FEN BİLİMLERİ ENSTİTÜSÜ

GIDA MÜHENDİSLİĞİ ANABİLİM DALI

GIDA MÜHENDİSLİĞİ PROGRAMI

BİTKİSEL YAĞ OLARAK DEĞERLENDİRİLEBİLEN YAĞLI TOHUM VE MEYVELERİN POSALARINDAN PROTEİN KONSANTRESİ VE İZOLATLARININ ÜRETİLMESİ VE BUNLARIN DEĞERLENDİRİLMESİ

YÜKSEK LİSANS TEZİ

ÖZGENUR COŞKUN

Danışmanı: Y.Doç.Dr. İBRAHİM GÜLSEREN

İSTANBUL

Şubat 2018

Her hakkı saklıdır

İstanbul Sabahattin Zaim Üniversitesi

INSTITUTE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF FOOD ENGINEERING

FOOD ENGINEERING PROGRAMME

PRODUCTION OF PROTEIN CONCENTRATES FROM OILSEED AND OIL FRUIT MEALS AND THEIR UTILIZATION

M.Sc. THESIS

ÖZGENUR COŞKUN

Advisor: Assistant Prof. İBRAHİM GÜLSEREN

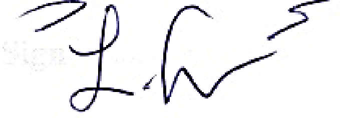
ISTANBUL
February 2018
All rights reserved.

APPROVAL PAGE

Özgenur Coşkun, a M.Sc. student of IZU Institute of Science and Technology with student ID 510315002, successfully defended the thesis/dissertation entitled "PRODUCTION OF PROTEIN CONCENTRATES FROM OILSEED AND OIL FRUIT MEALS AND THEIR UTILIZATION", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Advisor / President

Assistant Prof. İbrahim GÜLSEREN



Member

Assistant Prof. Banu METİN

Member

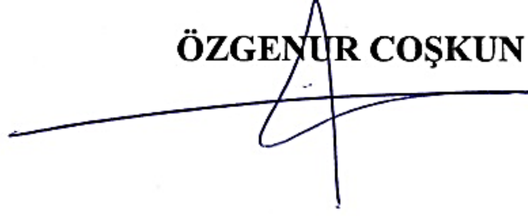
Associate Prof. Filiz ALTAY

DECLARATION

This master science thesis were prepared by me at Istanbul Sabahattin Zaim University, Natural Science Institute, Food Engineering Department. This study is totally original and I followed scientific ethics from the beginning to the end of my work. I declare that I refer to all the information and interpretations that I have obtained during this study and I do not infringe any patents or copyrights.

Sign

ÖZGENUR COŞKUN



FOREWORD

This study was funded by a grant from TÜBİTAK 3501 Programme (Grant No. 115O569; The Scientific and Technological Research Council of Turkey).

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

To my sincere advisor Yrd. Doç Dr. İbrahim GÜLSEREN, who shared his valuable knowledge, experience and support, guided me with his opinions and practical solutions during my thesis period,

To Yrd. Doç. Dr. Halime PEHLİVANOĞLU and Bilal ÇAKIR, whom I experienced them extensive knowledge and guidance throughout my master education period,

To my dear friend, Esra YILDIRIM whom I consult with, learn and benefit from whom was friendly and cooperative during my master education period, also Kübra YEŞİLYURT, Sinejan ÖZCAN and Feyza BAŞBUĞ thank you for listening and supporting me through the entire process.

To precious members of my family, especially my mother Zühre ÖZDEMİR who supported me either physically or mentally throughout my thesis study and whole life.

To my husband, Emre COŞKUN for his support, understanding and efforts making my life easier. Thank you for being there.

TABLE OF CONTENTS

APPROVAL PAGE .... Hata! Yer işareti tanımlanmamış.

DECLARATION .... Hata! Yer işareti tanımlanmamış.

FOREWORD .... **ii**

TABLE OF CONTENTS .... **iii**

ABBREVIATIONS .... **vii**

LIST OF TABLES .... **ix**

LIST OF FIGURES .... **xii**

LIST OF SYMBOLS .... **xv**

ABSTRACT (English/Turkish) .... **xvi**

1. INTRODUCTION **1**

2. LITERATURE REVIEW **3**

2.1 Functional properties of proteins 3

2.2 Sources of plant proteins 4

2.3 Fundamental information on the current samples 5

2.4 Enhancement of oilseed protein functionality 7

2.5 Applications on the utilization of plant proteins in baked foods 8

3. MATERIAL AND METHODS **11**

3.1 Materials 11

3.2 Preparation of protein concentrates 11

3.2.1 Alkali extraction-isoelectric precipitation method 11

3.2.2 Salt extraction method 12

3.2.3 Micellar precipitation method 12

3.2.4 Solvent extraction 12

3.3 Basic physicochemical analysis of the meals and protein concentrates .. 13

3.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) 13

3.5 Functional properties of protein concentrates 13
3.5.1 Solubility 13
3.5.2 Water and oil holding capacity 14
3.5.3 Emulsification activity 14
3.5.4 Foaming capacity 15
3.5.5 Drop shape tensiometry.. 15
3.5.6 Rheological analysis.. 15
3.6 Preparation of Maillard Conjugates 16
3.6.1 Native-PAGE or SDS-PAGE analysis 16
3.6.2 Foaming capacity and stability... 17
3.6.3 RP-HPLC-RID analysis 17
3.6.4 Drop shape tensiometry.. 18
3.7 Preparation of TGase enzyme treated protein hydrolysates 19
3.7.1 Preparation of black cumin, pumpkin seed and grape seed protein concentrates 19
3.7.2 Measurement of transglutaminase activity... 19
3.7.3 SDS-PAGE analysis of TGase treated protein hydrolysates 20
3.7.4 Foaming capacity and stability of TGase treated protein hydrolysates 20
3.8 Wheat bread manufacture.. 20
3.8.1 Wheat flour analysis... 22
3.8.2 Dough properties 22
3.8.3 Color parameters 22
3.8.4 Texture profile analysis.. 23
3.9 Gluten-free bread manufacture... 23
3.9.1 Gluten-free bread manufacture with functionality improved protein samples 25
3.9.2 Loaf volume analysis 27
3.9.3 Color analysis.. 27

3.9.4 Texture profile analysis 27

4. RESULTS AND DISCUSSION 28

4.1 Influence of aqueous and organic extraction on the functionality of black cumin protein concentrates 28

4.1.1 Influence of extraction methodologies on protein concentrates 28

4.1.2 Molecular weight analysis of protein concentrate (SDS-PAGE) 30

4.1.3 Functionality of the black cumin protein concentrates 33

4.2 Physicochemical characteristics and functionality of protein concentrates manufactured from pumpkin, pomegranate and grape seeds 46

4.2.1 Physicochemical properties of protein concentrates 46

4.2.2 SDS-PAGE analysis of protein concentrates 48

4.2.3 Functionality of protein concentrates 51

4.3 Enhancement of foaming characteristics of black cumin protein concentrates based on Maillard conjugation 64

4.3.1 Electrophoretic analysis 64

4.3.2 Foaming capacity and stability 66

4.3.3 RP-HPLC-RID Analysis 72

4.3.4 Drop shape tensiometry 75

4.4 Enhancement of foaming characteristics of current samples based on transglutaminase treatment 79

4.4.1 SDS-PAGE analysis 79

4.4.2 Effect of crosslinking on foaming capacity and stability 81

4.5 Effect of black cumin, grape seed and pumpkin seed protein concentrates on the quality of wheat bread 83

4.5.1 Chemical composition 83

4.5.2 Wheat flour analysis 85

4.5.3 Dough rheological properties 88

4.5.4 Bread characteristics 93

4.5.5 Texture profile analysis 95

4.5.6 Color parameters 97

4.6 Effect of black cumin, grape seed and pumpkin seed protein concentrates on the gluten-free bread characteristics 99

4.6.1 Gluten-free bread making 99

4.6.2 Loaf volume analysis 104

4.6.3 Texture parameters 106

4.6.4 Color parameters 110

5. CONCLUSION 113

REFERENCES 115

ACKNOWLEDGEMENTS 131

CURRICULUM VITAE 132

ABBREVIATIONS

BC: black cumin

GS: grape seed

PS: pumpkin seed

BCPC: black cumin protein concentrate

GSPC: grape seed protein concentrate

PSPC: pumpkin seed protein concentrate

BCPD: black cumin protein dispersion

GSPD: grape seed protein dispersion

PSPD: pumpkin seed protein dispersion

ND: Not detected.

AE-IP

SE: salt extraction

MP: micellar precipitation

EAI: Emulsifiying activity index

FC: Foaming capacity

WHC: Water holding capacity

OHC: Oil holding capacity

SDS-PAGE: Sodium dodecly sulfate polyacrylamide gel electrophoresis

AOAC: Official Methods of Analysis

NMKL: Nordic Committee on Food Analysis

CCD: charge coupled device

RP-HPLC-RID: Reversed phase-high pressure liquid chromatography-refractive index detector

TGase: Transglutaminase

TCA: Trichloroacetic acid

ICC: International Association for Cereal Science and Technology

AACCI: American Association of Cereal Chemists International

TPA: Texture profile analysis

UF/DF:Ultrafiltration/diafiltration

LIST OF TABLES

Table 3.1. Bread formulations (BC: black cumin; GS: grape seed; PS: pumpkin seed; BCPC: black cumin protein concentrate; GSPC: grape seed protein concentrate; PSPC: pumpkin seed protein concentrate)... 21

Table 3.2. Gluten-free bread formulations (BC: black cumin; GS: grape seed; PS: pumpkin seed; BCPC: black cumin protein concentrate; GSPC: grape seed protein concentrate; PSPC: pumpkin seed protein concentrate). 24

Table 3.3. Gluten-free bread formulations produced with protein concentrates with improved properties (BC: black cumin; GS: grape seed; PS: pumpkin seed; BCPD: black cumin protein dispersion; GSPD: grape seed protein dispersion; PSPD: pumpkin seed protein dispersion). 26

Table 4.1. Protein (%), moisture (%) and ash (%) contents of deoiled black cumin meals and black cumin protein concentrates before or after hexane extraction. The data represent the average of three independent experiments and their corresponding standard deviation. 29

Table 4.2. Protein (%), moisture (%) and ash (%) contents of pumpkin seed, pomegranate seed and grape seed protein concentrates. The data are the average of two independent experiments with standart deviation. ND: Not detected................ 47

Table 4.3. % Solubility of pumpkin, pomegranate, and grape seed protein concentrates manufactured manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP).The data are the average of three independent experiments with standart deviation....................................... 52

Table 4.4. % Water and oil holding capacity of pumpkin, pomegranate and grape seed protein concentrates manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP). The data represent the average of three independent experiments and their corresponding standard deviation.. 55

Table 4.5. Emulsifiying activity index (EAI) of pumpkin, pomegranate and grape seed protein concentrates manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP).................................. 57

Table 4.6. %Foaming capacity (FC) of pumpkin, pomegranate and grape seed protein concentrates manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) at varying pH values. 59

Table 4.7. Glucose binding (%) and fructose formation (%) characteristics in black cumin protein isolates due to Maillard reaction (100°C) as a function of time at a protein:glucose ratio of 1:2. 74
Table 4.8. Surface elasticity at the Maillard conjugate dispersion-air interface as a function of oscillatory frequency (Hz). Standar deviation was < 5% of the sample mean in all cases... 78
Table 4.9. Chemical composition of protein-fortified bread and flour. BCPC: black cumin protein concentrate enriched wheat flour/bread; GSPC: grape seed protein concentrate enriched wheat flour/bread; PSPC: pumpkin seed protein concentrate enriched wheat flour/bread. The data are the mean of the two measurements with the standard deviation (p<0.05). 84
Table 4.10. Analytical quality parameters of protein-fortified flours. BCPC: black cumin protein concentrate enriched wheat flour; GSPC: grape seed protein concentrate enriched wheat flour; PSPC: pumpkin seed protein concentrate enriched wheat flour. The data are the mean of the two measurements with the standard deviation (p<0.05). 87
Table 4.11. Extensograph parameters of protein-fortified flours. BCPC: black cumin protein concentrate enriched wheat flour; GSPC: grape seed protein concentrate enriched wheat flour; PSPC: pumpkin seed protein concentrate enriched wheat flour. The data are the mean of the two measurements with the standard deviation (p<0.05). 89
Table 4.12. Farinograph parameters of protein-fortified flours. BCPC: black cumin protein concentrate enriched wheat flour; GSPC: grape seed protein concentrate enriched wheat flour; PSPC: pumpkin seed protein concentrate enriched wheat flour. The data are the mean of the two measurements with the standard deviation (p<0.05). 92
Table 4.13. Color parameters for crumb. BCPC; black cumin protein concentrte enriched bread, GSPC: grape seed protein concentrate enriched bread, PSPC: pumpkin seed protein concentrate enriched bread. The data are the mean of the two measurements with the standard deviation (p<0.05). 98
Table 4.14. Loaf volume analysis results for gluten-free breads (BC: black cumin; GS: grape seed; PS: pumpkin seed). The data represent the average of two independent experiments and ± standart deviation (p<0.05). 105

Table 4.15. Color parameters of gluten-free bread crumb (BC: black cumin protein concentrates fortified gluten-free bread; GS: grape seed protein concentrates fortified gluten-free bread; PS: pumpkin seed protein concentrates fortified gluten-free bread). The data represent the average of two independent experiments with ± standart deviation ($p<0.05$). 112

LIST OF FIGURES

Figure 4.1. SDS-PAGE analysis of black cumin (lane numbers: 1-3 after hexane extraction AE-IP, SE and MP, respectively; 4-6: before hexane extraction AE-IP, SE and MP, respectively) protein concentrate dispersions (1%) manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP) methods. 32

Figure 4.2. Aqueous solubility (%) of black cumin protein concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE), or micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein isolate was added as a reference.. 34

Figure 4.3. Water holding capacity (WHC) of black cumin protein concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein isolate was added as a reference. 36

Figure 4.4. Oil holding capacity (OHC) of black cumin protein concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein isolate was added as a reference. 38

Figure 4.5. Foaming stability of black cumin protein concentrates prepared by **(A)** alkali extraction-isoelectric precipitation (AE-IP), **(B)** salt extraction (SE) and **(C)** micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein isolate was added as a reference. 40

Figure 4.6. Emulsification activity index of black cumin concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein isolate was added as a reference.. 42

Figure 4.7. Dynamic surface tension of black cumin protein concentrates (0.1%) prepared by alkali extraction-isoelectric precipitation (AE-IP) method as a function

of time before and after hexane extraction. A representative run was shown for each sample. Data for soy protein isolate was added as a reference. 45
Figure 4.8. SDS-PAGE analysis of pumpkin seed (lane numbers: 1-AE-IP; 2-SE; 3-MP), pomegranate seed (lane numbers: 4-AE-IP; 5-SE) and grape seed (lanes number 6-AE-IP; 7-SE) protein concentrates (1%) manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP) methods. 50
Figure 4.9. Surface tension of pumpkin seed, pomegranate seed and grape seed (0.1%) protein concentrate by alkali extraction-isoelectric precipitation method. 61
Figure 4.10. Heat induced gelation profile of pumpkin seed (A), pomegranate seed (B) and grape seed (C) (10%) protein concentrate dispersions prepared by alkali extraction and isoelectric precipitation method; G'- storage modulus, G''- loss modulus. 63
Figure 4.11. SDS-PAGE analysis of black cumin protein isolates (2%) (Lane 1). Native-PAGE analysis of black cumin-glucose Maillard reaction products (30 min, pH 7 at 100°C) as a function of protein: glucose ratio of 1:1, 1:2, and 1:4 (Lanes 2-4, respectively). Lane 5-7: 1:2 protein: glucose ratio 30 min at pH 3, 5 and 7 respectively. 65
Figure 4.12 Foaming capacity (%) and stability of foams prepared with black cumin protein concentrates (2%) and caseinate (2%) as a function of pH (3-7) and time (0-120 min). 67
Figure 4.13. Foaming capacity (%) and stability of foams prepared with black cumin protein isolates (2%) and glucose as a function of protein:sugar ratio (1:1, 1:2 and 1:4), pH (3-7), reaction duration (0-30 min) and time (0-120 min). 69
Figure 4.14. Foaming capacity (%) and stability of foams prepared with black cumin protein isolates (2%) and lactose or maltodextrin at a protein:sugar ratio of 1:2 as a function of pH (3-7), reaction duration (0-30 min) and time (0-120 min)................ 71
Figure 4.15. RP-HPLC-RID chromatogram of black cumin-glucose Maillard products prepared at a protein:glucose ratio of 1:2 held at (A) 25 °C, (B) 100 °C for 15 min, (C) 100 °C for 30 min. 73
Figure 4.16. Dynamic surface tension of black cumin protein conjugates and the corresponding untreated sample as a function of time (i.e., bubble age) at the air-conjugate dispersion interface. Heat treatment was carried out at 100°C for 15 min at

pH 7 and a protein:carbohydrate concentration ratio of 1:2. Drop shape tensiometry was utilized. Representative runs. 77
Figure 4.17. SDS-PAGE analysis of TGase treated black cumin, pumpkin seed and grape seed protein concentrates (Lane 1: black cumin, Lane 2: Pumpkin seed; Lane 3: grape seed protein concentrates). 80
Figure 4.18. Foaming capacity and stability of TGase treated black cumin, pumpkin seed and grape seed protein concentrates........ 82
Figure 4.19. Images of control and enriched breads with different fermentation times after baking. Duration of fermentation; A: 80 min, B: 110 min, C: 140 min. 94
Figure 4.20. Textural parameters of protein-fortified breads. BC; black cumin protein concentrte enriched bread, GS: grape seed protein concentrate enriched bread, PS: pumpkin seed protein concentrate enriched bread. The data are the mean of the two measurements, and the error bars represent the standard deviation ($p<0.05$). 96
Figure 4.21. Baked gluten-free breads enriched with black cumin, grape seed and pumpkin seed protein concentrate with different water levels (A: Equal water level; B: water level increased by 8%; C: water level increased by 15%)........ 101
Figure 4.22. Baked gluten-free breads enriched with modified protein concentrate of black cumin, grape seed and pumpkin seed (A: Maillard conjugation treatment, B: TGase treatment). 103
Figure 4.23. Texture parameters of control gluten-free bread and gluten-free breads enriched with proteins concentrates. BC: black cumin; GS: grape seed; PS: pumpkin seed protein concentrates. Presented data are mean values of two replications. Error bars mean standart deviations. 108
Figure 4.24. Texture parameters of control gluten-free bread and gluten-free breads enriched with modified protein concentrates. BC: black cumin; GS: grape seed; PS: pumpkin seed protein dispersion added. The data are the mean of the two measurements, and the error bars represent the standard deviation. 109

LIST OF SYMBOLS

%: Percent

$A_{\circ}$: absorbance of the diluted emulsion immediately after homogenization

N: the dilution factor

C: weight of protein per volume ($g.mL^{-1}$)

Φ: oil volume fraction of the emulsion.

Π: The surface pressure

°C: Celsius

mM: milimole

w/v: weight/volume

kDa: kilodalton

ε: Elasticity

γ: Interfacial tension

A: Area

h: hour

g: gram

ml: milliliter

ABSTRACT (English/Turkish)

In this thesis, protein concentrates were produced from cold press meals using appropriate aqueous and organic extraction methods. Among the main samples used in this context were black cumin seeds, pumpkin seeds, grape seeds, and pomegranate seeds. Protein production was carried out using 3 basic aqueous extraction methods. Furthermore oil molecules remaining in the samples were also removed by hexane extraction. The protein contents in the samples demonstrated a wide protein concentration range (about 25-90%).

