ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF *PHASEOLUS COCCINEUS L.* PROTEIN ISOLATE OBTAINED BY ISOELECTRIC PRECIPITATION METHOD

M.Sc. THESIS

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

İZOELEKTRİK ÇÖKTÜRME METODU İLE ELDE EDİLEN *PHASEOLUS COCCINEUS L.* PROTEİN İZOLATININ FİZİKOKİMYASAL VE FONKSİYONEL ÖZELLİKLERİ

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Seçil Bal Batır, a M.Sc student of İTU Graduate School of Science Engineering and Technology student ID 506121540, successfully defended the thesis/dissertation entitled "PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF *PHASEOLUS COCCINEUS L.* PROTEIN ISOLATE OBTAINED BY ISOELECTRIC PRECIPITATION METHOD", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my family,

FOREWORD

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September 2018

Seçil BAL BATIR
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ABBREVIATIONS

AOAC **:** Association of Official Analytical Chemists
ANOVA **:** Analysis of variance
ATR **:** Attenuated total reflection
AU **:** Absorbance Unit
CIE **:** International commission on illumination
DB **:** Dry basis
DSC **:** Differantial scanning calorimetry
DNA **:** Deoxyribonucleic acid
DPPH **:** 1,1-Diphenyl-2- picrylhydrazyl
DW **:** Dry weight
DB : Dry bases
EA **:** Emulsifying activity
ES **:** Emulsifying stability
FC **:** Foaming capacity
FS **:** Foaming stability
FT-IR **:** Fourier transform infrared
kDa **:** Kilo Dalton
HPH **:** High-pressure homogenization
HPP **:** High pressure processing
IEP **:** Isoelectric precipitation
LDL **:** Low density lipoprotein
OAC **:** Oil absorption capacity
OD **:** Optic Density
Mpa **:** Mega pascal
Nm **:** Nano meter
MWD **:** Molecular weight distribution
PCLF **:** *Phaseolus coccineus L.*flour
PCLP **:** *Phaseolus coccineus L.* protein
PCLPI **:** *Phaseolus coccineus L.* protein isolate
PSI **:** Pounds per square inch
PI **:** Isoelectric Point
WAC **:** Water absorption capacity
W/w : Weight/weight
W/v : Weight/volume
20 Psi : Equal to 20,000 pounds per square inch
40 Psi : Equal to 40,000 pounds per square inch

SYMBOLS

ml	**:** Milliliter
µL	**:** Microliter
mg	**:** Milligram
mV	**:** Millivolt
$\mathbf{C^{O}}$	: Degrees celcius
ζ	: Zeta
%	: Percentage

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PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF *PHASEOLUS COCCINEUS L.* PROTEIN ISOLATE OBTAINED WITH ISOELECTRIC PRECIPITATION METHOD

SUMMARY

Recently, due to ease of access to information consumers are more aware and conscious about their eating habits. Carbohydrates, lipids and, proteins are very important for a balanced diet and provide energy. However, proteins not only provide energy, but also contribute to construction of muscle and enzymes. If there is lack of protein in human diet, there might be several diseases occurs. On the other hand, proteins are like a key due to their functional and physicochemical properties in foods. Proteins interact with solvents, ions, polysaccharides, lipids and other proteins in food matrix and alter the physical structure of foods. Protein solubility, water and oil binding/absorption capacity, viscosity, foaming capacity and foaming stability, emulsification, and gelation are critical functional parameters, which are role on final product quality. The World population is growing dramatically and food security is one of the biggest concerns of agro-food industry and governments. Land plants are included in human diet to give energy and nutrients for sustainable life style for a long time ago. However, the usage of plant proteins less than expected level even if they are fairly cheap and more abundant than animal proteins.

Legumes are considerable interest of academia due to sufficient nutritional composition, and alternative to animal originated foods among plant proteins. Despite the thousands of species only soybeans, peas, chickpeas, broad beans, lentil, and cowpeas are comprehensively studied. To date, there was not enough studies in literature found regarding to *Phaseolus coccineus L*. (Cranberry Bean).

On the other hand, altering functional properties of proteins are gaining importance by novel and sustainable methods. Among them, microfluidization that is dynamic high-pressure advanced technique and alters several functional properties of protein isolates reported. It has been reported that microfluidization(HPH) is environmentally friendly way while comparing to conventional techniques.

The aim of this study is obtaining protein isolate from Cranberry bean (*Phaseolus coccineus L.*) by isoelectric precipitation. Then, subject to isolates microfluidization treatment and investigate the effects of microfluidization treatment on several functional and physicochemical properties of protein isolates.

Initially, the proximate composition analysis performed for PCLF (*Phaseolus coccineus L*. flour). Then, the flour prepared for protein extraction. The color values of PCLF *(Phaseolus coccineus L.* flour) and freeze PCLPI (*Phaseolus coccineus L.* protein isolate) measured instrumentally. After that, protein content of PCLPI was measured. Oil absorption capacity (OAC), water absorption capacity (WAC), emulsifying and foaming properties were of protein isolates also examined.

The analysis of composition showed that *Phaseolus coccineus L*. that was grown in Çanakkale province, contains 4.8% ash, 2.86% moisture, 27.01% protein and 1.53% lipid and 63.73% carbohydrate content on dry weight.

The protein content of *Phaseolus coccineus L*. protein isolate (PCLPI) were determined 83.53% with 2.37% standard deviation on dry weight.

According to color result of color analysis, *Phaseolus coccineus L*. flour shows L* value 89.763 which is higher than *Phaseolus coccineus L.* isolate with 81.751. However, the redness value and yellowness values of the isolates higher than the flour a* value of the flour is 0.173 and b* value is 6.907 while isolates a* value is 0.253 and b* value is 10.783.

Water absorption capacity (WAC) of freeze dried PCPLI was 2.63 g of water/g of protein on dry weight (dw). In this study oil absorption capacity (OAC) was detected 3.95 g of oil/g of protein isolate on dry weight (dw). Foaming capacity (FC) and foaming stability (FS) of freeze- dried *Phaseolus coccineus L*. isolate (PCLPI) were 247.400% and 132.000% respectively. The emulsifying activity of freeze-dried *Phaseolus coccineus L.* isolate were 50.957% and emulsion stability were 100%.

High pressure treatment carried out 20 Psi (pounds per square inch) - 4 or 6 Cycle levels and 40 Psi(pounds per square inch)- 4 or 6 cycle selected.

Investigating effect of microfluidizitaion or high-pressure homogenization (HPH) on foaming and emulsifying properties, turbidity, particle size, ζ- potential microstructure and secondary structure analysis were conducted. The highest foaming capacity (FC) and foaming stability (FS) observed for 40 Psi- 6 Cycle treatment. The highest foaming capacity and stability were 612.000% and the 182.500% for 40 Pi- 6 Cycle treatment. The lowest foaming capacity (FC) and foaming stability (FS) detected for control sample 235.000% and 88.100% respectively. The emulsifying activities were changing from 55.06% to 52.80%. Emulsifying stability were 100% for all samples, including control. However, High Pressure treatment statistically were not changed EA (emulsifying activiy) or ES (emulsifying stability).

Turbidity, zeta (**ζ)-** potential, particle size, measurements were conducted from pH 2 to pH 10. The lowest turbidity value detected as 0.138 nm au for 40 Psi-6 Cycle at pH 10, while the highest absorbance was between 2.639 and 2.853 nm au at pH 5 and all samples were statistically same. The lowest net charge was observed at pH 4 that was very close to isoelectric point 0.924 mV and belong to 40 Psi – 6 Cycle treatment. The highest zeta (**ζ)-** potential observed at pH 10 and all the samples were statistically same and the net charge were between -13,22 and -14,25 mV.

According results of statistical analysis, after or at neutral pH values microfluidization reduced particle diameter. However, when pH approach to isoelectric point of PCLPI, proteins showed agglomeration potential and particle size were increased. And the highest diameter was observed at pH 4 with 7920.00 nm which belonged 40 Psi- 4 Cycle treatment and, the lowest mean diameter belonged to 40 Psi- 6 Cycle with 61.138 nm at pH 8.

According light microscope images, microfluidization treatment broke into fragments and more homogenous particles by reducing big cluster to small clumps. Secondary structures of PCPLIs were imaged by FT-IR spectrum. PCPLI showed absorption band between amide I region at 1635.47 cm^{-1}. The FT-IR images support

that PCPLP was in amide I region and consist of β-sheet structure, ~ % 80 peptide bonds exist and C=O exhibited stretch vibrations.

As a result, HPH improved foaming, reduced particle diameter and turbidity and increased zeta (ζ)- potential at and after neutral pHs. However, more research proposed to be done by changing pressure and cycle number, to understand effect of microfluidization (HPH) on physicochemical and functional properties of proteins.

İZOELEKTRİK ÇÖKTÜRME METODU İLE ELDE EDİLEN *PHASEOLUS COCCINEUS L.* PROTEİN İZOLATININ FİZİKOKİMYASAL VE FONKSİYONEL ÖZELLİKLERİ

ÖZET

Son zamanlarda, bilgiye kolay ulaşım nedeniyle tüketiciler yeme alışkanlıkları hakkında daha farkında ve bilinçlilerdir. Karbonhidratlar, yağlar ve proteinler dengeli bir diyet için oldukça önemlidir. Ancak protein sadece enerji vermekle kalmaz, ayrıca vücutta kas ve enzim yapımına da katkı sağlar. Eğer, insan beslenmesinde protein eksikliği belirirse bazı hastalıklar ortaya çıkabilir. Diğer yandan proteinler gıdalarda fonksiyonel ve fizikokimyasal özelliklerinden dolayı gıda teknolojisi için bir anahtar gibidir. Proteinler gıda matrisi içindeki çözücüler, iyonlar, polisakkaritler, yağlar ve diğer proteinlerle etkileşerek su, yağ tutma kapasitesi, viskozitesi köpüklenme ve köpüklenme stabilitesi, emülsüfikasyon, jelleşme gibi özellikleriyle son ürün kalitesinde rol almaktadır. Dünya nüfusu belirgin olarak artmaktadır ve gıda güvencesi zirai - gıda endüstrisinin en büyük endişelerinden biridir. Hayvansal kaynaklı proteinler pahalı ve daha çok enerji ile işçilik gerektirir. Kara bitkileri sürdürülebilir yaşam biçimi için insan beslenmesine enerji ve besin vermek üzere uzun süreden beri dâhil olmuştur. Ancak bitkisel proteinlerin, kullanım seviyesi; daha ucuz ve bol olmasına rağmen beklenenin altındadır.

Baklagiller, nitelikli besinsel bileşimi nedeniyle hayvansal kaynaklı gıdalara alternatif oluşturmuş bu sebeple akademinin ilgisini önemli ölçüde kazanmıştır. Binlerce çeşit baklagiller arasından soya fasülyesi, bezelyeler, nohut, kuru bakla, mercimek ve börülce kapsamlı şekilde çalışılmıştır. Bu güne kadar Barbunya Fasülyesine(*Phaseolus coccineus L)* yönelik yeterince çalışmaya rastlanmamıştır.

Protein izolatlarının, yeni ve sürdürülebilir yöntemlerle fonksiyonel, fizokokimyasal ve diğer özelliklerini iyileştirmek önem kazanmaktadır. Yüksek basınçla homojenizasyon veya mikroakışkanlaştırma innovatif yöntemler arasında yer alan çevre dostu uygulamadır. Mikroakışkanlaştırma yönteminin protein izolatlarının bazı fonksiyonel özelliklerini geliştirdiği raporlanmıştır. Bu çalışmanın amacı barbunya fasülyesinden (*Phaseolus coccineus L*.) izoelektrik çöktürme ile protein izole etmek ve elde edilen izolatları mikroakışkanlaştırma (yüksek basınçlı homojenizasyon) tekniğine tabi tutarak, izolektrik çöktürme ile elde edilen izolatın ve mikroakışkanlaştırılan örneklerin bazı fonksiyonel ve fizikokimyasal özelliklerini belirlemektir.

İlk olarak, yöresel pazardan satın alınan ve Çanakkale bölgesinde yetişen Barbunya Fasulyesinin (*Phaseolus coccineus L*.) kimyasal kompozisyonu belirlenmiştir. Izoelektrik çöktürme metodu ile elde edilen Barbunya Fasulyesi (*Phaseolus coccineus L*.) izolatı dondurularak kurutulmuştur. Barbunya Fasulyesi (*Phaseolus coccineus L*.) unu ve elde edilen izolatın aletsel renk ölçümleri yapılmıştır. İzolatın protein miktarı belirlenmiştir. İzolatın yağ, su tutma kapasitesi, emülsüyon ve köpüklenme özellikleri test edilmiştir.

Kompozisyon analizi neticesinde, Çanakkale bölgesinde yetişen Barbunya fasulyesi (*Phaseolus coccineus L)* kuru ağırlık üzerinden %4,8 kül, %2,86 nem, %27,01 protein ve %63,73 karbonhidrat içerdiği tespit edilmiştir.

İzoelektik çöktürme yöntemi ile elde edilen *Phaseolus coccineus L.* protein izolatı (PCLPI) için protein değeri, %2,37'lik standart sapma ile %83,53 olarak hesaplanmıştır.

Aletsel renk değeri ölçümleri, Barbunya fasulyesi (*Phaseolus coccineus L.*) unu ve izolatı için yapılmıştır. Barbunya fasulyesi unu için L* değeri izolata göre yüksektir, değerler ise sırası ile 89,763 ve 81,751 olarak ölçülmüştür. Diğer yandan, izolatın kırmızılık ve sarılık değeri undan fazla olarak belirlenmiş, değerler takiben izolatta a* değeri 0,253 ve b* değeri 10,783 iken unda a* değeri 0,173 ve b* değeri ise 6,907 olarak tespit edilmiştir.

