging activity	LPE	-0.708 (0.001)	-	-	-	-		
İron chelating capacity	LPE	-	-0.635 (0.005)	-	-	0.679 (0.002)	-	
Emulsifying capacity (NTU)	LPE	-	-	-	-	-	-0.596 (0.009)	-
Emulsifying capacity (abs)	LPE	0.647 (0.004)	-	-	-	-	-0.639 (0.004)	-
Emulsion stability (NTU)	LPE	-		-		-		0.605 (0.008)
Emulsion stability (abs)	LPE	-	-0.694 (0.001)	-	0.718 (0.001)	-		
Water absorption capacity	LPE							
Oil absorptipon capacity	LPE		-	-	-0.729 (0.001)	-	0.624 (0.006)	-
Foaming capacity	LPE	-0.656 (0.003)	-	-		-	-	-
Foam stability	LPE	-0.655 (0.003)	-	-		-	0.733 (0.001)	-
Water soluble protein content	LHE	-	-	-	-	-	-	-
Free radical scavenging activity	LHE	-	-	-	-	-	-	-
İron chelating capacity	LHE				-0.676 (0.002)	-	-	-
Emulsifying capacity (NTU)	LHE	-	0.668 (0.002)	-	-	-	-	-
Emulsifying capacity (abs)	LHE			-	-	-	-	-
Emulsion stability (NTU)	LHE		-	-		-	0.643 (0.004)	-
Emulsion stability (abs)	LHE	-		-	-			-0.607 (0.008)
Water absorption capacity	LHE	-	-	-	0.617 (0.006)		-	
Oil absorptipon capacity	LHE	-	-	-	-	-	-	-
Foaming capacity	LHE	-0.804 (0.000)	-		-	-	-	
Foam stability	LHE	-0.751 (0.000)	-	-	-	-	-	

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Table. 6.18. (cont) Correlations between different measured parameters of lentil water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Lentil Protein Extracts (LPE)						
		Emulsifying capacity (NTU)	Emulsifying capacity (abs)	Emulsion stability (NTU)	Emulsion stability (abs)	Water absorption capacity	Oil absorptipon capacity	Foaming capacity
Total flavonoid content	LWE							
Free radical scavenging activity	LWE							
İron chelating capacity	LWE							
Water soluble protein content	LPE							
Free radical scavenging activity	LPE							
İron chelating capacity	LPE							
Emulsifying capacity (NTU)	LPE							
Emulsifying capacity (abs)	LPE	0.826 (0.000)						
Emulsion stability (NTU)	LPE		-					
Emulsion stability (abs)	LPE	-	-	0.911 (0.000)				
Water absorption capacity	LPE							
Oil absorptipon capacity	LPE	-	-	-	-	-		
Foaming capacity	LPE	-	-	-	-	-	0.744 (0.000)	
Foam stability	LPE		-0.635 (0.005)	-	-	-	0.735 (0.001)	0.717 (0.001)
Water soluble protein content	LHE	-	-	-	-	-	-	-
Free radical scavenging activity	LHE	-	-	-	-	-	-	0.738 (0.000)
İron chelating capacity	LHE	-	-	-	-	-		-
Emulsifying capacity (NTU)	LHE	-	-			-	-	-
Emulsifying capacity (abs)	LHE	-	-	-	-	-	-	-0.615 (0.007)
Emulsion stability (NTU)	LHE	-		-	-	-	0.645 (0.004)	-
Emulsion stability (abs)	LHE	-	-	-	-	-		-
Water absorption capacity	LHE	-	-	-	-	-	-0.837 (0.000)	
Oil absorptipon capacity	LHE	-		-	-	-	-	-
Foaming capacity	LHE	-	-	-	-	-	-	
Foam stability	LHE	-	-	-	-	-	-	0.614 (0.007)

