

DEVELOPING ENTERAL FEEDING FORMULATIONS WITH DIFFERENT
PROTEIN SOURCES, RHEOLOGICAL CHARACTERIZATION AND
MICROSTRUCTURAL ANALYSIS OF THESE FORMULATIONS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY



BY
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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

FEBRUARY 2021

Approval of the thesis:

**DEVELOPING ENTERAL FEEDING FORMULATIONS WITH
DIFFERENT PROTEIN SOURCES, RHEOLOGICAL
CHARACTERIZATION AND MICROSTRUCTURAL ANALYSIS OF
THESE FORMULATIONS**

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ABSTRACT

DEVELOPING ENTERAL FEEDING FORMULATIONS WITH DIFFERENT PROTEIN SOURCES, RHEOLOGICAL CHARACTERIZATION AND MICROSTRUCTURAL ANALYSIS OF THESE FORMULATIONS

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February 2021, 178 pages

People who underwent surgery or suffer from dysphagia disease cannot consume solid food materials. Liquid food formulations which are called as enteral feeding formulations are prepared for these people. The main objective of this study was to investigate the usage possibilities of different plant-based proteins in enteral nutrition products and to examine the effects of these proteins on these products such as rheology, stability and microstructure. In the first part of this study protein concentrates were extracted from lentil, chickpea, mung bean and quinoa. Extracted proteins and two commercial proteins which are pea protein concentrate and soy protein concentrate were characterized. In order to characterize these protein concentrates, proximate analysis, amino acid analysis, WHC, OBC, FTIR analysis and scanning electron microscopy analysis were carried out.

In the second part of this study, these proteins were used to formulate enteral nutrition products together with those currently used in the industry. After formulation step, particle size analysis and stability analysis were conducted.

Rheological and microstructural properties were also investigated by using rheometer and confocal laser scanning microscope (CLSM), respectively. All enteral products which were prepared with different protein and fat sources were compared with commercial enteral product.

Keywords: Enteral Feeding Products, Plant Proteins, Rheology, Microstructure



ÖZ

FARKLI PROTEİN KAYNAKLARI İLE ENTERAL BESLENME FORMÜLASYONLARININ GELİŞTİRİLMESİ, BU FORMÜLASYONLARIN REOLOJİK KARAKTERİZASYONU VE MİKROYAPISAL ANALİZİ

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Şubat 2021, 178 sayfa

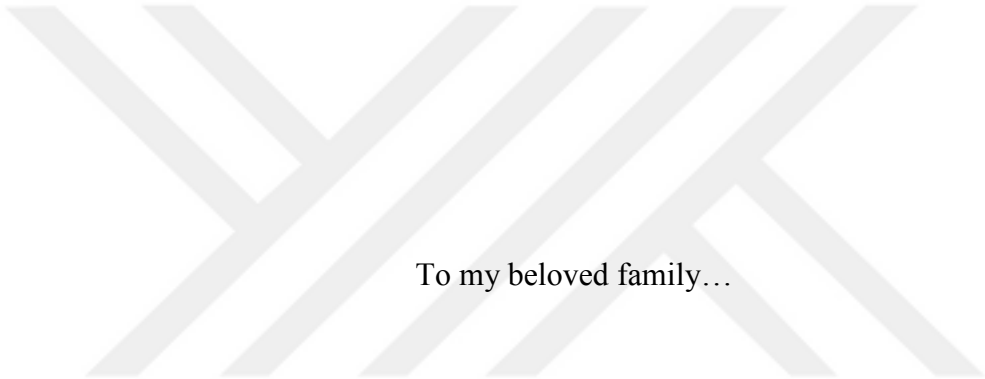
Ameliyat geçirmiş veya disfaji hastalığı olan kişiler katı gıda maddelerini tüketemezler. Bu kişiler için enteral beslenme formülasyonları olarak adlandırılan sıvı gıda formülasyonları hazırlanır. Bu çalışmanın temel amacı, farklı bitki bazlı proteinlerin enteral beslenme ürünlerinde kullanım olanaklarını araştırmak ve bu proteinlerin bu ürünler üzerindeki etkilerini (reoloji, stabilite ve mikroyapı) incelemektir. Bu çalışmanın ilk bölümünde mercimek, nohut, maş fasulyesi ve kinoaadan protein konsantreleri çıkarılmıştır. Ekstrakte edilmiş proteinler ile bezelye proteini konsantresi ve soya proteini konsantresi olan iki ticari protein karakterize edildi. Bu protein konsantrelerini karakterize etmek için proksimal analiz, amino asit analizi, WHC, OBC, FTIR analizi ve taramalı elektron mikroskobu analizi yapılmıştır.

Bu çalışmanın ikinci bölümünde, bu proteinler, endüstride halihazırda kullanılanlar (peynir altı suyu proteini, kazein ve soya proteini) ile birlikte enteral beslenme ürünlerini formüle etmek için kullanıldı. Formülasyon aşamasından sonra partikül

boyutu analizi ve stabilite analizi yapıldı. Reolojik ve mikroyapısal özellikler sırasıyla reometre ve konfokal lazer taramalı mikroskop (CLSM) kullanılarak araştırıldı. Farklı protein ve yağ kaynakları ile hazırlanan tüm enteral ürünler ticari enteral ürün ile karşılaştırıldı.

Anahtar Kelimeler: Enteral Beslenme Ürünleri, Bitki Proteinleri, Reoloji, Mikroyapı





To my beloved family...

ACKNOWLEDGMENTS

Firstly, I would like to acknowledge The Scientific and Technological Research Council of Turkey (TUBITAK, 2210/C Öncelikli Alanlara Yönelik Yurtiçi Yüksek Lisans Burs Programı) for funding my thesis.

Then, I am grateful to my thesis advisor, Prof. Dr. Behiç Mert for his continuous support and guidance for me. It would not be possible to complete this thesis without his motivation and support.

I would like to thank you Prof. Dr. Tülay Bakır for her kindness and introducing me to my master thesis topic which is enteral nutrition products.

I would like to thank you my friends Ülkü Ertuğrul, Eylül Uygun, Kamil Urgan, Gökçem Tonyalı Karşlı, Orçin Akgönül, Ozan Taş, Kaya Çağatay Akan and Zikrullah Bölükkaya for their encouragement and support.

I would like to thank my English Preparatory School friends Hacı Osman Öztürk, Emircan Elikkaya, Süleyman Aksoy, Göktuğ Söğütçü, Oğuzcan Yumrukaya and Nazlı Bozkurt, who always managed to make me laugh even if they never supported me.

I also would like to thank you my close friend Atınç Özgün Akgün for his continuous support and patience.

Finally, I would like to thank my dear family. I am grateful for every sacrifice that they've done for me. This accomplishment would not have been possible without their valuable support.

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LIST OF ABBREVIATIONS

ABBREVIATIONS

LPC: Lentil Protein Concentrate

CPC: Chickpea Protein Concentrate

MPC: Mung Bean Protein Concentrate

QPC: Quinoa Protein Concentrate

PPC: Pea Protein Concentrate

SPC: Soy Protein Concentrate

CO: Corn Oil

SFO: Sunflower Oil

WHC: Water Holding Capacity

OBC: Oil Binding Capacity

GIT: Gastro Intestinal Tract

PER: Protein Efficiency Ratio

AA: Amino Acids

EAA: Essential Amino Acids

FT-IR: Fourier Transform Infrared Spectroscopy

SEM: Scanning Electron Microscopy

CLSM: Confocal Laser Scanning Microscopy

HPLC: High Pressure Liquid Chromatography

LIST OF SYMBOLS

SYMBOLS

τ : Shear Stress

γ : Shear Rate

η : Shear Viscosity

K: Consistency Index

CHAPTER 1

INTRODUCTION

1.1 Medical Foods

Medical food has been defined as " a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation." by The US Food and Drug Administration. According to this definition it can be understood that all foods that fed sick patients should not be defined as medical foods. They should be formulated and processed for specific purposes. Special dietary uses, being designed to meet specific nutritional requirements of a specific conditions and usage of under medical supervision differentiate the medical foods from the other group of foods.

1.1.1 Enteral Nutrition

A patient with a reduced appetite, difficulty swallowing, or any operation that interferes with eating food, or a person with chronic illness, may not be able to provide daily nutritional requirements naturally. In this case, the person should be provided with external nutrition support. This method is called as enteral nutrition. Enteral foods can be defined as group of medical foods which provide complete or partial nutritional support for patients who have normal gut functions with accessible gastrointestinal tract but suffer from ingesting enough amount of food orally. These

foods can be delivered to the stomach, duodenum, or jejunum directly by mouth or with feeding tubes.

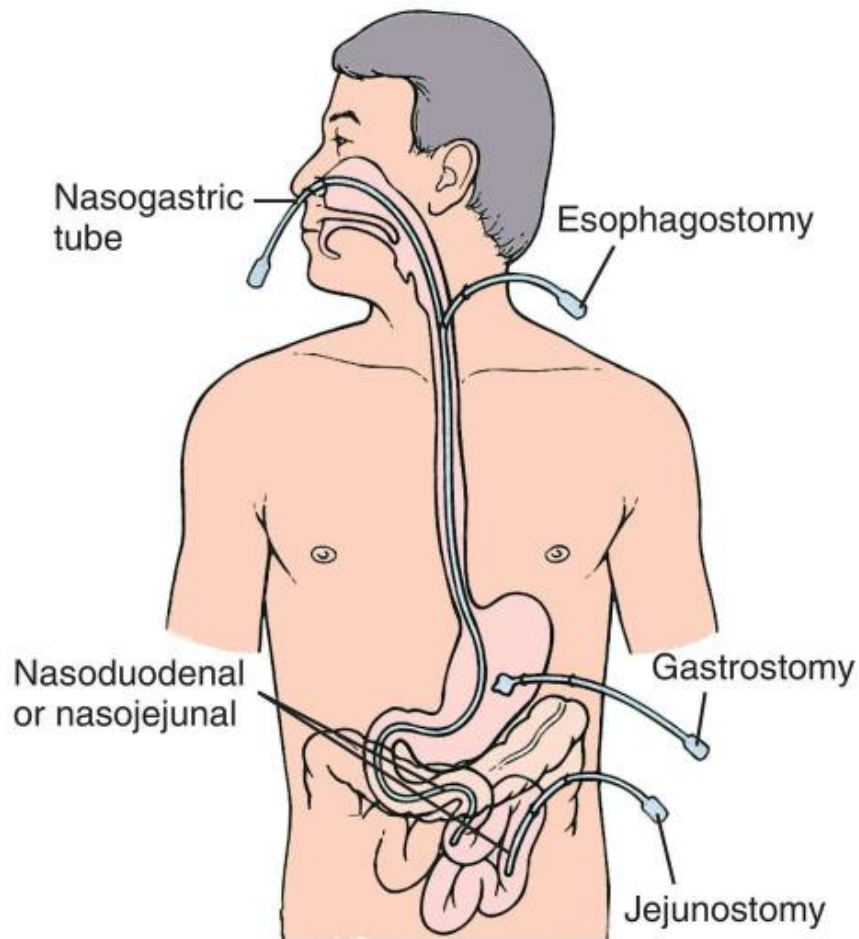


Figure 1.1. Placement of enteral feeding tubes (Kaur, 2009)

1.1.1.1 Types of Enteral Nutrition

The idea of enteral nutrition began with blenderized foods. Then, other enteral nutrition types which are polymeric, defined, modular and disease-specific enteral foods were developed.

1.1.1.1.1 Blenderized Formulae

As mentioned at the beginning of the section, the first enteral foods were prepared by mixing general foods such as rice and milk and delivered to the patient by mouth or by feeding tube. These foods have been emotionally satisfying because they give patients the feeling of eating normal food. Blenderized formulae can be prepared easily anywhere. But these foods may not be safe in terms of microbiology. In addition to microbiological problems, clogging of the feeding tube is an important problem of this type of enteral feeding.

1.1.1.1.2 Polymeric Formulae

Some of the disadvantages and difficulties of the previous formula have been overcome by the development of polymeric formulae. The preparation of the polymeric formula is carried out by mixing carbohydrates, dietary lipids in pure form and proteins in appropriate proportions. Generally, soy polysaccharides are added to the polymeric formula as a source of dietary fiber.

1.1.1.1.3 Defined Formulae

Defined enteral formulae contain chemically purified foodstuffs such as glucose, sucrose, maltodextrin, amino acid, medium-chain triglycerides, corn oil and sunflower oil. These formulas are specially designed to facilitate digestion and absorption. The chemical nature of these foodstuffs makes these formulae generally unpalatable. Therefore, artificial flavors are used to improve these formulations' palatability. To avoid the problem of removing human waste in space, NASA has tried the feasibility of using these formulas in space by astronauts. But this project was rejected due to taste, flavor and aroma problems. This example shows that even formulations with flavor, and flavor are not enough in terms of sensory properties. Most of the defined formulae are designed with lactose-free and fiber-free foodstuffs

for use of the patients who have weakened digestion and absorption system because of the disease they are suffering from such as short bowel syndrome and radiation enteritis.



Figure 1.2. Commercial Enteral Products

1.1.1.1.4 Disease-Specific Formulae

The kind of the nutritional support depends on the physiological condition and disease of the patient. Therefore, disease-specific formulations are developed to meet patients' specific needs.

Table 1.1 Disease specific formulations and patient needs (Kaur, 2009)

Physiological Conditions	Calorie	Protein	Fat	Vitamins and Minerals
Cancer		High Protein (1,2-2,0 g kg ⁻¹ day ⁻¹) animal-based protein sources are preferred	Low fat and low residue	high vitamin C, thiamine, carotene, folic acid, iron and zinc
Hepatic Diseases	High calorie (35-50 kcal kg ⁻¹ day ⁻¹)	Moderate (0,5-1 g kg ⁻¹ day ⁻¹)	Moderate containing MCT	Rich in Thiamine, B12, zinc adequate potassium and iron. Sodium restricted
Renal Diseases	High calorie (40-60 kcal kg ⁻¹ day ⁻¹)	Low Protein (0,6-0,8 g kg ⁻¹ day ⁻¹)		Calcium 300-500 mg/day Sodium up to 500 mg/day
Pulmonary Diseases	Moderate calorie (25-35 kcal kg ⁻¹ day ⁻¹) Low carbohydrate	High Protein (1,2-2,0 g kg ⁻¹ day ⁻¹)	Low fat	High in phosphorus
Burns		High Protein (1,5-2,0 g kg ⁻¹ day ⁻¹)	Rich in n-3 fatty acid	
Surgery/sepsis /trauma	Moderate calorie (25-35 kcal kg ⁻¹ day ⁻¹)	High Protein (1,0-2,5 g kg ⁻¹ day ⁻¹)	25-30% of total kcal day ⁻¹	Slightly higher than RDA
HIV/AIDS	50 kcal kg ⁻¹ day ⁻¹	1,0-1,2 g kg ⁻¹ day ⁻¹	Low fat rich in MCT	Rich in immunomodulatory component
Patients of Medicine and Surgery	25-35 kcal kg ⁻¹ day ⁻¹	1,0-2,0 g kg ⁻¹ day ⁻¹	Moderate level	all essential vitamins and minerals

1.1.2 Parenteral Nutrition

Total parenteral nutrition is defined as intravenous nutrition that provides all the nutrients and fluids necessary for the patient when patients cannot feed themselves by mouth. Paraenteral nutrition is usually provided to patients in critical diseases and psychological conditions. Sterile liquid foods with various effects are administered directly into the vascular route. The precise indication of parenteral nutrition support is that the patient has a dysfunctional gastrointestinal tract (GIT). Although total paraenteral nutrition is effective and life-saving, it is not desirable because it can damage intestinal function in long-term use. Another long-term use consequences are villus atrophy, reduction of disaccharide activity, cellularity decrease and all them may lead to curling ulcer. Since gastrointestinal tract (GIT) is not used, overgrowth of pathogenic microflora is observed in intestine.

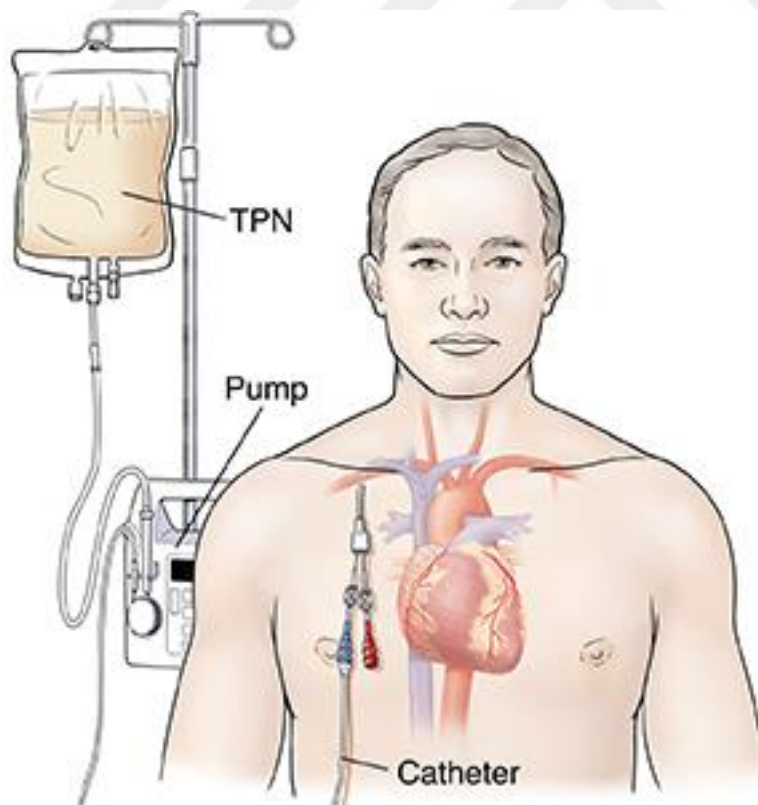


Figure 1.3. Paraenteral nutrition administration (Kaur, 2009)

Table 1.2 Comparison of enteral and paraenteral nutrition

Enteral Nutrition	Paraenteral Nutrition
Easy to apply, physiologically advantageous and adaptive to intestinal functions.	Difficult to apply and more mechanical process.
The normal condition of intestinal villi is maintained	Causes villus atrophy
Intestinal structure and functions are preserved and maintained.	Intestinal structure and functions are not preserved and maintained.
Septic complications are rare.	Causes serious infections and mainly septic shocks related to the catheter.
The mucosal barrier is preserved and bacterial translocation is prevented.	The cause of the breakdown of the mucosal barrier. Enables bacterial and endotoxin translocation.
Normal liver functions are maintained. Diseases such as gall bladder motility and lithiasis are prevented.	Affecting liver function can cause metabolic complications.
Cost effective	Quite expensive

1.2 Proteins

All living organisms contain highly complex and vital molecule for life which is protein. The term protein was coined from the Greek word *proteios* which means "holding the first place" by Swedish chemist Jöns Jacob Berzelius in 1838. Providing the building materials for synthesis of tissues, especially for the muscle tissues, is the main role of the proteins. Proteins have key roles in different biological processes. Transportation of biological molecules, growth of the cell and enzyme structures are examples which show vital importance of proteins for biological processes (Belitz et al., 2009).

Basically, proteins are synthesized only in ribosomes and contain 50-55% carbon, 6-7% hydrogen, 20-23% oxygen, 12-19% nitrogen and 0.2-3% sulfur. Elements such as P, Fe, Zn and Cu can be found in the structure of some proteins. Amino acids forming the protein structure are linked to each other by peptide bonds to form the polypeptide chain structure. Amino acids of different numbers and types form polypeptides of different structure. The protein structure is formed by combining one or more polypeptide chains.

The importance and functions of proteins for organism:

- The smallest structural unit of the organism is the cell, while the building blocks of the cell are proteins.
- Proteins are used in the formation and repair of body tissues.
- Viruses, most of the hormones and the structure of all enzymes are composed of proteins. In fact, protein-structured enzymes are involved in protein synthesis processes.

- Basically the organism consists of 20% dry matter, 90% of this dry matter is the protein structure. A good example of this is the fact that the 70% of dry weight of the liver, muscle and kidney tissues is protein.
- The body's needs for essential amino acids are met by proteins.
- Proteins are responsible for the transport and storage of the organism. Ions and small molecules carry out their functions by transporting and storing them with specific proteins. The transport of oxygen by hemoglobin in the red blood cell, by myoglobin in the muscle, and the storage of iron by complexing with ferritin in the liver are two good examples of these functions of proteins.
- Antibodies produced by the body against antigens are specific proteins. These proteins react with bacteria, viruses and other organisms by identifying them as foreign substances. In this way, proteins are involved in the immune system by separating the structures belonging to the organism or not.
- Providing mechanical support to the body is one of the tasks of proteins.
- The formation and transmission of nerve impulses is provided by proteins.
- Proteins are the controllers of metamorphosis and growth in living forms.

1.2.1 Amino Acids

The basic building blocks of proteins are amino acids. There are 20 amino acids identified to date. Amino acids are branched hydrocarbon chains. In the general formula of an amino acid, an amino group ($-\text{NH}_2$), a hydrogen atom ($-\text{H}$) and a specific R group are attached to the alpha carbon attached to the carboxyl group ($-\text{COOH}$). The different R groups carried by amino acids, differentiate these amino acids. Glycine is the simplest amino acid since the R group consists of only hydrogen atoms. For all other instances, the R group of amino acids has either an aliphatic or heterocyclic structure.

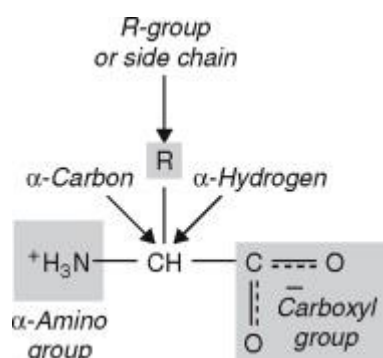


Figure 1.4. Amino acid structure (Belitz et al., 2009)

The chemical nature of the R group determines the physicochemical properties of amino acids such as solubility hydrogen bonding strength, chemical reactivity and net charge. In general, proteins have 20 kinds of side chains (R group). The ordering of these 20 kinds of amino acids in different numbers and order has led to the many and varied functions carried out by proteins within the organism.

Amino acids can be classified according to their chain and ring structures and their active groups, reaction properties in solution, number of carboxyl and amino groups in their molecules, and most importantly their biological importance and role. Amino acids can be differentiated into three groups in terms of physiological roles which are essential amino acids (valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, threonine, histidine), semi-essential amino acids (lysine and arginine) and nonessential amino acids (glycine, alanine, proline, serine, cysteine, tyrosine, asparagine, glutamine, aspartic acid and glutamic acid).

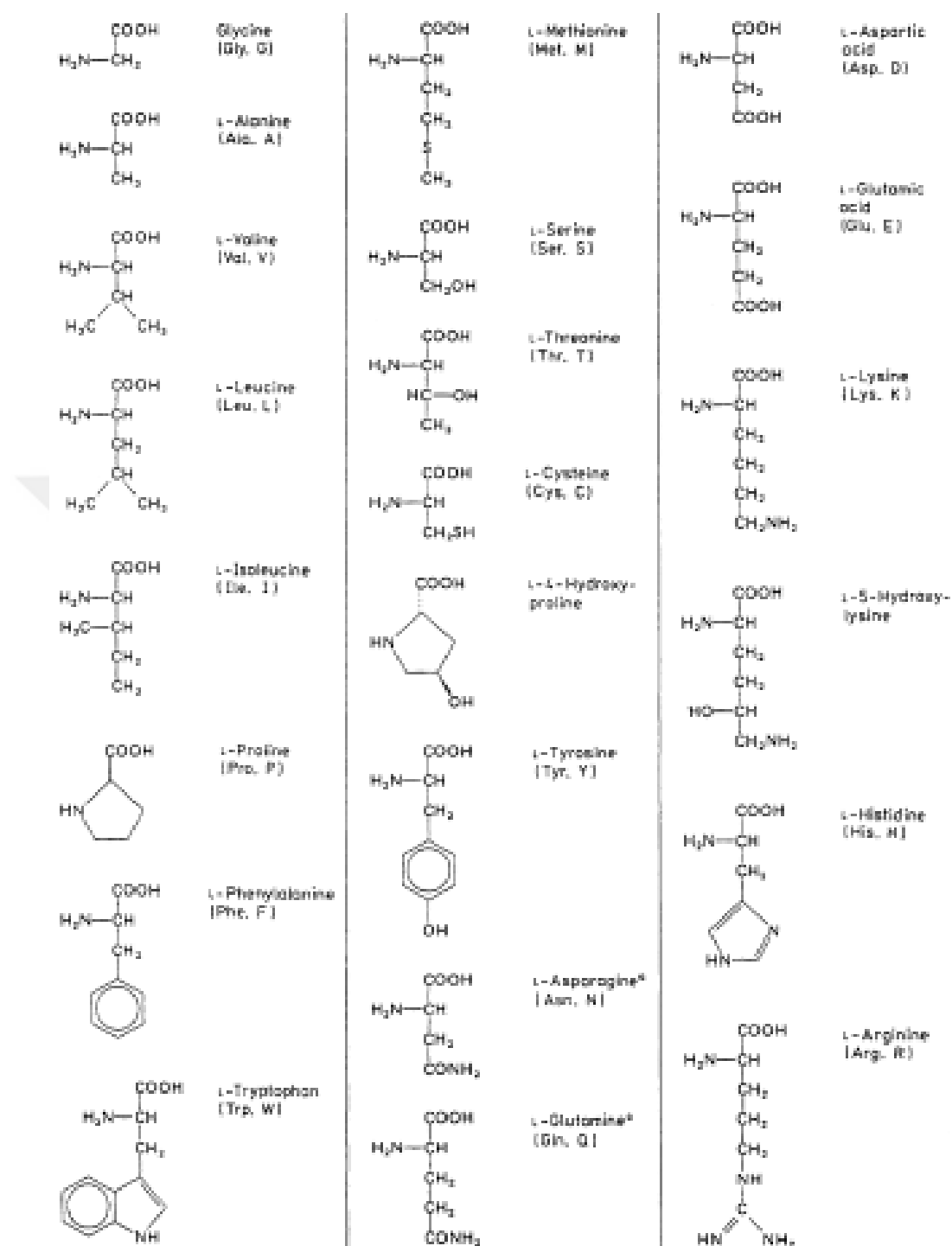


Figure 1.5. Amino acids with their one letter symbols (Belitz et al., 2009)

1.2.2 Protein Structure

Each protein has its own 3-dimensional natural structure. A protein loses its biological activity when it loses its 3-dimensional structure (denaturation). Primary, secondary, tertiary and quaternary structures are responsible for the formation of the unique 3-D structure of proteins.

The primary structure of proteins is determined by the polypeptide chain and amino acid sequence in that protein molecule. The amino acids in the chain are all L - structure. The number of peptide bonds is $n-1$. Although no protein has reached its characteristic 3-dimensional structure in the primary structure, the primary structure constitutes the transition to the 3-dimensional structure of the proteins.

In some parts of the primary structure, the formation of inter-chain bonds results in the secondary structure. In this way, the polypeptide chain itself folds along the axis. The helix curve formation of the polypeptide chain is usually caused by the H bonds formed between two electronegative atoms (mostly oxygen and nitrogen). In other words, the secondary structure indicates the periodic, spatial arrangement of amino acid residues in certain segments of the polypeptide chain. α - helix structures and β - sheets are two different secondary structures observed in proteins. The α - helix structure consists of a chain of proteins in the form of a helix, with the overlapping amino acids aligning with each other to form hydrogen bonds. β - sheet is the structure formed by the bonding of protein chains onto each other by layers of hydrogen bonding. These layers can stand in parallel as well as anti-parallel to each other.

Tertiary structure is formed by the formation of H bonds, ionic bonds, non-polar bonds, polar bonds, sulfhydryl bonds in polypeptide chains. Folds occur with

additional bonds in the structure. This structure is generally seen in globular proteins. The formation of globular or fibrous proteins depends on the difference in the tertiary structure. In proteins comprising more than one polypeptide chain, the polypeptide chains are joined by the same types of bonds that form the tertiary structure and form the quaternary structure.

1.2.3 Protein Sources

In today's world, protein is an important part of human nutrition. Milk, meat and egg can be classified as sources of animal proteins while cereal, legumes and oilseeds can be classified as sources of plant proteins. Not only these proteins from traditional sources but also any protein from different biological sources can be defined as food proteins. Green algae are good examples of non-traditional source of food proteins.

1.2.3.1 Milk Proteins

Milk is a liquid with high nutritional value secreted from mammary glands following the birth of female mammals. Milk contains all the nutrients necessary for the newborn to survive and complete its development. The highest component of milk in quantity is water and it differs greatly according to the species it belongs to. The main component of milk is 63-87% water. The macro and trace components of milk are distributed in different forms within the water phase, forming the peculiar characteristics of milk. Milk fat, protein, lactose, mineral substances and vitamins are considered as the most important elements in the composition of milk.

Milk proteins have been an important research topic from the very beginning of protein chemistry since it is easier to access, separate and isolate compared to other proteins. The first research project on milk proteins was published by Berzellius in

1814, while the term 'protein' was introduced in 1838. This shows how old milk proteins are the subject of study (Huppertz et al., 2018)

There are basically two classes of protein in bovine milk. These proteins are separated from each other according to their solubility properties at 20 °C and pH 4.6. Most of the milk proteins precipitate under these ambient conditions. This type of protein is called casein. On the other hand, proteins that are still dissolved under these ambient conditions are called serum proteins or whey proteins.

1.2.3.1.1 Whey Protein

The proteins which are not associated with casein and other non-protein milk particles such as somatic and microbial cells and membrane structures can be defined as whey proteins. β -Lactoglobulin, α -lactalbumin, immunoglobulin and serum albumin are known as the major whey proteins while lactoferrin, osteopontin, lactoperoxidase, lysozyme and proteose pepton 3 are known as the minor whey proteins (Boland & Zealand, 2008).

Whey protein has anti-carcinogenic effect, antioxidant activity and antimicrobial effect. It is also used for weight management so whey protein is considered as important part of human nutrition (Akal, 2017). Whey protein have been used as encapsulation agent for value-added food ingredients and drugs by enhancing their bioavailability, solubility, bioaccessibility and transport. (List, 2014)Whey protein can be used as texture modifier, gelling agent thickening agent, foaming agent and surface active component (de Castro et al., 2017).

The different food system applications of whey protein have been investigated including infant formula (Fenelon et al., 2019), nutrition bar (Lu & Zhou, 2019),

energy drinks (Mehmood et al., 2019), baked products (Luis et al., 2020) and edible films (Pérez et al., 2016).

1.2.3.1.2 Caseinate

Casein is defined as the heterogeneous group of phosphoproteins precipitated at pH 4.6 and 20 °C of skim milk. α_{s1} -casein, α_{s2} -casein, β -casein, γ -casein and κ -casein are known as the major fractions of casein. When acid is added until milk reaches the isoelectric point of the casein, pH 4.6, the casein is chemically purified and precipitated until it is completely purified from calcium and phosphorus salts. This method is mostly used to obtain commercial casein.

It contains polar and apolar residues that do not show uniform distribution in the primary structure of the casein. Accordingly, casein may have hydrophilic and hydrophobic character. Therefore, casein is a good emulsifier. Since the casein does not have tertiary structure it cannot be denaturalized (Belitz et al., 2009).

Casein is important part of human nutrition like other milk protein which is whey protein. It has decreasing effect on the risk of cardio vascular diseases (Shang et al., 2018). Consumption of casein phosphor-peptides (CPP) also increases the absorption and accumulation of calcium ion in bones (especially tibia) due to the mineral-binding properties of CPP's. This concept have been proven by animal studies (Tsuchita et al., 2001). Bioactive and nutraceutical compounds can be delivered through nanoencapsulation process by manipulating the casein micelles (Qi, 2009).

1.2.3.2 Plant-Based Proteins

The increasing world population and the trend towards protein-rich nutrition have increased the orientation to non-animal protein sources in the food industry. In recent years, production methods have been developed for the production of protein that can be used as fillers in processed meat products or in beverages enriched with protein. During the development of new methods, it is important to maintain the nutritional value, sensory and functional properties of the products.

In recent years, vegetable proteins have gained great importance due to changing consumer preferences, personal choices such as religious, cultural or vegetarianism / veganism, reduced confidence in products of animal origin, and lower cost, sustainable production. At present, functional properties of some plant proteins have been utilized in food formulations and processing, and scientific studies on plant proteins have increased in recent years. According to the literature, vegetable proteins are a powerful alternative to whey isolate and / or soy protein isolate which is frequently used commercially in terms of some functional properties. There is a wide variety of sources in the production of plant protein isolates. The plant source to be used to provide a desired functional property in the product, as well as the extraction method or other processes to be applied, may vary.

1.2.3.2.1 Lentil Protein

Lentil (*Lens culinaris*) is included in the legumes family and is edible pulse. In the developing countries, it is considered as an important source of dietary protein. Besides being an important source of protein, lentils are also an excellent source of vitamins, minerals, complex carbohydrates, and dietary fiber (Matina Joshi et al., 2017). According to FAO statistics, the yield of lentil is 1153 kg.ha⁻¹ and the global production is 7,59 million tons in 2017 (2019). Although lentil is known as drought-enduring plant (grown worldwide), four countries which are USA, Australia, Canada

and Turkey are producing more than 75% of global production (Alexander, 2015). Many scientific studies have been conducted on the effects of lentil consumption on health. As a result of these studies, it has been proved that lentil consumption has a positive effect on reducing the risk of diseases such as cardiovascular diseases, cancer, hypertension, cholesterol and gastrointestinal disorders (Matina Joshi et al., 2017). Due to these reasons, lentil consumption per capita in the world and lentil production increased significantly in parallel with this consumption (FAOSTAT, 2019). The content of lentil seeds has been investigated by many different researchers. Generally, lentils contain 21-31% protein and 62-69% carbohydrates. A large part of the carbohydrate content is starch. The protein content of lentils is about twice the protein content of other grain products. The essential amino acid content of lentil protein is 39.3 grams per 100 grams of protein. While this is evidence that the lentil contains all essential amino acids, lentils are limited in terms of sulfur-containing amino acids like all other legumes (Shekib et al., 1986). The different food system applications of lentil flour have been investigated including breads (Turfani et al., 2017), cakes (De la Hera et al., 2012), noodles (Ciudad-Mulero et al., 2020) and infant formulas (Le Roux et al., 2020) in terms of nutritional value, bioavailability, texture and sensory properties.

1.2.3.2.2 Chickpea Protein

Chickpea (*Cicer arietinum* L.) is a member of the legume family and one of the edible seeds. When compared with wheat flour, chickpea flour has more protein, fat and dietary fiber content. In addition, chickpea flour is rich in minerals, both in terms of macro nutrients such as sodium, potassium and calcium, and micronutrients such as copper, iron and zinc (Rachwa-Rosiak et al., 2015). This seed is usually grown in temperate and semi-dry climates. Production of chickpea is quite common in Asian, European and Australian continents and the number one manufacturer of this legume is India. According to the statistics of FAO, 64.5% of the total chickpea production in the world in 2017 belongs to India alone. Australia (14.2%), Myanmar (3.74%)

Ethiopian (3.36%) and Turkey (3.33%) is among the five largest producers of chickpeas (2019). Due to its wide possibilities and applications, chickpea is a plant that has been known and used for a long time in Asia. In addition to the ability of chickpea leaves to stop bleeding, these leaves can also be applied as a treatment in cases of bone dislocation when cooked. Moreover, extract can be given as a treatment for diarrhea and digestive disorders. It is known that to cure cough, head and throat aches and increase body weight chickpea grains have been used in Egypt.