Dondurularak kurutulmuş Barbunya fasulyesi protein izolatı (PCPLI) için su tutma kapasitesi, kuru ağırlık üzerinden 2,63 g su/g protein olarak ölçülmüştür. Yağ tutma kapasitesi ise kuru ağırlık üzerinden 3,95 g yağ/g protein olarak belirlenmiştir. Barbunya fasulyesi *(Phaseolus coccineus L)* protein izolatının köpüklenme kapasitesi ve stabilitesi sırasıyla %247,000 ve %132,000 olarak ölçülmüştür. Barbunya fasulyesi (*Phaseolus coccineus L*) izolatının emülsiyon aktivitesi %50,957 kararlılığı ise, %100 olarak tespit edilmiştir.

Yüksek basınçlı homojenizasyon uygulaması 20-Psi (İnç karede pound) 4 veya 6 çevrim ve 40- Psi (İnç karede pound) 4 veya 6 çevrim; basınç ve döngülerinde yapılmıştır.

Yüksek basınç homojenizasyonun proteinler üzerindeki etkilerini incelemek için bazı fonksiyonel özellikler, bulanıklık, parçacık boyutu, ζ- (zeta) potansyeli, mikro ve ikincil yapıları araştırılmıştır. Yüksek basınçla homojenize edilen örneklerin köpüklenme kapasitesi ve stabilitesi kontrol numunesinde en düşük olarak tespit edilmiş ve değerler sırayla; %235,000 ve %88,100 olarak saptanmıştır. En yüksek değer 40 Psi- 6 Cycle uygulamasına ait olup köpüklenme kapasitesi %612,000, köpüklenme stabilitesi %82,500 olup, 40 Psi- 6 Cycle uygulamasına aittir.

Emülsiyon aktiviteleri %55,06 ve %52,8 aralığındadır. Emülsiyon kararlılığı tüm örnekler için %100 olarak belirlenmiştir. Ancak, yüksek basınç homojenizasyon uygulamasının emülsiyon aktivitesi ve stabilitesi üzerinde etkisi tespit edilememiştir.

Mikroakışkanlaştırılan örneklerin zeta (**ζ) -** potansiyeli, parçacık boyutu ve bulanıklık analizleri pH 2'den pH 10'a kadar ölçümlenmiştir. En düşük net yük, izoelektrik noktayı kapsayan pH 4'te ölçülmüştür, bu değer 0,924 mV olup 40 Psi- 6 Cycle uygulamasına aittir. En yüksek net yük ise -13,22 ve -14,25 mV ve pH 10'da olup kontrol ile diğer numunler arasında istatiksel farklılık gözlemlenmemiştir Bu çalışmada, en düşük bulanıklık değeri 0,138 nm au olarak 40 Psi-6 Cycle ait olup pH 10'da gözlemlenmiştir. En yüksek bulanıklık değeri ise 2,639 ve 2,853 nm au olup pH 5'tedir ve bu pH'da tüm numuneler istatiksel farlılık yoktur. Parçacık boyutu analizlendiğinde nötr pH ve sonrasında yüksek basınçlı homojenizasyon uygulamasının parçacık boyutunu istatiksel olarak indirgediği tespit edilmiştir. Ancak izoelektrik noktaya yakın pH değerlerinde, yüksek basınçlı homojenizasyon uygulamasının parçacıklar üzerinde kümelenme potansiyeline sebep olduğu ve parçacık boyutunda artış olduğu saptanmıştır. Elde edilen sonuçlara göre, en büyük çap 7920,700 nm olup, pH 4'te 40 Psi- 4 Cycle uygulamasına aittir. En düşük

ortalama çap ise 40 Psi- 6 Cycle uygulaması için ve 61,138 nm olup, fakat pH 8 değerindedir.

Yüksek basınçlı homojenizasyona tabi tutulan örnekler ve kontrol numunesinin mikroyapıları incelendiğinde, yüksek basınçlı homojenizasyon uygulamasının partikülleri daha küçük parçalara ayırdığı ve partiküllerin homojen dağılım gösterdiği gözlemlenmiştir.

Proteinlerin ikincil yapıları, fourier dönüşümlü kizilötesi (FT-IR) spektroskopisi kullanılarak incelenmiştir. FT-IR görüntüsü Barbunya fasülyesi protein izolatı (PCPLI) için absorpsiyon bandı 1635,47 cm-1'de vermiş, bu değerin amid I bölgesinde ve β-sheet yapısında olup, enerjinin %80'i peptid bağlarındaki C=O esneklik titreşimlerindedir. Yüksek basınçla homojenizasyon işlemi Barbunya fasulyesi izolatının ikincil yapılarında değişime sebep olmamıştır.

Sonuç olarak, yüksek basınçlı homojenizsayon uygulaması köpüklenme özelliklerini geliştirmiş, nötr pH ve sonrasında; partikül boyutunu ve bulanıklık değerini indirgemiş, zeta potansyelini artırmıştır. Ancak, yüksek basınçla homojenizasyon uygulamasının, farklı basınç ve çevrimlerde proteinlerin fizikokimyasal ve fonksiyonel özellikleri üzerindeki etkisi araştırılması önerilmektedir.

1. INTRODUCTION

In recent years, massive information on nutrition, medicine, and plant technology and biotechnology available due to ease to access rapidly and as a result it opens new doors about food and health concepts (Popescu & Golubev, 2012).

Proteins have important for nutrition, food technology, and food processing. During the preparation and production, storage, consumption; proteins give structural and desired properties to final products. Proteins interact with solvents, ions, polysaccharides and other proteins in food matrix and changes physical structure of foods (Demirci, 2011).

As told above, food proteins are important during processing, storage, preparation and consumption, which are called functional properties and effective on physical and chemical behavior of food system. Water and oil absorption, foaming, emulsification, gelation are among several critical functional properties of food proteins (Nakai & Modler, 1996).

The protein trend is internationally has gained too much popularity. More than last five years, all around the world new product launches with protein claims tripled. On the other hand, the plant-based protein segment is blooming, consumption rate regarding to protein rich products grown exponentially in last decade. Raising trends such as healthy living and weight management are boosted in the market. Consumers are ever more aiming for a responsible life style with a focus on health and wellness, including “natural”, “free from” and “sustainable” goods (Yavuz et al., 2016; Beneo, 2017). This exponentially, growing consumption of protein has urged the food industry and academia to look for sustainable sources of highly nutritious protein with low allergenic potential for food applications. Plants are highly existing in the nature, low cost of production and labor force comparing to animal sources, also due to reference of some customers based on their religious, choice’s or beliefs, (like as vegan, vegetarian) (Karaca et al. 2011).

Land plants are had included in human diet to give energy and nutrients for sustainable life style. However, the usage of plant proteins is less than expected level even if they are fairly cheap and abundant than animal proteins. In general plant, proteins are used as animal feed to them get back milk, egg, meat. When considering steps of obtaining protein from animal origin (conversion to plant animal protein), it is an inefficient method. The reason is maximum 15% of plant-sourced protein feeds turns to animal proteins for human consumption, the rest of 85% wasted. As result, food related animal protein production is in charge of food related environmental pollution. On the other hand, animal protein production needs 100 times water than the same amount of water during plant protein production.

The world population is growing dramatically and food security is one of the biggest concerns of agri-food industry and governments. Intelligent and effective conversation of plant sources to plant proteins can help to find energy efficient and environmentally friendly solutions to supply food for human nutrition.

As an example, the development of new meat analogue products, protein shakes and protein bars are very popular in recent years, with some of the most promising alternatives based on proteins from plant origin, as chickpea, soybean, pea, and the dairy substitutes market has expanded.

Plant protein-based meat and dairy substitutes might provide the same quality but lower cost products by lowering greenhouse gas emissions and destruction of forestland (Day, 2013).

Pulses gives energy and may have potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease, and lowering of LDL cholesterol. Pea, chickpea, bean and lentil have 17–30% protein content with variable quantity of essential amino acids. In the past decade, the proteins from a number of pulses have been confirmed to be a valuable protein source potentially developed as a kind of food ingredients, due to their good functional properties and nutritional feature (Liu et al., 2014).

Even though, there are many researches available regarding to pulses and *Phaseolus vulgaris* legume varieties, such as chickpea, pea, soybean, faba bean however there are limited or rare studies have been conducted regarding to beans, especially cranberry bean (*Phaseolus coccineus L.*) in the literature. Species belonging to the

family Leguminosae are commonly grown all around the World but also Turkey. Approximately, production of fresh beans are 638,532 tones, production of cranberry bean is 88,362 tons in 2017 in Turkey (Bügem, 2017).

Cranberry bean was stated as pinto bean (*Phaseolus vulgaris L. var. Pinto)* by Turkish scientists, that is an agricultural product in Turkey, and farmers grow and maintain local varieties in Turkish "Barbunya Fasülye" (Balkaya & Ergün, 2007). The cranberry bean, also recognized as scarlet runner bean, termed (*Phaseolus coccineus L.*). Cranberry bean takes its name from spotted red and ivory pinto markings, and can be grown easily in Nigeria (Aremu et al. 2010). It has been reported that cranberry beans are low in fat and full of with nutrients, and they do not have with flatulence-producing enzymes (Aremu et al., 2010). The cranberry bean is known for its creamy texture and flavor similar to chestnuts, and is a popular in northern Italy and Spain (Chen et al., 2016).

Protein isolation by novel methods is gaining interest to alter functional, physicochemical, and other properties. Microfluidization is an advanced dynamic high-pressure technique, which uses the combination of ultrahigh pressure and high frequency vibration, instant pressure, intense shear and cavitation, and for these reasons; it provides more energy density than conventional valve homogenization. It was stated that microfluidization both alter structure of globular proteins and transforms resoluble heat applied protein aggregates to smaller soluble aggregates. This technique applied to soy protein isolates and improved the solubility and functional attributes of soy protein isolates (Shen & Tang, 2018).

In the literature, there are limited studies investigating the effect of microfluidization on protein isolates from beans *Phaseolus vulgaris* species. Our objective is for this study to obtain protein extract from cranberry bean by isoelectric precipitation and determine their physicochemical and functional characteristics. After that, the obtained isolates will subject to high-pressure homogenization (microfluidization) and the effects of microfluidization on functional properties and physicochemical properties of isolates (*Phaseolus coccineus L.*) will be investigated.

2. LITERATURE STUDY

2.1 Pulses and Legumes

The family Leguminosae, acknowledged as legumes, involves 13.000 different species. The family is the third largest family inside flowering plants and the second in economic importance following the grasses (Sathe, 1996).

Despite the thousands of species only soybeans, dry beans, peas, chickpeas, broad beans, lentil, and cowpeas are widely grown for commercial purposes. The total world value for leguminous crops is thought to be approximately two billion US dollars per annum.

Soybean is by far the most widely produced legume. The soybean is undoubtedly the world's most valuable crop, used as feed by billions of livestock, as a source of dietary protein and especially its oil is used by millions of people and in the industrial manufacture of thousands of products. Because of this popularity, soybean is usually kept separate from the others (Sathe, 1996; Sparvoli et al., 2015). In the following, soybeans will not be used further to compare the composition of cowpeas with other legumes, as soybeans are very extensively studied and thus well known. Pea, chickpea, bean and lentil contain 17–30% protein with varying concentrations of essential amino acids.

Pulses supply energy, dietary fiber, protein, minerals and vitamins required for human health. Soluble and insoluble fiber, resistant starch, oligosaccharides, phenolic compounds, bioactive peptides, enzyme inhibitors, lectins and folate are bio actives materials, which are gained attention of scientists. Based on experimental and epidemiological tests, these components are direct relationship prevention and/or regulation of chronic degenerative diseases (Vega et al., 2012). The most related bioactive property of peptides is the ACE inhibition. ACE (angiotensin I-converting enzyme) is a critical enzyme to control of the blood pressure pathway. It catalyzes the conversion of angiotensin I to angiotensin II. Angiotensin II has a dual responsibility in raising blood pressure: acts as a vasoconstrictor, and degrade a

vasodilator, named bradykinin, thus contributing to elevate blood pressure (Los Basso, et al. 2018).

The main proteins in pulses are albumin and globulin. Albumin is water soluble. However, globulins are salt soluble and approximately establishes 70% of legumes (Karaca et al., 2011). Other minor proteins found in pulses include prolamins and glutelins. Prolamins are alcohol soluble and are characterized by a high proportion of proline and glutamine. Glutelins, on the other hand, are soluble in dilute acid or alkali detergents, and in the presence of chaotropic or reducing agents (Boye et al. 2010). Legumin is a hexameric protein with an average molecular weight of 300–400 kDa whereas vicilin is a trimeric protein with a molecular weight between 150 and 180 kDa (DerbyshireWright &Boulter, 1976). Disulfide bridges link the α- and β-chains of legumin, and hydrophilic α-chains are located at the surface of the molecule while hydrophobic sections are located at the interior, minimizing their contact with water (Karaca et al., 2011). Table 2.1 is present proximate % composition of legumes (Sparvoli et al., 2015; Bouker et al. 2015; USDA, 2016).

Table 2.1 : Proximate % composition of legumes (Sparvoli et al., 2015; Bouker *et al.* 2015; USDA, 2016).

