ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF NATURAL SCIENCES

VALORIZATION OF FUNCTIONAL PROTEIN FROM A PLANT BASED FOOD WASTE: SOUR CHERRY KERNEL, AND ITS PHYSICOCHEMICAL CHARACTERISTICS

M.Sc. THESIS

Hatice Saadiye ERYILMAZ

Department of Food Engineering

Food Engineering Programme

JUNE 2016

ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF NATURAL SCIENCES

VALORIZATION OF FUNCTIONAL PROTEIN FROM A PLANT BASED FOOD WASTE: SOUR CHERRY KERNEL, AND ITS PHYSICOCHEMICAL CHARACTERISTICS

M.Sc. THESIS

Hatice Saadiye ERYILMAZ
(506131512)

Department of Food Engineering

Food Engineering Programme

Thesis Advisor: Prof. Dr. Beraat ÖZÇELİK
Thesis Co-Advisor: Dr. Aslı CAN KARAÇA

JUNE 2016

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

BİR BİTKİSEL GIDA ATIĞI OLARAK VİŞNE ÇEKİRDEĞİNDEN ELDE EDİLEN PROTEİNLERİN FİZİKOKİMYASAL VE FONKSİYONEL ÖZELLİKLERİNİN BELİRLENMESİ

YÜKSEK LİSANS TEZİ

Hatice Saadiye ERYILMAZ
(506131512)

Gıda Mühendisliği Anabilim Dalı

Gıda Mühendisliği Programı

Tez Danışmanı: Prof. Dr. Beraat ÖZÇELİK
Eş Danışman: Dr. Aslı CAN KARAÇA

HAZİRAN 2016

Hatice Saadiye ERYILMAZ, a M.Sc. student of ITU Institute of Natural Sciences with student ID 506131512, successfully defended the thesis/dissertation entitled "VALORIZATION OF FUNCTIONAL PROTEIN FROM A PLANT BASED FOOD WASTE: SOUR CHERRY KERNEL, AND ITS PHYSICOCHEMICAL CHARACTERISTICS", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Beraat ÖZÇELİK** Istanbul Technical University	.....
Co-advisor :	**Dr. Aslı CAN KARAÇA** Aromsa A.S.	.....
Jury Members :	**Assoc. Prof. Dr. Esra ÇAPANOĞLU GÜVEN** Istanbul Technical University	.....
	Assist. Prof. Dr. Hatice Funda KARBANCIOĞLU GÜLER Istanbul Technical University	.....
	Assist. Prof. Dr. Derya KAHVECİ Yeditepe University	.....

Date of Submission : 2 May 2016
Date of Defense : 8 June 2016

FOREWORD

This project would not have been possible without the support of many people.
I want to present my symphathy,

To my sincere advisor Prof. Dr. Beraat ÖZÇELİK, who shared her valuable knowledge, experience and support, guided me with her opinions and practical solutions during my thesis period,
To my sincere co-advisor Dr. Aslı CAN KARAÇA, whom I experienced her extensive knowledge and guidance throughout my master education period,
To my colleagues Kadriye KASAPOĞLU and Evren DEMiRCAN, whom I consult with, learn and benefit from; whom were friendly and cooperative during my master education period,
To precious members of my family, who supported me either physically or mentally throughout my thesis study and whole life.

June 2016

Hatice Saadiye ERYILMAZ
(Chemist)

TABLE OF CONTENTS

FOREWORD ..... **vii**
TABLE OF CONTENTS ..... **ix**
ABBREVIATIONS ..... **xi**
SYMBOLS ..... **xiii**
LIST OF TABLES ..... **xv**
LIST OF FIGURES ..... **xvii**
SUMMARY ..... **xix**
ÖZET ..... **xxiii**
1. INTRODUCTION **1**
2. LITERATURE REVIEW **3**
2.1 Functional Properties of Sour Cherry 3
2.2 Sour Cherry Kernel 5
2.2.1 Chemical composition of sour cherry kernel 6
2.2.2 Functional properties of sour cherry kernel 6
2.3 Proteins 7
2.3.1 Sour cherry kernel proteins 8
2.3.2 Functional properties of proteins 8
2.4 Fruit and Vegetable Based Food Wastes 9
2.4.1 Functional properties of proteins from fruit and vegetable wastes 10
2.4.1.1 Determination of protein content 15
2.4.1.2 Extraction of protein 18
2.4.2 Functional health effects 20
3. MATERIALS AND METHODS **23**
3.1 Materials 23
3.2 Sample Preparation 23
3.3 Proximate Analysis 23
3.4 Defatting of Sour Cherry Kernel Flours 23
3.5 Determination of Thermal Properties 24
3.6 Surface Charge and Isoelectric Point Determination 24
3.7 Experimental Design for Protein Extraction and Statistical Analysis 25
3.8 Determination of Solubility 28
3.9 Determination of Bulk Density and Color Characteristics 28
3.10 Determination of Emulsifying Properties 29
3.11 Determination of Foaming Properties 29
3.12 Determination of Water Absorption Capacity 30
3.13 Determination of Oil Absorption Capacity 30
3.14 Determination of Gelation Properties 30
3.15 Determination of *in vitro* Protein Digestibility 30
4. RESULTS AND DISCUSSION **33**
4.1 Proximate Composition 33
4.2 Thermal Properties 33
4.3 Surface Charge and Isoelectric Precipitation Point Determination 35
4.4 Experimental Design for Protein Extraction and Statistical Analysis 36
4.4.1 Model fitting 37

4.4.2 Interpretation of response surface model 38
4.4.3 Verification of predictive model 39
4.5 Protein Solubility..... 43
4.6 Bulk Density and Color Characteristics 44
4.7 *in vitro* Protein Digestibility..... 45
4.8 Functional Properties..... 45
5. CONCLUSION..... 49
REFERENCES..... 51
CURRICULUM VITAE..... 61

ABBREVIATIONS

%	: Percent
AACC	**:** American Association of Cereal Chemists
AB	: Aril Bagasse
ANOVA	: Analysis of Variance
AOAC	**:** Association of Official Agricultural Chemists
Arg	**:** Arginine
Asp	: Aspargine
BBD	: Box-Behnken Design
BHA	: Butylated hydroxyanisole
BHT	**:** Butylated hydroxytoluene
BSA	: Bovine Serum Albumin
CC	**:** Creaming Capacity
CS	: Creaming Stability
CV	**:** Coefficients of Variation
DPPH	: 2,2-Diphenyl-1-picrylhydrazyl
DSC	: Differential Scanning Calorimeter
EC	: Emulsion Capacity
EDTA	: Ethylene diamine tetraacetic acid
ES	: Emulsifying Stability
EU	: European Union
F	**:** Flour
FA	**:** Foaming Activity
FAO	: Food and Agricultural Organization
FDA	: Food and Drug Administration of United States
FS	: Foaming Stability
GA	**:** Gelation Ability
Glu	**:** Glutamine
HO-1	: Heme oxygenase-1
HPA	: Human pancreatic alpha-amylase
HSA	: Human salivary alpha-amylase
IVD	**:** In *vitro* Digestibility
Leu	: Leucine
LGC	: Least Gelation Concentration
Mt	: Million tones
mL	: mililiter
MTT	: Tetrazolium Dye Colorimetric Assays
NASS	: National Agricultural Statistics Services of United States
OHC	: Oil Holding Capacity
PC	: Protein Concentrate
Phe	: Phenylalanine
PI	: Protein Isolate
PS	**:** Protein Solubility
RA	: Rheumatoid Arthritis

ROX : Reactive Oxidant Species
RSM : Response Surface Methodology
SA : Swelling Ability
SC-CO_2 : Supercritical Carbondioxide
SCKF : Sour Cherry Kernel Flour
SCKPI : Sour Cherry Kernel Protein Isolate
SCSE : Sour Cherry Seed Extract
SD **:** Standard Deviation
Ser **:** Serine
SH **:** Surface Hydrophobicity
T2DM : Type-2 Diabetes
TCA : Trichloroacetic acid
Tt : Thousand tones
Tyr **:** Tyrosine
UE : Electrophoretic Mobility
USP **:** United States Pharmacopeia standard for enzyme activity
UV : Ultraviolet
v/v **:** Volume by Volume
w/v **:** Weight by Volume
w/w **:** Weight by Weight
WFB **:** Whole Fruit Bagasse
WHC : Water Holding Capacity
WSI **:** Water Solubility Index

SYMBOLS

b_0	: the fixed response at the central point
b_n; b_{nn}	: the linear and quadratic coefficients
b_{nm} , b_{nnm} , b_{nmm}	: the cross product coefficients
DH	: transition enthalpy
F	: Fischer statistical test value
p	: probability
pI	: Isoelectric point
R^2	: regression coefficients
T	: temperature
t	: time
T_c	: conclusion temperature
T_o	: onset temperature
T_p	: peak temperature
W	: weight
W_F	: weight of flour
W_{PI}	: weight of protein isolate
X_i	: coded factors
x_i	: uncoded factors
X_n, X_m	: input variables
α	: particle radius
ε	: permittivity
η	: viscosity
κ	: Debye length

LIST OF TABLES

Page

Table 2.1 : Functional properties of proteins from fruit and vegetable wastes. **11**
Table 3.1 : Experimental domain of the Box-Benkhen Design. **26**
Table 3.2 : Box-Benkhen design for independent variables x1 (pH), x2 (solid/solvent (g/mL) ratio), and x3 (extraction time(h)), and coded variables(X1, X2, X3). **27**
Table 4.1 : Proximate composition of sour cherry kernel flour. **33**
Table 4.2 : Transition temperatures and enthalpies of of sour cherry kernel protein isolate. **33**
Table 4.3 : Box-Benkhen design for independent variables x_1 (pH), x_2 (solid/solvent (g/mL) ratio), and x_3 (extraction time(h)), and response of protein yield..... **36**
Table 4.4 : ANOVA for response surface quadratic model: Estimated regression model of relationship between response variable (yield) and independent variables (X_1, X_2, X_3)..... **37**
Table 4.5 : Color characteristics and bulk density of sour cherry kernel. **44**
Table 4.6 : The functional properties of sour cherry kernel protein isolate. **46**

LIST OF FIGURES

Page

Figure 4.1 : The DSC profile of sour cherry kernel protein isolate in 1:1 (w/w) distilled water slurry. **34**
Figure 4.2 : Surface charge on sour cherry kernel flour at different pH values..... **35**
Figure 4.3 : Response surfaces: (i) three-dimensional plot and (ii) contour plot for extraction yield as a function of pH (8-11) and solid to solvent ratio (1/10-1/30 g/ml) at time 2h. **40**
Figure 4.4 : Response surfaces: (i) three-dimensional plot and (ii) contour plot for extraction yield as a function of pH (8-11) and extraction time (1-3h) at ratio 1/20g/ml..... **41**
Figure 4.5 : Response surfaces: (i) three-dimensional plot and (ii) contour plot for extraction yield as a function of solid to solvent ratio (1/10-1/30g/ml) and extraction time (1-3h) at pH 9.5..... **42**
Figure 4.6 : Solubility of sour cherry kernel protein isolate (SCKPI) at varying pH's (2.0–10.0)..... **43**

VALORIZATION OF FUNCTIONAL PROTEIN FROM A PLANT BASED FOOD WASTE: SOUR CHERRY KERNEL, AND ITS PHYSICOCHEMICAL CHARACTERISTICS

SUMMARY

Sour cherry fruit has been considered as a functional food because of its high content of antioxidant compounds. It has large amounts of water-soluble vitamins including C, B_1, B_2, B_3, B_6. In addition, gallotannins, melatonin and other low molecular weight phenolic compounds are also responsible for the high water-soluble antioxidant properties. The anthocyanins, hydroxycinnamic acids and other flavonoids found in sour cherry fruits, such as isorhamnetin rutinoside and quercetin, have been reported to possess various phytotherapeutic activities.

According to National Agricultural Statistics Services of United States (NASS), almost 99% of the produced sour cherry fruit (1.4 million tonnes) has been processed into jam, juice, or canned food worldwide. Turkey is among the top four sour cherry producer countries of the world. In Turkey, forty percent of the total annual sour cherry crop has been processed into juice due to consumer preferences. During sour cherry juice processing, the seed is removed since the hard kernel shell is not edible and its existence is prohibited by authorities such as FDA. As far as the production yield of sour cherry fruit and its processes considered, it is observed that high amounts of sour cherry seeds arise as a waste every year generating huge disposal problems.

There are recent studies on utilizing the sour cherry seed on areas other than food such as biofuel generation, animal feed production, and activated carbon generation for removal of hazardous compounds from industrial wastewater. However, these options can be utilized for less valuable by products of food industry such as the non-edible hard shells of fruits. In addition, the researches investigating the process effect on sour cherry juices enlightened the potent nutritional value of sour cherry seed and kernel, exclusively and defined sour cherry kernel as a nontoxic low-cost plant material.

A seed of sour cherry contains 76.5% hard shell and 23.5 % edible kernel w/w. The chemical composition of the sour cherry kernel as investigated in previous studies is 46.6% total carbohydrates, 29.3% protein, 17% total lipids, 3.9 % moisture, 3.1% ash w/w and 30.25% dietary fiber by weight. Thus, the sour cherry kernel can be evaluated and further utilized in terms of its rich protein, lipid, and dietary fiber content. Moreover, sour cherry kernel has a number of functional properties, some of which have still being investigated. Among the many, dermaprotective, cardioprotective, anti-inflammatory, anti-diabetes, antioxidant, and therapeutic effects can be stated as important ones. The protein fraction of sour cherry representing 29.3% w/w makes it a valuable plant protein. Plant proteins play significant role in human nutrition, especially where average protein intake is less than the essential amount. Because of inadequate supplies of food proteins, there has been a growing interest for plant proteins, as new protein sources, to be used as both

functional food ingredients and nutritional supplements. In addition to their nutritional value, proteins offer great potential as functional food ingredients providing useful properties when incorporated into foods. In order to utilize a by-product as a protein source it should both present high protein content and quality based on well-balanced essential amino acids, and be free of allergic or toxic substances. Several protein products (flour, concentrates, or isolates), depending on their protein content, can be added to food products in order to improve their functional properties. Sour cherry kernel protein is a nontoxic plant protein rich in conditionally essential amino acids such as glutamic acid, arginine, aspartic acid, and serine. It is a good source of essential amino acids such as lysine, which is limited in most cereals, and phenylalanine, as well. The amino acid composition of sour cherry kernel was evaluated previously as follows: Glutamic acid 27.96 % Arginine 9.30 %, Proline 8.25%, Aspartic acid 7.55 %, Phenylalanine 7.05 %, Glycine 6.52 %, Lysine 5.28 %, Alanine 4.57 %, Serine 4.49 %.

Although the scientists have investigated the sour cherry kernel flour and its functional properties coming from protein constituents, to the best of our knowledge, the protein fraction of sour cherry has not been investigated by researchers in detail; it would be beneficial to analyze sour cherry kernel proteins and proceed to its valorization as a plant based food waste. Therefore, the aims of this study were the optimization of protein extraction conditions from sour cherry kernel based on the protein yield at first, and secondly to investigate the physicochemical and functional properties of the protein isolate which were produced in the optimum extraction conditions.

Sour cherry kernel flours were defatted prior to extraction, thermal properties of dispersions containing defatted sour cherry kernel flour were evaluated by a differential scanning calorimeter, the protein denaturation transition of sour cherry kernel was observed as an endothermic peak at 80.75^0C. The overall surface charge of the defatted flour samples was determined by measuring the electrophoretic mobility (UE) of defatted sour cherry kernel flour solutions (0.01%, w/w) at pH 2-11. The isoelectric point of defatted sour cherry kernel flour, the point at which zeta potential is zero, was found to be at around pH 4.2, by simultaneous zeta potential measurement at different solutions from pH 2 to 11. Then, sour cherry kernel protein isolate was extracted by isoelectric precipitation technique using the parameters of pH, solid/solvent ratio, and extraction time with respect to Box-Benhken experimental design of Response Surface Methodology (RSM). In the first set of experiments, the effects of three variables, X_1 (pH, 8 to 11), X_3 (solid/solvent ratio, 1:30 to 1:10 g:100 mL), X_3 (extraction time, 1-3h), at three levels on sour cherry kernel protein extraction were investigated in order to determine the conditions giving highest protein yield by RSM. A total of seventeen experimental designs (i.e. twelve factorial points and five central points) were carried out for three factor experiment. Runs at the central point of design were applied to estimate the possible pure error. Also, the protein yield was used as the response variable corresponding to the combination of the independent variables. The maximum yield (79.64%) was found under the experimental conditions of X_1 (pH) = 9.5, X_2 (solid/solvent ratio) = 1/ 30 g/mL, and X_3 (time)= 3h. Data from extractions were fitted to RSM by means of a reduced cubic model of ANOVA. The response surface model was evaluated by Design-Expert® 8.0.5 to determine a set of experimental conditions for the optimum protein yield. Secondly, the verification experiment was carried out with optimum conditions from software, the predicted (62.28%) and experimental yield (63.76%)

were consistent with each other, in that, the experimental result was within the range of 95% confidence level. After extracting proteins at optimum conditions, the desired functional properties including water and oil absorption, emulsifying, foaming properties; as well as digestibility and solubility, are investigated. Sour cherry kernel protein isolate exhibited functional properties that are comparable to other extensively used plant proteins such as soy but it has yellowish color and acrid taste. In particular, with a foaming capacity of 375%, least gelation concentration of 6.0 g/100 g of protein, and oil absoption capacity of 3.56g/g protein; sour cherry kernel protein isolate can be a good alternative to soy protein isolate in fortification of bakery and dairy food products with low amounts so that sensory properties of products are not changed.

BİR BİTKİSEL ATIK OLARAK VİŞNE ÇEKİRDEĞİ PROTEİNLERİNİN FİZİKOKİMYASAL VE FONKSİYONEL ÖZELLİKLERİNİN BELİRLENMESİ

ÖZET

Vişne meyvesi yüksek oranda antioksidan madde bulundurması sebebiyle fonksiyonel gıdalar arasında önemli bir yer tutmaktadır. C, B_1, B_2, B_3, ve B_6 vitaminleri gibi suda çözünen vitaminler yönünden zengindir. Bunun yanı sıra, gallotanin, melatonin ve diğer düşük moleküler ağırlıklı fenolik maddeler de vişnenin suda çözünen antioksidan aktivitesinin yüksek olmasına yol açar. Vişne meyvesinde bulunan antosiyaninlerin, hidroksisinamikasitlerin, izoramnetin rutinosit ve kuersetin gibi flovanoidlerin hareket modlarına ve hedef bölgelere bağlı olarak çeşitli fitoterapik aktiviteler gösterdikleri rapor edilmiştir.

Vişnede bulunan fenolikler hakkında çeşitli kaynaklar farklı bilgiler vermektedir. Bunun sebebi vişne türleri arasındaki farklılık, antioksidanlar arasında sinerjik etkiye yol açan fitokimyasal etkileşimler, meyveye uygulanan proses koşullarının farklılığı olabilmektedir. Genel itibariyle vişnede antosiyanin türü olarak siyanidin 3-glikozit, siyanidin 3- rutinozit, siyanidin 3- glikozil rutinozit; flavonoid olarak kuersetin 3-glikozit, kuersetin 3- rutinozit; ayrıca kateşin ve epikateşin bulunmaktadır. Vişne meyvesinin fonsiyonel özellikleri arasında antioksidan, antienflamatuar, antikarsinojen, antidiabetik, antinörodejeneratif aktiviteler ve dolaylı yoldan ortaya çıkan sinerjik etkileşimler sayılabilir.

Vişnenin kütlece % 65.9'nu etli yenebilir kısım, %15.3'ni posa, %6.8'ni çekirdek oluşturmaktadır. 100 g çekirdeği alınmış vişnede 86.1 g su, 12.2 g karbonhidrat (1.6g diyet lifi), 1g protein, 0.3 g yağ, ve 0.3 g kül bulunur. Vişne çekirdeğinin ise kütlece %76.5'ini sert kabuk, %23.5'ini yenebilir çekirdek içi oluşturur.