The different food system applications of chickpea flour have been investigated including bread (Shrivastava & Chakraborty, 2018), pasta (Goñi & Valentín-Gamazo, 2003) and yoghurt (Hussein et al., 2020).

1.2.3.2.3 Mung Bean Protein

Mung bean (*Vigna radiate* (L.)) is a member of the legume family, is one of the edible grain products and is consumed by most households in Asia. Approximately 8.5% of the total area of grain produced in the world belongs to mung beans. It is grown by most Asian countries (mainly China, India, Bangladesh and Pakistan) due to its short growth cycle of approximately 70 days and its relatively drought-resistant nature. Mung beans have a balanced nutrient distribution that includes dietary fiber, protein, minerals and vitamins. In addition, it contains a significant amount of bioactive substances such as flavonoid, phenolic acid and tannins.

The different food system applications of mung bean have been investigated including dough (Meng et al., 2019) and antioxidant edible film (Rompothi et al., 2017).

1.2.3.2.4 Quinoa Protein

Quinoa (*Chenopodium quinoa* wild) is an herbaceous flowering plant belonging to the Chenopodiaceae family and is classified as a pseudo-cereal due to botanical reasons. In the Andes region (Ecuador, Bolivia, Peru), called the mother grain by the Incas, the agricultural history of the quinoa dates back 5000 to 7000 years. Taking place of the corn, another very important agricultural product for the Incas, quinoa was considered sacred by this society (Ceyhun Sezgin & Sanlier, 2019). In general, quinoa seeds are characterized by high protein, fat content and lower carbohydrate content than other major grains such as wheat, corn, barley and rice (Dakhili et al., 2019). According to FAO statistics, the yield of lentil is 847 kg.ha⁻¹ and the global production is 146735 tons in 2017. Peru (53,6%), Bolivia (45,51%) and Ecuador (0,8%) are three largest producers of quinoa (2019).

Quinoa may have positive effects on human health since quinoa contains dietary fibers and phytochemicals such as saponins, phytosterols and phytoecdysteroid. It is a known fact that quinoa has a lowering effect on plasma and liver cholesterol (Noratto et al., 2019).

Research results show that the average protein content of the quinoa seed is 12-23%, depending on the variety of the seed. Although this value is higher than most grains, it is lower than oilseeds and legumes (Dakhili et al., 2019).

The different food system applications of quinoa have been investigated including sourdough (Rizzello et al., 2016), yoghurt (Ujiroghene et al., 2019), pasta (Lorusso et al., 2017) and bread (Xu et al., 2019).

1.2.3.2.5 Pea Protein

Pea (*Pisum Sativum*) is a member of the legume family and grown for human consumption and stock-feed mostly in Asian countries. According to FAO statistics, the yield of pea is 7735,5 kg.ha⁻¹ and the global production is 21,22 million tons in 2018. China India and United States are three leading producers of pea (2019). Pea ,like other legumes, becomes important source of dietary proteins where consumption of animal proteins is restricted due to non-availability and economical reasons (Boye et al., 2010). Studies show that consumption of pea reduces the risk of cardio vascular diseases (Flight & Clifton, 2006). Consumption of pea protein is also important for athletes since pea protein promotes muscle thickness, strength and performance both for regular trainings and high-intensity functional trainings (Babault et al., 2015; Banaszek et al., 2019). The protein amount of peas ranges from 23.1% to 30.9%, and a significant part of the total protein is albumin (15-25%) and globulin (50-60%) proteins (Xu et al., 2017).

The different food system applications of pea have been investigated including snack (Philipp et al., 2017; Saldanha do Carmo et al., 2019), pasta (Mercier et al., 2011), bread (Millar et al., 2019) and sponge cake(Bustillos et al., 2020).

1.2.3.2.6 Soy Protein

Soybean (*Glycine maxis*) is an annual plant of about 1-1.5 m tall, belonging to the legume family. Soybeans have always been an important food source. Therefore, the harvested area and the yield of production increased. According to FAO statistics, the harvested area of soybean is 124,92 million ha and the global production is 348,7 million tons in 2018 (2019).

Soy is particularly important nutritional source for the human diet, with its rich amino acid profile proteins, mineral content (phosphorus, potassium, calcium and sulfur) and vitamin content mostly consisting of A and B vitamin groups. Although soy protein is lack of important essential amino acid which is methionine, its amino acid profile is similar to beef. Therefore, consumption of soy protein as animal protein substitution may decrease the risk of chronic kidney disease (renal failure) and cardio vascular diseases (Friedman & Brandon, 2001; Sacks et al., 2006). Besides its beneficial aspects, it is known that soy is one of the major allergens. Therefore, it should be consumed carefully (Messina, 2016).

Soy protein concentrate is obtained from the oil extracted soybean by applying wet processing method (alkali solubilization followed by isoelectric precipitation). This protein isolates can be used as meat substitutes by texturizing and flavor addition. Soy protein is also used for improving processing qualities such as water binding capacity and stabilization (Belitz et al., 2009). Therefore, the different food system applications of soy protein have been investigated including yoghurt (Rinaldoni et al., 2012), cheese (Li et al., 2013; Rinaldoni et al., 2014), bread (Masure et al., 2019), sourdough (Omedi et al., 2016), noodle (Guo et al., 2018) and infant formulas (Nguyen et al., 2015).

1.2.4 Protein Extraction Methods

Wet milling and dry milling are two important methods for producing protein-rich ingredients from the raw materials such as cereals. Wet milling processes can be considered as mainstream method for producing isolates of plant proteins and alkali extraction is known as the first step of this established method. This step is followed by isoelectric precipitation and one of the drying methods which can be freeze drying or spray drying. Dry milling process includes two important steps which are disintegration (flaking grinding and milling) and fractionation (air classification,

sieving). Both processes have advantages and disadvantages in terms purity, chemical usage, functionality, time, energy and cost. Although wet milling requires too much water and energy (mixing and drying steps), the resulting ingredient can be defined as an isolate which contains high amount of protein ($\geq 80\%$). On the other hand, dry fractionation's energy requirements much lower but the resulting ingredient's protein content is between 30%-60%. Although energy consumption of dry milling process is increased, application of pre-processing methods such as supercritical CO₂ extraction, solvent extraction and moisture adjustment can increase purity of proteins concentrates. Wet milling process requires too much chemical than dry milling process. Dry milling process can be defined as mature technology so this process needs no chemical usage. However, for wet milling process, NaOH (to solubilize proteins in alkali environment) and HCl (to precipitate proteins in acidic environment) are used. Therefore, salt (NaCl) production is observed and filtration steps can be needed to obtain pure protein isolates. In terms of functionality both of the processes can be preferred since functionality of material depends on further process conditions and applications. Processing time is one of the parameters and wet milling process requires too much time than dry milling process. Since the last step of wet milling process is drying (mostly freeze drying for quality issues) takes 48 hours to complete the process. Lab scale, pilot scale and industrial scale facilities are available for those processes.

1.2.5 Protein Characterization Techniques

1.2.5.1 Kjeldahl Analysis

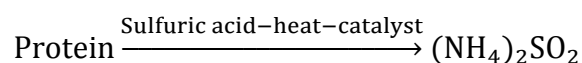
Proteins are considered as an ample component in all cells and most of them are important part of cell structure and biological functions. One of the complex protein types is food proteins. Purification and characterization of many of them have been accomplished. Just as proteins have many roles in living organisms and biological activities, food proteins have many roles in food structure and functionality as well.

Therefore, analysis of protein content in food sample is important for three reasons. The first reason is correct nutrition labeling. In today's world, consumers want to know what they eat and the composition of foodstuff. The second reason is the functional property investigation. Different food proteins have unique functional properties in different foods. Foaming function of egg albumen, lattice network formation of gliadin and glutenin in bread making and the coagulation function of casein in the production of cheese products can be given as examples of food proteins' functional properties. The last reason is the biological activity determination of food samples. Enzymes and enzyme inhibitors which are important topic of food science have protein structures. Tenderization of meat and ripening of fruits occur due to the enzymatic activities.

In order to measure protein content of food sample, many methods have been established and developed. Determination of nitrogen (Kjeldahl and Dumas method), aromatic amino acids (ultraviolet 280 nm absorption method), peptide bonds (Biuret method), dye-binding capacity (anionic dye-binding method and Bradford dye-binding method), ultraviolet absorptivity (Ultraviolet 280 nm Absorption Method) and light scattering properties (Lowry and Biuret methods) of proteins are the basic principles of protein determination methods. Precision, accuracy, sensitivity, speed and cost of the analysis are the important factors for which method should be performed. However, what is actually being measured is the main concern for the selection of the appropriate method. Official Methods of Analysis of AOAC International recommends infrared spectroscopy, Kjeldahl and Dumas methods as protein determination methods which are commonly used for quality assurance and nutrition labeling purposes in food industry. In order to measure protein content of cereals, Kjeldahl method is recommended by AOAC International.

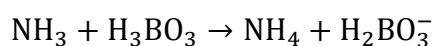
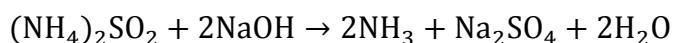
Apart from the sample preparation, Kjeldahl procedure consists of three important steps which are digestion, neutralization followed by distillation and titration.

Complete breakdown of all organic matter occurs in the first step which is digestion. As a result of the reaction between nitrogen and sulfuric acid, nonvolatile ammonium sulfate is formed.

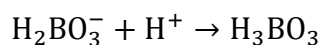


During digestion, liberation of protein nitrogen occurs to form ammonium, organic matter oxidized by sulfuric acid and combines with ammonium, carbon dioxide and water are formed due to digestion of carbon and hydrogen elements.

In order to neutralize sulfuric acid, sodium thio-sulfate which contains alkali is used. Due to distillation of ammonia into a boric acid solution, ammonium and borate ions are formed.



Titration is performed with standardized HCl and borate anions which are proportional the amount of nitrogen.



Kjeldahl method is also known as crude protein method and as a principle total organic nitrogen is found. Therefore, percent nitrogen should be converted to percent

crude protein by multiplying the conversion factor. Conversion factor depends on the amount of nitrogen which is found in protein. Since 16% of the most of the proteins is nitrogen, conversion factor $(100/16)$ is 6.25. Kjeldahl method has many advantages such as being applicable to all types of foods, accurate, inexpensive and can measure small quantities of protein. On the other hand, measurement of total nitrogen content instead of protein nitrogen content, being time consuming and usage of corrosive reagent are disadvantages of this method.

1.2.5.2 Soxhlet Extraction

One of the principal component of the human diet is fat. It is also preferred by the consumer due to its texture, aroma, flavor and palatability. Fat-soluble vitamins which are vitamin A, D, E and K are carried by fats. Vegetable, animal and marine are the sources of fats and oils which can be manufactured in combination of these in food industry. Fats play essential roles for the texture and structure of food products as follows:

- Addition or modification of texture and flavor
- Aeration of doughs and batters
- Contribution to flakiness and tenderness
- Fat is heat transfer medium in frying
- Prevention of sticking
- Emulsification
- Providing satiety

The largest segment of edible oils are oils which are used in spreads, dressings, margarines and frying oils. Fruits and many vegetables do not contain fats and oils in their compositions.

In order to maintain quality assurance, precise and accurate quantitative and qualitative analysis of lipids is critical. Lack of accuracy and precision in analysis could result in undesirable functionality and poor quality of food products. Solvent extraction methods, non-solvent wet extraction methods and instrumental methods are the three main fat analysis methods. Three types of solvent extraction methods are Goldfish method (continuous), Soxhlet method (semi-continuous) which is mostly used for analysis of cereal fat and Mojonnier method (discontinuous) which can be used to analyze both solid and liquid products. The main principle of non-solvent wet extraction methods which are Gerber Milk Fat Method and Babcock Method is similar. Both of them use sulfuric acid for digestion of protein and carbohydrates. Fat is not digested and maintained in a liquid state due to the generated heat during reaction. The difference between these two methods is the application of isoamyl alcohol in Gerber method which prevents the charring of the sugar. Gerber method has variety dairy applications in food industry since Gerber method is simpler and faster method than Babcock Method. Infrared methods, nuclear magnetic resonance and Foss-Let Method are the instrumental methods which are compared with extraction methods. These methods are considered as rapid and non-destructive. Their chemical consumption and sample preparation requirements are much less than the conventional methods. On the other hand, various compositions require establishment of different calibration curves and the equipment for measurement are expensive. Although instrumental methods have drawbacks, nuclear magnetic resonance and mid-IR spectroscopy are widely used for quality assurance purposes and research & development applications in food industry.

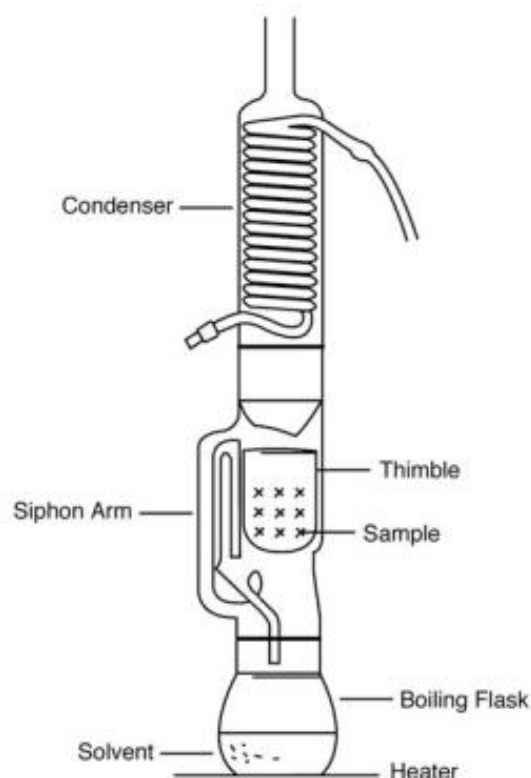


Figure 1.6. Soxhlet extraction apparatus (“Food Analysis,” 2017)

In order to measure fat content of cereal products, Soxhlet extraction is official method by AOAC (AOAC Method 920.39C for cereal fat). In the conventional application of Soxhlet extraction, food sample is placed in a thimble part which is gradually filled by condensed fresh solvent from a boiling flask. Aspiration of liquid from the thimble into the boiling flask occurs when it reached the overflow level. Extracted analyte is carried into the bulk liquid by this way. This process is repeated until the extraction is fully accomplished. System has a continuous attributes due to the solvent recirculation through the sample whereas the Soxhlet extractor assembly is a batch system. Therefore, Soxhlet extraction system can be considered as semi-continuous system.

When it is compared with other conventional techniques, Soxhlet extraction has many advantages:

- The fresh portion of solvent contact with the food sample repeatedly which makes much more efficient mass transfer is taking place.
- Because of the heat application, solvent flask could reach the extraction cavity.
- Filtration is not required when leaching step is accomplished.
- The yield of the extraction (accuracy of the analysis) can be increased by parallel extraction system which are inexpensive and easy to set up basic equipment.

However, Soxhlet extraction also has serious drawbacks compared to conventional techniques for solid sample preparations:

- Soxhlet extraction cannot be considered as green process because of the large amount of solvent waste and long process time requirement.
- When extraction is completed, evaporation and concentration steps become inevitable.
- Thermal decomposition of the sample can take place since extraction occurs at boiling temperature of the solvent.
- Agitation which would increase mass transfer cannot be provided by using conventional Soxhlet extractor.
- The automation of Soxhlet extraction is not easy and solvent selection is restricted.

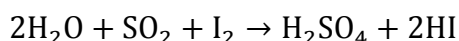
1.2.5.3 Moisture Analysis

Almost all foods like all living things contain some amount of water. Despite the fact that water has no contribution in calories, it is important part of human nutrition and essential for life. Moisture content greatly affects the texture although the food material is same. When texture of grape and raisins is compared, it can be understood that water plays a key role for the texture of foods. It can be given turgor or crisp texture to fruit and vegetables by changing the moisture content. Having low moisture content and keeping out of the moisture is important to maintain quality for some food products such as salt, sugar and potato chips.

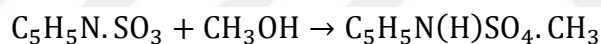
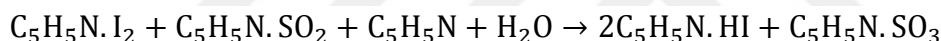
Unique properties of water should be known since water has so many functions in terms of processing and quality. Usage or modification of water is involved most of the food processing techniques. Drying, freezing, emulsification, thickening of starch and bread making can be given as a few examples. Moisture content has important effect on food quality since growth of microorganisms cannot occur without water. Therefore, food processes such as freezing and dehydration are applied to inhibit growth of microorganisms and to increase shelf life.

In order to analyze moisture content of food sample four main methods are established which are oven drying methods, distillation procedures, Karl Fischer titration (chemical method) and physical methods. Different methods based on different principles to determine moisture content of foodstuffs. Removal of moisture (oven drying methods), electrical properties of water (the dielectric method), relationship between specific gravity and moisture content (hydrometric methods), reflection of light (the refractive index method), measurements of absorption at different wavelengths (near infrared analysis) and the amount of titrant used (Karl Fischer method) can be given as examples.

The most suitable method for low-moisture food samples which contain high amount of protein and sugar is Karl Fischer titration method. Important examples of these foods are roasted coffee, oils and fats (AOAC Method 984.20), chocolate (AOAC Method 977.10) and dried fruits and vegetables (AOAC Method 967.19 E-G). The reduction of iodine by SO₂ in the presence of water is the main principle of Karl Fischer titration method.



In order to dissolve the SO₂ and iodine, this reaction is modified by the addition of methanol and pyridine.



According to this reaction, for each mole of water, 3 mol of pyridine, 1 mol of iodine, 1 mol of methanol and 1 mol of SO₂ are used. In a volumetric Karl Fischer titration ml of each Karl Fischer reagent (mgH₂O / mL) spent is the amount (mg) of water in the sample. Karl Fischer titration method is widely used in food industry since the method is accurate and rapid. Moisture content is found without heat treatment by applying this method. However, Karl Fischer titration has some sources of errors and major difficulties such as:

- Particle size of the sample (fineness of grind) is important while preparation of some food (especially cereal grains) because of incomplete moisture extraction.

- Atmospheric moisture is another source of error. Therefore, external air must not pass through the reaction chamber.
- All glassware must be dried carefully to avoid moisture adhering to inner parts of the titration unit.
- Moisture content may be overestimated due to the reaction between certain food component and chemicals which are used for titration. The reactions between unsaturated fatty acids with iodine; carbonyl compounds with methanol and the oxidation of ascorbic acid by Karl Fischer Reagent can be given as specific examples.

1.3 Lipids

Organic substances, whose common feature is insolubility in water, can be briefly described as lipid. Lipids are a very large class of organic substances that do not produce saponification reactions such as hydrocarbons, sterols, colorants and antioxidants, as well as saponification agents such as glycerides, fatty acids and waxes. Lipids, which have such a wide range, are handled beyond the concept of fats or oils and are investigated under lipid chemistry.

It is possible to classify lipids according to their physical properties at room temperature (fats and oils), according to their polarity (neutral and polar lipids), their essentialness for humans (essential fatty acids and non-essential fatty acids) and their structure (simple and complex). Due to exceptions in other classification methods, it is preferred to classify lipids according to their structure.

1.3.1 Corn Oil

Corn (*Zea mays*) which is also called as Indian corn or maize, is edible grain. It has played an important role for the history of mankind as a food source, biodiesel and

raw material for the industry. Corn contains higher oil content among commercial cereals since it has relatively large germ. In fact, corn is not known as an oilseed due to its lipid content in the kernel which is between 3% and 5%. However, large amount of germ is generated by the large industrial dry and wet milling processes. This large amount of germ is channeled to animal feedstock and oil crushing industries. The weight of the germ is between 9% and 11% of the whole kernel but it contains 80% of the lipids found in the corn seed. Endosperm (15%) and bran (2-5%) contain the rest of the lipid content. The extraction of crude oil and refining of the crude oil are two main stages of industrialization of corn oil. Corn oil is known as corn germ oil since commercial corn is extracted from germ (Moreau, 2011).

In order to determine the quality and physicochemical characteristics of vegetable oils (including corn oil), American Oil Chemists' Society has validated analyses that provide important information on characteristics of finished products and the efficiency of the refining process. Free fatty acids, solid fat content (sfc), iodine value, peroxide index and oxidative stability index (osi) can be given as examples to validated analyses.

Many clinical studies have supported the statement that corn oil may lower cholesterol. The prevention of oxidation of low density lipoprotein (LDL) is achieved by the antioxidant properties of tocopherols which is found in corn oil (Barrera-Arellano et al., 2018).

Most of the corn oil was produced for the domestic uses such as preparation of fried food and salad dressing. Packaged for consumers, institutional products, and oil for industrial frying are three product category of cooking oil. Due to partially hydrogenated fats are restricted for institutional frying, corn oil application for this purpose extended dramatically. The application of corn oil was extended to

formulate industrialized products since its availability increased at lower cost. Shortenings, margarines, mayonnaise and technical fats can be given as industrialized products examples (List, 2014). Corn oil is also used as ingredient in the formulations of snacks, baking mixes and bakery products (Zhao et al., 2020).

1.3.2 Sunflower Oil

Sunflower (*Helianthus annuus* L.) is known as one of the most ancient species in North America. It was cultivated and domesticated in central North America even before maize which dates back to 3000 BC. This seed was introduced to Europe by Spanish explorers at the beginning of the sixteenth century (Grompone, 2011).

Vegetable oils, pharmaceuticals, livestock feeds and biofuels are provided from oil seeds. After soybean, palm and rapeseed, sunflower is the fourth most important oil seed crop. Sunflower seeds can be considered as advantageous among the other seeds due to their lower amount of allergenic, anti-nutritional and toxic compounds (Karefyllakis et al., 2019).

Regular sunflower oil contains high amount of linoleic acid (18:2) and oleic acid (18:1). Oleic acid composition is much higher for mid-oleic and high-oleic sunflower oils (Raß et al., 2008). Physicochemical characteristics of sunflower oil can be determined by American Oil Chemists' Society's validated analyses that provide important information on characteristics of finished products. Free fatty acids, solid fat content (sfc), iodine value, peroxide index and oxidative stability index (osi) can be given as examples to validated analyses (Grompone, 2011).

Sunflower oil has anti-oxidant properties with total cholesterol and low-density lipoprotein (LDL) reducing effects (Bester et al., 2010). Due to its high oleic acid composition, sunflower oil may reduce the risk of heart attack (Guo et al., 2017). Sunflower oil has different food applications such as salad oil, cooking oil, frying oil shortening and margarine (Salas et al., 2015).

1.4 Microfluidizer

Microfluidization is the one of the important novel techniques which develops stability, texture and taste is used in food industry. Enhancing the stability, color, taste and textural properties is provided by the reduction of particle size and the production of more uniform food samples. Ultra high pressure, instantaneous pressure drop, high shear, high frequency vibration and cavitation are the concepts which is combined by microfluidization phenomena to reduce particle size and produce more uniform samples. The main principle of microfluidization is that the liquid is separated in two jet streams, which enter microchannels where these jet streams are facing high shear forces. After this, the jet streams are canalized in an area where liquid/liquid contact occurs and these liquids are subjected to turbulence and cavitation because of the pressure drop of homogenized liquid while the liquid is passed out of the reaction chamber.

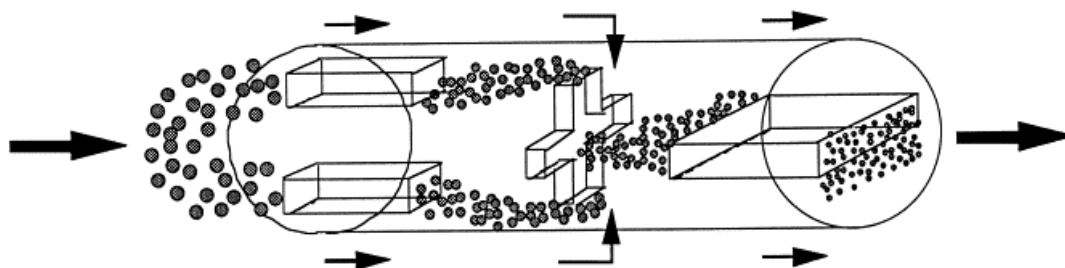


Figure 1.7. Schematic representation of reaction chamber (Lagoueyte & Paquin, 1998)

The different food system applications of microfluidization are researched including milk (Bucci et al., 2018; Kumar et al., 2019), yoghurt (Demirkesen et al., 2018), honey (Villalobos-castillejos et al., 2020), mozzarella cheese (Van Hekken et al., 2007), cheddar cheese (Lemay et al., 1994), corn gluten meal (Ozturk & Mert, 2019), gluten free corn breads (Ozturk & Mert, 2018), whey protein (Koo et al., 2018), soy protein isolate (Chen et al., 2016), fish oil (García-Márquez et al., 2017), wheat bran (Koo et al., 2018), ketchup (Mert, 2012), high oleic palm oil (Ricaurte et al., 2016), potato starch (He et al., 2020).

1.5 High Performance Liquid Chromatography

Analytical characterization of a sample by using a tool which identify the components qualitatively and quantitatively is needed to know chemical composition of a sample for many different fields such as food, cosmetic and pharmaceutical. In order to analyze compounds of different properties and molecular weights, high performance liquid chromatography (HPLC) can be applied as a separation technique.

Analytical separations were performed by conventional methods such as distillation, extraction and precipitation until the 1950s. Open-column chromatography was begun to apply in the laboratories commercially before 1970s. Mikhail Tswett who is a botanist invented and named chromatography in 1903. He tried to separate plant pigments by using a glass column which filled with finely divided calcium carbonate. The name chromatography derived from two Greek words which are "chroma" meaning color and "graphein" meaning write since he observed the preparation of color zones at glass column as a result of his experiment.

Modern HPLC equipment composed of six main sections which are solvent supply system, pumping system, sampling system, separation system, detection system and

data processing system. Solvents which are necessary for the mobile phase of HPLC are provided by solvent supply system. These solvents (mobile phase) are delivered by using pumping system from solvent container. Samples which are injected from sampling system are carried by mobile phase into the separation system. In order to avoid air in mobile phase, degasser is used before and during the chromatographic run since air in mobile phase can reduce the flow stability and sensitivity of the analysis. Generation of high pressure must be maintained by pumps to overcome the resistance through chromatographic column.

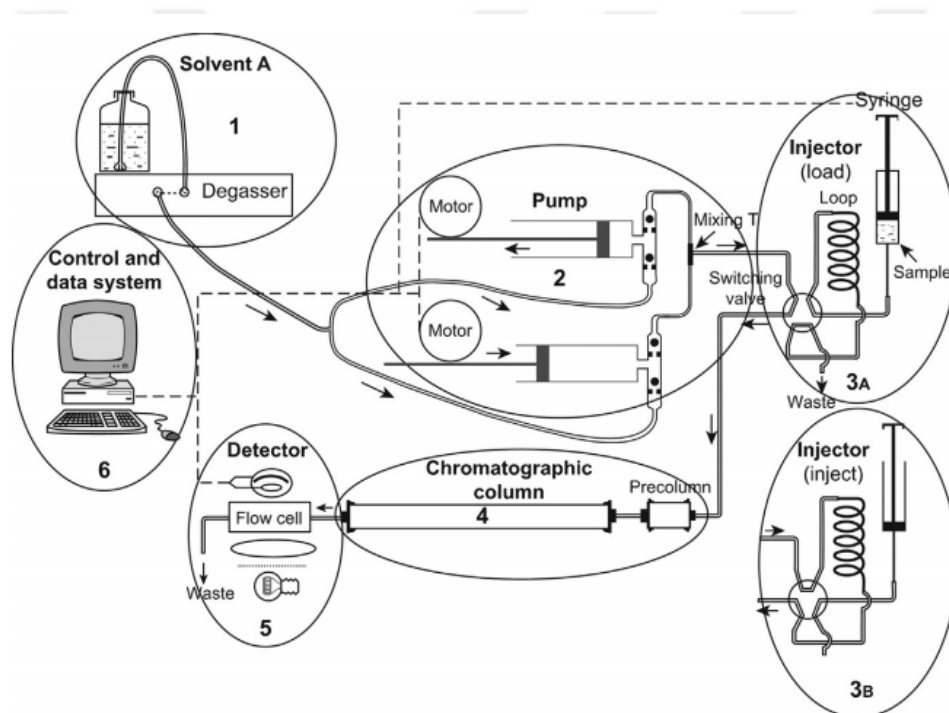


Figure 1.8. Six main sections of HPLC system (Nie & Nie, 2019)

Differences in adsorption/desorption abilities of components which are dispersed between mobile phase and stationary phase is the main principle of separation in HPLC. Different components of the sample are carried by mobile phase through stationary phase. Differential interactions are observed between analytes and phases.

Analytes which have stronger interactions with stationary phase have a tendency to move slower since the stationary phase is fixed phase. On the other hand, analytes which have lower interactions with stationary phase have a tendency to move faster. The time for the analytes passing through chromatographic column and the peak broadening are determined by these interactions between phases and analytes. As a consequence, in a chromatographic run individual compounds are eluted in the column and reach the detector at different time ranges. The information which is provided by the detector is shown in chromatograms after data processing (Nie & Nie, 2019)

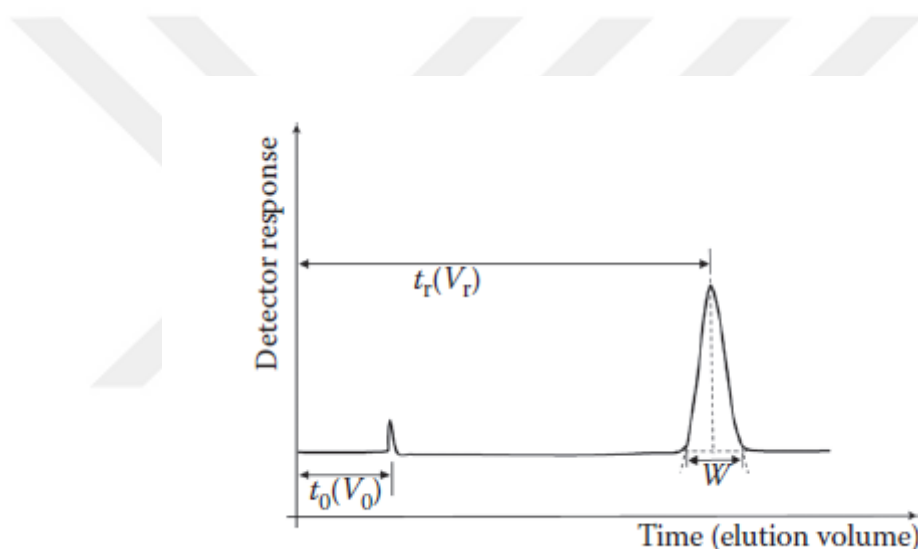


Figure 1.9. Scheme of simple chromatogram (“Food Analysis by HPLC,” 2013)

In a chromatogram, x-axis gives the retention time which is required travel time of individual compounds from injection to the detector and y-axis gives the detector response. The concentration of compound is found by comparing the calibration curve and the area under the peak.

High pressure which reduces the analysis time gives the name "HPLC". After many improvements at detectors and columns which were essential developments to achieve high performance, instruments could work up to 400 bar pressure ("Food Analysis by HPLC," 2013). Column technology was improved by using adsorbent whose particle size much smaller (1,8 μm) and instrumentation was developed at the beginning of 2000s. Therefore, much higher pressures (1000 bar) can be applied to the instrument while delivering the solvent. As a consequence, sensitivity, speed and resolution have been improved. The new technology is named as ultra-performance liquid chromatography (UPLC).

Nowadays, compounds are detected online after the separation was performed. These methods are called as continuous HPLC methods. In order to analyze complex mixtures of compounds in terms of separation, identification and quantification, HPLC system can be coupled with different detection systems. Mass spectrometry (MS), ultraviolet-visible (UV-Vis) spectroscopy, Fourier transform Raman (FTR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy can be given as examples to these detection systems.

Minor and specific compounds are detected and discovered by the coupling of HPLC with mass spectrometry (MS). These analyzers are important analytical tool for characterizing food products which is important to assure the quality of food products.

The analysis of a composition of food product is carried out by performing chromatographic techniques (especially by using HPLC). The normal composition of food product (proteins, amino acids, fats, carbohydrates and organic acids), food additives (colors, sweeteners, preservatives and flavors), chemical residues (pesticides, herbicides, antibiotics and fertilizers), toxins (mycotoxin) and food

adulteration can be identified by the application of high-performance liquid chromatography (HPLC). Complex mixtures are broken down to individual compounds which can be identified and quantified by applying suitable detectors (Lozano-Sánchez et al., 2018).

1.6 Microstructure

1.6.1 Scanning Electron Microscopy

Manfred Von Ardenne invented scanning electron microscopy (SEM) which is form of surface microscopy uses the electrons to create images of objects in three-dimensional form in 1930s. These three-dimensional images provide the information about objects' morphology, composition and topology. This technique has been used for different industrial and commercial applications such as material science, forensic science and food science.

All scanning electron microscope system includes four main section. Beam of electrons are created by electron column, electron beam-sample interaction occurs in a sample chamber, different signals obtained from sample-beam interaction are monitored by detector and images are constructed utilizing signals by viewing system.

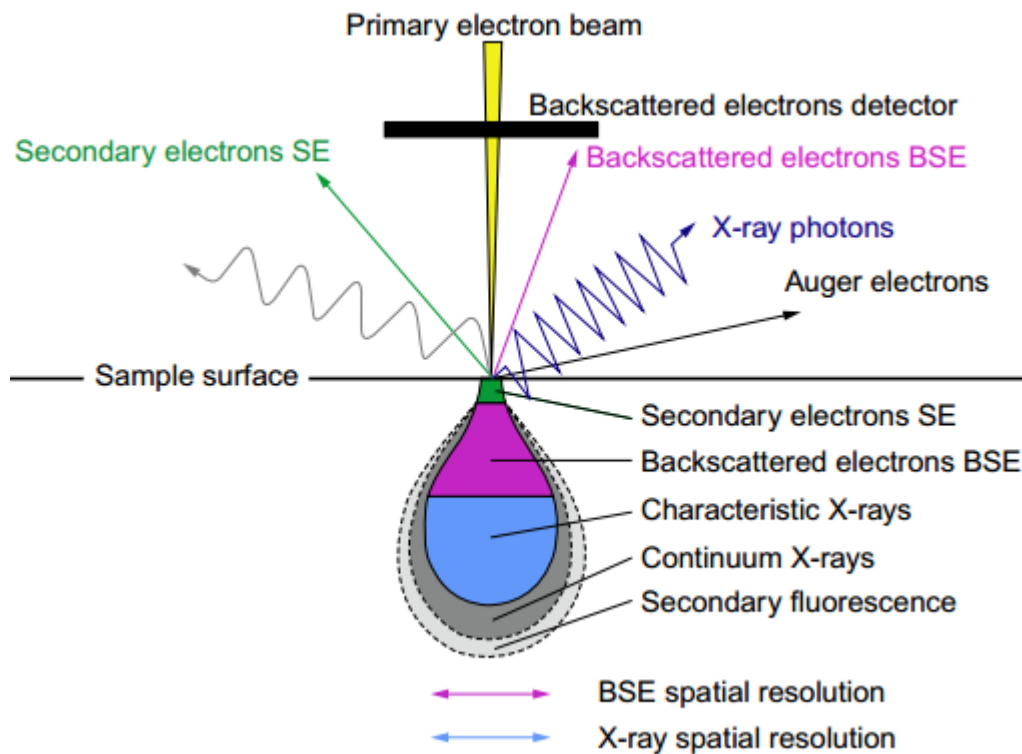


Figure 1.10. Schematic representation of working principle of scanning electron microscopy (Sharma & Bhardwaj, 2019)

The evaluation of scanning electron microscope begins with the interaction of sample and primary electron beam. Some of these electron beams are scattered inelastically (secondary electrons), others scattered elastically (back-scattered electrons). Topographic image is built by collecting the former with specific detector. In order to combine chemical composition and topological features of the sample, X-rays which are generated in a chamber are collected. This technique is called as Energy Dispersive X-Ray Spectroscopy (EDS) (Metilli et al., 2020).