Species	Protein	Fat	Ash	Carbohydrates
Cowpea	25-36	1-1.3	2-3.5	56-68
Lentil	20.6-32	1-2.1	2.5-3.4	54-58
Pea	21.9-31	1.3-3	2-3.3	52-62
Chickpea	16-28	3.1-7	2-3.5	54-66
Common bean	20.9-30.1	1.3-2.5	1.8-3.2	54-64
Faba bean	24.3-32.3	1.1-4	2.2-2.8	57-60
Pigeon bean	15.9-24.1	1.2-1.6	4-4.5	57-58
Mung bean	23.3-27.7	0.7-2.4	3.2-4	61-62

If legumes are regularly in daily diet, they provide so many bioactive components such as enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds (Singha et al., 2017). Lectins of vegetable origin possess a great variety of biologic activities including cell agglutination, mitosis, toxicity and inhibition of cell growth. Several types of lectin have shown to induce cell death of cancer cells, suggesting that they may have an application for cancer treatment. Carcinogenic cells

secrete or express glucoconjugates with abnormal glycan structure and lectins may detect such changes (Popescu et a. 2012). Free radicals and metal ions damage biological systems. Antioxidant deficiency leaded to the occurrence of hypertension and other degenerative diseases. Antioxidants due to their radical scavenging as well as metal chelating activity, substances are like phytic acid which has antioxidant and DNA protective that against to cancer. Bioactive peptides may be coded in a larger protein's amino acid sequence, and usually consist of 3-20 amino acids released from the original protein after degradation.

Phenolic compounds, flavonoids and phenolic acids provide antioxidant and other specific properties and galactooligosaccharides may employ prebiotic activity (Vega et al., 2010). On the other hand, phenolic compounds not only function like bioactive compounds and are also responsible of particular color, taste and flavor of foods (Kumar et al., 2017). Specifically, beans are broadly spread in the world among the pulses, they are rich in multiple bioactive compounds which are under specific attention due to increasing demand on new style of nutrition is towards improving health. The protein present in the beans meets the minimal need of human requirements endorsed by the World Health Organization and Food and Agriculture Organization. Thus, 100 g of dry common beans serve in human provides about 9–25 g of protein, which is almost 20% of the recommended daily consumption for a normal adult. The bioactives consist of soluble and insoluble fiber, resistant starch, oligosaccharides, phenolic compounds, bioactive peptides, enzyme inhibitors, lectins and folate. Bioactive peptides have also been isolated from various proteins using some digestive enzymes and different combinations of proteinases, including Alcalase®, chymotrypsin, pancreatin, pepsin and thermolysin, as well as enzymes from bacterial and fungal sources. Low saturated fat content complimented by their high essential nutrients and phytochemical composition could account for the many health benefits associated with bean consumption (Chen et al., 2016). Figure 2.1 shows health promoting effects of polyphenol-rich dry common beans.

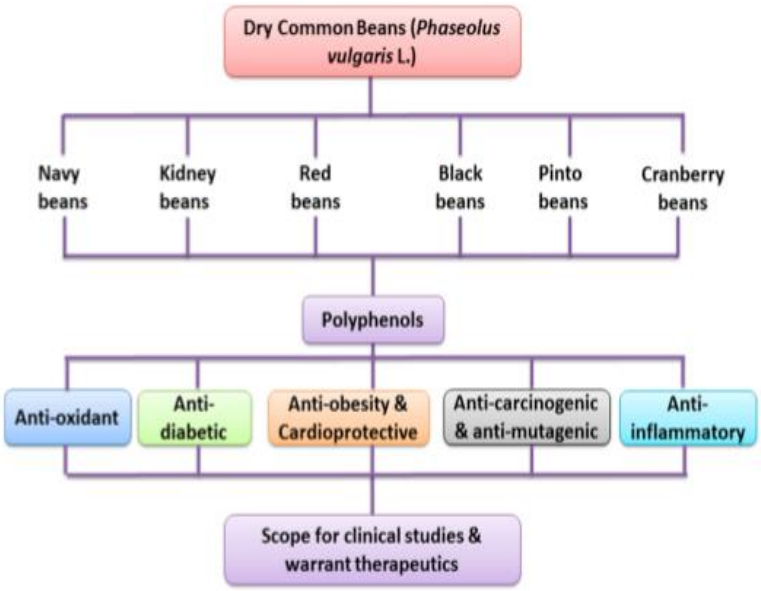

Figure 2.1 : Health promoting effects of polyphenol-rich dry common beans (Kumar et al., 2017).

2.1.1 Cranberry bean (*Phaseolus Coccineus L.*)

The genus *Phaseolus* includes several wild and cultivated species, such as *P.coccineous L.*, known as also known as Scarlet runner bean a species of family Fabaceae, gets its name from its spotted cranberry red and ivory pinto markings, and can be grown easily in Nigeria. Its introduction into southern Colombia (Antioquia and Narino) and Europe may have occurred in the 17th century before reaching other parts of the world, such as the Ethiopian Highlands. It has been found in archaeological remains only in southern Mexico, in Durango and Pueblo, and wild only in Tamaulipas (Aremu et al. 2010 & 2005). According to the Ontario Bean Growers Association, cranberry beans are the second most produced variety of dry beans in Ontario covering 14.000 acres of land (Chen et al., 2016).

On the other hand, in Turkish culture beans are important for by replacing animal protein as an economical alternative and consumed indulgently by consumers (Bügem, 2017). The cranberry bean is known for its creamy texture and flavor similar to chestnuts, and is a favorite in northern Italy and Spain. It has been reported that cranberry beans are low in fat and loaded with nutrients, and do not contain flatulence-producing enzymes (Aremu et al., 2010).

Catechins and procyanidins represent almost 70% of total phenolic compounds in cranberry beans (seed coat). The total essential amino acid (TEAA) values

approximately 48.31% in red specks scarlet runner bean. The major amino acids exist in the dry beans are lysine (6.5–7.5 g/100 g protein) and tyrosine with phenylalanine (5.0–8.0 g/100 g protein) (Kumar et al, 2017). Table 2.2 presents, amino acid profile of *Phaseolus coccineus* as compared with conventional protein sources of Food and Agriculture Organization of the United Nations (FAO) (g per 100 gr protein).

Table 2.2 : Amino acid profile of *Phaseolus coccineus* as compared with conventional protein sources of Food and Agriculture Organization of The United Nations (FAO) (g per 100-gr protein).

Source	FAO	Egg	*Phaseolus coccineus*	Soybean
lle	4.0	6.6	3.8	5.3
Leu[a]	7.0	8.8	6.6	7.7
Val[a]	5.0	7.2	3.3	5.3
Lys[a]	5.5	5.3	3.1	6.4
Phe[a]	6.0	5.8	4.0	5.0
Tyr		4.2	3.3	3.7
Met	3.5	3.2	1.9	1.3
Cys		2.3	0.3	1.9
Try	1.0	1.7	nd	1.4
Thr[a]	4.0	5.0	3.2	4.0
Ala			3.4	5.0
Arg[a]		6.2	3.9	7.4
Asp[a]		11.0	4.8	1.3
Glu		12.6	13.3	19.0
Gly		4.2	2.9	4.5
His[a]		2.4	2.0	2.6
Pro		4.2	3.0	5.3
Ser		6.9	3.1	5.8

*The Values belong to *Phaseolus coccineus obtained from the article* (Aremu, 2016).
[a]Essential amino acid. "nd = not determined."

2.2 Proteins

2.2.1 General information about proteins

Proteins are complex macromolecules that establish approximately 50% of a living cell. “Protein” name comes from Greek word “proteios” which means “initial” and “holding the first”. In Latin language the word “protein” is correspondence of “essential element for living organisms” (Saldamlı, 2007).

Proteins consist of from organic polymers, which are carbon, hydrogen, oxygen, and nitrogen. In addition to these polymers, some proteins contain also metal ions such as copper, iron, phosphorus, or zinc. There are 20 different kinds of amino acids in the structure of proteins. The amino acids contain at least one amino group (-NH2) and at least one carboxyl group (-COOH) (Demirci, 2011). Amino acid is made of an amino group (NH2), a carboxyl group (-COOH), a hydrogen atom and distinguishing group (-R group) all bonded to single carbon atom which is called α- carbon. R group is determinates of physicochemical properties of amino acid and specifications of relevant protein. Amino acids are linked by peptide bonds and this bond is called covalent bond. A peptide bond is formed between α- carboxyl group and α- amino group of two amino acids. Because of this chemical reaction water will occur. In this binding style, more than 100 amino acids can build up polypeptides chains which have different molecular weight and dissimilar from each. The amino acid sequence, order number, and length of polypeptide effect to physical, structural, biological and functional properties of proteins (Fıratgil-Durmuş, 2008).

Proteins are necessary for nutrition and also food technology and food processing. The main tasks of proteins to provide energy like as carbohydrates and fats while contributes to formation of muscle and some enzymes for body. Human body needs nitrogenous compound and for this reason, it is essential. If malnutrition occurs, proteins are decomposing for provide energy. Consequently, Marasmus and Kwashiorkor diseases arise (Demirci, 2011).

During the preparation and production, storage, consumption of proteins gives structural and desired properties. Proteins interact with solvents, ions, polysaccharides, lipids and other proteins in food matrix and changes physical structure of foods. Protein solubility, water and oil binding/absorption capacity, viscosity, foaming and foaming stability, emulsification, gelation are critical

functional parameters, which are role on final product quality (Demirci, 2011). Also, denaturation changes hydrophilic/hydrophobic ratio on protein surface and this situation effects protein solubility (Dündar, 2016). Table 2.3 presents functional roles of food proteins in food systems that are influence, the functional properties of proteins in foods.

Table 2.3 : Functional roles of food proteins in food systems (Schwenke 1996).

Function	Mechanism	Food System	Protein Source
Solubility &Viscosity	Hydrophicity, water binding, hydrodynamic size, shape	Beveregas, soups, gravies, salad dressings	Whey proteins
Water Binding	H-bonding, ion hydration	Meat sausages, cakes, breads	Muscle proteins, egg proteins, milk protein
Gelation	Water entrapment, immobilization, network formation	Meats, gels, cakes, baked goods, cheeses	Muscle proteins, egg proteins, whey proteins
Cohession, adhession	Hydrophobic, ionic and H-bonding	Meats,sauces, pasta, baked goods	Muscle protein
Emulsification	Adsorption at interfaces,film formation	Sausages, bologna, soups, cakes, dressings	Muscle proteins, egg proteins, milk proteins
Foaming	Interfacial adsorpsion, film formation	Whipped toppings, ice cream, cakes, desserts	Egg proteins, milk protein
Fat and Flavour Binding	Hydrophobic bonding, entrapment	Sitimulated meats, baked goods, doughnuts	Milk proteins, egg proteins

2.3 Classification of Proteins

2.3.1 Classification of Proteins according to chemical composition

According to chemical composition, proteins are in two different categories, simple and conjugated proteins. Simple proteins only have one amino acid. Conjugated protein has amino acid plus organic inorganic groups. Globulins, albumins are examples of simple protein. On the other hand, phosphoproteins, lipoproteins, nucleoproteins belong to conjugated protein class (Owusu-Apenten, 2004).

2.3.1.1 Classification of proteins by source

Proteins from animal origin are (meat, fish, poultry, eggs) are common. Plant proteins are less common comparing to animal sourced ones. In addition, emerging sources of dietary protein include leaf and microbiological proteins, algae (Chlorella, Scenedesmus, Spirulina spp.), yeasts, and bacteria (single-cell proteins) (Owusu-Apenten, 2004; Tahergorabi, and Hosseini, 2017).

2.3.1.2 Classification of proteins by shape

Proteins can be classified according to shape as globular and fibrous. Globular proteins are look like sphere-shaped and this of result of polypeptides are folded into globular or spherical shape. Globular proteins are soluble in water or salted water and sensitive to heat, acid and alkali. Globular proteins have specific catalytic activity and they are building blocks of enzymes. Casein, hemoglobin, ovalbumin, myoglobin are examples of globular proteins. Fibrous proteins are and neighbor amino acids cross-linked each wrapped tightly, insoluble in water and not sensitive to alkali and acids. However, fibrous proteins are instable to heat. Their role is building structure like tendon, muscle, skin, cell organelles. Gluten, elastin and collagen are under group of fibrous proteins (Gögüş and Fadıloğlu 2005; Owusu-Apenten 2005; Fıratgil Durmuş, 2008).

2.3.2 Classification of protein by solubility

Thomas Burr Osborne categorized proteins in five classes according to their solubility in range of solvents. Albumins soluble in water, globulin soluble with dilute salt, prolamin soluble in ethanol-water solvent, glutelin soluble in with dilute

alkali and residue protein soluble in alkali, urea, and disulfide reducing agents (Owusu-Apenten 2004).

Amino acids behave as amphoteric molecule. It means, it has positive and negative charges the same molecule and accordingly pH of medium it both shows as acid or base characteristic. This constitution called as zwitter ion or dipolar ion. The pH when molecules are in dipolar form, this pH called as isoelectric pH. Dipolar ions are neuter, as consequence they cannot move in electrical field. Proteins show minimum solubility at isoelectric point and they can be precipitated very easily. Different amino acids and proteins show different isoelectric point (Fıratgil Durmuş, 2008).

2.4 Protein Structure

Each protein has a specific 3–dimensional shape. The biological activity of protein alters on its three dimensional shape. Protein structure usually declared as having four levels organization primary, secondary, tertiary, and quaternary structure. Proteins with similar primary structure have similar conformations and functions. Primer structure is a kind of transition to three-dimensional shape. Protein denaturation expressed as destruction of secondary, tertiary or quaternary however primary structure and amino acid arrangement remains the same. Denaturation alters physical or biological aspects without rupture of peptide bonds; as result of denaturation, liberalization occurs between cross-linked bonds. Denaturation process might be reversible however, if the disulfide bonds were destroyed, denaturation cannot be reversible (Gögüş & Fadıloğlu 2005; Lin et al. 2012).