Türkiye vişne üretiminde Polonya, Rusya ve Ukrayna ile birlikte dünyadaki ilk dört ülkeden biridir. Amerika Birleşik Devletleri Ulusal Tarım İstatistikleri Servisi'nin belirttiğine göre dünya çapında her yıl üretilen yaklaşık 1,4 milyon ton vişne meyvesinin %99'u reçel, meyve suyu ve konserve gibi bir proses ürününe dönüştürülmektedir. Türkiye'de ise yıllık üretilen vişnenin %40'ı tüketici tercihlerinden dolayı meyve suyu olarak işlenmektedir. Tüm bu prosesler sırasında, sert çekirdek kabuğunun yenilebilir olmaması ve mevzuatta çekirdeğe izin verilmemesi sebebiyle vişne çekirdeğinin kabuğundan mekanik yollarla ayrılması gerekmektedir. Üretim miktarı ve proses koşulları göz önünde bulundurulduğunda her yıl azımsanmayacak miktarda vişne çekirdeği atığının ortaya çıktığı ve bu durumun atık yönetimi açısından ciddi sorunlar oluşturduğu görülmektedir.

Yakın zamanda vişne çekirdeğinin gıda haricindeki alanlarda değerlendirilmesiyle ilgili çeşitli araştırmalar yapılmıştır. Bunların arasında hayvan yemi üretimi, biyoyakıt üretimi, endüstriyel atıksu arıtımında kullanılmak üzere aktif karbon üretimi sayılabilir. Ancak, bu çalışmaların besleyici yönü bulunmayan sert çekirdek kabukları gibi yan ürünlerle değerlendirilmesi daha isabetli olacaktır. Zira vişne

suyunda proses etkisini araştıran çalışmalar vişne çekirdeğinin içinin besin ögesi potansiyelini ortaya çıkarmıştır. Bunun yanısıra bazı araştırmacılar tarafından vişne çekirdeği toksik olmayan makul fiyatlı bitkisel gıda maddesi olarak tanımlanmıştır.

100g kabuklu vişne çekirdeği 76.5g sert kabuk ve 23.5g yenebilir çekirdek içi ihtiva eder. Vişne çekirdeğinin kimyasal kompozisyonu yapılan araştırmalarda kütlece %46.6 toplam karbonhidrat, %29.3 protein, %17 toplam yağ, %3.9 nem, ve %3.1 kül olarak tesbit edilmiştir. Diyet lifinin % 30.25 olarak bulunması ve şeker oranının %2.91 gibi düşük bir seviyede olması dikkat çekmiştir. Böylece, kabuksuz vişne çekirdeğinin atık olarak değerlendirilebileceği ve kendisinden zengin protein, yağ ve diyet lifi bileşenleri yönünden faydalanılabileceği anlaşılmıştır. Vişne meyvesi gibi vişne çekirdeği de sayısız fonksiyonel özelliğe sahiptir. Bunların bir kısmıyla ilgili araştırmalar hala sürmektedir. Pek çok fonksiyonel özellik arasında dermoprotektif, kardioprotektif, antienflamatuar, antidiabetik, antioksidan, ve tedavi edici etkileri gıda ve ilaç sektörü nazarında önemli atfedilir.

Bitkisel proteinler insan beslenmesinde, bilhassa gelişmekte olan ülkelerde ortalama protein alımının zaruri olan miktarın altına düşmesiyle, oldukça önemli yer tutmaktadır. Gıda proteinlerinin dünya nüfusunun ihtiyacını karşılacayacak düzeyde yeterli miktarda bulunmaması, yeni protein kaynağı olarak fonksiyonel gıda bileşeni ve ek besin halinde kullanılmak üzere, bitki kaynaklı proteinlere olan yönelimi artırmıştır. Proteinler içerdikleri besin ögelerinin yanı sıra eklendikleri gıdaya kazandırdıkları yararlı özelliklerle fonksiyonel gıda olarak kullanılma potansiyeline sahiptirler.

Bir gıda yan ürününü protein kaynağı olarak değerlendirebilmek için dengeli esansiyel aminoasitler nazarında yüksek miktarda ve değerde (kalitede) protein içermesi; ayrıca alerjen ve toksik maddeler bulundurmaması, bulundurduğu takdirde etkili bir ön işlemle bu alerjen ya da toksik maddelerden arındırılmış olması gerekmektedir. İçerdiği protein miktarıyla ilişkili olarak, un, konsantre ya da izolat gibi farklı türde protein ürünleri, fonksiyonel özelliklerini geliştirmek maksadıyla gıda maddelerine katılabilmektedir.

Vişne çekirdeği proteini toksik olmayan, bunun yanında glutamik asit, arginin, aspartik asit, ve serin gibi şartlı esansiyel aminoasitler yönünden zengin olan bir bitkisel gıda proteinidir. Pek çok tahılın mahrum olduğu esansiyel aminoasit: lisin ve fenil alanin gibi esansiyel aminoasitleri de hatrı sayılır miktarda bulundurur. Vişne çekirdeğinin aminoasit kompozisyonu bir çalışmada kütlece şöyle tesbit edilmiştir: % 27. 96 Glutamik asit, % 9.30 arginin, %8.25 prolin, %7.55 aspartik asit, %7.05 fenil alanin, % 6.52 glisin, %5.28 lisin, %4.57 alanin, ve %4.4.9 serin.

Vişne çekirdeği ununun protein bileşeni dolayısıyla ortaya koyduğu fonksiyonel özellikleri inceleyen çalışmalar mevcut olmakla beraber, araştırdığımız kadarıyla, salt protein bileşeni (29.3%) ve özellikleri daha önce incelenmemiştir. Vişne çekirdeği proteinlerini analizlemek ve bir bitkisel gıda atığı olarak değerlendirilmesine katkı sağlamak faydalı olacaktır. Dolayısıyla, bu çalışmada vişne çekirdeği proteinini optimum koşullarda ekstrakt ettikten sonra fizyokimyasal ve fonksiyonel özelliklerini belirlemek ve değerlendirmek amaçlanmıştır.

Sıvı azotla öğütülmüş vişne çekirdeği unlarının yağları ekstraksiyon öncesi toplu olarak ayrılmıştır. Suda çözündürülmüş yağsız vişne çekirdeği ununun ısıl özelliklerine diferansiyel taramalı kalorimetre (DSC) cihazı ile bakılmış; vişne çekirdeği ununun protein denaturasyonu sıcaklığı endotermik pik olarak 80.75^0C' de gözlemlenmiştir. Yağsız vişne çekirdeği ununun kapsamlı yüzey yükü suyla

hazırlanan kütlece % 0.01'lik çözeltiden pH 2-11 arasında alınan elektroforetik hareketlilik (UE) ölçümü ile hesaplanmıştır. Zeta potansiyelinin sıfır olduğu nokta olan izoelektrik çökme noktası, % 0.01'lik çözeltiden pH 2-11 arasında anlık zeta potansiyeli ölçümü yapılarak, pH 4.2 'de bulunmuştur. Daha sonra Tepki Yüzeyi Metodolojisi (RSM)'nin Box-Benkhen deneme deseni aracılığıyla belirlenen pH, katı madde /çözelti oranı, ekstraksiyon süresi gibi parametrelerle izoelektrik çökelme yöntemi kullanılarak vişne çekirdeği proteini ekstrakt edilmiştir.

Deneylerin ilk setinde RSM 'de vişne çekirdeğinden protein ekstraksiyonu için en yüksek verimi veren kombinasyonu bulmak maksadıyla üç seviyede üç degişkenin etkisi incelenmiştir. Bunlar: X_1 (pH, 8 to 11), X_2 (katı madde/ çözelti oranı, 1:30 to 1:10 g:100 mL), X_3 (ekstraksiyon süresi, 1-3s) dir. Toplam 17 deneme deseni (12 faktoriyel nokta ve 5 merkezi nokta) üç faktörlü deney için kullanılmıştır. Merkezi noktadaki tekrarlar muhtemel saf hatayı tahmin etmek için uygulanmıştır. Ayrıca, protein verimi bağımsız değişken kombinasyonlarına tepki değişkeni olarak seçilmiştir. En yüksek protein verimi (% 79.64), X_1 (pH): 9.5, X_2 (katı madde/ çözelti oranı): 1/ 30 g/mL, ve X_3 (süre):3s olduğu deney koşullarında elde edilmiştir. Ekstraksiyonlardan elde edilen datalar ANOVA azalan kübik modelince RSM'e uygun bulunmuştur ($p<0.05$). RSM modeli Design-Expert® 8.0.5 programı tarafından deneme koşul setlerinin optimum verimini belirlemek için kullanılmıştır.

İkinci aşamada yazılım programından elde edilen optimum koşullarda doğrulama deneyi gerçekleştirilmiştir. Optimum koşullarda elde edilen deneysel veri %95 güven aralığı içinde olduğu için tahmini verim değeri (%62.28) ile deneysel verim değeri (%63.76) birbiriyle tutarlı bulunmuştur.

Doğrulanma işleminin ardından yeniden optimum koşullarda ekstrakte edilen protein izolatları çeşitli fizyokimyasal ve fonksiyonel özelliklerini belirlemek üzere incelenmiştir. *In vitro* protein sindirilmesi ve çözünürlüğün yanısıra su ve yağ tutma kapasitesi ve stabilitesi, emülsifikasyon ve köpük oluşturma kapasitesi ve stabilitesi gibi fonksiyonel özellikler de incelenmiştir. Vişne çekirdeğine duyusal analiz yapılmamış olmakla birlikte Hunter parametrelerinden elde edilen sarımtırak rengi ve kekremsi tadı dolayısıyla kayısı çekirdeğine oldukça benzediği gözlemlenmiştir. Vişne çekirdeği protein izolatı, gıda sektöründe yaygın olarak kullanılan soya proteini başta olmak üzere diğer bitkisel kaynaklı atık proteinlerinlerine (kayısı çekirdeği, domates posası ve çekirdeği, karpuz çekirdeği, nar posası ve çekirdeği gibi) benzer fonksiyonel özelliklere sahiptir. Vişne çekirdeği protein izolatı, bilhassa düşük asgari jelleşme konsantrasyonu, yüksek köpük oluşturma potansiyeli, ve yüksek yağ tutma kapasitesiyle (sırasıyla 6.0g/100g protein, 375%, ve 3.56g/g protein), soya proteininden üstün bulunmuştur. Diğer yandan soya proteinine kıyasla suda daha iyi çözünen ve *in vitro* sindirilebilirliği yüksek (95.72%) bir bitkisel proteindir. Bu veriler ışığında, gıda sektöründe ürünün duyusal özelliklerini değiştirmeyecek küçük miktarlarda fırıncılık ve süt ürünleri sahasında, dondurma ve puding, şekerleme ve bisküvi gibi ürünlerde tekstür artırıcı ve fonksiyonel zenginleştirici olarak kullanılabileceği kanaatine varılmıştır.

1. INTRODUCTION

Sour cherry (*Prunus cerasus L.)* is a red fruit with seeds very similar to sweet cherry (*Prunus avium*) in texture but different in acidic taste and flavor. It is a species of *Prunus* with subspecies of *cerasus* and a member of *Rosaceae* family. It has been produced worldwide around 1.4 million tons annually according to FAO statistics [1]. Its harvesting area covers Europe and southwest Asia, particularly Anatolia and Balkans [2]. Turkey is among the top four producer countries of sour cherry worldwide; which are Ukraine, Russia, Poland, and Turkey; with a production of almost 200 Tt per year [1].

A sour cherry consists of a fleshy edible part (~65.9 w %), a peel (~15.3 w %), and a kernel (~6.8 w %) [3]. A 100g seedless sour cherry has a chemical composition of 86.1g water, 12.2g total carbohydrates (in which 1.6g dietary fiber), 1g protein, 0.3g total lipids, and 0.3g ash. Moreover, a seed of sour cherry contains 76.5% hard shell and 23.5 % edible kernel w/w [4].

According to National Agricultural Statistics Services of United States (NASS), almost 99% of the produced sour cherry fruit has been processed into jam, juice, or canned food worldwide. In Turkey, forty percent of the total annual sour cherry crop has been processed into juice due to consumer preferences for this type of juice [5]. During processes of juice, jam, and canning, the seed should be removed by mechanical devices since the hard kernel shell is not edible and the regulations by authorities such as FDA inhibit the presence of sour cherry seed in these products. As far as the production yield of sour cherry fruit and its processes considered, it is observed that high amounts of sour cherry seeds arise as a waste every year generating huge disposal problems [6].

There are recent studies on utilizing the sour cherry seed on areas other than food such as animal feed production, biofuel generation, and activated carbon generation for removal of hazardous compounds from industrial wastewater [7-9]. However, these options can be utilized for less valuable by products of food industry such as

the non-edible hard shells of fruits [8]. Additionally, the researches investigating the process effect on sour cherry juices head light on the potent nutritional value of sour cherry seed and kernel, exclusively [10]. Some scientists define sour cherry kernel as a nontoxic low-cost plant material [11] having passion for the day its being valorized.

Although the scientists have investigated the sour cherry kernel flour and its functional properties thanks to protein constituents, to the best of our knowledge, the protein fraction of sour cherry representing 29.3% w/w [12] has not been investigated purely by researchers. It would be beneficial to analyze sour cherry kernel proteins and proceed to its valorization as a plant based food waste. Therefore, the aim of this study is to extract protein from sour cherry kernel at optimum conditions and to investigate the physicochemical and functional properties of that protein.

2. LITERATURE REVIEW

2.1 Functional Properties of Sour Cherry

Sour cherry fruit can be considered as a “functional food” because of its high content of antioxidant compounds. Sour cherries accumulate large amounts of water-soluble vitamins (C, B1, B2, B3, B6) [13] of which the ascorbic acid has the largest superoxide anion eliminating capacity. In addition, gallotannins, melatonin and other low molecular weight phenolic compounds are also responsible for the high water-soluble antioxidant properties. The anthocyanins, hydroxycinnamic acids and other flavonoids found in sour cherry fruits, such as isorhamnetin rutinoside and quercetin, have been reported to possess various phytotherapeutic activities that are based on their modes of action at different target sites [14, 15]. The phenolic substances within sour cherry fruit; though differs from one study to another due to various reasons such as being member of different cultivars, phytochemical interactions leading to synergistic effects between antioxidants, and the processes applied to fruit. They can be named as cyaniding 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-glucosylrutinoside as anthocyanins; and catechin, epicatechin, quercetin-3-glucoside, quercetin-3-rutinoside as flavonoids [16]. Antioxidant, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-neurodegenerative activities, and synergistic activities thereby, can be stated among various functional properties of sour cherry fruit.

Anthocyanins, which are bioactive phytochemicals, are widely distributed in plants and especially enriched in sour cherries responsible for color and leading to high antioxidant activity in metabolic reactions, due to their ability to scavenge oxygen free radicals and other reactive species (ROX) [17]. This feature makes anthocyanins a potential tool for use in studies on oxidative stress and its related pathologies. For example, it has been reported in animal studies that sour cherry-enriched diets reduce fasting blood glucose and fatty liver by reducing oxidative stress and inflammation with consuming physiologically relevant amounts of the whole fruit [18]. Moreover, recent studies have revealed that anthocyanins from sour cherry exhibit *in vitro*

antioxidant activities comparable to those from commercial products, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), and superior to vitamin E at 2M concentration. The derivatives of cyanidin in sour cherry showed better anti-inflammatory activity than aspirin in an anti-inflammatory assay. Thus, it is proposed that the production of a "natural aspirin" could be a pharmaceutical alternative for digestive tract ulcer patients or allergetic people to aspirin and to non-steroidal anti-inflammatory compounds [19, 20].

In a study testing the potential of anthocyanins to inhibit intestinal tumor development in mice and growth of human colon cancer cell lines was investigated [21]. Mice consuming the sour cherry diet have similar colonic tumor numbers and volume with controls; however, anthocyanins and cyanidin from sour cherry fruit have reduced cell growth of human colon cancer cell lines. Researchers indicate that sour cherry anthocyanins and cyanidin have anti-carcinogenic effect; they may reduce the risk of colon cancer [21].

Furthermore, anthocyanins are noted for anti-diabetic activity. A condition associated with insulin resistance; type 2 diabetes, can be prevented by consuming sour cherries since they include different types of anthocyanins capable of increasing insulin secretion, such as pelargonidin-3-galactoside and its aglycone, pelargonidin [22]. Matsui et al. [23] studied alpha-glucosidase inhibitory activity of anthocyanin extracts. In *in vitro* and animal studies, anthocyanin extracts were found to have potent alpha-glucosidase inhibitory activity, suppressing the increase in glucose level after nourishment. Homoki et al. [16] investigated inhibitory effect of anthocyanins from sour cherry extracts on human salivary a-amylase (HSA), which is objective of drug producers to treat diabetes, obesity and dental caries. They indicate that anthocyanins decreased the blood sugar level by two serial effects: improving insulin sensitivity of cells [24], and inhibiting starch hydrolysis. Human pancreatic a-amylase (HPA) having homologous active sites with HSA proposed to exhibit the same inhibitory effect by anthocyanins from sour cherry extract [16].

Kim et al. [14] have found that sour cherries are rich in phenolics, especially in anthocyanins, with a strong antineurodegenerative activity. Sufficient dose of sour cherry phenolics, mainly anthocyanins protected neuronal cells (PC 12) from cell-damaging oxidative stress implying that they can serve as a good source of biofunctional phytochemicals in diet [14]. For example, the antioxidant, melatonin

(N-acetyl-5-methoxytryptamine), has been identified in fresh-frozen fruits of ‘Balaton’ and ‘Montmorency’ variety of sour cherries, suggesting that sufficient consumption of sour cherry could alter the blood melatonin levels and provide protection against oxidative damage and related diseases [25].

The biological effectiveness of sour cherries may be due to phytochemical interactions that accomplish complementary effects. Thus, it is not surprising that whole cherry fruit products or mixtures of sour cherry secondary metabolites could be biologically more active than individual components. Such a synergistic effect refers to cases when combinations of bioactive substances exert effects at target sites that are greater than the sum of individual components [26]. Kirakosyan et al. [17] examined ten sour cherry products either dried, powdered, concentrate, and frozen forms of two Hungarian sour cherry cultivars, mainly ‘Balaton’ and ‘Montmorency’. However the experimental antioxidant values of sour cherry products did not match with reference standards which can be explained by synergistic action with respect to antioxidant activity between the main polyphenolics present in sour cherries [17].

Another important parameter leading to an increase in antioxidant activity is latent on the process or storage conditions that the raw sour cherry fruit is subjected to. Some anthocyanin derivatives may be rapidly formed after processes, in turn, may affect bioavailability and bioactivity. For example, during the juice processing of sour cherry, the hard seed shell is cracked prior to pitting to allow the transport of tannin species from seed shell to pitted fruit. Although the purpose of the cracking is to increase flavor of sour cherry juices, the sour cherry nectar exhibits an increased antioxidant activity making it more bioavailable than the unprocessed sour cherry thanks to cracking [10].

2.2 Sour Cherry Kernel

The researches investigating the process effect on sour cherry juices head light on the potent nutritional value of sour cherry seed and kernel, exclusively [10]. Some scientists define sour cherry kernel as a nontoxic low-cost plant material [11]. In recent studies, the sour cherry seed has been utilized on areas other than food ranging from animal feed production, to biofuel generation, and activated carbon generation for removal of hazardous compounds from industrial wastewater [7-9]. However, these options can be utilized for less valuable by products of food industry such as

the non-edible hard shells of fruits [8]. Sour cherry kernel is palatable food component with its chemical composition and encouraging functional properties.

2.2.1 Chemical composition of sour cherry kernel

A seed of sour cherry contains 76.5% hard shell and 23.5 % edible kernel w/w [27]. The chemical composition of the sour cherry kernel has been investigated by Yilmaz and Gokmen [12]. They have found that a sour cherry kernel without a shell contains 46.6% total carbohydrates, 29.3% protein, 17% total lipids, 3.9 % moisture, and 3.1% ash w/w. Within the total carbohydrates, 30.25% represents the dietary fiber and the sugar amount is as low as 2.91% by weight. Thus, the sour cherry kernel can be evaluated and further utilized in terms of its rich protein, lipid, and dietary fiber content. A few researchers have performed lipid analysis in sour cherry kernels. Bak et al. have indicated that sour cherry kernels comprise 32–36% oil, which is rich in γ-sitosterol, β-tocopherol and unsaturated fatty acids, with high content of oleic acid (50–53%) and linoleic acid (35–38%) [28]. Yilmaz and Gokmen analyzed the effect of extraction technique on fatty acid composition of sour cherry kernel lipids. They indicate that the oil extracted from sour cherry seed kernel either by hexane or SC-CO_2 methods is rich in certain bioactive compounds like polyunsaturated fatty acids, tocopherols, β -carotene and phenolic compounds [12].