When it is compared with other microscopy techniques, scanning electron microscopy (SEM) has many advantages:

- Sample preparation is relatively simple than the other methods.
- It has wide range of magnification and high depth of field.
- Technique has high resolution due to its short wavelength which is $> 1.4 \text{ nm}$
- Image analysis and quantification can be made since electronic data represents the image. (Secondary electron is used for topology imaging.)

However, scanning electron microscopy (SEM) also has serious drawbacks compared to other microscopy techniques:

- Specimens must be insulated before the examination. The evaluation of hydrated samples without changing their state is difficult to apply.
- The instrument is expensive and large. It should be placed in an area free of vibration, electric field and magnetic field.
- Voltage and currents to electromagnetic coils must be kept steady. Cool water circulation must be applied.
- Operating personnel must have special training.
- The application is limited to solid and inorganic samples which can tolerate vacuum.
- There is a risk of radiation exposure (Sharma & Bhardwaj, 2019).

The application scanning electron microscopy (SEM) in food technology enables researchers to identify structure of food materials. Therefore, the different food systems have been investigated by using this technique including yoghurt (Vianna et al., 2017), ice cream (Cavender & Kerr, 2020), chocolate (Tran et al., 2017), protein isolates (Tyuftin et al., 2020), gels (L. Wang et al., 2020), cheese (McMahon et al., 1996).

1.6.2 Confocal Laser Scanning Microscopy

Confocal laser scanning electron microscopy is a powerful tool to investigate microstructure and interactions between food components. It is also important to performance and functionality evaluation of processed foods (Tromp et al., 2003).

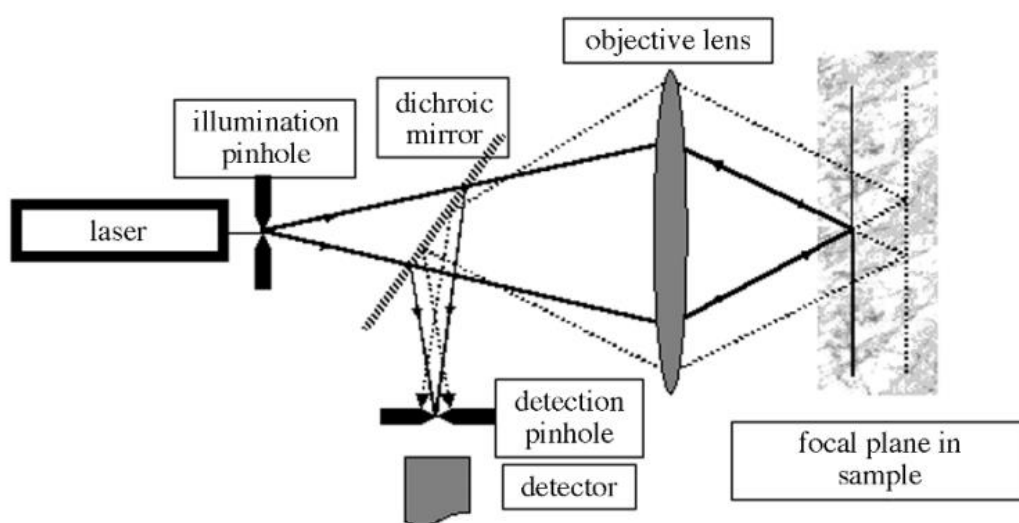


Figure 1.11. The working principle of confocal microscope (Tromp et al., 2003)

Confocal microscopy has common points with fluorescence microscopy which is one of the form optical microscopy. Focusing of illumination and detection systems on a single volume of sample is the key feature of confocal imaging. Wide field illumination is employed by conventional light microscope which means volume of sample below and above the focus plane is illuminated simultaneously and uniformly. Because of out-of-focus blur, resolution and specimen contrast decrease. Therefore, blurred sample images are obtained. On the other hand, in CLSM, small aperture is implanted in front of the emitted light detector which enables to reduce out-of-focus light and enhance the signal to noise ratio (Auty, 2013).

When it is compared with conventional light microscopy technique, confocal laser scanning microscopy (CLSM) has many advantages:

- CLSM can form 3D images of bulk samples (thick samples) thanks to optical sectioning and digital reconstruction technology.
- CLSM enables to obtain improved resolution.
- CLSM detects much sensitive images due to multiple fluorochrome probes.
- Minimal sample preparation is needed to carry out analysis.
- Not only static samples but also dynamic processes can be studied by controlling the environmental conditions and using fast acquisition rates.

However, confocal laser scanning microscopy (CLSM) also has serious drawbacks:

- Food samples usually should be labelled with proper fluorochrome, which may include multiple processing steps and solvent that may affect the sample.
- In order to make sure even emission and illumination signal across the field of view, sample surface must be flat.
- Proper resolution of colloidal food systems like interfacial films and casein micelles cannot be achieved since the best diffraction-limited lateral resolution is about 200 nm.

The application of confocal laser scanning microscopy (CLSM) in food technology enables researchers to identify structure of food materials and interactions between them. Therefore, the different food systems have been investigated by using this technique including milk (Yiran Wang et al., 2019), cheddar cheese (Khanal et al., 2018), yoghurt (H. T. H. Nguyen et al., 2018), ice cream (Y. Guo et al., 2018),

chocolate (Do et al., 2011), bread (Ben Halima et al., 2015) and dough (Bousquieres et al., 2014).

1.7 Objectives of the Study

In today's world with the increase in the population, the demand for all food sources, especially protein, has increased. Animal proteins cannot sustainably meet this increasing demand. At the same time, it is a big concern for consumers since animal foods contain high amounts of saturated fat and cholesterol. The use of vegetable proteins has gained great importance in many areas, especially environmental systems, agriculture and food systems, for a more sustainable world and healthier population. The main objective of this study is to investigate the application of plant proteins to enteral nutrition formulation and rheological, microstructural characterization of these formulations.

Qualitative and quantitative analysis of the extracted proteins is another objective of this study. Microstructural analysis is one of the important methods to analyze and qualitative comparison of extracted protein samples. In order to do this analysis, Scanning Electron Microscopy (SEM) was performed. For the quantitative analysis of proteins, protein, fat, carbohydrate, ash and moisture contents of extracted samples were found by proper techniques. Moreover, amino acid profiles of each proteins were determined by using high performance liquid chromatography (HPLC).

Since the enteral nutrition formulations are administrated to patients through the feeding tube, the flow behavior of the formulation is so important in order not to clog the feeding tube. Therefore, flow behavior of each enteral formulations was determined compared with the existing commercial product by using rheometer.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Yayla brand lentil, chickpea, quinoa and mung bean were bought from the local stores for the protein extraction part. Sodium hydroxide pellets and hydrochloric acid were bought from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

In addition to the use of extracted proteins which are lentil protein, chickpea protein, quinoa protein and mung bean protein in the enteral solution preparation part, the pea protein concentrate was supplied from the Roquette Co. (Lestrem, France) and the soy protein concentrate was bought from Alfasol (Turkey). As a carbohydrate source maltodextrin with DE value of 18 was purchased from Tito (Turkey). In addition, Evin brand corn oil and sunflower oil were used as fat source. Emulsifier soy lecithin was obtained from Pendik Nişasta Sanayii A.Ş. (Turkey). Mineral premix, vitamin-trace elements premix and choline, L-carnitine and taurine premix were supplied from Evarom Food Additives (İstanbul Turkey). Mineral premix contains calcium, phosphorus and magnesium. Vitamin-trace elements premix includes cyanocobalamine B12, biotin, pyridoxine hydrochloride (vitamin B6), pantothenic acid (Calcium-D-pantothenate) (vitamin B5), niacinamid, nicotinamide, niacin (vitamin B3), riboflavin (vitamin B2), thiamine hydrochloride (vitamin B1), pteroylmonoglutamic acid, folic acid, ascorbic acid (vitamin C), phyloquinone (vitamin K), dl-alpha-tocopheryl acetate (vitamin E), cholecalciferol (vitamin D3),

beta-carotene, retinol (vitamin A) vitamins and Fe, Cu, Zn, Mn, Cr, Se, Mo, I trace elements. The last premix includes choline, L-carnitine and taurine.

2.2 Methods

2.2.1 Protein Extraction

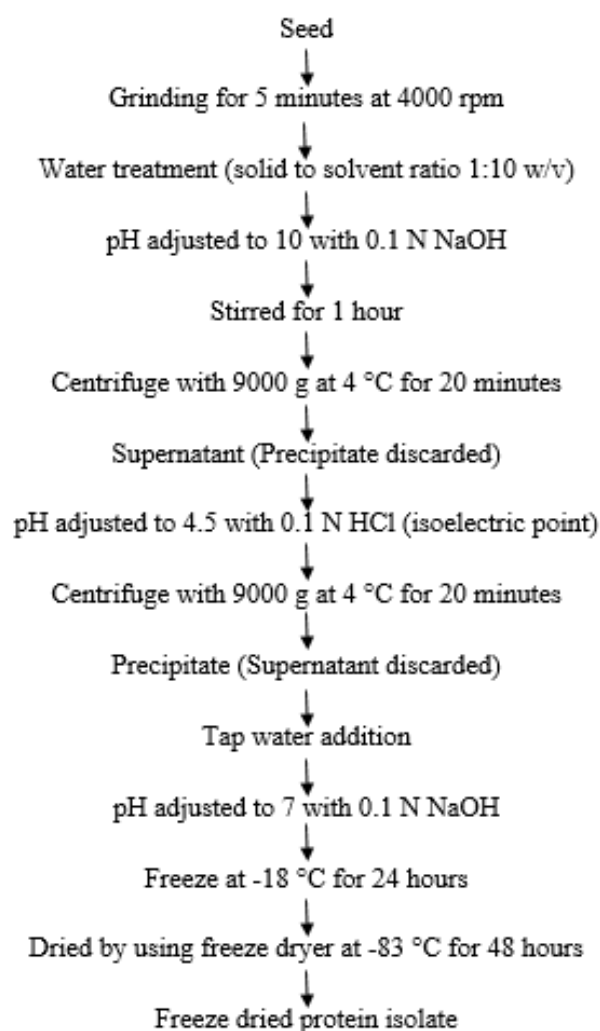


Figure 2.1. Protein extraction process

2.2.2 Enteral Formulation Preparation

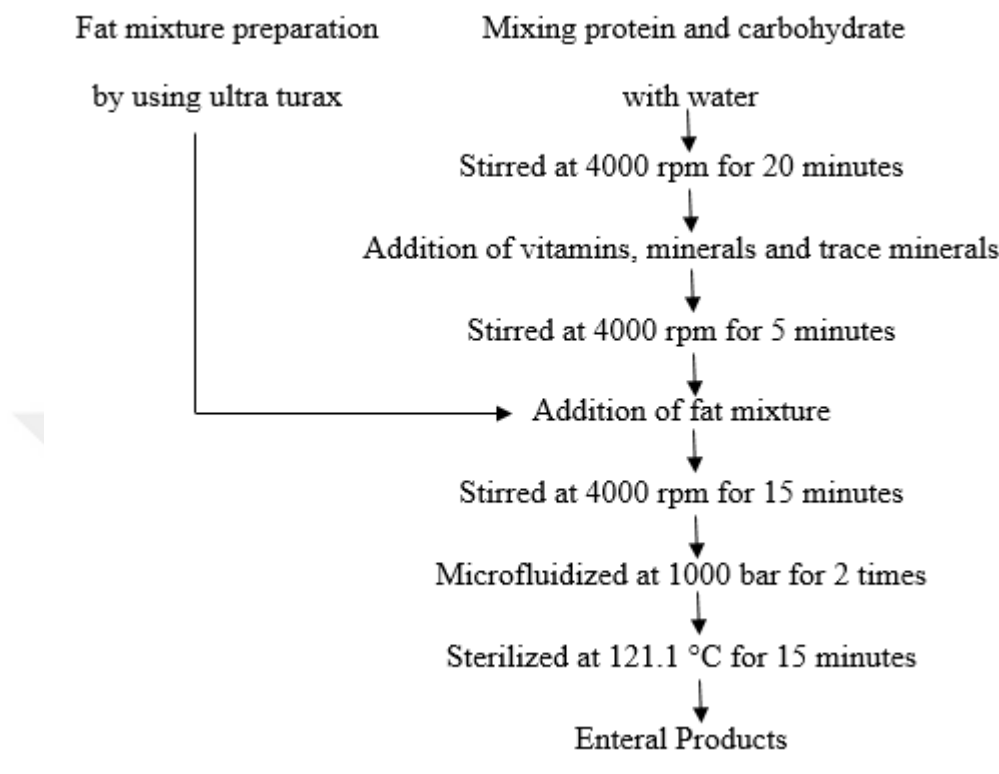


Figure 2.2. Enteral formulation preparation process

2.2.3 Protein Extraction

Protein Yield (PY)

$$= \frac{\text{Isolate protein content (\%)} \times \text{Isolate protei}}{\text{The amount of extracted plant (gram)} \times \text{Protein content of plant} \left(\frac{\text{g}}{100 \text{ g}} \right)} \times 100$$

2.2.4 Kjeldahl Analysis

Apart from the sample preparation, Kjeldahl procedure includes three important steps which are digestion, distillation and titration. It is important have homogenous samples which are grounded to pass a 20-mesh screen for sample preparation step. For the digestion process, catalyst tablet and anti-foam tablet were firstly put into Kjeldahl tubes. After 1-gram sample was carefully weighed, 25 ml of sulfuric acid was added after protective measures were taken. The prepared Kjeldahl tube was digested in the digestion equipment for 3 hours. After the digestion process was completed and the equipment cooled down, the Kjeldahl tube was placed in the distillation device. At the same time, 50 ml of boric acid and 4 drops of methyl red were prepared and placed in the distillation apparatus. The original color of boric acid and methyl red in the flask which was is pink turned to yellow at the end of distillation process. Titration, the last step of the Kjeldahl analysis, began by filling the previously prepared 0.1 N HCl solution into the burette. Then, the consumption required for the yellow solution to turn pink with 0.1 N HCl was recorded.

The percent nitrogen content and crude protein content of the samples were calculated as follow:

$$\text{Nitrogen \%} = \frac{(V_1 - V_0) \times N \times 0,014}{m} \times 100$$

$$\text{Protein \%} = \text{Nitrogen \%} \times f$$

V_1 : volume of HCl which is used for titration

V_0 : volume of HCl which is used for blank

N : normality of HCl

m : mass of the sample

f : conversion factor

Conversion factor taken as 6,25 for lentil (Jarpa-Parra et al., 2014) and chickpea (J. I. Boye et al., 2010) whereas it is taken as 5,54 for quinoa (Föste et al., 2015) and 5,71 for mung bean (Du et al., 2018).

2.2.5 Soxhlet Extraction Analysis

Soxhlet extraction method is semi-continuous method for fat analysis in food materials. In this method, fat content is measured by the weight of removed fat from the foodstuff. For the fat analysis of cereal products AOAC Method 920.39C procedure is recommended. In this study this procedure was followed with some modifications.

Since the analyzed samples were freeze dried protein concentrates, further drying of the samples was not required. Approximately 2 grams of sample were weighed very precisely (0.0001 grams) on dried kraft paper, which allows rapid flow of hexane. Dried boiling flasks were weighed with the same precision and 250-300 ml of hexane was placed in them. Condenser, Soxhlet flask and boiling flask were assembled. Extraction was performed at a rate of 5-6 drops per second by condensation for four hours in a Soxhlet extractor by heating solvent in the boiling flask. After the extraction process, boiling flasks with extracted fat were dried at 100 °C for 30 minutes in an air oven. The samples were allowed to cool down in dessicator until the weight was measured (“Food Analysis,” 2017).

$$\text{Fat \% (dry weight basis)} = \frac{\text{weight of dried flask} - \text{tare weight of dried flask}}{\text{original sample weight}} \times 100$$

2.2.6 Ash Content Analysis

The inorganic residue remaining because of the ignition or complete oxidation of organic materials in foodstuffs is called as ash. The amount of ash represents the total amount of minerals in foods. Dry ashing and wet ashing are two important techniques to measure ash content of food materials. In this study dry ashing procedure was followed.

Since the analyzed samples were freeze dried protein concentrates, different pre-treatment technique was not required. Firstly, 5-10 grams of samples were weighed on the tared crucibles. Then, after taking the necessary protective measures, the crucibles were placed in the muffle furnace. In the next step, samples were ignited at 550-600 °C for 12 hours. After the ignition was finished, the muffle furnace was waited to cool down and the crucibles were carefully removed to avoid the losing ash. The removed samples were quickly transferred to the desiccators by using a safety tong. The desiccator was closed and the crucibles were allowed to cool until the weight was measured ("Food Analysis," 2017).

The ash content of protein concentrate samples was calculated as follow:

$$\text{Ash\% (wet weight basis)} = \frac{\text{weight after ashing} - \text{tare weight of crucible}}{\text{original sample weight}} \times 100$$

2.2.7 Moisture Content Analysis

Moisture content is one of the key characteristics of foods and it should be determined accurately. It also has an importance in terms of microbiological stability.

The Karl Fischer titration method was used to determine moisture content of protein concentrates. This method was selected since heat is not applied, accurate results are obtained so it can be used for determining of moisture of high protein foodstuff. Automated Karl Fischer titration unit (Mettler Toledo, USA) was used for moisture determination of extracted protein concentrates (LPC, CPC, MPC and QPC) and commercial protein concentrates (PPC and SPC).

2.2.8 Fourier transform infrared (FTIR) spectroscopy Analysis

Fourier transform infrared (FTIR) spectroscopy analyses of extracted protein concentrates were performed by using a FTIR spectrometer (IR-Affinity1, Shimadzu Corporation, Kyoto, Japan) with Attenuated Total Reflectance (ATR) attachment. This analysis was carried out with 32 number of scans and 4 cm⁻¹ resolution at 600-4000 cm⁻¹ frequency range.

2.2.9 Amino Acid Profile Analysis

Amino acid profile analysis was performed at Central Research Institute of Food and Feed Control of the Ministry of Agriculture and Forestry (Bursa, Turkey) to determined amino acid composition of extracted protein samples.

In order to carry out amino acid profile analysis, 0.5 grams of homogenized protein concentrate samples were weighed separately. To optimize the recovery of cystine,

methionine and tyrosine, 0.1 g of sodium sulfite was added to each sample. 5 ml of 6 N HCl was added to solution and stirred. To prevent oxidation, 250 μ L of 2 nM phenol solution was added. This mixture was kept in the oven at 110 °C for 24 hours. After 24 hours, samples were cooled at room temperature and added to the centrifuge tube. The pH is adjusted between 6.7 and 7.3 using a 10 N NaOH solution. The sample is completed to 50 ml by adding pure water to the centrifuge tube. Samples were centrifuged at 4000 rpm for 5 minutes. After centrifugation, it was taken from the clear part of the sample and filtered through a 0.45 micron filter. Chromatographic analysis was performed by using HPLC (Agilent, California, USA) with ZORBAX Eclipse-AAA 4.6 x 75 mm, 3.5 μ m column (“Food Analysis by HPLC,” 2013; Henderson et al., 2000).

2.2.10 Water Holding Capacity

The water holding capacity (WHC) of sample was determined by following the procedure from Gong et al (2015). Approximately 1 g of sample (W_0) was weighed together with centrifugal tube (W_1). 10 ml of distilled water was added to centrifuge tube and mixed by using vortex for 5 minutes. The samples were stand for 30 minutes at room temperature and centrifuged at 3000 $\times$ g for 20 minutes. After the removal of supernatant, centrifuge tube containing sediment was weighed (W_2). The water holding capacity (WHC) of protein samples were calculated (gram of water per gram of protein) by given equation.

$$\text{Water Holding Capacity (WHC)} = \frac{W_2 - W_1}{W_0}$$

2.2.11 Oil Binding Capacity

The oil binding capacity (OBC) of sample was determined by following the procedure from Gong et al (2015) with slight modifications. Approximately 1 g of

sample (W_0) was weighed together with centrifugal tube (W_1). 5 ml of corn oil was added to centrifuge tube and mixed by using vortex for 5 minutes. The samples were stand for 30 minutes at room temperature and centrifuged at $3000\times g$ for 20 minutes. After the removal of supernatant, centrifuge tube containing sediment was weighed (W_2). The oil binding capacity (OBC) of protein samples were calculated (gram of oil per gram of protein) by given equation.

$$\text{Oil Binding Capacity (OBC)} = \frac{W_2 - W_1}{W_0}$$

2.2.12 Scanning Electron Microscopy Analysis

Microstructural analysis of extracted plant proteins and dried enteral nutrition formulations was carried out by using scanning electron microscope (SEM) at Central Laboratory of METU (Ankara, Turkey). Six different protein isolates and enteral formulations which were prepared by using these proteins were analyzed.

All twelve samples were frozen at $-18\text{ }^{\circ}\text{C}$ for 24 hours, after that they freeze dried in freeze-drier (Christ, Alpha 2-4 LD plus, Germany) at $-83\text{ }^{\circ}\text{C}$ for 48 hours. In order to make these samples electrically conductive, freeze dried samples were covered with a layer of gold-palladium mixture by Sputter Coater Device (Polaron Range, East Sussex, England). Finally the images of twelve samples were recorded with a scanning electron microscope (QUANTA 400F Field Emission SEM, Eindhoven, Holland) at magnification levels of $250\times$, $500\times$, $1000\times$, $2500\times$ and $5000\times$ with accelerating voltage of 30 kV.

2.2.13 Particle Size Analysis

Particle size measurements were taken with Malvern Metasizer 3000 system (Malvern Instruments Limited, Worcestershire, UK). Measurement of particles are made to find out mean particle size of enteral formulations which were prepared with different protein and oil types.

2.2.14 Creaming Index

In order to understand separation state and aggregation of enteral nutrition formulations, creaming index analysis were used. The larger creaming index (CI) implies more aggregation and the larger flocculation. Evaluation of creaming index (CI) was carried out by using the different method which was established by modifications of previous studies (Chanamai & McClements, 2000; Hong et al., 2018; Shen & Tang, 2012). Each enteral formulation was filled into plastic rectangular tubes (50 mm*15 mm*5 mm) All the samples were centrifuged with 6000×g at 20 °C for 20 minutes. After the centrifugation process, photos of each rectangular tubes were taken. Number of pixels of enteral formulation (NP_E) and supernatant part (NP_S) was found by the application of Adobe Photoshop Program. Creaming index (CI) was calculated as:

$$\text{Creaming Index (CI)} = \frac{NP_S}{NP_E} \times 100$$

2.2.15 Rheology Analysis

The rheological analysis were performed by using rheometer (Kinexus dynamic rheometer, Malvern, Worcestershire, UK), using cup & bob coaxial cylinder geometry (9.15 mm gap) The prepared enteral formulation and existing commercial sample were placed between the cylinders. Corresponding shear stress (Pa), shear

viscosity (Pa.s) data were obtained by the application of shear rate properties between 0,1-100 s⁻¹ at 25 °C and 37 °C which are room temperature and human body temperature respectively. All measurement was taken as triplicate.

2.2.16 Confocal Microscopy Analysis

In order to observe the interaction of fat and protein structures in enteral nutrition solution, Confocal Laser Scanning Microscope (Zeiss LSM 880, UK) at METU Molecular Biology and Biotechnology R&D Center. Two different enteral formulation samples were analyzed in two-dimensional form which were prepared with MPC and SPC. Corn oil was used as a fat source. The samples were excited with an Argon laser at 488 nm, with light emitted back at 543 nm. Nile Red was obtained from Sigma-Aldrich (USA) used to stain fat structures and fluorescein isothiocyanate (FITC) was obtained from Thermo Fisher Scientific (UK) was used to stain protein structures.

For staining and for the preparation of Nile Red and FITC, method by Kirtil & Oztop was followed (2016).

2.2.17 pH Analysis

Digital pH meter (InoLab pH 720, Germany) was used to analyze pH of enteral nutrition formulations which were prepared with different protein and oil types. In order to calibrate pH meter probe, distilled water (pH 7.00) was used. pH data of all samples were taken 24 hours after the production as triplicate.

2.2.18 Microfluidization

Microfluidization process was performed by using microfluidizer equipment (M110-Y, Microfluidics, USA). All samples were microfluidized at 1000 bar pressure through a chamber of 200 μm size.

2.2.19 Statistical Analysis

In order to determine whether there was significant difference between extracted proteins and enteral nutrition formulations which were prepared by using different protein and fat sources, analysis of variance (ANOVA) was performed ($p < 0.05$). Tukey Single Range Tests were performed by using MINITAB Statistics Software (2016) when significant difference was obtained.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Analysis of Extracted Proteins

3.1.1 Protein Extraction Results

Extraction yield and protein yield are the most important indicators of the extraction efficiency. In this study, four different plant-based proteins (LPC, CPC, MPC and QPC) were extracted. Extraction yield results and protein yield results were reported as the amount of extract versus 100 gram flour and ratio of extracted protein amount of 100 gram flour versus protein amount of the 100 gram seed, respectively.

In this study, extraction yields were found as 12.15 gr for LPC, 12.89 gr for CPC, 11.57 gr for MPC and 8.57 gr for QPC. The extraction yield depends mainly on pH and solute to solvent ratio. Jarpa-Parra et al. conducted similar experiment with lentil protein to find out optimum yield by changing alkali solubility pH and solid to solvent ratio (2014). In that study, 12.35 – 16.50 g extract was extracted from 100 g lentil flour and it was found that protein content decreasing while the extraction yield increasing due to the effect of high alkali application. Similar extraction yields were found except for QPC. In order to increase extraction yield of quinoa protein, alkali solubility pH can be increased but protein content of quinoa seed is also low. Therefore, alkali solubility pH of quinoa protein can be decreased to increase protein content and to find optimum point.

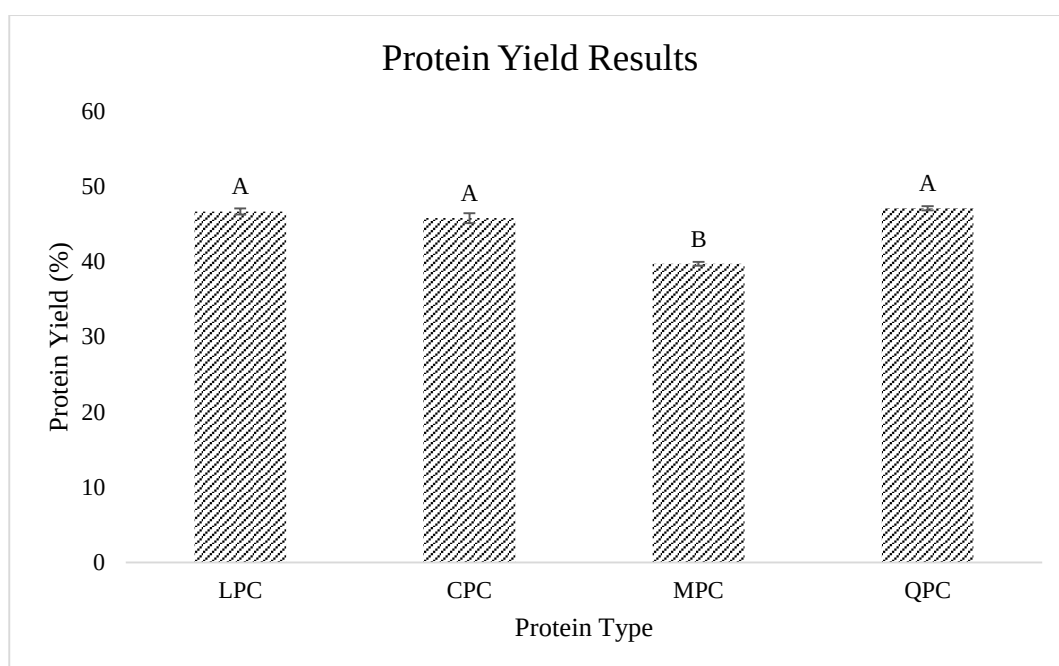


Figure 3.1. Protein yield results (%) of LPC, CPC, MPC and QPC

The protein yields of extracted proteins were given in Figure 3.1. The highest protein yield was found as 47.11 % for QPC while the lowest protein yield was found as 39.73 for MPC. The effect of alkali pH on protein yield of quinoa protein was studied by Ruiz et al. In that study, protein yield was found as 36.3% for pH 8 and 52% for pH 11 (2016). In this study, alkali solubility pH was adjusted as 10. Therefore, no difference was found between experimental results and literature results. Despite the protein content of QPC is less than others, protein yield of QPC is the highest. The reason may be quinoa seed's protein content (14 g protein per 100 g quinoa seed flour) is half of the other seeds' which were used in this study.

Although protein content of mung bean seed is high, protein yield of MPC is the lowest due to low extraction yield. Since mung bean seed's protein content is high,

alkali solubility pH can be increased up to optimum point to increase both extraction and protein yields.

3.1.2 Kjeldahl Analysis

Kjeldahl analysis were applied to find out protein content of proteins which were used to formulate enteral nutrition. Average protein contents of four extracted proteins and two commercial proteins were given in the Table 3.1. The highest protein content was found as 88.33 ± 0.038 % for LPC while the lowest protein content was found as 64.6 ± 1.32 % for QPC. Protein content of LPC and MPC are significantly different than the other commercial and extracted proteins.

Different researches were conducted to understand the effect of extraction parameters on protein content of concentrates. In those studies protein contents were found as 84.12 ± 0.06 % for lentil protein (Ko et al., 2017), 78.0 ± 2.1 % for chickpea protein (Sánchez-Vioque et al., 1999), 74.58 ± 0.71 % for mung bean protein (Li et al., 2010) and 67.93 ± 0.24 % for quinoa protein (Föste et al., 2015). In this study, protein contents of extracted proteins were higher or similar to literature findings since same method and similar parameters were applied.

Protein content of the concentrates depends on method of extraction (dry milling, isoelectric precipitation, salt extraction and enzyme extraction) and extraction parameters such as pH, stirring time, solvent to solute ratio, temperature and centrifugal force ($\times g$). Therefore, different protein contents can be obtained by applying different methods and changing the parameters. In this study, isoelectric precipitation was selected as a method and parameters were determined according to literature review and preliminary studies.

Table 3.1 Average Protein Content (%) of LPC, CPC, MPC, QPC, PPC, SPC

Protein Type	Average Protein Content (%)
LPC	88.33±0.038 ^A
CPC	74.59±0.22 ^C
MPC	85.82±0.004 ^A
QPC	64.6±1.32 ^D
PPC	80.68±0.108 ^B
SPC	79.59±0.088 ^B

3.1.3 Soxhlet Extraction Analysis

Average fat contents of four extracted proteins and two commercial proteins were given in Table 3.2. The highest fat content was found as 20.33±1.32 g fat per 100 g protein concentrate for QPC while the lowest fat content was found as 0.86±0.004 g fat per 100 g protein concentrate for MPC.

In this study, defatting with hexane was not applied since these protein powders were used in enteral nutrition. Fat content were separated after the first centrifugation physically. Therefore, huge difference was observed between defatted protein concentrates and non- defatted protein concentrates. The studies were conducted by different researchers to characterize different protein isolates. In those studies fat contents were found as 0.42±0.24 g per 100 g protein for defatted lentil protein (Ko et al., 2017) and 4.49±0.37 g per 100 g protein for non- defatted lentil protein (Alonso-Miravalles et al., 2019). These studies show the importance of defatting process on fat content. Another study which was conducted by Zar Oo et al. to characterize defatted mung bean protein concentrates reported fat content as 0.3±0.02 g per 100 g protein (2017). Toews & Wang studied on functional properties of chickpea protein concentrate. Fat content of non-defatted chickpea protein was

found as 14.6 ± 0.62 g fat per 100 g protein while it was found as 0.66 ± 0.21 g fat per 100 g protein for defatted chickpea protein (2013). In this study, fat content of chickpea protein concentrate was found as 9.84 ± 0.22 g fat per 100 g protein which is lower than the literature findings. Another reason of high fat content of chickpea protein may be the color of supernatant. The yellowish color of chickpea protein's supernatant made it difficult to physical separation of fat layer.

After grinding of seed, the first step of wet milling is water treatment. When quinoa flour is treated with water, gel formation was observed because of the high starch content of quinoa flour (Wu et al., 2017). This carbohydrate-based gel made it difficult to physical separation of fat layer. The huge difference between fat contents of quinoa protein and the other proteins may be explained by this gel formation.

Table 3.2 Average Fat Content (%) of LPC, CPC, MPC, QPC, PPC, SPC

Protein Type	Average Fat Content (%)
LPC	1.62 ± 0.04^C
CPC	9.84 ± 0.22^B
MPC	0.86 ± 0.01^C
QPC	20.33 ± 1.32^A
PPC	2.29 ± 0.11^C
SPC	2.14 ± 0.09^C

3.1.4 Ash Content Analysis

Average ash contents of four extracted proteins and two commercial proteins were given in Table 3.3 as wet basis. The highest ash content was found as 7.79 ± 0.34 g ash per 100 g protein concentrate for QPC while the lowest ash content was found as 4.36 ± 0.10 g ash per 100 g protein concentrate for LPC. There is no significant difference between LPC, CPC and MPC whose ash contents were lower than the others.

The effect of isoelectric extraction technique on physicochemical properties of lentil, chickpea, pea, and soy proteins was studied by Karaca et al.. In that study, ash contents were found as 3.63 ± 0.09 % for lentil protein, 3.05 ± 0.33 % for chickpea protein, 5.59 ± 0.34 % for pea protein and 2.09 ± 0.15 % for soy protein (2011). In this study, ash contents were found higher than the study was conducted by Karaca et al.. The reason of this difference is pH adjustment of alkali extraction processes. In that study, pH was adjusted to 9 for alkali extraction while it was adjusted to 10 for alkali extraction in this study. Therefore, more ash was formed due to more chemical usage. Another study was conducted by Brishti et al. on the isolation of mung bean protein. Although protein curds were washed twice before the freeze drying, ash content was found as 4.38 ± 0.28 g ash per 100 g protein (2017).