2.5 Functional Properties of Food Proteins

Functional properties of food proteins expressed as the physical and chemical properties, which are influence on protein behavior during production, storage, and consumption of food products. Protein structure, shape and surface hydrophobicity, hydrophobic/hydrophilic ratio, etc.), aa acid composition, extraction and drying processes are several factors which mainly determinates functional properties. Solubility, water and oil absorption capacity, foaming and emulsifying, gelling are important functional features of food proteins and takes role constitution of food texture, organoleptic characteristics for confectionery, beverage, sauce and meat

products (Yavuz et al., 2016; Boye et al. 2010; Demirci, 2011). The factors (instrinsic, environmental, processing) are influencing functional properties of proteins are indicated at Table 2.4.

Table 2.4 : Factors influence the functional properties of proteins in foods (Schwenke,1996).

Intrinsic	Environmental Factors	Processing Treatment
Composition of protein(s)	pH	Heating
Conformation of proteins	Oxidation-reduction status	Drying
Mono or multicomponent	Salts	pH
	Water	Ionic strength
	Carbohydrates	Reducing agents
	Lipids	Storage conditions
	Surfcatans	Chemical modification
	Flavours	

2.5.1 Protein solubility

Protein solubility is a physicochemical property and it has affected by many parameters including pH, temperature, ionic strength, hydrophobic residues, denaturation, temperature, electrostatic repulsion, condition and methods for protein extraction (Ahmed, 2013; Moure et al., 2016; Feyzi et al., 2017). The first condition for the proteins to be functional is solubility (Yavuz et. al., 2016). Solubility of protein is the amount of dispersed nitrogen under specific conditions. Pulse proteins generally show high solubility trend at high pH values and less soluble at very low pHs. Solubility dramatically decreases close to the isoelectric point generally between pH 4 and pH 6 for most pulses. Usually, minimum protein solubility occurs at the PI. Mostly, protein solubility curve is "U" shape for most pulse proteins (Hull, 1996; Fıratgil- Durmuş, 2008).

2.5.2 Water and oil binding capacity

Protein can interact with water and oil because of its hydrophilicity and hydrophobicity in food matrix (Ahmed, 2013). Water binding or holding capacity can be expressed as the total water absorbed by a food material. Water binding capacity is very important in food chain and new product development as well. If in the formulation there is not sufficient water is hold in food matrix serum separation occurs but in case of reverse condition, the sample get dry. The determination of water holding capacity can be measure by suspending water the protein powder then emptying the water by centrifuging technique. Water holding capacity can be predicted with amino acid composition, which is related to proteins and very close

relationship with amino acid profiles. Additionally, ionic charge residues, pH, temperature, ionic strength, and protein concentration change water-holding capacity (Hürriyet, 2014; Fıratgil Durmuş, 2008).

Oil binding capacity is effects quality parameters of foods. Hydrophobic amino acids in protein configuration can be promote for oil binding ability. Fat or oil binding capacity is calculated as the weight of oil absorbed per weight of protein powder or legume flour.

Fat/oil binding property of protein powder is also related to particle dimensions of the powders. Comparing to high having density protein powders with having low density and smaller particle size ones; low density and smaller particle size protein powders absorb much more fat than higher dense protein powders (Hürriyet, 2014; Fıratgil Durmuş, 2008). Most importantly, oil-binding mechanism gives information about protein microstructures (Hürriyet, 2014).

2.5.3 Emulsifying properties

Emulsion is two phase systems and prepared by solving or mixing two immiscible liquids as tiny (0.1 and 100 μm) droplets through the matrix of second liquid. It called emulsifying agent (Al-Malah et al., 2000, Dündar, 2016). In addition, emulsion formation is correlated with surface activity and emulsifiers decrease interface tension and helps to enhancing stabile oil-water and air- water surface development (Hürriyet, 2014). Emulsifying property is important for many food applications such as soups, salad dressings, mayonnaise, and cakes. Emulsifying characteristic is highly influenced by the pH, ionic atmospheres, protein type, protein quantity, solubility, viscosity, variation in processing pre-treatment of the proteins and thermal processing of emulsion-based foods also external factors (Fıratgil Durmuş, 2008; Day, 2013; Hürriyet, 2014). Among them, solubility is the one of the important attribute of proteins among the other emulsifying features. Even the protein has less solubility; it forms weak emulsion stability (Ahmed, 2013).

2.5.4 Foaming properties

In food industry foaming ability is important for whipped cream, cake, bread, ice creams, mousses (Hürriyet, 2014). Foams are also two-phase systems; air cells are continuously separate from liquid phase with thin layer. There are three steps during

the foam formation. The initial step is starts with diffusion of globular proteins to air/water surface that is decreasing surface tension. The next step is orientation polar parts that close to water then the folded shape proteins unfold at surface. The last step is polypeptides influence each other to establish film by denaturation and coagulation (Fıratgil Durmuş, 2008, Hürriyet, 2014). Foaming capacity and stability are terms, which indicate foaming ability and durability (Boye et al., 2010). Protein type, protein extraction method, processing temperature and time, protein quantity and pH are several important factors influence foaming properties (Ahmed 2013).

2.5.5 Other properties

Gelling properties of proteins are very important for food products in comminuted sausage products, oriental textured foods, like tofu, fruit gels, fillings for pastry, bread doughs (Zhang et. al., 2016; Fıratgil- Durmuş, 2008).

Thermal properties are another important physicochemical parameter of proteins that are effect protein functionality. Differential scanning calorimetry (DSC) is very useful and effective technique to investigate thermal transformations and denaturation (Hürriyet, 2014; Rui et al., 2010). Denaturation temperatures for bean proteins reported in the literature range from 84 °C to 91 °C, with kidney beans (Tang, 2008). Infrared spectroscopy is one of the oldest and well designed and helps to analysis of secondary structure of analyses proteins. It is non-destructive while requiring less sample preparation and it is easy to adapt different conditions (Kong, 2007). Fourier transform infrared (FT-IR) spectroscopy technique gives information to understand changes of protein secondary structure. The principle of FT-IR is the absorption capacity of infrared radiation by sample and wavelength (Hürriyet, 2014).

2.6 Protein Extraction Methods

To be able to study on structural and functional properties of proteins, protein should be separated from the other proteins and non- proteins. The first stage of protein obtaining is extraction of raw material in appropriate solvent to get protein rich product (Fıratgil Durmuş, 2008).

According to Boye et al., (2010) alkaline extraction/isoelectric precipitation, acid extraction, water extraction, salt extraction (micellization) and ultrafiltration,

methods are used to extracting pulse proteins. Alkaline extraction by isoelectric precipitation is going to described briefly in following sub-section.

2.6.1 Alkaline extraction/ isoelectric precipitation

Aqueous alkaline extraction followed by isoelectric precipitation is another commonly used procedure for the extraction of legume proteins. The technique bases on the solubility of legume proteins, which is high at alkaline pH and low at pH values close to their isoelectric point (pH 4–5). Figure 2.2 shows schematic diagram of alkaline extraction and isoelectric precipitation process for production of pulse proteins extracts (Boye et. al., 2010).

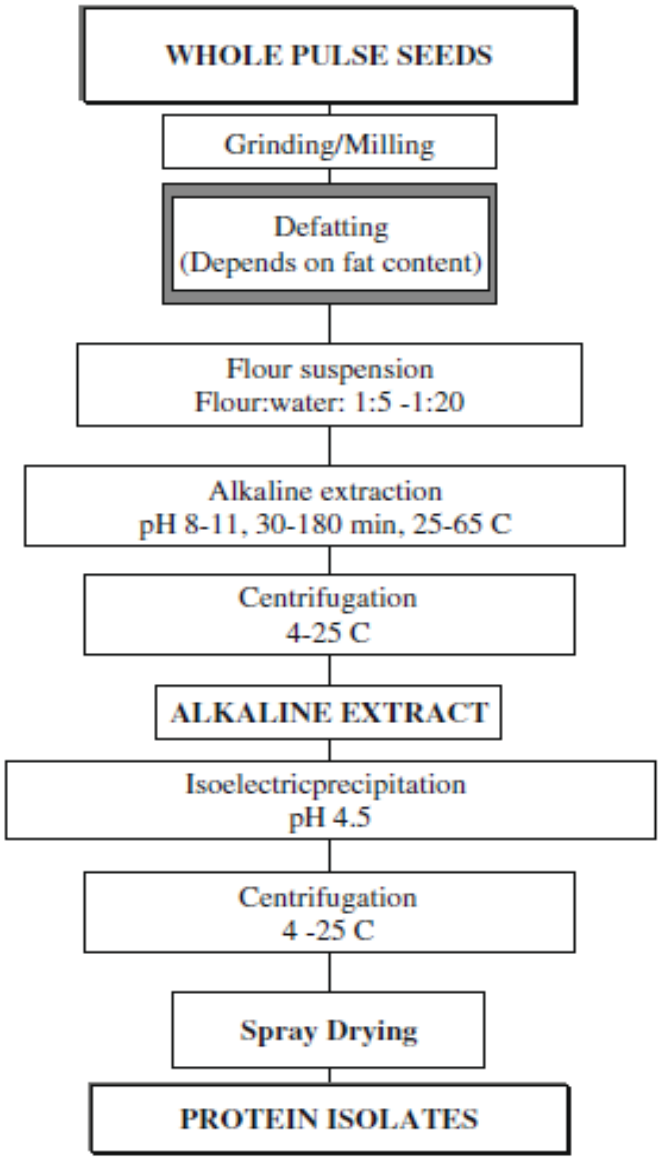

Figure 2.2 : Schematic diagram of alkaline extraction and isoelectric precipitation process for production of pulse protein (Boye et al., 2010).

2.7 Microfluidization (High Pressure Homogenization)

Microfluidization is a mechanical technique applying high-pressure 150-200 MPa, or 350-400 MPa for ultrahigh pressure system able to generate high velocity- micro streams (Mc Carthy et al., 2016; Stang, 2001). Particles or droplets pass through a microchannel in the interaction chamber and while treatment carried on, particles are under upper high shear stress and force as a result rapid pressure release size reduction and loosened microstructure of solid particles come up. Even it is non–thermal process, temperature increases, due to of the heat of compression and enhanced by shear effects and partial conversion of mechanical energy into heat. The result is a total temperature increase of 17-21°C per 100 MPa. This needs to be controlled in case of heat sensitive applications (Stang, 2001). Recently, microfluidization have been used for improving rheological characteristics of fruit juices; improving the properties of polysaccharides) and proteins. Moreover, in food industry microfluidization treatment is used for homogenization and deagglomeration purpose as well (He, et. al. 2016).

The microfluidization technique successfully improved the solubility and surface proteins of soybeans isolates (Shen and Tang, 2017). Koo et al., (2017) investigated effect of microfluidization on denaturated whey protein fibrils and the results showed that rheological properties of the protein fibril solutions altered. Sarıcaoğlu et al., (2017) studied effects of high-pressure homogenization on deboned chicken meat proteins and the findings showed that high-pressure homogenization a valuable technique, which could improve functional and rheological properties of chicken meat proteins. High-pressure treatment altered surface hydrophobicity and the reactive sulfhydryl group content of sarcoplasmic proteins at exceeding 200 MPa. Dong, et al., (2011); Sarıcıoğlu et al., (2017) reported that the HPH treatment improved the functional properties such as emulsifying activity index, foaming capacity, and water-holding capacity of peanut protein. Also, HPH has been improved total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of potato peel (Zu, et al 2016). Figure 2.3 shows picture of microfluidizer which is used in this study.

Figure 2.3 : Microfluidics, USA. Model: 1101 Serial: 2011169.

2.8 Fourier Transform Infrared (FT-IR) Spectroscopy

Infrared spectroscopy is one of the oldest and well-designed technique that helps to analysis of secondary structure of proteins. It is non-destructive while requiring less sample preparation and it is easy to adapt different conditions (Kong & Yu, 2007). The principle of FT-IR is the absorption capacity of infrared (IR) radiation by sample and wavelength (Hürriyet, 2014). Polypeptides and proteins show nine specific IR absorption bands. This specific bands names are amid, A, B and I-VII. If FT-IR technique has applied appropriately, it can provide information about secondary structure, analysis conformational changes, structural dynamics and stability studies of proteins (Kong & Yu, 2007).

3. MATERIALS AND METHODS

3.1 Materials

Cranberry beans (*Phaseolus coccineus L.*) were bought from local market, which were grown in Çanakkale province at Turkey.