2.2.2 Functional properties of sour cherry kernel

As well as sour cherry fruit, sour cherry kernel has a number of functional properties, some of which have still being investigated. Among the many, dermaprotective, cardioprotective, anti-inflammatory, anti-diabetes, antioxidant, and therapeutic effects can be stated as important ones making it a valuable product through perspective of food and medicine.

In a dermatoxicological study by Tosaki et al. [29], mice and guinea pigs have either consumed sour cherry seed kernel or have been dermally threated with the oil of that kernel. Researchers have pointed out that the sour cherry kernel is a nontoxic material suitable for oral consumption and dermal care in a daily food or healthcare dosage. The oil was found to be protective against UV damage of skin, also [29].

Sour cherry kernel is investigated for its bioactive compounds, as well; researchers have pointed out that the kernels contain anthocyanidins, hydroxycinnamates, and

flavanoids which are responsible for its cardioprotective effect as a functional food [30]. Juhasz et al. investigated the protective effect of sour cherry seed extract (SCSE) against cardiovascular disease and inflammation in hypercholesterolemic rabbit hearts [31]. They have demonstrated that SCSE have a strong anti-inflammatory activity preserving tissues through induction of heme oxygenase-1 (HO-1), a critical host antioxidant defense enzyme [31]. Czompa et al. have isolated hearts from rats, made them suffer from cardiovascular homeostasis. After application of sour cherry seed extract treatment, they observed an improvement on postischemic cardiac functions meaning that the SCSE have cardioprotecting effect via the same HO-1 mechanism [11].

Mahmoud et al. [32] have evaluated SCSE in peripheral blood human leukocytes from rheumatoid arthritis (RA) patients for its capacity to inhibit the proteins that are diagnostic biomarkers for inflammatory pathologies. They have found a modulatory effect of SCSE in RA via induction of HO-1; reducing oxidative stress, in turn, strengthening regulation of pro-inflammatory signaling pathways [32]. This result was consistent with the previous study of same authors in which the anti-diabetes and anti-inflammatory effect of SCSE on peripheral blood mononuclear cells from type 2 diabetes (T2DM) patients were analysed [33]. These studies have designated the therapeutic use of sour cherry (*Prunus cerasus*) seed extract as phytochemical inducers of some enzymes such as HO-1. They indicate that combinations of dietary phytochemicals may be configured to synergistically strengthen immune regulatory mechanisms that normally prevent inflammation from a number of systems leading to disease including the cardiovascular and central nervous system, the lungs and the kidneys, as well. There is a potential use of dietary phytochemical formulations as tools for the clinical application of HO-1 in therapeutic reduction of oxidative stressors, with resultant improved treatment of inflammatory pathologies [34].

2.3 Proteins

Proteins are highly complex polymers formed by twenty different amino acids consisting of an α-carbon atom covalently bond to a hydrogen atom, an amino group, a carboxyl group, and a side-chain R group [35]. The structure and function differences among proteins arise from the sequence in which the amino acids are linked together via amide bonds. Proteins are important food components present

mostly in milk, meats (including fish and poultry), eggs, cereals, legumes and oilseeds [36]. Plant proteins play significant role in human nutrition, particularly in developing countries where average protein intake is less than the essential amount. Because of inadequate supplies of food proteins, there has been a growing interest for plant proteins, as new protein sources, to be used as both functional food ingredients and nutritional supplements [37]. In addition to their nutritional value, proteins offer great potential as functional food ingredients providing useful properties when incorporated into foods. In order to utilize a by-product as a protein source it should both present high protein content and protein value (quality) based on well-balanced essential amino acids. An additional requirement to utilize a material for food purposes is the absence of allergic or toxic substances or the application of a proper pretreatment for the efficient removal. Several protein products (flour, concentrates, or isolates), depending on their protein content, can be introduced to food products in order to improve their functional properties [38].

2.3.1 Sour cherry kernel proteins

Sour cherry kernel protein is a nontoxic plant protein rich in conditionally essential amino acids such as glutamic acid, arginine, aspartic acid, and serine [29]. It is a good source of essential amino acids such as lysine, which is limited in most cereals, and phenylalanine, as well. The amino acid composition of sour cherry kernel was evaluated by Yilmaz and Gokmen as follows: Glutamic acid 27.96 % Arginine 9.30 %, Proline 8.25%, Aspartic acid 7.55 %, Phenylalanine 7.05 %, Glycine 6.52 %, Lysine 5.28 %, Alanine 4.57 %, Serine 4.49 % [12]. It has not been investigated through sensory analysis, however; the observations in this study indicate that sour cherry kernel is a very similar product with apricot kernel with its yellowish color and somewhat acrid taste and can be used in bakery industy to fortifiy the functional properties of the products [39].

2.3.2 Functional properties of proteins

Proteins have a great number of functional properties in food systems such as solubility, water absorption, water binding, emulsification, fat absorption, foaming, gelation, creaming, modifying viscosity, adhesion, elasticity, plasticity, color and flavor binding, catalysis, and fiber formation. Depending on the food systems and the types of proteins involved, such functions may be desirable as in the case of egg

proteins as a foaming agent, or undesirable, such as enzymatic browning of fruits and vegetables. The distinctive functional properties of various proteins make them crucial for the production of some foods. For example, wheat gluten is a unique protein for dough since it makes the dough elastic and plastic [40]. Functional properties of proteins may depend on some intrinsic factors such as protein amino acid composition and protein amino acid sequence; and extrinsic factors including pH, temperature, and ionic strength. Protein solubility or insolubility is an important factor for understanding the performance of functionality of the protein in food systems since protein insolubility may also limit other functional properties of proteins [40]. The water-holding capacity is the ability of a moist protein to retain water when subjected to an external centrifugal gravity force or compression. It consists of the sum of bound water, hydrodynamic water and, mainly, physically trapped water [41]. The emulsifying capacity is an ability of protein to act as an agent that facilitates solubilization or the dispersion of two immiscible liquids, and emulsifying stability (ES) is the ability to maintain the integrity of an emulsion [42]. Oil absorption capacity represents the ability of proteins to interact with lipid materials, which is important in food formulation and processing since many properties of foods involve the interaction of proteins and lipids such as fat entrapment and flavor absorption [43]. Foaming ability is among the specifications of food proteins; foams are double phase colloidal systems with a continuous liquid or aqueous phase and a dispersed gas or air phase that formed by proteins. For example, egg white is noted for recipes that require whipping or foaming [40, 43].

2.4 Fruit and Vegetable Based Food Wastes

For the last few decades food waste is a topic of concern worldwide as great amount of food that should have been eaten turns into waste through the food chain [44]. According to EU statistics, about 42% of food waste (excluding the agricultural food loss) is produced by households, 39% losses occur in the food manufacturing industry, 14% pertains to food sector such as ready to eat food, catering and restaurants; while 5% is lost along distribution chain [45]. Moreover, food waste is expected to increase up to 126 Mt by 2020 if the identification, quantification and characterization of the residues are not performed [46]. Food waste sources and high-added value ingredients should be classified, the stages for recovery should be

detected, and the conventional and emerging technologies should be applied for processing these wastesv[47].

Food industries produce large amount of vegetable and fruit waste. The most promising sources of valuable compounds from fruits and vegetables so far are: olives, exotic fruits and tomatoes, which can provide several valuable compounds [44]. Mirabella et al. investigated articles related to food waste evaluation coming from dairy, meat, and fruit and vegetable industry from years 2010 to 2014 [44]. They indicate that the food waste is utilized after a transformation that allows extracting active ingredients with high added value. The wastes from fruit-and vegetables processing generally contain large amounts of suspended solids, and present high biochemical and chemical oxygen demand, which influence possible recovery solutions and costs treatment. Waste organic composition includes about 75% sugars and hemicellulose, 9% cellulose and 5% lignin [48]. Biomolecules potentially extractable from the targeted wastes include sugars, polysaccharides, ethanol, proteins and derivatives, fibers, natural flavor compounds, phytochemicals [49]. Wastes mainly consist of hydrocarbons and relatively small amounts of proteins and fat, with moisture content of 80-90%; the wastewaters contain dissolved compounds, pesticides, herbicides and cleaning chemicals [50]. There are many researches on antioxidants, fiber, phenols, polyphenols and carotenoids extraction, due to their high possibilities of application and potentials. Hence, it would be beneficial to focus on the ones extracting protein.

2.4.1 Functional properties of proteins from fruit and vegetable wastes

Researches involving vegetable and fruit based protein extraction have widely focused on soybean, corn, and wheat proteins [51]. In recent studies these protein isolates, especially soy protein isolate, have been used as a reference for validation of experimental protein extraction processes [51-53]. By using **"×"** symbol to indicate that the functional property have been analyzed in the corresponding article, Table 2.1 summarizes some studies evaluating proteins of fruit and vegetable based wastes and their corresponding functional properties in recent studies till today. Lee et al. [49] investigated the functional properties of two Australian cultivars of lentil protein isolate by using alkaline extraction method at different temperature and pH conditions.

Table 2.1 : Functional properties of proteins from fruit and vegetable wastes.

Fruit / Vegetable	Functional Property														Ref
	WHC	WSI	OHC	SA	EC	ES	ESI	FA	FS	CC	CS	SH	PS	GA	
Apricot kernel PI	×				×	×		×	×				×		[54]
Tomato seed meal PI	×		×		×	×		×	×						[55]
Sour cherry kernel and wheat F	×		×		×	×		×	×						[56]
Passion fruit seed and pulp F	×		×	×											[42]
Pomegranate bagasse F	×		×		×	×									[57]
Bitter melon seed and soy PI					×	×		×	×			×	×		[52]
Canola, flaxseed, and soy PI					×	×				×	×	×	×		[58]
Soy PI and Hydrolsate															[59]
Chickpea PI			×			×		×	×				×		[43]
Lentil PI	×				×	×		×	×						[60]
Lentil PI								×	×			×	×	×	[61]
Chickpea, faba bean, lentil, and pea PI					×	×				×	×	×	×		[62]
Cowpea PC			×	×	×		×	×		×	×				[63]
Arachin (Peanut) PI							×	×	×					×	[64]

They preferred lentil because it is considered as one of the best and cheapest sources of vegetable proteins having 25 g protein in a 100g whole fat seed [65] as determined by Kjeldahl method. Foaming capacity and stability, water holding capacity, emulsifying capacity and stability were among the studied functional properties. All the functional properties of protein such as emulsion activity (46% to 41%), emulsion stability (89% to 80%), and foaming capacity (50% to 30%), were decreased by increasing pH and temperature; whereas foaming capacity vice versa (20% to 50%). The highest values obtained for green lentil at pH 9.5 and 40^0C was 62.4% foaming stability. The water holding capacity (~3%) did not change slightly between conditions of distilled water at 22 ^{0}C and at pH 9.5 and 40^0C [60].

Sharma et al. [51] have investigated the functional properties of apricot kernel protein under optimized extraction conditions [66]. The protein solubility is among the functional properties studied, the least soluble fraction of proteins (<20%) obtained at pH 2, whereas the maximum solubility (87%) was obtained at pH 8. The coagulation-isoelectric precipitation performed at pH 4 gave highest protein isolate yield (24.3%), with an extraction efficiency of 71.3% and protein content of 68.8% w/w in the protein isolate. Gandhi et al. prepared protein isolate from soy meal by extraction with 0.2 M NaOH in meal to water ratio of 1:20 at pH 9 and coagulation of proteins at pH 4.5 by using 1 M H_2SO_4. The treatment yielded 45% protein with 91% protein in the isolate [67]. Similar procedure followed and results obtained for apricot kernel. Other functional properties were water absorption capacity (1.4g/g proteins), oil absorption capacity (1.4g/g proteins), emulsification capacity (5.5mL/ proteins), foaming stability (3h), and foaming capacity (21% increase). These values found consistent with peach, soybean, and pea protein isolate studies [67, 68]; thus, apricot kernel protein isolate can be utilized as an emulsification, foaming, or fortification agent.

Viuda-Martos et al. [69] determined the chemical, physicochemical and functional properties of pomegranate juice extraction bagasses of two kinds, one included the arils and peels (whole fruit bagasse), the other one included the arils alone (arils bagasse). The protein content was determined with Kjeldahl method according to AOAC [70]. In chemical composition (g/100g dry) of pomegranate juice, proteins represented 12.6 in arils bagasse (AB) and 10.9 in pomegranate juice whole fruit bagasse (WFB). The functional properties involved water and oil holding capacity,

emulsifying capacity and stability. WFB showed more water holding capacity (4.9g/g dry total), less emulsion capacity (30.7mL/100mL) and less emulsion stability (90.7mL/100mL) than that of AB (4.5g/g dry total, 37.3mL/100mL, 93.8 mL/100mL, respectively); whereas they showed similar oil holding capacities (5.9g/g dry total). The researchers deduce that the low protein content may be result of the low emulsion properties which may be increased further via increasing protein extraction yield [69].

Mune Mune et al. [63] have utilized response surface methodology (RSM) to optimize protein extraction parameters of pH(7-11) and NaCl (0.0-0.5M) concentration, in order to obtain cowpea protein concentrate with high functional properties. The protein content of the cowpea protein concentrates was between 71% (for NaCl concentration 0.4M and pH 11) and 86% (for NaCl concentration 0.1M and pH 9) in a previous study of Mune Mune et al. [71]. Water solubility index, water absorption capacity, oil holding capacity, emulsifying activity, emulsifying stability, and foaming ability were the desired functional properties. The optimum condition was found to be pH 8.43 and 0.25 M NaCl concentration giving following results: Water solubility index of 17.20%, water absorption capacity of 383.62%, oil holding capacity of 1.75 g/g, emulsifying activity of 0.15, emulsifying stability of 19.76 min, and foaming ability of 67.20% [63].

Horax et al. [52] have investigated the effect of pH (6-10) and NaCl concentration (0.0-2.0M) on the yield and functional properties of bitter melon seed protein extracts in comparison to soy protein isolate by using RSM. Optimum conditions for protein extraction from bitter melon seeds were at a pH 9.0 and 1.3M NaCl, whereas the protein content (90.2%) was not significantly different from soy protein isolate (90.0%) as determined by Kjeldahl method. Bitter melon seed protein isolate had a single denaturation temperature (113.1 ^{0}C) while soy protein isolate had two denaturation temperatures (78.0 and 94.8 ^{0}C). Surface hydrophobicity of bitter melon seed protein isolate (690), which is an important parameter leading to isoelectric point determination, was significantly higher than that of soy (399). The solubility of bitter melon seed protein (~65%) was lower than that of soy (~87%) at pH levels other than isoelectric range of pH 4.5-5.0. Bitter melon seed protein isolate had lower emulsifying activity (0.36 vs. 0.73), foaming capacity (39.6 vs. 61.0 mL), and foaming stability (21.5 vs. 25.5 min) than had soy protein isolate.

The effect of extrinsic factors such as solid/solvent ratio (1/10-1/25) and pH (7-11) on the functional properties of lentil protein isolate was examined by using Response Surface Methodology (RSM) to optimize its alkaline extraction in the study of Jarpa Parra et al. [61]. After performing the yield optimization, temperature and time kept constant at 60 min and 22^0C; then were examined protein solubility, gelling and foaming properties. At pH 9.0 and ratio 1:10 (g/ mL), optimum extraction yield of 14.5% with a protein content of 82 % w/w was obtained. The protein solubility was changed upon pH changes, though the isoelectric point stay same (pH 4-5) for protein samples extracted at pH's 7-9. During gelation measurements, denaturation temperature was measured by differential scanning calorimeter (DSC) as 85^0C, and least gelation concentration (LGC) was evaluated as 8-10 g/100 g of protein. LGC value was lower than that of pea and chickpea (10-12%), and close to that of soy (10%) indicating a good gelation ability of lentil protein isolate. The foaming capacity has been increased upon decrease in pH, the optimum result was obtained for solution pH 3 (680%). Therefore, for these functional properties involved, it has been deduced that environmental pH has a great effect on protein solubility and surface charge, and their gelling and foaming properties of lentil proteins [61].

Yust et al. [43] have studied the functional properties of chickpea protein isolate and hydrolysates representing enzymatic modification. The protein content of chickpea isolates was 89.3% and the average of hydrolysates was 88.5% w/w. The studied functional properties were nitrogen solubility, oil adsorption, emulsifying activity and stability, foaming activity and stability. The nitrogen solubility of isolates and hydrolysates showed similar curve against pH changes, in that, the isoelectric point did not change, however the solubility increased upon 10% rise in the degree of hydrolysis about ~40%. Chickpea protein isolates showed oil adsorption of 308g oil/ 100g, emulsifying activity of 44.7% v/v, emulsion stability of 76.5%, and no foaming activity or stability; whereas hydrolysis from 1% to 10% showed an average oil adsorption of 600g oil/100g, emulsifying activity of 50% v/v, emulsion stability of 46%. Foaming capacity and stability increased from 0 to 120% and 40%, respectively. It was clear that the hydrolysis have a positive effect on these parameters with corresponding data [43].

The optimization parameters of arachin protein extraction, such as temperature (45-65), pH (8.5-10.5), and solid/solvent ratio (1/10-1/20), from defatted peanut cakes

was studied by Zhao et al. [64] by using response surface methodology. The optimum extraction conditions were found to be temperature 56.0 ◦C, pH 8.7, and solid/solvent ratio 1/14, giving the arachin yield of 31.0% w/w. Researchers also examined the effect of ultra-high pressure on functional properties of arachin protein like solubility, emulsifying activity, emulsifying stability and surface hydrophobicity. The effect of high pressure on these properties was obvious. The surface hydrophobicity was increased upon pressure (0 to 600MPa) from 100 to 300. The solubility (90% vs 60%), emulsion activity ($40m^2/g$ vs $90m^2/g$) and emulsion stability index (80min vs 90min) of unmodified arachin protein isolate were improved with pressure up to 300MPa. It is indicated that modified arachin protein can be replaced with soy protein with its better flavour in meat, milk and flour products to have cost-effective and nutritionally improved products [64].

2.4.1.1 Determination of protein content

In order to determine the protein content of fruit and vegetable based protein extracts, various methods can be used sharing similar principles like using indicator dyes upon protein denaturation etc. The difference in methods stems from the sensitivity of the measurement. For example, a common Kjeldahl procedure can be used in determining 0.3g protein, whereas micro-Kjeldahl method can be sufficiently used in measurement of microgram levels. The methods include hereafter can be named as Biuret, Lowry, Bradford, Kjeldahl, micro-Kjeldahl, and nitrogen analyser, some using bovine serum albumin as a standard of protein existence. These methods have been used sometimes to measure the protein content of protein isolates or concentrates directly [42]; whereas the experimental procedures, such as *in vitro* digestibility or protein solubility, may involve the measurement of protein content of the residue [59, 62].

In a study, the chemical and technological properties of pulp and seeds and albedo obtained from passion fruit by-products was investigated. Protein content determined by estimating the nitrogen content using the Kjeldahl method was 0.35% in pulp and seeds, and 1.49% for albedo. The functional properties covered water holding capacity (13.00 and 1.80), oil holding capacity (2.03 and 1.43), and swelling (37.00 and 5.00), for the pulp-seed and albedo respectively [42].

Siow et al. [72] studied the functional properties of cumin seed protein isolate. The defatted cumin seed powder was suspended in phosphate buffer solution (pH 8) and incubated at designated time, temperature and buffer-to-sample ratio with constant agitation at 200 rpm. After centrifugation of the slurry at 5000 g for 30 min, the supernatant was collected and. The protein content of the sample was determined using Bradford assay expressed as mg protein per gram of sample [73]. The bovine serum albumin (BSA) was used as a standard since the principle of this assay involves degeneration of albumin fraction.