Before the protein extraction process, these seeds contain inorganic materials naturally. Extra filtration process was not applied before drying process. Moreover, these proteins were extracted by wet milling which includes chemicals such as HCl and NaOH, therefore, salt and ash formation at the end-product was expected. QPC has the highest ash content among all studied proteins. The reason may be the high mineral content of quinoa seed flour (Elsohaimy et al., 2015). Commercial plant proteins' ash contents are also high. Both of the suppliers claim that ash content can be up to 10%. Therefore, this was also expected.

According to Codex Alimentarius, ash content must not exceed 8% on dry weight basis (1989). In this study average ash content was measured as wet weight basis. However, ash contents (dry weight basis) of all extracted protein concentrates are lower than limit value. Only PPC's ash content is higher than 8%.

Table 3.3 Average Ash Content (%) of LPC, CPC, MPC, QPC, PPC, SPC

Protein Type	Average Ash Content (%)
LPC	4.36±0.10 ^C
CPC	5.17±0.13 ^{BC}
MPC	4.98±0.07 ^{BC}
QPC	7.79±0.34 ^A
PPC	7.76±0.31 ^A
SPC	5.54±0.01 ^B

3.1.5 Moisture Content Analysis

Average moisture contents of four extracted proteins and two commercial proteins were given in Table 3.4. The highest moisture content was found as 7.02±0.019 g moisture per 100 g protein concentrate for SPC while the lowest moisture content was found as 1.93±0.049 g moisture per 100 g protein concentrate for MPC. Biological stability of protein concentrates depends on the moisture contents of these. Therefore, low moisture content with good functional properties are desirable.

Tontul et al. studied on drying methods of chickpea protein. In that study, moisture content of freeze dried chickpea protein was found as 2.86 g moisture per 100 g of protein (2018). Another study was conducted by Ko et al. to isolate and characterize lentil protein. Moisture content of freeze dried lentil protein was found as 5.20 ±0.04

g moisture per 100 g protein isolate (2017). Moisture content of freeze dried mung bean protein was found 4.56 ± 0.11 g moisture per 100 g protein isolate at another study (Brishti et al., 2017). In this study, moisture contents of extracted proteins (except for chickpea protein) were found lower or similar to previous studies.

Increased surface area relative to the volume results in moving of more water molecules at the same amount of time. The difference between moisture content of CPC and rest of the extracted proteins may be caused by the surface area differences of protein samples before drying.

Soy protein and pea protein bought from different suppliers (spray dried). High moisture contents of these two proteins may be caused by spray drying method.

Table 3.4 Average Moisture Content (%) of LPC, CPC, MPC, QPC, PPC, SPC

Protein Type	Average Moisture Content (%)
LPC	4.91 ± 0.04^C
CPC	6.97 ± 0.04^A
MPC	1.93 ± 0.05^D
QPC	1.98 ± 0.07^D
PPC	6.00 ± 0.11^B
SPC	7.02 ± 0.02^A

3.1.6 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FT-IR spectroscopy is an effective technique to evaluate food samples since this technique non-destructive, rapid and quite simple (Rodriguez-Saona & Allendorf,

2011). Characterization of minor food components in the level of ppb (parts per billion) and quality control applications can be achieved by using FT-IR spectroscopy (Anjos et al., 2015).

In this study, the structural differences between six different protein samples which are LPC, CPC, MPC, QPC, PPC and SPC were examined in the wavelength range of 600-4000 cm^{-1} .

Table 3.5 Characteristics peaks of protein concentrates and their functional groups (Kong & Yu, 2007)

Wavelength (cm^{-1})	Assignment	Functional groups
1600-1700	C=O Stretching	Amide I
1480-1600	N-H Bending	Amide II
1200-1400	C-N Stretching	Amide III
~ 3300	N-H stretching	Amide A
~ 3100	N-H stretching	Amide B
~1050	C-O/C-C Stretching	Carbohydrates

The wavelengths of important peaks which were obtained from samples were demonstrated in Table 3.5.

In this study, amide I, amide II, amide III, amide A and amide B bands were observed (Figure 3.2). The observation of these bands was expected since these bands indicate protein structures. According to studies, Amide I and Amide II peaks are important

peaks for protein structures. Amide I band represents C=O stretching which can be observed at the frequency range of 1600-1700 cm^{-1} . On the other hand, Amide II band represents N-H bending which can be observed at the frequency range of 1480-1600 cm^{-1} . Also, amide A and amide B represent N-H stretching and can be observed at the frequency range of 3100 cm^{-1} and 3300 cm^{-1} , respectively (Kong & Yu, 2007).



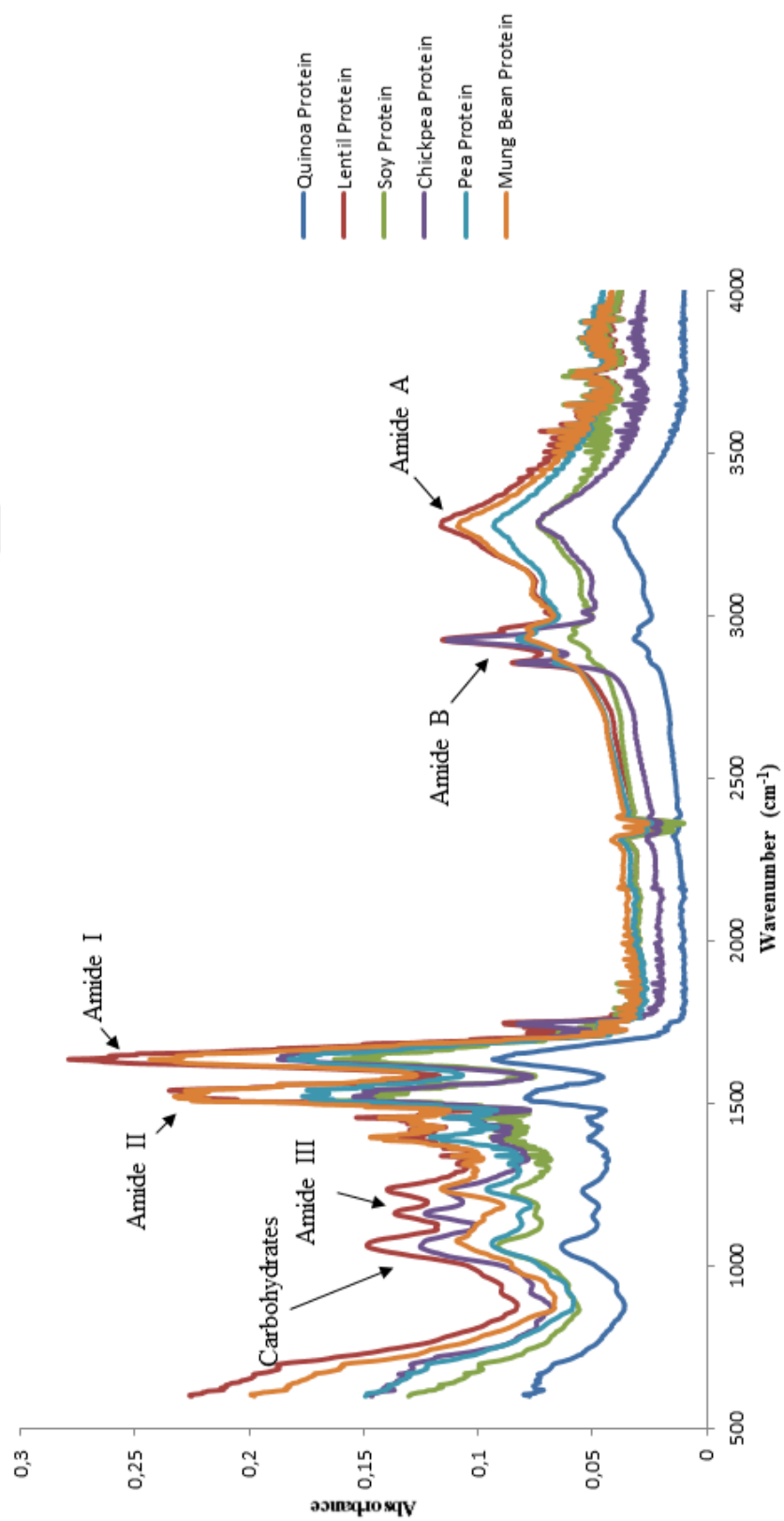


Figure 3.2. FTIR spectrum of protein concentrates

Different protein concentrates were characterized by different researchers using FT-IR equipment. Same proteins bands and similar intensities were found for lentil protein (de la Rosa-Millán et al., 2018), chickpea protein (Xu et al., 2017), mung bean protein (Du et al., 2018) , quinoa protein (Wang et al., 2020), pea protein (Liu et al., 2020) and soy protein (Wang et al., 2011).

Since FT-IR can be used to analyze samples both qualitatively and quantitatively, the correlation between Kjeldahl results and FT-IR peak areas was expected (Kong & Yu, 2007). In this study, any contradiction was found between FT-IR results and Kjeldahl results. Overall, important peak which are associated with protein structures were observed. The high intensity of these bands indicates the high amount of protein which was found in samples.

3.1.7 Water Holding Capacity (WHC)

Water holding capacities of six different protein concentrates were given in Figure 3.3 as a percentage. SPC has the highest WHC with 213.74 ± 4.74 % and CPC has the lowest WHC with 140.29 ± 1.87 %.

In the study of Li et al., sixteen different mung bean cultivars' protein isolates were characterized and WHC of these protein were found between 2.78 ± 0.04 g water per gram protein and 1.03 ± 0.09 g water per gram protein (2010). Aydemir & Yemenicioğlu studied on functional properties of lentil protein isolates of different cultivars. In that study, the average water absorption capacity of lentil protein isolate was found as 1.04 ± 0.52 g water per gram protein (2013). WHCs of soy bean protein isolate and pea protein isolate were found as 1.3 ± 0.1 g water per gram protein and 1.7 ± 0.1 g water per gram protein respectively (Fernández-Quintela et al., 1997). These studies show that the results obtained in this study are similar to the results found in the literature.

Although all of the protein concentrates were processed at the same conditions, differences in WHC may be due to seed type (Boye et al., 2010). Polar amino acids such as serine, threonine and tyrosine are known as primary sites for water-protein interactions. Therefore, availability of these amino acids is important for WHC property of protein concentrates (Maninder Kaur & Singh, 2007). Polar amino acid content of SPC is higher than the other protein concentrates and not surprisingly it has the highest WHC. Fat content of protein concentrates is also important parameter for WHC (Shevkani et al., 2019). In this study, QPC and CPC have the highest fat contents. The low WHC of CPC can be explained by high fat content. However, the lowest WHC value was expected from QPC since its fat content is the highest and polar amino acid content is the lowest. On the contrary, QPC has higher WHC than PPC and CPC. The reason of relatively high WHC may be due to starch content of QPC (Li & Zhu, 2018).

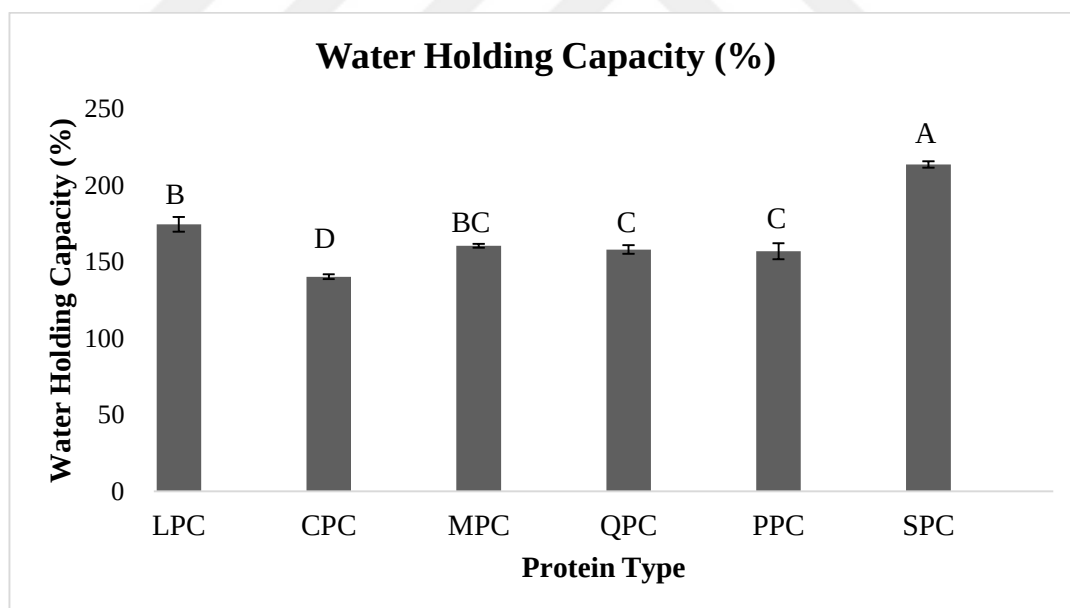


Figure 3.3. Water holding capacities (%) of LPC, CPC, MPC, QPC, PPC, SPC

3.1.8 Oil Binding Capacity (OBC)

High oil binding capacity is desirable functional property for the food industry applications since it retains flavor compound and enhances mouth feel (Li et al., 2010). Oil binding capacities of six different protein concentrates were given in Figure 3.4 as a percentage. MPC has the highest OBC with 138.81 ± 1.42 % and PPC has the lowest OBC with 102.09 ± 4.77 %.

Functional properties of sixteen different mung bean cultivars were studied by Li et al. In that study, OBC of these protein were found between 3.38 ± 0.12 g oil per gram protein and 1.00 ± 0.12 g oil per gram protein (2010). Another research was conducted by Boye et al. to investigate the effects of processing methods on functional properties of different pulse proteins. Oil binding capacities (in terms of percentage) of lentil protein and chickpea protein were found as 124.8% and 135.3%, respectively (2010). Oil binding capacity (in terms of percentage) of pea protein and soy protein were also reported as 152% and 159%, respectively (Fuhrmeister & Meuser, 2003). Literature studies show that results which were reported in this study similar to those obtained in the literature.

Hydrophobic interaction can be formed by non-polar amino acid side chains and hydrocarbon chains of lipid. Therefore, the differences between oil binding capacities may be caused by the variation in the non-polar amino acid side chains (Jitngarmkusol et al., 2008). Non-polar amino acid content of MPC is higher than the other protein concentrates and not surprisingly it has the highest OBC.

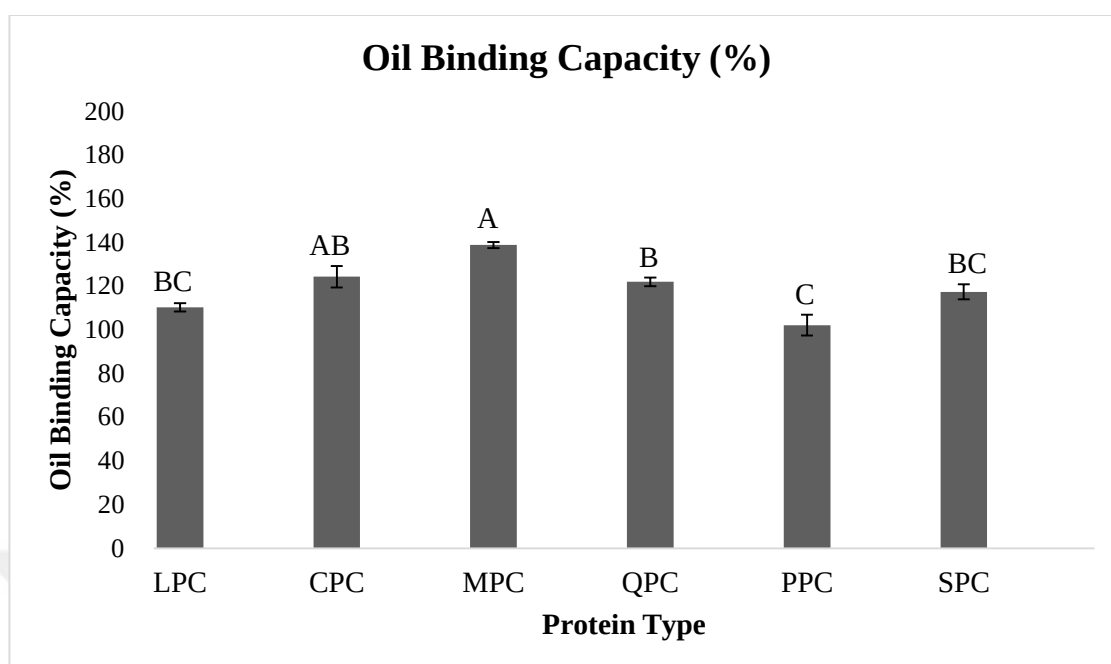


Figure 3.4. Oil binding capacities (%) of LPC, CPC, MPC, QPC, PPC, SPC

3.1.9 Amino Acid Profile Analysis

The structure and functionality of the protein mainly influenced by its amino acid composition. Moreover, nutritional quality depend on amino acid composition and the amount of essential amino acids (Mauer, 2003).

In this study, six different protein concentrates' amino acid compositions were determined. Amino acid profiles and total amount of essential amino acids of each protein were given as g amino acid per 100 g protein concentrate in the Table 3.6. The concentration of glutamic acid which was between $8.83 \pm 0.01\%$ (QPC) and $15.22 \pm 0.01\%$ (SPC) was found as the highest concentration of amino acid for each protein investigated. Glutamic acid's concentration was followed by aspartic acid's concentration which was between $5.35 \pm 0.01\%$ (QPC) and $9.55 \pm 0.01\%$ (MPC). On the other hand, the concentration of methionine which was between $0.57 \pm 0.00\%$ (LPC) and $1.33 \pm 0.00\%$ (QPC) was found as the lowest concentration of amino acid for each protein investigated. It was expected since plant-based proteins especially

pulse proteins such as LPC and CPC known to be deficient in sulfur-containing amino acids (Samaranayaka, 2017). Wongsiri et al. reported relative deficiency of sulfur-containing amino acids in mung bean protein isolate (2015). Another study was conducted by Dhawan et al. to investigate amino acid composition of chickpea protein. In that study, the reason of the relative deficiency of sulfur-containing amino acids were explained as the high amount of glutelins and globulins which are deficient in terms of methionine are the major constituent parts of chickpea protein (1991).

Nutritional value and muscle protein synthetic response are mainly dependent on the amount of essential amino acids (Atherton et al., 2010; Volpi et al., 2003). WHO/FAO/UNU essential amino acid requirements for adults is at least 27.7% essential amino acid of total amino acid consumption. It should be noted that this requirement is recommended when adult protein consumption of 0.66 g/kg body weight/day (WHO/FAO/UNU Expert Consultation, 2007). In this study, the amounts of essential amino acids of six different plant-based proteins were determined. They were found between 24.19 % (QPC) and 34.54 % (MPC) which were represented in Figure 3.7. Only QPC cannot meet WHO/FAO/UNU amino acid requirements with 24.19% EAA. Important study was conducted by Gorissen et al. to investigate protein contents, amino acid profiles and the amount of essential amino acids of fifteen different proteins including plant-based and animal-based proteins. In that study, pea (30%), corn (32%), potato (37%) and brown rice (28%) proteins can meet WHO/FAO/UNU amino acid requirements while soy (27%), oat (21%), lupin (21%), wheat (22%), hemp (23%) and microalgae (23%) proteins are below this requirements. It was also found that the amount of essential amino acids in animal-based proteins such as whey (43%), milk (39%), casein (34%) and egg (32%) are well above the WHO/FAO/UNU requirements (2018). These studies show some of the plant-based proteins especially MPC (34.54%) which was extracted in this study can be compared with animal-based proteins such as casein and egg in terms of the amount of EAAs.

Leucine and lysine have muscle protein synthesis effects with other essential amino acids (Dijk et al., 2018; Lorieau et al., 2019). The muscle protein synthesis effects of leucine supplementation has been studied for animal studies (Areta et al., 2014; Rieu et al., 2007) and human studies (Casperson et al., 2012; Luiking et al., 2016) and positive results have been reported. According to WHO/FAO/UNU amino acid requirements, leucine and lysine concentration should be above 5.9% and 4.5% respectively (2018). In this study, leucine and lysine concentration of each protein were represented in Figure 3.5 and Figure 3.6, respectively. MPC has the highest leucine and lysine content with 6.66 ± 0.01 g amino acid per 100 g protein concentrate and 6.56 ± 0.01 g amino acid per 100 g protein concentrate respectively. LPC, PPC and SPC can also meet WHO/FAO/UNU's leucine and lysine requirements. However, only QPC cannot meet both of the amino acid requirements.

Table 3.6 Amino acid composition of LPC, CPC, MPC, QPC, PPC, SPC and total amount of essential amino acids (Σ EAA).

Results were given as g amino acid/100 g protein concentrate.

	LPC	CPC	MPC	QPC	PPC	SPC
Histidine	2.21±0.01 ^e	2.37±0.00 ^c	2.98±0.01 ^a	2.20±0.00 ^e	2.28±0.01 ^d	2.49±0.01 ^b
Isoleucine	3.75±0.01 ^c	3.44±0.01 ^d	3.75±0.01 ^c	2.85±0.01 ^e	3.79±0.01 ^b	3.82±0.01 ^a
Leucine	6.25±0.01 ^b	5.87±0.01 ^e	6.66±0.01 ^a	4.40±0.01 ^f	6.07±0.00 ^d	6.13±0.01 ^c
Lysine	5.96±0.01 ^c	5.22±0.01 ^e	6.56±0.01 ^a	3.84±0.01 ^f	6.35±0.01 ^b	5.71±0.01 ^d
Methionine	0.58±0.00 ^f	0.86±0.00 ^d	1.01±0.00 ^c	1.33±0.00 ^a	0.81±0.00 ^e	1.05±0.00 ^b
Phenylalanine	4.04±0.00 ^c	4.53±0.00 ^b	5.04±0.00 ^a	2.61±0.00 ^f	4.01±0.00 ^d	3.93±0.00 ^e
Tyrosine	2.32±0.00 ^c	1.85±0.00 ^f	2.28±0.00 ^d	1.87±0.00 ^e	2.57±0.00 ^b	2.66±0.00 ^a
Threonine	2.69±0.00 ^c	2.40±0.00 ^f	2.47±0.00 ^d	2.41±0.00 ^e	2.87±0.00 ^b	2.98±0.00 ^a
Valine	3.40±0.00 ^c	2.88±0.00 ^e	3.77±0.00 ^a	2.68±0.00 ^f	3.47±0.00 ^b	3.29±0.00 ^d
Aspartic Acid	8.91±0.01 ^c	8.90±0.01 ^c	9.56±0.01 ^a	5.35±0.01 ^e	8.62±0.01 ^d	8.95±0.01 ^b
Glutamic Acid	11.34±0.01 ^e	12.87±0.01 ^c	14.59±0.01 ^b	8.83±0.01 ^f	12.67±0.01 ^d	15.22±0.01 ^a
Serine	4.26±0.00 ^b	4.06±0.00 ^d	4.44±0.00 ^a	2.96±0.00 ^f	3.93±0.00 ^e	4.19±0.00 ^c
Glycine	2.82±0.00 ^c	2.62±0.00 ^f	2.69±0.00 ^e	2.77±0.00 ^d	2.92±0.00 ^b	3.12±0.00 ^a
Arginine	6.35±0.01 ^b	6.52±0.01 ^a	5.62±0.01 ^e	4.99±0.01 ^f	6.06±0.01 ^c	5.80±0.01 ^d
Alanine	3.09±0.00 ^d	3.07±0.00 ^e	3.27±0.00 ^b	2.76±0.00 ^f	3.21±0.00 ^c	3.33±0.00 ^a
Proline	3.00±0.00 ^c	2.78±0.00 ^d	3.19±0.00 ^b	2.28±0.00 ^f	2.66±0.00 ^e	3.65±0.00 ^a
ΣEAA	31.21±0.04^d	29.43±0.04^e	34.54±0.04^a	24.19±0.03^f	32.21±0.04^b	32.05±0.04^c

Different letters in the same row represent statistical difference by Tukey's Test (p<0.05)

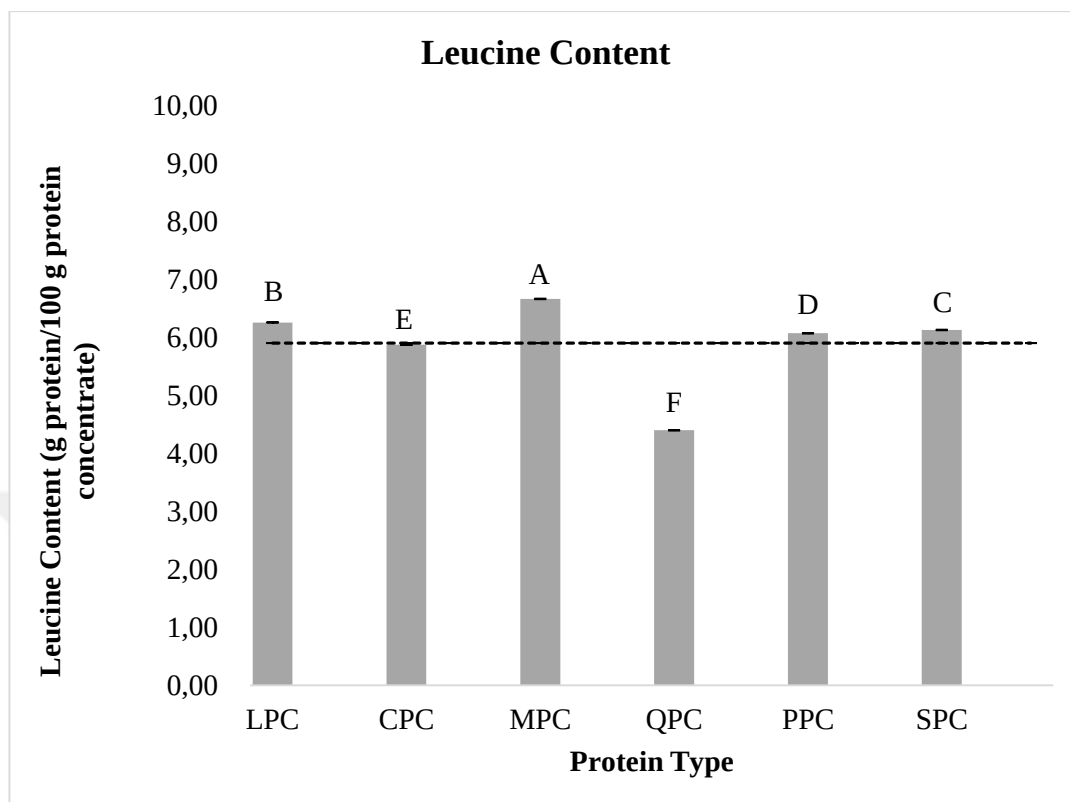


Figure 3.5. Leucine content (g Leucine/100 g protein concentrate) of LPC, CPC, MPC, QPC, PPC and SPC. Line represents Leucine requirements (5.9 g Leucine/100 g protein concentrate) for adults (WHO/FAO/UNU Expert Consultation, 2007)

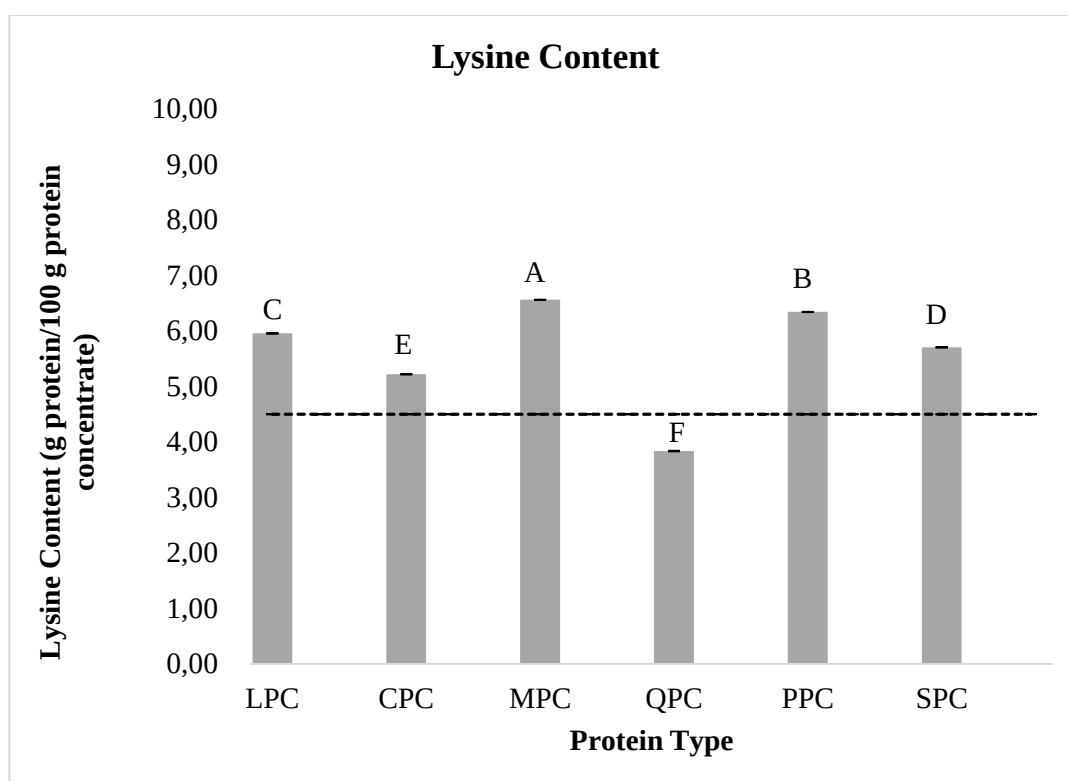


Figure 3.6. Lysine content (g lysine/100 g protein concentrate) of LPC, CPC, MPC, QPC, PPC and SPC. Line represents lysine requirements (4.5 g lysine/100 g protein concentrate) for adults (WHO/FAO/UNU Expert Consultation, 2007)

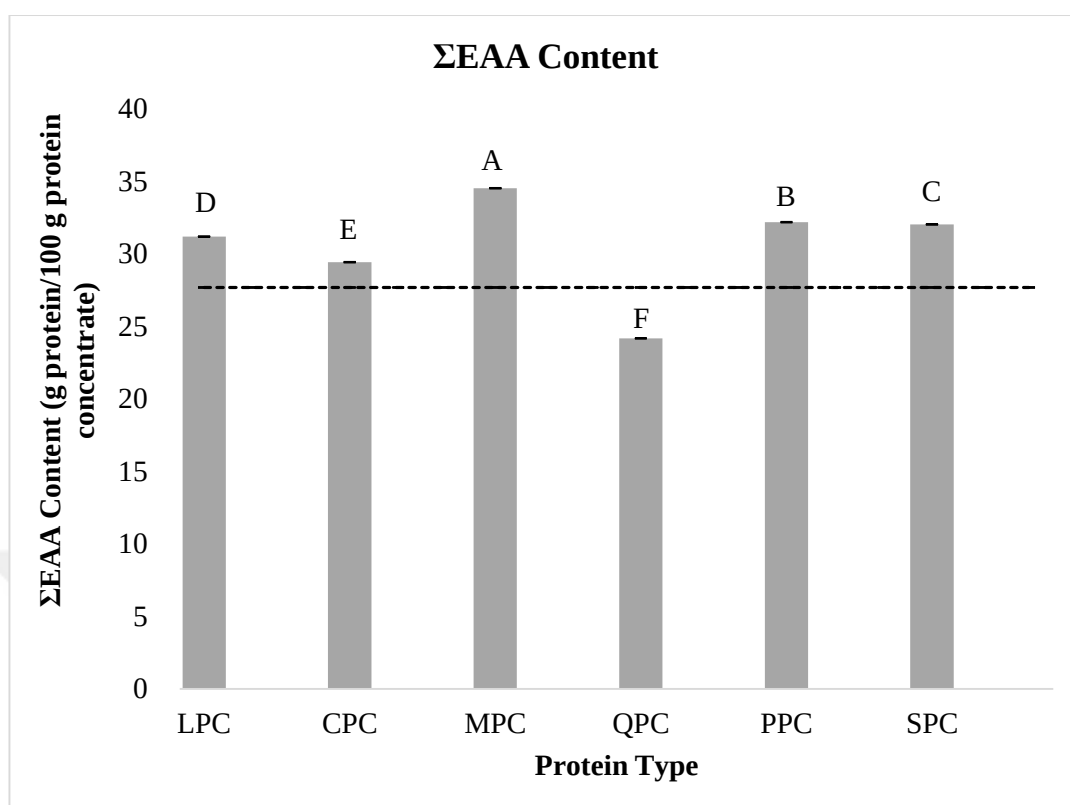


Figure 3.7. Σ EAA content (g Σ EAA /100 g protein concentrate) of LPC, CPC, MPC, QPC, PPC and SPC. Line represents Σ EAA requirements (27.7 g Σ EAA /100 g protein concentrate) for adults (WHO/FAO/UNU Expert Consultation, 2007)

3.1.10 Protein Efficiency Ratio

Protein efficiency ratio (PER) can be described as the amount of weight which is gained by the test subject divided by its particular food protein consumption. In order to find experimental PER, test subject which is growing animal is needed. On the other hand, good predictions can be made by using equations (Alsmeyer, R.H.; Cunningham, A.E.; Happich, 1974). As it is emphasized previous section, leucine plays important role for muscle growing with other essential amino acids so these equations are mainly based on leucine content (Chavan et al., 2001).

$$\text{PER} - 1 = -0,684 + 0,456 \times \text{Leu} - 0,047 \times \text{Pro}$$

$$\text{PER} - 2 = -0,468 + 0,454 \times \text{Leu} - 0,105 \times \text{Tyr}$$

$$\text{PER} - 3 = -1,816 + 0,435 \times \text{Met} + 0,780 \times \text{Leu} + 0,211 \times \text{His} - 0,944 \times \text{Tyr}$$

(Alsmeyer, R.H.; Cunningham, A.E.; Happich, 1974)

Table 3.7 Protein efficiency ratios (PER) of LPC, CPC, MPC, QPC, PPC and SPC.

	LPC	CPC	MPC	QPC	PPC	SPC
PER-1	2.027±0.001 ^b	1.863±0.001 ^e	2.204±0.002 ^a	1.213±0.001 ^f	1.959±0.001 ^c	1.938±0.001 ^d
PER-2	2.127±0.001 ^b	2.003±0.001 ^e	2.317±0.002 ^a	1.332±0.001 ^f	2.018±0.001 ^d	2.034±0.001 ^c
PER-3	1.585±0.002 ^c	1.888±0.002 ^b	2.296±0.003 ^a	0.894±0.002 ^f	1.329±0.001 ^e	1.431±0.002 ^d

Different letters in the same row represent statistical difference by Tukey's Test (p<0.05)

Protein quality can be described by using protein efficiency ratios (PER). Normally, PER below 1.5 indicates a protein of low quality; between 1.5 and 2.0 indicates intermediate quality; between 2.0 and 3.0 indicates good quality; above 3.0 indicates excellent quality (Friedman, 1996). According to this information, only MPC can be described as good quality protein with PER value between 2.204±0.002 and 2.317±0.002. The reason of high PER value of mung bean protein is the high leucine concentration which is 6.66 %. On the other hand, quinoa protein can be classified as poor-quality protein due to its low leucine concentration with PER value between 0.894±0.002 and 1.332±0.001.