3.2 Methods

3.2.1 Determination of chemical composition of *Phaseolus coccineus L.* flour

Moisture, ash, protein and lipid content of dried flour determined in accordance with AOAC methods. Total nitrogen content was determined by using Kjeldahl method. Method of Barbano et al., (1990); Firestone, (1990) was used with slight modifications. The chemicals were hydrochloric acid, sodium hydroxide, hexane, sulfuric acid, copper sulfate and of potassium sulfate, boric acid, which were analytical grade.1g of sucrose was used as blank. 0.2 g of sample, 0.3 g of copper sulfate and 15 g of potassium sulfate was weighed and transferred into Kjeldahl digestion tubes and boiling stones were added. Then 25 ml of sulfuric acid (95-98%) was added. Digestion tubes were placed into digestion unit and they were heated at for 30 minutes then heated at 10 one hour. After the digestion step; test tubes were cooled to room temperature and 50 ml of distilled water was added. 25 ml of boric acid with 2 drop of methyl red and 3 drops of methyl blue indicators were put into erlenmeyer flask. Then, erlenmeyer flask and digestion tube were placed into distillation unit. After completing of distillation step, distillate was titrated with 0.2 N hydrochloric acid solutions and consumption was recorded. Total protein nitrogen content calculated with equation (3.1), 6.25 used as transition factor to converting nitrogen to protein, as and (3.2).

$$\text{Nitrogen (\%)} = \frac{0.014\text{g nitrogen x (mL HCL,sample} - \text{mL HCl,blank)x normality HCl}}{\text{g sample}} \times 100 \qquad (3.1)$$

$$\text{Protein (\%)} = \text{\% N} \times 6.25 \qquad (3.2)$$

Moisture content was determinate by drying samples at 105 ^{O}C until constant weight, approximately five hours (Firestone, 1990).

Soxhlet extraction method was used for Crude lipid analysis (Firestone, 1990).

Crude ash measurements were conducted according to AOAC Method 923.03, 1990 (Firestone, 1990). All the experiments were duplicated with two parallels. Carbohydrate content was estimated based on percent differential from 100%.

3.2.2 Protein Isolation from defatted *Phaseolus coccineus L.* flour

The protein isolates obtained by following the protocol with simple changes that was used by (Rui, et al., 2011). Sample was defatted by using method of (Stone et al at., 2015). Cranberry bean flours which was freeze dried and sieved was mixed with hexane that is three times of cranberry bean flour sample weight. Then slurry was mixed with magnetic stirrer at 500 rpm for 40 minutes. After the mixing step, hexane was decanting and this procedure was repeated extra two times. After the final defatting step, mixture was filtered by using whatman no.1 filter paper and samples were dried by air at the fume hood for 18 hours. Defatted flours weighed into the beakers 16 g then distilled water added until 240 g to obtain 15-fold ratio. pH increased to 9 with 0.5 M or NaOH and stirred 1 hour at room temperature. It was shaken 1 hour 40 rpm and 40 ^{O}C with shaking water bath (Classic C76, New Bruswink Scientific, USA). The samples centrifuged at 11000 rpm at 4 ^{O}C for 30 minutes. The centrifuged samples are stored 4 ^{O}C overnight to precipitation of starches. The supernatants were collected and pH decrease to with 1 M HCI to 4.5 and centrifuged at 11000 rpm at 4 ^{O}C for 30 minutes. Sediments were collected and washed 2 times 1:5 (w:w) distilled water and centrifuged 5000 rpm 10 minutes then excess of water removed by titling of falcon tube. Isolates were collected and freeze-dried.

3.2.3 Determination of protein content of PCLPI

Total nitrogen content of freeze-dried isolates was determined by using Kjeldahl method Firestone and 1990, Barbano et al., (1990) was used with slight modifications. Total protein nitrogen content calculated with equation (3.1), 6.25 used as transition factor to converting nitrogen to protein, as equation (3.2).

3.2.4 Color measurements

For measuring instrumental color values, Minolta CR–400 reflectance colorimetry used both freeze-dried PCLF and PCLPI samples. Color parameters measured according to CIE systems L*, a* and b* values determined. All measurements were duplicated. According to CIE system following values represent the colors in brackets L* = 0 (black), L* =100(white), a* value (+a*= red, -a* =green) and b* value (+b* =yellow, -b* =blue).

3.2.5 Microfluidization (High Pressure Homogenization)

Protein isolates obtained from Cranberry Bean was treated with microfluidizer according to method used by Sarıcıoğlu et al., (2018) with slight changes. Samples were diluted in distillated water as 2% (w/w) suspension. Then, suspension stirred with magnetic stirrer for 90 minutes, pH adjusted with 0.5 M HCI and NaOH to pH 8. The microfluidizer adjusted 20.000 or 40.000 Psi, 2% (w/w) protein solutions were loaded to chamber pipe at ambient temperature and passed chamber 4 cycle or 6 cycle. PCLPI 2% solution kept as control without any treatment for investigating effects of microfluidization. Sodium azide was added at 0.02% to all samples preserves them until further analysis.

3.2.6 Measurement of water absorption (WAC) and oil absorption (OAC)

The weights of experiment tubes are recorded and 0.1 g dry PCLPI weighted in experiment tube then 10 ml distilled water added and pH adjusted to 8 by using 0.1 M NaOH. Experiment tubes are subject to vortex to mix water and isolated properly for 1 minute then stored 30 minutes at room temperature. After that, tubes taken to centrifuge and the device adjusted to 5200 rpm for 20 minutes at room temperature, then supernatant removed.

Oil absorption capacity measured regarding to the method (Kumar et al., 2014). The weights of experiment tubes were recorded and 0.1 g dry PCLPI weighted in experiment tube then 10 ml sunflower oil added. Experiment tubes were subject to vortex to mix oil and isolated properly for 1 minute then stored 30 minutes at room temperature. After that, tubes taken to centrifuge and the device adjusted to 5200 rpm for 20 minutes at room temperature, then supernatant removed. Water absorption capacity measured regarding to the method (Kumar et. al., 2014).

The water absorption capacity was determined by using below equation (3.3).

$$\text{WAC/OAC (g of water/g of oil)} = \frac{W_2 - W_1}{W_0} \quad (3.3)$$

Where, Wo is weight of dry sample (g), W_1 is weight of tube and dry sample (g), W_2 is weight of tube and sediment (g) respectively.

3.2.7 Measurement of emulsifying and foaming properties

Emulsifying capacity and stability of freeze-dried PCLPIs determined by method of with slight changes (Chau, Cheung & Wong, 1997). 0.1 gr sample taken into beaker them mixed with 10 ml water pH adjusted to 8 by using 0.1 M NAOH. The mixture homogenized for 2 minutes, at room temperature high-speed blender (Ultra-Turrax T18 basic, IKA, Staufen, Germany). Then 10 ml sunflower oil added and again homogenized 2 minutes. Then slurry transferred to falcon tube and centrifuged at 3300 rpm at 5 minutes at 20 ^{O}C. To determinate emulsifying stability, after the emulsification, samples were heated at 80 ^{O}C for 30 minutes, and then falcon tubes centrifuged at 1200 g for 5 minutes.

To be able to compare and measure emulsifying capacity and stability of microfluidized samples as dilution/suspension form, the protocol used which were used by Shevkani et al., (2015); Chau, Cheung & Wong, (1997) with some modifications was followed. 2 gr of microfluidized solutions added to beaker and filled until 10 ml with distilled water and homogenized for 2 minutes, at room temperature high-speed blender (Ultra-Turrax T18 basic, IKA, Staufen, Germany). Then 10 ml sunflower oil added and again homogenized 2 minutes. After that, the same procedure as above followed for ES. The only difference was sample preparation between freeze-dried and microfluidized samples, the rest of the stages were same. Emulsion activities of samples were estimated by using below equations (3.4) and emulsion stability (3.5).

$$\text{EA}(\%) = \frac{\text{Height of emulsified layer}}{\text{Height of contents of tube}} \times 100 \quad (3.4)$$

$$\text{ES}\ (\%) = \frac{\text{Height of remaining emulsified layer}}{\text{Height of remaining emulsified layer}} \times 100 \quad (3.5)$$

Foaming capacity PCLPIs are measured according to method of Jarpa-parra et al., (2014). 0.1 g of PCLPI was mixed with 20 ml of distilled water and volume of mixture was recorded as volume before homogenization. Then it was homogenized

by using ultra turrax high-speed blender (Ultra-Turrax T18 basic, IKA, Staufen, Germany) for 2 minutes, and then volume was recorded. For the determining foaming stability after the homogenization step, the sample was kept at room temperature for 30 minutes and volume was recorded. To compare foaming properties of microfluidized samples as dilution/suspension form, the protocol used which were used by Shevkani et al., (2015) with some modifications. 2 gr of microfluidized solutions added to beaker and filled until 20 ml to obtain 2 mg/ml dilution. High-speed homogenizer (Ultra-Turrax T18 basic, IKA, Staufen, Germany) was used to mix protein solution, pH 8 for 2 minutes. For the determining foaming stability after the homogenization step, it was kept at room temperature for 30 minutes and volume was recorded. Foaming capacity was determined by equation (3.6). Foaming stability was defined by equation (3.7).

$$\text{FC}\ (\%) = \frac{\text{volume after whipping(ml)} - \text{volume before whipping(ml)}}{\text{volume before whipping(ml)}} x100 \qquad (3.6)$$

$$\text{FS}(\%) = \frac{\text{volume after standing(ml)} - \text{volume before whipping(ml)}}{\text{volume before whipping(ml)}} \times 100 \qquad (3.7)$$

3.2.8 Measurements of turbidity and microstructure

Microfluidized samples are diluted 1% (w/v) with deionized water, then pH adjusted from 2 to 10 with 0.1 and 0.5 M HCI or NaOH. Turbidity of microfluidized and control 1%(w/w) dilutions were measured spectrophotometrically as absorbance units (au) at λ 630 nm and ambient temperature, 300 μL sample placed in a 96- well microplate(Italy) then transferred Synergy HT (Biotek Inc) spectrophotometry. The measurements were duplicated. The results were recorded.

Microstructure of control and microfluidized samples are observed by a light microscope Olympus CX21 (Olympus, Japan) with 20-fold magnitude. Samples were adjusted 2% (w/w) to pH 8 placed between microscopic slides More than 5 images were taken. Among these, one of the most representative one were shown.

3.2.9 Zeta (ζ) potential and particle size measurements

Microfluidized samples were diluted 1%(w/v) with deionized water, then pH adjusted intervals of 2-10 with 0.1 and 0.5 M HCI or NaOH. A commercial instrument, which combined dynamic light scattering and microelectrophoresis (Nano-ZS, Malvern Instruments,Worcestershire, UK) is used to measure the droplet

size of particles and the ζ-potential of the samples. The measurements were duplicated.

3.2.10 Fourier transform infrared (FTIR) spectroscopy measurements

To determine effect of microfluidization on structural and composition changes and dynamics of cranberry bean isolate, FT-IR spectroscopy used as tool the method followed according to (L'Hoccine et al., 2007) with slight modifications. The measurements done putting sample of 100 µl(microliter) 2% protein isolate solution to ATR component. The active molecule groups were determined at where wavelengths pikes and vibrations formed.

3.2.11 Statistical data analysis

All measurements were reported means and standard deviations. For multiple comparisons, data were subject to the statistical analysis using Minitab software Versions 18, Minitab Pennsylvania, USA) for the analysis of variance (ANOVA) and Tukey tests.

4. RESULTS AND DISCUSSION

4.1 Chemical Composition of *Phaseolus Coccin*eus L. Flour

The results of proximate analysis showed that PCLF compose of 4.8 ± 0.09% ash, 2.86 ± 0.12% moisture, 27.01 ± 0.40% protein, and 1.53 ± 0.13% lipid and 63.73% carbohydrate content is by calculated by difference (Table 4.1). Table 4.1 provides the experimental results of PCLF proximate composition within dry basis.

Table 4.1 : Proximate composition of PCLF.

% Composition in Dry Basis				
Ash	Moisture	Protein	Fat	*Carbohydrate
4.82±0.09	2.86±0.12	27.01±0.4	1.53±0.13	63.73

*Calculated by percent differential from 100%. Data presented in this table consist of average values ± standard deviations.

Karaca et al. (2011) studied proximate analysis of several legumes. Chickpea flour had 16.71% protein, 5.74% moisture, 3.77% lipid, 2.72 % ash, 71.66% carbohydrate. Faba bean flour had 23.94% protein, 6.37% moisture, 0.73% lipid, 2.89% ash, 66.07% carbohydrate. Lentil flour had 18.43% protein, 6.22% moisture, 0.73% lipid, 2.56% ash, 72.066% carbohydrate. Pea Flour had 18.76% protein, 6.77% moisture, 0.89% lipid, 2.73% ash, 70.85% carbohydrate. Soy flour proximate composition was 45.41% protein, 6.23% moisture, 0.59% lipid, and 6.27% ash. The proximate composition of PCLF was close to previous work done by (Rui et. al., 2011). Rui et al., (2011) reported *Phaseolus vulgaris* legume varieties chemical composition; protein content of beans had been ranged between 22.36- 28.50%, low content of lipid 1.16-1.70%, ash content ranging around 4.25-5.09% and carbohydrate around 65.06-72.11% with dry basis. Especially, Cranberry Bean had the lowest protein content among other beans with 22.36%, red kidney bean has 27.07% following, and great northern bean has highest protein content 28.5% with dry basis.

Phaseolus coccineus L. is very abundant source of protein on the other hand it has low lipid content. There is slight difference between two *Phaseolus coccineus L*. or cranberry beans protein composition, which is growth in Canada the other is focus of

this study which growth in Turkey at Çanakkale province, the reason can be different geographical location and growth conditions or soil nitrogen, nutrition content. It might be seasonal differences as well.

4.2 Protein Content of PCLPI

In this study, protein content of PCLPI determined 83.53% with dry basis the standard deviation is 2.37%. Wani et al. (2015) reported nine kidney bean protein isolates protein quantities are 83.96–89.25% span, French yellow 76.96%, Cotendor 83.96%, Master bean 77.20% and Local red 80.40% had protein contents. Rui et al., (2011) also stated of *Phaseolus vulgaris* variabilities protein isolates which were obtained by isoelectric precipitation, great northern bean protein isolate had protein content of 89.25% (d.b.) were the highest protein although, cranberry bean protein isolate had the lowest 83.96%, (d.b.). Protein content cranberry bean isolate (PCPLI) in this study which is extracted by IEP is consistent with the earlier researched done by (Rui et al., 2011).