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Table. 6.18. (cont) Correlations between different measured parameters of lentil water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Lentil Protein Extracts (LHE)	Lentil Hydrocolloids Extracts (LHE)					
		Foam stability	Water soluble protein content	Free radical scavenging activity	İron chelating capacity	Emulsifying capacity (NTU)	Emulsifying capacity (abs)	Emulsion stability (NTU)
Total flavonoid content	LWE							
Free radical scavenging activity	LWE							
İron chelating capacity	LWE							
Water soluble protein content	LPE							
Free radical scavenging activity	LPE							
İron chelating capacity	LPE							
Emulsifying capacity (NTU)	LPE							
Emulsifying capacity (abs)	LPE							
Emulsion stability (NTU)	LPE							
Emulsion stability (abs)	LPE							
Water absorption capacity	LPE							
Oil absorptipon capacity	LPE							
Foaming capacity	LPE							
Foam stability	LPE							
Water soluble protein content	LHE	-						
Free radical scavenging activity	LHE	0.779 (0.000)						
İron chelating capacity	LHE	-	-	-				
Emulsifying capacity (NTU)	LHE	-	-	-	-			
Emulsifying capacity (abs)	LHE	-	-	-	-	0.894 (0.000)		
Emulsion stability (NTU)	LHE	0.629 (0.005)	-	-	-	-	-	
Emulsion stability (abs)	LHE	-	-	-	-	-	-	0.863 (0.000)
Water absorption capacity	LHE	-	-	-	-	-	-	-
Oil absorptipon capacity	LHE		-	-	-	-	-	-
Foaming capacity	LHE	-	-	-		-	-	-
Foam stability	LHE		-	0.598 (0.009)	0.614 (0.007)	-	-	-

(cont. on next page)

Table. 6.18. (cont) Correlations between different measured parameters of lentil water extracts, protein extracts and hydrocolloids extracts (P<0.01)

		Lentil Hydrocolloids Extracts (LHE)			
		Emulsion stability (abs)	Water absorption capacity	Oil absorptipon capacity	Foaming capacity
Total flavonoid content	LWE				
Free radical scavenging activity	LWE				
İron chelating capacity	LWE				
Water soluble protein content	LPE				
Free radical scavenging activity	LPE				
İron chelating capacity	LPE				
Emulsifying capacity (NTU)	LPE				
Emulsifying capacity (abs)	LPE				
Emulsion stability (NTU)	LPE				
Emulsion stability (abs)	LPE				
Water absorption capacity	LPE				
Oil absorptipon capacity	LPE				
Foaming capacity	LPE				
Foam stability	LPE				
Water soluble protein content	LHE				
Free radical scavenging activity	LHE				
İron chelating capacity	LHE				
Emulsifying capacity (NTU)	LHE				
Emulsifying capacity (abs)	LHE				
Emulsion stability (NTU)	LHE				
Emulsion stability (abs)	LHE				
Water absorption capacity	LHE	-			
Oil absorptipon capacity	LHE	-	-		
Foaming capacity	LHE	-	-	-	
Foam stability	LHE	-	-	-	0.888 (0.000)

CHAPTER 7

CONCLUSIONS

Technologically important conclusions for use of chickpea and lentil protein extracts in the food, drug and cosmetics industries:

1. Protein extracts of chickpeas and lentils showed considerable emulsifying and foaming capacity in almost the same range, but **emulsions and foams formed by chickpea proteins are mostly more stable than those of lentil proteins.**

2. The lentil protein extracts are highly soluble and showed poor water absorption characteristics. In contrast, **chickpea protein extracts showed a moderate water absorption capacity.**

3. Lentil protein extracts showed good oil absorption capacity. However, **chickpea protein extracts are good oil absorbers** with almost two fold better oil adsorption capacity.