The effect of acid and alkali pH's on soy protein isolate to investigate its potential as a meat processing ingredient was analysed by Jiang et al. [74] The pH's were adjusted to pH 1.5 with 2 M HCl, or pH 12 with 2 M NaOH. After holding for 1 h at room temperature to unfold, neutralized to pH 7 with 2 M HCl, and kept for 1 h to allow partial refolding. Then the protein from the treatments at both pH values were precipitated at pH 4.5, washed 3 times, and solubilized at pH 7.0 to remove salts coming from the pH adjustments. These protein isolates were put in protein gel systems with small amounts like 0.25-0.75%. The amount of protein present in lyophilized soy protein isolate measured by Biuret procedure calibrated to Kjeldahl method, whereas the amount of myofibrillar protein was determined using bovine serum albumin as standard in order to determine the small amounts sensitively [75]. As a result, pH treatment was found to be effective in enhancement of soy protein as an alternative method to preheating for meat products [74].

Chen et al. [59] studied the effect of oxidant reagent, trichloroacetic acid (TCA), on *in vitro* digestion process of soy protein isolates. Isoelectric precipitated soy protein isolates dissolved in deionized water at pH 7, freeze-dried and stored at 40^0C until use. Pepsin (2% w/w, protein basis) was added to suspensions of soy protein (3% w/v, in deionized water) adjusted to pH 2.0, and incubated at 37 C for 1 h. Then pancreatin (2% w/w, protein basis) was added to medium adjusted at pH 7, incubated at 37 C for 2 h, and then submerged in a boiling water bath for 5min to stop the digestion. Aliquots of soy protein isolate digests were removed at 0, 1 h pepsin digestion, and 2 h pancreatin digestion for the measurement of the hydrolysis degree, TCA-soluble peptide yield and antioxidant activity. A 20% (w/w) trichloroacetic acid (TCA) added to equal volume of the digest sample was kept for 30 min at 4 C. After centrifuge for 10 min, the peptide content in the supernatant was determined by

Lowry method using BSA as the standard protein [76]. TCA soluble peptide yield was calculated as the ratio of peptide in the supernatant to total protein before digestion, expressed as a percentage. As the oxidant reagent increased to 5 mM, TCA soluble peptide yield showed a significant decrease, from 30.6% to 26.7% [59].

Karaca et al. [62] investigated the surface hydrophobicity, emulsion capacity, emulsion activity/stability indices, creaming stability, and protein solubility of chickpea, faba bean, lentil, and pea protein isolates relative to a soy protein isolate produced by isoelectric precipitation and salt extraction methods. The protein composition (as is basis) from flours, isoelectric precipitated protein isolates, and salt extracted protein isolates were 16.71%, 85.40%, and 81.63% for chickpea; 23.94%, 84.14%, and 81.98% for faba bean;18.43%, 81.90%, 74.71% for lentil; 18.76%, 88.76%, 81.09% for pea; 45.41%, 87.59%, 72.64% for soy; 31.93%, 75.31%, 93.10% for canola; 25.41%, 89.25%, 87.39% for flaxseed, respectively, as determined by Kjeldahl method using 6.25 protein conversion factor. In order to analyse protein solubility, 1.0% protein/ phosphate buffer (pH 8.00) solutions w/v were prepared and pH adjusted to 7.00 with either 0.1M NaOH or 0.1M HCl. The nitrogen content in the supernatant from centrifuge was determined using a micro-Kjeldahl digestion-distillation unit to measure the small quantities of soluble protein sensitively. The percent protein solubility of protein isolates by isoelectric precipitation was measured as 91.20% for chickpea, 89.65% for faba bean, 90.73% for lentil, 61.42% for pea, and 96.53% soy; and by salt extraction was found to be 30.16% for chick pea, 52.54% for faba bean, 89.88% for lentil, 38.12% for pea, and 96.79% for soy [62]. Among five different protein isolates studied, all of them showed similar creaming stability (avg 98.52%) with soy protein isolate (95.76%), whereas pea protein isolate had the lowest emulsion capacity and stability (477.78 g oil/g protein, 12.40 min, respectively) stemming from its low solubility. All proteins carried a net negative charge at neutral pH, and had surface hydrophobicity between 53.0 and 84.8 (pea protein isolate). The isoelectric precipitated chickpea and lentil protein isolates showed high emulsifying activity (504.43g oil/g protein, 484.44g oil/g protein, respectively) and stability (82.94 and 86.79 min, respectively) comparable to the soy (520.00g oil/g protein and 85.97 min). These functional properties indicate that chickpea and lentil protein isolates can be good replacers of soy in stabilizing oil-in-water emulsions [62].

Jarpa-Parra et al. studied the optimization of lentil protein isolates. The suspensions prepared from 50 mg sample in 10 mL distilled water adjusted to pH 2-12 using 0.1 mol/L HCl or NaOH solutions. After stirred for 1 h at 22 C and centrifuged at 8000 g for 15 min, the amount of protein in the freeze dried supernatant was determined with the nitrogen analyser with bovine serum albumin as standard. Solubility was expressed as the percentage of the protein in the supernatant per total protein in the extract. The nitrogen analyser used to determine protein content of the extracts was calibrated with analytical grade EDTA and measurements were made using a protein conversion factor of 6.25 similar to Kjeldahl method [61].

2.4.1.2 Extraction of protein

Due to some intrinsic properties of proteins such as amino acid composition and surface hydrophobicity, different extraction methods favour different yields of fruit and vegetable based protein isolates or concentrates [40, 52, 58]. These methods mentioned here can be classified as alkaline extraction, salt extraction, acid precipitation, isoelectric precipitation, ethanol precipitation, and enzymatic extraction. Each method has its own principle, advantage, and disadvantage; it has been given here brief information about the procedure of these methods.

Isoelectric-precipitated chickpea, faba bean, lentil, pea, canola, flaxseed, and soy protein isolates were prepared in a similar way for each isolate in two different studies of Karaca et al. [58, 62]. Defatted meals were mixed with a 1.0M NaOH solution at 1:10 ratio (w/v), stirred for 20 min, and centrifuged for 30 min at 8 °C. The supernatants were filtered through a 110mm Whatman#1 filter paper, adjusted to pH 4.00-5.00, which is isoelectric point of target protein, with 0.1MHCl. After a second centrifugation, the precipitate dispersed in water and dialyzed at 4 °C for 24 h with a 6–8 kDa cutoff dialysis tubes to reach 2.0–2.5mS/cm conductivity. The dialysis was carried out to discard the effect of salt coming from pH adjustments. A third centrifugation was to collect the protein, and then it was freeze-dried [58, 62].

Salt extraction method was carried out for chickpea, faba bean, lentil, pea, canola, flaxseed, and soy protein isolates in a similar way to each other. Defatted meals of samples were mixed with Tris–HCl or phosphate buffer (pH 7.0-8.0) containing 0.1M NaCl at 1:10 ratio (w/v) and stirred for 2 h at ambient temperature. After centrifuge for 1 h at 4 °C, the supernatant was recovered. A second centrifuge step

for 30 min was used to further clarify the supernatant of insoluble residues, followed by dialysis (6–8 kDa cut off) against water at 4 °C for 72 h. As in the case of isoelectric precipitation, a third centrifuge was applied to collect the proteins. Then, they were freeze-dried to obtain homogeneous particles [58, 62].

In the isoelectric precipitation method, proteins were extracted under dilute alkali (pH 8.0–10.0) conditions due to their high solubility at high pH, and were precipitated at pH close to their isoelectric point (4.0–5.0). For the salt extraction method, various salt concentrations were used to solubilise the proteins of interest. Removal of salt during the dialysis step resulted in protein precipitation due to disruption of hydration layer around surface of proteins. In two different studies by Karaca et al. isoelectric precipitation method was found to be more effective than salt extraction at isolating legume proteins, since it gave higher protein concentration yields [58].

Shao et al. investigated the effect of different extraction conditions on the functional properties of tomato seed meal proteins from hot and cold break processes [55]. The protein content of defatted hot and cold press tomato meals were 38.66 and 41.42 %w/w, respectively, as determined by Kjeldahl method by using protein conversion factor of 6.25. In the alkaline extraction step, the seed meals were mixed with either 1% NaOH (pH 13), or distilled water at pH 9 or 11. After mixing for 1 h, they were centrifuged for 10 min and supernatant collected and vacuum dried. In the acid precipitation step, the pH of collected supernatant from alkaline extraction was adjusted to isoelectric point: 3.9. After 30 min centrifuge, the precipitate collected and dried. Acidic precipitation gave results with higher protein content (48% for hot break, 72% for cold break) than alkaline extraction (avg 20% for hot break, 48% for cold break) up to 25%, while the high alkaline pH pretreatment allowed higher yields of protein extraction (avg 35.5% for hot break and 47.5% for cold break) in all the samples. Emulsion capacity and stability, foaming capacity and stability, water (avg 8g/g) and oil absorption (avg 6g/g) were among the functional properties studied. Researchers pointed out than cold break processes gave higher yield of proteins than hot break due to protein denaturation. Tomato seed meal protein isolates showed better foaming properties than commercial soy protein isolate and worse emulsion properties due to lower protein content [55].

Kammerer et al. studied de-oiled sunflower press cake as a nontoxic source of food protein [77]. Sunflower seeds contain about 20% of protein, while protein contents of de-oiled expeller resulting from oil processing can reach to 50% w/w. They proposed a novel extraction method including isoelectric precipitation of mild-acidic extracts and subsequent removal of phenolic substances from de-oiled sunflower seed. In comparison to conventional alkaline extraction, the novel extraction ensured a lighter color, nutritionally and functionally better sunflower protein isolates being free of phenolics. Referring to previous studies they indicate that sunflower proteins are a highly valuable food ingredient having well-balanced amino acid composition but lacking lysine. In addition, only few clinical reports have indicated allergenic reactions toward sunflower constituents [78, 79]. The combination of isoelectric protein precipitation with an adsorptive and anion exchange discoloration of the sunflower protein extracts allowed a natural antioxidant generation from the polyphenol enriched eluate [77].

In the study of Ma et al. [80] the peanut protein concentrate (PPC) was isolated from defatted peanut flour (DPF) by ethanol precipitation and physical separation procedures. Protein extraction was carried out with ethanol solution by connecting it to a water bath. After continuous oscillation at 210 g/min for a in for a selected period, solution was centrifuged at1500g/min for10min and the precipitate was extracted with different levels of ethanol concentration (85 mL/100 mL optimum) and extract temperature (36.35 ^{0}C optimum), then oven dried for 60min at 40^{0}C.

Finally, Moura et al. [81] have investigated the effect of two commercial endoproteases on the protein extraction yield from extruded soybean flakes during enzyme assisted aqueous extraction processing. It was a purification and optimization process leading to almost 85-90% extraction yields.

2.4.2 Functional health effects

Functional health effects of plant waste flour and proteins were investigated in many studies [64, 72, 82]. The most pronounced effect among them can be stated as the antiproliferative, anticarcinogenic, and antidiabetic activities.

Li et al. investigated the antiproliferative activity of peels, pulps and seeds of 61 fruits [82]. The *in vitro* antiproliferative activities of the peels, pulps and seeds of these fruits on four cancer cell lines, which are human lung, breast, hepatoma, and

colon cancer cell line was analysed using MTT colorimetric assay. The results revealed that different fruits and different parts of one fruit exhibited different antiproliferative capacities. Remarkable inhibitory effects toward the four cancer cell lines, and decreased viability of them in a dose-dependent manner were observed for honey peach, salak, orange, and Peru ground cherry fruit wastes. Researchers indicate that these fruit wastes can be utilized as a dietary supplement through prevention and treatment of cancer [83].

Siow et al. investigated the effect of solid/solvent ratio (1:10-1:30), temperature (20-40^{O}C), and time (0.5-1.5h) on the extraction yield of cumin seed protein isolate. The optimum conditions were found to be 0.6 h, 26.3^{0}C, and 1/10 mL/g giving the protein yield of 44.98mg/g. Some physicochemical properties such as amino acid composition, and potential bioactivities of cumin seed protein isolate such as antioxidant and antidiabetic activity were evaluated. The amino acid composition of cumin seed protein consists of Tyr, Glu, Asp, Arg, Leu and Phe, indicating a high quality of natural protein. In addition, cumin seed protein isolate demonstrated high free radical scavenging activity (47.7 %DPPHsc/mg) and reducing power (12.4 mM/mg) as an antioxidant. Although it showed a relatively low a-amylase inhibition activity (6.7%) as an indicator of antidiabetic effect; results indicate that it can be utilized as a potent nutraceutical or functional and health-promoting foods [72].

Yilmaz and Gokmen [56] have investigated functional properties of sour cherry kernel flour including water and oil absorption capacity, foaming stability and capacity, and emulsion stability and capacity, in comparison to wheat flour. They have found that sour cherry kernel flour have water absorption capacity 130.42%, oil absorption capacity 117.05%, foaming capacity 9.43%, foaming stability 83.33%, emulsion capacity 44.69%, and emulsion stability of 44.66%. They have not performed protein extraction; they have used defatted sour cherry kernel flour with a protein content of around 35.3-40.0%. In order to name the obtained extracts as a protein concentrate or isolate, protein concentration should be at least 65% or 90% as in dry bases, respectively [84]. Therefore, it is necessary to analyze physicochemical and functional properties of sour cherry kernel protein with a proper extraction process yielding higher protein content in the pursuit of efficient valorization.

3. MATERIALS AND METHODS

3.1 Materials

Pitted sour cherry kernels were supplied from a local fruit and vegetable waste flour processing company, Hedef Un A.S., located in Izmir, Turkey. Double distilled water was used as the solvent throughout the study. All chemicals used in this study were analytical reagent grade (Sigma–Aldrich Co. LLC).

3.2 Sample Preparation

Some of the kernels, which were not seperated from the seeds, were discarded prior to analysis. Kernel skins were not removed to allow valorization of sour cherry kernel as a waste with all of its edible portions since the skin coat was rich in both dietary fiber and protein content [85]. Kernels were ground with liquid nitrogen with the help of IKA T10 basic disperser (IKA Werke, Staufen, Germany) and stored at deep freezer at -80^{0}C until use so that lipid oxidation risk would be avoided.

3.3 Proximate Analysis

The moisture content (Method 925.09, 1990), the oil content using the Soxhlet method (Method 960.39, 1990), the total protein content using the Kjeldahl method with multiplying the nitrogen content by a factor of 6.25 (Method 950.48, 1990), and total ash content (Method 923.03, 1990) of sour cherry kernel were determined according to Association of Official Analytical Chemists methods (AOAC) [86]. Dietary fiber analysis (Method 32-07.01) was performed using American Association of Cereal Chemists methods (AACC) [87]. Carbohydrate content was calculated by subtracting the total percentage of other components.

3.4 Defatting of Sour Cherry Kernel Flours

Sour cherry kernel flour samples were defatted prior to extraction according to Stone et al. [88] with minor modifications. In brief, sour cherry kernel flour samples were

mixed with hexane (1:3, w/v) for 40 min using a magnetic stir plate at 500 rpm followed by hexane decanting. After repeating 3 times, the mixture was filtered through Whatman #1 filter paper (Whatman International Ltd., Maidstone, United Kingdom), then dried under a fume hood at room temperature for 16 h. The defatted flour was stored at 4^0C for further analysis.

3.5 Determination of Thermal Properties

Thermal properties of dispersions containing sour cherry kernel protein isolate were evaluated by a differential scanning calorimeter (DSC Q10, New Castle, USA) according to Tan et al. with minor modifications [53]. 0.5 g of sour cherry kernel protein isolate was dissolved in 0.5 mL of distilled water and stirred for 20 min to obtain a 1:1 slurry (w/w) and equilibrated at 5 ± 1 ^{0}C for 16h. Measurements were performed in triplicate. Fifteen mg of the slurry was prepared in an aluminum pan by using a microliter pipette. After the pan was hermetically sealed, the samples were submitted to heating over a range of temperatures from 27 ^{0}C to 157^{0}C at 5^0 C/min with the flow rate of 50 mL/min nitrogen. An empty sealed hermetic pan was used as a reference. Thermal stability was expressed as denaturation peak temperature (T_p, ^{0}C). The onset (T_o), peak (T_p), conclusion (T_c) and enthalpy (DH) were determined using Universal Analysis 2000 data analysis software (TA Instruments Ltd., New Castle, DE) [89]. T_o is the intersection temperature of a tangential line from the low temperature side of the peak and the baseline; T_p is the temperature at the tip of the peak, T_c is the intersection temperature of the tangential line from the high temperature side of the peak and the baseline, and DH (J/g) is the area under the peak bound by the baseline.

3.6 Surface Charge and Isoelectric Point Determination

The electrophoretic mobility of defatted sour cherry kernel flour samples under pH 2-11 (adjusted using 0.1 mol/L HCl or NaOH) at 22^o C was measured by laser Doppler velocimetry using a Zetasizer NanoS (Stabino™ Zeta Potential Nano-Flex™ Particle Size Distribution Instrument) by performing basic to acidic titrations and vice versa. The overall surface charge of the defatted flour samples was determined by measuring the electrophoretic mobility (UE) of defatted flour solutions (0.01%, w/w) at pH 2-11 [58].

Electrophoretic mobility (i.e., velocity of a particle within an electric field) was related to the zeta potential using the Henry equation (3.1):

$$\boldsymbol{U_E} = 2\varepsilon \times \boldsymbol{\xi} \times \boldsymbol{f(\kappa\alpha)/3\eta} \quad (3.1)$$

where, ε is the permittivity, f(κα) is a function related to the ratio of particle radius (α) and the Debye length (κ), and η is the dispersion viscosity. For this study, the Smoluchowski approximation f(κα) equalled 1.5.

3.7 Experimental Design for Protein Extraction and Statistical Analysis

Sour cherry kernel protein isolate was extracted according to Siow, Horax, and Jarpa-Parra et al. with slight modifications using isoelectric precipitation method [52, 61, 72]. The protein content of defatted sour cherry kernel flour was 43.58% as determined by Kjeldahl method prior to experiments. Dispersions of defatted sour cherry kernel flour was suspended in distilled water were adjusted to desired pH (8.0-11.0) with 0.1 mol/L NaOH and subsequently incubated at designated time and solid-to-solvent ratio (1/30 to 1/10, w/v) using magnetic stirrer at room temperature. The resulting slurry was then centrifuged at 5000 rpm for 30 min after incubation. Since the protein constituents will have higher solubility at alkaline pH's, and the effect of pH was found significant in a number of studies extracting protein [53, 61]; the pH of suspension is selected as one of the parameters. Other important parameters of protein extraction were extraction time and solid/solvent ratio; whereas extraction temperature is not among the parameters because proteins are prone to denaturation [52] and phytate formation [53] upon rise in temperature which reduces yield of protein extraction. Moreover, the optimum temperature for protein extraction was found to be around room temperature in previous studies [53, 72]. The levels of these parameters were chosen with respect to experimental design explained hereafter. In order to obtain increased yields, the extraction and centrifugation procedures were repeated twice on the residues. The extracts were combined and the pH was adjusted to 4.2 (pI of sour cherry kernel protein) with 0.1 mol/L HCl to precipitate the proteins. The precipitate was recovered by centrifugation at 3000g at 5^0C for 20 min, followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged at 3000g for 15 min. Then the

washed precipitate was freeze-dried as defatted sour cherry kernel protein isolates and the dry powder was stored in plastic bags at 4 ^{0}C until further analysis.

Two sets of extraction experiments were performed to assess the effects of different variables at three levels. In the first set of screening experiments, the effects of three variables on sour cherry kernel protein extraction: X_1 (extraction time, 1-3h), X_2 (pH, 8 to 11), X_3 (solid/solvent ratio, 30 to 10 g:100 mL), were investigated by using Response Surface Methodology in order to determine the conditions yielding highest protein content in an efficient way. A Box-Behnken design (BBD) was used in this regard. As shown in Table 3.1, the designated parameters were selected based on single factor experiment results, using steepest ascent techniques.

Table 3.1 : Experimental domain of the Box-Benkhen Design.