The physicochemical properties and technical functionalities of the protein concentrates produced were evaluated. In this context, evaluated functional properties were **solubility, emulsion and foam-forming capacity, oil and water retention, swelling capacity and surface activity at air-water interfaces**. For example, surface tension was 37.6, 45.5, 47.5 and 56.1 $mN.m^{-1}$ for AE-IP treated black cumin, pumpkin, pomegranate and grape seed protein concentrate at a protein concentration of 0.1% after 10,000 s of adsorption.

Maillard conjugation and transglutaminase (TGase) treatments have been utilized to improve the functionality of the evaluated samples. The extent of glucose binding was dependent on reaction conditions and increased up to 85% and there was a limited extent of increase in foaming characteristics in foaming capacity. The protein concentrates and their Maillard or TGase treated products were utilized in bread production in addition to the functionality tests.

Base on the support of local company, protein concentrates were used in normal and gluten-free bread formulations and the properties of the bread formulations were evaluated. Consequently, bread products that were both protein-enriched and improved in technical properties were generated. For example, in all cases, the loaf volume of gluten-free breads that enriched with black cumin, grape seed and pumpkin seed protein concentrate significantly improved when water level increased by 15%. It was therefore possible to test the byproduct of a national company in formulations of another national company.

Keywords: Manufacture of cold press oils; oil seed and oil fruit meals; protein concentrates; functional properties of proteins; membrane processes; bread product.

Özet

Bu tezde uygun sulu ve organik ekstraksiyon yöntemleri kullanılarak soğuk pres posalarından protein konsantreleri üretimi gerçekleştirilmiştir. Kullanılan başlıca numuneler arasında çörek otu, kabak çekirdeği, üzüm çekirdeği, nar çekirdeği, posaları olarak sıralanabilir. 3 temel sulu ekstraksiyon yöntemi ile protein üretimi yapılmış ve uygun durumlarda numunelerde kalmış olan yağ molekülleri de hegzan ekstraksiyonu yoluyla uzaklaştırılmıştır. Numunelerdeki protein içeriği geniş bir aralıkta seyretmektedir (yaklaşık %25-90).

Üretilen protein konsantrelerinin ve izolatlarının fizikokimyasal özellikleri ve teknik fonksiyonellikleri incelenmiştir. Bu bağlamda, incelenen fonksiyonel özellikler; **çözünürlük, emülsiyon ve köpük oluşturma kapasitesi, yağ ve su tutma, şişme kapasitesi ve hava-su ara yüzeylerinde yüzey aktivitesidir.** Örneğin; AE-IP yöntemiyle elde edilmiş çörek otu, kabak çekirdeği, nar çekirdeği ve üzüm çekirdeği protein konsantrelerinin %0,1 protein konsatrasyonu ve 10.000 s adsorpsiyondan sonra hava-su yüzey gerilimi sırasıyla, 37.6, 45.5, 47.5 ve 56.1 $mN.m^{-1}$ olarak bulunmuştur.

Numunelerin fonksiyonelliklerinin iyileştirilmesi amacıyla Maillard konjugasyonu ve transglutaminaz muamelesi gibi yöntemlerden yararlanılmıştır. Maillard konjugasyonunda glukoz bağlanma derecesi, reaksiyon koşullarına bağlı olarak % 85'e kadar yükselmiştir.Aynı şekilde transglutaminaz enzim muamelesi ile köpük kapasitesinde gelişme gözlenmiştir.

Yerel bir firmanın desteği ile firmanın tesislerinde protein konsantreleri normal ve glütensiz ekmek formülasyonlarında kullanılmış ve ekmeklerin özellikleri değerlendirilmiştir. Bu çalışmalar sonunda gerek proteince zenginleştirilmiş, gerekse de teknik özellikleri iyileştirilmiş bazı ekmek ürünleri ortaya çıkarılmıştır. Örneğin, çörek otu, üzüm çekirdeği ve kabak çekirdeği protein konsantresi ile zenginleştirilmiş glütensiz ekmeklerin somun hacmi, su seviyesi % 15 arttığında önemli ölçüde iyileşmiştir. Bundan sonrak çalışmalarda farklı gıda formulasyonlarında ilgili proteinlerin kullanılması söz konusu olabilir.

Anahtar Kelimeler: Soğuk pres yağ üretimi; yağ bitkisi posaları; protein izolat ve konsantreleri; proteinlerin fonksiyonel özellikleri; membran prosesleri; ekmek ürünleri.

1. INTRODUCTION

Processing of oilseeds generates significant amounts of byproducts globally such as oilseed meals. For example, thirty to thirty-five million metric tons of oilseeds are being processed in the EU, mostly incuding soybeans (approx. 50%), rapeseeds (approx. 33%) and sunflower seeds (approx. 18%) (Commission of the European Communities, 2002). While in many cases, it is practical to utilize meals in feed or fertilizer applications, these products are mostly of low commercial value (Oreopoulo and Tzia, 2006). Furthermore prior to drying, microbial degradation is an issue. Also, their bulk volumes render processing, transportation and utilization rather difficult. Consequently, high value end products are necessary to rationalize their exploitation (Oreopoulo and Tzia, 2006). Accordingly, the increasing global demand for novel protein sources also coincide with the increasing extents of oilseed meal accumulation.

Current availability of commercial plant protein products include proteins manufactured from legumes, cereals and oilseeds (Moure et al. 2006). However, the global demand for protein is constantly increasing and novel resources are being highly sought after (Markiewicz, 2010; Aiking et al. 2006; Day, 2013). It would be quite advantageous to utilize renewable raw materials in plant protein production. According to Day (2013), the conversion efficiency of plant proteins to animal proteins is roughly 15%, which automatically implies cost efficiency and improved sustainability. Currently the plant protein demand of the food industry is sufficient to consume the commercially available plant proteins (Day, 2013). With the utilization of novel resources, it could be possible to target novel uses. In that sense, valorization of industrial waste and/or byproducts in plant protein manufacture is a viable strategy.

Most manufactured food products are made of heterogeneous mixtures where dispersed phase(s) are contained in an aqueous medium. The presence, composition, size, concentration of dispersed particles, their charge characteristics, and interactions with the continuous medium determine the microstructural attributes of the food matrix (Dickinson, 2012).

The aim of this study was to investigate the physicochemical and functional properties of black cumin, pumpkin, pomegranate and grape seeds protein concentrates using multiple aqueous protein isolation methodologies with or without the application of an organic extraction step. The industrial processing was carried out at <40°C which preserved the quality of proteins, after which the meals were immediately collected and processed gently based on aqueous extraction techniques. The physicochemical and functional properties of the protein concentrates were investigated, since technical functionality studies on these protein systems are relatively scarce in this field. The simple methodologies utilized here are applicable to industrial settings and appropriate for the utilization of industrial by-product streams in order to reduce costs in the processing of cold press valuable oils.

Maillard conjugation between black cumin proteins and certain carbohydrates (glucose, lactose and maltodextrin) was utilized to enhance the foaming characteristics of these protein concentrates. Commercial TGase enzyme was also used to enhance foaming capacity of black cumin, pumpkin seed and grape seed protein concentrates. All samples were compared to untreated samples.

Finally, the objective of this study was to evaluate the effect of black cumin, grape and pumpkin seed protein concentrates and their functionality improved counterpart on starch based gluten-free flour and also develop wheat bread formulations with black cumin, grape seed and pumpkin seed protein concentrates addition without compromising bread quality characteristics. The influence on the textural properties of the gluten-free and wheat bread was investigated as well as the final bread quality.

2. LITERATURE REVIEW

2.1 Functional properties of proteins

Proteins are highly functional biomolecules both in the sense of technical and biological functionality. In addition to their potential bioactivities, it is the technical characteristics of protein systems that enable their commercial utilization. The major functional properties of proteins are related to their hydration, structural/rheological and interfacial/surface related characteristics. Novel protein products should be able to compare favorably with animal proteins to partially or fully replace them (Siebert, 2003; Moure et al. 2006). Consequently functional properties such as water and oil holding capacity, solubility, foam and emulsion formation capacity, and ability in lowering surface/interfacial tension are usually monitored.

Proteins are functional biopolymers that enable the stabilization dispersed phases. Among the major functional properties of proteins, interfacial and surface related characteristics take place including the ability to stabilize foams (Campbell, 1999). Although most of the commercial protein products utilized by the food industry have originated from animal resources (i.e., dairy proteins), plant proteins become increasingly available.

The ability of proteins to stabilize interfaces is due to their amphiphilic nature. While the hydrophilic head group has a higher affinity to the aqueous phase, the hydrophobic portions interact with less polar phases such as air bubbles or oil phases. Protein molecules rearrange on the surface of air or oil interfaces, with their hydrophobic moieties (with amino acids such as phenylalanine, leucine, and isoleucine) adsorbing at the surface and the hydrophilic portions protruding into solution (Hasenhuettl, 2008) which in turn causes partial unfolding of the proteins at the interface (Haynes and Norde, 1995). Once proteins are adsorbed at the interface, through intermolecular interactions they create films with different viscoelastic properties (Martin et al. 2002). In the case of protein-sugar complexes, in addition to the already existing polar residues of proteins, hydrophilic sugar molecules further protrude into the aqueous phases and generating strong steric protection which extends the stability of emulsions and foams (Dickinson and Izgi, 1996; Dickinson, 1995). In various studies, molecular size and environmental conditions applied were shown to

modulate the effectiveness of such complexes (Miquelim et al. 2010). In that sense, the valorization of deoiled meals requires the comprehensive analysis on the functional characteristics.

2.2 Sources of plant proteins

Plant proteins represent an alternative source to animal proteins for utilization in food and other commercial applications. Currently, there is a variety of globally available commercial plant protein products primarily manufactured from legumes, cereals and oilseeds (Moure, Sineiro, Dominguez, & Parajo, 2006). Due to the rapidly increasing global protein demand, the exploration of alternative sources is necessary. The drawback of using plant proteins include the sulfur amino acid deficiency of some plants and the presence of antinutritive agents in the final products (Castriotta & Canella, 1978). However, supplementation with other proteins could generally solve these problems (Moure et al. 2006). While cereals are generally deficient in lysine and rich in methionine, pulses are poor in methionine but rich in lysine, which could facilitate complementarity between the two sets of products (Chardigny & Walrand, 2016).

Oilseeds contain considerably higher amounts of protein compared to cereals (Potter & Hotchkiss, 1995) which renders them useful in protein manufacture. After the extraction of oil, proteins are highly concentrated in the deoiled meals, and consequently plant protein concentrates can be produced from these inexpensive by-products. Protein content coud account for up to 60% of the meals (Radha, Kumar, & Prakash, 2007). In 2004/2005 period, total protein meal amount accounted up to 207 million metric tons globally (Ash & Dohlman, 2006) which underlines the importance of their utilization. Soybeans, rapeseed, cottonseed, sunflower seed and peanut meals were the most abundant protein meals in this period and accounted for 69%, 12.4%, 6.9%, 5.3% and 2.8%, respectively (Ash & Dohlman, 2006). In addition to economic reasons, manufacture of seed protein products also bring in environmental advantages such as the reduction of waste (Tekeli, 2014). Low cost protein sources are needed to replace the high cost protein sources in animal feeds (Tekeli, 2014) and in foods.

While oilseeds contain significant amounts of oil (i.e., in most cases, 17–47%), the extent of oil recovery depends on the means of oil manufacture such as cold press technologies or solvent extraction. Especially, oilseed meals generated via

desolventization represent significant sources of protein both due to high protein content, and the extent of availability (Visser and Thomas, 1987; Friedman, 1996). However, solvent usage and thermal treatments might affect the nutritional properties of protein mixtures (Zeng et al. 2013), since meal proteins need to be converted into edible-grade products. The presence of phenolic substances (i.e., chlorogenic, quinic, and caffeic acids), reducing sugars (glucose, fructose), or toxic factors (i.e., gossypol) could limit the potential utilization of meal proteins in foods (Wolf et al. 1982; Liadakis et al. 1993).

2.3 Fundamental information on the current samples

Black cumin (*Nigella sativa*) is a valuable and annually flowering medicinal plant from *Ranunculaceae* family (Baydar, 2009) which is native to the East Mediterranean countries, South Europe and Asia Minor (Baytop, 1999; Baydar, 2009). Currently, black cumin is also cultivated in the Middle East, North Africa and Asia (Durani, Chand, Zaka, Sultan, Khattak & Durrani, 2007). According to Commodity Trade Statistics Database, the global consumption of black cumin was estimated to be 187,000 tonnes. While the global market of spices and culinary herbs is approximately worth more than $2.8 billion, 2.8% of this budget is occupied by black cumin. India cultivates more than 85% of the global production of black cumin, whereas approximately 3.5% and 2.8% are generated by Syria and Turkey, respectively (Anon. 2014).

Black cumin seeds are composed of approximately 21% protein, 35% carbohydrates and 35 to 38% oil by weight (Baydar, 2009). Consequently, in the deoiled meal, protein content can be anticipated to be >30%. Black cumin seed hydrolysates were previously shown to contain 15 different amino acids including 9 essential amino acids which render the seeds a valuable amino acid/protein source (Haq, Remo, & Al-Sedairy, 1996; Babayan, Kootungal, & Halaby, 1978). Black cumin seeds can be utilized in medicinal applications and also used as spice or nutritional supplements. Further utilization in industrial applications are unknown to our group. Due to the difficulty of consuming black cumin seeds, protein products generated from this valuable resource could increase its global consumption and its extent of manufacture in our country and elsewhere.

Due to their health promoting effects such as anti-carcinogenic activity, prevention of protein malnutrition and inhibition of blood coagulation, pumpkin seed proteins have a good potential in utilization in food formulations (Yadav et al. 2010; Tomar et al. 2014; Bucko et al. 2015). Furthermore antidiabetic (Quanhong et al. 2003), antifungal (Wang and Ng, 2003), antibacterial and antiinflammatory (Caili et al. 2006) and antioxidant activities (Nkosi et al. 2006) were demonstrated. Their utilization in food formulations as well as in nutrition supplements has been previously documented (El-Soukkary, 2001; El-Adawy and Taha, 2001; Giami and Barber, 2004). In the previous studies, protein content of pumpkin (*Cucurbita* sp.) seed has been found to be approx. 36 % (Quanhong et al. 2005), whereas protein concenration significantly increases due to deoiling approx. to a range of 60–65% (Pericin et al. 2009). However, the extent of studies on pumpkin seed protein functionality remains relatively limited.

Pomegranate (*Punica granatum Linn.*) is a shrub belonging to the family Punicaceae and is mainly cultivated in the tropics and subtropical regions such as China, Japan, USA and Mediterranean countries. Pomegranate seeds contain a large amount of flavonoids and anthocyanins (Promprom et al. 2010), which enables their utilization in the manufacture of medicines, cosmetics and functional foods. The protein content of pomegranate seeds might be as high as 120 g/kg (Kimberly and Roberts, 1905). While biological activities of other seed ingredients have been widely investigated (de Nigris et al. 2007; Mirmiran et al. 2010; Schubert et al. 1999), the information about its protein composition and characteristics are largely unknown (Yang et al. 2011).

Grape (*Vitis vinifera* L.) seeds constitute another abundant source of seed proteins, since grapes are among the most heavily cultivated fruits at 69 million tons annually (FAOSTAT, 2011). The seeds account for approximately 2-3% of the total harvest and its protein content is approx. 10-13% (Fantozzi et al. 1979; Harborne et al. 1975; Fantozzi et al. 1981). In winemaking, roughly 13% of grape weight is converted to grape pomace which is the major by-product and about 38–52% of the pomace is represented by grape seeds on a dry weight basis (Maier et al. 2010; Schieber et al. 2002). Consequently, both after winemaking and cold press oil processing, grape seeds could be utilized as a source of plant proteins that demonstrate nutritional and technical value (Igartuburu et al. 1991; Zhou et al. 2011). The protein content of the grape seed

was reported in a few studies, while the protein values ranged widely between 8.44% (Igartuburu et al. 1991) and 25.9% (Fazio et al. 1983). However, investigations on the characterization of grape seed protein components and their functionality are rather limited (Gianazza et al. 1989; Zhou et al. 2010; Gazzola et al. 2014).

2.4 Enhancement of oilseed protein functionality

Modification of proteins is possible by changing the composition (by removing or inserting various constituents) or the size of the molecule based on physicochemical and/or enzymatic methods. The isolated protein can be subjected to chemical, thermal or enzymatic treatments in order to improve functional properties (Moure et al., 2006). Chemical modifications are commonly used in studies performed to characterize the relationships between structure, stability and functional properties of isolates (Dua et al., 1996; El-Adawy et al., 2000; Gruener & Ismond, 1997). Chemical modification can be achieved through alkylation (which affects Lys, Cys, Me, His and Try), oxidation (Cystine, Cys, Me, His, and Try), acylation (acting on Lys and Tyr), esterification and amide formation (on Glu and Asp). An alternative option is the enzyme modification through cross links of proteins, peptides and amines.

Transglutaminase (TGase) has been successfully applied in several food systems (Kuraishi et al., 2001). TGase catalyses the reaction between an ε-amino group on protein bound lysine residues and a ɤ-carboxyamide group on protein-bound glutamine residues, leading to the covalent crosslinking of the proteins (Folk and Finlayson, 1977). Transglutaminase introduces covalent cross links between proteins, peptides and various amines. This enzyme has the ability to induce cross linking and gelation of food proteins (Anuradha and Prakash, 2009). Motoki et al. (1992) proposed transglutaminase for improving the gelation properties of vegetable proteins. Other physical treatments such as high-pressure, unfolding, and exposing hydrophobic sites improve the functional properties of the proteins (Molina et al., 2002).

The addition of sugar enhanced the whipping properties of oilseed proteins (Khalil et al., 1985).In the previous literature, the formation of protein-carbohydrate complexes has been widely studied due to their biological and technical significance. Composition of the protein-carbohydrate mixture, molecular size of both entities, and environmental factors such as pH, ionic strength and temperature determine the type of complexes that might be formed. The aqueous mixture of proteins and

carbohydrates could be co-soluble, form soluble or insoluble complexes, or totally phase separate. Especially when soluble complexes forms, due to their limited surface charge, the stability has to be closely monitored. In products such as acidic milk drinks, emulsions and foams, such complexes could be utilized. As well as capitalizing on electrostatic forces, in many studies Maillard conjugates of proteins and simple sugars or polysaccharides were exploited (Li et al. 2009; Qi et al. 2010; Zhu et al. 2008; Gu et al. 2010; Li et al. 2013).

2.5 Applications on the utilization of plant proteins in baked foods

Bread is the most staple food that is widely consumed in the World and wheat is the most common grain that is used for bread production. Wheat endosperm constitutes the largest portion of wheat(83%, 85%). The aleuron layer surrounding the endosperm is rich in protein. Essential amino acids are localized in the aleurone layer. However, flour production is based on the utilization of the endospermprotein, vitamins and minerals rich in rushes (2-3%) and bran (13-15%) seperated from the wheat since they reduce the bread quality of the flour for industrial production. Since wheat bran is largely discarded during wheat grinding, wheat flour becomes poorer in terms of essential amino acids (Delcour and Hoseney, 2010).

Gluten proteins affect the dough properties including knead ability and. These proteins form a thin film layer during dough kneading, keep the CO_2 gas produced by the bread yeast during the dough fermentation, allowing the gas to remain in the dough and to raise the dough. Therefore, gluten quality and quantity are important for bread formation. Celiac disease is the reaction of body to gluten proteins. Gluten-digest peptidase enzyme inability synthesize or ineffectively synthesize results with this disease. Celiac disease may require a long-term diet (Marco and Rosell, 2008b; Demirci 2009). Therefore, a variety of studies have been conducted for the production of gluten-free bread formulations.