4.3 Color

Color values expressed as CIE unit for PCLF and PCLPI. Table 4.2 presents L*, a*and b* values of PCLF and PCLPI.

Table 4.2 : Color Values Expressed as CIE unit.

	PCLF	PCLPI
L*	89.763 ± 0.781^{a}	81.751 ± 0.834^{a}
a*	0.173 ± 0.116^{a}	0.214 ± 0.253^{a}
b*	6.97 ± 0.248^{b}	10.783 ± 0.593^{a}

*Means with standard deviations different letters within a line (a, b, c, d, e) are significantly different (Tukey, p =0.05). PCLF (*Phaseolus coccineus L. flour*), PCLPI (*Phaseolus coccineus L. isolate*).

Phaseolus coccineus L. flour shows L* value 89.763 which is higher than *Phaseolus coccineus L.* isolate with 81.751. However, the redness value and yellowness values of isolates higher than the flour a* value of flour is 0.173 and b* value is 6.907 while isolates a* value is 0.253 and b* value is 10.783. Figure 4.1 shows physical appearance of PCLF and PCLPIs. So, there is slight difference between the flour and isolate.

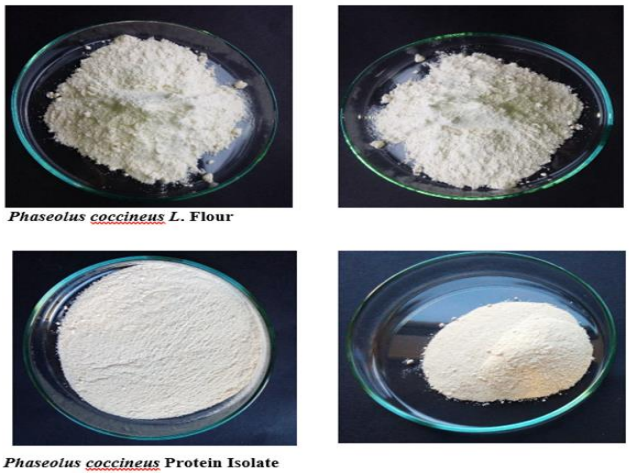

Figure 4.1 : Visual appearance of PCLF and PCLPIs.

4.4 Functional Properties of Freeze-Dried PCLPI and Microfluidized Samples

Functional specifications of freeze-dried PCLPI and Microfluidized Samples are given independently in Table 4.3 and Table 4.4 respectively. Table 4.3 provides information regarding to WAC, OAC, EA, ES, FC, FC of freeze dried PCLPI.

Table 4.3 : Functional properties of freeze-dried PCLPI.

WAC (g of water/g of protein isolate dw)	OAC (g of oil/g of protein isolate dw)	EA (%)	ES (%)	FC (%)	FS (%)
2.63±0.020	3.95±0.060	50.957±0.000	100	247±0.000	142±10.000

*Data presented in this table consist of average values ± standard deviations. PCLPI (*Phaseolus coccineus L. isolate*). WAC (Water absorption capacity), OAC (Oil absorption capacity), EA (Emulsion activity), ES (Emulsion activity), FC (Foaming Capacity), FS(Foaming Stability).

Table 4.4 shows information regarding to WAC, OAC, EA, ES, FC, FC of microfluidized samples with control.

Table 4.4 : Functional properties of Microfluidized samples with control.

	EA (%)	ES (%)	FC (%)	FS (%)
Control	52.98±3.25a	100±0.000a	235.000±8.240c	88.110±26.200b
20 Psi 4 Cycle	52.86±3.78a	100±0.000a	437.900±58.200b	178.300±36.400a
20 Psi 6 Cycle	52.80±2.38a	100±0.000a	397.500±70.900b	122.500±63.400ab
40 Psi 4 Cycle	55.06±2.19a	100±0.000a	488.900±28.800b	136.300±25.700ab
40 Psi 6 Cycle	54.90±3.92a	100±0.000a	612.500±47.900a	182.500±23.600a

*Means with standard deviations different letters within a column (a, b, c, d, e) are significantly different (Tukey, p = 0.05). EA(Emulsion activity), ES(Emulsion activity), FC (Foaming Capacity), FS(Foaming Stability). Psi (Pounds per square inch) presents pressure level.

4.4.1 WAC and OAC of freeze-dried PCPLI

According to results of the present study WAC of freeze- dried PCPLI is 2.63 g of water/g of protein given Table 4.3.

Chau et al. (1997) reported WAC of *Phaseolus angularis, Phaseolus calcaratus,* and Dolichos *lablab* seeds concentrates and compared with soybean, WACs were 5.05 g of water/g of protein, 5.28 g of water/g of protein, 5.08 g of water/g of protein and 3.46 g of water/g of protein respectively. African yam bean (*Sphenostylis stenocarpa* hochst. Ex a. Rich.) obtained by isoelectric and calcium precipitation and (WAC) was in the range of 2.0–2.33 g water/g protein Arogundade e*t al*. (2013). The values are very close to PCPLI obtained in this study to African yam bean (*Sphenostylis stenocarpa* hochst. Ex a. Rich.).

WAC is functional property of proteins that are important for soups, sauces, bakery products. PCPLI can be possible source for food systems due to its water absorption capacity.

In this study OAC detected 3.95 ± 0.06 g of oil/g of protein isolate on dw. Lupin protein isolate had 2.7–2.9 g oil/g protein OAC, mung bean protein isolate had 1.00–1.38 g oil/g protein OAC reported (Wani et al, 2015). OAC of Black gram bean, defatted lupin seed, and winged bean protein concentrates were reported to be 3.48, 3.89 and 4.01 g oil/g protein respectively (Chau et al., 1997). OAC of freeze-dried PCLPI close to defatted lupin and winged bean protein concentrates.

During food manufacturing and storing; OAC is plays very important role like as mayonnaise or similar products due to emulsifying capacity. EA has close correlation with oil absorption capacity. Additionally, meat extending, or replacement fat absorption of proteins are by providing mouth feel and retention of flavor (Saroğlu, 2016). PCPLI can be potential use in food industry as a functional ingredient due to its OAC.

4.4.2 Foaming and emulsifying properties of freeze-dried PCLPI and microfluidized samples

FC and FS result of PCLPI in freeze-dried and microfluidized samples are given in Table 4.4 and 4.5. FC, FS of freeze dried PCLPI is 247.400 ± 0.200% and 132.000 ± 0.101% subsequently. The values similar to the *Phaseolus coccineus L*. protein

isolates which were extracted by isoelectric precipitation and ultrafiltration Makri et al. (2005) and stated FC ~ 2.30, 4.00 and FS ~ 0.63 and 0.83 respectively (the data in original paper not given as %). *Spirulina Plantensis* protein under different conditions investigated and FC ranged 250% to 400% and FS were between 85% and 88.88% Saroğlu, (2017).

Microfluidized samples showed remarkable FC. The control sample is statistically different from microfluidized samples with lowest value. The highest FC observed 40 Psi-6 Cycle treatment which is 612.000 ± 47.900 %. The lowest FC and FC detected with control sample 88.100 ± 26.200 %, 235.000 ± 8.240% respectively.

Foams are helps to improve smoothness, lightness, and flavor of foods. Especially, applications requiring high foaming capacity and stability like as breads, cakes, marshmallow, whipped toppings, ice cream, and deserts (Ahmed, et. al., 1999; Omosuli, et al 2011). Experimental results based on current study support that, PCLPI might be great potential to find application in food products such as marshmallows, coffee whiteners, cakes, whipped toppings due to remarkable foaming properties.

The emulsifying activity of freeze dried PCPI were 50.957 ± 0.000 and stability was 100%. On the other hand, microfluidized samples with control had statistically same emulsifying activity and stability. The emulsifying activities were changing from 55.06 ± 2.1% to 52.80 ± 2.38%. This results are similar earlier researches for Lima bean protein isolates and canola protein isolates and rapeseed protein isolates 50-57%, %56.1%, 50.0% respectively (Yavuz, 2016; Hürriyet, 2016). Lafarga et al., (2018) published data regarding to Ganxet bean and the highest EC was at pH 8.0 and calculated as 69.4% and emulsion stability ~ 88%. However, EA values in that study lower than wheat protein isolate with 87%, soy protein isolates 87.5% and 69.4%.

The emulsifying stabilities were 100% for all samples in this study. In the literature wheat protein emulsion stability was 79% and rapeseed emulsion stability was 65% reported (Yavuz, 2016; Hürriyet, 2016). Microfluidization were not influenced EA or stability in this study. On the other hand, it stated that HPH negatively affected on EA and ES of Faba bean proteins (Yang, et al. 2018). However, PCLPI could be promising emulsifying agent food, which needs high emulsification and emulsion

stability such as meat analogs, soups, salad dressings, mayonnaise, and cakes, plant based beverages.

4.5 Turbidity of Microfluidized Samples

Turbidity has correlation with solubility and visual appearance of the different protein fractions. Therefore, to understand effect of different pH values on solubility turbidity measurement is such a useful and practical assay (Schmidt et. al., 2018).

Nahid et al., (2010) worked pigeon pea and hyacinth bean proteins. Turbidity of pigeon pea and hyacinth bean measured at OD 500 nm pH 2, pH 4, pH 6, pH 10, pH 12. Pigeon pea showed absorbance at 0.30, 0.70, 0.42, 0.26, 0.30, 0.24 nm au subsequently. However, hyacinth bean turbidity results are higher with 0.56, 1.5, 0.70, 0.24, 0.19, 0.19 nm au than pigeon pea. In this study, at pH 2 the least turbidity observed at 40 Psi-6 Cycle treatment with 0.29 6± 0.001 nm au and the highest value has been detected 0.736 ± 0.018 nm at 40 Psi-4 Cycle. Control has the lowest mean 1.084 ± 0.032 nm au at pH 3 and the highest value has been detected 1.423 ± 0.0521 nm au at 40 Psi-4 Cycle treatment. At pH 4 the least turbidity observed which are belong to both 40 Psi- 4 Cycle and 40 Psi- 6 Cycle treatments has the lowest mean 2.066 ± 0.016 and 2.112 ± 0.0104 nm au and the highest value has been detected 2.475 ± 0.059 nm au at 20 Psi-6 Cycle at pH 4. There is not any significant statistical difference between any samples at pH 5. Microfluidization were not influenced turbidity of PCPLI at closer isoelectric point. Control and 40 Psi-6 Cycle has statistically closer each other with higher turbidity and the rest of the tree treatments are exhibited at pH 6 the low turbid values. The highest turbidity belongs to control sample with 0.439 ± 0.085 nm au and the all microfluidization treatment lowered the turbidity of PCLPI at pH 7. The highest turbidity belonged to control sample with 0.326 ± 0.005 nm au and the lowest value has been detected 0.168 ± 0.003 nm au that was 40 Psi-6 Cycle at pH 8. All of the samples are different from each other at pH 8. The highest turbidity belongs to control sample with 0.328 ± 0.012 nm au and the lowest value has been detected 0.163 ± 0.020 nm au that is 40 Psi-6 Cycle at pH 9. Finally, at pH 10 the highest turbidity belonged to control sample mean with 0.304 ± 0.007 nm au and the lowest value has been detected 0.138 ± 0.009 nm au at 40 Psi-6 Cycle treatment.

Briefly, microfluidiziation influenced PCLPI suspensions, the least absorbance observed for 40 Psi-6 Cycle treatments with 0.296 ±0 .001 nm au at pH 2. When pH increased 4 the least absorbance observed which were belong to both 40 Psi-4 Cycle and 40 Psi-6 Cycle have the lowest mean 2.066 ± 0.016 nm au and 2.112 ± 0.0104 nm au, pH 6 lowest turbidity values belong to 20 Psi-6 Cycle and 40 Psi–4 Cycle respectively 2.470 ± 0 .015, 2.512 ± 0.027 nm au. The lowest value detected 0.138 ± 0.009 nm au at 40 Psi- 6 Cycle at pH 10. The data presented in this thesis are slightly different from literature. Microfluidization lowered the turbidity after neutral pH and far from the isoelectric point. Turbidity provides information the amount of colloidal material present in the suspension. The increase in turbidity is a reflection of an increase in flocculation degree. Hence, the maximum turbidity is supposed to be in the range of the minimal solubility of the proteins (Gerzhova et al., 2016). In respect to this information, solubility increase after neutral pHs and declined closed isoelectric point by effect of microfluidization for all treatments. Table 4.5 presents experimental results of turbidity values of control and microfluidized samples with control pH ranged from 2.0 to 10.0 at different pressure and cycles.

Table 4.5 : Changes in the turbidity of control PCLPI (1%) solution against to microfluidized PCLPI (1%) solution at different pressure and cycles with pH ranged from 2.0 to 10.0.