X_i	Factor Levels		
	-1	0	1
pH	8.0	9.5	11
Solid/solvent ratio	1/10	1/20	1/30
Time	1	2	3

A total of seventeen experimental designs (i.e. twelve factorial points and five central points) were carried out for three factor experiment (Table 3.2). Three experimental replicates of each condition were performed and the mean values were stated as experimental responses. Experimental runs were randomised to minimise the effects of unexpected variability in the observed responses.

The variables were coded according to the equation 3.2

$$x = \frac{X_i - X_0}{\Delta x} \tag{3.2}$$

where x is the coded value, X_i was the corresponding actual value, X_o was the actual value in the centre of the domain, and ΔX is the increment of X_i corresponding to a variation of 1 unit of x.

Runs at the central point of design were applied to estimate the possible pure error. In addition, the protein yield was used as the response variable corresponding to the combination of the independent variables.

Table 3.2 : Box-Benkhen design for independent variables x1 (pH), x2 (solid/solvent (g/mL) ratio), and x3 (extraction time(h)), and coded variables (X1, X2, X3).

		Coded Factors			Uncoded Factors			Response
		1	2	3	1	2	3	
Std	Run	X_1	X_2	X_3	x_1:pH	x_2:Ratio	x_3:Time	Yield %
6	1	1	0	-1	11	1:20	1	-
17	2	0	0	0	9.5	1:20	2	-
12	3	0	-1	1	9.5	1:30	3	-
1	4	-1	1	0	8	1:10	2	-
5	5	-1	0	-1	8	1:20	1	-
4	6	1	-1	0	11	1:30	2	-
16	7	0	0	0	9.5	1:20	2	-
2	8	1	1	0	11	1:10	2	-
10	9	0	-1	-1	9.5	1:30	1	-
15	10	0	0	0	9.5	1:20	2	-
13	11	0	0	0	9.5	1:20	2	-
8	12	1	0	1	11	1:20	3	-
9	13	0	1	-1	9.5	1:10	1	-
3	14	-1	-1	0	8	1:30	2	-
11	15	0	1	1	9.5	1:10	3	-
7	16	-1	0	1	8	1:20	3	-
14	17	0	0	0	9.5	1:20	2	-

Protein content of the isolates was determined using a Kjeldahl method. The protein yield was calculated as follows (3.3):

$$Y(\%, \mathrm{w/w}) = \frac{W_{PI} \times \%Protein_{PI}}{W_F \times \%Protein_F} \times 100 \quad (3.3)$$

where W_{PI} and W_F are defined as weight of extracted sour cherry kernel protein isolate, and sour cherry kernel flour, respectively.

Data from extractions were fitted to a response surface design by means of a reduced cubic model of ANOVA. The response surface model was evaluated by Design-Expert® 8.0.5 (Stat Ease, Inc., Minneapolis, MN, USA, 2010) to determine a set of experimental conditions for the optimum protein yield. The response in terms of protein yield (Y) were analyzed by RSM to fit the following statistical model (3.4):

$$Y = b_0 + \sum_{n=1}^{3}(b_n X_n + b_{nn} X_n^2) + \sum_{n<m}^{3}(b_{nm} X_n X_m + b_{nnm} X_n X_m^2 + b_{nmm} X_n^2 X_m) \quad (3.4)$$

where b_0 is the value of the fixed response at the central point of the experiment, which is the point (0,0,0); b_n; b_{nn} and b_{nm} , b_{nnm} , b_{nmm} are the linear, quadratic, cross product coefficients, respectively, and X_n, X_m are the input variables.

At the second set of experiment, an optimum extraction condition was selected with respect to yield response by RSM. Additional confirmation experiments were subsequently conducted to verify the validity of the experimental model. Based on the optimum condition, the protein isolate from SCKF was extracted using the optimized pH, time and solid-to-solvent ratio to evaluate its characteristics under the effect of external factors and desired functional properties. These investigated properties were solubility, emulsion, foaming, water and oil absorption, gelation, and *in-vitro* protein digestibility.

3.8 Determination of Solubility

The protein solubility profile, at various pH's, was determined using a method described by Chee et al. [90] with some modifications. One hundred milligram of sample was dispersed in 10mL of distilled water. Then, the solution pH was adjusted to pH 2-10 by using either 1 M HCl or 1 M NaOH. The protein contents in the solution before centrifugation, and in the supernatants after centrifugation at 10.000 g for 30 min, were determined by Bradford method with respect to equation 3.5:

$$\text{Solubility \%} \left(\frac{\text{g}}{100\text{g}}\right) = \frac{\text{Protein content of supernatant}}{\text{Protein content of sample}} \times 100 \quad (3.5)$$

3.9 Determination of Bulk Density and Color Characteristics

The bulk density and color measurements were performed according to Turan et al. with some modifications [91]. The sour cherry kernel protein isolates were placed into a 10 ml graduated cylinder by constant tapping until there was no further change in volume. The contents were weighed and the bulk density of samples was calculated. Results of duplicate treatments were reported as g/ml.

Color parameters (Hunter L, a, and b values) were measured for sour cherry kernel F and PI using a hand colorimeter Minolta Chroma Meter- 400 (Ramsey, New Jersey,

USA). A white tile is calibrated L = 96.82, a = +0.02 and b = +2.08 as reference [91, 92]. Triplicate measurements for samples were reported with mean ±SD.

3.10 Determination of Emulsifying Properties

The emulsifying activity (EA) and emulsion stability (ES) were determined according to Tan et al. [53] with slight modifications. Briefly, 10mL 1% (w/v) protein isolate suspensions were prepared in distilled water prior to homogenization (Ultra Turrax T25, Staufen, Germany) at speed 6 for 2 min at room temperature. The slurries were then added with 10 mL of vegetable oil and then homogenized for 2 min. The emulsions were centrifuged at 1200 g for 5 min. The percentage of emulsifying activity was determined as in equation 3.6:

$$\text{EA}\ (\%) = \frac{\text{Height of emulsified layer}}{\text{Height of the contents of the tube}} \times 100 \tag{3.6}$$

For the emulsion stability analysis, the emulsion was re-centrifugated after heating at 80 °C for 30 min, and then ES was calculated as in equation 3.7:

$$\text{ES}\ (\%) = \frac{\text{Height of remaining emulsion layer}}{\text{Height of the original emulsified layer}} \times 100 \tag{3.7}$$

3.11 Determination of Foaming Properties

The foaming capacity (FC) and stability (FS) were measured according to Jarpa-Parra et al. [61] with slight modifications. Protein isolates of 20mL 1% (w/v) was mixed for 2 min with a homogenizer at speed 6. Volumes were recorded before and after homogenization using a graduated cylinder. The foaming capacity was calculated using the equation 3.8.

$$\text{FC}\ (\%) = \frac{\text{Volume after whipping(mL)} - \text{Volume before whipping(mL)}}{\text{Volume after whipping(mL)}} \times 100 \tag{3.8}$$

For the determination of FS, foam volume changes in the graduated cylinder were recorded at 10, 30, and 60 min after standing at room temperature according to equation 3.9. All analyses were performed in triplicate.

$$\text{FS}\ (\%) = \frac{\text{Volume after standing(mL)}}{\text{Volume after whipping(mL)}} \times 100 \tag{3.9}$$

3.12 Determination of Water Absorption Capacity

The water absorption capacity (WAC) of sour cherry kernel protein isolate (SCKPI) was determined by vortex mixing 1 g of SCKPI and 10 mL of distilled water for 30 s and left for 30 min. The mixture was centrifuged (4000 rpm, 20 min at 25 ^{0}C), and the weight of the supernatant was determined. The weight (g) of water absorbed per gram of SCKPI was reported. Measurements were done in triplicate [93].

3.13 Determination of Oil Absorption Capacity

The oil absorption capacity (OAC) was determined by vortex mixing 1 g of sour cherry kernel protein isolate (SCKPI) and 10 mL of refined vegetable oil (olive oil, density of 0.89 g/ mL) for 30 s, and allowed to stand for 30 min. The mixture was centrifuged (4000 rpm, 20 min at 25 ^{0}C), and the weight of the supernatant was determined. The weight (g) of oil absorbed per gram of SCKPI was reported. Measurements were done in triplicate [93].

3.14 Determination of Gelation Properties

The gelation properties of sour cherry kernel protein isolates (SCKPI) were determined by employing the standard methods of Yuliana et al. [94]. Suspensions with a protein fraction of 2-20 % were prepared with an interval of fraction of two in distilled water. Samples were heated for 1 h in boiling water followed by rapid cooling under ice bath, and then cooled further at 4°C for 2 hours. The least gelation concentration (LGC), which is the concentration above which the sample did not fall down or slip when the test tube was inverted, was determined as g/100g protein bases.

3.15 Determination of *in vitro* Protein Digestibility

The *in vitro* protein digestion determination of sour cherry kernel flour (SCKF) and protein isolates (SCKPI) was performed as described previously by Świeca et al. [95]. Simulated saliva solution was prepared by dissolving 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, and 8 g NaCl, 100 mg of mucin in 1 L of distilled water. The solution was adjusted to pH 6.75 and α-amylase (EC. 3.2.1.1.) was added to obtain 200 U/mL of enzyme activity. For gastric digestion 300 U/mL of pepsin (from porcine stomach

mucosa, pepsin A, EC 3.4.23.1) was prepared in 0.03 mol/L NaCl, pH 1.2. Further, simulated intestinal juice was prepared by dissolving 0.05 g of pancreatin (activity equivalent 4 USP) and 0.3 g of bile extract in 35 mL 0.1 mol/L $NaHCO_3$. The sour cherry kernel flour and protein isolates were subjected to simulated gastrointestinal digestion as follows: 1 g of sample was homogenized in a Stomacher laboratory blender for 1 min to simulate mastication in the presence of 15 mL of simulated salivary fluid; and subsequently, the samples were shaken for 10 min at 37 °C. The samples were adjusted to pH 1.2 using 5 mol/L HCl; and subsequently, 15 mL of simulated gastric fluid was added. The samples were shaken for 60 min at 37 °C. After digestion with the gastric fluid, the samples were adjusted to pH 6.0 with 0.1 mol/L of $NaHCO_3$ and then 15 mL of a mixture of bile extract and pancreatin was added. The extracts were adjusted to pH 7 with 1 mol/L NaOH and finally 5 mL of 0.12 mol/L NaCl and 5 mL of 0.12 mol/L KCl were added to each sample. Once prepared, the samples were submitted for *in vitro* digestion for 120 min, at 37 °C and in darkness. Thereafter, samples were centrifuged and supernatants were collected. The digestion was replicated twice.

The proteins content from the supernatants was determined with the Bradford method [73] using BSA (bovine serum albumin) as the standard protein. The *in vitro* protein digestibility was evaluated on the basis of total soluble protein content and the content of protein determined after digestion *in vitro* with respect to equation (3.10),

$$\text{PD}\ (\%) = \ 100\% - (\frac{\text{Pr}}{\text{Pt}} \times 100\%) \tag{3.10}$$

where PD (%) represents *in vitro* digestibility of protein; Pt, total protein content; Pr, content of proteins after *in vitro* digestion.

4. RESULTS AND DISCUSSION

4.1 Proximate Composition

The proximate composition of sour cherry kernel flour is demonstrated as a mean ± SD of duplicate or triplicate measurements in Table 4.1. The kernels were found to be rich in lipids, proteins, carbohydrates with their corresponding concentrations of 39.40%, 35.48%, and 17.27%, respectively (Table 4.1). Dietary fibers were determined as the major form of carbohydrates (15.66%) confirming that sour cherry kernels can be useful in the intestinal regulation, the intestinal absorption of glucose and reduction of cholesterol levels [84]. The lipid composition was nearly consistent with the findings of Bak et al. [28], who expressed the oil content of sour cherry kernel as 32-36%, but significantly different than that of Yılmaz et al. (17.00%) [12]. The protein content of Yılmaz et al. was lower than this study as well, 29.34%, which may be explained by the varietal and geographic regional differences of sour cherries.

Table 4.1 : Proximate composition of sour cherry kernel flour.

Composition	% (w/w)
Carbohydrate	17.27±1.40
Oil	39.40±0.21
Protein	35.48±1.02
Ash	2.20±0.07
Dietary Fiber	15.66±0.21
Moisture	5.70±0.09

4.2 Thermal Properties

DSC results for dispersions containing defatted sour cherry kernel flours at 1:1 (w/w) proportion with distilled water were displayed in Figure 4.1. The plot showed that the DSC heat flow as a function of sample temperature with an endothermic response oriented upwords. DSC thermograms allowed analysis of transition temperatures (i.e. onset, To; peak, Tp; conclusion, Tc), as well as transition enthalpies (DH) for defatted sour cherry kernel flour (Table 4.2). Data reported as mean ±standard deviation (SD) of triple measurements and shown in Table 4.2.

Table 4.2 : Transition temperatures and enthalpies of of sour cherry kernel protein isolate.

	T_0 (^{0}C)	T_P (^{0}C)	T_C (^{0}C)	Total Entalphy(W/g)
1	82.91±0.87	83.16±2.13	90.73±1.42	1.56±0.16
2	99.39±1.17	109.38±3.12	122.51±3.12	4.35±0.65

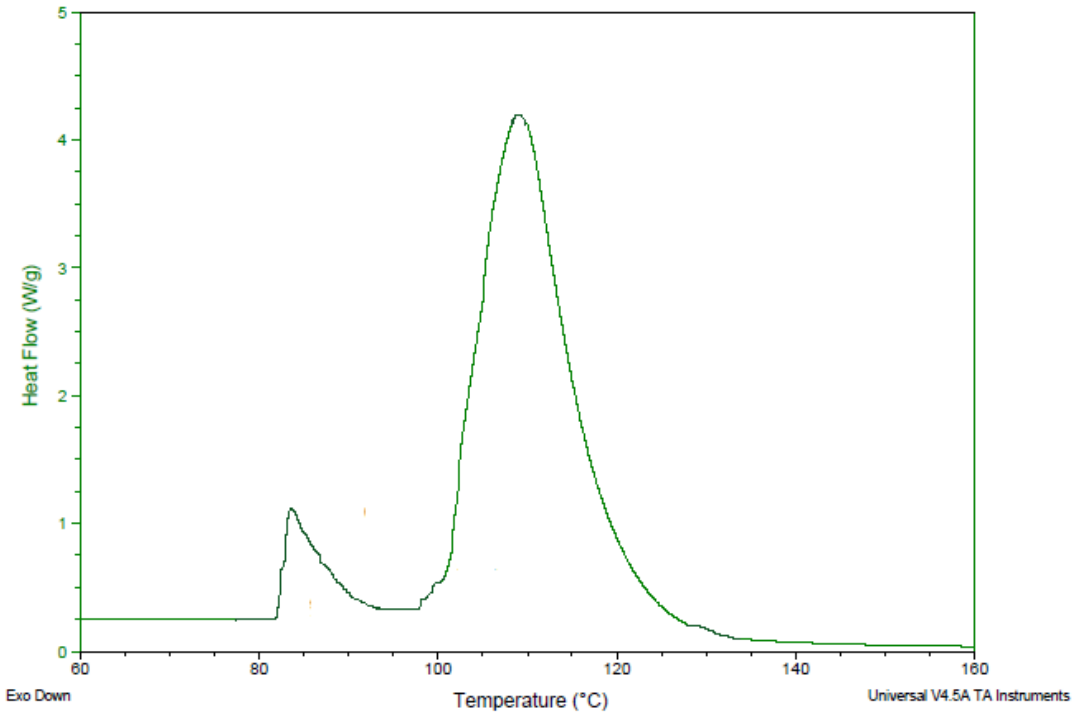


Figure 4.1 : The DSC profile of sour cherry kernel protein isolate in 1:1 (w/w) distilled water slurry.

The protein denaturation transition of sour cherry kernel protein isolate (protein content: 88.28%) was observed as doublet, an endothermic peak at 83.16^{0}C with an onset temperature of 82.91^{0}C and a conclusion temperature of 90.73^0 C, a second endothermic peak at 109.38^0 C with an onset temperature of 99.39^{0}C and a conclusion temperature of 122.51^0 C, as shown in Table 4.2. A DSC thermogram of defatted sour cherry kernel (with a protein content of 43.58%) exhibited a wider peak (data not shown) at the interval of first transition temperature starting at 61.12^{0}C possibly due to starch gelatinization [96]. For the best of our knowledge, there is no study regarding the starch constituent of sour cherry kernel, however starch present in low amounts in nut and kernel flours (2.70% in hazelnut) can undergo gelatinization upon heating and affect the thermal properties [97]. The high denaturation temperatures of proteins indicate high thermal stability that may be beneficial during processing of some food products [98]. Tan et al. indicated that soy protein isolate has a single denaturation point at 92.5 ^{0}C under similar experimental

conditions [53]. That is why the data of this study demonstrates lower thermal stability than that of soy protein with respect to first transition temperature. On the other hand, there are researches indicating doublet denaturation temperatures for soy (75^0C and 94.8^0C) implying that the singlet or doublet peaks can be altered from one variety to another [52]. Sour cherry kernel protein can be safely utilized in food processing applications below 80^0C. Since assessment of the thermal stability of globular proteins is determined by the T_p [53, 96], the thermographic data would be better understood if the protein constituents or fractions like globulin, albumin, glutelin are analysed in further studies.

4.3 Surface Charge and Isoelectric Precipitation Point Determination

The isoelectric point of defatted sour cherry kernel flour, the point at which zeta potential is zero, was found to be at around pH 4.2, by simultaneous zeta potential measurement at different solutions from pH 2 to 11. When pH was deviated from the isoelectric point, the net charge on protein chains increased. Similar z charges observed between different starting points of pH, as the pH was gradually increased from pH 2.5 to 10. The highest charge reached to +30-40 at pH 2.5 and -30-40 at neutral and alkaline pH as shown in Figure 4.2.

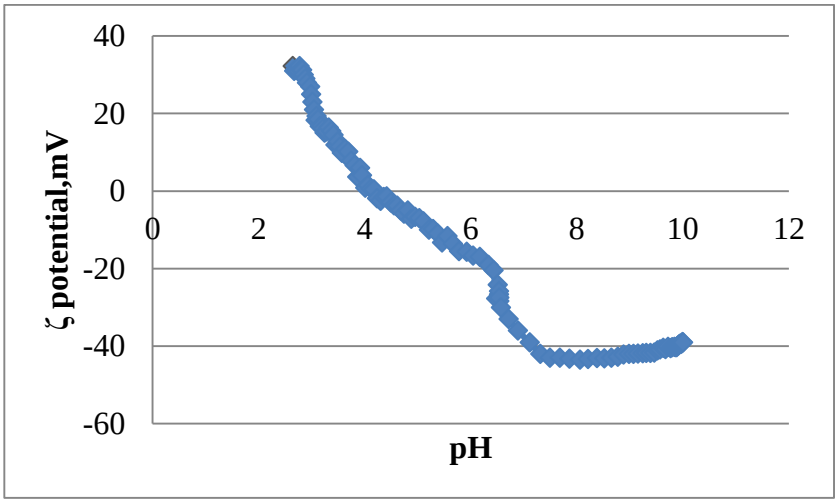


Figure 4.2 : Surface charge on sour cherry kernel flour at different pH values.

Since previous studies do not involve the isoelectric precipitation phenomena of sour cherry kernel, the isoelectric point of defatted sour cherry kernel flour (pH 4.2) was compared with other similar products in the literature. The experimental isoelectric point of sour cherry kernel is in the interval of pH 4.0 and 5.0 [99, 100] which is the

range of isoelectic precipitation for majorty of proteinaceous food such as soybean (pH 4.5), apricot kernel (pH 4.0), and bitter melon seed (pH 4.0), hence, it is deduced to be consistent with the literature [52, 58, 62, 66].

4.4 Experimental Design for Protein Extraction and Statistical Analysis

The effects of three process variables (i.e. pH (X_1), solid/solvent ratio (X_2) and time (X_3)) were studied during experimentation. The response variable was protein extraction yield. The results of 17 runs using BBD were presented in Table 4.3 that include the design with uncoded variables and observed responses (The coded variables can be followed at Table 3.2). The extraction yield seemed to be varied depending on the conditions given. The protein yield response of the extracted samples ranged between 42.53% and 79.64% (Table 4.3). The maximum yield (79.64%) was found under the experimental conditions of X_1 (pH) = 9.5, X_2 (solid/solvent ratio) = 1/ 30 g/mL, and X_3 (time)= 3h (Figure 4.3).