In recent years, studies have been carried out bothenriching bread with vitamins and minerals and also addition of proteins. Addition of proteins to bread products targetd to prevent the widespread occurence of the diseases caused protein deficiencies, malnutrition, disorders of protein metabolism, and gluten allergy (Stelten et al., 2014). Soy, tomato seed, legume, rye, maize, oats, barley, rice, almond, hazelnut

concentrates and hydrolysates which are rich in amino acids as well as animal proteins such as fish, chicken, milk, whey and egg albumin were used for enrichment of bread and other cereal products. Breads enriched with different protein sources were evaluated not only nutritional side but also physical, chemical, rheological, sensory and functional characteristics (Marco and Rosell, 2008a; Marco and Rosell, 2008b; Acevedo-Pacheco and Sema-Saldiva, 2016; Gambus et al. 2011; Delcour et al. 1998; Andersson, 2015; Madenci and Bilgiçci, 2014; Oliete et al. 2008; Storck et al. 2013). Dough which was enriched with wheatgrass flour has enhanced extensibility and raising attributes (Marti et al. 2016). Pea and lentil protein extracts were used in bread and cake formulations andsensory, rheological properties were analyzed and it has been proposed that pea and lentil proteins could be replaced with soy and animal proteins (Bildstein et al. 2008).

Viscoelastic properties of quinoa flour and bran added gluten-free bread were determined and it was found that the addition of 10% bran increased the bread volume by 7.4% compared to control without causing an adverse change in taste (Föste et al. 2014). Inulin added gluten-free bread enriched with bovine plasma protein. The addition of the enhancers improved moisture retention of the loaves after cooking and an increase of loaf volume and lightness of crumb with respect to the control was observed (Rodriguez et al. 2015). Flavour from peptides characterised in the study of gluten-free breads made with greenmussel protein hydrolysates enriched buckwheat, rice and chickpea flour. They concluded that protein hydrolysates from green mussel can be a used as an alternative natural flavouring agent (Vijaykrishnaraj et al. 2016). Protein, mineral, dietary fiber ratio increased as well as structural, technological and sensory parameters were developed with a corn flour addition to gluten-free bread (Korus et al. 2015). 5%, 7.5%, 10% soy protein isolate and whey added to the bread and emulsifiers such as glycerol monostearate, sodium sterarol-2-lactylate, lecithin added to the rice flour, especially, 7.5% soy and whey developed cooking and quality parameters of bread (Sarabhai et al. 2015). Gluten-free bread baked with chesnut flour at different ratio then physicochemical properties (color, texture, chemical composition), antioxidant capacity and in vitro digestion were investigated during the storage of bread for three days finally there was no o significant difference in the change of parameters (Paciulli et al. 2016). Concerning other gluten-free grains, in order to improve our fundamental understanding of the observed effects on their flours

of protein-altering treatments, there is a clear need to investigate how these treatments affect the functionality and structure of their isolated proteins.

The previous literature, Ziobro et al (2013) reported that strong influence of applied plant protein preparations on dough properties. Thus it seemed necessary to adjust the level of water used in baking experiments, so as to obtain the dough suitable for bread baking. To this end consistency of the control sample (which was found to be appropriate for baking in our previous studies) was checked on Brabender Faringoraph E (Brabender, Duisburg, Germany), and water absorption of other dough mixes was determined taking into account the established value (i.e. 100 farinograph units).

Farinograph and extensograph measurements are generally used to determine the bread making properties of bread flour and structural attribıtes of the dough. Farinograph analysis gives information about the characteristics of kneading dough and baking bread. Dynamometer shows resistance to the kneader pallets during kneading of the dough. Water absorption (%) and rheological properties of dough during kneading (stability and degree of softening)and dough forming properties of gluten proteins determined with this analysis. Resistance to extension and extension ability can be tested in the extensograph analysis. The ability of the dough to retain carbon dioxide during fermentation is related to extension ability and resistance to extension and its important for bread making properties of flour. Extensograms give information about overall quality of the flour and its response to flour additives (Delcour and Hoseney, 2010; Şahin et al. 2013). Therefore, taking these factors into consideration, essential measurements were carried out in this thesis.

3. MATERIAL AND METHODS

3.1 Materials

Cold press deoiled black cumin, pumpkin, pomegranate and grape seed meals were generously donated by Oneva (Neva Foods Ltd., İstanbul, Turkey), a local manufacturer of cold press oils. In all cases, the maximum temperature observed by the cold press samples was lower than 40°C. Commercial gluten-free flour containing, corn starch, rice flour, sugar, leavening agents (sodium bicarbonate, sodium acid pyrophosphate), thickeners (pectin, xanthan gum) was used. Salt, compressed yeast (Marmara Maya, Istanbul, Turkey), sunflower seed oil. The material for wheat bread dough and bread making consisted of wheat flour, salt and compressed yeast (Marmara Maya, Turkey) were purchased from local supermarkets. All chemicals used were of reagent grade and purchased from Sigma-Aldrich, except for sodium dodecyl sulfate (SDS) which was purchased from Merck (Millipore Corp, Germany).

3.2 Preparation of protein concentrates

Three different aqueous extraction techniques were utilized in order to extract proteins from the deoiled meals (black cumin, pumpkin seed, grape seed, pomegranate seed). In addition, solvent extraction was utilized to remove remaining black cumin oil from the samples.

3.2.1 Alkali extraction-isoelectric precipitation method

Alkali extraction–isoelectric precipitation (AE-IP) technique was based on the solubilization of protein molecules at basic pH, which was followed by the isoelectric precipitation at acidic pH values. Protein concentrates from deoiled black cumin meals were produced using the method of Boye et al. (2010) with slight modifications. Briefly, 50 g of deoiled meal was dispersed in water (1:15, w/v) and the pH of the medium was adjusted to pH 9.5 using 1.0 N NaOH. The dispersions were stirred at 500 rpm for 1 h at 22±1°C. Immediately afterwards, the dispersions were centrifuged at 13500xg for 15 min at 4°C using a CR22N high-speed refrigerated centrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan). The supernatant containing the solubilized proteins was collected and the medium pH was adjusted to pH 4.5 in order to induce

isoelectric precipitation. To ensure the completion of protein precipitation, the supernatant was centrifuged under identical conditions as before. The pellet was collected and immediately frozen at −20 °C. Frozen samples were lyophilized using a Teknosem TRS 2/2V freeze drier (Teknosem Corp., İstanbul, Turkey).

3.2.2 Salt extraction method

Salt extraction (SE) methodology detailed in Liu et al. (2009) was used with slight modifications. 50 g of deoiled meal was mixed with 500 ml of 0.1 M sodium phosphate buffer (pH 8) containing 6.4% KCl. The dispersions were kept stirred (500 rpm, 1 h) at the ambient temperature (22±1°C). Dissolved proteins were recovered by centrifugation at a rate of 13500xg for 15 min at 4°C. The supernatant was collected and diafiltrered using a Sartorius Masterflex Ultrafiltration System (10 kDa cutoff; Sartorius Sedium Biotech GmbH, Goettingen, Germany) against deionized water, until the conductivity decreased to approx. 20 $\mu s.cm^{-1}$. Immediately afterwards, the extract was frozen at −20 °C and kept frozen until lyophilization.

3.2.3 Micellar precipitation method

Micellar precipitation (MP) was performed according to the method of Lampart-Szczapa (1996) with slight modifications. 50 g of deoiled meal was suspended in 500 ml of 1.0 N NaCl solution and kept stirred for 2 h at room temperature (500 rpm). The suspension was centrifuged at 13500xg for 15 min at 4°C. and the supernatant was diluted 10x with cold deionized water (4 °C), which was followed by refrigerated storage (4 °C) for 18 h. Immediately afterwards, the dispersion was centrifuged again under similar conditions. Finally, the pellet was collected and stored at −20 °C until lyophilization.

3.2.4 Solvent extraction

Soxhlet extraction system was used for the removal of black cumin oil from the samples (Behr Labortechnik, R106S, Düsseldorf, Germany). Firstly, the samples were treated with hexane (208752, Sigma-Aldrich Corp.) for 7 h at a sample to hexane ratio of 1:50. In order to remove hexane, the samples were kept at 80°C overnight and dried at 55°C until constant weight was reached. Consequently, all samples were subjected to basic physicochemical analysis and functionality tests.

3.3 Basic physicochemical analysis of the meals and protein concentrates

The protein, moisture and ash contents of the raw material and the protein concentrate were determined according to AOAC Official Methods 920.87 (%N × 6.25), 925.10 and 923.03 respectively (AOAC, 2003). Fat content analysis were determined according to NMKL 960 (1968).

3.4 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was carried out based on the protocols of Laemmli (1970) under reducing conditions using a Bio-Rad Mini Protean Tetra Cell System (Bio-Rad Laboratories Inc., USA). Firstly, lyophilized protein concentrate (1%) were dispersed in deionized water. Immediately afterwards, protein samples and 2x Laemmli loading buffer containing 0.004% Bromophenol blue, 10% 2-mercaptoethanol, 20% glycerol, 4% SDS and 0.125 M Tris-HCl (pH 6.8) were mixed 1:1 in centrifuge tubes (1.5 ml). Samples were heated 5 min at 100°C, cooled, and loaded on a Mini-Protean TGX Stain-Free Precast Gel (12%). Precision Plus protein standards from the same manufacturer were used as the reference sample (Catalog number; 161363). Gel electrophoresis was carried out for 45 min using Tris/Glycine/SDS running buffer at 200 V (constant). Imaging was carried out by transferring the gel to a stain-free tray and using Gel Doc EZ System. The images were analyzed using the Image Lab Software (Bio-Rad Laboratories, Inc, USA).

3.5 Functional properties of protein concentrates

The functional attributes of the protein concentrates were tested and compared to that of a commercial soy protein isolate under identical conditions (Jem Nutrimax, Sonic Biochem, India).

3.5.1 Solubility

Protein solubility (%) was determined by dispersing 0.2 g protein (w/v) in 19 ml of 0.1 N NaCl solution, adjusting the pH to 7 using 0.5 N HCl or NaOH as necessary, and keeping the dispersion stirred (500 rpm) for 1 h at 50°C. Total solution volume then was brought to 20.0 g with 0.1 N NaCl. The mixtures were left to stand

for 10 min to observe the extent of precipitation. The solution was then centrifuged at 4200×g for 10 min at the ambient temperature. Percent solubility was determined in the supernatant using an appropriate protein analysis kit based on a modified Lowry method (TP0300, Sigma Aldrich Corp.). For all the standards and samples, absorbance was measured at 750 nm.

3.5.2 Water and oil holding capacity

1 g protein cocentrate was added to 10 ml of distilled water (or oil) in a 15 ml centrifuge tube. The contents were stirred for 30 s every 5 min on a vortex stirrer (Vortex, Genie 2-Mixer, Scientific Industrial Inc., Bohemia, NY, USA) and after 30 min the tubes were centrifuged at 3000xg for 20 min at the ambient temperature. Once the free water or oil portion was withdrawn, water/oil holding capacity was calculated from the percentage of increase in sample weight due to water or oil holding (Tsaliki, Pegiadou, & Doxastakis, 2004).

3.5.3 Emulsification activity

Emulsification activity and emulsion stability (o/w) were determined based on the method proposed by Pearce and Kinsella (1978) and Beuschel, Culbertson, Partridge, & Smith, 1992). 5 ml of protein concentrate dispersion (1%) at a defined pH value was utilized to homogenize 15 ml soy oil (S7381, Sigma-Aldrich) using an ultrasonic homogenizer (Hielscher Model UP200Ht; full power, 60 s, 1:1 pulse with 1 sec pulses) at the ambient temperature. A small aliquot from the emulsions (80 µl) was diluted to 10 ml with 0.1% sodium dodecyl sulfate (SDS) and sample absorbance was measured at 500 nm (Optima SB-3000 UV/VIS spectrophotometer). Emulsion activity index (EAI) was calculated according to the method of Karaca, Low, & Nickerson, 2011):

$$EAI(m^2/g) = \frac{2\ x\ 2.303\ x\ A_{\circ}\ x\ N}{C\ x\ \varphi\ x\ 1000}$$

where, $A_{\circ}$ is the absorbance of the diluted emulsion immediately after homogenization, N is the dilution factor, c is the weight of protein per volume ($g.mL^{-1}$), φ is the oil volume fraction of the emulsion.

3.5.4 Foaming capacity

Foaming capacity of protein concentrates and the stability of correspond foams were determined according to the method of Sathe and Salunkhe (1981). Briefly, 50 ml of 1% (w/v) protein concentrate solution prepared at a defined pH value was whipped for 3 min in a Waring lab blender (Model no: 8011ES, Waring Products Division, Torrington, CT, USA) at “high stir” setting and then poured into a 100 ml graduated cylinder. Immediately prior to the whipping, pH of protein solutions was adjusted to pH 7 using 0.1 N HCl or NaOH. Once total volume of the foam was studied, % increase in sample volume was calculated based on the comparison of foam volumes at a given time and at t=0.

3.5.5 Drop shape tensiometry

The surface tension ($mN.m^{-1}$) at the air-aqueous solution interface was determined using drop shape tensiometry (25°C) (Biolin Scientific, Attension Theta, Espoo, Finland). An air bubble was automatically formed at the tip of an inverted syringe which was immersed in a quartz cuvette containing the protein dispersion (0.1%) prepared in 100 mM sodium phosphate buffer (pH 7). The shape of the droplet was automatically analyzed to record the changes in the surface tension over time, as the cuvette and syringe assembly were monitored by a CCD (charge coupled device) camera and high quality image acquisition was utilized (Gülseren, Güzey, Bruce, & Weiss, 2007). Surface tension was calculated based on the Young- Laplace equation using Attension Theta OneAttension version 2.6 (r5305) software. All the measurements were carried out in triplicate. The surface pressure (π) was calculated as the difference in the surface tension of the buffer (72.3 mNm^{-1}) and the protein solution at the air–water interface, as a function of time.

3.5.6 Rheological analysis

An Anton Paar rheometer (MCR 302, Austria) fitted with a temperature controlled Peltier system (H–PTD 200) was used to monitor temperature dependence of the rheological characteristics of protein concentrates prepared by the AE-IP method. 10% aqueous protein dispersions were adjusted to pH 7 using 1 M NaOH. Immediately afterwards, centrifugation was performed to remove the undissolved matter. Approximately 1 mL of protein dispersion was placed on the lower plate of the

parallel-plate geometry. The diameter of the upper parallel plate was 25 mm and the gap distance between the two parallel plates was 1 mm. A solvent trap cover was used, and light silicon oil was applied to the exposed part to minimize evaporation during heating. Also the head was charged with water so as not to contact the sample and in order to saturate the medium with water vapor. The rheometer was operated at constant angular frequency of 1 Hz and strain range of 1-10% depending on sample behaviour within the linear viscoelastic region of the protein dispersions. The heating protocol involved a linear temperature ramp from 25°C to 85°C at a heating rate of 5°C.s^{-1}, holding at 85°C for 2 minutes and thereafter cooling to 25 °C at a cooling rate of 5 °C.s^{-1}, and finally holding at 25 °C for 2 minutes. Shear strain and modulus values (G' and G'') of the samples were investigated as a function of time and temperature (Sun & Arntfield, 2010). Anton Paar, Turkey is also acknowledged for their support in rheological analysis.

3.6 Preparation of Maillard Conjugates

In order to prepare Maillard conjugates of black cumin protein and glucose, 3 different protein:glucose ratios (1:1, 1:2, 1:4) were selected. For comparative purposes, lactose and maltodextrin was also used at a protein:carbohydrate concentration ratio of 1:2. Black cumin, pumpkin seed and grape seed protein concentrates were dispersed in 750 ml of deionized water in order to generate a final protein concentration of 1% and dispersion pH was adjusted to pH 12 using 10 M NaOH (Li et al. 2009). The dispersion was kept stirred for 1 h to ensure complete hydration. Thermal processing was carried out at 100°C for 0-30 min using a water-bath. Immediately afterwards, the samples were cooled in an ice bath and then thus formed conjugates were frozen at -20°C and lyophilized (Teknosem TRS 2/2V lyophilizer, Teknosem, İstanbul, Turkey).

3.6.1 Native-PAGE or SDS-PAGE analysis

Native-PAGE electrophoresis was carried out in order to analyze Maillard conjugates of black cumin proteins and carbohydrates on an as is basis. Native-PAGE analysis was carried out based on the protocols of Laemmli (1970) under non-reducing conditions using a Bio-Rad Mini Protean Tetra Cell System (Bio-Rad Laboratories Inc., USA). Firstly, lyophilized conjugates were dispersed in sodium phosphate buffer (pH 7). Immediately afterwards, protein samples and 2x sample buffer containing 1%

Bromophenol blue, 25% glycerol and 62.5 mM Tris-HCl (pH 6.8) were mixed 1:1 in Eppendorf tubes (1.5 ml). Samples loaded on a Mini-Protean TGX Stain-Free Precast Gel (12%). Precision Plus protein standards from the same manufacturer were used as the reference sample. Gel electrophoresis was carried out for 45 min using Tris/Glycine running buffer at 200 V (constant). Imaging was carried out by transferring the gel to a stain-free tray and using Gel Doc EZ System. The images were analyzed using the Image Lab Software (Bio-Rad Laboratories, Inc, USA). For SDS-PAGE analysis, same gel was utilized along with reducing conditions, SDS addition and heating of samples (100°C, 5 min), as appropriate.

3.6.2 Foaming capacity and stability

Foaming capacity of the conjugates and the stability of foams prepared by these conjugates were determined according to Niu et al. (2011). Briefly, 50 ml of 2% (w/v) Maillard conjugate dispersions prepared at defined pH values were whipped for 3 min using a Waring lab blender at a “high stir” setting and then poured into a 100 ml graduated cylinder. To ensure pH stability, 100 mM sodium citrate buffer was used for pH 3 and pH 5 samples, whereas 100 mM sodium phosphate buffer was utilized for pH 7. Total volume of the foam was studied as a function of time, and % increase in sample volume was calculated based on the following equations at a given time and at t=0. Heat treated black cumin protein concentrate dispersions without any carbohydrates and caseinate dispersions were also analyzed as references.

$$Volume\ increase\ (\%) = \frac{(Volume\ after\ whipping - Volume\ before\ whipping)\ ml}{Volume\ before\ whipping\ (ml)} x100$$

3.6.3 RP-HPLC-RID analysis

The RP-HPLC-RID analyses of the samples were performed on an Shimadzu LC-20AD HPLC system (Shimadzu Scientific Instruments) which consisted of a pump, thermostated column compartment and refractive index detector under previously described conditions and procedures of the manufacturer (Inertsil application-Analysis of Sugars, Data No. LB180-0871, https://www.gls.co.jp/viewfile/?p=LB180). The Inertsil, InertSustain NH_2 Column

(4.6 mm ID × 250 mm, 5 µm pore size) was used. The mobile phase was composed of an isocratic flow of 85% acetonitrile and 15% HPLC water for 25 min.

HPLC analyis was carried out at a column temperature of 40°C, at an eluent flow rate of 1 $ml.min^{-1}$, and using RID (Refractive Index Detector) detection. At least 5 concentration levels of all standard ranging between 0-2% were injected into the HPLC column. The corresponding peak areas were plotted against concentrations. Statistical analysis showed that all standard solutions had good linearity within the concentration range examined as shown by the high correlation coefficients (r^2> 0.9998). The amounts of sugars in the extracts were calculated as % of sample, on the basis of peak areas and by using calibration curves constructed for each standard.

After the redispersion of Maillard conjugates, equilibrium dialysis (1:1 by volume) was carried out for 72 h (Product No: D977, Sigma-Aldrich Corp., 14 kDa cutoff). In order to determine the extent of free (i.e., unreacted) sugars, samples were withdrawn from the dialysis permeate and filtered through 0.45 mm PTFE membranes (Isolab, Germany) prior to injection into the HPLC system.