Mitchell et al. studied experimental PER of animal-based and plant-based proteins. In that study, experimental PER of soy protein isolate and chickpea were found as 2.0 ± 0.10 and 1.6 ± 0.05 respectively (1989). These results are similar to predicted PER values of this study which are 2.034 ± 0.001 for SPC and 2.003 ± 0.001 for CPC.

U.S. Dairy Export Council published Reference Manual for U.S. Whey and Lactose Products to complete review of these dairy product. In that manual PER value of whey protein concentrate, cow milk and casein were given as 3.2, 3.1 and 2.5 respectively (2004). Whey and cow milk proteins are classified as excellent quality proteins. Only MPC which was extracted in this study has comparable trend with casein. On the other hand, PER values of whey, cow milk, beef and egg proteins are well above the MPC (Hoffman & Falvo, 2004; Sarwar, 1997).

3.1.11 Scanning Electron Microscopy Analysis

The SEM micrographs of four extracted proteins (LPC, CPC, MPC and QPC) which were represented in Figure 3.8, Figure 3.9, Figure 3.10, Figure 3.11 and commercial proteins (PPC, SPC) which were represented in Figure 3.12 and Figure 3.13 were quite different. Smooth surface structure and plate-shaped morphology without pores and fissures were observed for extracted and freeze-dried protein concentrates. In addition, extracted protein concentrates have large size distribution. On the other hand, wrinkled surface morphology with smaller size distribution was observed for commercial protein concentrates. The huge difference between interior microstructure and the surface morphologies of extracted proteins and commercial proteins which were represented by SEM micrographs were caused by the differences of drying environments. Extracted proteins were prepared by freeze-drying method while commercial proteins were prepared by spray-drying method.

Different studies were conducted to investigate functional and microstructural properties of plant protein concentrates and similar plate-shaped morphologies were observed for lentil protein (M. Joshi et al., 2011), chickpea protein (Yuntao Wang et al., 2020), mung bean protein (Zar Oo et al., 2017) and quinoa protein (Mir et al., 2019).

The reason of freeze-dried extracted protein concentrates were observed bulk plate-shaped instead of individual particle is process conditions and molecular reactions during the process. Because of the low pressure and temperature of freeze-drying environment, solute particles come into close contact with each other. Electrostatic interactions, hydrophobic interactions and even covalent linkages can be enhanced by freeze drying. These interactions can lead solute aggregation (Gong et al., 2015).

In the spray-drying process, particles experience hot and dry air. These particles shrink because of the high flux of moisture which leaves particles. The complex interaction between the change in the surface tension at interface and moisture diffusivity of protein concentrates is the main reason of the shape and irregular surface morphology. Collapsed particles of protein concentrates are formed due to interaction between rapidly decreasing diffusivity (moisture removal difficulty) and increasing surface tension. Lan et al. studied on pea protein isolate which was prepared by different amorphous matrix carriers and different drying methods. Same result was observed in that study (2019). Similar effect of drying method on rice protein isolate was also observed in the study of Zhao et al. (2013).

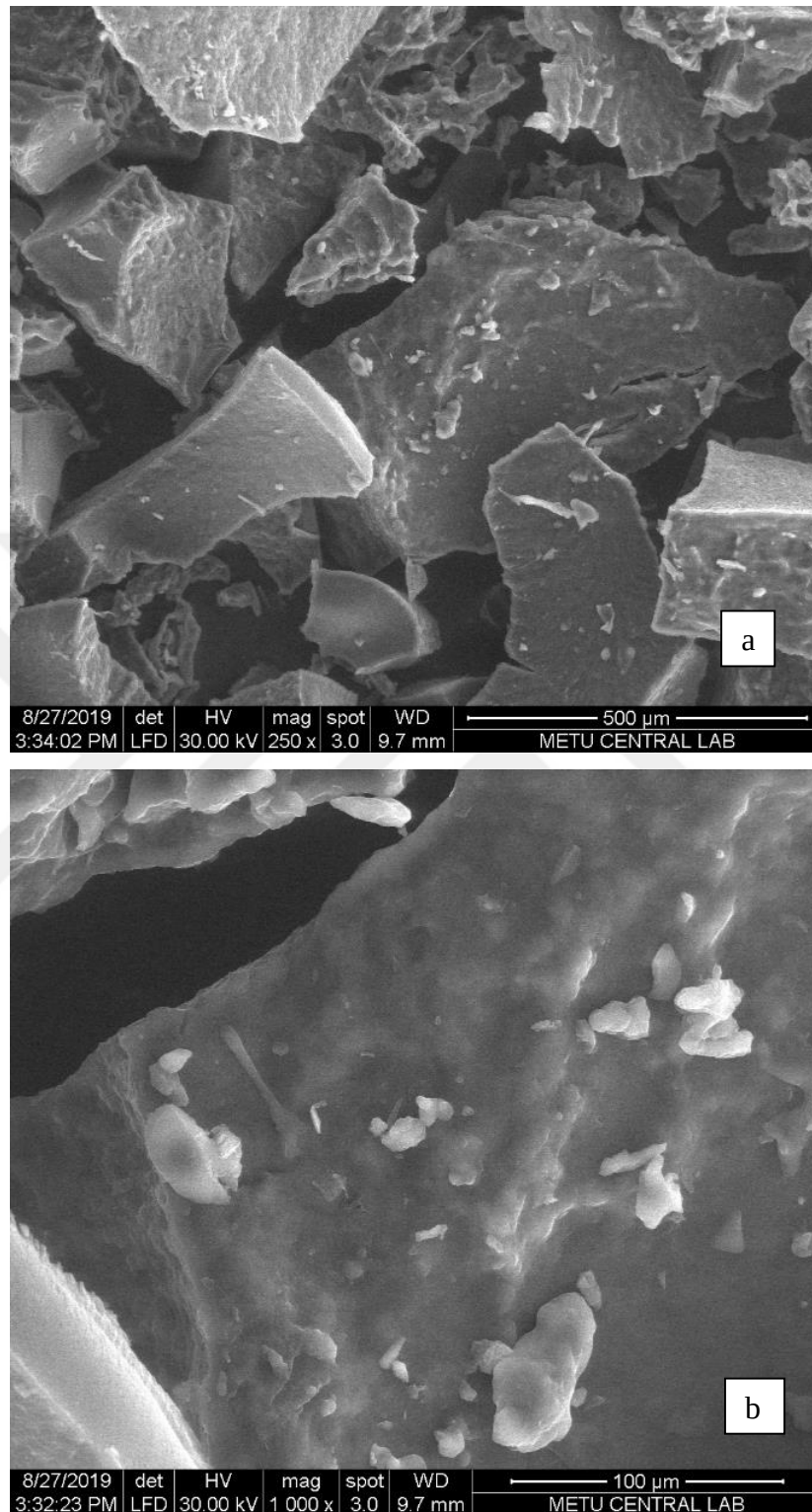


Figure 3.8. Scanning electron microscope images of LPC Magnification: 250 $\times$ (a) and 1,000 $\times$ (b)

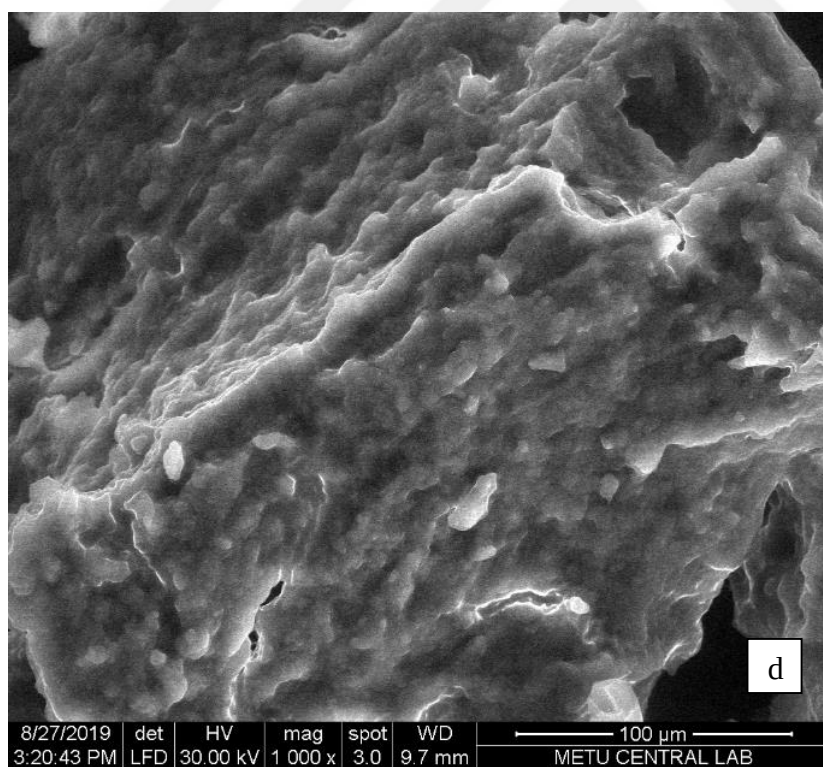
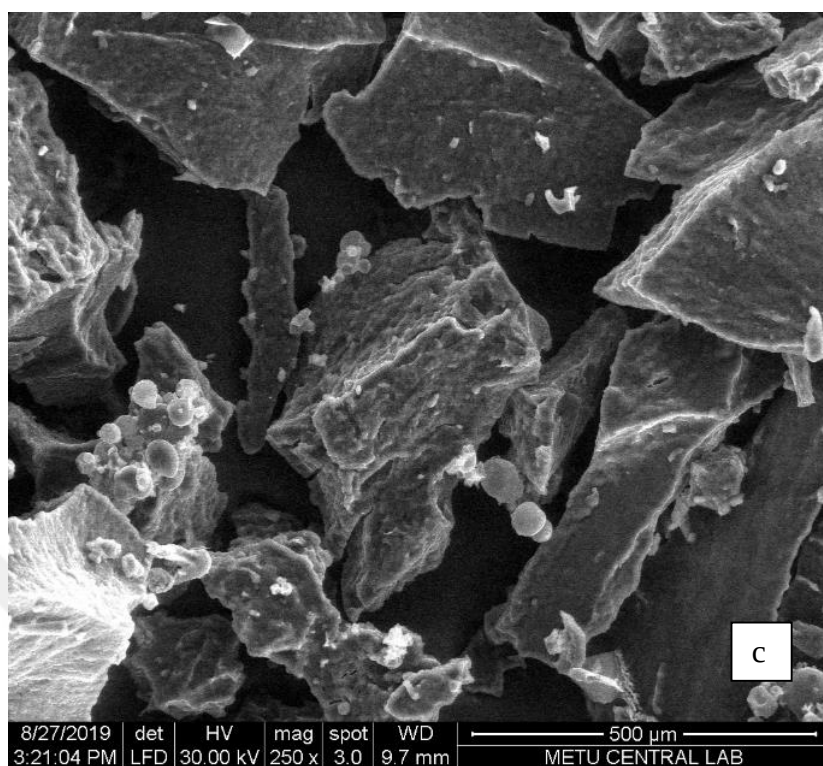


Figure 3.9. Scanning electron microscope images of CPC Magnification: 250 $\times$ (c) and 1,000 $\times$ (d)

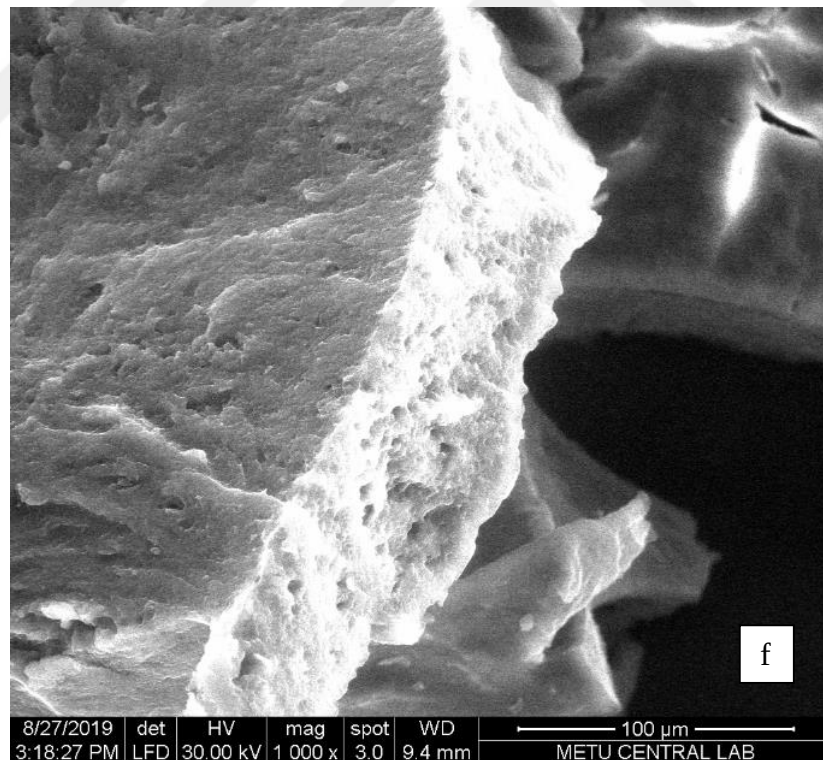
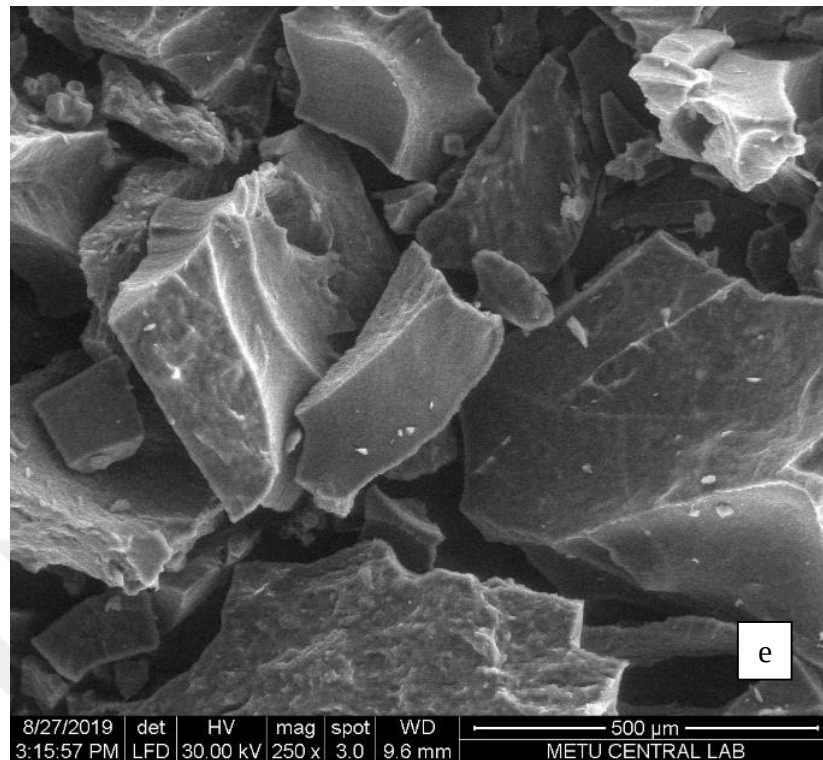


Figure 3.10. Scanning electron microscope images of MPC Magnification: 250× (e) and 1,000× (f)

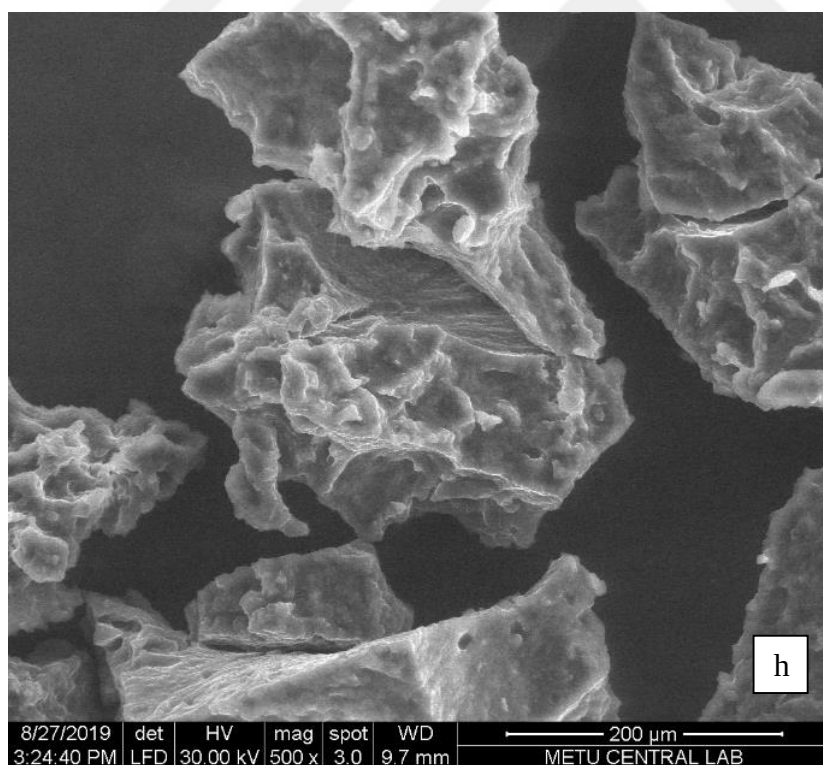
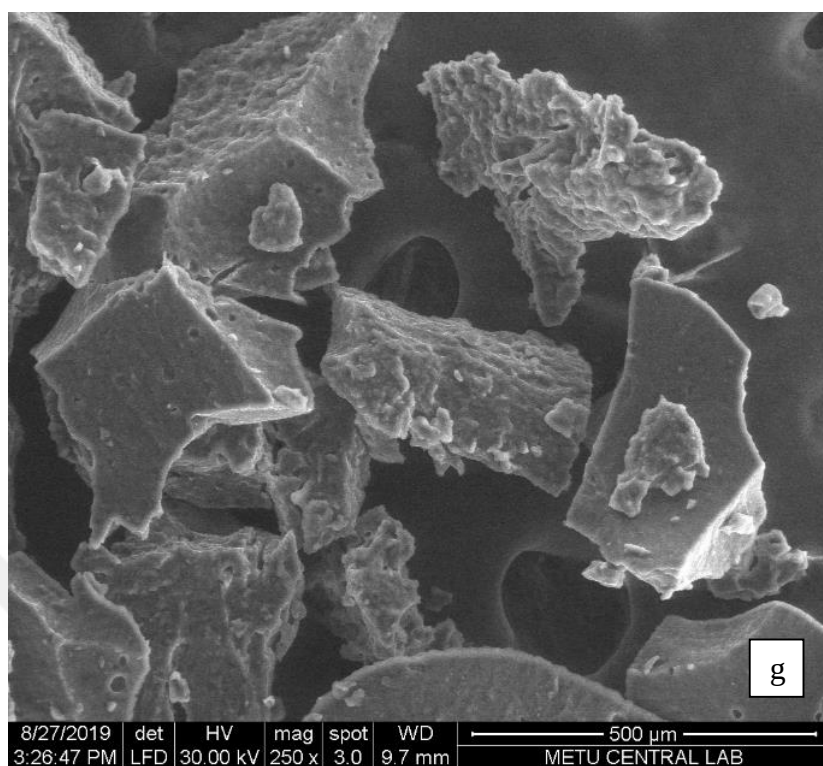


Figure 3.11. Scanning electron microscope images of QPC Magnification: 250× (g) and 500× (h)

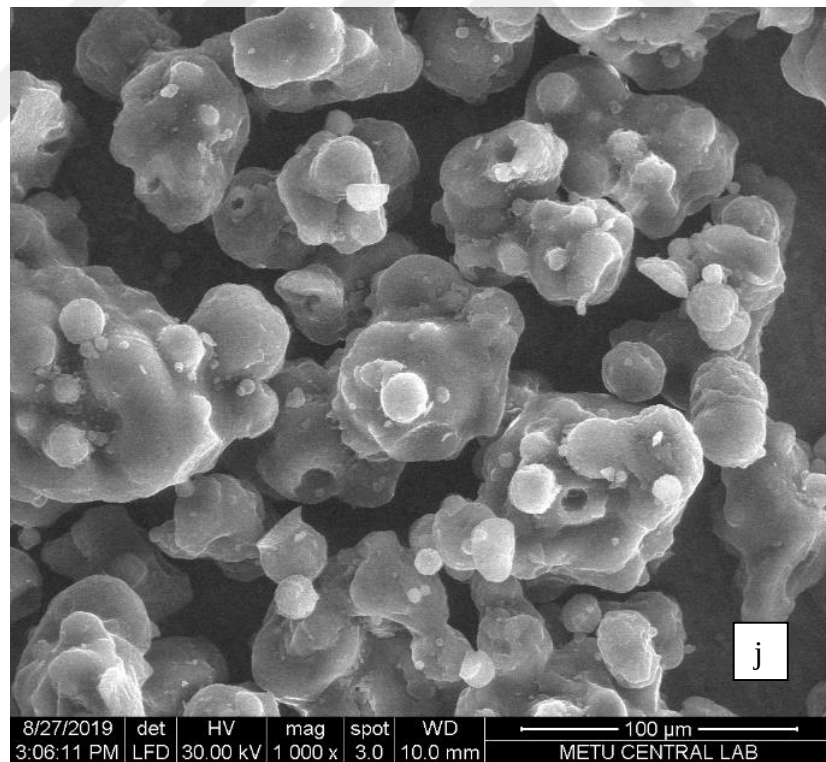
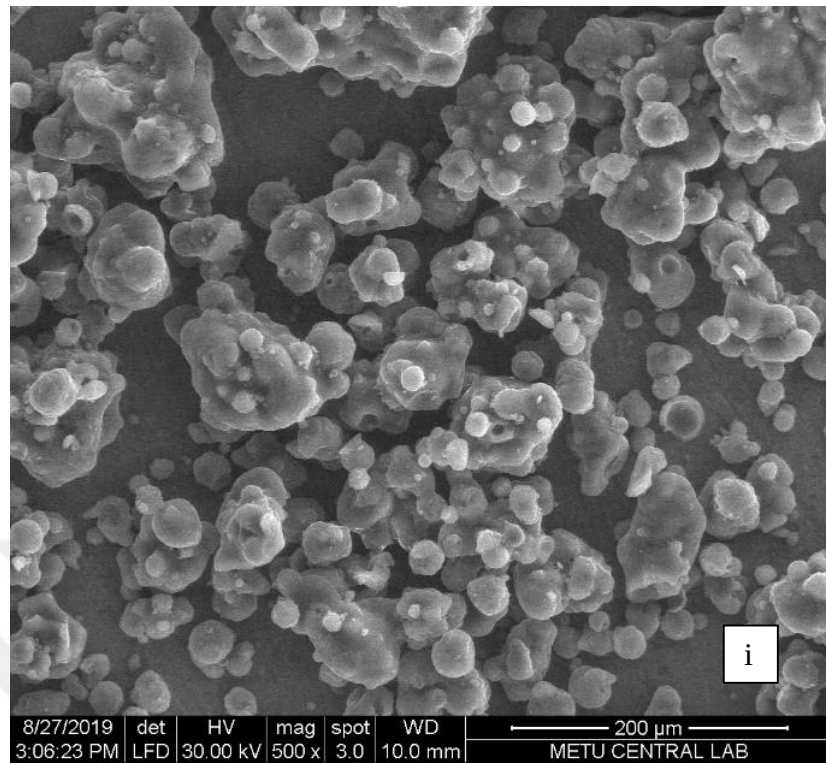


Figure 3.12. Scanning electron microscope images of PPC Magnification: 500× (i) and 1,000× (j)

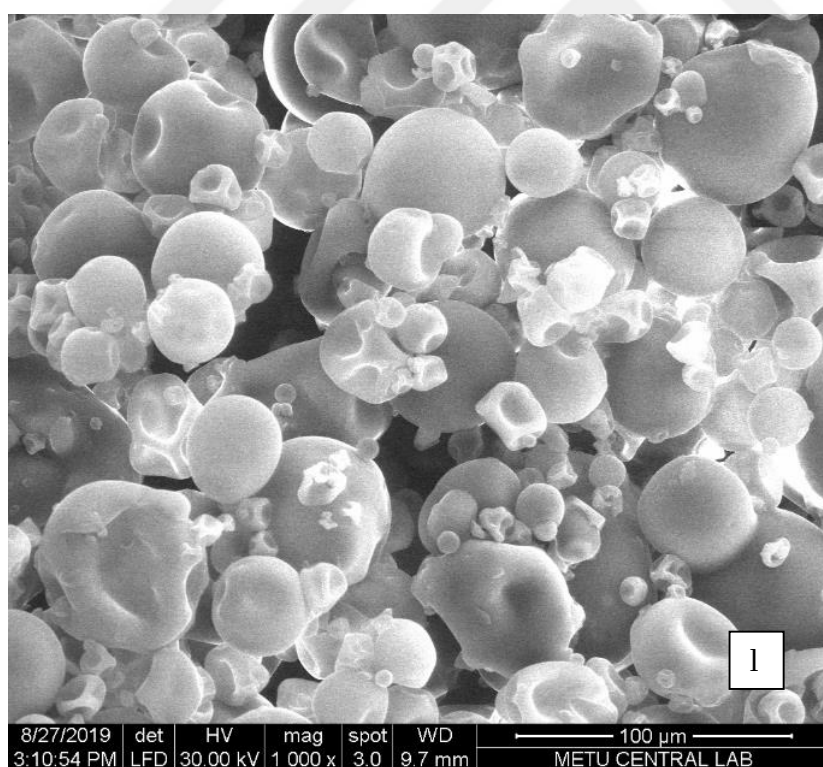
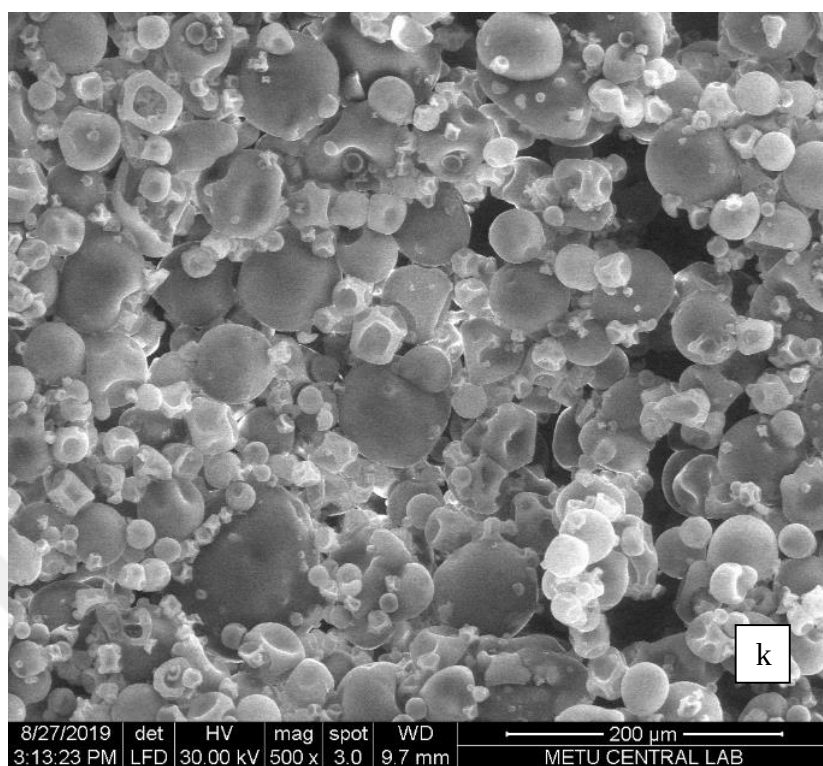


Figure 3.13. Scanning electron microscope images of SPC Magnification: 500× (k) and 1,000× (l)

3.2 Analysis of Enteral Nutrition

3.2.1 Particle Size Analysis

Particle size is considered as one of the important physical quality parameters of food materials. Critical attributes of final product such as stability changes as a result of particle size reduction. Homogenization pressure and time are the critical parameters for particle size reduction.

Volume based mean diameter $D[4,3]$ results of six different protein concentrates were given in Figure 3.14 as μm . MPC with sunflower oil has the smallest particle size distribution with $2.551 \pm 0.149 \mu\text{m}$ and LPC with corn oil has the largest particle size distribution with $9.085 \pm 0.169 \mu\text{m}$.

Significant difference between enteral products which were prepared with corn oil and sunflower oil was observed. The particle size of enteral products which were prepared with corn oil is larger than those which were prepared with sunflower oil. Özogul et al. compared nanoemulsion which were prepared with commercial oils. Same trend in particle size was found in that study (2016). The reason may be free fatty acid composition of these oils. Sunflower oil has higher linoleic acid (18:2) concentration than corn oil whereas corn oil has higher oleic acid (18:1) concentration than sunflower oil. Therefore, sunflower oil has more double bond structure and fatty acids cannot be stacked closely due to these structures (Kim et al., 2010).

Particle size of enteral products which were prepared with LPC is larger than the all the other enteral products. The reason of this may be gelation characteristics of LPC. Least gelation concentration is the determinant parameter for gelation at the same

pH values. According to literature studies, LPC has the lowest gelation concentration among all protein concentrates used in this study (Brishti et al., 2017; Fernández-Quintela et al., 1997; M. Joshi et al., 2011; Maninder Kaur & Singh, 2007; Ogungbenle, 2003). Especially with the increasing pressure, this characteristic leads to aggregation of LPC (Primožic et al., 2018; Saricaoglu, 2020).

Mean particle size of commercial sample was also measured as a control (Figure 3.15 and 3.16) and it was expected that the largest particle size was obtained from commercial samples since microfluidization is more effective process than conventional homogenization in terms of particle size reduction (Mao et al., 2010). However, the particle size of commercial samples was $7.081 \pm 0.108 \mu\text{m}$ and was not the largest one. Although processing parameters of commercial sample have not been shared by supplier, large scale high pressure homogenizers might be used for particle size reduction to obtain more stable products (Bi et al., 2020; Warncke & Kulozik, 2020).

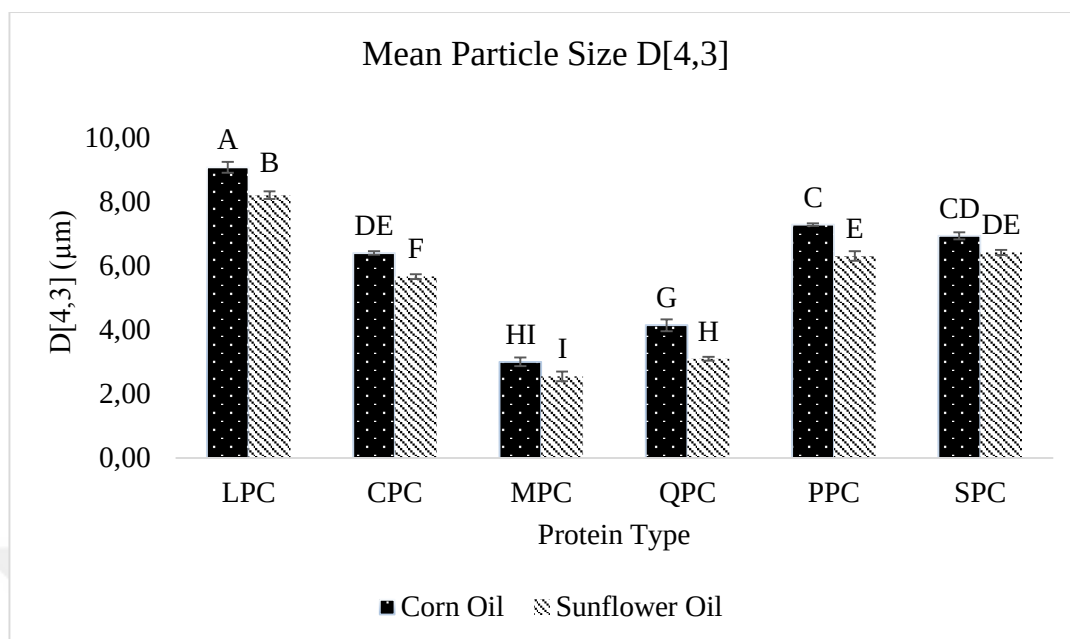


Figure 3.14. Mean particle sizes (µm) of enteral formulations prepared with corn oil and sunflower oil

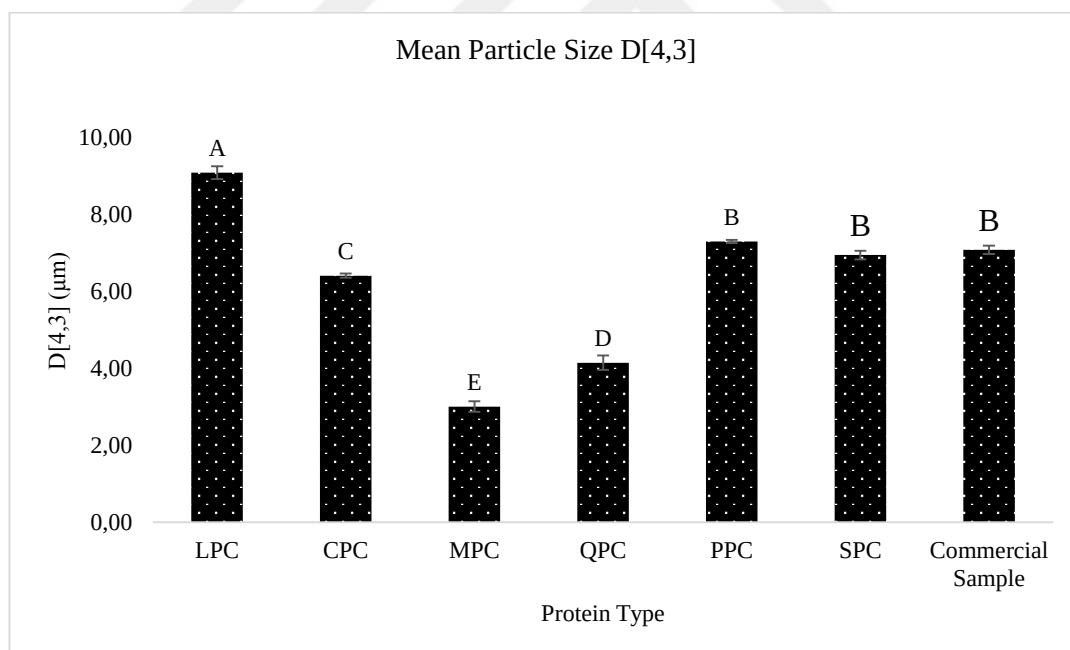


Figure 3.15. Mean particle sizes (µm) of commercial sample and enteral products which were prepared with corn oil

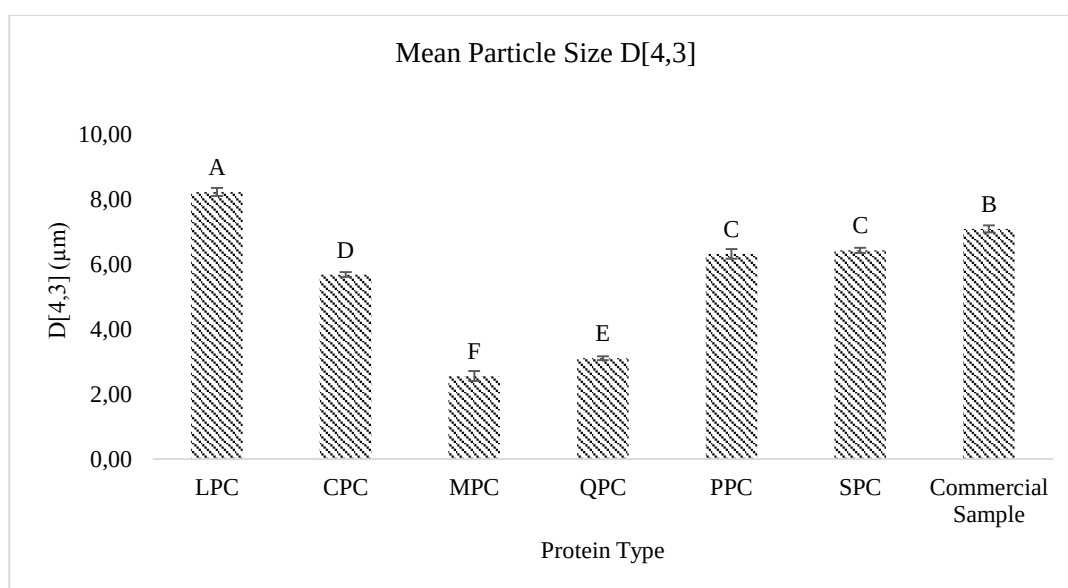


Figure 3.16. Mean particle sizes (μm) of commercial sample and enteral products which were prepared with sunflower oil

3.2.2 Creaming Index

Stability is one of the important quality parameters of enteral products. Phase separation should not be observed at the end of the shelf life. In order to evaluate stability, these products were examined carefully but significant phase separation was not observed for three months. Therefore, accelerated stability test (creaming index) was performed to evaluate stability of enteral formulations which is practical method used in food industry to predict long-term stability (McClements, 2007).