Table 4.3 : Box-Benkhen design for independent variables X1 (pH), X2 (solid/solvent (g/mL) ratio), and X3 (extraction time(h)), and response of the predicted and experimental protein yield (%).

		Uncoded Factors			Response	
		1	2	3		
Std	Run	x_1:pH	x_2:Ratio	x_3:Time	$Yield_{pred}$	$Yield_{exp}$
6	1	11	1:20	1	42.53	42.53
17	2	9.5	1:20	2	49.19	48.46
12	3	9.5	1:30	3	79.64	79.64
1	4	8	1:10	2	45.30	45.30
5	5	8	1:20	1	49.10	49.10
4	6	11	1:30	2	59.35	59.35
16	7	9.5	1:20	2	49.19	46.39
2	8	11	1:10	2	46.85	46.85
10	9	9.5	1:30	1	59.93	59.93
15	10	9.5	1:20	2	49.19	49.49
13	11	9.5	1:20	2	49.19	48.84
8	12	11	1:20	3	51.94	51.94
9	13	9.5	1:10	1	61.86	61.86
3	14	8	1:30	2	44.85	44.85
11	15	9.5	1:10	3	25.39	25.39
7	16	8	1:20	3	50.35	50.35
14	17	9.5	1:20	2	49.19	52.75

4.4.1 Model fitting

Table 4.4 presents the results of fitting reduced cubic models to the data. The results of analysis of variance (ANOVA) indicate that the contribution of reduced cubic model was significant. The fitted model for extraction yield in coded variables is given in equation 4.1.

$$Y = 49.19 - 1.24X_1 + 13.08X_2 - 4.19X_3 + 3.24X_1X_2 + 2.04X_1X_3 + 14.05X_2X_3 - 4.16X_1^2 + 4.06X_2^2 + 3.46X_3^2 - 10.07X_1^2X_2 + 6.86X_1^2X_3 + 5.26X_1X_2^2 \quad (4.1)$$

The significance of each coefficient was determined using the F-test and P-value under 95% confidence level (Table 4.4). The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller.

Table 4.4 : ANOVA for response surface model: Estimated regression model of relationship between response variable (yield) and independent variables (X1, X2, X3).

Source	Sum of Squares	DF	Mean Square	F-value	P-Value
Model	1923.24	12	160.27	30.16	0.0024
X_1-pH	*6.20*	*1*	*6.20*	*1.17*	*0.3409*
X_2-Ratio	*684.35*	*1*	*684.35*	*128.76*	*0.0003*
X_3-Time	*70.22*	*1*	*70.22*	*13.21*	*0.0221*
X_1 X_2	*41.93*	*1*	*41.93*	*7.89*	*0.0484*
X_1 X_3	*16.65*	*1*	*16.65*	*3.13*	*0.1515*
X_2 X_3	*789.05*	*1*	*789.05*	*148.46*	*0.0003*
X_1^2	*72.93*	*1*	*72.93*	*13.72*	*0.0208*
X_2^2	*69.52*	*1*	*69.52*	*13.08*	*0.0224*
X_3^2	*50.28*	*1*	*50.28*	*9.46*	*0.0371*
X_1^2 X_2	*202.71*	*1*	*202.71*	*38.14*	*0.0035*
X_1^2 X_3	*93.98*	*1*	*93.98*	*17.68*	*0.0136*
X_1 X_2^2	*55.28*	*1*	*55.28*	*10.40*	*0.0321*
Pure Error	21.26	4	5.31		
Core Total	1944.50	16			
R^2	0.9891				
R_{Adj}^2	0.9563				
CV %	4.54				

It is obvious that the variable with the largest effect on extraction yield was linear term of solid/solvent ratio (X_2) followed by two way interaction term of extraction time and solid/solvent ratio (X_2X_3) and quadratic effects of all of the three parameters (X_1^2, X_2^2, X_3^2). These results shown in Table 4.4 suggested that the change of extraction time and solid/solvent ratio had significant effects ($p < 0.05$) on the extraction yield of protein unlike the effect of pH. Although the effect of linear

term of pH seemed to be insignificant ($p > 0.05$), as well as two-way interaction term of pH and time (X_1X_3); the rest of all the variables with pH demonstrated significant effects ($p < 0.05$) on protein yield. The insignificance of the change in pH on protein yield may be because of high solubility of sour cherry kernel protein at the interval of alkaline pH's (8-11). Since the solubility of sour cherry kernel protein is above 85% (section 4.5) for each pH values studied here, a high yield of extraction can be performed without significant differences between conditions related to pH. Moreover, previous RSM studies for plant proteins indicated that both pH and solid/solvent ratio were important factors affecting protein yield [61, 72, 101].

The predicted values (Table 3.1) of yield were calculated using the regression models and compared with experimental values. The value of the determination coefficient R^2 was 0.9891 while the value of adjusted determination coefficient R^2_{adj} was 0.9563, indicating a high degree of correlation between the experimental and predicted values indicating a reasonable fit of the reduced cubic model to the experimental data [61, 100]. The coefficients of variation (CV) for protein yield, which is a standard deviation expressed as a percentage of the mean, was 4.54%, indicating good precision and high reliability and reproducibility when below 10% [53, 61]. Therefore, pH solid/solvent ratio, and extraction time were preserved as variables for the next stage of optimization, which is verification.

4.4.2 Interpretation of response surface model

Three-dimensional (3-D) and counter plots for extraction yield as a function of pH and solid/ solvent ratio, pH and extraction time, and solid/solvent ratio and extraction time under constant level of third factor at room temperature can be followed at Figure 4.3-5. It can be seen that the yield changed from 45% to 65% as a function of pH and solid/ solvent ratio when extraction time was 2h and increasing the solid/solvent ratio would increase the extraction yield at pH around 9.0 (Figure 4.3). When the ratio is kept constant (Figure 4.4) the yield changed from 45% to 55% as a function of pH and time, increasing the single factor of extraction time or pH did not increase the yield enormously but with higher yield around pH 8.5-9.0 again. At Figure 4.5, when the pH is kept constant at 9.5, the range of yield was 30% to 70% with a maximum at 1/30 ratio and minimum at 1/10 ratio at 3h of extraction both. In consistence with ANOVA data, the response surface data indicate that the ratio is the most important factor having an effect on the protein yield, while extraction time and

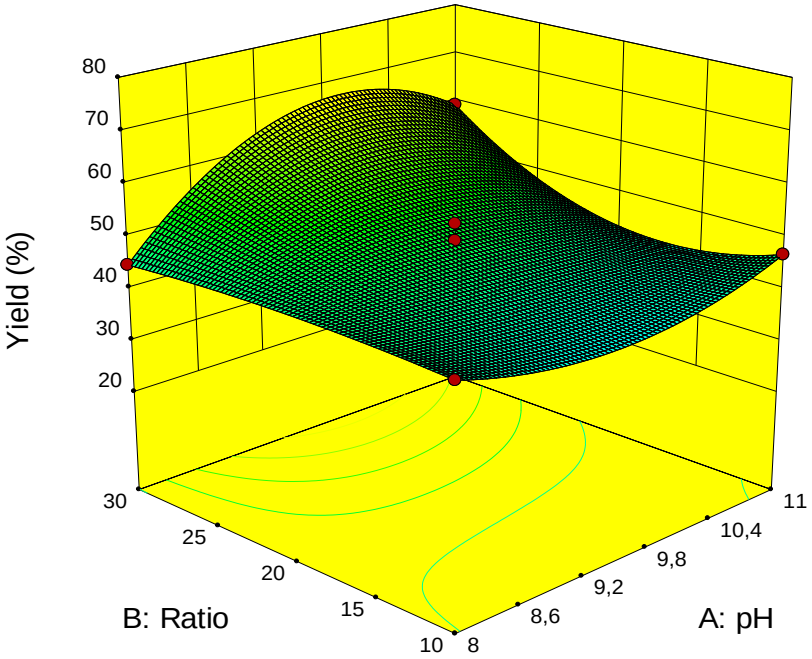
pH do not have a direct effect. The pH of the solution should have significant effect on the protein yield response according to previous studies of protein extraction [52, 53, 61]; however, the response surfaces demonstrate vice versa. The isoelectric precipitation method of protein extraction relies on high solubility of protein constituents at alkaline pH's [58]. Moreover, the solubility of sour cherry kernel protein was high above 85% and did not change drastically within the interval of pH 8-11. That is why the change in pH did not significantly affect the protein yield, which can be selected as pH 8.5 to have solubility around 90%. The extraction time has been reported to be unleading factor of protein extraction in that undesired protein−phytic acid complexes can be formed and solubility of protein can be lowered with increase in extraction time [102]. Therefore, it is suggested that extraction at pH 8.5 with shorter extraction period (<2 h) and higher ratio (1/10g/ml) would produce higher yield of SCK protein extraction to reduce wasting time and solvent.

4.4.3 Verification of predictive model

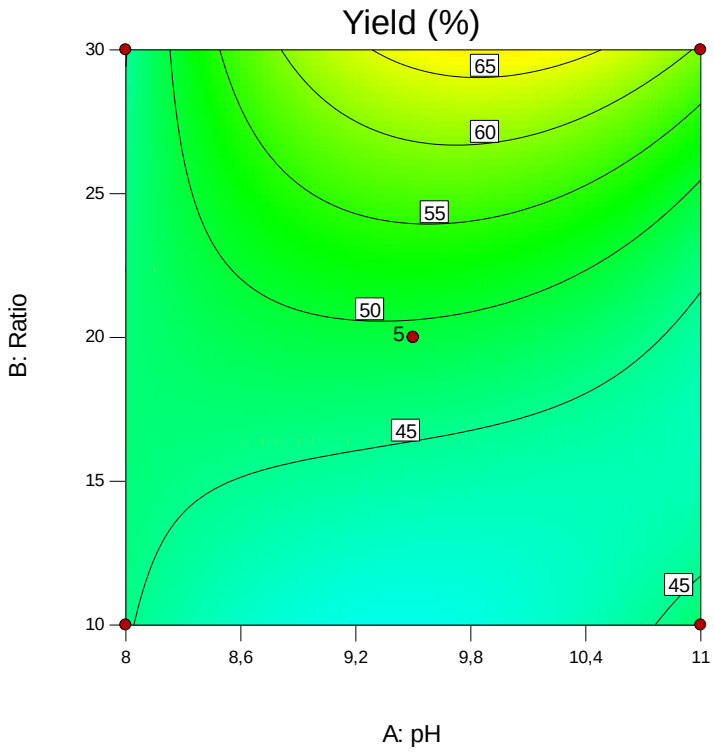
Based on the above findings, an optimisation study was performed to evaluate the optimal operating conditions for the extraction with high extraction yield of protein. One combination of parameters to maximise the response taking into consideration of the efficiency, the energy conservation and the feasibility of the experiment was determined. The verification experiment was carried out with protein extracts at optimum conditions. The optimum condition for sour cherry kernel protein extraction was pH (8.5), solid/solvent ratio (1/10), and time (1h) as determined by the help of Design Expert® 8.0.5 (Stat Ease, Inc., Minneapolis, MN, USA, 2010) with corresponding counter and response surface plots as shown in Figure 4.3. The predicted (62.28%) and experimental yield (63.76%) were consistent with each other, in that, the experimental result was within the range of 95% confidence level.

Moreover, the protein content of optimally extracted sour cherry kernel protein isolate determined by Kjeldahl method was 88.28% (w/w) which indicates the high potencial of sour cherry kernel to be utilized in foods since it ensures one of the criteria of protein suplementation that is high protein content [38]. The protein content of the optimally extracted SCKPI was higher than that of apricot kernel

(68.8%) [66] and very close to soy (90%-82.3%) [67, 103] under similar extraction conditions.

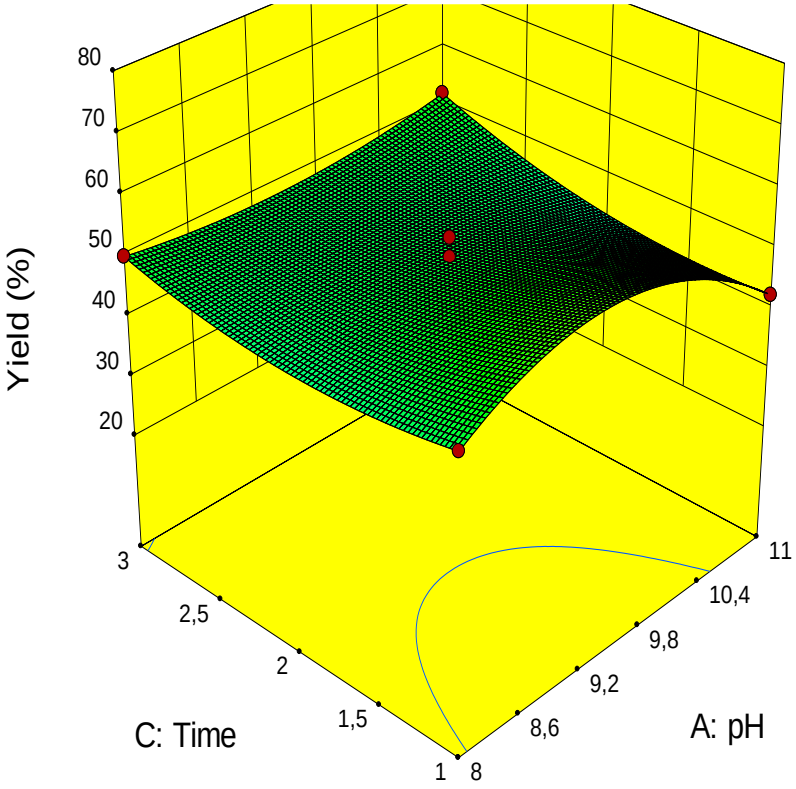


(i)

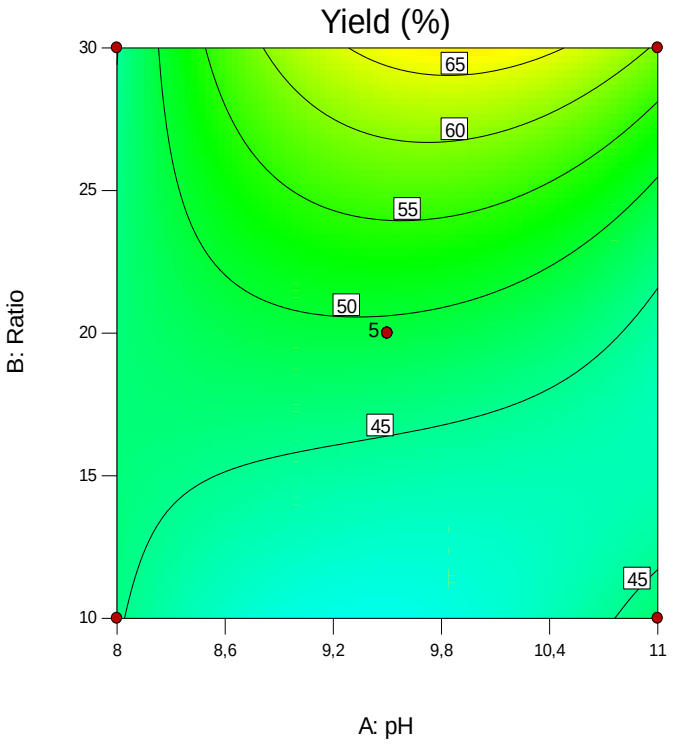


(ii)

Figure 4.3 : Response surfaces: (i) three-dimensional plot and (ii) contour plot for extraction yield as a function of pH (8-11) and solid to solvent ratio (1/10-1/30 g/ml) at time 2h.

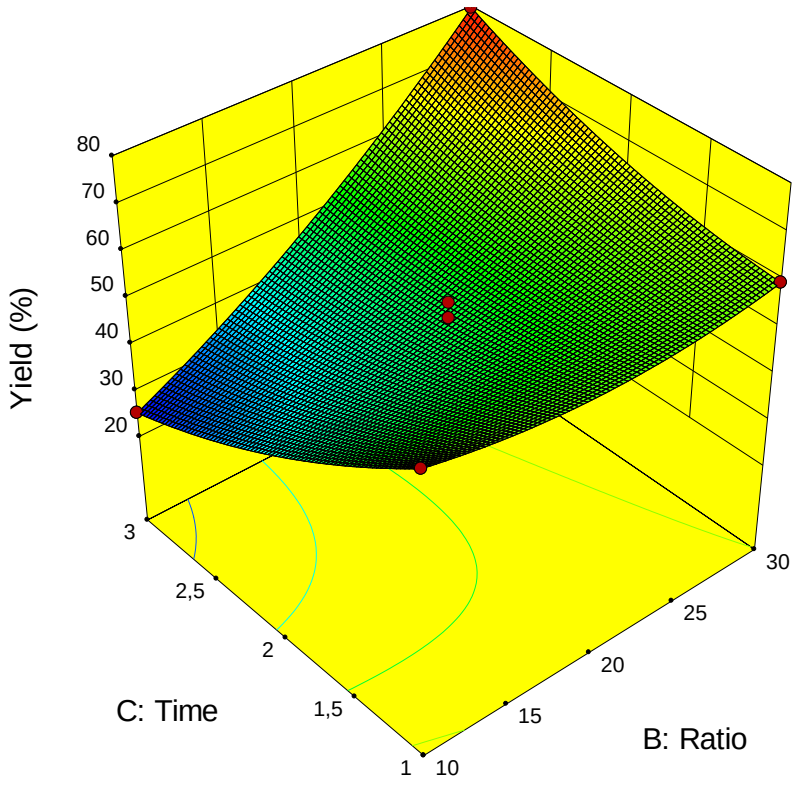


(i)

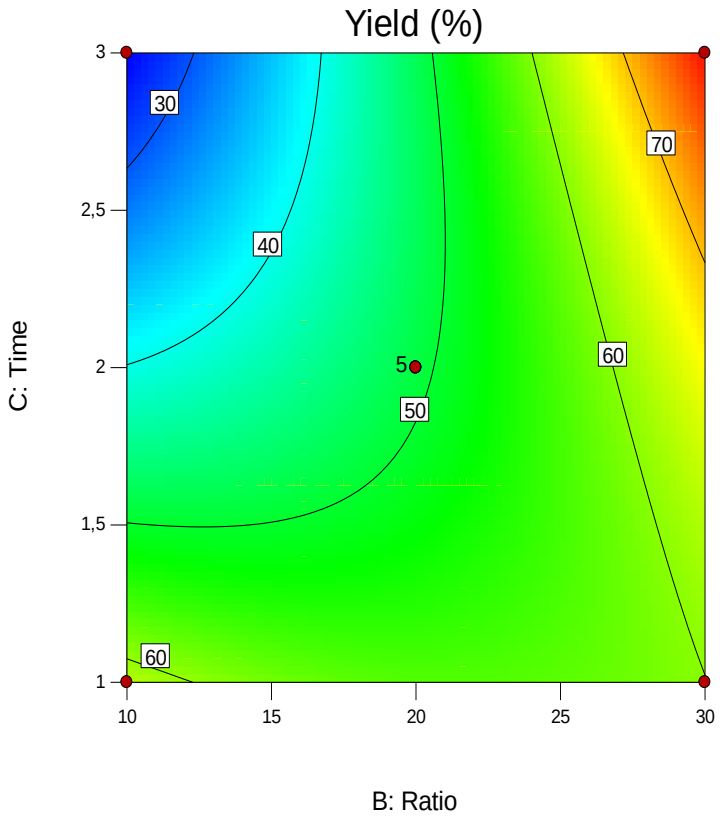


(ii)

Figure 4.4 : Response surfaces: (i) three-dimensional plot and (ii) contour plot for extraction yield as a function of pH (8-11) and extraction time (1-3h) at ratio 1/20g/ml.



(i)



(ii)

Figure 4.5 : Response surfaces: (i) three-dimensional plot and (ii) contour plot for extraction yield as a function of solid to solvent ratio (1/10-1/30g/ml) and extraction time (1-3h) at pH 9.5.