3.6.4 Drop shape tensiometry

The surface tension ($mN.m^{-1}$) at the air-aqueous solution interface was determined using drop shape tensiometry (25°C) (Biolin Scientific, Attension Theta, Espoo, Finland). An air bubble was automatically formed at the tip of an inverted syringe which was immersed in a quartz cuvette containing the protein or Maillard conjugate dispersion. The shape of the droplet was automatically analyzed to record the changes in the surface tension over time, as the cuvette and syringe assembly were monitored by a CCD (charge coupled device) camera and high quality image acquisition was utilized (Potter & Hotchkiss, 1995). Surface tension was calculated based on the Young- Laplace equation using Attension Theta OneAttension version 2.6 (r5305) software. All the measurements were carried out in triplicate. The surface pressure (π) was calculated as the difference in the surface tension of the buffer (72.3 mNm^{-1}) and the protein solution at the air–water interface, as a funcion of time.

For the interfacial elasticity measurements, equilibration duration of 3000 s was used prior to analysis, when necessary, the adsorption process was allowed to take place continuously overnight. In most cases, however, the difference in measured elastic modulus from a 4 h or an overnight equilibration was negligible. Once the

equilibrium was attained, dilational elasticity was determined at a strain amplitude range of 0-0.5 ($\Delta A/A$ = 0 to 0.5, A being the bubble surface area) and a sinusoidal oscillation frequency (x = 100 mHz), unless otherwise stated. This extent of dilation lies within the linear viscoelastic range (Qi et al., 2010)The method is based on the automatically controlled, sinusoidal compression–expansion of the aqueous droplet at a defined oscillatory frequency and amplitude of dilation. The number of data points shown was reduced for clarity. The interfacial modulus of dilational elasticity was calculated from the change in interfacial tension relative to the change in droplet surface area (Qi et al., 2010).

$$\varepsilon = \frac{d\gamma}{\mathrm{dln(A)}}$$

To enable accurate measurements of elasticity in Maillard conjugates (section 2.2.2), their dispersions were treated with α-amylase (Product No: A1031, Sigma-Aldrich Corp., 4 h, 20°C) prepared in sodium phosphate buffer (100 mM, pH 7). After the treatment, the samples were subjected to overnight extensive dialysis in buffer solution to ensure the removal of non-protein substrates.

3.7 Preparation of TGase enzyme treated protein hydrolysates

3.7.1 Preparation of black cumin, pumpkin seed and grape seed protein concentrates

Alkali extraction–isoelectric precipitation (AE-IP) technique which described in section 3.2.1 was used to obtain protein concentes.

3.7.2 Measurement of transglutaminase activity

Commercial TGase activity was determined according to the method of Zeeb et al. (2013) with some modifications using Z-Gln-Gly as a substrate. A mixture of 12 mg/ml Z-Gln-Gly, 100 mM hydroxylamine, 10 mM glutathione (reduced) and 5 mM CaCl2 was prepared in Tris buffer (200 mM, pH 6.0). The reaction cocktail was incubated at 37 °C for 5 min in a thermo mixer. 30 µl enzyme solution (100 mg/ml) was added to initiate the reaction. The reaction was stopped by addition of TCA (12%

(w/v), 500 µl) after 10 min. Finally, a $FeCl_3$ solution (5% (w/v), 500 µl) prepared in hydrochloric acid (100 mM) was added to the solution and the absorbance was spectrophotometrically measured at 525 nm (Optima SB-3000 UV/VIS, spectrophotometer). TGase enzyme (Sigma code: T5398) was used as a reference and both enzyme were treated same. One unit of TGase activity was defined according to reference enzyme at 37 °C and pH 7.0. A transglutaminase activity of 0.1 unit/mg was measured.

3.7.3 SDS-PAGE analysis of TGase treated protein hydrolysates

The method specified in Section 3.4 was used.

3.7.4 Foaming capacity and stability of TGase treated protein hydrolysates

Foaming capacity and stability of TGase treated black cumin, pumpkin seed and grape seed protein concentrate were determined and compared to untreated samples. Three different times were studied to determine the optimum conditions for TGase enzyme. Black cumin, pumpkin seed and grape seed protein concentrate dispersions (2%) were prepared by stired in sodium phosphate buffer (100 Mm, pH 9) at 50 °C for 1 hour. After the activity of the TGase enzyme was determined, 100 mg of TGase enzyme was added to the dispersions and treated for 2, 4 and 18 hour (37 °C, pH 7). Foam capacity were determined according to Sathe ve Salunkhe (1981) as mentioned in section 3.5.4. Dispersions were observed for 120 min to determine foaming stability.

3.8 Wheat bread manufacture

For the production of reference bread samples, 430 g water, 200 g ice, 1000 g wheat flour, 15 g salt and 25 g press yeast were used. In addition, the protein content was enriched with protein concentrates of black cumin, pumpkin seed and grape seed. 1.5% each on the basis of pure protein. Protein content of black cumin, grape and pumpkin seed protein concentrates were 54.7%, 30.1% and 82.9% respectively. Since the protein contents of the protein concentrates varied between sample, the amount of protein concentrates added different for each bread. Formulations were given in Table 3.1.

Table 3.1. Bread formulations (BC: black cumin; GS: grape seed; PS: pumpkin seed; BCPC: black cumin protein concentrate; GSPC: grape seed protein concentrate; PSPC: pumpkin seed protein concentrate).

Ingredient	Blank	BC	GS	PS
Water	430 g	430 g	430 g	430 g
Ice	200 g	200 g	200 g	200 g
Wheat flour	1000 g	1000 g	1000 g	1000 g
Salt	15 g	15 g	15 g	15 g
Pressed yeast	25 g	25 g	25 g	25 g
BCPC	-	27.4 g	-	-
GSPC	-	-	49.83 g	-
PSPC	-	-	-	18.07 g

Firstly, all ingredients except salt and yeast were added to the mixing vessel and mixed for 4 minutes (Diosna, Dierks & Söhne, D-49074, Osnebrück, Germany). In the second step, salt and yeast were also added to the mixing vessel and bread dough was prepared. The doughs were rolled. (Ekmasan roller, Annex 37, Bursa, Turkey) and then dough was fermented (Tecnomac, Juniorlev, Italy) As detailed above, a total of 12 bread formulations were produced, based on 3 separate protein concentrates and a control sample manufactured at 3 different fermentation time (80,110,140 min). After baking, the loaves cooled at ambient temperature. Preparations were based on the procedures of Polen Food Company (Istanbul, Turkey), where all the baking experiments took place.

3.8.1 Wheat flour analysis

Wheat flour analyzes were conducted to determine the effect of enrichment on the quality of wheat flour such as falling number, fungal falling number, sedimentation, delayed sedimentation, wet gluten and gluten index of wheat flour and wheat flour enriched with protein concentrates were analyzed according to standart ICC method (1968) (Perten, 1500, Huddinge, Sweden), fungal falling number method (Perten Instruments, 1995) (Perten, FN 1900, Huddinge, Sweden) Zeleny (ICC Standard No. 116/1) (40 cycle/minutes, Erkaya, Zeleny 120, Turkey), standart ICC method 137-1 (2000) (Perten GM 2200, Huddinge, Sweden), respectively.

3.8.2 Dough properties

Physical properties of the dough samples were determined using the following methods: farinograph AACCI method no. 54.21 (Brabender, Farinograph-E, 810114, Germany), extensograph AACCI method no. 54.10 (Brabender, Extensograph-E, 860702, Germany) (American Association of Cereal Chemists International (2000), Approved Methods, St. Paul, MN, USA).

3.8.3 Color parameters

The crumb color of breads were determined using Hunter lab Colorflex EZ spectrophotometer (Hunter Associate Laboratory, Murnau, Germany). The instrument was calibrated using a white standard calibration plate and the color was expressed in

CIE-Lab space as L* (whiteness/ darkness), a* (redness/greenness) and b* (yellowness/blueness) (Jafari et al. 2017).

3.8.4 Texture profile analysis

Texture profile analysis (TPA) of bread crumb of one loaf from each batch was performed, using texture analyzer TA-XT2plus (Stable Micro Systems, England), at the test speed rate 1 mm/s. Sample of bread crumb, taken from the center of the loaf with a height 2 cm was pressed to reach 25% strain by a P/236 R (AACC 36 mm cylinder probe with radius) for 60 s. The resulting firmness and springiness of the crumb were used as indicators of textural changes. The calculations were performed using the instrumental software, Texture Exponent (Stable Micro Systems, England). The analysis was performed 2 h after the completion of baking process (Ziobro et al., 2013)

3.9 Gluten-free bread manufacture

Preliminary farinograph analyses indicated a strong influence of protein concentrate addition on dough properties. Therefore it was necessary to adjust the water level used in baking experiments in order to obtain dough characteristics suitable for baking. To test the influence of water level, three different levels were used for bread making. Firstly, 300 ml of water, 300 g of gluten-free flour (Sinangil, Turkey), 3.6 g of salt and 12 g of pressed yeast (Marmara Maya, Turkey) were used for the production of gluten-free bread enriched with black cumin, grape and pumpkin seed protein concentrates. Thereafter, the water level was increased by 8% or 15% (i.e., by 24 ml or 45 ml), while the amount of all the components were kept constant (3 levels x 3 proteins and control). Thus, 3 different trial sets were formed and a total of 12 breads were produced. The protein content was enriched with 1.5% each. Protein content of black cumin, grape and pumpkin seed protein concentrates were 54.7%, 30.1% and 82.9%, respectively. Since the protein contents of the protein concentrates varied between sample from one to another, the amount of concentrate added was different for each bread. Formulations were given in Table 3.2.

Table 3.2. Gluten-free bread formulations (BC: black cumin; GS: grape seed; PS: pumpkin seed; BCPC: black cumin protein concentrate; GSPC: grape seed protein concentrate; PSPC: pumpkin seed protein concentrate).

Ingredient	Control	BC	GS	PS
Water	300 ml	300 ml	300 ml	300 ml
Water level increased by 8%	300 ml	324 ml	324 ml	324 ml
Wter level increased by 15%	300 ml	345 ml	345 ml	345 ml
Gluten-free flour	300 g	300 g	300 g	300 g
Salt	3.6 g	3.6 g	3.6 g	3.6 g
Compressed yeast	12 g	12 g	12 g	12 g
Sunflower seed oil	18 g	18 g	18 g	18 g
BCPC	-	8.22 g	-	-
GSPC	-	-	15 g	-
PSPC	-	-	-	5.42 g

For the production of gluten-free bread, all the ingredients were placed in the same set-top mixer (Kenwood set top mixer, major titanium, palette beater). After 3 minutes of fast and 1 minute of slow mixing, 300 gr was taken from the dough and placed in the cake molds and kept in the fermenter for 45 minutes (Tecnomac, Juniorlev, Italy). The bottom was baked at 220°C and the top at 230°C for 30 min, A total of 12 gluten-free breads were produced, including 3 untreated protein concentrates and a control at 3 different water ratios. After baking, the loaves were removed from the pans, and cooled at ambient temperature. Procedures of Polen Food Co. (Istanbul, Turkey) were used to produce all gluten-free breads.

3.9.1 Gluten-free bread manufacture with functionality improved protein samples

Maillard conjugation and transglutaminase (TGase) treatment were performed to improve the properties of protein concentrates of black cumin, grape seed and pumpkin seed. In this context, for the Maillard conjugation optimum conditions were used based on the previous studies (1:2 protein:glucose ratio pH 7 ,15 min 100 °C) (Özdemir, Çakır and Gülseren, 2017). Firstly protein concentrates (1.5%) were stirred for 1 hour at pH 12 in water to dissolve. Then glucose was added to the solution to facilitate protein:glucose interactions. The resulting mixture was then allowed to stand at 100° C water bath for 15 minutes in order to allow the Maillard reaction. Immediately after the reaction mixture was removed from the water bath, the mixture was placed in an ice-bath to rapidly lower the temperature. The resulting mixture was used in the manufacture of gluten-free bread samples.

In the case of Tgase treated protein concentrates, firstly protein dispersions (1.5%) were prepared for the TGase treatment, Then, 50 mg of commercial TGase enzyme (0.1 unit/mg) was used for each % protein and the solution was kept stirred at 37 °C for 16 hours. The resulting mixture was used in gluten-free bread manufacture. Formulations were given in Table 3.3.

Table 3.3. Gluten-free bread formulations produced with protein concentrates with improved properties (BC: black cumin; GS: grape seed; PS: pumpkin seed; BCPD: black cumin protein dispersion; GSPD: grape seed protein dispersion; PSPD: pumpkin seed protein dispersion).

Ingredient	Control	BC	GS	PS
Water	300 ml	-	-	-
Gluten-free flour	300 g	300 g	300 g	300 g
Salt	3.6 g	3.6 g	3.6 g	3.6 g
Compressed yeast	12 g	12 g	12 g	12 g
Sunflower seed oil	18 g	18 g	18 g	18 g
BCPD	-	345 ml	-	-
GSPD	-	-	345 ml	-
PSPD	-	-	-	345 ml

3.9.2 Loaf volume analysis

Loaf volume of gluten-free breads were determined by a displacement technique. According to this method, a certain amount of hemp seeds were placed into a cabinet until overflow. Then the hemp seeds were removed from the container, the bread was placed into the cabinet, and the gaps were once again filled with hemp seeds. The loaf volume was calculated from the reduction in seed volume (ml). (Hamid and Luan, 2000).

3.9.3 Color analysis

The method specified in Section 3.8.3 was used

3.9.4 Texture profile analysis

The method specified in Section 3.8.4 was used.

4. RESULTS AND DISCUSSION

4.1 Influence of aqueous and organic extraction on the functionality of black cumin protein concentrates

4.1.1 Influence of extraction methodologies on protein concentrates

Prior to all further analyses, deoiled cold press meals were analyzed for their compositional characteristics. Protein, fat, ash and moisture contents (%) of black cumin meal were found to be 26.5, 22.3, 7 and 6%, respectively. Using three different protein isolation techniques (AE-IP, SE or MP), protein concentrates were prepared from the deoiled meals. Consequently, protein, moisture and ash content of all samples were determined immediately after freeze-drying (Table 4.1). Based on the AE-IP method, the protein contents of the concentrate prepared from deoiled meals were 54.7% for black cumin concentrate (Table 4.1) in the absence of hexane extraction. Consequently, the extent of improvement in the protein contents of black cumin meals was approximately 107% after AE-IP processes. Solvent extraction represented a further 20% improvement in protein content. In the case of SE method, although the procedure was significantly more labor intensive and probably requires a more advanced configuration in the case of an industrial scale-up, the extent of protein recovery was considerably higher than AE-IP method (Table 4.1). For the SE processed black cumin meals, protein concentration was as high as 67.8%.

Table 4.1. Protein (%), moisture (%) and ash (%) contents of deoiled black cumin meals and black cumin protein concentrates before or after hexane extraction. The data represent the average of three independent experiments and their corresponding standard deviation.

Sample/Preparation Method	Solvent extraction	Protein Content (%)	Fat Content (%)	Moisture (%)	Ash (%)
Soy protein concentrate	-	88.7±0.4		4.64±0.1	6.61±0.1
Deoiled meal	-	26.47±0.1	22.3±0.5	6.03±0.8	7.04±0.7
Deoiled meal	+	37.04±0.1		6.69±0.8	7.55±0.4
AE-IP	-	54.7±0.5	21.56±0.1	0.84±0.1	1.32±0.1
AE-IP	+	65.83±0.1		6.87±0.3	4.32±0.4
SE	-	67.82±2.1	18.14±0.6	7.02±0.1	3.81±0.1
SE	+	72.83±0.6		6.89±0.5	8.37±0.3
MP	-	85.49±2.9	18.24±0.4	9.56±0.2	1.85±0.8
MP	+	90.3±1.2		2.26±0.88	3.5±0.6

Boye et al. (2010) reported that UF/DF process yielded protein concentrates with slightly higher protein contents compared with the AE-IP process for pea, chickpea and lentil protein concentrates. Studies conducted by Fuhrmeister and Meuser (2003) also found that wrinkled pea concentrates prepared by ultrafiltration had higher protein content (70–80%) and lower fat content (2.3%) than concentrates obtained by isoelectric precipitation (68% and 3.8%, respectively). In our study for SE treated black cumin protein concentrates had higher protein contents than AE-IP treated protein concentrates. For the MP treatment, the protein content was the highest (approx. 85%), which was further increased to 90% after solvent extraction.

In most cases, the moisture and ash contents of the SE treated black cumin protein concentrates had higher ash and moisture content than AE-IP and MP treated black cumin protein concentrates, possibly due to the salt content in the system (Table 4.1). In addition, the ash and moisture contents of the AE-IP samples were generally lower compared to the other two methodologies which could imply that AE-IP was more efficient in the removal of fiber and other non-protein hydrocolloids in the samples. Sosulski and McCurdy (1987) indicated that strong alkali or acid used in isoelectric precipitation methods may result in salt formation and a subsequent higher ash level in the protein concentrate relative to the flour. Since no dialysis was applied after the AE-IP treatment, this could lead to relatively higher ash contents in the samples.

In all cases, aqueous extraction lead only to the removal of a small portion of oil in the system, while the protein contents significantly increased with further deoiling (Table 4.1). The influence of hexane extraction on moisture and ash contents was less clear.

4.1.2 Molecular weight analysis of protein concentrate (SDS-PAGE)

Molecular weight distribution of protein concentrates from black cumin seeds was analyzed by SDS-PAGE before and after hexane extraction (Figure 4.1). First of all, the major bands were found to lay between 15-40 kDa for the black cumin samples. For the oil extracted AE-IP samples (Lane 1), there were also other faint bands between 10-15 kDa and >40 kDa. To some extent, the major bands on the other two lanes (SE, MP) were comparable to the Lane 1 bands. In any case, it was obvious that the manufacturing methodologies affected the protein composition in

the concentrates. In addition to the fact that some bands did not appear in all lanes, the thickness of the common bands were generally different as well. Previously Haq et al. (1999) reported that *Nigella sativa* proteins ranged between 10-200 kDa. These investigators further fractionated the proteins by Rotofor technology and mostly found the bands around 25, 40, 65 and 200 kDa. Since the major bands in the current study were located around 15 kDa and 40 kDa in most cases, our findings are generally coherent with the previous findings.

After the oil extraction, especially for AE-IP samples broadening of the bands were observed which could imply that broader bands could represent more hydrophobic proteins, whereas the influence of solvent extraction on the variety of protein bands was mostly weak. Meanwhile when the samples applied to Lane 5 were treated with hexane, smearing between 15 an 20 kDa clearly diminished (see Lane 2) possibly indicating the loss of more hydrophilic proteins.