Preliminary study was conducted for different gravitational forces and time combinations. Phase separation was observed at 6000×g for 20 minutes centrifugation. In order to evaluate creaming index, rectangular tubes (Figure 3.17) were used so 2D image processing was applied by using Adobe Photoshop CS (2019). Application of this method enabled to obtain more accurate and precise stability results. Similar 2D image processing method was applied by Krebs et al. to investigate coalescence kinetics of emulsions (2013). Creaming index were

calculated as the area of cream layer versus the area of all enteral product (including cream layer).

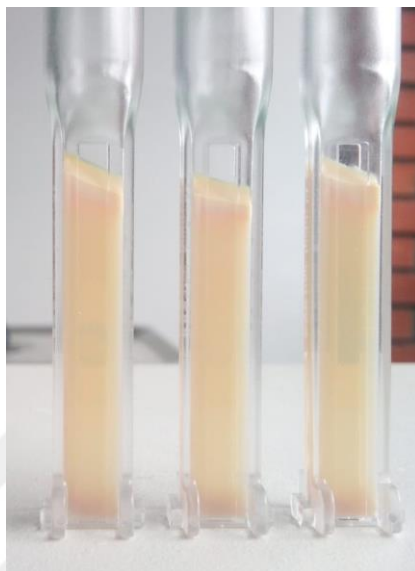


Figure 3.17. Rectangular tubes after centrifugation

Twelve different enteral formulations were compared with each other and the results were demonstrated at Figure 3.18. Lower creaming index means higher stability. The lowest creaming index was found as 9.381 ± 0.098 % (LPC with sunflower oil). On the other hand, the highest creaming index was found as 12.318 ± 0.150 % (PPC with corn oil).

Significant phase separation was not observed for three months (long-term stability test). The reason of this result is the application of microfluidizer. The use of microfluidizer has reduced the particle size which leads to obtain more stable enteral products. The effect of microfluidization on particle size reduction and stability have been studied by many researchers and same results were reported (Bai et al., 2019; Bucci et al., 2018; He et al., 2021; Mert, 2012).

Although significant difference between creaming indexes of enteral products which were prepared with corn oil and sunflower oil was not observed, enteral products which were prepared with sunflower oil always showed more stable behavior. Enteral products which were prepared with sunflower oil also have smaller particle size distribution. More stable enteral products may be obtained due to these particle size differences.

Although the enteral products which were prepared with LPC have the largest particle size distribution, these enteral products have the lowest creaming index (the highest stability) with significant difference ($p > 0.05$). This finding can be explained by two reasons. The first reason is the emulsion stability index of this plant-based protein concentrate since emulsion stability is an ability to preserve the structure of the emulsion against changes. Emulsion stability index (ESI) of proteins has been studied by many researchers. Karaca et al. studied emulsifying properties of legume proteins and they found that the ESI 86.79 ± 4.14 (min) for lentil protein, 82.94 ± 3.18 (min) for chickpea protein (2011). The second reason may be the aggregated protein structures in enteral products which were prepared with LPC. This aggregated protein structures may entrap oil droplets (Primožic et al., 2017). Therefore, more stable enteral products can be obtained.

It was stated that high creaming index means high phase separation and low stability in this study. All enteral formulations were compared with commercial sample and results were demonstrated at Figure 3.19 (enteral products with corn oil) and 3.20 (enteral products with sunflower oil). It was found that the commercial sample's stability was the highest with 12.663 ± 0.199 % creaming index. There was no significant difference between commercial sample and enteral products which were prepared with SPC for both of the oil types. Similar stability of commercial sample and enteral products prepared with SPC was expected since SPC is the one of the major protein sources of industrial products. Enteral products which were prepared

with LPC and MPC showed more stable behavior than commercial sample. Significant difference between these products prepared with both of the oil types and commercial samples was found in terms of stability.

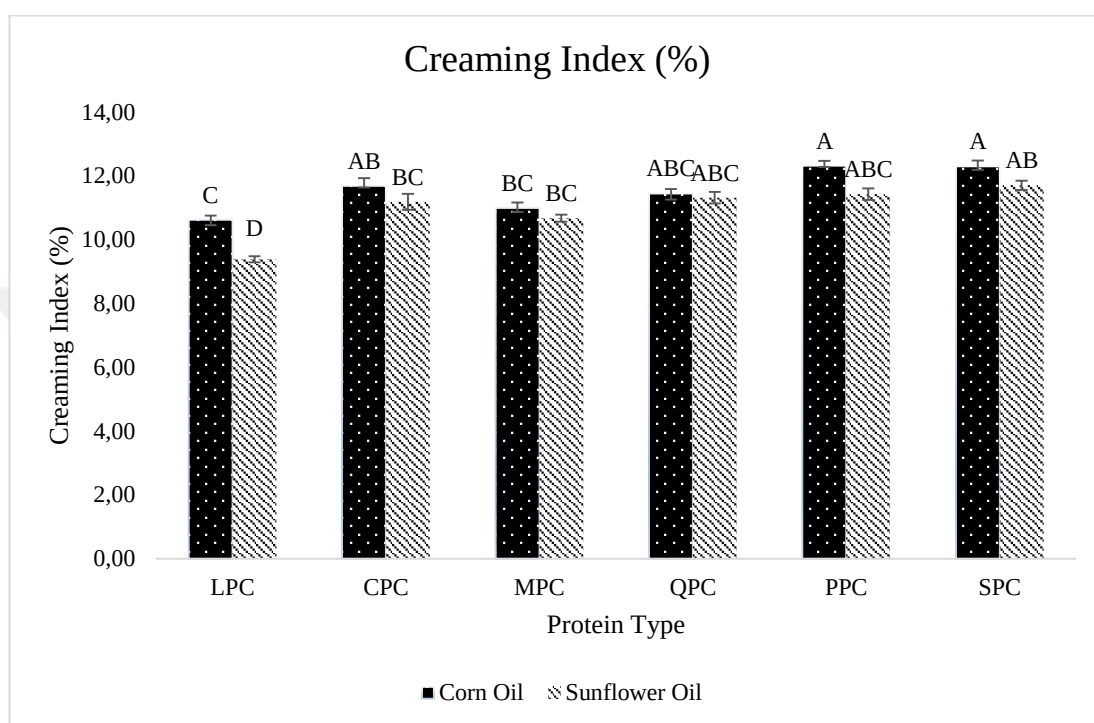


Figure 3.18. Creaming index (%) of enteral formulations prepared with corn oil and sunflower oil

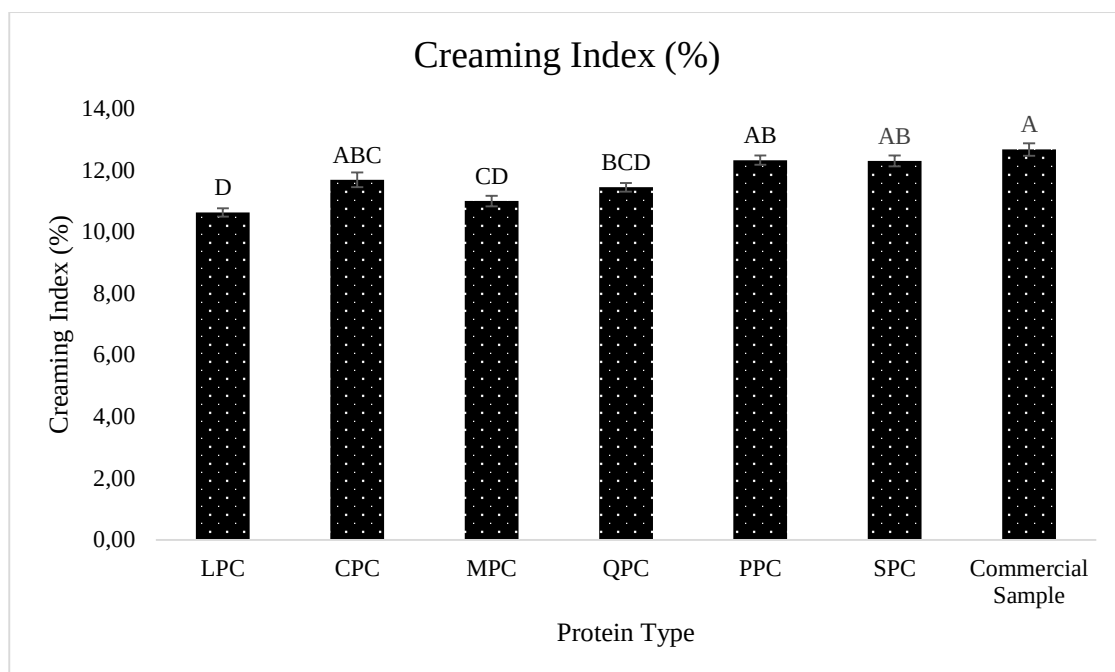


Figure 3.19. Creaming index (%) of commercial sample and enteral products which were prepared with corn oil

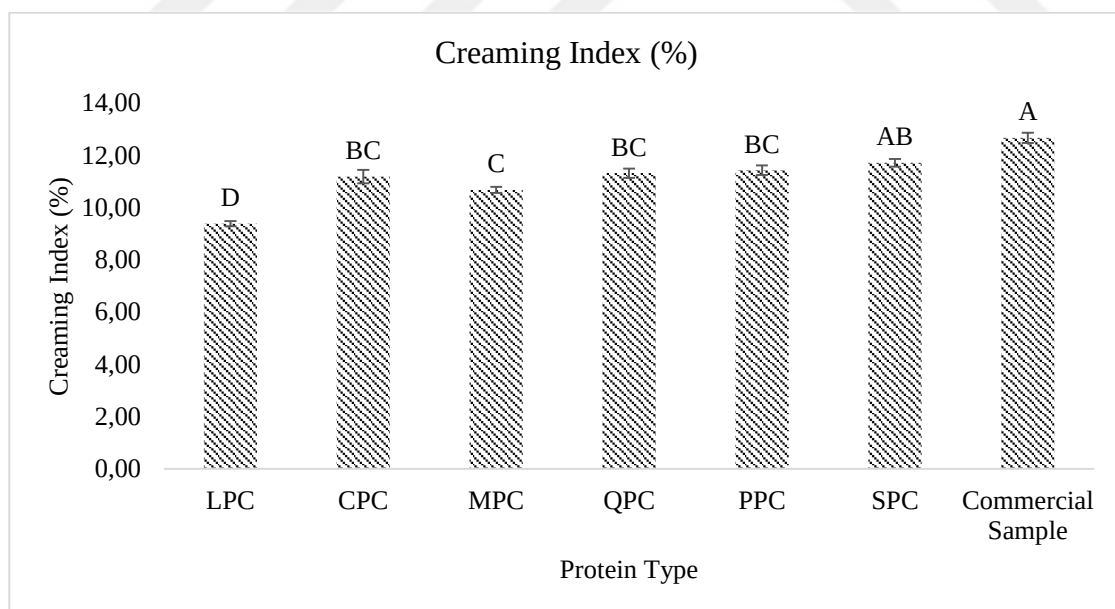


Figure 3.20. Creaming index (%) of commercial sample and enteral products which were prepared with sunflower oil

3.2.3 Rheology Analysis

Rheological properties of enteral nutrition formulations were discussed as analysis of flow behaviors. Enteral products which were prepared by using different plant-based proteins were compared commercial formulation to understand effect of different protein sources on rheological behavior of enteral products. Since enteral products are applied with feeding tubes to the patients, flow inside the tube through body, rheological experiments were carried out at two different temperatures which are 25 °C (storage – outside temperature) and 37 °C (human body temperature).

In this study, ready-to-hang commercial sample was selected as a reference sample. Enteral products were designed to be application of feeding tubes. Therefore, Newtonian behavior and Power-Law distribution were expected. The shear stress (τ) versus shear rate ($\dot{\gamma}$) data of all enteral products which were prepared with LPC were fitted well to Power Law Model. The shear stress (τ) versus shear rate ($\dot{\gamma}$) data were fitted well to Newtonian Model for the rest of the enteral products including commercial samples. Power Law (Equation 3.1) and Newtonian (Equation 3.2) models are given below.

$$\tau = K(\dot{\gamma})^n \quad \text{Equation 3.1}$$

$$\tau = \eta \dot{\gamma} \quad \text{Equation 3.2}$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($Pa.s^n$), η is shear viscosity (Pa.s) and n is flow behavior index.

The Power Law parameters of enteral products which were prepared with LPC were represented in Table 3.8. Shear viscosity values (Pa.s) of enteral products which showed Newtonian behavior were represented in Table 3.9.

Table 3.8 Power Law Parameters for enteral products prepared with LPC

Protein Type	Oil Type	Temperature (°C)	K (Pa.s ⁿ)	n
LPC	Corn Oil	25°C	0,411	0,526
LPC	Corn Oil	37°C	0,263	0,553
LPC	Sunflower Oil	25°C	0,202	0,636
LPC	Sunflower Oil	37°C	0,128	0,662

Table 3.9 Shear Viscosity (Pa.s) values of enteral products prepared with CPC, MPC, QPC, PPC and SPC with corn oil and sunflower oil.

Protein Type	Oil Type	Temperature (°C)	Average Shear Viscosity (Pa.s)
CPC	Corn Oil	25°C	0,01040 ^A
CPC	Corn Oil	37°C	0,00786 ^{DEFG}
CPC	Sunflower Oil	25°C	0,00991 ^{AB}
CPC	Sunflower Oil	37°C	0,00757 ^{EFG}
MPC	Corn Oil	25°C	0,00841 ^{DE}
MPC	Corn Oil	37°C	0,00644 ^{IJ}
MPC	Sunflower Oil	25°C	0,00832 ^{DEF}
MPC	Sunflower Oil	37°C	0,00640 ^J
QPC	Corn Oil	25°C	0,00941 ^{BC}
QPC	Corn Oil	37°C	0,00743 ^{FGH}
QPC	Sunflower Oil	25°C	0,00937 ^{BC}
QPC	Sunflower Oil	37°C	0,00737 ^{GHI}
PPC	Corn Oil	25°C	0,00868 ^{CD}
PPC	Corn Oil	37°C	0,00654 ^{HJ}
PPC	Sunflower Oil	25°C	0,00809 ^{DEFG}
PPC	Sunflower Oil	37°C	0,00623 ^J
SPC	Corn Oil	25°C	0,00784 ^{DEFG}
SPC	Corn Oil	37°C	0,00607 ^J
SPC	Sunflower Oil	25°C	0,00779 ^{DEFG}
SPC	Sunflower Oil	37°C	0,00603 ^J

Shear stress (τ) versus shear rate ($\dot{\gamma}$) graphs of all enteral products including commercial sample for 25 °C and 37 °C were represented in Figures 3.21, 3.22, 3.23, 3.24, 3.25, 3.26 and 3.27.

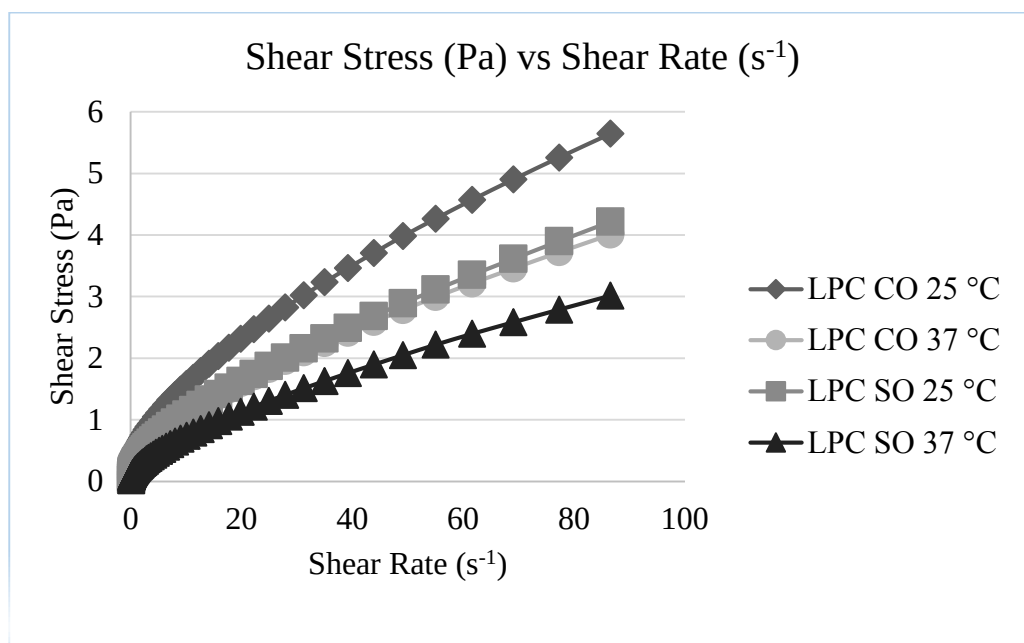


Figure 3.21. Shear stress (Pa) vs shear rate (s⁻¹) graph of enteral products for 25 °C and 37 °C which was prepared with LPC, CO and SO

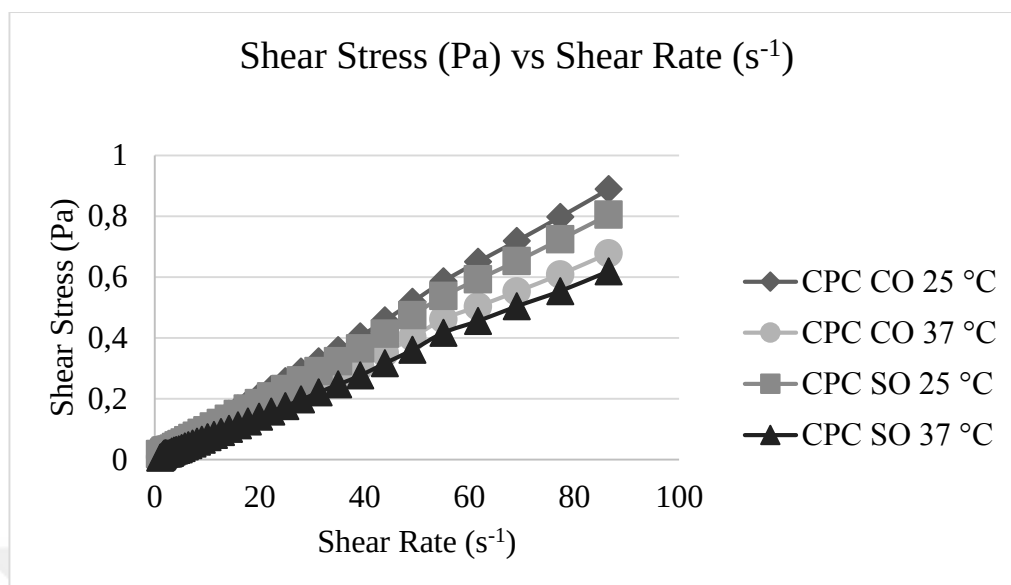


Figure 3.22. Shear stress (Pa) vs shear rate (s^{-1}) graph of enteral products for 25 °C and 37 °C which was prepared with CPC, CO and SO

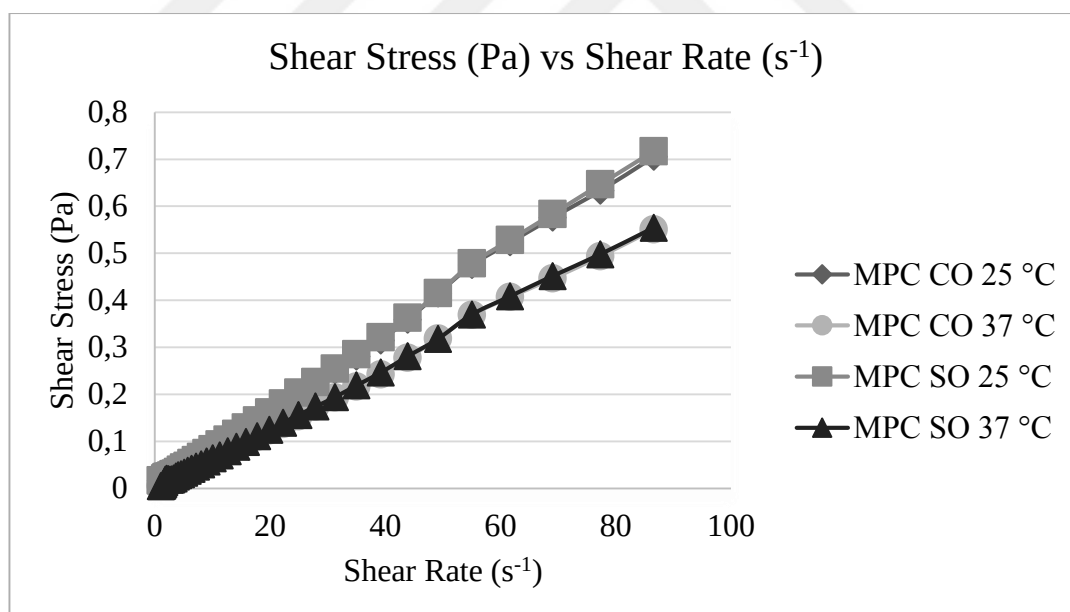


Figure 3.23. Shear stress (Pa) vs shear rate (s^{-1}) graph of enteral products for 25 °C and 37 °C which was prepared with MPC, CO and SO

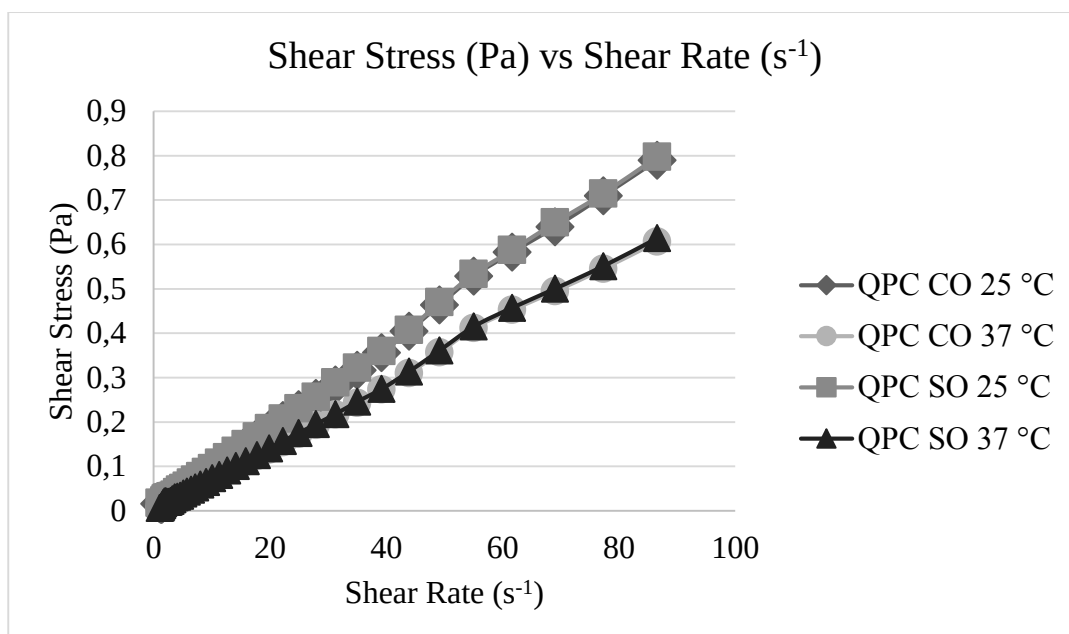


Figure 3.24. Shear stress (Pa) vs shear rate (s⁻¹) graph of enteral products for 25 °C and 37 °C which was prepared with QPC, CO and SO

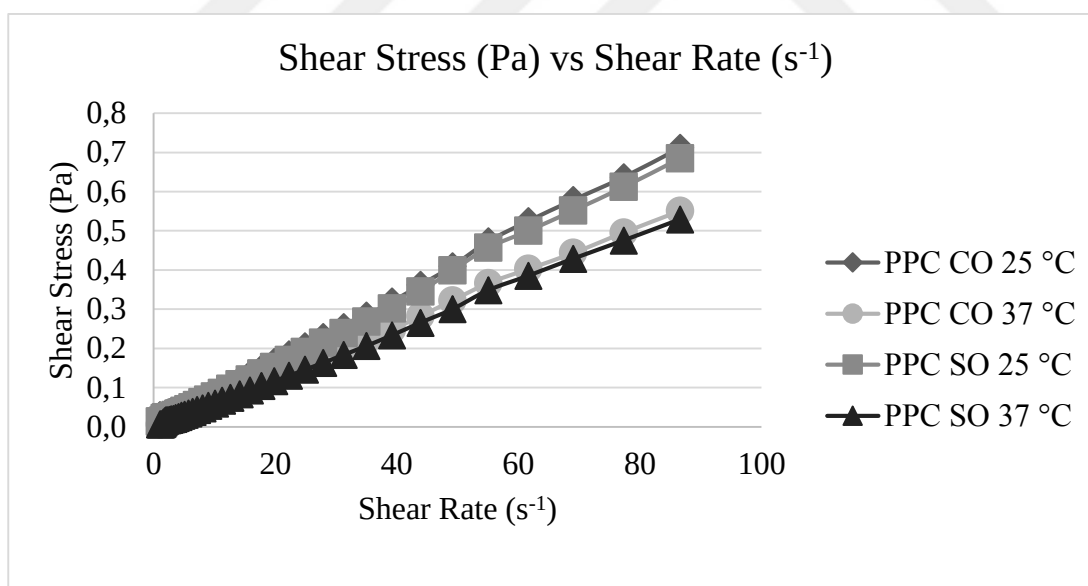


Figure 3.25. Shear stress (Pa) vs shear rate (s⁻¹) graph of enteral products for 25 °C and 37 °C which was prepared with PPC, CO and SO

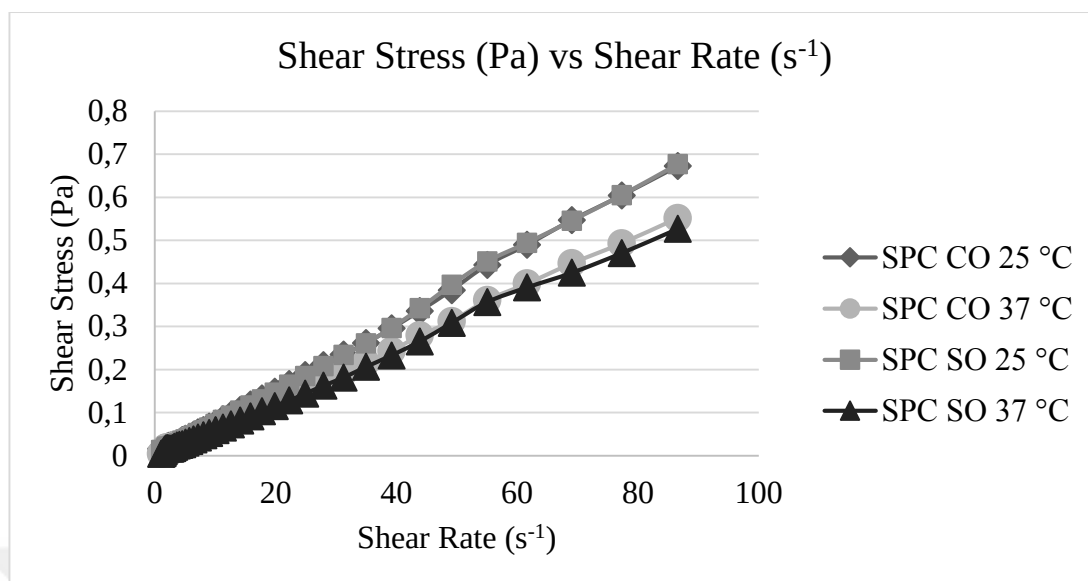


Figure 3.26. Shear stress (Pa) vs shear rate (s⁻¹) graph of enteral products for 25 °C and 37 °C which was prepared with SPC, CO and SO.

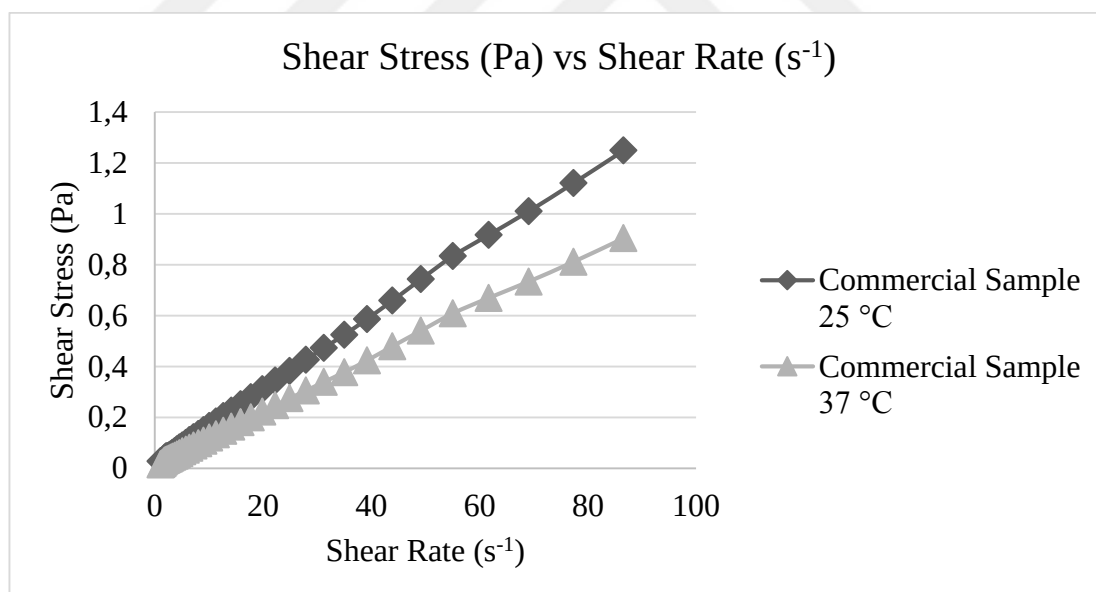


Figure 3.27. Shear stress (Pa) vs shear rate (s⁻¹) graph of commercial enteral products for 25 °C and 37 °C.

According to shear stress (Pa) vs shear rate (s^{-1}) graphs, Table 3.9 and statistical data, it can be found out that there is significant difference between shear viscosities of enteral products which were prepared with corn oil and sunflower oil ($p>0.05$). The reason of this difference may be the differences between fatty acid composition of corn oil and sunflower oil. Kim et al. conducted a research about the effect of fatty acid composition on rheological behavior. It was found that the ratio of major components of fatty acids which are 18:2 (linoleic acid) and 18:1 (oleic acid) have important impact on the flow behaviors (2010). The increase in the portion of linoleic acid and the decrease in the portion of oleic acid cause more double bond structure in the environment and decrease in viscosity. Fatty acid molecules cannot be stacked closely and crystalline state packing cannot be formed due to the presence of double bond since a kink in the straight chain is formed by each double bond which has cis configuration (Abramovič & Klofutar, 1998). The amount of linoleic acid (18:2) is high in sunflower oil while the amount of oleic acid (18:1) is high in corn oil which cause decrease in viscosity of sunflower oil and increase in viscosity of corn oil (Kim et al., 2010). In another study, it was stated that higher viscosity was obtained with increasing saturated and monounsaturated fatty acid composition and decreasing polyunsaturated fatty acid composition. Corn oil has higher saturated (especially high amount of palmitic acid) and lower polyunsaturated fatty acid composition than sunflower oil (Yalcin et al., 2012). Therefore, corn oil has higher shear viscosity than sunflower oil. Enteral products are complex systems which are contributed by many components and oil is one of them. This study and literature studies show that oil type and fatty acid composition may affect the shear viscosity of enteral product.

All enteral products which were prepared with LPC showed Power Law distribution. On the other hand, rest of the enteral products including commercial sample showed Newtonian distribution. The reason of this may be gelation capacities of these plant-based proteins. Gelation capacities of these protein concentrates can be compared by using least gelation concentrations. Different researchers have studied on functional and gelation properties of these proteins. In those studies least gelation

concentrations were found as 11% for lentil protein isolate (Joshi et al., 2011), 14%-18% for different cultivars of chickpea protein isolates (Kaur & Singh, 2007), 12% for mung bean protein isolate (Brishti et al., 2017), 16% for quinoa flour (Ogunbenle, 2003), 18% for pea protein isolate and 16% for soy protein isolate (Fernández-Quintela et al., 1997). These literature studies show that lentil protein has higher gelation capacity for the same pH (lower gelation concentration means higher gelation capacity). Although same amount of protein concentrates were used in enteral products, only LPC gave higher viscosity to these products.

Temperature is one of the key parameters for the rheology of emulsions (Zhu et al., 2020). The significant difference between shear viscosity values (Pa.s) of enteral products at 25°C and at 37°C was observed. According to results, shear viscosity values of all enteral product at 37°C were lower than the shear viscosity values of the same products at 25°C. When temperature is increased, thermal movement among the molecules also increases and reduction of intermolecular bond forces is observed. This makes flux between molecules easier and causes decrease in viscosity (Fujiu et al., 2011; Santos et al., 2004) .