4. The **lentil proteins showed superior solubility and free radical scavenging capacity than chickpea proteins**.

5. The general technological functions of chickpea proteins are superior to those of lentil proteins. Thus, **chickpea proteins are suggested as soy and whey protein alternatives for functional proteins used in the food, drug and cosmetics industries.**

6. Due to their high solubility and free radical scavenging capacity, **lentil proteins may be more suitable ingredients in functional nutritive foods** than chickpea proteins which need improvement of solubility by enzymatic hydrolysis.

Technologically important conclusions for use of obtained hydrocolloids extracts in the food industry:

7. The hydrocolloids extracts from chickpeas and lentils showed **low free radical scavenging and iron chelating capacities.**

8. The chickpea and lentil hydrocolloids extracts showed **similar and comparable emulsifying capacities with protein extracts.** The emulsion stability of lentil hydrocolloids extracts is comparable to those of their protein extracts. However, chickpea hydrocolloids extracts show lower emulsion stability than their protein extracts.

9. The lentil hydrocolloids extracts showed little foaming capacity, but their foams are mostly more stable than those of their protein extracts. **The chickpea hydrocolloids extracts show a considerable foaming capacity and foam stability**, comparable to those of the protein extracts.

10. The lentil and hydrocolloids extracts showed much more water absorption capacity than the protein extracts. Both extract showed also comparable oil absorption capacities with protein extracts. **Particularly, chickpea hydrocolloids extracts are good oil and water absorbers.**

11. The hydrocolloid extracts of chickpeas and lentils are crude and contain both proteins and carbohydrates. Thus, these extracts are not suitable for cosmetics and drug industry which mostly needs pure ingredients. However, **hydrocolloids extracts can be used in the food industry as cheap source of natural ingredients to develop legume based foods and to obtain a specific functionality.**

Conclusions important for use of data in agronomy and molecular biology:

12. The outstanding Turkish chickpea cultivars are Gökçe and Cevdetbey. Gökçe showed the highest antioxidant activity based on free radical scavenging and iron chelating capacities of legume water extracts, highest protein solubility and protein foam stability and considerably high protein water and oil absorption capacities than other cultivars. Cevdetbey cultivar had the highest protein antioxidant activity, highest protein foaming capacity and stability and considerably high water and oil absorption capacities. **Thus, these two cultivars can be used in breeding studies to improve the nutritional and technological properties of Turkish chickpeas. The extensive growth of these cultivars is suggested to provide suitable legume cultivars for industrial production of proteins.**
13. The outstanding Turkish lentil cultivar is Alidayı. Alidayı showed highest protein free radical scavenging capacity, protein emulsion stability, protein foaming capacity and foam stability and oil absorption capacity. This cultivar also showed considerably high antioxidant activity in legume water extracts, protein iron chelating capacity and protein emulsifying capacity. **Thus, this cultivar can be used in breeding studies to improve nutritional and technological properties of Turkish lentils. The extensive growth of this cultivar is suggested to provide suitable legume cultivars for industrial production of protein extracts.**

14. For lentil cultivars, considerable variation was observed for total flavonoids content, protein free radical scavenging capacity, protein iron chelating capacity, protein emulsion stability, protein foaming capacity and protein foam stability. For chickpeas, considerable variation was observed for free radical scavenging capacity and iron chelating capacity of legume water extracts and protein extracts, protein emulsion stability, foaming capacity and foam stability, and protein water absorption capacity. **This variation in functional properties suggests the diversity of genes responsible for these properties. Thus, this study showed the possibility for improvement of functional properties of**

chickpeas and lentils by use of breeding programs or biotechnological methods which employ molecular techniques. This study identified monitored the insufficient or lacking technological functions of chickpeas and lentils and is a reference study for future breeding programs.

Nutritionally important conclusions:

15. **Turkish chickpeas and lentils are good sources of antioxidant phenolic compounds and proteins and show sufficient antioxidant activity** based on free radical scavenging and iron chelating capacities. The consumption of legumes can make a contribution to increase antioxidant level in blood and cell and prevent many human diseases including cancer, cardiovascular diseases, aging and Parkinson disease.

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