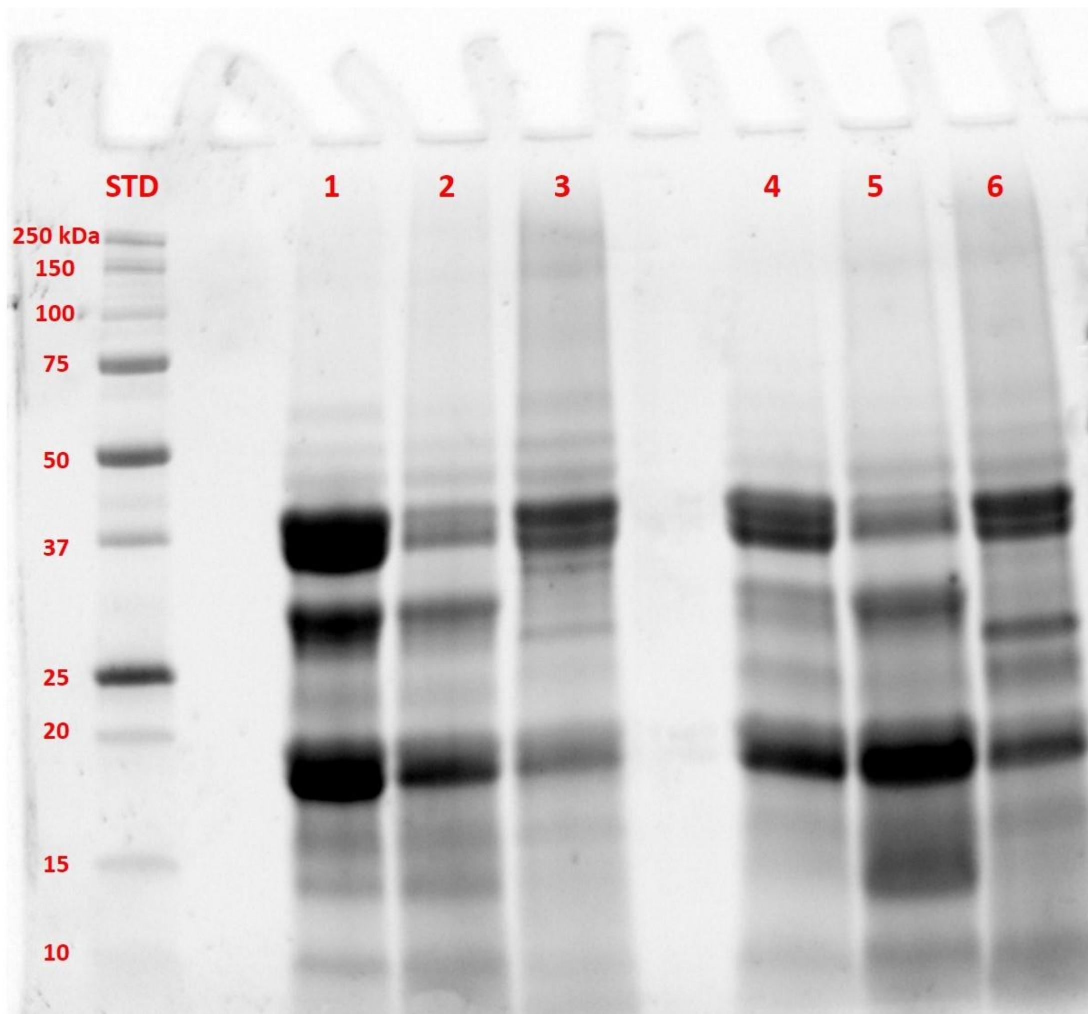


Figure 4.1. SDS-PAGE analysis of black cumin (lane numbers: 1-3 after hexane extraction AE-IP, SE and MP, respectively; 4-6: before hexane extraction AE-IP, SE and MP, respectively) protein concentrate dispersions (1%) manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP) methods.

4.1.3 Functionality of the black cumin protein concentrates

Various functional properties of the protein concentrates were tested including solubility, water and oil holding capacities, emulsification and foaming capacities, and surface activity.

4.1.3.1 Solubility

Amino acid composition and the distribution of their hydrophilic/hydrophobic characteristics throughout the protein molecules influence aqueous solubility of proteins. Consequently protein solubility also has a bearing on the other functional properties such as foam and emulsion formation, gelation and thickening in food dispersions (Damodaran, 1997).

Solubility (pH 7.0) characteristics of the current protein concentrates were presented (Figure 4.2). Solvent extraction clearly increased protein solubility in all cases. Prior to solvent extraction, AE-IP, SE and MP processed concentrates demonstrated approximately 12.1, 12.4 and 10.4% aqueous solubility, respectively, while after hexane extraction the numbers rose to 32.2%, 37% and 73%. In the previous literature, Karaca et al. (2011) reported that extraction method significantly affected pea protein concentrate solubility with AE-IP resulting in higher solubility than SE, which was attributed to the differences in surface characteristics of the proteins that were induced by each extraction method. For example, hydrophobic interactions between proteins reduce the extent of solvent-protein interactions, which in turn lowers solubility. Changes in solubility may also be attributed to conformational changes (Adebowale, Schwarzenbolz, & Henle, 2011) and the stability/destabilization of the native structure (Fuhrmeister & Meuser, 2003). The solubility of the current concentrate were higher than the reference protein source (i.e., soy protein concentrate) in all cases (4.13%). Stone et al. (2015) found the solubility of soy protein as 14.9% upon 1% preparation at pH 7. However, functional properties of soy concentrates and sensitivity to various treatments differ due to processing conditions, which affect the level of protein denaturation and solubilization.

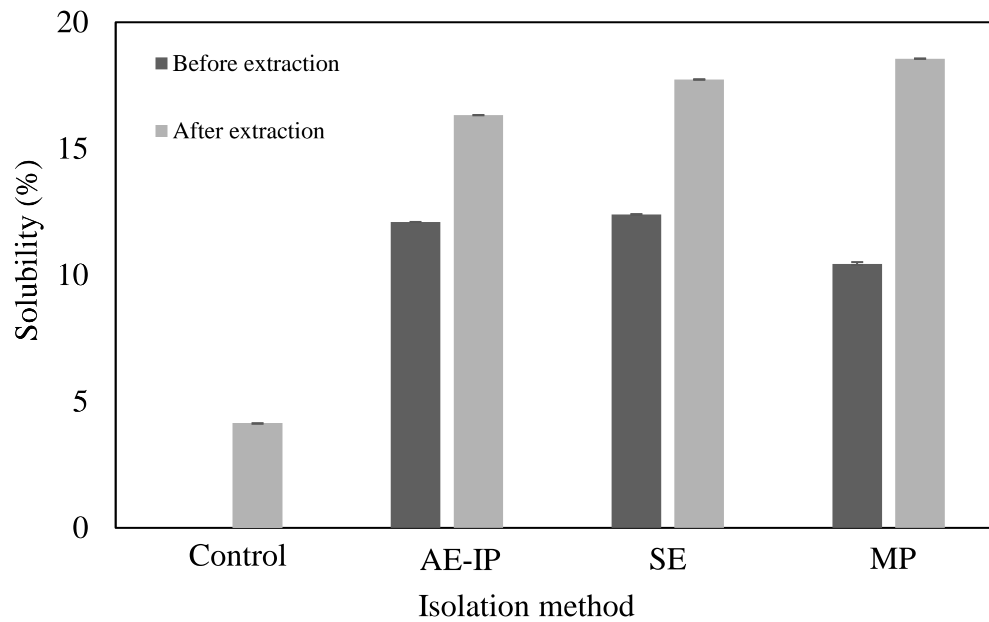


Figure 4.2. Aqueous solubility (%) of black cumin protein concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE), or micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein concentrate was added as a reference.

4.1.3.2 Water and oil holding capacities

Water holding capacity (WHC) is among the most critical characteristics of food proteins. A pronounced WHC inhibits the separation of water due to gravitational forces. Environmental conditions as well as amino acid content and conformational characteristics of proteins determine the interactions between water and proteins (Damodaran, 1997) which in turn influence the sensory and textural attributes of foods (Lawal, 2004). Both the oil and water absorption capacity of the proteins in the oilseed meals were shown to increase due to deoiling of seeds (Moure et al. 2006). Water or oil holding capacity of proteins are defined as the amount of water or oil that can be absorbed by 1 g of a certain protein product (Stone et al. 2015).

Water holding capacity (WHC) data for the current samples were presented on Figure 4.3. Once again, solvent extraction was shown to enhance the WHC of the protein concentrates. Prior to solvent extraction AE-IP, SE and MP processed samples demonstrated WHC values of 119.2, 5.78 and 110 (g/100 g water), respectively, whereas after the extraction these values increased to 131.3, 8.02 and 120.5 (g/100 g water). In all cases, soy protein concentrate had a higher WHC than the current samples (202 g/100g water) (Figure 4.3). In various studies, extraction technologies were shown to affect the WHC values of proteins (Sumner et al. 1981; Adebowale et al. 2011; Paredes-Lopezet al. 1991). These authors attributed the higher WHC to greater hydrogen bonding with water by side chains and polar groups exposed on the protein by the micelle method, whereas the isoelectric technique resulted in protein structures that limited the interaction of proteins with water. Proteins with higher amounts of hydrophilic groups near the surface abide more water (Stone et al. 2015). Based on the moisture content data (Table 4.1), it is likely that AE-IP black cumin samples contained little or no fibers or other non-protein hydrocolloids. Their elevated WHC could be in part due to the presence of hydrophilic glycoproteins.

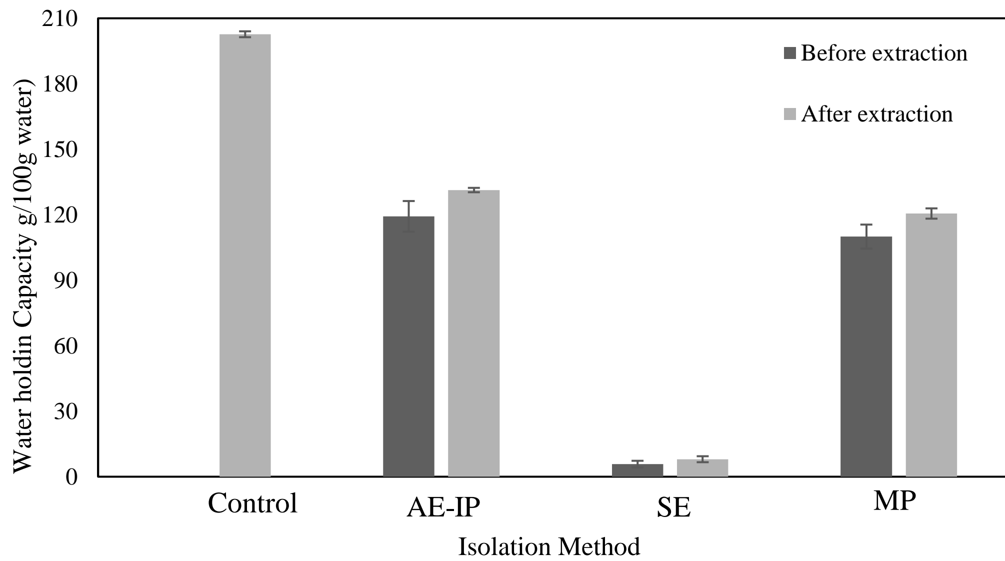


Figure 4.3. Water holding capacity (WHC) of black cumin protein concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein concentrate was added as a reference.

The oil holding capacity (OHC) values for all samples are presented in Figure 4.4. Salt extraction resulted in significantly ($p<0.05$) higher OHC values than either AE-IP or MP samples (Figure 4.4). Once again, extraction techniques were shown to have a bearing on the OHC values of protein concentrates according to Sumner et al. (1981), Adebowale et al. (2011), Abdel-Aal et al. (1986) and Paredes-Lopez et al. (1991). Furthermore, solvent extraction enhanced OHC values in all cases (Figure 4.4). Prior to hexane extraction, OHC for AE-IP, SE and MP samples were 162, 210 ve 194 (g/100 g oil), respectively. After hexane extraction, these values increased to 232, 292 and 264 (g/100 g oil), while for the commercial soy protein concentrate, OHC was 119 (g/100 g oil). Consequently, OHC values of the current concentrates were higher than the soy protein concentrate in all cases. (Figure 4.4).

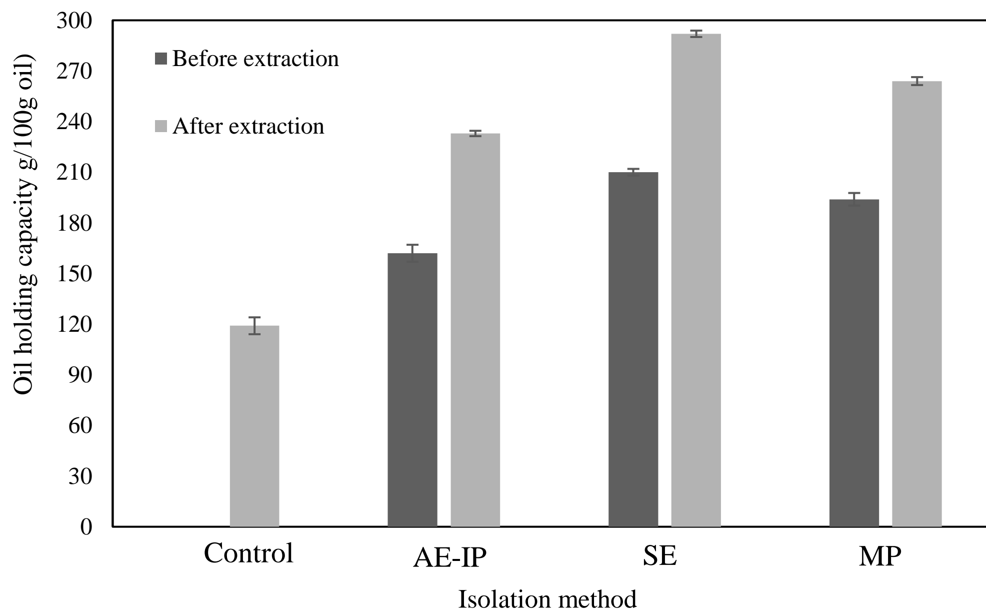


Figure 4.4. Oil holding capacity (OHC) of black cumin protein concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein concentrate was added as a reference.

4.1.3.3 Foaming capacity

During foam formation, aqueous phases surround air droplets where air is the non-polar phase. Theoretically, the amphiphilic character of proteins renders them as good foaming agents and inhibits the coalescence of gas bubbles (Zhou et al. 2011). Foaming capacity (FC) is the ability of a protein to form a stable foam under well-defined experimental conditions such as pH, temperature, and ionic strength. The effect of extraction methods on the FC values of the current samples is summarized in Figure 4.5. However, the FC values demonstrated a broad range between 30-100% for black cumin protein concentrates which was clearly influenced by extraction conditions. Prior to hexane extraction, the foam stabilizing capacities for AE-IP, SE and MP samples were 60%, 40% and 30%, respectively. After the extraction, all of the foaming capacity values significantly (Figure 4.5), all of which were once again higher than that of the commercial soy protein concentrate utilized here. Although a little higher, Deng et al. (2014) determined the corresponding value for soybean protein samples as 20.23% at pH 7.5. Consquently oil extraction enhanced the foaming capacity in all cases, which could be due to the solvent induced and thermal denaturation of the proteins. These changes could also affect the OHC and WHC values as well, as detailed above.

On Figure 4.5, foam stability was also demonstrated as a function of time. Stability of foams was highly dependent both on the extraction method and over a storage duration of 2 h, there was significant reduction in foam volume in all cases.

FC values of other plant protein products prepared from Indian chickpeas (Kaur & Singh, 2007), winged beans (Sathe, Deshpande, & Salunkhe, 1982a), mucuna beans (Adebowale & Lawal, 2003), lupin seeds (Sathe, Deshpande, & Salunkhe, 1982b), and pigeon peas (Akintayo, Oshodi, & Esuoso, 1999) have been reported to range between 25-80%, which were coherent with our current results for black cumin protein concentrates.

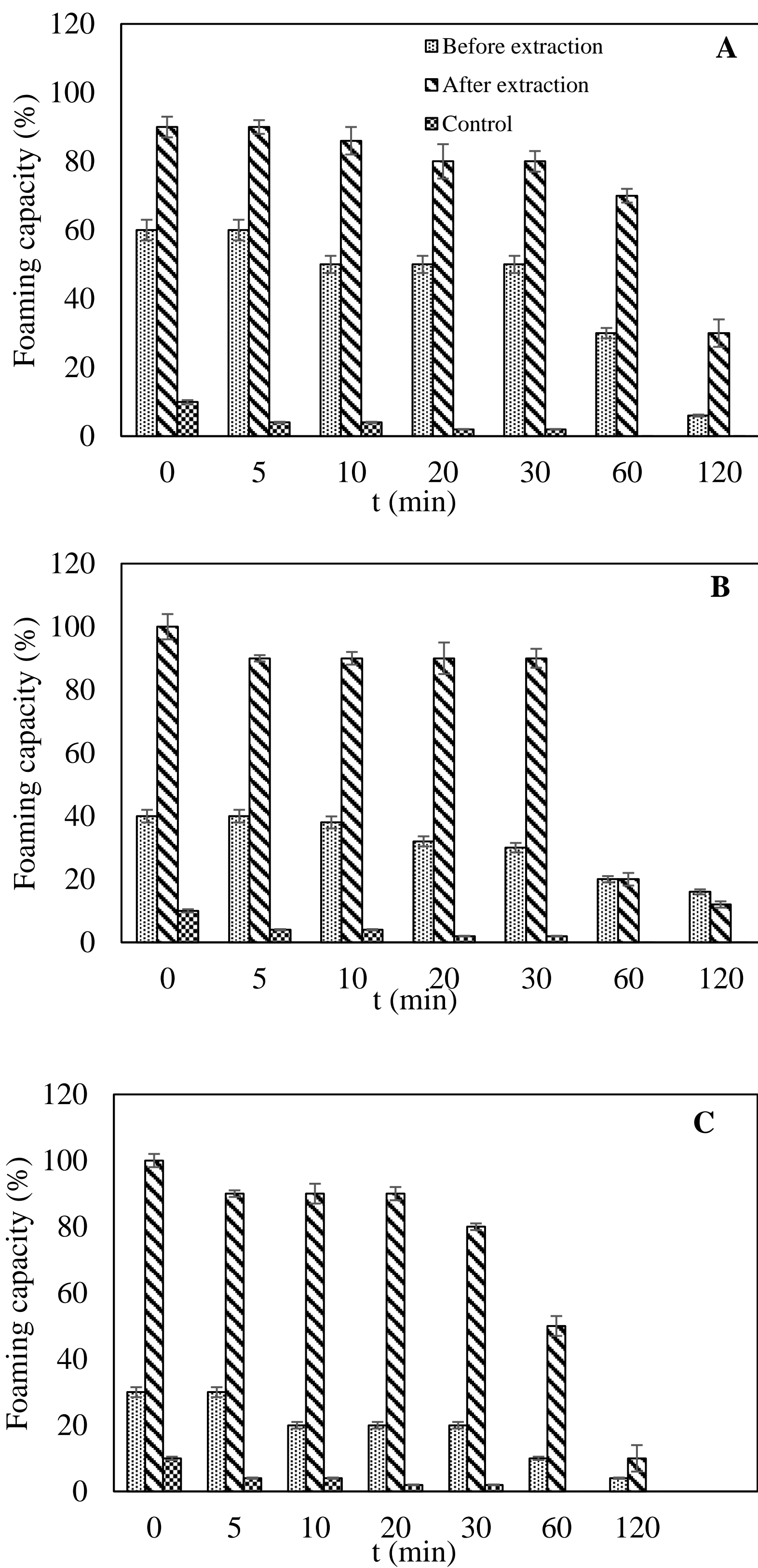


Figure 4.5. Foaming stability of black cumin protein concentrates prepared by **(A)** alkali extraction-isoelectric precipitation (AE-IP), **(B)** salt extraction (SE) and **(C)** micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein concentrate was added as a reference.

4.1.3.4 Emulsification activity index and emulsion stability

Proteins are the primary agents in the stabilization of food foams and emulsions due to their abilities in lowering surface/interfacial tension, interfacial adsorption at the freshly formed surfaces and the formation of a viscoelastic film at the interfaces (Graham & Philips, 1976; Hettiarachchy & Ziegler, 1994; Damodaran, 1997).

Emulsification capacity (EC) may be defined as the ratio of the amount of oil that can be emulsified by a certain amount of protein on a g per g basis. Based on EC values and the physicochemical characteristics of the dispersed phases, emulsification activity index (EAI) may be defined. EAI refers to the amount of surface area that can be stabilized by a certain amount of protein ($m^2.g^{-1}$). Prior to hexane extraction, AE-IP, SE and MP samples had EAI values of 4.2, 2.0.2, and 11 $m^2.g^{-1}$, respectively. While after the extraction the values increased in all cases to 24.7, 21.6, and 25.3 $m^2.g^{-1}$ (Figure 4.6).