Commercial samples' shear viscosity values were also compared with other enteral products' shear viscosity values and results were demonstrated at Table 3.10. Enteral products which were prepared with corn oil were used for this comparison since their shear viscosity values higher than the enteral products which were prepared with sunflower oil. However, it was found out that shear viscosity values of commercial samples were higher significantly ($p > 0.05$). The reason for this may be the carrageenan content of commercial sample. Although concentration of carrageenan was not declared by the supplier, it should be low since this concentration was not enough to make commercial sample into Power Law liquid.

Table 3.10 Shear viscosity values (Pa.s) of commercial samples and enteral products which were prepared with corn oil.

Protein Type	Temperature	Average Shear Viscosity (Pa.s)
Commercial Sample	25	0,01532 ^A
Commercial Sample	37	0,01105 ^B
CPC	25	0,01040 ^B
CPC	37	0,00786 ^{DE}
MPC	25	0,00841 ^D
MPC	37	0,00644 ^F
QPC	25	0,00941 ^C
QPC	37	0,00743 ^E
PPC	25	0,00868 ^{CD}
PPC	37	0,00654 ^F
SPC	25	0,00784 ^{DE}
SPC	37	0,00607 ^F

Both of the commercial sample and enteral products which prepared with plant-based proteins other than LPC are not suitable to consume by dysphagia patients since they cannot consume Newtonian liquids. The reason of this is the risk of quick flow of the Newtonian liquid to larynx even before the entrance of airway has closed which leads to aspiration for these patients (O’Leary et al., 2010). Therefore, they consume pre-thickened formulations with gum-based thickeners to change bolus rheology and to avoid the risk of aspiration (Leonard et al., 2014). The purpose of this study prepare and evaluate enteral nutrition products which are administrated by feeding tubes. As it was stated previously, any type of thickener was not added. However, these enteral products can be consumed by these patients after addition of commercial thickener by a physician (Seo & Yoo, 2013).

3.2.4 Confocal Microscopy Analysis

Confocal laser scanning microscope (CLSM) was used to observe microstructure of enteral products. The protein structures appear in green and fat structures appear in red since proteins and fats were stained by Nile Red and FITC, respectively. Enteral products which were prepared with LPC, SPC and MPC were represented in Figures 3.28, 3.29 and 3.30 respectively.

Production process of enteral products includes high pressure and high temperature systems. The application of microfluidizer generates high pressure (100 MPa) and high shear. This pressure and shear make particles finer (Mao et al., 2010). Any contradiction between CLSM images and particle size results was not observed.

High temperature (121.1 °C) was applied for 15 minutes to sterilize enteral products. This treatment needed to make sure that to obtain enteral products which are microbiologically safe and stable. Similar study was conducted by Li et al. to investigate the effects of high temperature (120 °C) and high pressure (30 MPa) on soy protein isolate emulsion with corn oil. In that study, it was found that at higher temperature more stable emulsion was obtained since aggregated state and surface hydrophobicity/hydrophilicity was balanced due to this heat treatment. Similar CLSM images were obtained in this study (2020). Another reason of having more stable products may be explained as the inhibition of flocculation due to weakened short-range interactions by heat-induced aggregation (Dickinson, 2010). Silva et al. conducted another research by using pea protein isolate with heat treatment (90 °C). However, in that study gelation of pea protein isolate was observed since emulsion was containing 12 % (w/w) pea protein isolate (2019).

Aggregated protein structures of enteral product which was prepared with LPC was demonstrated in Figure 3.28. Sizes of these aggregates were measured as 10 μm . This image supports the particle size data of LPC and the higher stability of enteral products with LPC.

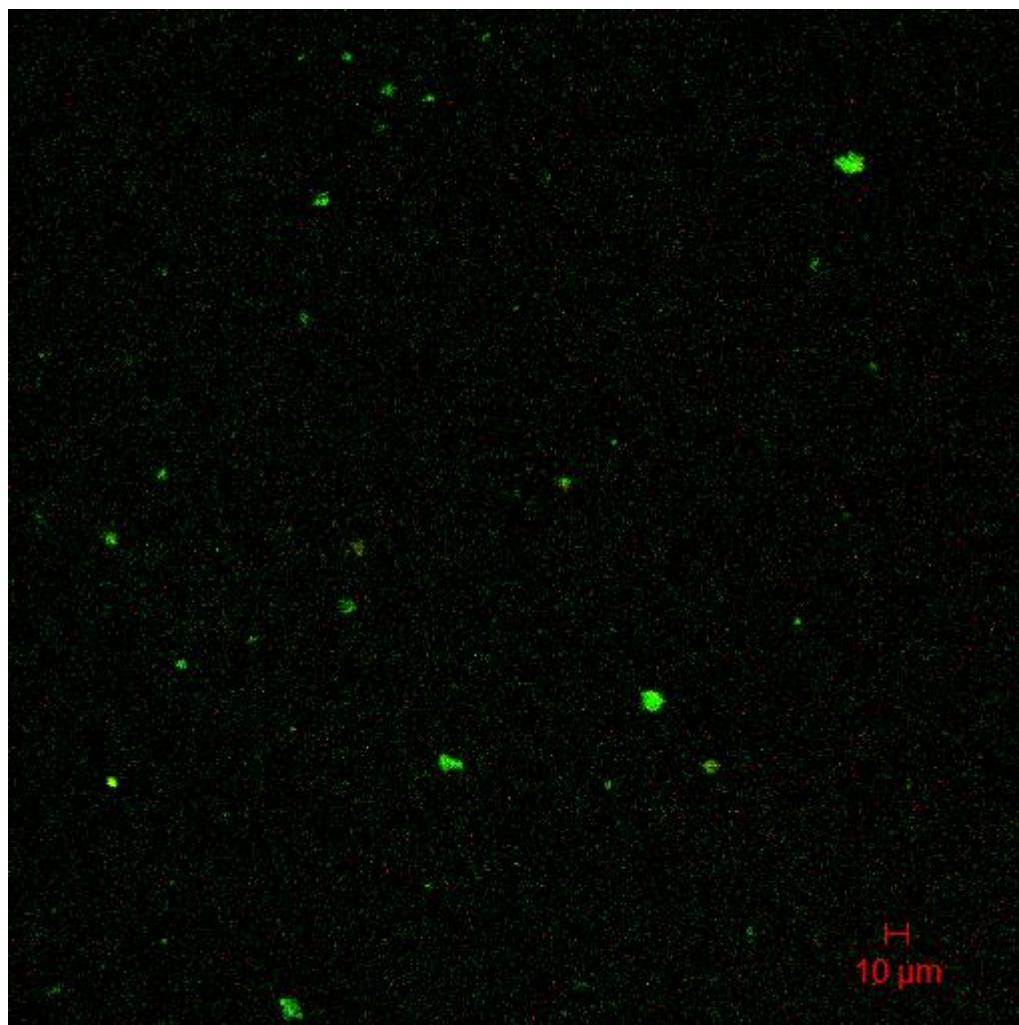


Figure 3.28. Confocal laser scanning microscope image of enteral formulation prepared with LPC and corn oil.

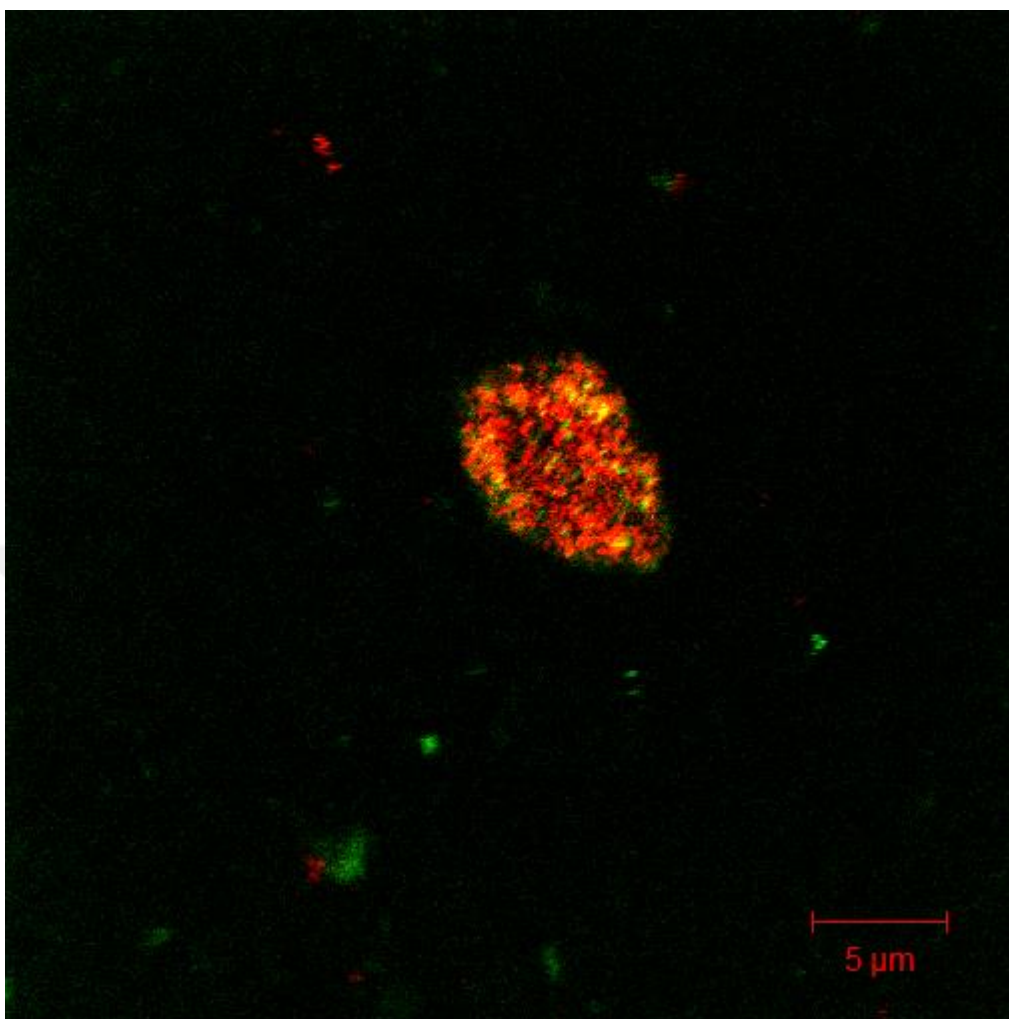


Figure 3.29. Confocal laser scanning microscope image of enteral formulation prepared with SPC and corn oil.

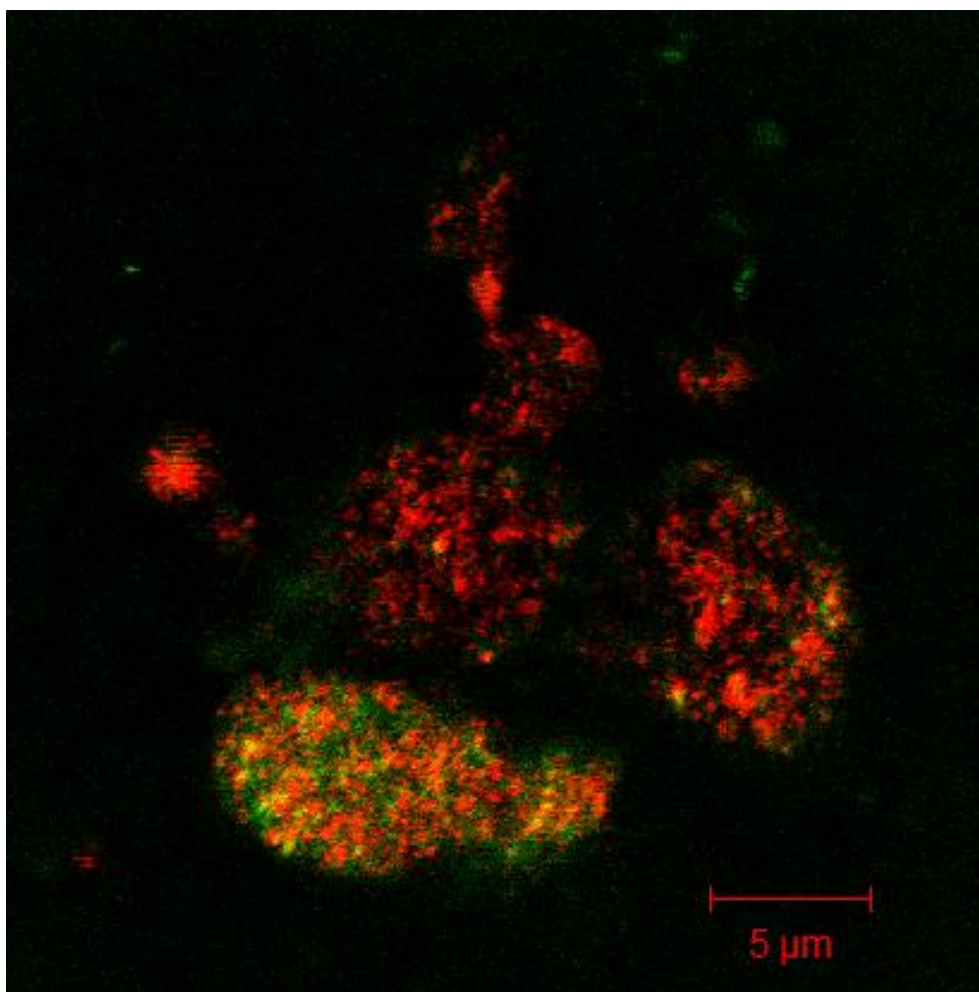


Figure 3.30. Confocal laser scanning microscope image of emulsion formulation prepared with MPC and corn oil.

3.2.5 pH Analysis

In this study, pH value of all enteral formulations which were prepared with different protein sources were measured. Lowest pH value of these formulations is 6.76 (QPC) and highest pH value is 7.08 (SPC). All of the samples were compared with control sample which is commercial enteral nutrition product whose pH value was measured as 7.01. There was no significant difference between commercial product and enteral formulations which were prepared with MPC, PPC and SPC.

pH values of enteral nutrition formulation are important since these formulations are consumed by patients who are suffering from critical diseases. It should not be lower than 4.6 since most of the proteins coagulates (isoelectric point) at that pH which will obstruct the feeding tube. Henriques et al. studied on pH values of enteral products which were prepared in the hospital. pH values of six different formulations ranged between 6.1 and 6.6. In that study these products classified as low acidic (2017).

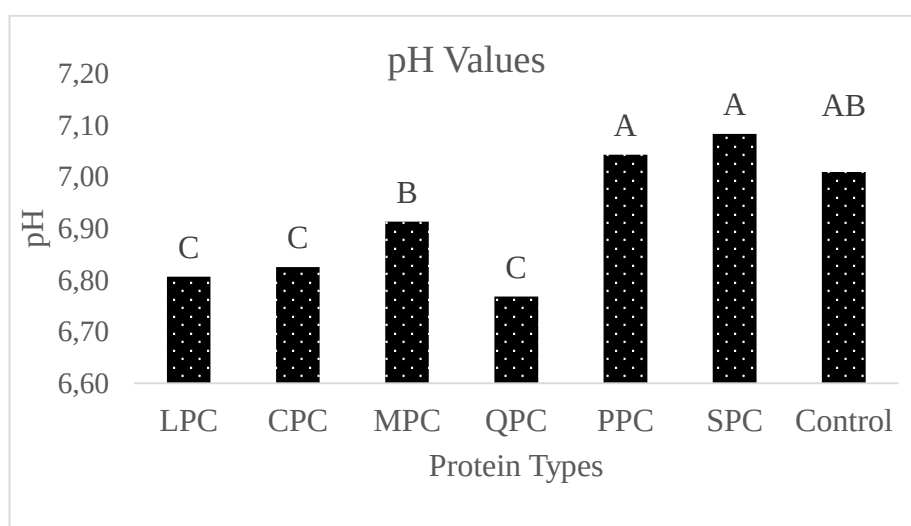


Figure 3.31. pH values of commercial enteral formulation and enteral formulations prepared with LPC, CPC, MPC, QPC, PPC and SPC

Oil type did not affect the pH value significantly for the same protein type. Significant difference was not observed. No pH increasing or decreasing factor was observed between corn oil and sunflower oil in prepared enteral nutrition products.

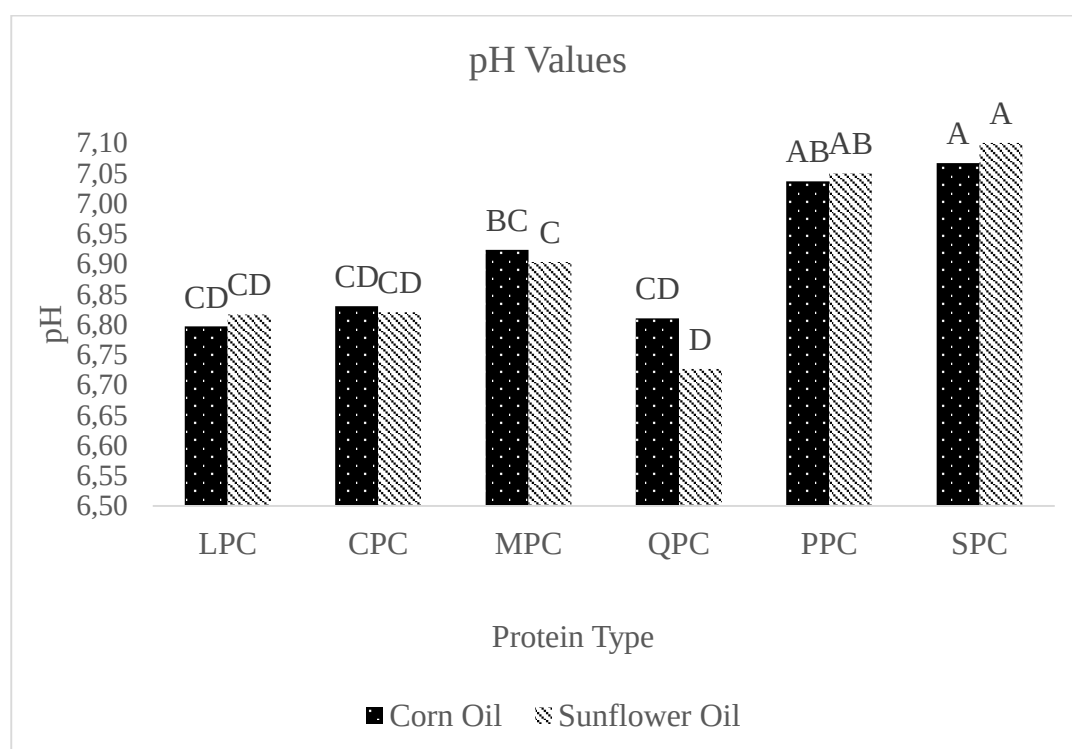


Figure 3.32. pH values of enteral formulations prepared with LPC, CPC, MPC, QPC, PPC and SPC with corn oil and sunflower oil

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In the first part of this study, four different proteins were extracted from lentil, chickpea, mung bean and quinoa. These extracted protein concentrates and two commercial protein concentrates which are pea protein concentrate and soy protein concentrate were characterized. Proximate analysis, amino acid analysis, WHC, OBC and FTIR analysis were carried out. According to these analyses, LPC and MPC showed better characteristics. LPC and MPC also have better nutrition characteristics due to their protein content, amino acid content and essential amino acid content (especially leucine and lysine). Microstructure of these protein concentrates were observed by scanning electron microscopy. Because of different processing types, different micrographs of extracted protein concentrates (freeze-dried) and commercial ones (spray-dried) were observed. In the second part of this study, these protein concentrates were used to formulate different enteral products. Microfluidizer was used for particle size reduction and autoclave was used for sterilization of these products. Enteral products which were prepared with LPC and MPC have better stability. All enteral products except for prepared with LPC showed Newtonian behavior. On the other hand, enteral products which were prepared with LPC have Power Law flow distribution. Enteral products which were prepared with corn oil had higher shear viscosity due to saturated and monounsaturated fatty acid composition of corn oil. Microstructure of these products were observed with confocal laser scanning microscopy (CLSM) but any difference due to protein type or oil type was not observed.

Enteral products market is growing and production volume increase day by day. Different types of products are launched for different diseases and conditions. There is also an effort in the industry to make existing formulations more functional and more sustainable. Plant-based proteins have an important effect in order to make more sustainable production. However, in order to solve the current problems of patients regarding with enteral products and to provide healthier solutions, it is necessary to select the plant-based proteins with the best functional properties and investigate their effects on these products. In this study, five different plant-based proteins were selected and their effect on enteral nutrition products was investigated. In future studies, in vitro research should be carried out, and then the effects on real patients should be observed in clinical trials. Enteral nutrition is a multi-disciplinary field which food engineers, medical doctors and nutritionists should work together. Multi-disciplinary research will be very beneficial in order to obtain more functional and sustainable enteral products.

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APPENDICES

A. Statistical Results

Table A.1 Kjeldahl Analysis ANOVA Results

General Linear Model: Protein Content versus Protein type

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	6	C; L; M; P; Q; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	5	1090,35	218,070	122,22	0,000
Error	12	21,41	1,784		
Total	17	1111,76			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
1,33577	98,07%	97,27%	95,67%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	78,934	0,315	250,71	0,000	
Protein type					
C	-4,349	0,704	-6,18	0,000	1,67
L	9,396	0,704	13,35	0,000	1,67
M	6,886	0,704	9,78	0,000	1,67
P	1,741	0,704	2,47	0,029	1,67
Q	-14,331	0,704	-20,36	0,000	1,67

Comparisons for Protein Content

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
L	3	88,3300	A
M	3	85,8200	A
P	3	80,6750	B
S	3	79,5933	B
C	3	74,5850	C
Q	3	64,6033	D

Means that do not share a letter are significantly different.

Table A.2 Soxhlet Analysis ANOVA Results

General Linear Model: Fat Content versus Protein type**Method**

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	6	C; L; M; P; Q; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	5	883,01	176,603	195,15	0,000
Error	12	10,86	0,905		
Total	17	893,87			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,951282	98,79%	98,28%	97,27%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,178	0,224	27,55	0,000	
Protein type					
C	3,658	0,501	7,30	0,000	1,67
L	-4,558	0,501	-9,09	0,000	1,67
M	-5,325	0,501	-10,62	0,000	1,67
P	-3,889	0,501	-7,76	0,000	1,67
Q	14,155	0,501	28,23	0,000	1,67

Comparisons for Fat Content**Tukey Pairwise Comparisons: Protein type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein type	N	Mean	Grouping
Q	3	20,333	A
C	3	9,836	B
P	3	2,289	C
S	3	2,136	C
L	3	1,620	C
M	3	0,853	C

Means that do not share a letter are significantly different.

Table A.3 Ash Content Analysis ANOVA Results

General Linear Model: Ash Content versus Protein type

Method

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	6	C; L; M; P; Q; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	5	32,703	6,5407	54,24	0,000
Error	12	1,447	0,1206		
Total	17	34,150			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,347246	95,76%	94,00%	90,47%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5,9316	0,0818	72,47	0,000	
Protein type					
C	-0,766	0,183	-4,18	0,001	1,67
L	-1,573	0,183	-8,59	0,000	1,67
M	-0,956	0,183	-5,23	0,000	1,67
P	1,826	0,183	9,98	0,000	1,67
Q	1,856	0,183	10,14	0,000	1,67

Comparisons for Ash Content

Tukey Pairwise Comparisons: Protein type

Grouping Information Using the Tukey Method and 95% Confidence

Protein type	N	Mean	Grouping
Q	3	7,78723	A
P	3	7,75738	A
S	3	5,54488	B
C	3	5,16600	B C
M	3	4,97531	B C
L	3	4,35867	C

Means that do not share a letter are significantly different.

Table A.4 Moisture Content Analysis ANOVA Results

General Linear Model: Moisture Content versus Protein type**Method**

Factor coding (-1; 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein type	Fixed	6	C; L; M; P; Q; S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein type	5	81,8728	16,3746	1494,49	0,000
Error	12	0,1315	0,0110		
Total	17	82,0043			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0,104674	99,84%	99,77%	99,64%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4,7995	0,0247	194,53	0,000	
Protein type					
C	2,1669	0,0552	39,28	0,000	1,67
L	0,1059	0,0552	1,92	0,079	1,67
M	-2,8705	0,0552	-52,03	0,000	1,67
P	1,2026	0,0552	21,80	0,000	1,67
Q	-2,8238	0,0552	-51,19	0,000	1,67

Comparisons for Moisture Content**Tukey Pairwise Comparisons: Protein type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein type	N	Mean	Grouping
S	3	7,01857	A
C	3	6,96643	A
P	3	6,00210	B
L	3	4,90543	C
Q	3	1,97570	D
M	3	1,92900	D

Means that do not share a letter are significantly different.

Table A.5 Water Holding Capacity Analysis ANOVA Results

General Linear Model: Water Holding Capacity versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	9533.1	1906.63	58.09	0.000
Error	12	393.8	32.82		
Total	17	9927.0			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
5.72890	96.03%	94.38%	91.07%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	167.36	1.35	123.94	0.000	
Protein Type					
C	-27.07	3.02	-8.97	0.000	1.67
L	7.23	3.02	2.39	0.034	1.67
M	-6.80	3.02	-2.25	0.044	1.67
P	-10.46	3.02	-3.47	0.005	1.67
Q	-9.27	3.02	-3.07	0.010	1.67

Regression Equation

Water Holding Capacity = 167.36 - 27.07 Protein Type_C
+ 7.23 Protein Type_L
- 6.80 Protein Type_M
- 10.46 Protein Type_P
- 9.27 Protein Type_Q
+ 46.38 Protein Type_S

Comparisons for Water Holding Capacity**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	213.741	A
L	3	174.591	B
M	3	160.560	B C
Q	3	158.098	C
P	3	156.901	C
C	3	140.290	D

Means that do not share a letter are significantly different.

Table A.6 Oil Binding Capacity Analysis ANOVA Results

General Linear Model: Oil Binding Capacity versus Protein Type**Method**

Factor coding (-1, 0, 1)

Factor

Information	Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S	

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	2381.1	476.21	13.98	0.000
Error	12	408.8	34.07		
Total	17	2789.9			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
5.83682	85.35%	79.24%	67.03%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	119.15	1.38	86.61	0.000	
Protein Type					
C	5.19	3.08	1.69	0.118	1.67
L	-8.83	3.08	-2.87	0.014	1.67
M	19.67	3.08	6.39	0.000	1.67
P	-17.05	3.08	-5.54	0.000	1.67
Q	2.83	3.08	0.92	0.375	1.67

Regression Equation

Oil Binding Capacity = 119.15 + 5.19 Protein Type_C - 8.83 Protein Type_L + 19.67 Protein Type_M - 17.05 Protein Type_P + 2.83 Protein Type_Q - 1.81 Protein Type_S

Fits and Diagnostics for Unusual Observations

Obs	Oil Binding Capacity	Fit	Resid	Std Resid
6	134.04	124.34	9.71	2.04 R

Comparisons for Oil Binding Capacity**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	138.818	A
C	3	124.338	A B
Q	3	121.984	B
S	3	117.342	B C
L	3	110.325	B C
P	3	102.099	C

Means that do not share a letter are significantly different

Table A.7 Yield ANOVA Results

General Linear Model: Yield versus Protein Type

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	4	C, L, M, Q

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	3	106.766	35.5886	64.39	0.000
Error	8	4.422	0.5527		
Total	11	111.188			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.743447	96.02%	94.53%	91.05%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	44.830	0.215	208.89	0.000	
Protein Type					
C	0.970	0.372	2.61	0.031	1.50
L	1.849	0.372	4.98	0.001	1.50
M	-5.101	0.372	-13.72	0.000	1.50

Regression Equation

Yield = 44.830 + 0.970 Protein Type_C
+ 1.849 Protein Type_L - 5.101 Protein Type_M
+ 2.282 Protein Type_Q

Comparisons for Yield

Tukey Pairwise Comparisons: Protein Type

Grouping Information Using the Tukey Method and 95% Confidence

Protein Type	N	Mean	Grouping
Q	3	47.1119	A
L	3	46.6794	A
C	3	45.7999	A
M	3	39.7289	B

Means that do not share a letter are significantly different.

Table A.8 Histidine vs Protein Types ANOVA Results

General Linear Model: Histidine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	1.27981	0.255961	12286.14	0.000
Error	12	0.00025	0.000021		
Total	17	1.28006			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0045644	99.98%	99.97%	99.96%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.42185	0.00108	2251.15	0.000	
Protein Type					
C	-0.05555	0.00241	-23.09	0.000	1.67
L	-0.20865	0.00241	-86.73	0.000	1.67
M	0.55605	0.00241	231.15	0.000	1.67
P	-0.13875	0.00241	-57.68	0.000	1.67
Q	-0.21765	0.00241	-90.48	0.000	1.67

Regression Equation

Histidine = 2.42185 - 0.05555 Protein Type_C
 - 0.20865 Protein Type_L
 + 0.55605 Protein Type_M - 0.13875 Protein Type_P
 - 0.21765 Protein Type_Q
 + 0.06455 Protein Type_S

Comparisons for Histidine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	2.9779	A
S	3	2.4864	B
C	3	2.3663	C
P	3	2.2831	D
L	3	2.2132	E
Q	3	2.2042	E

Means that do not share a letter are significantly different.

Table A.9 Isoleucine vs Protein Types ANOVA Results

General Linear Model: Isoleucine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	2.15307	0.430614	17224.57	0.000
Error	12	0.00030	0.000025		
Total	17	2.15337			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.005	99.99%	99.98%	99.97%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.56728	0.00118	3026.94	0.000	
Protein Type					
C	-0.12338	0.00264	-46.82	0.000	1.67
L	0.18162	0.00264	68.92	0.000	1.67
M	0.18582	0.00264	70.51	0.000	1.67
P	0.22082	0.00264	83.79	0.000	1.67
Q	-0.72138	0.00264	-273.75	0.000	1.67

Regression Equation

Isoleucine = 3.56728 - 0.12338 Protein Type_C
+ 0.18162 Protein Type_L
+ 0.18582 Protein Type_M
+ 0.22082 Protein Type_P
- 0.72138 Protein Type_Q
+ 0.25652 Protein Type_S

Comparisons for Isoleucine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	3.8238	A
P	3	3.7881	B
M	3	3.7531	C
L	3	3.7489	C
C	3	3.4439	D
Q	3	2.8459	E

Means that do not share a letter are significantly different.

Table A.10 Leucine vs Protein Types ANOVA Results

General Linear Model: Leucine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	9.15248	1.83050	51563.29	0.000
Error	12	0.00043	0.00004		
Total	17	9.15291			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0059582	100.00%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.89680	0.00140	4198.93	0.000	
Protein Type					
C	-0.02550	0.00314	-8.12	0.000	1.67
L	0.35750	0.00314	113.84	0.000	1.67
M	0.76580	0.00314	243.87	0.000	1.67
P	0.17300	0.00314	55.09	0.000	1.67
Q	-1.50100	0.00314	-477.99	0.000	1.67

Regression Equation

Leucine = 5.89680 - 0.02550 Protein Type_C
+ 0.35750 Protein Type_L + 0.76580 Protein Type_M
+ 0.17300 Protein Type_P - 1.50100 Protein Type_Q
+ 0.23020 Protein Type_S

Comparisons for Leucine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	6.6626	A
L	3	6.2543	B
S	3	6.1270	C
P	3	6.0698	D
C	3	5.8713	E
Q	3	4.3958	F

Means that do not share a letter are significantly different.

Table A.11 Lysine vs Protein Types ANOVA Results

General Linear Model: Lysine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	14.6212	2.92424	53930.72	0.000
Error	12	0.0007	0.00005		
Total	17	14.6219			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0.0073636	100.00%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.60683	0.00174	3230.46	0.000	
Protein Type					
C	-0.38293	0.00388	-98.67	0.000	1.67
L	0.35157	0.00388	90.59	0.000	1.67
M	0.95777	0.00388	246.79	0.000	1.67
P	0.73977	0.00388	190.62	0.000	1.67
Q	-1.76873	0.00388	-455.75	0.000	1.67

Regression Equation

Lysine = 5.60683 - 0.38293 Protein Type_C
+ 0.35157 Protein Type_L + 0.95777 Protein Type_M
+ 0.73977 Protein Type_P - 1.76873 Protein Type_Q
+ 0.10254 Protein Type_S

Comparisons for Lysine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	6.56460	A
P	3	6.34660	B
L	3	5.95840	C
S	3	5.70937	D
C	3	5.22390	E
Q	3	3.83810	F

Means that do not share a letter are significantly different.

Table A.12 Methionine vs Protein Types ANOVA Results

General Linear Model: Methionine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	0.982825	0.196565	49141.23	0.000
Error	12	0.000048	0.000004		
Total	17	0.982873			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.002	100.00%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.938967	0.000471	1991.85	0.000	
Protein Type					
C	-0.07827	0.00105	-74.25	0.000	1.67
L	-0.36287	0.00105	-344.25	0.000	1.67
M	0.07273	0.00105	69.00	0.000	1.67
P	-0.13297	0.00105	-126.14	0.000	1.67
Q	0.39413	0.00105	373.91	0.000	1.67

Regression Equation

Methionine = 0.938967 - 0.07827 Protein Type_C
 - 0.36287 Protein Type_L
 + 0.07273 Protein Type_M
 - 0.13297 Protein Type_P
 + 0.39413 Protein Type_Q
 + 0.10723 Protein Type_S

Comparisons for Methionine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
Q	3	1.3331	A
S	3	1.0462	B
M	3	1.0117	C
C	3	0.8607	D
P	3	0.8060	E
L	3	0.5761	F

Means that do not share a letter are significantly different.

Table A.13 Phenylalanine vs Protein Types ANOVA Results

General Linear Model: Phenylalanine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	9.92733	1.98547	124091.58	0.000
Error	12	0.00019	0.00002		
Total	17	9.92752			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0.004	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	4.02790	0.00094	4272.23	0.000	
Protein Type					
C	0.50380	0.00211	238.97	0.000	1.67
L	0.01260	0.00211	5.98	0.000	1.67
M	1.01490	0.00211	481.41	0.000	1.67
P	-0.01660	0.00211	-7.87	0.000	1.67
Q	-1.41980	0.00211	-673.47	0.000	1.67

Regression Equation

Phenylalanine = 4.02790 + 0.50380 Protein Type_C
+ 0.01260 Protein Type_L
+ 1.01490 Protein Type_M
- 0.01660 Protein Type_P
- 1.41980 Protein Type_Q
- 0.09490 Protein Type_S

Comparisons for Phenylalanine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	5.0428	A
C	3	4.5317	B
L	3	4.0405	C
P	3	4.0113	D
S	3	3.9330	E
Q	3	2.6081	F

Means that do not share a letter are significantly different.