	Turbidity (AU/630 nm)				
pH	Control	20 Psi 4 Cycle	20 Psi 6 Cycle	40 Psi 4 Cycle	40 Psi 6 Cycle
2.0	0.416±0.049^{b}	0.420±0.018^{b}	0.380±0.018^{bc}	0.736±0.018^{c}	0.296±0.001^{a*}
3.0	1.084±0.032^{c}	1.363±0.053^{a}	1.227±0.059^{b}	1.423±0.0521^{a}	1.123±0.021^{c}
4.0	2.205±0.061^{bc}	2.301±0.061^{b}	2.475±0.059^{a}	2.066±0.016^{c}	2.112±0.0104^{c}
5.0	2.768±0.093^{a}	2.639±0.0207^{a}	2.853±0.134^{a}	2.651±0.038^{a}	2.767±0.059^{a}
6.0	2.852±0.224^{a}	2.540±0.032^{bc}	2.470±0.015^{c}	2.512±0.027^{c}	2.740±0.027^{ab}
7.0	0.439±0.085^{a}	0.228±0.005^{b}	0.251±0.003^{b}	0.223±0.003^{b}	0.190±0.03^{b}
8.0	0.326±0.005^{a}	0.226±0.007^{b}	0.242±0.009^{c}	0.202±0.048^{d}	0.168±0.003^{e}
9.0	0.328±0.012^{a}	0.214±0.000^{c}	0.234±0.012^{b}	0.221±0.010^{bc}	0.163±0.020^{d}
10.0	0.304±0.007^{a}	0.208±0.006^{c}	0.234±0.012^{b}	0.200±0.030^{c}	0.138±0.009^{d}

*Means with standard deviations different letters within a line (a, b, c, d, e) are significantly different (Tukey, p = 0.05). Psi (Pounds per square inch) presents pressure level.

4.6 Microstructure

The microstructure of protein isolate with 2% dilution form observed with light microscopy with magnitude of 20-fold ratio to evaluate effect of high-pressure microfluidization at different pressure and cycles on physical structure of PCLPI. Control sample has heterogeneous and compose group of different size and irregular shapes. Microfluidization treatment broke into fragments and more homogenous particles by reducing big cluster to small clumps (Figure 4.4).

Between the images 40 Psi-6 Cycle treatment, particles are smaller sizes, comparing the rest of the three treatments. 20 Psi-6 Cycle and 40 Psi-4 Cycle applications have similarities that might be different cycle effect even with different pressures. 20 Psi-4 Cycle has homogeneous shaped distribution but there are several large size particles observed. The findings are consistent with the earlier studies in literature. Hu, et al. (2011), had been published research effect of effects of microfluidization treatment on peanut protein isolates. Microfluidization caused peanut protein isolates formed smaller sized clusters due disruption of huge particle colonies. Figure 4.4 shows microstructure of protein samples that are 2% dilution form, determined by light microscopy magnitude of 20- fold ratio.

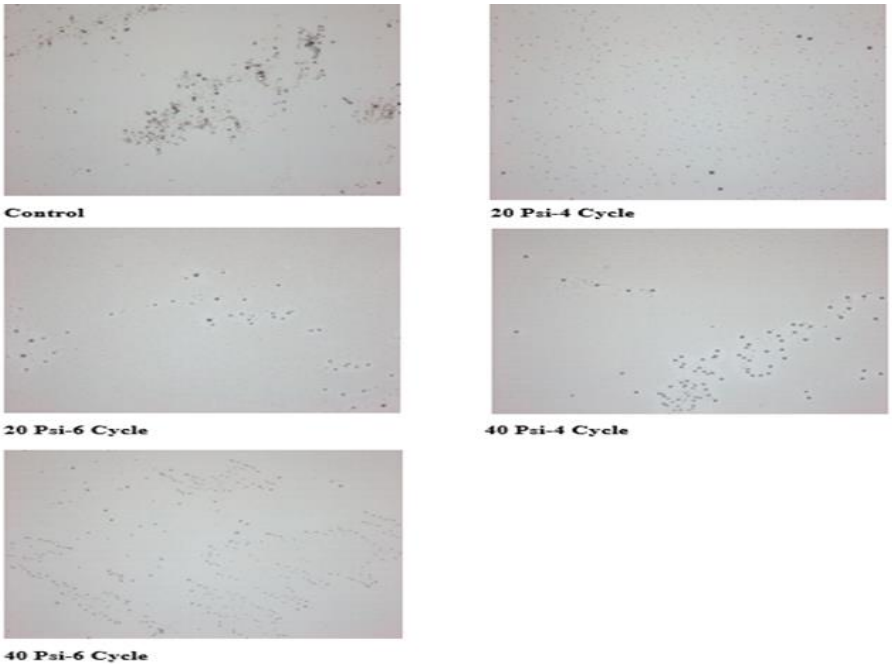

Figure 4.2 : Microstucture of protein samples that are 2% dilution form, determined by optical/light microscopy magnitude of 20-fold ratio. Psi (Pounds per square inch) presents pressure level.

4.7 Zeta (ζ) Potential

Proteins charges in the alkaline medium pHs and zeta potential values are negative on the other hand, below the isoelectric point values positive. Increasing the ζ-potential can lead higher tendency of dispersed particles, thus particles avoid crashing each other and colloidal system remains constant against unstable equilibrium processes such as flocculation, coalescence (Shariffa et al., 2016; Yin et al., 2009). In other words, escalating the surface charge on colloidal particles might strengthen the inter-particle electrostatic repulsion and disrupt existing protein aggregates and avoid agglomeration means higher zeta potential provides more stable dispersions (Chen et al., 2016; Song et al., 2013).

Table 4.6 indicates changes in the zeta potential of PCLPI (1%) solution with control against to micro fluidized PCLPI at different pressure and cycles. According the results of present study, net charge positive was positive at pH 2, control sample showed minimum ζ- potential that was 7.732 ± 0.338 mV, 20 Psi-6 and 4 Cycle treatments showed the highest ζ- potential values with 9.788 ± 0.168 mV and 9.928 ± 0.168 mV. At pH 4, minimum net charge was 0.924 ± 0.098 mV that belonged to 40 Psi-6 Cycle treatment and the other treatments including control was the statistically same with higher ζ- potential values. Net charge was negative at pH 5 and control sample showed maximum ζ- potential which was -7.868 ± 0.515 mV. The lowest values belonged to 20 Psi-4 Cycle, 40 Psi-4 Cycle and 40 Psi-6 Cycle respectively net charges were 5.533 ± 0.474, mV, -5.580 ± 0.159 mV and -5.463 ± 0.523 mV at pH 5. 40 Psi-6 Cycle showed highest ζ- potential, which was -11.875 ± 0.660 mV pH 6. And, the rest of the three treatments with control exhibited at pH 6 the lower ζ-potential. The minimum net charge was -11.067 ± 0.910 mV and belonged to 20 Psi-4 Cycle treatment at pH 7. Control sample with 20 Psi-4 Cycle, 20 Psi-6 Cycle, 40 Psi-6 Cycle statistically were the same at pH 7. In the literature black bean protein isolate shows ~ -12 mV net charge at pH 7 (Jiang et al. 2014) reported. The lowest net charge was -11.070 ± 0.410 mV and belonged to 40 Psi-4 Cycle treatments at pH 8. Control sample with 20 Psi-4 Cycle, 20 Psi-6 Cycle, 40 Psi-6 Cycle treatments were statistically do not any difference at pH 8.

The minimum net charge belonged control sample -12.033 ± 0.419 mV at pH 9. The highest values belonged to 20 Psi-4 and 6 Cycle 13.300 ± 0.497 mV, -13.800 ±

0.852 mV at pH 9. There was not any difference between each sample pH 10. Microfluidization were not influenced any samples pH 10 because PCPLI most probably reached the maximum solubility. To sum up, minimum net charge was observed at between pH 4 and 5 which, was in range of isoelectric point. In this study, beyond the isoelectric point, 40 Psi–6 Cycle can provide more stable colloidal system than control and the other HPH treatments as seen in Table 4.6.

Table 4.6 : Changes in the zeta potential of control PCLPI (1%) solution against to micro fluidized PCLPI (1%) solution at different pressure and cycles with pH ranged from 2.0 to 10.0.

	Zeta Potential (mV)				
pH	Control	20 Psi 4 Cycle	20 Psi 6 Cycle	40 Psi 4 Cycle	40 Psi 6 Cycle
2.0	7.732±0.338d	9.788±0.168ab	9.928±0.054a	8.395±0.401cd	8.957±0.651bc
3.0	2.633±0.188d	4.790±0.167c	6.908±0.474a	4.780±0.292c	6.010±0.349b
4.0	-1.530±0.357b	-1.620±0.151b	-1.423±0.151b	-1.228±0.227b	0.924±0.098a
5.0	-7.868±0.515c	-5.533±0.474a	-6.538±0.207b	-5.580±0.159a	-5.463±0.523a
6.0	-8.883±0.1791a	-8.258±0.288a	-8.790±0.369a	-8.697±0.605a	-11.875±0.660b
7.0	-12.367±0.386b	-11.067±0.9100b	-11.733±0.967b	-11.100±0.327a	-12.225±0.591b
8.0	-12.93±0.31b	-13.83±0.74b	-13.470±0.60b	-11.070±0.410a	-13.60±0.92b
9.0	-12.033±0.419a	-13.300±0.497ab	-13.800±0.852ab	-12.600±0.906b	-13.55±0.412b
10.0	-13.225±0.492a	-14.133±0.953a	-13.968±0.403a	-13.533±0.801a	-14.25±0.624a

*Means with standard deviations different letters within a line (a, b, c, d, e) are significantly different (Tukey, p =0.05). Psi (Pounds per square inch) presents pressure level.

4.8 Particle Size

It has been reported that application of microfluidization causes clusters disrupted and sites exposed if, multiple passes are performed. Chen et al. (2016) reported that the highest zeta potential of chicken myofibrils occurred for samples with smaller particle dimension (pressurized at 103 MPa). According to Porto et al. (2018) usually, at low pressures, a macroscopic damage of groups arises, but with no shift in the protein unit. However, at higher pressure, the protein network is destroyed, and causing to the development of small fragments. Sarıcıoğlu et al. (2018) applied high-pressure homogenization intervals of 0 to 150 MPa to hazelnut meal proteins. 0 MPa showed highest molecular size and 150 Mpa has the lowest size briefly. In general, this might be the higher the homogenization pressure, the less the mean particle sizes

obtained. In present study, the particles are aggregated at pH 5, pH 6 which are closer to isoelectric point and statistically there is difference between control sample and microfluidized samples. On the other hand, until and beyond pH 5 and pH 6 lower sized particles observed. Table 4.7. shows effect of microfluidization on particle size with different pressure and cycles. The lowest diameter belonged to 40 Psi-6 Cycle which had the lowest mean 104.768 ± 1.179 nm at pH 2. All the samples were statistically different from each other. At pH 3 the smaller diameter belonged to 20 Psi-6 Cycle has the lowest mean 751.700 ± 35.300 nm and the highest value detected 6950.000 ± 356 nm at 40 Psi-4 Cycle. The smallest diameter belonged to control sample had the lowest mean 3151.300 ± 112.900 nm and the highest value detected 7920.700 ± 198.100 nm at 40 Psi-4 Cycle treatment at, pH 4. The lowest diameter belonged to control sample had the lowest mean 1203.330 ± 32.60 nm and the highest value detected 5912.300 ± 68.800 nm at 40 Psi-4 Cycle treatment at pH 5. Due to pH 5 was closer to isoelectric point of PCLPI proteins showed agglomeration potential with HPH treatment this related to declining of electrostatic repulsion between protein molecules to (Koo et al., 2018). The least diameter belonged to control sample had the lowest mean 825.600 ± 15.970 nm and the highest value detected 1905.300 ± 58.300 nm at 20 Psi-4 Cycle at pH 6. All the samples were statistically different from each other at pH 7. At pH 7 the highest diameter belonged to control sample mean with 93.478 ± 0.284 nm and the lowest value detected 63.843 ± 0.194 or 64.543 ± 0.251 nm at 40 Psi-6 Cycle 40 Psi-4 Cycle pH 7. At pH 8 the highest diameter belonged to control sample mean with 93.032 ± 0.671 nm and the lowest value detected 61.138 ± 0.024 nm at 40 Psi-6 Cycle. The highest diameter belonged to control sample and mean diameter was 94.183 ± 1.252 nm while the lowest value detected 63.810 ± 0.441 and 62.277 ± 0.363 at 40 Psi-4 Cycle and 40 Psi-6 Cycle consequently at pH 9. The highest diameter belonged to control sample mean with 93.070 ± 1.147 nm and the lowest value has been detected 63.253 ± 0.163 nm at 40 Psi-6 Cycle at pH 10. After or at neutral pH values, microfluidization reduced particle diameter. However, when pH closer to isoelectric point of PCLPI and proteins showed agglomeration potential and particle size were increased. And the highest diameter observed pH 4 with 7920.700 ± 198.100 nm which belonged 40 Psi- 4 Cycle treatment and, lowest mean diameter belonged to 40 Psi- 6 Cycle with 61.138 ± 0.024 nm at pH 8. Table 4.7. provides more detailed data about effect of microfluidization on particle size with different pressure and cycles.

Table 4.7 : Changes in the particle size of control PCLPI (1%) solution against to micro fluidized PCLPI (1%) solution at different pressure and cycles with pH ranged from 2.0 to 10.0.