In some studies, extraction method was found to affect the EAI values, for example, in faba bean, chickpea and fenugreek protein concentrate (Abdel-Aal et al. 1986), whereas the technique was not influential in pea protein concentrate (Karaca et al. 2011). Based on the data obtained from pulse proteins, however, the current EAI values were comparable (Boye et al. 2010). Fuhrmeister and Meuser (2003) reported higher EAI values (10.1 - 27.4 $m^2.g^{-1}$) for wrinkled pea protein concentrate prepared by ultrafiltration. Time dependence of EAI values were also investigated throughout a brief storage period of 90 min. The emulsions formed with the current protein concentrates were generally stable (data not shown). In most cases, the EAI values of the current samples were higher than that of the commercial soy protein concentrate (17.6 $m^2.g^{-1}$) (Figure 4.6). All of the studies on OHC, WHC, emulsification and foaming characteristics were carried out a constant protein concentration of 1%. Some of the discrepancies between the current samples and the literature data might be attributed to differences in concentrations, pH and processing techniques.

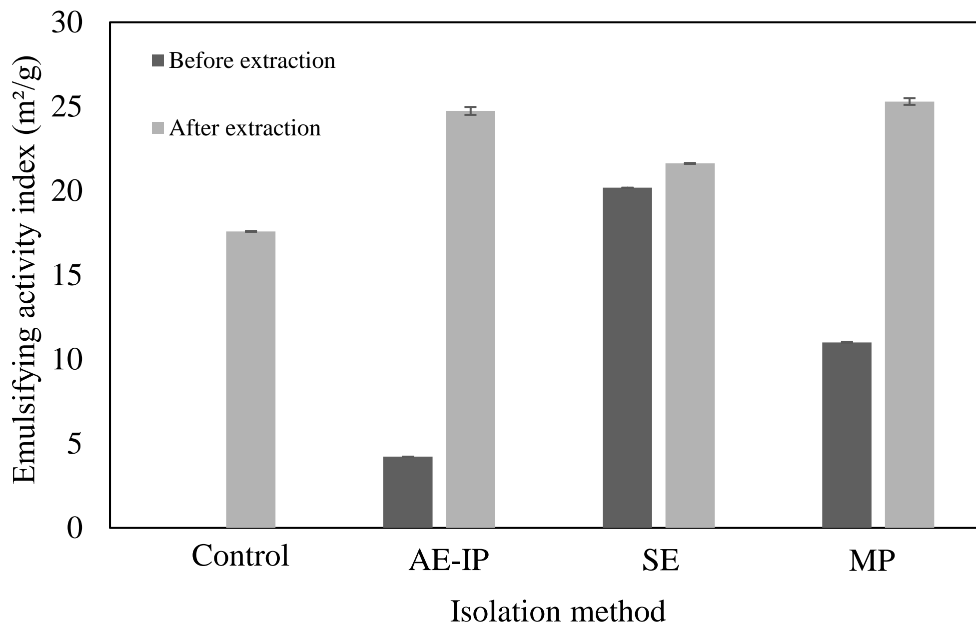


Figure 4.6. Emulsification activity index of black cumin concentrates prepared by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) before and after hexane extraction. The data represent the average of three independent experiments with standard deviation. Data for soy protein concentrate was added as a reference.

4.1.3.5 Drop shape tensiometry

The surface tension at the air-protein solution surface was determined using drop shape tensiometry (Figure 4.7). The surface tension values for black cumin protein concentrates (AE-IP) before and after oil extraction were approximately 37.6 and 37.5 $mN.m^{-1}$ respectively, at a protein concentration of 0.1% after 10,000 s. The surface pressure exerted by black cumin proteins were superior to that of soy protein (γ =49 $mN.m^{-1}$) at the same protein concentration after 10,000 s. Although the equilibrium values for the samples before and after hexane extraction was comparable, hexane extracted sample reached to this level of surface pressure sooner, which could imply both some differences in adsorption behavior as well as a further concentration dependence of the plateau value. Interactions of the remaining oil in the system in part might negatively increase the extent of interactions between the proteins and the air phase. These findings demonstrated that the protein concentrates prepared here demonstrated significant surface activity as also demonstrated by the functionality tests. Also, the size range of the proteins investigated here (Figure 4.1) was mostly comparable to that of highly surface active food proteins. To the best of our knowledge, there were no previous studies that measured the surface tension for black cumin protein concentrates. Also, as the long term kinetics of the black cumin protein concentrates were investigated (i.e., $\Delta\Pi$ vs $t^{-0.5}$) (Gülseren et al. 2007), maximum surface pressure values of 36.7 (AE-IP, no hexane extraction) and 35.8 (AE-IP, with hexane extraction) $mN.m^{-1}$ were predicted for as t approached infinity.

The initial stages of protein adsorption are generally limited by diffusional characteristics (Kozhevnikor, Danilenko, Braudo, & Schwenker, 2001). Following adsorption, protein unfold and rearrange at the interfaces (MacRitchie, 1978; Graham and Phillips, 1979). This process is characterized by the rapid increase in surface pressure. In order for surface tension to reach the steady state, penetration, unfolding and molecular rearrangements in the adsorbed film have to be completed (MacRitchie, 1978). In the previous literature, Tsoukala et al. (2006) measured surface pressure for broad bean legumin protein dispersions (0.05% $w.v^{-1}$) aprox. 15 $mN.m^{-1}$ and for lupin protein concentrate solutions (0.05% $w.v^{-1}$) aprox. 22 $mN\ m^{-1}$ at 10 min. Our results were comparable to these findings.

Hexane extraction clearly enhanced the functional characteristics in most cases, either due to the enhancement of protein-solvent interactions in the absence of

oil or the partial denaturation of the proteins. Aqueous extraction with no heating or hexane extraction generated protein concentrates with poor functional characteristics. With the exception of water holding capacity, all functional properties were found to be comparable or superior to the commercial soy protein concentrate sample utilized here. Although the water holding characteristics were these proteins were relatively weak, significant emulsion and foam formation capacities were shown to exist, which could be instrumental in the production of many food products.

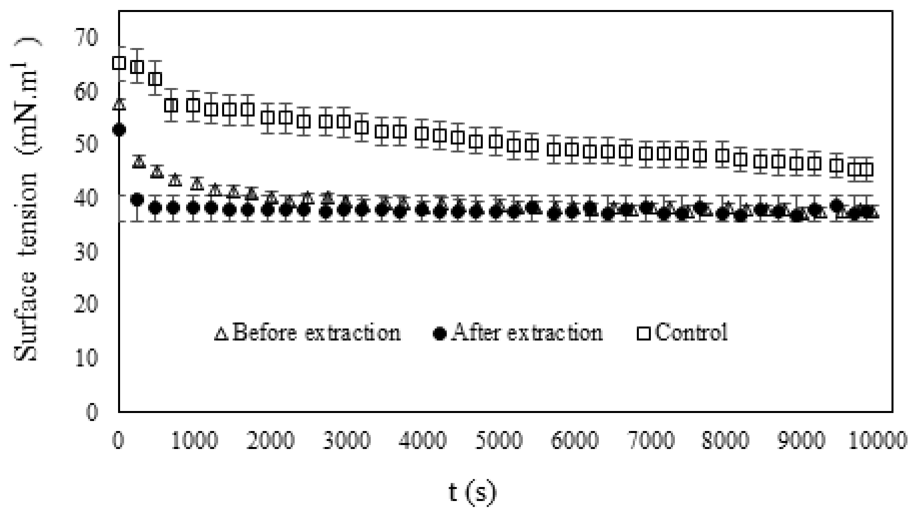


Figure 4.7. Dynamic surface tension of black cumin protein concentrates (0.1%) prepared by alkali extraction-isoelectric precipitation (AE-IP) method as a function of time before and after hexane extraction. A representative run was shown for each sample. Data for soy protein concentrate was added as a reference.

4.2 Physicochemical characteristics and functionality of protein concentrates manufactured from pumpkin, pomegranate and grape seeds

4.2.1 Physicochemical properties of protein concentrates

Prior to all operations, deoiled cold press meals were analyzed for their initial protein content based on the Kjeldahl method. Using three different protein separation techniques, all three meals were subjected to AE-IP, SE or MP treatments. The protein, moisture and ash content of all samples were determined immediately after freeze-drying (Table 4.2). In all cases, pumpkin seed concentrates contained more proteins than the other concentrates. Based on the AE-IP method, the protein contents of the concentrates prepared from deoiled meals were approx. 83%, 45.8%, and 30.1% for pumpkin, pomegranate and grape seed samples, respectively. In the case of SE method, although the procedure was more labor intensive and possibly less suitable to an industrial scale-up, the extent of protein recovery was considerably lower in all cases compared to the AE-IP samples (Table 4.2). Finally in the case of MP treatment, although the efficiency of protein recovery for the other samples was generally lower compared to the other extraction methods, the protein content of the MP pumpkin protein concentrates were slightly higher compared to the AE-IP pumpkin samples. It was possible to generate approx. 86.6% protein containing pumpkin protein concentrate without further purification. However, it is worth noting that in the case of pomegranate seed and grape seed meals (MP), the protein recovery efficiency was considerably low compared to pumpkin samples and protein content could not be analyzed.

Table 4.2.Protein (%), moisture (%) and ash (%) contents of pumpkin seed, pomegranate seed and grape seed protein concentrates. The data are the average of two independent experiments with standart deviation. ND: Not detected.

Preparation Method	Sample	Protein (%)	Moisture (%)	Ash (%)
Control	Soy protein concentrate	88.75±0.4	4.64±0.17	6.61±0.02
AE-IP	Pomegranate seed	45.81±17.8	14.30±7.1	2.75±0.1
	Pumpkin seed	82.99±1.3	5.67±1.04	1.71±0.04
	Grape seed	30.1±14.9	5.71±1.2	5.45±0.3
SE	Pomegranate seed	20.77±0.5	7.96±1.3	17.13±0.07
	Pumpkin seed	66.06±1.3	8.95±0.2	12.07±0.1
	Grape seed	37.05±14.1	17.94± 2.6	12.59±2.06
MP	Pomegranate seed	ND	ND	ND
	Pumpkin seed	86.59±0.3	11.00±0.2	4.06±0.3
	Grape seed	ND	ND	ND

In the previous investigations, protein content in pomegranate seeds was found to vary between approx. 12-20% (Gölükçü et al. 2007). Similarly, according to Bucko et al. (2015) and Rezig et al. (2013), protein content in pumpkin seeds were in the order of 31-94%. Protein content of grape seeds were previously found to be 10-13% (Castriotta and Canella, 1977; Fantozzi, 1981; Nowshehri, Bhat, & Shah, 2015; Pesavento, Bertazzo, Flamini, & Vedova, 2008). In addition, Gazzola et al. (2014) and Zhou et al. (2011) manufactured grape seed concentrates with protein contents of 40% or 64.85%, respectively, based on aqueous extraction techniques.

In most cases, SE treated samples have higher moisture and ash contents than AE-IP treated samples (Table 1). Sosulski and McCurdy (1987) indicated that strong alkali or acid used in isoelectric precipitation methods may result in salt formation and a subsequent higher ash level in the protein concentrates relative to the meal or flour. Although extensive diafiltration was carried out, salts remaining in the system could contribute to higher ash contents in the concentrates.

4.2.2 SDS-PAGE analysis of protein concentrates

Molecular weight distribution of protein concentrates from deoiled pumpkin, pomegranate and grape seeds was analyzed by SDS-PAGE (Figure 4.8). In the previous literature, pumpkin seed proteins were shown to be represented by a 12S globulin (325 kDa), called cucurbitin (Rezig et al. 2013; Bucko et al. 2015) which contains of six 54 kDa subunits. These subunits are linked by disulfides, which are approx. 33 kDa and 22 kDa in size (Colman et al., 1980; Marcone et al., 1998). Some studies also demonstrated the presence of 4.8 kDa, 7.9 kDa, and 12.5 kDa proteins (Fang et al. 2010). In our analysis, for pumpkin seed protein concentrates the major bands laid between 25-37 kDa, 20-25 kDa and finally around 10 kDa (especially AE-IP and SE), which were mostly coherent with the previous literature. There were some faint bands around 50-75 kDa for SE and MP treated pumpkin seed protein concentrates.

Although more faint, for pomegranate seed protein concentrates, the major bands laid around 15, 25 and 35 kDa. There were faint bands and some smearing at lower and higher molecular weights (Figure 4.8). The protein concentrate obtained by AE-IP method had more intense bands than the SE sample. Yang et al. (2011) reported that water soluble storage proteins of pomegranate seed protein concentrate had the

highest amount of polypeptides in a molecular weight range of 10-25 kDa (Yang et al. 2011), which were comparable to the current findings.

For the grape seed samples, although quite faint, the bands were located mostly between 20-40 kDa. Manufacturing techniques affected the composition of samples in all cases (Figure 4.8). Previously, Gazzola et al. (2014) showed that under non-reducing conditions, there were various proteins/polypeptides in the size range of 25 to 65 kDa. Under reducing conditions, however, the bands changed significantly. According to Zhou et al. (2010) and Zhou (2011), there were two main bands around 160 and 300 kDa. In addition, these investigators demonstrated the presence of other bands between 20-43 kDa. Consequently, our findings were partly coherent with both sets of investigations.

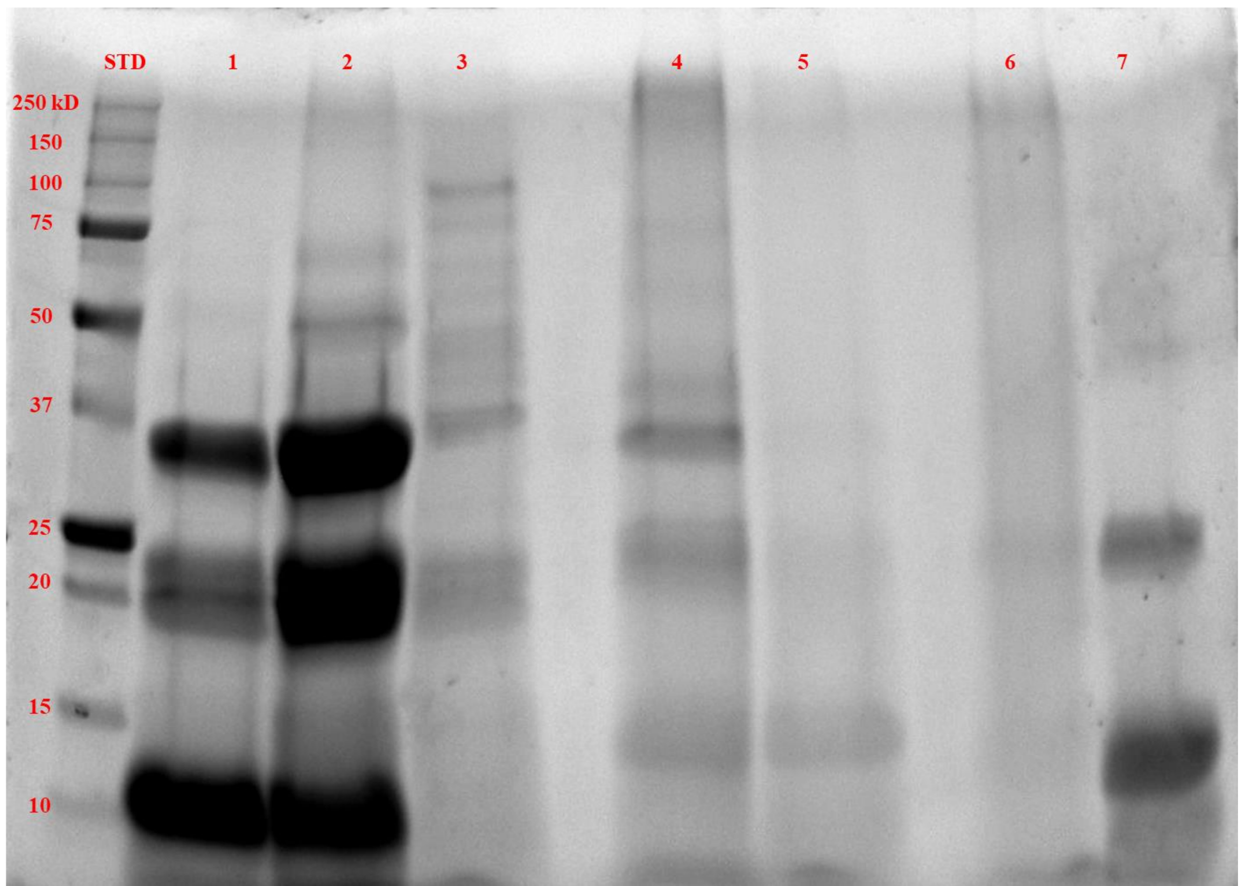


Figure 4.8. SDS-PAGE analysis of pumpkin seed (lane numbers: 1-AE-IP; 2-SE; 3-MP), pomegranate seed (lane numbers: 4-AE-IP; 5-SE) and grape seed (lanes number 6-AE-IP; 7-SE) protein concentrates (1%) manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP) methods.

4.2.3 Functionality of protein concentrates

4.2.3.1 Solubility

The solubility characteristics of proteins are among the most important functional properties since many functional performances of proteins depend upon their capacity to go into solution (Radha et al. 2007). Therefore, solubility of pumpkin, pomegranate and grape seed protein concentrates were evaluated (Table 4.3). The solubility (%) of AE-IP, SE and MP pumpkin seed concentrates were found to be aprox. 5, 40 and 12.3%, respectively. Bucko et al. (2015) reported that solubility of pumpkin seed protein concentrate (PSPI) was ~60% at pH 7. While they have utilized an alkali extraction-isoelectric precipitation method, differences in solubility could be attributed to extraction procedures since in our samples, no organic extraction was carried which could imply the presence of slightly higher extents of residual oil.

In the case of AE-IP and SE methods, the solubility of grape seed protein concentrates were found to be approx. 92.5 and 31.7%, respectively. In the previous literature, Zhou et al. (2011) and Castriotta and Canella (1977) reported that grape seed protein concentrate solubility was aprox. 18% and 15%, respectively, at pH 7. Finally, AE-IP and SE pomegranate protein concentrate solubility was found to be 40.8 and 28.3%, respectively. To the best of our knowledge, no data existed in the literature on the solubility of these proteins.

Table 4.3. % Solubility of pumpkin, pomegranate, and grape seed protein concentrates manufactured manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP).The data are the average of three independent experiments with standart deviation.

Preparation method	**Sample**	**Solubility(%)**
Control	Soy	4.13±0.01
AE-IP	Pomegranate seed	40.81±0.01
	Pumpkin seed	4.95±0.03
	Grape seed	92.52±0.07
SE	Pomegranate seed	28.28±0.1
	Pumpkin seed	39.99±0.04
	Grape seed	31.65±0.22
MP	Pumpkin seed	12.32±0.02

4.2.3.2 Water and oil holding capacity

In food applications water holding capacity (WHC) is related to the ability of protein molecules to retain water against gravity. This property clearly relates to the amino acid composition and spatial distribution of amino acids in the protein molecules. Especially the increased number of charged amino acids tends to influence the WHC values (Kuntz & Kauzmann, 1974). In addition, factors such as molecular conformation, hydrophobicity, pH, temperature, ionic strength and protein concentration affect WHC (Damodaran, 1997). Fat absorption capacity is the binding of fat by nonpolar side chains of proteins (Sathe, 1982). Fat absorption is usually measured by adding an excess liquid fat to protein dispersions, and determining the amount of bound oil.

Water and oil holding capacities of the protein concentrates were investigated (Table 4.4). Consequently AE-IP, SE and MP pumpkin seed protein concentrates were found to have WHC values of approx. 175.8, 90.6 and 81.2 (100 g water/g protein), respectively. AE-IP and SE pomegranate seed protein concentrates were found to be 178.3 and 12.2 (100 g water/g sample), respectively. Finally AE-IP and SE grape seed protein concentrates were found to be 258 and 157.7 (100 g water/g sample), respectively. In all cases, AE-IP samples had higher WHC values than the SE samples. The primary interactions related to WHC include the protein–water interactions (Stone et al., 2015). Consequently, the amount of protein within the protein concentrates may have a bearing on the WHC values observed. Since the protein contents of AE-IP samples were higher than the SE samples, WHC and protein content values were found to be coherent. WHC values obtained here were mostly lower compared to the WHC value of a commercial soy protein concentrate (202.68 (100 g water/g protein)) (Table 4.4).