4.5 Protein Solubility

The protein solubility profile of sour cherry kernel protein isolate has been shown at Figure 4.6 over a range of pH's (2.0–10.0). SCKPI had the lowest solubility at pH's between 4.0 and 5.0 which is around the isoelectric point of sour cherry protein (pI 4.2) as well as many plant based proteins such as apricot kernel, bitter melon seed, red pepper seed, and soy [66, 100, 104], while the solubilities increased at pH's above 5.0 and below 4.0. Like that of soy PI, solubility profiles of sour cherry kernel PI exhibited a U-shaped curve (Figure 4.6).

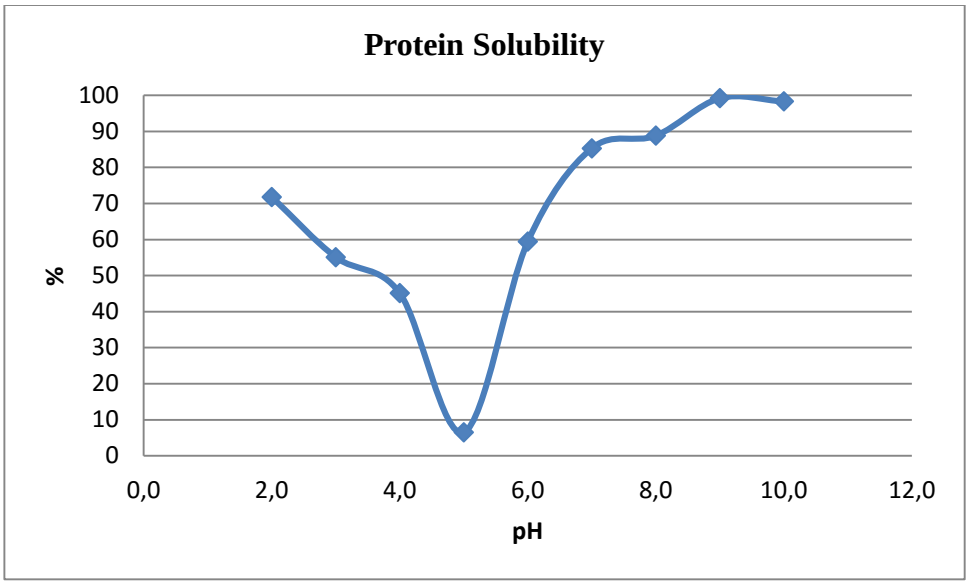


Figure 4.6 : Solubility of sour cherry kernel protein isolate (SCKPI) at varying pH's (2.0–10.0).

At pH 3.0, the sour cherry kernel proteins demonstrated moderate solubility (55.1%), whereas the solubility increased through more acidic pH's, it reached up to 71.8% at pH 2.0. At pH 7.0, neutral pH, sour cherry kernel PI showed high solubility of 85.3%, which is very close to that of soy PI (86.7%) under similar experimental conditions [52]. At alkaline pH's the highest solubility values, more than 95%, has been observed for sour cherry kernel protein. With the maximum value of 99.2% at pH 9, sour cherry kernel protein exhibited superior solubility profile at alkaline pH's than that of many plant proteins, even soy protein (86.7-90.1%) [52] which is favorable for its potential applications. The protein solubility is a phenomena having relation with interactions of proteins with solvents and themselves, and the surface hydrophobic–hydrophilic balance of them, as well [105]. The interactions such as hydrogen bonds and electrostatic interactions stem from intra and inter-molecular

bondings in the presence of charged, polar, and nonpolar groups of protein amino acid residues on the surface of the protein structure; whereas, nonpolar amino acid residues of the native protein stays in the protein interior [106]. Thus, the superior solubility profile of sour cherry kernel protein isolate can be associated with its rich content of polar Asp(7.55 %), Gly(6.52 %), and Ser(4.49 %) aminoacid residues as determined by previous studies [12].

4.6 Bulk Density and Color Characteristics

Bulk density and Hunter color parameters (L, a, b) of a protenecous sample can be important in physicochemical aspects. The bulk density value obtained sour cherry kernel protein isolate was 0.32±0.00 g/ml (Table 4.5). The low bulk density of sour cherry kernel is very close to that of raw hazelnut flour (0.31g/ml) [91] determined under the same experimental procedure. The bulk density of a sample has been affected by many parameters like particle size obtained by different drying and grinding procedures. Moreover, it has been used especially for food quality control purposes in the detection of raw materials or contaminants [107]. Since the methods of drying (with freeze-drying) and grinding (with liquid nitrogen) in this study are those noted for generating very finite particles [108], they promote unexceptionally lower bulk densities. Color characteristics and bulk density of sour cherry kernel is shown in Table 4.5 with data indicating mean and SD of triplicate and duplicate measurements, respectively.

Table 4.5 : Color characteristics and bulk density of sour cherry kernel.

	Color measurements			Bulk Density
	L	a	B	(g/ml)
SCKPI	90.32±0.11	0.12±0.01	10.97±0.20	0.32±0.00
SCKF	83.77±0.90	1.99±0.15	11.86±0.34	

Hunter L value is for lightness to darkness (0 represents black, 100 represents white), a (redness to greenness) and b (yellowness to blueness) are for the color opponent dimensions [109]. It is observed that the color of sour cherry kernel gets darker and the yellowness gets lower upon protein extraction. Since the change in color parameters has paramount importance in thermal functional properties [109, 110] with respect to nutritional changes and consumer acceptance; these data can be

beneficial to explain the relationship between thermal degradation kinetics and color of sour chery kernel protein in future studies, as well as gelation and starch related properties.

4.7 *in vitro* Protein Digestibility

Performing *in vitro* digestion with a multi-enzyme system for the estimation of protein digestibility mimicking the metabolic activities throughout the human body will give an insight information on the bioactivity of sour cherry kernel protein [59]. After being digested by alpha-amylase, pepsin, and pancreatin, simultaneously; the protein content of sour cherry kernel was almost vanished. The PD% values for SCKF and SCKPI was found to be 93.91% and 95.72%, respectively; which is higher than that of soy PI(85.2%) [53] upon digestion by chymotrypsin and pancreatin enzymes. This result can be explained by the protein composition of sour cherry kernel like storage proteins that are easily digested and utilized throughout the bodily functions [111]. In addition, the digestibility value of sour cherry kernel is very close to that of apricot kernel, whose *in vitro* protein digestibility depended barely on the enzyme system involved. When the system involved pancreatin, pepsin, or trypsin only, the kernel flour had low digestibility (around 32.3% and 66.3% for apricot kernel F and PI, respectively). As in this study, if the system involved pepsin and pancreatin, the apricot kernel demonstrated very high digestibility (96.4% and 98.1% for apricot kernel F and PI, respectively) [112]. Moreover, since the enzymes used in this study are the same with human salivary, gastrointestinal, and pancreatic system ones so that the *in vitro* digestion experiment efficiently lead to high bioactivity and bioavailability of SCKPI. The highly digestible SCKPI can be used in bread formulations without need of modification such as deamidation, hydrolysis, high pressure as in the case of many other plant proteins such as soy [59], wheat [113], and lentil [65].

4.8 Functional Properties

In this study, sour cherry kernel protein samples, extracted at optimum conditions described in section 4.4.2, were tested for their water and oil absorption, emulsion and foaming, and gelation properties. The results of the functional property

experiments of sour cherry kernel protein isolate can be followed at Table 4.6 with data representing mean±SD of triplicate measurements.

Table 4.6 : The functional properties of sour cherry kernel protein isolate.

WAC	OAC	EA	ES	FC	FS			LGC
					10min	30min	60min	
(g/g)	(g/g)	(%)	(%)	(%)	(%)	(%)	(%)	(g/100g)
1.72±0.04	3.56±0.09	44.86±1.17	96.58±2.42	450±40.8	90.86±0.68	83.38±1.30	75.91±3.04	6.0±0.0

The water absorption capacity of sour cherry kernel PI (1.72g/g proteins) was comparable to apricot kernel PI (1.4g/g proteins) [66], but significantly lower than soy PI (6.13 g/g protein) [53]. The position of polar amino acid groups can be responsible for protein–water interaction [106] in that the more polar groups such as Gly and Ser [12] in sour cherry kernel on surface, the more the water absorption capacity. The oil absorption capacity of sour cherry kernel PI was found as 3.56g/g protein, which was lower than that of tomato seed meal PI (6g/g protein) [55]; however higher than many other protein isolates including apricot kernel (1.4g/g protein) [66], cowpea (1.75 g/g protein) [63], even soy PI (1.51g/g protein) [53]. Since OAC is required in most food applications such as in bakery products giving rise to flavor retention and improved palatability, sour cherry kernel protein can be added in smaller amounts more efficiently than most of the plant based protein isolates.

Emulsifying activity and emulsion stability values of sour cherry kernel PI was 44.86% and 96.58%, respectively, which are comparable to that of soy PI (47.4% and 100%) under similar experimental conditions [53]. These EA values may be affected by surface hydrophobicity and solubility of sour cherry kernel protein [105]. Highly soluble protein would have a good EA as argued in previous researches [52, 114]. That is why the sour cherry kernel protein have similar solubility profile and EA with soy protein isolate of different studies [58, 104]. A higher solubility of sour cherry kernel protein at alkaline pH's (above 85%) may help the proteins to hydrate sufficiently, in turn, show good emulsion activities [115]. Moreover, the surface hydrophobic– hydrophilic balance of sour chery kernel protein may favor the interactions of oil and aqueous medium so that the stable emulsions were formed between them giving ES value comparable to that of soy protein [58, 104].

The foaming capacity (FC) and stability (FS) values of sour cherry kernel PI extracted at optimum conditions (pH 8.5, solid/solvent ratio: 1/10, 1h) were 450% and 76% (at 60min), respectively. The FS measurements were performed at 10, 30, and 60 min. At 10 min, the FS value was 91% and it decreased upto 83% at 30 min and 76% at 60 min. The FC of sour cherry kernel PI (450%) was significantly higher than soy PI (120%), significantly lower than lentil PI (600%) under similar experimental conditions [103]. Different methods of foaming practices yielded cowpea PI (67.2%) [63], tomato seed meal PI (30.0%) [55], apricot kernel PI (21.5%) [66] representing lower foaming capacities. All samples of SCKPI demonstrated high FS regardless of the protein indicating more than 83% of the formed foams remained stable after standing for half an hour at room temperature. This FS value can compete with that of lentil PI (80%) [61] and soy PI(91%) [103]; and it is much higher than many of the plant proteins obtained from different foaming methods such as chickpea, pea (35-40%) [55, 103, 116]. Foam forming capacity of a protein is related to its ability to reach, adsorb, and unfold rapidly at the liquid-gas interface. A protein must be able to associate through protein-protein interactions and become part of the film to create foam. The foaming capacity of a protein can be related to its high solubility and unique amino acid composition with balanced hydrophilic and hydrophobic segments and high surface charge so that it can be adsorbed at the air-water interface with hydrophobic segments oriented towards the air and vice versa. In food industry, many products with oil-in-water or water-in-oil emulsions contain emulsifiers or stabilizers to decrease the interfacial tension between oil and water phase increasing the stability of the system. For example, soy lecithin is the most common emulsifier and stabilizer in the production of emulsions and foam products in food industry. It is the most used emulsifier in confectionary such as in chocolates and coatings by a level of about 0.1−2% [96].

Thanks to its emulsion and foaming properties, sour cherry kernel PI can be used as a stabilizer, emulsifier, or foaming agent in cakes, dairy products, and confectionary, as an alternative to soy. While determining the amount of sour cherry kernel protein isolate as an emulsifier or fortification agent, the sensory properties should be taken into consideration. Since it has a yellowish color and somewhat acrid taste, sour cherry kernel protein should be added to food products in smaller amounts than soy. The sensory attributes of a food product has been a matter of paramount importance

especially in dairy industry, where Maillard reactions and such darkening agents is undesirable for the sake of consumer preferences. That is why sour cherry kernel protein can be added to dairy products like fruity yoghurts or ice creams where the color and taste can be masked by other colorful and sweet tasted ingredients. On the other hand, some functional properties of sour cherry kernel allow addition in lower amounts that soy such as gelation and oil holding capacity, which can suppress the undesirable sensory properties.

Gelation properties of optimally extracted sour cherry kernel PI was determined by evaluating the least gelation concentration (LGC) since lower LGC values represent higher gelation capacity [117]. LGC value of 6.0 g/100 g of protein in the extract was observed for all suspensions of 2-20% in three replicates. Sour cherry kernel protein isolates showed much lower LGC value than that of soy PI (9-11 g/100 g) [118], chickpea and pea PI (12-14g/100g) [116, 117], and lentil PI (8-10g/ 100g) [61]; whereas a LGC value was in the interval of that of whey PI (5-9 g/100 g) under similar gelling conditions [119]. The lower LGC value of sour cherry kernel PI indicates the possibility of obtaining desirable textural properties in food products such as puddings and ice creams by using lower concentrations than soy protein.

Overall, functional properties of SCKPI were comparable with most of plant based proteins isolates, even the most common soy PI. The solubility, oil absorption, and gelation properties of SCKPI are superior to that of SPI. Thus, sour cherry kernel protein can be used instead of soy to improve dairy and bakery food products such as puddings, ice creams, and biscuits but in smaller amounts than soy in order to ensure the sensory preferences. When the industrial scale production considered, the annual production rate of a crop and the price of its protein constituent should be given attention. Despite the fact that soybean has been produced (278 Mt) much more than sour cherry (1.4 Mt), and used in more than 2000 commercial food products, worldwide [52]; the production rate in Turkey is very close in between those two crops (180 Tt for soy and 200 Tt for sour cherry) [1]. Moreover, Turkey is among the top producers of sour cherry, although the annual domestic production price of sour cherry crop (1064.12 USD/tonne) is twice of soy (523.11 USD/tonne) [1], the waste disposal costs of juice processing industry can overcome this price [6]. With its aforementioned functional properties and high digestibility, sour cherry kernel protein can be preferred over soy.

5. CONCLUSION

In conclusion, the study of single factors (i.e. pH, solid/solvent ratio, extraction time) had a significant impact on the yield of sour cherry kernel protein isolate. Implementation of RSM was optimized the yield of sour cherry kernel protein isolate. The model was successfully verified and the most efficient extraction conditions were corresponding to pH 8.5, solid/solvent ratio 1:10g/mL, and extraction time of 1 h giving a protein yield of 63.76%, and a protein content of 88.28%. Sour cherry kernel protein has both high protein content and protein quality with respect to content of limited aminoacids like Lys. Through comparison with soy protein isolate, it is deduced that the physicochemical and functional properties of sour cherry kernel protein isolate can be valorised as a promising proteinaceous food ingredient among plant based food wastes. A special focus should be given on fortification of food products that require improved functionality on oil absorption, foaming, and gelation as in confectionary, dairy, and bakery. The added amount of our cherry kernel protein should be arranged in a manner that would not alter the sensory attributes of the product. With its high solubility and *in vitro* digestibility, the sour cherry kernel protein can be used efficiently in food industry. The protein and starch constituents of sour cherry kernel that are responsible for thermal degradation and ease of digestion should be analysed further in future studies.

REFERENCES

[1] **FAOSTAT**. *World List of Annual Production Quantity and Price of Sour Cherry and Soybean Crops*. 2013 May 2015 [cited 2016 June 6]; Available from: http://faostat3.fao.org/download/Q/QC/E.

[2] **TURKSTAT**. *The production of sour cherry crop has been increased*. 2014 2015 [cited 2016 June 6]; Available from: http://www.turkstat.gov.tr/PreTablo.do?alt_id=1001.

[3] **Chaovanalikit, A.a.W., R.E.,**, *Total Anthocyanins and Total Phenolics of Fresh and Processed Cherries and Their Antioxidant Properties.* Food Chemistry and Toxicology 69, 2003: p. FCT67-FCT72.

[4] **SELFNutritionData**. *Nutritional Information of Red Raw Sour Cherries and Their Kernel*. 2015 2015 [cited 2016 July 6]; Available from: http://nutritiondata.self.com/.

[5] **Damar, İ. and A. Ekşi**, *Antioxidant capacity and anthocyanin profile of sour cherry (Prunus cerasus L.) juice.* Food chemistry, 2012. **135**(4): p. 2910-2914.

[6] **Barrett, D.M., L. Somogyi, and H.S. Ramaswamy**, *Processing fruits: science and technology*. 2004: CRC Press.

[7] **Angin, D.**, *Production and characterization of activated carbon from sour cherry stones by zinc chloride.* Fuel, 2014. **115**: p. 804-811.

[8] **Parfitt, J., M. Barthel, and S. Macnaughton**, *Food waste within food supply chains: quantification and potential for change to 2050.* Philosophical Transactions of the Royal Society of London B: Biological Sciences, 2010. **365**(1554): p. 3065-3081.

[9] **Wadhwa, M. and M. Bakshi**, *Utilization of fruit and vegetable wastes as livestock feed and as substrates for generation of other value-added products.* RAP Publication, 2013. **4**.

[10] **Toydemir, G., et al.**, *Investigating the transport dynamics of anthocyanins from unprocessed fruit and processed fruit juice from sour cherry (Prunus cerasus L.) across intestinal epithelial cells.* Journal of agricultural and food chemistry, 2013. **61**(47): p. 11434-11441.

[11] **Czompa, A., et al.**, *Cardioprotection afforded by sour cherry seed kernel: the role of heme oxygenase-1.* Journal of cardiovascular pharmacology, 2014. **64**(5): p. 412-419.

[12] **Yılmaz, C. and V. Gökmen**, *Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions.* Industrial Crops and Products, 2013. **49**: p. 130-135.

[13] **Ferretti, G., et al.**, *Cherry antioxidants: from farm to table.* Molecules, 2010. **15**(10): p. 6993-7005.

[14] **Kim, D.-O., et al.**, *Sweet and sour cherry phenolics and their protective effects on neuronal cells.* Journal of Agricultural and Food Chemistry, 2005. **53**(26): p. 9921-9927.

[15] **Jayaprakasam, B., et al.**, *Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits.* Journal of Agricultural and Food Chemistry, 2005. **53**(1): p. 28-31.

[16] **Homoki, J.R., et al.**, *Anthocyanin composition, antioxidant efficiency, and α-amylase inhibitor activity of different Hungarian sour cherry varieties (Prunus cerasus L.).* Food chemistry, 2016. **194**: p. 222-229.

[17] **Kirakosyan, A., et al.**, *Chemical profile and antioxidant capacities of tart cherry products.* Food Chemistry, 2009. **115**(1): p. 20-25.

[18] **Seymour, E.M., et al.**, *Altered hyperlipidemia, hepatic steatosis, and hepatic peroxisome proliferator-activated receptors in rats with intake of tart cherry.* Journal of medicinal food, 2008. **11**(2): p. 252-259.

[19] **Blando, F., C. Gerardi, and I. Nicoletti**, *Sour cherry (Prunus cerasus L) anthocyanins as ingredients for functional foods.* BioMed Research International, 2004. **2004**(5): p. 253-258.

[20] **Wang, H., et al.**, *Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries.* Journal of natural products, 1999. **62**(2): p. 294-296.

[21] **Kang, S.-Y., et al.**, *Tart cherry anthocyanins inhibit tumor development in Apc Min mice and reduce proliferation of human colon cancer cells.* Cancer letters, 2003. **194**(1): p. 13-19.

[22] **Zhang, Y., et al.**, *Insulin secretion and cyclooxygenase enzyme inhibition by cabernet sauvignon grape skin compounds.* Journal of agricultural and food chemistry, 2004. **52**(2): p. 228-233.

[23] **Matsui, T., et al.**, *α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity.* Journal of Agricultural and Food Chemistry, 2001. **49**(4): p. 1948-1951.

[24] **Guo, H., et al.**, *Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1.* The Journal of nutritional biochemistry, 2012. **23**(4): p. 349-360.

[25] **Burkhardt, S., et al.**, *Detection and quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (Prunus cerasus).* Journal of Agricultural and Food Chemistry, 2001. **49**(10): p. 4898-4902.

[26] **Cseke, L.J., et al.**, *Natural products from plants*. 2006: CRC press.

[27] *SELFNutritionData*, in *Fruit and Fruit Juices*. 2015.

[28] **Bak, I., et al.**, *Isolation and analysis of bioactive constituents of sour cherry (Prunus cerasus) seed kernel: an emerging functional food.* Journal of medicinal food, 2010. **13**(4): p. 905-910.