	Particle Size Nm Average				
pH	Control	20 Psi 4 Cycle	20 Psi 6 Cycle	40 Psi 4 Cycle	40 Psi 6 Cycle
2.0	161.550±4.350^{b}	146.00±1.606^{c}	126.233±1.611^{d}	2209.33±7,42^{a}	104.768±1.179^{e}
3.0	1185.700±21.500^{c}	1880.33±21.800^{b}	751.700±35.300^{d}	6950.00±356^{a}	837.43±34.800^{cd}
4.0	3151.300±112.900^{d}	6913,500±54.800^{c}	7626.000±854.00^{ab}	7920.700±198.100^{a}	4847.000±299.000^{c}
5.0	1203.330±32.60^{c}	4238.000±248.00^{b}	4276.000±526.000^{b}	5912.300±68.800^{b}	4325.000±264.000^{a}
6.0	825.600±15.970^{d}	1905.30±58.300^{a}	1413.400±55.900^{c}	1419.700±24.400^{c}	1605.000±52.000^{b}
7.0	93.478±0.284^{a}	69.567±0.364^{c}	72.328±1.505^{b}	64.543±0.251^{d}	63.843±0.194^{d}
8.0	93.032±0.671^{a}	69.843±0.204^{b}	70.230±0.161^{b}	62.728±0.145^{c}	61.138±0.024^{d}
9.0	94.183±1.252^{a}	69.960±0.335^{c}	73.718±0.981^{b}	63.810±0.441^{d}	62.277±0.363^{d}
10.0	93.070±1.147^{a}	71.880±0.331^{b}	72.958±0.732^{b}	65.193±0.403^{c}	63.253±0.163^{d}

*Means with standard deviations different letters within a line (a, b, c, d, e) are significantly different (Tukey, $p < 0.05$). Psi (Pounds per square inch) presents pressure level.

4.9 Fourier Transform Infrared (FTIR) Spectroscopy Measurements

Fourier transform infrared (FTIR) spectroscopy technique gives information to understand changes of protein secondary structure. In the literature the bands including 1620–1640 cm-^{1}, 1641–1649 cm-^{1}, and 1650-1660 cm-^{1} were associated with β-sheet, random coil, and α-helix, correspondingly. The bands at 1618 cm-^{1} - 1682 cm-^{1} have been stated to be related to the aggregated structures of the proteins (Timilsena, et al., 2016). The secondary structural components of proteins are in amide I region mostly due to the band at showed pike at 1700-1610 cm-^{1}. In this area, ~ % 80 pepdide bonds C=O exhibits stretch vibrations, vibrations with some N–H bending and to a little extent to C–H stretching vibrations (Emir, 2014; Liu et al., 2013). The FT-IR image of control and microfluidized samples were shown in Figure 4.2. The major pike observed between amide I region was 1635.47 cm-^{1}and the bands occur between 1600 and 1691 cm-^{1} were related to β-turn, excluding the bands at 1672–1678 cm-^{1}, which were assigned to β-sheet. As a result, control and microfluidized samples were in amide I region and have β-sheet structure and ~ % 80 pepdide bonds C=O exhibits stretch vibrations. Microfludization application with 20

Psi- 4 Cycle and 6 Cycle and with 40 Psi- 4 Cycle and 6 Cycle is not effected secondary structure of proteins.

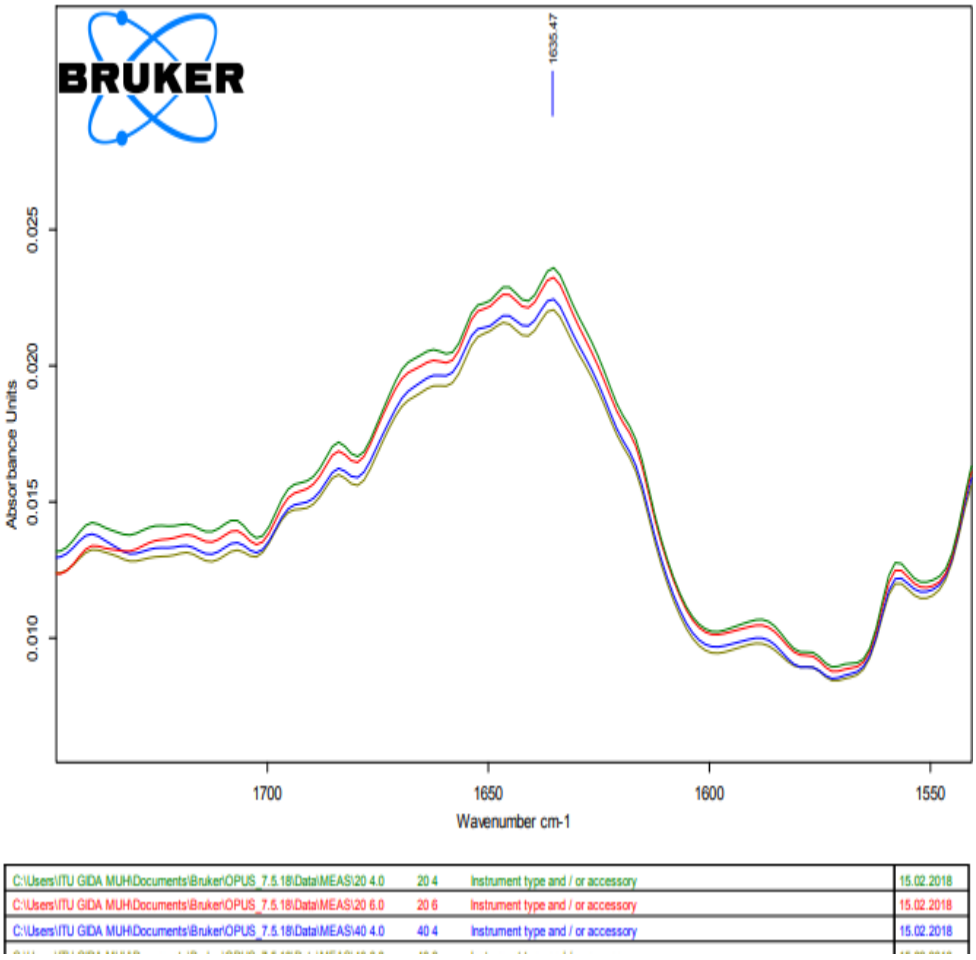

Figure 4.3 : FT-IR images of control and microfluidized samples Psi (Pounds per square inch) presents pressure level.

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5. CONCLUSIONS AND RECOMMENDATIONS

The aim in this study is to obtain protein isolate from Cranberry bean (*Phaseolus coccineus L.*) by isoelectric precipitation then, investigate effects of microfluidization (HPH) on functional and physicochemical properties of PCPLIs.

Legumes are considerable interest of academia due to sufficient nutritional composition, and alternative to animal originated foods. Functional parameters are very important in food industry, plant based protein are started to replace animal proteins and searching functional properties of plant proteins are important. On the other hand, altering functional properties of proteins are gaining importance by novel and sustainable methods. *Phaseolus coccineus L.* (Cranberry Bean) has high protein content with like as other legumes. To date, there was not enough studies in literature found regarding to *Phaseolus coccineus L.* (Cranberry Bean). Microfluidization is advanced dynamic high-pressure technique which uses the combination of ultrahigh pressure and high frequency vibration, instant pressure, intense shear and cavitation and for these reasons it provides more energy density than conventional valve homogenization. It has been reported that microfluidization(HPH) is environmentally friendly way while comparing to conventional techniques. This technique is used to improve several functional parameters of proteins according to literature.

In this Master thesis, initially, proximate composition analyzed for *Phaseolus coccineus L* which is grown in Çanakkale *Phaseolus coccineus L*. contains 4.8% ash, 2.86% moisture, 27.01% protein, 1.53 % lipid and 63.73% carbohydrate on dry weight.

Secondly, protein was extracted by IEP from PCLF. The protein content was determined 83.53% with 2.37% standard deviation on dry basis. According to result of color measurement, *Phaseolus coccineus L*. flour L* value 89.763 which is higher *than Phaseolus coccineus L*. isolate with 81.751 determined. However, the redness value and yellowness values of isolates higher than the flour. a* value of flour is 0.173 and b* value is 6.907 while isolates a* value is 0.253 and b* value is 10.783.

WAC of freeze dried PCPLI is 2.63 g of water/g of protein. In this study OAC was detected 3.95 g of oil/g of protein isolate. FC, FS of freeze dried PCLPI was 247.400% and 132.000%. The emulsifying activity stability of freeze dried PCLPI were 50.957% and 100% respectively.

HPH treatment conducted by adjusting micro fluidizer to 20.000 and 40.000 Psi levels. Effect on microfluidizitaion (HPH) on functional properties, turbidity, particle size, ζ- potential microstructure and secondary structure were accomplished.

FC of control sample is statistically different from microfluidized samples with the lowest value. The highest FC and FS observed at 40 Psi- 6 Cycle treatment, which was 612.000%, 182.500% consequently. The lowest FC and FS detected with control sample, 235.000% and 88.100% respectively. The emulsifying activities ranged from 55.06% to 52.80% and emulsifying stabilities for all samples were 100%. Microfluidization statistically were not changed EA or ES.

Turbidity, ζ- potential and particle size measurements were conducted from pH 2 and pH 10. Minimum net charge was observed at pH 4. After the neural or at neuter pH values microfluidization reduced particle diameter, increased net charge and lowered turbidity. However, when pH closer to isoelectric point of PCLPI showed agglomeration potential, particle size and turbidity were increased and minimum net charge approached to 0 by HPH treatment. As a result, microfluidization had positive impact until isoelectric or beyond the isoelectric point on ζ- potential, turbidity and particle diameter. However, more research should be done by changing pressure and cycle number, to understand effect of microfluidization (HPH) on solubility and other physicochemical and functional properties of proteins.

According light microscope images, microfluidization treatment broke into fragments and more homogenous particles by reducing big cluster to small clumps obtained. Secondary structure of PCPLI was imaged by FT-IR spectrum. The FT-IR images support PCPLP is in amide I region, consist of β-sheet structure and ~ % 80 peptide bonds have C=O exhibits stretch vibrations. Microfluidization application was not affected secondary structure of proteins.

This study supports that, protein isolate extracted isoelectric precipitation from PCLF which have possible application as a functional ingredient during food

manufacturing. Especially, foaming capacity and emulsifying stabilities are promising to find application during food processing.

PCLPI could be promising emulsifying agent food, which needs high emulsification, and emulsion stability such as meat analogs, soups, salad dressings, mayonnaise, and cakes, plant based beverages.

Experimental studies support that, PCLPI might be great potential to find application in food products such as marshmallows, coffee whiteners, cakes, whipped toppings due to impressive foaming properties. However, more research proposed to be done by changing pressure and cycle number, to understand effect of microfluidization (HPH) on physicochemical and functional properties of proteins.

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APPENDICES

APPENDIX A: The visual appreance of microfluzided and control solutions pH ranges 2-10.

APPENDIX A:

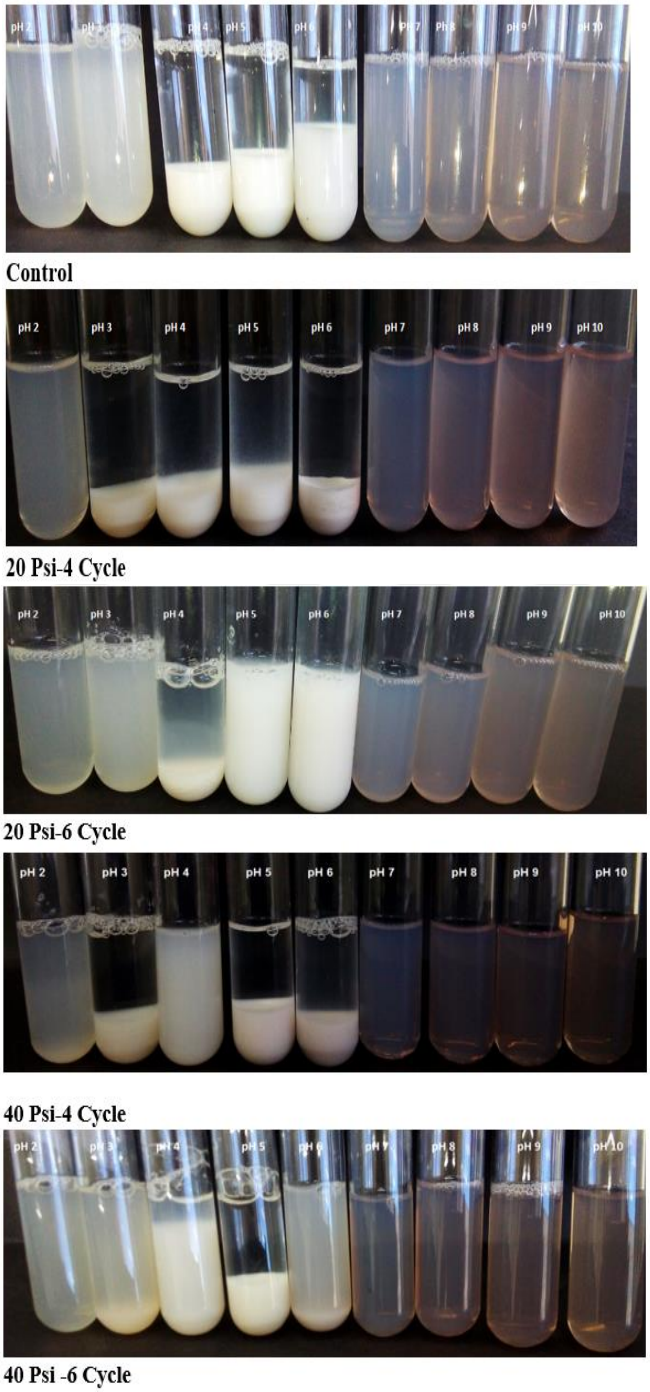
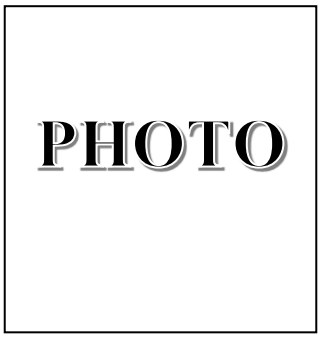


Figure A.1 : The visual appreance of microfluzided and control solutions pH ranges 2-10.

CURRICULUM VITAE



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