The oil holding capacity (OHC) values for all samples were also presented in Table 4.4 AE-IP pumpkin, pomegranate and grape seed protein concentrates demonstrated OHC values of approx. 337.3, 182.2 and 414.5 (100 g oil/g protein), respectively. In the same order OHC values for the SE samples were 364.5, 240.4 and 1131.3 (100 g oil/g protein), respectively. Finally, MP sample for pumpkin represented an OHC value of 267.6 (100 g oil/g protein). As a reference, OHC value of commercial soy protein concentrate was 119 (100 g oil/g protein). Consequently, OHC values of the current concentrates were higher than the soy protein concentrate in all cases

(Table 4.4), while WHC values were generally lower. This finding could be attributed to the relatively low abundance of polar amino acids in the protein concentrates and the presence of considerable extents of non-polar side chains, which may bind the hydrocarbon units of oils, thereby resulting in higher oil absorption (Lazos, 1991).

Table 4.4. % Water and oil holding capacity of pumpkin, pomegranate and grape seed protein concentrates manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP). The data represent the average of three independent experiments and their corresponding standard deviation.

Preparation method	Sample	Water Holding Capacity (100 g water/g sample)	Oil Holding Capacity (100 g oil/g sample)
Control	Soy protein concentrate	202.68±1.34	119.05±5
AE-IP	Pomegranate seed	178.27±8.5	337.27±34.2
	Pumpkin seed	175.75±11.9	182.16±15.3
	Grape seed	257.96±5.08	414.53±38.6
SE	Pomegranate seed	12.21±0.4	364.50±35.3
	Pumpkin seed	90.63±33.8	240.37±0.43
	Grape seed	157.66±4.13	1131.28±10.83
MP	Pumpkin seed	81.15±5.2	267.63±76.3

4.2.3.3 Emulsifiying activity

Oil-in-water emulsions (o/w) were prepared using soybean oil (10 ml) and protein concentrate dispersions (1% w/v) at pH 7. The results were summarized on Table 4.5 in terms of emulsifying activity (EAI), which describes the ability of a protein to form an emulsion. This values presents an estimate for the maximum extent of interfacial area that can be stabilized by a certain amount of protein (Karaca et al., 2011).

EAI data for the current samples were presented on Table 4.5. For AE-IP pumpkin, pomegranate and grape seed protein concentrates, EAI values were approx. 6.1, 15.2 and 22.3 (m^2/g), respectively. In the case of SE samples, EAI values were found as approx. 21, 31.3 and 20.2 (m^2/g) for pumpkin, pomegranate and grape seed protein concentrates, respectively. Finally EAI value was approx. 1.6 (m^2/g) for MP pumpkin seed protein concentrates prepared by micellar precipitation (Table 4.5). Protein concentrates produced by salt extraction had significantly higher solubilities, which contributed to their higher EAI when compared to alkali extracted concentrates (Table 4.3). Solubility plays an important role as highly insoluble proteins tend to perform as poor emulsifiers and lead to coalescence. According to Kato and Nakai (1980), the emulsifying properties are correlated to the presence of hydrophobic residues on the protein surface. In most cases, the EAI values of the current samples were higher than that of the commercial soy protein concentrate (17.6 $m^2.g^{-1}$) (Table 4.5), while Fuhrmeister and Meuser (2003) reported an EAI of 18.6 m^2/g for soy protein concentrate.

Table 4.5. Emulsifiying activity index (EAI) of pumpkin, pomegranate and grape seed protein concentrates manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) or micellar precipitation (MP).

Preparation method	Sample	EAI (m^2/g)
Control	Soy protein concentrate	17.59±0.02
AE-IP	Pomegranate seed	15.24±0.01
	Pumpkin seed	6.05±0.01
	Grape seed	22.30±0.01
SE	Pomegranate seed	31.26±0.01
	Pumpkin seed	21.02±0.03
	Grape seed	20.22±0.01
MP	Pumpkin seed	1.57±0.003

4.2.3.4 Foaming capacity

Foaming capacity (FC, %) of the pumpkin, pomegranate and grape seed protein concentrates were listed on Table 4.6. In general, the current protein concentrates were found to perform as limited foaming agents. None of the grape seed protein concentrates demonstrated significant foaming capacity within the pH range studied here (pH 3-7).

Pomegranate seed protein concentrates demonstrated foaming capabilities only for AE-IP samples at pH 3 and pH 5, whereas SE samples were quite poor in that sense. While SE and AE-IP pumpkin protein concentrates demonstrated a foaming capacity of 40 and 25% at pH 3, MP samples once again did not form or stabilize significant foam volumes. Consequently, the lowest FC range for all samples occurred between 2-40%, whereas foam formation at pH 5 was quite limited. This finding could be due to the low solubility of proteins at this pH (Aluko and Yada, 1995).

Table 4.6. %Foaming capacity (FC) of pumpkin, pomegranate and grape seed protein concentrates manufactured by alkali extraction-isoelectric precipitation (AE-IP), salt extraction (SE) and micellar precipitation (MP) at varying pH values.

Preparation method	**Sample**	**Foaming Capacity (%)**		
		pH=3	**pH=5**	**pH=7**
Control	Soy protein concentrate			10±1
AE-IP	Pomegranate seed	35	ND	2
	Pumpkin seed	25	ND	ND
	Grape seed	ND	ND	ND
SE	Pomegranate seed	ND	ND	ND
	Pumpkin seed	40	30	ND
	Grape seed	ND	ND	ND
MP	Pumpkin seed	ND	ND	ND

4.2.3.5 Drop shape tensiometry

The surface tension at the air-protein solution surface was determined using drop tensiometry (Figure 4.9). After 10,000 s, equilibrium surface tension values were approximately 45.5, 47.5 and 56.1 mN.m^{-1} for AE-IP pumpkin, pomegranate and grape seed samples, respectively, at a protein concentration of 0.1%, which implied grape seed proteins were comparatively less surface active compared to pumpkin and pomegranate samples. Also, as the long term kinetics of the protein concentrates were investigated (i.e., $\Delta\Pi$ vs $t^{-0.5}$) (Gülseren et al. 2007), maximum surface pressure values of 30 (γ = 42.3 mN.m^{-1}), 27.8 (γ = 44.5 mN.m^{-1}), and 21 (γ = 51 mN.m^{-1}) mN.m^{-1} were predicted for as t approached infinity for pumpkin, pomegranate and grape seed protein concentrates. Long-term prediction essentially yielded similar results. Commercial soy protein concentrate was analyzed under similar conditions and a surface tension value of 47.9 mN.m^{-1} was attained. Bucko et al. (2015) reported the surface pressure for pumpkin protein concentrates as 23.11 mN/m. This finding roughly corresponds to a surface tension of 50 mN.m^{-1} at pH 5, which was comparable to the current findings. Based on these findings, surface activity of the current protein concentrates were found to be comparable to the commercial soy protein concentrate sample.

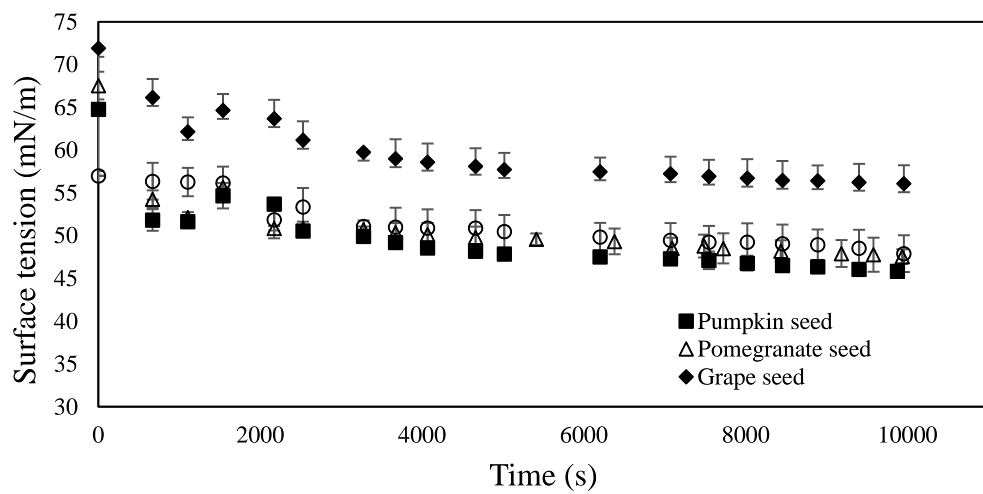


Figure 4.9. Surface tension of pumpkin seed, pomegranate seed and grape seed (0.1%) protein concentrate by alkali extraction-isoelectric precipitation method.

4.2.3.6 Rheological characteristics

In order to determine the thermal gelation characteristics of the protein concentrates (AE-IP), a temperature controlled rheometer system with a parallel plate geometry was utilized. The protein dispersions (10%) were heated to 80°C from 25°C, held at 80°C and cooled back to 25°C. Heating and cooling rate was 5°C.s^{-1} in all cases. G' values for pumpkin and pomegranate seed protein samples were found to become higher than G'' after approx. 10 and 5 minutes of heating, respectively (Figure 4.10). In addition, during the heating and cooling experiments, loss moduli values were larger than or roughly equal to the storage moduli which indicated the formation of weaker gels, since G″ measures the viscous contribution to the system and represents the molecular interactions that do not lead to the formation of 3D-gel networks (Sun & Arntfield, 2010). In the case of grape seed protein concentrates, however, G'' values were found to be higher than G' in all cases indicating the absence of gel formation.

The physicochemical characteristics, molecular weight distributions and functional properties of these protein concentrates were determined. Although the water holding capacities of these proteins were relatively weak, oil holding and emulsion formation characteristics were significant which could be instrumental especially in the production of foods and other industrial products. Emulsification activity generally increased with the solubility of protein concentrates. Dynamic surface tension analyses indicated that that all of the current protein samples lowered surface tension significantly at air/protein dispersion interface, while pumpkin proteins were the most effective in that sense. The thermal gelling characteristics of pumpkin and pomegranate protein concentrates were weak, but it must be noted that we have worked at a very limited range of protein concentrations, pH values, temperatures and ionic strengths. Under the current circumstances, grape seed proteins were not found to form 3D-gels. Finally, studies on enhancing the functional characteristics of the current proteins studied.

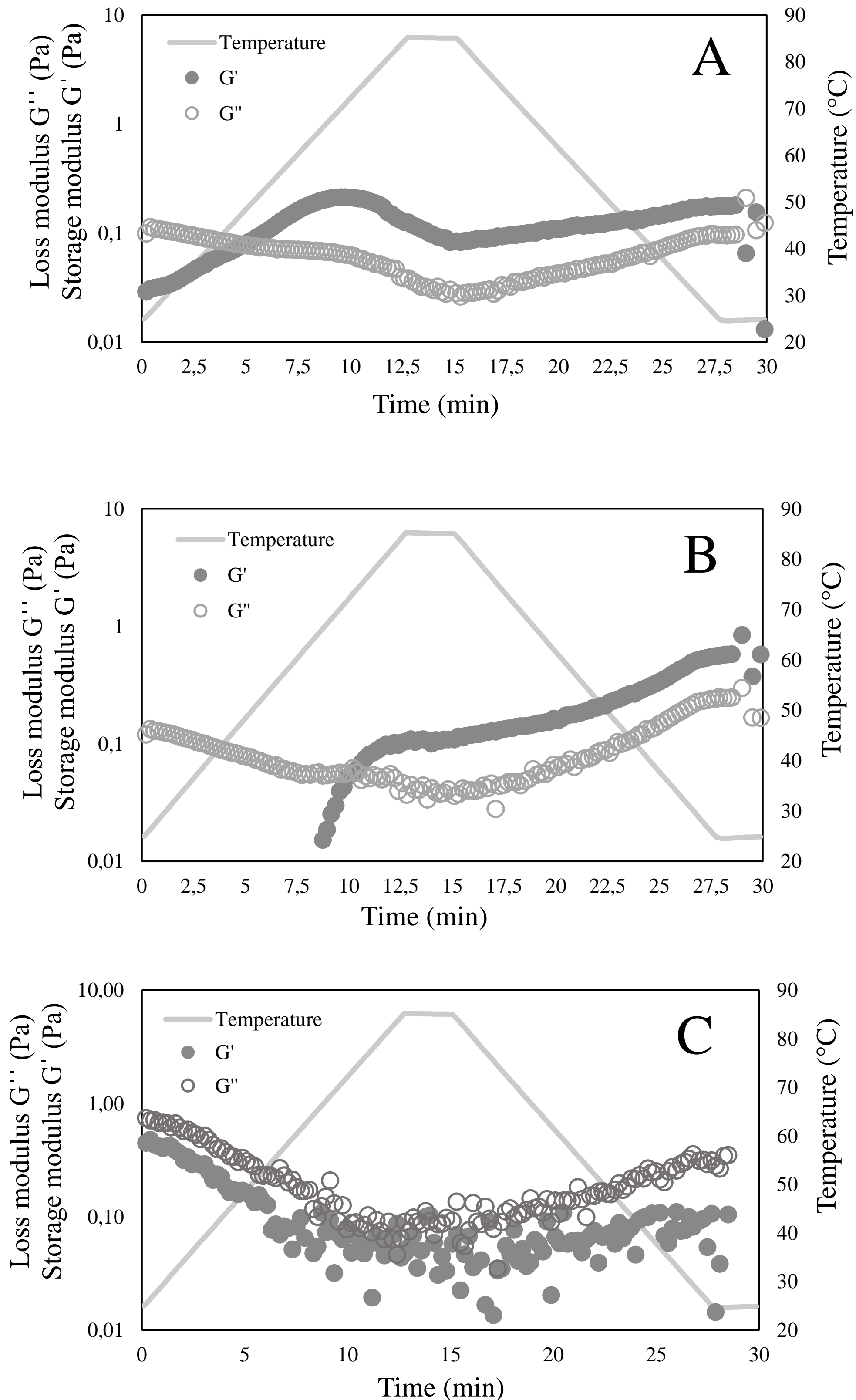


Figure 4.10. Heat induced gelation profile of pumpkin seed (A), pomegranate seed (B) and grape seed (C) (10%) protein concentrate dispersions prepared by alkali extraction and isoelectric precipitation method; G'- storage modulus, G''- loss modulus.

4.3 Enhancement of foaming characteristics of black cumin protein concentrates based on Maillard conjugation

4.3.1 Electrophoretic analysis

The molecular weight distribution of proteins in the black cumin protein concentrates was investigated using native- and SDS-PAGE methods (Figure 4.11). Firstly, on Lane 1, SDS-PAGE analysis of the untreated protein concentrates were shown. The major bands appeared between 15 and 25 kDa, around 37 kDa. In order to analyze the molecular weight distribution of Maillard reaction conjugates accurately, Native-PAGE analysis was also carried out. Although there was smearing and some faint spots were observed throughout the gel, the major bands were shown to lie between 75 and 250 kDa in all cases (Lanes 2-4) for protein:glucose ratios of 1:1 to 1:4. For the 1:4 samples, the size of the band was considerably smaller. At a protein: glucose ratio of 1:2 and as a function of pH, once again the distribution of molecular weights were investigated (Lanes 5-7). Smearing was once again the case at Lanes 5 and 6 between 25 and 37 kDa, 20 kDa and 10-15 kDa (Figure 4.11). At pH 7 (Lane 7), while a broad band was observed between 75 and 250 kDa, it did not exist at pH 3 (Lane 5) or pH 5 (Lane 6). This observation could be related to the potential isoelectric precipitation of proteins at pH 3 or 5. Similarly in the previous literature, the major bands of complexes only appeared at pH 7, whereas the likelihood of Maillard reaction taking place was considerably less at lower pH (i.e., pH 3 and 5) (Guan et al., 2010; Lertittikul et al., 2007).

Conjugate formation results in a decreased band intensity compared to pure protein (Kato, 2002). Since the bands appears at higher molecular weight spots, these findings indicate covalent binding between the proteins and carbohydrates. In the case of polysaccharides with high molar mass, these bands appear at the border between the stacking gel and the resolving gel. Kato (2002) indicating the higher molecular weight of the conjugates.

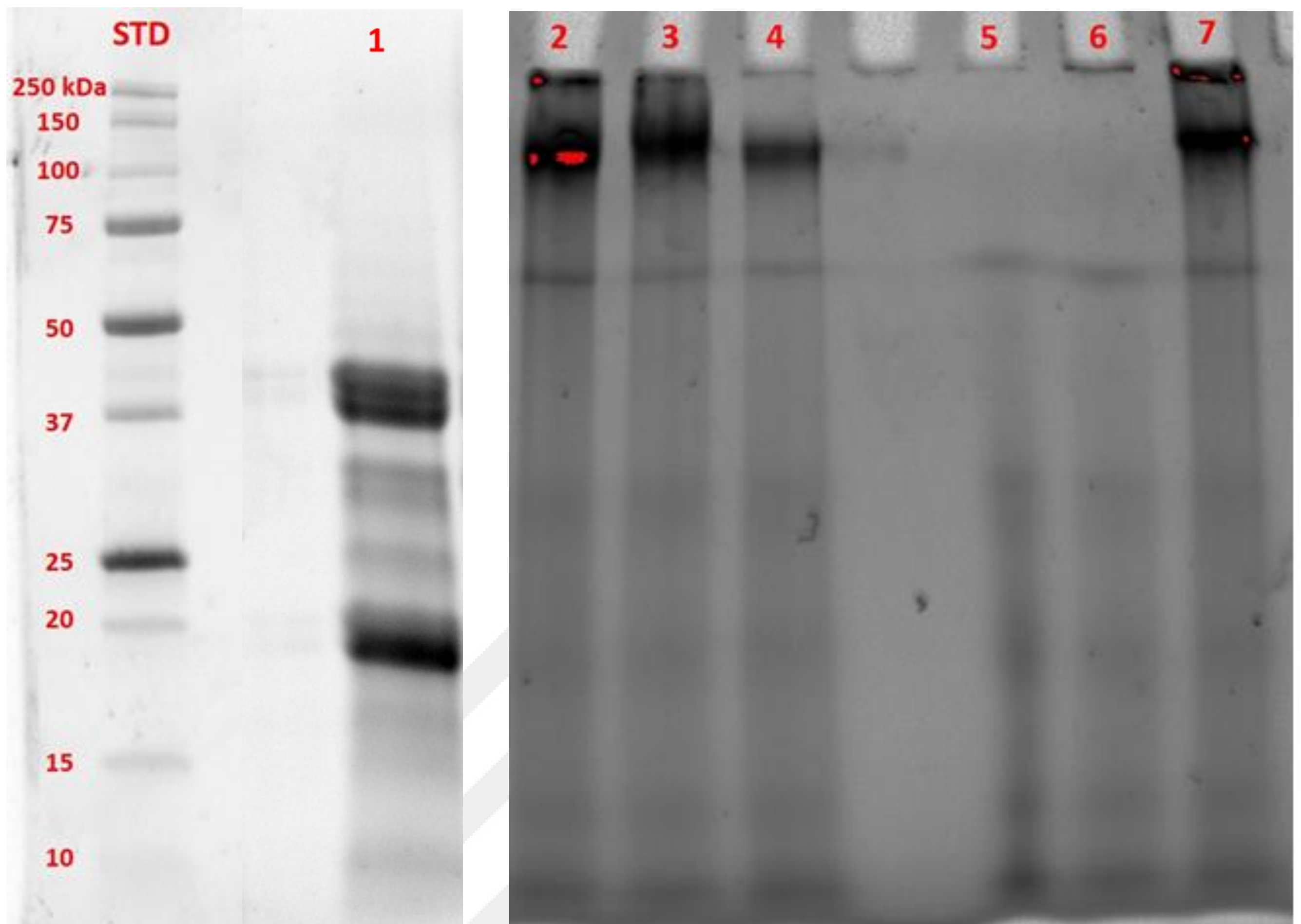


Figure 4.11. SDS-PAGE analysis of black cumin protein concentrates (2%) (Lane 1). Native-PAGE analysis of black cumin-glucose Maillard reaction products (30 min, pH 7 at 100°C) as a function of protein: glucose ratio of 1:1, 1:2, and 1:4 (Lanes 2-4, respectively). Lane 5-7: 1:2 protein: glucose ratio 30 min at pH 3, 5 and 7 respectively.