Table A.14 Tyrosine vs Protein Types ANOVA Results

General Linear Model: Tyrosine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	1.73415	0.346829	38536.56	0.000
Error	12	0.00011	0.000009		
Total	17	1.73425			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.003	99.99%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.25870	0.00071	3194.28	0.000	
Protein Type					
C	-0.40600	0.00158	-256.78	0.000	1.67
L	0.06600	0.00158	41.74	0.000	1.67
M	0.02180	0.00158	13.79	0.000	1.67
P	0.30720	0.00158	194.29	0.000	1.67
Q	-0.39070	0.00158	-247.10	0.000	1.67

Regression Equation

Tyrosine = 2.25870 - 0.40600 Protein Type_C
+ 0.06600 Protein Type_L + 0.02180 Protein Type_M
+ 0.30720 Protein Type_P - 0.39070 Protein Type_Q
+ 0.40170 Protein Type_S

Comparisons for Tyrosine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	2.6604	A
P	3	2.5659	B
L	3	2.3247	C
M	3	2.2805	D
Q	3	1.8680	E
C	3	1.8527	F

Means that do not share a letter are significantly different.

Table A.15 Threonine vs Protein Types ANOVA Results

General Linear Model: Threonine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	0.927450	0.185490	46372.48	0.000
Error	12	0.000048	0.000004		
Total	17	0.927498			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.002	99.99%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.63847	0.00047	5597.03	0.000	
Protein Type					
C	-0.24077	0.00105	-228.41	0.000	1.67
L	0.05473	0.00105	51.92	0.000	1.67
M	-0.16397	0.00105	-155.55	0.000	1.67
P	0.23133	0.00105	219.46	0.000	1.67
Q	-0.22417	0.00105	-212.66	0.000	1.67

Regression Equation

Threonine = 2.63847 - 0.24077 Protein Type_C
+ 0.05473 Protein Type_L
- 0.16397 Protein Type_M
+ 0.23133 Protein Type_P
- 0.22417 Protein Type_Q
+ 0.34283 Protein Type_S

Comparisons for Threonine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	2.9813	A
P	3	2.8698	B
L	3	2.6932	C
M	3	2.4745	D
Q	3	2.4143	E
C	3	2.3977	F

Means that do not share a letter are significantly different.

Table A.16 Valine vs Protein Types ANOVA Results

General Linear Model: Valine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	2.39952	0.479905	53322.73	0.000
Error	12	0.00011	0.000009		
Total	17	2.39963			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.003	100.00%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.24877	0.00071	4594.45	0.000	
Protein Type					
C	-0.36427	0.00158	-230.38	0.000	1.67
L	0.15033	0.00158	95.08	0.000	1.67
M	0.52403	0.00158	331.43	0.000	1.67
P	0.21883	0.00158	138.40	0.000	1.67
Q	-0.56627	0.00158	-358.14	0.000	1.67

Regression Equation

Valine = 3.24877 - 0.36427 Protein Type_C
+ 0.15033 Protein Type_L + 0.52403 Protein Type_M
+ 0.21883 Protein Type_P - 0.56627 Protein Type_Q
+ 0.03733 Protein Type_S

Comparisons for Valine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	3.7728	A
P	3	3.4676	B
L	3	3.3991	C
S	3	3.2861	D
C	3	2.8845	E
Q	3	2.6825	F

Means that do not share a letter are significantly different.

Table A.17 Aspartic Acid vs Protein Types ANOVA Results

General Linear Model: Aspartic versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	34.4985	6.89971	107807.90	0.000
Error	12	0.0008	0.00006		
Total	17	34.4993			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0.008	100.00%	100.00%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	8.38142	0.00189	4444.92	0.000	
Protein Type					
C	0.51968	0.00422	123.25	0.000	1.67
L	0.53108	0.00422	125.96	0.000	1.67
M	1.17358	0.00422	278.34	0.000	1.67
P	0.23458	0.00422	55.64	0.000	1.67
Q	-3.03112	0.00422	-718.89	0.000	1.67

Regression Equation

Aspartic = 8.38142 + 0.51968 Protein Type_C
+ 0.53108 Protein Type_L + 1.17358 Protein Type_M
+ 0.23458 Protein Type_P - 3.03112 Protein Type_Q
+ 0.57218 Protein Type_S

Comparisons for Aspartic**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	9.5550	A
S	3	8.9536	B
L	3	8.9125	C
C	3	8.9011	C
P	3	8.6160	D
Q	3	5.3503	E

Means that do not share a letter are significantly different.

Table A.18 Glutamic Acid vs Protein Types ANOVA Results

General Linear Model: Glutamic Acid versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	79.9728	15.9946	159945.66	0.000
Error	12	0.0012	0.0001		
Total	17	79.9740			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.01	100.00%	100.00%	100.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	12.5881	0.0024	5340.66	0.000	
Protein Type					
C	0.28565	0.00527	54.20	0.000	1.67
L	-1.24365	0.00527	-235.97	0.000	1.67
M	1.99815	0.00527	379.12	0.000	1.67
P	0.08325	0.00527	15.80	0.000	1.67
Q	-3.75535	0.00527	-712.53	0.000	1.67

Regression Equation

Glutamic = 12.5881 + 0.28565 Protein Type_C
 Acid - 1.24365 Protein Type_L
 + 1.99815 Protein Type_M
 + 0.08325 Protein Type_P
 - 3.75535 Protein Type_Q
 + 2.63195 Protein Type_S

Comparisons for Glutamic Acid**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	15.2200	A
M	3	14.5862	B
C	3	12.8737	C
P	3	12.6713	D
L	3	11.3444	E
Q	3	8.8327	F

Means that do not share a letter are significantly different.

Table A.19 Serine vs Protein Types ANOVA Results

General Linear Model: Serine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	4.16621	0.833242	52077.60	0.000
Error	12	0.00019	0.000016		
Total	17	4.16640			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.004	100.00%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.97163	0.00094	4212.55	0.000	
Protein Type					
C	0.08517	0.00211	40.40	0.000	1.67
L	0.29207	0.00211	138.54	0.000	1.67
M	0.46537	0.00211	220.74	0.000	1.67
P	-0.04123	0.00211	-19.56	0.000	1.67
Q	-1.01583	0.00211	-481.85	0.000	1.67

Regression Equation

Serine = 3.97163 + 0.08517 Protein Type_C
+ 0.29207 Protein Type_L + 0.46537 Protein Type_M
- 0.04123 Protein Type_P - 1.01583 Protein Type_Q
+ 0.21447 Protein Type_S

Comparisons for Serine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	4.4370	A
L	3	4.2637	B
S	3	4.1861	C
C	3	4.0568	D
P	3	3.9304	E
Q	3	2.9558	F

Means that do not share a letter are significantly different.

Table A.20 Glycine vs Protein Types ANOVA Results

General Linear Model: Glycine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	0.489468	0.097894	10877.06	0.000
Error	12	0.000108	0.000009		
Total	17	0.489576			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.003	99.98%	99.97%	99.95%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.82192	0.00071	3990.79	0.000	
Protein Type					
C	-0.20542	0.00158	-129.92	0.000	1.67
L	-0.00432	0.00158	-2.73	0.018	1.67
M	-0.13512	0.00158	-85.46	0.000	1.67
P	0.09818	0.00158	62.10	0.000	1.67
Q	-0.05362	0.00158	-33.91	0.000	1.67

Regression Equation

Glycine = 2.82192 - 0.20542 Protein Type_C
 - 0.00432 Protein Type_L - 0.13512 Protein Type_M
 + 0.09818 Protein Type_P - 0.05362 Protein Type_Q
 + 0.30028 Protein Type_S

Comparisons for Glycine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	3.1222	A
P	3	2.9201	B
L	3	2.8176	C
Q	3	2.7683	D
M	3	2.6868	E
C	3	2.6165	F

Means that do not share a letter are significantly different.

Table A.21 Arginine vs Protein Types ANOVA Results

General Linear Model: Arginine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	4.56537	0.913073	36522.93	0.000
Error	12	0.00030	0.000025		
Total	17	4.56567			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.005	99.99%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	5.89108	0.00118	4998.75	0.000	
Protein Type					
C	0.62972	0.00264	238.96	0.000	1.67
L	0.45832	0.00264	173.92	0.000	1.67
M	-0.27218	0.00264	-103.29	0.000	1.67
P	0.16822	0.00264	63.83	0.000	1.67
Q	-0.89738	0.00264	-340.53	0.000	1.67

Regression Equation

Arginine = 5.89108 + 0.62972 Protein Type_C
+ 0.45832 Protein Type_L - 0.27218 Protein Type_M
+ 0.16822 Protein Type_P - 0.89738 Protein Type_Q
- 0.08668 Protein Type_S

Comparisons for Arginine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
C	3	6.5208	A
L	3	6.3494	B
P	3	6.0593	C
S	3	5.8044	D
M	3	5.6189	E
Q	3	4.9937	F

Means that do not share a letter are significantly different.

Table A.22 Alanine vs Protein Types ANOVA Results

General Linear Model: Alanine versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	0.616434	0.123287	13698.53	0.000
Error	12	0.000108	0.000009		
Total	17	0.616542			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.003	99.98%	99.98%	99.96%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	3.12165	0.00071	4414.68	0.000	
Protein Type					
C	-0.05415	0.00158	-34.25	0.000	1.67
L	-0.03265	0.00158	-20.65	0.000	1.67
M	0.15145	0.00158	95.79	0.000	1.67
P	0.08675	0.00158	54.87	0.000	1.67
Q	-0.35815	0.00158	-226.51	0.000	1.67

Regression Equation

Alanine = 3.12165 - 0.05415 Protein Type_C
 - 0.03265 Protein Type_L + 0.15145 Protein Type_M
 + 0.08675 Protein Type_P - 0.35815 Protein Type_Q
 + 0.20675 Protein Type_S

Comparisons for Alanine**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	3.3284	A
M	3	3.2731	B
P	3	3.2084	C
L	3	3.0890	D
C	3	3.0675	E
Q	3	2.7635	F

Means that do not share a letter are significantly different.

Table A.23 Proline vs Protein Types ANOVA Results

General Linear Model: Proline versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels Values
Protein Type	Fixed	6 C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	3.34462	0.668923	41807.70	0.000
Error	12	0.00019	0.000016		
Total	17	3.34481			

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0.004	99.99%	99.99%	99.99%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.92767	0.00094	3105.26	0.000	
Protein Type					
C	-0.14797	0.00211	-70.19	0.000	1.67
L	0.07523	0.00211	35.69	0.000	1.67
M	0.26413	0.00211	125.29	0.000	1.67
P	-0.26927	0.00211	-127.72	0.000	1.67
Q	-0.64737	0.00211	-307.07	0.000	1.67

Regression Equation

Proline = 2.92767 - 0.14797 Protein Type_C
+ 0.07523 Protein Type_L + 0.26413 Protein Type_M
- 0.26927 Protein Type_P - 0.64737 Protein Type_Q
+ 0.72523 Protein Type_S

Comparisons for Proline**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	3	3.6529	A
M	3	3.1918	B
L	3	3.0029	C
C	3	2.7797	D
P	3	2.6584	E
Q	3	2.2803	F

Means that do not share a letter are significantly different.

Table A.24 ΣΕΑΑ vs Protein Types ANOVA Results

General Linear Model: ΣΕΑΑ versus Protein Type**Method**

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Protein Type	Fixed	6	C, L, M, P, Q, S

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein Type	5	189.142	37.8284	28288.80	0.000
Error	12	0.016	0.0013		
Total	17	189.158			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0365680	99.99%	99.99%	99.98%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	30.6056	0.0086	3550.87	0.000	
Protein Type					
C	-1.1729	0.0193	-60.85	0.000	1.67
L	0.6028	0.0193	31.28	0.000	1.67
M	3.9349	0.0193	204.17	0.000	1.67
P	1.6026	0.0193	83.15	0.000	1.67
Q	-6.4156	0.0193	-332.88	0.000	1.67

Regression Equation

ΣΕΑΑ = 30.6056 - 1.1729 Protein Type_C
 + 0.6028 Protein Type_L + 3.9349 Protein Type_M
 + 1.6026 Protein Type_P - 6.4156 Protein Type_Q
 + 1.4480 Protein Type_S

Comparisons for ΣΕΑΑ**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
M	3	34.5405	A
P	3	32.2082	B
S	3	32.0536	C
L	3	31.2084	D
C	3	29.4327	E
Q	3	24.1900	F

Means that do not share a letter are significantly different.

Table A.25 Protein efficiency ratio (PER) ANOVA Tables

General Linear Model: PER-1 versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for PER-1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	1,74013	1,74013	0,34803	54235,88	0,000
Error	12	0,00008	0,00008	0,00001		
Total	17	1,74021				

S = 0,00253316 R-Sq = 100,00% R-Sq(adj) = 99,99%

Test for Equal Variances: SRES1 versus Protein Type

95% Bonferroni confidence intervals for standard deviations

Protein Type	N	Lower	StDev	Upper
CPC	3	0,526219	1,23192	19,0649
LPC	3	0,432045	1,01145	15,6530
MPC	3	0,714567	1,67286	25,8888
PPC	3	0,337871	0,79098	12,2410
QPC	3	0,526219	1,23192	19,0649
SPC	3	0,526219	1,23192	19,0649

Bartlett's Test (Normal Distribution)
Test statistic = 1,00; p-value = 0,962

Levene's Test (Any Continuous Distribution)
Test statistic = 0,23; p-value = 0,943

General Linear Model: PER-1 versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for PER-1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	1,74013	1,74013	0,34803	54235,88	0,000
Error	12	0,00008	0,00008	0,00001		
Total	17	1,74021				

S = 0,00253316 R-Sq = 100,00% R-Sq(adj) = 99,99%

Table A.25 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

Protein			
Type	N	Mean	Grouping
MPC	3	2,2	A
LPC	3	2,0	B
PPC	3	2,0	C
SPC	3	1,9	D
CPC	3	1,9	E
QPC	3	1,2	F

Means that do not share a letter are significantly different.

General Linear Model: PER-2 versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for PER-2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	1,68172	1,68172	0,33634	58516,21	0,000
Error	12	0,00007	0,00007	0,00001		
Total	17	1,68179				

S = 0,00239748 R-Sq = 100,00% R-Sq(adj) = 99,99%

Test for Equal Variances: SRES1 versus Protein Type

95% Bonferroni confidence intervals for standard deviations

Protein				
Type	N	Lower	StDev	Upper
CPC	3	0,525669	1,23063	19,0450
LPC	3	0,426602	0,99871	15,4558
MPC	3	0,723804	1,69448	26,2234
PPC	3	0,327534	0,76678	11,8665
QPC	3	0,525669	1,23063	19,0450
SPC	3	0,525669	1,23063	19,0450

Bartlett's Test (Normal Distribution)

Test statistic = 1,12; p-value = 0,953

Levene's Test (Any Continuous Distribution)

Test statistic = 0,25; p-value = 0,930

Table A.25 (Continued)

General Linear Model: PER-2 versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for PER-2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	1,68172	1,68172	0,33634	58516,21	0,000
Error	12	0,00007	0,00007	0,00001		
Total	17	1,68179				

S = 0,00239748 R-Sq = 100,00% R-Sq(adj) = 99,99%

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
MPC	3	2,3	A
LPC	3	2,1	B
SPC	3	2,0	C
PPC	3	2,0	D
CPC	3	2,0	E
QPC	3	1,3	F

Means that do not share a letter are significantly different.

General Linear Model: PER-3 versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for PER-3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	3,49016	3,49016	0,69803	52060,22	0,000
Error	12	0,00016	0,00016	0,00001		
Total	17	3,49032				

S = 0,00366171 R-Sq = 100,00% R-Sq(adj) = 99,99%

Table A.25 (Continued)

Test for Equal Variances: SRES1 versus Protein Type

95% Bonferroni confidence intervals for standard deviations

Protein				
Type	N	Lower	StDev	Upper
CPC	3	0,508908	1,19139	18,4377
LPC	3	0,427614	1,00108	15,4925
MPC	3	0,761934	1,78375	27,6048
PPC	3	0,316175	0,74019	11,4550
QPC	3	0,478762	1,12082	17,3456
SPC	3	0,539054	1,26197	19,5299

Bartlett's Test (Normal Distribution)
Test statistic = 1,39; p-value = 0,925

Levene's Test (Any Continuous Distribution)
Test statistic = 0,32; p-value = 0,891

Test for Equal Variances: SRES1 versus Protein Type

General Linear Model: PER-3 versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for PER-3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	3,49016	3,49016	0,69803	52060,22	0,000
Error	12	0,00016	0,00016	0,00001		
Total	17	3,49032				

S = 0,00366171 R-Sq = 100,00% R-Sq(adj) = 99,99%

Grouping Information Using Tukey Method and 95,0% Confidence

Protein			
Type	N	Mean	Grouping
MPC	3	2,3	A
CPC	3	1,9	B
LPC	3	1,6	C
SPC	3	1,4	D
PPC	3	1,3	E
QPC	3	0,9	F

Means that do not share a letter are significantly different.

Table A.26 Particle size ANOVA Results

General Linear Model: D [4,3] versus Protein Type; Fat Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC
Fat Type	fixed	2	Corn Oil; Sunflower Oil

Analysis of Variance for D [4,3], using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	694,900	696,787	139,357	690,88	0,000
Fat Type	1	25,175	25,175	25,175	124,81	0,000
Protein Type*Fat Type	5	2,107	2,107	0,421	2,09	0,069
Error	167	33,686	33,686	0,202		
Total	178	755,868				

S = 0,449122 R-Sq = 95,54% R-Sq(adj) = 95,25%

Unusual Observations for D [4,3]

Obs	D [4,3]	Fit	SE Fit	Residual	St Resid
1	10,0000	8,9764	0,1200	1,0236	2,37 R
15	7,2000	8,2160	0,1160	-1,0160	-2,34 R
61	3,9300	3,0073	0,1160	0,9227	2,13 R
83	3,4700	2,5513	0,1160	0,9187	2,12 R
84	3,6600	2,5513	0,1160	1,1087	2,56 R
101	3,2400	4,1493	0,1160	-0,9093	-2,10 R
102	3,1300	4,1493	0,1160	-1,0193	-2,35 R
103	2,9000	4,1493	0,1160	-1,2493	-2,88 R
142	5,1400	6,3140	0,1160	-1,1740	-2,71 R
146	7,2000	6,3140	0,1160	0,8860	2,04 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
LPC	29	8,6	A
PPC	30	6,8	B
SPC	30	6,7	B
CPC	30	6,0	C
QPC	30	3,6	D
MPC	30	2,8	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Fat Type	N	Mean	Grouping
Corn Oil	89	6,1	A
Sunflower Oil	90	5,4	B

Table A.26 (Continued)

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	Fat Type	N	Mean	Grouping
LPC	Corn Oil	14	9,0	A
LPC	Sunflower Oil	15	8,2	B
PPC	Corn Oil	15	7,3	C
SPC	Corn Oil	15	6,9	C D
SPC	Sunflower Oil	15	6,4	D E
CPC	Corn Oil	15	6,4	D E
PPC	Sunflower Oil	15	6,3	E
CPC	Sunflower Oil	15	5,7	F
QPC	Corn Oil	15	4,1	G
QPC	Sunflower Oil	15	3,1	H
MPC	Corn Oil	15	3,0	H I
MPC	Sunflower Oil	15	2,6	I

Means that do not share a letter are significantly different.

Table A.27 Particle size (corn oil vs commercial sample) ANOVA Results

General Linear Model: D [4,3] versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	7	Commercial Sample; CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for D [4,3], using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	6	362,277	362,277	60,379	284,05	0,000
Error	97	20,619	20,619	0,213		
Total	103	382,895				

S = 0,461046 R-Sq = 94,62% R-Sq(adj) = 94,28%

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
LPC	14	9,0	A
PPC	15	7,3	B
Commercial Sample	15	7,1	B
SPC	15	6,9	B
CPC	15	6,4	C
QPC	15	4,1	D
MPC	15	3,0	E

Means that do not share a letter are significantly different.

Table A.28 Particle size (sunflower oil vs commercial sample) ANOVA Results

General Linear Model: D [4,3] versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	7	Commercial Sample; CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for D [4,3], using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	6	386,408	386,408	64,401	351,10	0,000
Error	98	17,976	17,976	0,183		
Total	104	404,383				

S = 0,428281 R-Sq = 95,55% R-Sq(adj) = 95,28%

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
LPC	15	8,2	A
Commercial Sample	15	7,1	B
SPC	15	6,4	C
PPC	15	6,3	C
CPC	15	5,7	D
QPC	15	3,1	E
MPC	15	2,6	F

Means that do not share a letter are significantly different.

Table A.29 Creaming Index ANOVA Results

General Linear Model: Creaming Index versus Protein Type; Fat Type

Factor	Type	Levels	Values
Protein Type	fixed	6	CPC; LPC; MPC; PPC; QPC; SPC
Fat Type	fixed	2	Corn Oil; Sunflower Oil

Analysis of Variance for Creaming Index, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	5	49,1450	49,1450	9,8290	22,33	0,000
Fat Type	1	10,1568	10,1568	10,1568	23,07	0,000
Protein Type*Fat Type	5	3,6103	3,6103	0,7221	1,64	0,157
Error	96	42,2581	42,2581	0,4402		
Total	107	105,1702				

S = 0,663467 R-Sq = 59,82% R-Sq(adj) = 55,22%

Table A.29 (Continued)

Unusual Observations for Creaming Index

Creaming					
Obs	Index	Fit	SE Fit	Residual	St Resid
27	13,1100	11,6814	0,2212	1,4285	2,28 R
28	9,6677	11,1802	0,2212	-1,5124	-2,42 R
29	9,9154	11,1802	0,2212	-1,2647	-2,02 R
36	12,7359	11,1802	0,2212	1,5557	2,49 R
96	13,9094	12,2958	0,2212	1,6135	2,58 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein			
Type	N	Mean	Grouping
SPC	18	12,0	A
PPC	18	11,9	A
CPC	18	11,4	A B
QPC	18	11,4	A B
MPC	18	10,8	B
LPC	18	10,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Fat Type	N	Mean	Grouping
Corn Oil	54	11,6	A
Sunflower Oil	54	10,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein				
Type	Fat Type	N	Mean	Grouping
PPC	Corn Oil	9	12,3	A
SPC	Corn Oil	9	12,3	A
SPC	Sunflower Oil	9	11,7	A B
CPC	Corn Oil	9	11,7	A B
QPC	Corn Oil	9	11,4	A B C
PPC	Sunflower Oil	9	11,4	A B C
QPC	Sunflower Oil	9	11,3	A B C
CPC	Sunflower Oil	9	11,2	B C
MPC	Corn Oil	9	11,0	B C
MPC	Sunflower Oil	9	10,7	B C
LPC	Corn Oil	9	10,6	C
LPC	Sunflower Oil	9	9,4	D

Means that do not share a letter are significantly different.

Table A.30 Creaming index (corn oil vs commercial sample) ANOVA Results

General Linear Model: Creaming Index versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	7	Commercial Sample; CPC; LPC; MPC; PPC; QPC; SPC

Analysis of Variance for Creaming Index, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	6	30,6162	30,6162	5,1027	10,85	0,000
Error	56	26,3363	26,3363	0,4703		
Total	62	56,9525				

S = 0,685778 R-Sq = 53,76% R-Sq(adj) = 48,80%

Unusual Observations for Creaming Index

Obs	Creaming Index	Fit	SE Fit	Residual	St Resid
18	13,1100	11,6814	0,2286	1,4285	2,21 R
51	13,9094	12,2958	0,2286	1,6135	2,50 R
63	11,3130	12,6634	0,2286	-1,3504	-2,09 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
Commercial Sample	9	12,7	A
PPC	9	12,3	A B
SPC	9	12,3	A B
CPC	9	11,7	A B C
QPC	9	11,4	B C D
MPC	9	11,0	C D
LPC	9	10,6	D

Means that do not share a letter are significantly different.

Table A.31 Creaming index (sunflower oil vs commercial sample) ANOVA

Results

General Linear Model: Creaming Index versus Protein Type

Factor	Type	Levels	Values
Protein Type	fixed	7	Commercial Sample; CPC; LPC; MPC; PPC; QPC; SPC

Table A.31 (Continued)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Protein Type	6	54,3765	54,3765	9,0627	19,95	0,000
Error	56	25,4342	25,4342	0,4542		
Total	62	79,8107				

S = 0,673931 R-Sq = 68,13% R-Sq(adj) = 64,72%

Unusual Observations for Creaming Index

Obs	Creaming Index	Fit	SE Fit	Residual	St Resid
10	9,6677	11,1802	0,2246	-1,5124	-2,38 R
18	12,7359	11,1802	0,2246	1,5557	2,45 R
63	11,3130	12,6634	0,2246	-1,3504	-2,13 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
Commercial Sample	9	12,7	A
SPC	9	11,7	A B
PPC	9	11,4	B C
QPC	9	11,3	B C
CPC	9	11,2	B C
MPC	9	10,7	C
LPC	9	9,4	D

Means that do not share a letter are significantly different.

Table A.32 Shear viscosities ANOVA Results

General Linear Model: Shear Viscos versus Protein Type; Oil Type; ...

Factor	Type	Levels	Values
Protein Type	fixed	5	CPC; MPC; PPC; QPC; SPC
Oil Type	fixed	2	Corn Oil; Sunflower Oil
Temperature	fixed	2	25; 37

Table A.32 (Continued)

Analysis of Variance for Shear Viscosity (Pa*s), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS
F				
Protein Type	4	0,0000327	0,0000327	0,0000082
89,09				
Oil Type	1	0,0000006	0,0000006	0,0000006
6,50				
Temperature	1	0,0000616	0,0000616	0,0000616
671,13				
Protein Type*Oil Type	4	0,0000005	0,0000005	0,0000001
1,32				
Protein Type*Temperature	4	0,0000008	0,0000008	0,0000002
2,07				
Oil Type*Temperature	1	0,0000000	0,0000000	0,0000000
0,44				
Protein Type*Oil Type*Temperature	4	0,0000000	0,0000000	0,0000000
0,12				
Error	40	0,0000037	0,0000037	0,0000001
Total	59	0,0000999		

Source	P
Protein Type	0,000
Oil Type	0,015
Temperature	0,000
Protein Type*Oil Type	0,278
Protein Type*Temperature	0,103
Oil Type*Temperature	0,509
Protein Type*Oil Type*Temperature	0,973
Error	
Total	

S = 0,000303013 R-Sq = 96,33% R-Sq(adj) = 94,58%

Unusual Observations for Shear Viscosity (Pa*s)

Obs	Shear Viscosity (Pa*s)	Fit	SE Fit	Residual	St Resid
8	0,009370	0,009913	0,000175	-0,000543	-2,20 R
43	0,008704	0,008094	0,000175	0,000610	2,46 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
CPC	12	0,0	A
QPC	12	0,0	B
MPC	12	0,0	C
PPC	12	0,0	C
SPC	12	0,0	D

Table A.32 (Continued)

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Oil Type	N	Mean	Grouping
Corn Oil	30	0,0	A
Sunflower Oil	30	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
25	30	0,0	A
37	30	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	Oil Type	N	Mean	Grouping
CPC	Corn Oil	6	0,0	A
CPC	Sunflower Oil	6	0,0	A B
QPC	Corn Oil	6	0,0	B
QPC	Sunflower Oil	6	0,0	B
PPC	Corn Oil	6	0,0	C
MPC	Corn Oil	6	0,0	C D
MPC	Sunflower Oil	6	0,0	C D
PPC	Sunflower Oil	6	0,0	C D
SPC	Corn Oil	6	0,0	D
SPC	Sunflower Oil	6	0,0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	Temperature	N	Mean	Grouping
CPC	25	6	0,0	A
QPC	25	6	0,0	B
PPC	25	6	0,0	C
MPC	25	6	0,0	C
SPC	25	6	0,0	C D
CPC	37	6	0,0	D
QPC	37	6	0,0	D
MPC	37	6	0,0	E
PPC	37	6	0,0	E
SPC	37	6	0,0	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Table A.32 (Continued)

Oil Type	Temperature	N	Mean	Grouping
Corn Oil	25	15	0,0	A
Sunflower Oil	25	15	0,0	A
Corn Oil	37	15	0,0	B
Sunflower Oil	37	15	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	Oil Type	Temperature	N	Mean	Grouping
CPC	Corn Oil	25	3	0,0	A
CPC	Sunflower Oil	25	3	0,0	A B
QPC	Corn Oil	25	3	0,0	B C
QPC	Sunflower Oil	25	3	0,0	B C
PPC	Corn Oil	25	3	0,0	C D
MPC	Corn Oil	25	3	0,0	D E
MPC	Sunflower Oil	25	3	0,0	D E F
PPC	Sunflower Oil	25	3	0,0	D E F G
CPC	Corn Oil	37	3	0,0	D E F G
SPC	Corn Oil	25	3	0,0	D E F G
SPC	Sunflower Oil	25	3	0,0	D E F G
CPC	Sunflower Oil	37	3	0,0	E F G
QPC	Corn Oil	37	3	0,0	F G H
QPC	Sunflower Oil	37	3	0,0	G H I
PPC	Corn Oil	37	3	0,0	H I J
MPC	Corn Oil	37	3	0,0	I J
MPC	Sunflower Oil	37	3	0,0	J
PPC	Sunflower Oil	37	3	0,0	J
SPC	Corn Oil	37	3	0,0	J
SPC	Sunflower Oil	37	3	0,0	J

Means that do not share a letter are significantly different.

Table A.33 Shear viscosity (corn oil vs commercial sample) ANOVA Results

General Linear Model: Shear Viscosity versus Protein Type; Temperature

Factor	Type	Levels	Values
Protein Type	fixed	6	Control; CPC; MPC; PPC; QPC; SPC
Temperature	fixed	2	25; 37

Table A.33 (Continued)

Analysis of Variance for Shear Viscosity (Pa*s), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
P					
Protein Type	5	0,0001573	0,0001573	0,0000315	383,53
0,000					
Temperature	1	0,0000538	0,0000538	0,0000538	655,59
0,000					
Protein Type*Temperature	5	0,0000065	0,0000065	0,0000013	15,85
0,000					
Error	24	0,0000020	0,0000020	0,0000001	
Total	35	0,0002195			

S = 0,000286369 R-Sq = 99,10% R-Sq(adj) = 98,69%

Unusual Observations for Shear Viscosity (Pa*s)

Obs	Shear Viscosity (Pa*s)	Fit	SE Fit	Residual	St Resid
9	0,008892	0,008408	0,000165	0,000484	2,07 R
32	0,015820	0,015323	0,000165	0,000497	2,12 R
33	0,014820	0,015323	0,000165	-0,000503	-2,15 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Protein Type	N	Mean	Grouping
Control	6	0,0	A
CPC	6	0,0	B
QPC	6	0,0	C
PPC	6	0,0	D
MPC	6	0,0	D E
SPC	6	0,0	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
25	18	0,0	A
37	18	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Table A.33 (Continued)

Protein Type	Temperature	N	Mean	Grouping
Control	25	3	0,0	A
Control	37	3	0,0	B
CPC	25	3	0,0	B
QPC	25	3	0,0	C
PPC	25	3	0,0	C D
MPC	25	3	0,0	D
CPC	37	3	0,0	D E
SPC	25	3	0,0	D E
QPC	37	3	0,0	E
PPC	37	3	0,0	F
MPC	37	3	0,0	F
SPC	37	3	0,0	F

Means that do not share a letter are significantly different.

Table A.34 pH ANOVA Results

General Linear Model: pH Results versus Protein Type; Oil Type

Method

Factor (-1;
coding 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein	Fixed	6	C; L; M;
Type			P; Q; S
Oil	Fixed	2	C; S
Type			

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein	5	0,514433	0,102887	51,37	0,000
Type					
Oil Type	1	0,000544	0,000544	0,27	0,607
Protein	5	0,013156	0,002631	1,31	0,292
Type*Oil Type					
Error	24	0,048067	0,002003		
Total	35	0,576200			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0447524	91,66%	87,83%	81,23%

Coefficients

Table A.34 (Continued)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,90667	0,00746	925,98	0,000	
Protein Type					
C	-0,0817	0,0167	-4,90	0,000	1,67
L	-0,1000	0,0167	-6,00	0,000	1,67
M	0,0067	0,0167	0,40	0,693	1,67
P	0,1367	0,0167	8,19	0,000	1,67
Q	-0,1383	0,0167	-8,29	0,000	1,67
Oil Type					
C	0,00389	0,00746	0,52	0,607	1,00
Protein Type*Oil Type					
C C	0,0011	0,0167	0,07	0,947	1,67
L C	-0,0139	0,0167	-0,83	0,413	1,67
M C	0,0061	0,0167	0,37	0,717	1,67
P C	-0,0106	0,0167	-0,63	0,533	1,67
Q C	0,0378	0,0167	2,27	0,033	1,67

Comparisons for pH Results**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean Grouping
S	6 7,08333	A
P	6 7,04333	A
M	6 6,91333	B
C	6 6,82500	C
L	6 6,80667	C
Q	6 6,76833	C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Oil Type**Grouping Information Using the Tukey Method and 95% Confidence**

Oil Type	N	Mean Grouping
C	18 6,91056	A
S	18 6,90278	A

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Protein Type*Oil Type**Grouping Information Using the Tukey Method and 95% Confidence**

Table A.34 (Continued)

Protein Type*Oil		
Type	N	Mean Grouping
S S	3 7,10000	A
S C	3 7,06667	A
P S	3 7,05000	A B
P C	3 7,03667	A B
M C	3 6,92333	B C
M S	3 6,90333	C
C C	3 6,83000	C D
C S	3 6,82000	C D
L S	3 6,81667	C D
Q C	3 6,81000	C D
L C	3 6,79667	C D
Q S	3 6,72667	D

Means that do not share a letter are significantly different.

General Linear Model: pH Results versus Protein Type

Method

Factor (-1;
coding 0; +1)

Factor Information

Factor	Type	Levels	Values
Protein	Fixed	7	C; Control; L;
Type			M; P; Q; S

Table A.34 (Continued)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein	6	0,54213	0,090354	46,16	0,000
Type					
Error	32	0,06263	0,001957		
Total	38	0,60476			

Model Summary

S	R-sq	sq(adj)	sq(pred)
0,0442413	89,64%	87,70%	84,97%

Coefficients

Table A.34 (Continued)

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6,92095	0,00730	948,35	0,000	
Protein Type					
C	-0,0960	0,0169	-5,67	0,000	1,76
Control	0,0857	0,0228	3,76	0,001	2,33
L	-0,1143	0,0169	-6,75	0,000	1,76
M	-0,0076	0,0169	-0,45	0,656	1,76
P	0,1224	0,0169	7,23	0,000	1,76
Q	-0,1526	0,0169	-9,02	0,000	1,76

Regression Equation

pH = 6,92095 - 0,0960 Protein Type_C
 Results + 0,0857 Protein Type_Control
 - 0,1143 Protein Type_L
 - 0,0076 Protein Type_M
 + 0,1224 Protein Type_P
 - 0,1526 Protein Type_Q
 + 0,1624 Protein Type_S

Comparisons for pH Results**Tukey Pairwise Comparisons: Protein Type****Grouping Information Using the Tukey Method and 95% Confidence**

Protein Type	N	Mean	Grouping
S	6	7,08333	A
P	6	7,04333	A
Control	3	7,00667	A B
M	6	6,91333	B
C	6	6,82500	C
L	6	6,80667	C
Q	6	6,76833	C

Means that do not share a letter are significantly different.