[29] **Bak, I., et al.**, *Evaluation of systemic and dermal toxicity and dermal photoprotection by sour cherry kernels.* Phytotherapy Research, 2011. **25**(11): p. 1714-1720.

[30] **Bak, I., et al.**, *Cardioprotective mechanisms of Prunus cerasus (sour cherry) seed extract against ischemia-reperfusion-induced damage in isolated rat hearts.* American Journal of Physiology-Heart and Circulatory Physiology, 2006. **291**(3): p. H1329-H1336.

[31] **Juhasz, B., et al.**, *Cardioprotective effects of sour cherry seed extract (SCSE) on the hypercholesterolemic rabbit heart.* Current pharmaceutical design, 2013. **19**(39): p. 6896-6905.

[32] **Mahmoud, F., et al.**, *Sour cherry (prunus cerasus) seed extract increases heme oxygenase-1 expression and decreases proinflammatory signaling in peripheral blood human leukocytes from rheumatoid arthritis patients.* International immunopharmacology, 2014. **20**(1): p. 188-196.

[33] **Mahmoud, F.F., et al.**, *Sour Cherry Seed Kernel Extract Increases Heme Oxygenase-1 Expression and Decreases Representation of CD3+ TNF-α+ and CD3+ IL-8+ Subpopulations in Peripheral Blood Leukocyte Cultures from Type 2 Diabetes Patients.* Phytotherapy Research, 2013. **27**(5): p. 767-774.

[34] **Haines, D., et al.**, *Role of haeme oxygenase-1 in resolution of oxidative stress-related pathologies: focus on cardiovascular, lung, neurological and kidney disorders.* Acta physiologica, 2012. **204**(4): p. 487-501.

[35] **R., F.O.**, ed. *Food Chemistry*. Amino acids, peptides, and proteins, ed. D. S. 1998. 321-400.

[36] **Ozdal, T., E. Capanoglu, and F. Altay**, *A review on protein–phenolic interactions and associated changes.* Food Research International, 2013. **51**(2): p. 954-970.

[37] **Khalid, E., E. Babiker, and A.E. Tinay**, *Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration.* Food Chemistry, 2003. **82**(3): p. 361-366.

[38] **Oreopoulou, V. and C. Tzia**, *Utilization of plant by-products for the recovery of proteins, dietary fibers, antioxidants, and colorants*, in *Utilization of by-products and treatment of waste in the food industry*. 2007, Springer. p. 209-232.

[39] **Durmaz, G. and M. Alpaslan**, *Antioxidant properties of roasted apricot (Prunus armeniaca L.) kernel.* Food Chemistry, 2007. **100**(3): p. 1177-1181.

[40] **Sathe, S.K.**, *Protein solubility and functionality.* Food proteins and peptides: chemistry, functionality, interactions, and commercialization, 2012: p. 95-124.

[41] **Alfredo, V.-O., et al.**, *Physicochemical properties of a fibrous fraction from chia (Salvia hispanica L.).* LWT-Food Science and Technology, 2009. **42**(1): p. 168-173.

[42] **López-Vargas, J.H., et al.**, *Chemical, physico-chemical, technological, antibacterial and antioxidant properties of dietary fiber powder obtained from yellow passion fruit (Passiflora edulis var. flavicarpa) co-products.* Food Research International, 2013. **51**(2): p. 756-763.

[43] **Yust, M.d.M., et al.**, *Improvement of functional properties of chickpea proteins by hydrolysis with immobilised Alcalase.* Food Chemistry, 2010. **122**(4): p. 1212-1217.

[44] **Mirabella, N., V. Castellani, and S. Sala**, *Current options for the valorization of food manufacturing waste: a review.* Journal of Cleaner Production, 2014. **65**: p. 28-41.

[45] **Monier, V., et al.**, *Preparatory study on food waste across EU 27*. 2011: European Commission.

[46] **Rosentrater, K.**, *Laboratory analysis of an electrostatic dust collection system.* Agricultural Engineering International: CIGR Journal, 2004.

[47] **Galanakis, C.M.**, *Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications.* Trends in Food Science & Technology, 2012. **26**(2): p. 68-87.

[48] **Kosseva, M.R.**, *Sources, Characterization, and Composition of Food Industry Wastes.* 2013: p. 37-60.

[49] **Baiano, A.**, *Recovery of biomolecules from food wastes--a review.* Molecules, 2014. **19**(9): p. 14821-42.

[50] **Pham, T.P.T., et al.**, *Food waste-to-energy conversion technologies: Current status and future directions.* Waste Management, 2015. **38**: p. 399-408.

[51] **Webb, M., H. Naeem, and K. Schmidt**, *Food protein functionality in a liquid system: a comparison of deamidated wheat protein with dairy and soy proteins.* Journal of Food Science, 2002. **67**(8): p. 2896-2902.

[52] **Horax, R., et al.**, *Protein extraction optimisation, characterisation, and functionalities of protein isolate from bitter melon (Momordica charantia) seed.* Food Chemistry, 2011. **124**(2): p. 545-550.

[53] **Tan, E.-S., N. Ying-Yuan, and C.-Y. Gan**, *A comparative study of physicochemical characteristics and functionalities of pinto bean protein isolate (PBPI) against the soybean protein isolate (SPI) after the extraction optimisation.* Food chemistry, 2014. **152**: p. 447-455.

[54] **Sharma, G.M., et al.**, *Functional properties of select edible oilseed proteins.* J Agric Food Chem, 2010. **58**(9): p. 5457-64.

[55] **Shao, D., et al.**, *Characteristics of Isolation and Functionality of Protein from Tomato Pomace Produced with Different Industrial Processing Methods.* Food and Bioprocess Technology, 2013. **7**(2): p. 532-541.

[56] **Yılmaz, C.**, *Vişne Çekirdeği Atıklarının Gıda İngrediyeni Olarak Değerlendirilmesi*, in *Food Engineering Department*. 2013, Hacettepe Üniversitesi: Ankara.

[57] **Viuda-Martos, M., et al.**, *Chemical, physico-chemical and functional properties of pomegranate (Punica granatum L.) bagasses powder co-product.* Journal of Food Engineering, 2012. **110**(2): p. 220-224.

[58] **Karaca, A.C., N. Low, and M. Nickerson**, *Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction.* Food Research International, 2011. **44**(9): p. 2991-2998.

[59] **Chen, N., M. Zhao, and W. Sun**, *Effect of protein oxidation on the in vitro digestibility of soy protein isolate.* Food Chem, 2013. **141**(3): p. 3224-9.

[60] **Lee, H., et al.**, *Chemical and functional quality of protein isolated from alkaline extraction of Australian lentil cultivars: Matilda and Digger.* Food Chemistry, 2007. **102**(4): p. 1199-1207.

[61] **Jarpa-Parra, M., et al.**, *Optimization of lentil protein extraction and the influence of process pH on protein structure and functionality.* LWT - Food Science and Technology, 2014. **57**(2): p. 461-469.

[62] **Karaca, A.C., N. Low, and M. Nickerson**, *Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction.* Food Research International, 2011. **44**(9): p. 2742-2750.

[63] **Mune Mune, M.A., S.R. Minka, and I.L. Mbome**, *Optimising functional properties during preparation of cowpea protein concentrate.* Food Chem, 2014. **154**: p. 32-7.

[64] **Zhao, J., et al.**, *Optimization of arachin extraction from defatted peanut (Arachis hypogaea) cakes and effects of ultra-high pressure (UHP) treatment on physiochemical properties of arachin.* Food and Bioproducts Processing, 2015. **95**: p. 38-46.

[65] **Ahmed, J., S.K. Varshney, and H.S. Ramaswamy**, *Effect of high pressure treatment on thermal and rheological properties of lentil flour slurry.* LWT - Food Science and Technology, 2009. **42**(9): p. 1538-1544.

[66] **Sharma, P., B. Tilakratne, and A. Gupta**, *Utilization of wild apricot kernel press cake for extraction of protein isolate.* Journal of food science and technology, 2010. **47**(6): p. 682-685.

[67] **Gandhi, A., et al.**, *Effect of incorporation of defatted soyflour on the texture of chapatis.* Journal of texture studies, 2000. **31**(6): p. 609-614.

[68] **Fernández-Quintela, A., et al.**, *Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain.* Plant Foods for Human Nutrition, 1997. **51**(4): p. 331-341.

[69] **Viuda-Martos, M., et al.**, *Chemical, physico-chemical and functional properties of pomegranate (Punica granatum L.) bagasses powder co-product.* Journal of Food Engineering, 2012. **110**(2): p. 220-224.

[70] **Lynch, J.M., D.M. Barbano, and J.R. Fleming**, *Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: collaborative study.* Journal of AOAC International, 1997. **81**(4): p. 763-774.

[71] **Mune Mune, M.A., S.R. Minka, and I.L. Mbome**, *Response surface methodology for optimisation of protein concentratepreparation from cowpea [Vigna unguiculata (L.) Walp]*. Food Chemistry, 2008. **110**(3): p. 735-741.

[72] **Siow, H.-L. and C.-Y. Gan**, *Functional protein from cumin seed (Cuminum cyminum): Optimization and characterization studies.* Food Hydrocolloids, 2014. **41**: p. 178-187.

[73] **Bradford, M.M.**, *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.* Analytical biochemistry, 1976. **72**(1-2): p. 248-254.

[74] **Jiang, J. and Y.L. Xiong**, *Extreme pH treatments enhance the structure-reinforcement role of soy protein isolate and its emulsions in pork myofibrillar protein gels in the presence of microbial transglutaminase.* Meat Sci, 2013. **93**(3): p. 469-76.

[75] **Gornall, A.G., C.J. Bardawill, and M.M. David**, *Determination of serum proteins by means of the biuret reaction.* J. biol. Chem, 1949. **177**(2): p. 751-766.

[76] **Classics Lowry, O., et al.**, *Protein measurement with the Folin phenol reagent.* J biol Chem, 1951. **193**: p. 265-275.

[77] **Kammerer, D.R., et al.**, *Recovery of polyphenols from the by-products of plant food processing and application as valuable food ingredients.* Food Research International, 2014. **65**: p. 2-12.

[78] **Kelly, J. and S. Hefle**, *2S methionine-rich protein (SSA) from sunflower seed isan IgE-binding protein*.* Allergy, 2000. **55**(6): p. 556-559.

[79] **Tkachuk, R. and G. Irvine**, *Amino acid compositions of cereals and oilseed meals.* Cereal Chemistry, 1969. **46**: p. 206-218.

[80] **Ma, T., Q. Wang, and H. Wu**, *Optimization of extraction conditions for improving solubility of peanut protein concentrates by response surface methodology.* LWT - Food Science and Technology, 2010. **43**(9): p. 1450-1455.

[81] **de Moura, J.N., et al.**, *Enzyme-assisted aqueous extraction of oil and protein from soybeans and cream de-emulsification.* Journal of the American Oil Chemists' Society, 2008. **85**(10): p. 985-995.

[82] **Li, F., et al.**, *Antiproliferative activity of peels, pulps and seeds of 61 fruits.* Journal of Functional Foods, 2013. **5**(3): p. 1298-1309.

[83] **Mohapatra, D., S. Mishra, and N. Sutar**, *Banana and its by-product utilization: an overview.* J Sci Ind Res, 2010. **69**(5): p. 323-329.

[84] **Rodrigues, I.M., J.F. Coelho, and M.G.V. Carvalho**, *Isolation and valorisation of vegetable proteins from oilseed plants: Methods, limitations and potential.* Journal of Food Engineering, 2012. **109**(3): p. 337-346.

[85] **Özdemir, K.S., et al.**, *Hazelnut skin powder: A new brown colored functional ingredient.* Food Research International, 2014. **65**: p. 291-297.

[86] **Helrich, K.**, *Official methods of Analysis of the AOAC. Volume 2*. 1990: Association of Official Analytical Chemists Inc.

[87] **Committee, A.A.o.C.C.A.M.**, *Approved Methods of the American Association of Cereal Chemists*. Vol. 1. 2000: Amer Assn of Cereal Chemists.

[88] **Stone, A.K., et al.**, *Functional attributes of pea protein isolates prepared using different extraction methods and cultivars.* Food Research International, 2015. **76**: p. 31-38.

[89] **Menegassi, B., A.M.R. Pilosof, and J.A.G. Arêas**, *Comparison of properties of native and extruded amaranth (Amaranthus cruentus L. – BRS Alegria) flour.* LWT - Food Science and Technology, 2011. **44**(9): p. 1915-1921.

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

[91] **Turan, D., E. Capanoglu, and F. Altay**, *Investigating the effect of roasting on functional properties of defatted hazelnut flour by response surface methodology (RSM).* LWT - Food Science and Technology, 2015. **63**(1): p. 758-765.

[92] **Özdemir, M. and O. Devres**, *Analysis of color development during roasting of hazelnuts using response surface methodology.* Journal of Food Engineering, 2000. **45**(1): p. 17-24.

[93] **Sridaran, A., A.A. Karim, and R. Bhat**, *Pithecellobium jiringa legume flour for potential food applications: Studies on their physico-chemical and functional properties.* Food Chemistry, 2012. **130**(3): p. 528-535.

[94] **Yuliana, M., et al.**, *Isolation and characterization of protein isolated from defatted cashew nut shell: Influence of pH and NaCl on solubility and functional properties.* LWT-Food Science and Technology, 2014. **55**(2): p. 621-626.

[95] **Świeca, M., B. Baraniak, and U. Gawlik-Dziki**, *In vitro digestibility and starch content, predicted glycemic index and potential in vitro antidiabetic effect of lentil sprouts obtained by different germination techniques.* Food chemistry, 2013. **138**(2): p. 1414-1420.

[96] **Turan, D., F. Altay, and E.Ç. Güven**, *The influence of thermal processing on emulsion properties of defatted hazelnut flour.* Food chemistry, 2015. **167**: p. 100-106.

[97] **Correia, P. and M.L. Beirão-da-Costa**, *Effect of drying temperatures on starch-related functional and thermal properties of chestnut flours.* Food and Bioproducts Processing, 2012. **90**(2): p. 284-294.

[98] **Acevedo, B.A., et al.**, *Gelation, thermal and pasting properties of pigeon pea (Cajanus cajan L.), dolichos bean (Dolichos lablab L.) and jack bean (Canavalia ensiformis) flours.* Journal of Food Engineering, 2013. **119**(1): p. 65-71.

[99] **Pickardt, C., et al.**, *Isoelectric protein precipitation from mild-acidic extracts of de-oiled sunflower (Helianthus annuus L.) press cake.* European Food Research and Technology, 2011. **233**(1): p. 31-44.

[100] **Firatligil-Durmus, E. and O. Evranuz**, *Response surface methodology for protein extraction optimization of red pepper seed (Capsicum frutescens).* LWT - Food Science and Technology, 2010. **43**(2): p. 226-231.

[101] **Mizubuti, I.Y., et al.**, *Response surface methodology for extraction optimization of pigeon pea protein.* Food Chemistry, 2000. **70**(2): p. 259-265.

[102] **Eromosele, C., et al.**, *Extractability of African yam bean (Sphenostylis stenocarpa) protein in acid, salt and alkaline aqueous media.* Food Hydrocolloids, 2008. **22**(8): p. 1622-1628.

[103] **Boye, J., F. Zare, and A. Pletch**, *Pulse proteins: processing, characterization, functional properties and applications in food and feed.* Food Research International, 2010. **43**(2): p. 414-431.

[104] **Horax, R., N. Hettiarachchy, and P. Chen**, *Extraction, quantification, and antioxidant activities of phenolics from pericarp and seeds of bitter melons (Momordica charantia) harvested at three maturity stages (immature, mature, and ripe).* Journal of agricultural and food chemistry, 2010. **58**(7): p. 4428-4433.

[105] **Moure, A., et al.**, *Functionality of oilseed protein products: A review.* Food Research International, 2006. **39**(9): p. 945-963.

[106] **Damodaran, S.**, *Food protein: An overview.* Food proteins and their applications, ed. S.D.A. Paraf. 1997, New York: Marcel Dekker.

[107] **Barbosa-Cánovas, G.V., et al.**, *Bulk properties.* Food Powders: Physical Properties, Processing, and Functionality, 2005: p. 55-90.

[108] **George, R.**, *Freezing proceseses used in the food industry.* Trends in Food Science & Technology, 1993. **4**(5): p. 134-138.

[109] **Ma, Z., et al.**, *Thermal processing effects on the functional properties and microstructure of lentil, chickpea, and pea flours.* Food Research International, 2011. **44**(8): p. 2534-2544.

[110] **Doporto, M.C., et al.**, *Physicochemical, thermal and sorption properties of nutritionally differentiated flours and starches.* Journal of Food Engineering, 2012. **113**(4): p. 569-576.

[111] **Damodaran, S.**, *Structure-function relationship of food proteins.* Protein functionality in food systems, 1994: p. 1-37.

[112] **El-Aal, M.A., M. Hamza, and E. Rahma**, *In vitro digestibility, physico-chemical and functional properties of apricot kernel proteins.* Food chemistry, 1986. **19**(3): p. 197-211.

[113] **Qiu, C., et al.**, *Effect of citric acid deamidation on in vitro digestibility and antioxidant properties of wheat gluten.* Food Chem, 2013. **141**(3): p. 2772-8.

[114] **El Nasri, N.A. and A. El Tinay**, *Functional properties of fenugreek (Trigonella foenum graecum) protein concentrate.* Food Chemistry, 2007. **103**(2): p. 582-589.

[115] **Tang, C.-H. and X. Sun**, *A comparative study of physicochemical and conformational properties in three vicilins from Phaseolus legumes: Implications for the structure–function relationship.* Food Hydrocolloids, 2011. **25**(3): p. 315-324.

[116] **Boye, J., et al.**, *Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques.* Food Research International, 2010. **43**(2): p. 537-546.

[117] **Zhang, T., B. Jiang, and Z. Wang**, *Gelation properties of chickpea protein isolates.* Food Hydrocolloids, 2007. **21**(2): p. 280-286.

[118] **Hua, Y., et al.**, *Heat induced gelling properties of soy protein isolates prepared from different defatted soybean flours.* Food Research International, 2005. **38**(4): p. 377-385.

[119] **Puyol, P., M. Perez, and D. Horne**, *Heat-induced gelation of whey protein isolates (WPI): effect of NaCl and protein concentration.* Food Hydrocolloids, 2001. **15**(3): p. 233-237.

CURRICULUM VITAE

Name Surname **:** Hatice Saadiye Eryılmaz

Place and Date of Birth **:** 08.08.1990 İstanbul

E-Mail **:** saadiyyee@yahoo.com

EDUCATION

- **B.Sc.** **:** 2013, Bosphorus University, Faculty of Natuıral Sciences, Department of Chemistry
- **Visitor Student** **:** 2011-2012, University of Houston, College of Liberal Arts and Social Sciences, Department of Health and Human Nutrition
- **High School** **:** 2007, Arda Asalet Anatolian High School

COMPUTATIONAL PROGRAMS AND SOFTWARES

- Word, Excel, Powerpoint: Good
- SPSS, Design Expert, Minitab: Average
- Spartan: Average

TRAININGS

- Research Student, Computational Chemistry Lab at Bosphorus University-2010-2011
- Internship at "Hıfzıssıhha Institute" as a Food Quality Control Lab Asistant-2010
- Intership at "İBB Halk Ekmek" as a Food Quality Control Lab Asistant-2009

PERSONAL INTERESTS, ACTIVITIES AND HOBBIES

- Taking online courses on food science, nutrition and health (including certificates): www.coursera.com
- Pariticipating congress and panels on food safety, process conditions, and regulations by "Halal and Healthy Food Platform"(including certificates): www.helalvesaglikli.com
- Playing musical instruments: Nay (good), Organ (good)
- Language Skills: English (Advanced), Deutsch (Intermediate), Arabic (Pre-inter), Chinese (Beginner).
- Art skills: A painting competition certificate on "Water and natural disasters" in 2005.

- Participating environmental activities (with certificate):
  -by a foundation on environmental and cultural issues “ÇEKÜD” such as tree planting organizations.
  -AVRASYA Marathone several times.