4.3.2 Foaming capacity and stability

In order to understand the influence of Maillard conjugation on the foaming characteristics of black cumin protein concentrates, foaming capacity and stability of foams prepared with untreated proten concentrates were prepared (Figure 4.12) and compared to that of conjugated proteins (Figure 4.13). At the time of preparation, foam capacity of pH 5 or pH 7 samples were higher than pH 3 samples (2% protein in all cases). As time passed, the stability of the foams rapidly decreased. At pH 3, the stability was the lowest at the time of preparation, while at longer durations, the foam volumes were relatively unchanged. However, in the case of pH 5 samples, the collapse was rapid and for the pH 7 samples, approx. 90% of the foam volume was lost after 2 hours of storage at the ambient temperature. When the sample performances were compared to sodium caseinate stabilized foams prepared at comparable conditions, although the performance of caseinate was slightly better than the pH 7 samples, in most cases the differences were in the range or 10%.

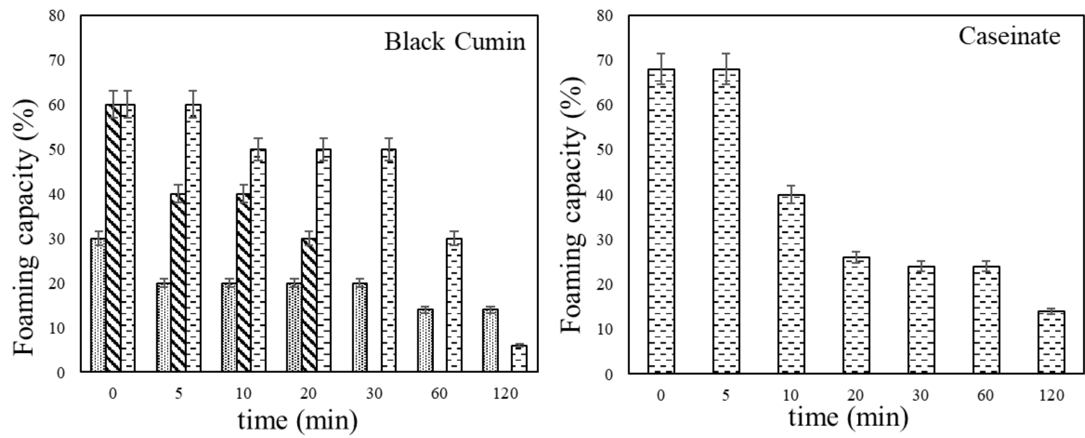


Figure 4.12 Foaming capacity (%) and stability of foams prepared with black cumin protein concentrates (2%) and caseinate (2%) as a function of pH (3-7) and time (0-120 min).

Influence of protein:glucose ratio (1:1 to 1:4) and heating duration (0-30 min at 100°C) was monitored (pH 7) (Figure 4.13). Especially at a ratio of 1:2 and 15 min of treatment, the foaming capacity of the black cumin proteins were significantly enhanced. For 1:1 and 1:2 ratios, 15 min treatments were generally more effective than the 30 min treatments where the stability values were lower. It has to be considered that at the highest glucose concentration, the sample viscosity is slightly higher than the other samples which in turn could positively affect the sample viscosity. However, the foaming capacity was not improved. The maximum improvement among the samples took place at protein:glucose ratio of 1:2 for 15 min process at pH 7, which represented a 23% improvement in foaming capacity at the time of preparation. These findings were coherent with the previous data (for example, (Dickinson and Izgi, 1996; Dickinson, 1995)) Since proteins demonstrate pronounced surface activity and ability to form thick viscoelastic layers, they are efficient stabilizers of foams. If macromolecules attached to the interface can also be strongly solvated by the aqueous medium, it is clear that foam stability should be significantly improved and protein-polysaccharide complexation essentially falls under this classification (Dickinson and Izgi, 1996; Dickinson, 1995).

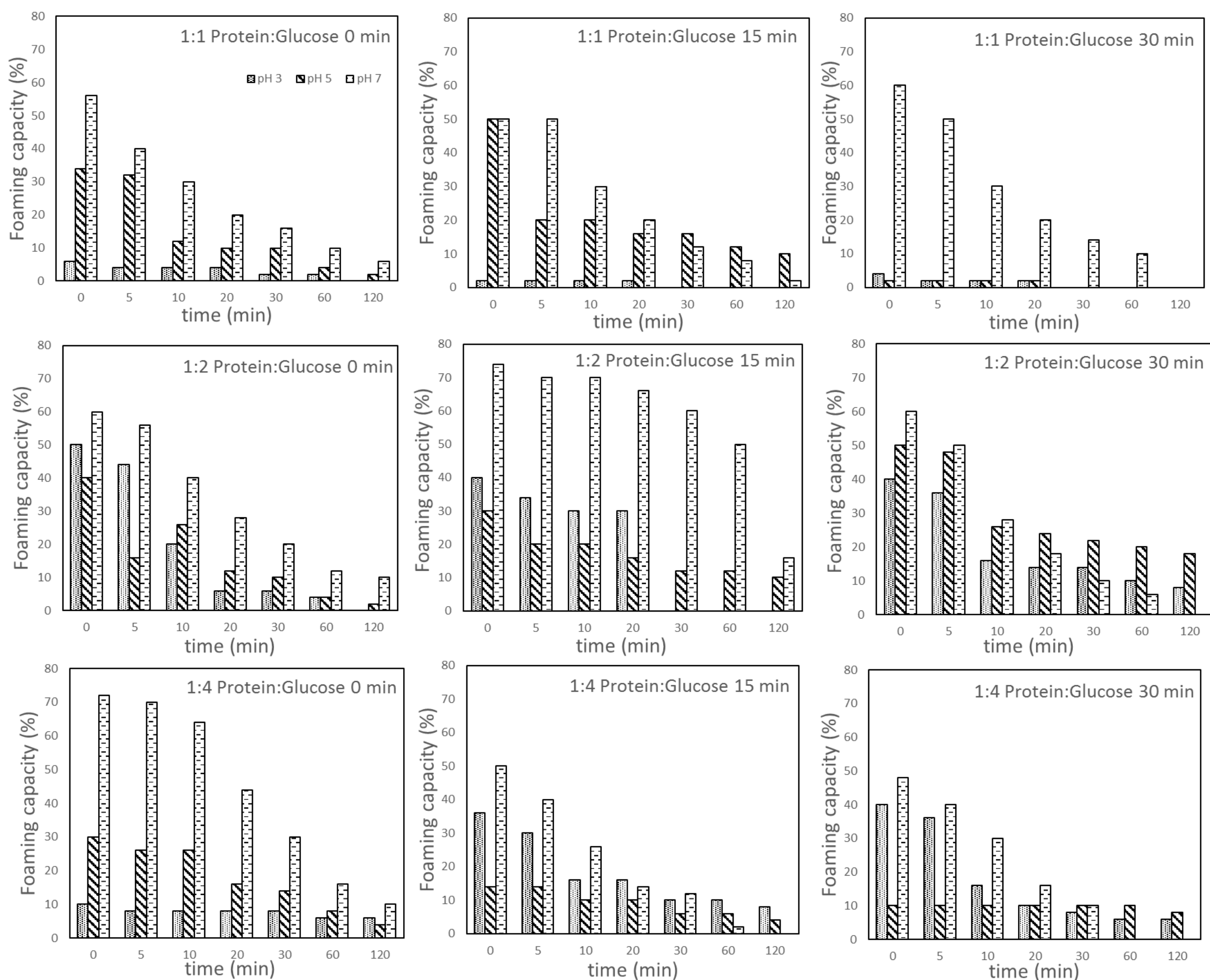


Figure 4.13. Foaming capacity (%) and stability of foams prepared with black cumin protein concentrates (2%) and glucose as a function of protein:sugar ratio (1:1, 1:2 and 1:4), pH (3-7), reaction duration (0-30 min) and time (0-120 min)

Based on the same protein:carbohydrate ratio (1:2), the influence of thermal treatment (0-30 min at 100°C) on Maillard conjugation was studied for lactose and maltodextrin as well (Figure 4.14). The foaming capacity of the samples processed at a protein:lactose ratio of 1:2 (30 min, 100 °C and pH 7) was found to be 70% which represented a further 16% increase compared to the unprocessed sample. Maltodextrin conjugation under similar conditions did not significantly improve the performance of the unprocessed sample. Consequently, the foaming capacities of protein concenrates-lactose or maltodextrin conjugates were considerably lower compared to glucose. Several investigations have shown that the reaction mechanisms of monosaccharides and disaccharides differ and that reaction products obtained from monosaccharides are different from those obtained from disaccharides (Li et al., 2009; Kato et al., 1988). A previous report indicated a linear correlation between the DS and the saccharide size. The lower reactivity of the maltodextrin and dextran have been reported to be related to steric hindrance effects (Kato, 2002). After 30 minutes of treatment, the foaming capacity and stability values were significantly improved, especially at pH 7. Possibly due to the larger molecular weights of these molecules, the Maillard reaction rate was considerably lower (Li et al., 2009). Meanwhile the interfacial behavior at the interface remains to be investigated.

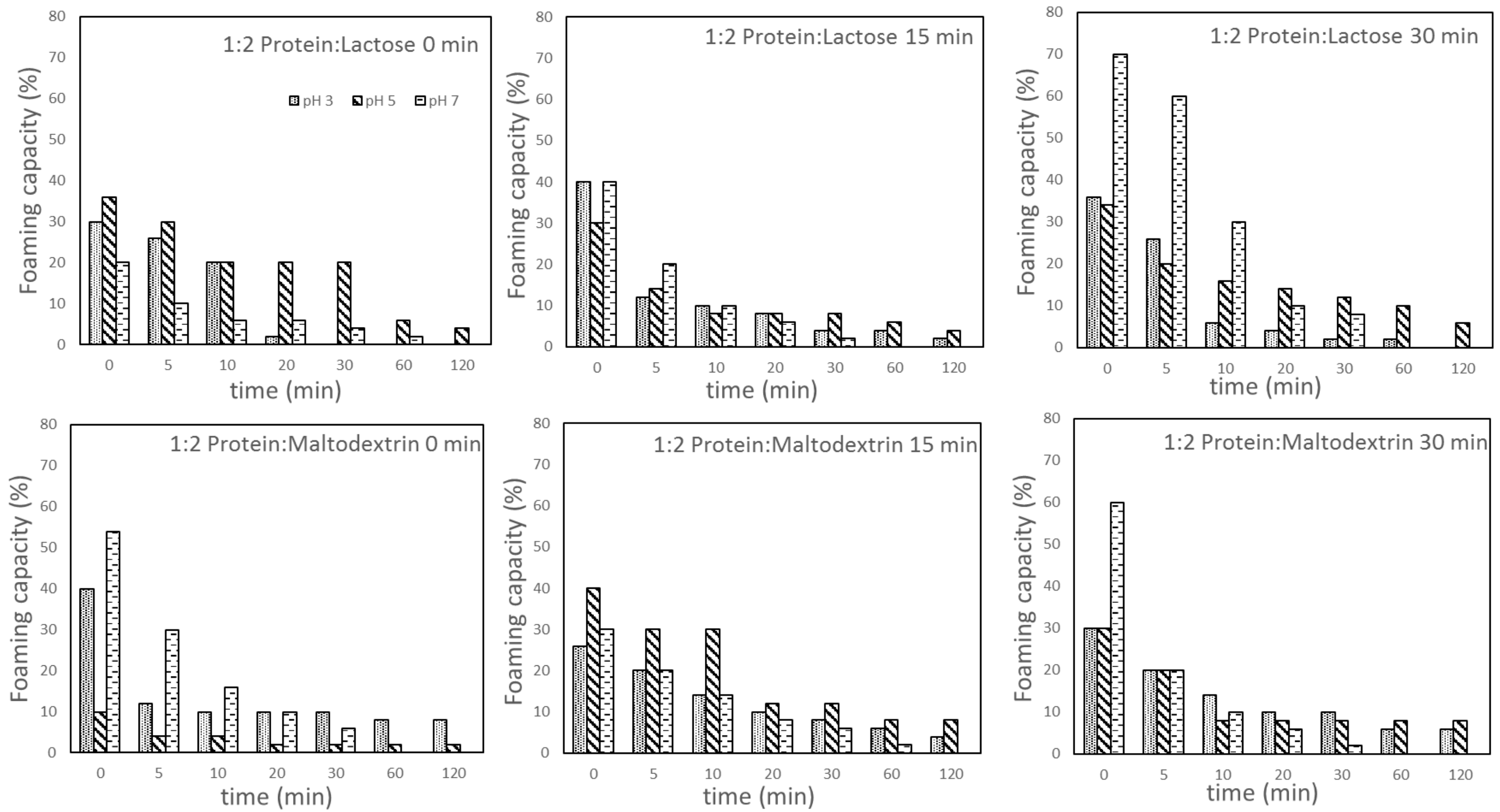


Figure 4.14. Foaming capacity (%) and stability of foams prepared with black cumin protein concentrates (2%) and lactose or maltodextrin at a protein:sugar ratio of 1:2 as a function of pH (3-7), reaction duration (0-30 min) and time (0-120 min).

4.3.3 RP-HPLC-RID Analysis

Using HPLC-RID methods, the glucose binding characteristics of black cumin protein concentrates were determined (Figure 4.15 and Table 4.7). As shown on Figure 4.15A, there was a tiny amount of fructose (approx. 0.013%) in the protein samples and they were mixed with a high concentration of glucose (approx. 1.3%). As the thermal processing was carried out, a considerable amount of glucose was isomerized to fructose during thermal processing, which was coherent with the previous work of Suzuki and Tsumura (1972) on isomerization. Meanwhile a certain portion of glucose (Table 4.7) was in all cases conjugated to the proteins. Conjugation efficiency was as high as 85% after 30 min of processing which is considerably higher than that at 15 min (75%). Since more glucose was conjugated which in turn decreased the foaming efficiency, the interfacial packing characteristics of the glucose molecules had to be altered at the air-water interface. The increasingly hydrophilic and glucose bearing molecular surfaces could have a difficulty at penetrating the interface thus rendering the foams less stable.

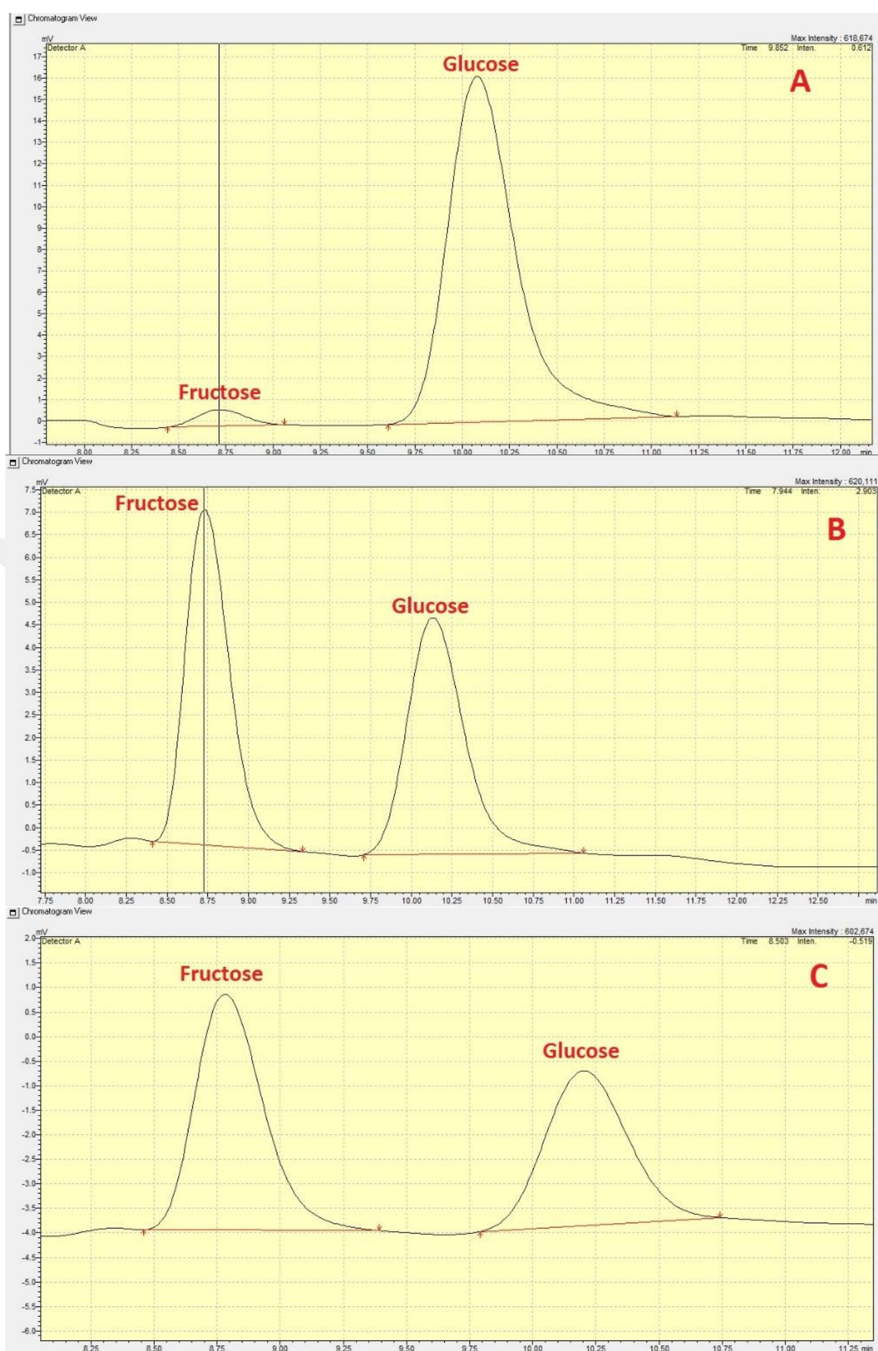


Figure 4.15. RP-HPLC-RID chromatogram of black cumin-glucose Maillard products prepared at a protein:glucose ratio of 1:2 held at (A) 25 °C, (B) 100 °C for 15 min, (C) 100 °C for 30 min.

Table 4.7. Glucose binding (%) and fructose formation (%) characteristics in black cumin protein concentrates due to Maillard reaction (100°C) as a function of time at a protein:glucose ratio of 1:2.

Time (min)	Concentration after HPLC		Binding (%)
	Fructose (%)	**Glucose (%)**	
0	0.013±0.01	1.319±0.02	34.0
15	0.345±0.04	0.491±0.07	75.5
30	0.230±0.04	0.289±0.04	85.5

4.3.4 Drop shape tensiometry

In this context, surface adsorption characteristics of the Maillard conjugates were studied (Figure 4.16) in order to elucidate the interactions between air bubbles and the conjugates using drop shape tensiometry.

The surface tension of Maillard conjugates were investigated by means of drop shape tensiometry and the data were compared to the untreated reference (i.e., black cumin protein concentrate) (Figure 4.16). In the case of the reference, after 3000 s of adsorption, the equilibrium surface tension was approx. 39.6 mN/m, while for the treated samples, the corresponding value was approx. 32 mN/N. However, the kinetics of adsorption between the treated samples were quite different. The time necessary to reach equilibrium increased with the the molecular weight of the carbohydrate unit. Glucose and lactose behaved roughly similar, whereas in the first few hundred seconds, the tension values for maltodextrin treated samples were considerably higher. In all cases, the surface pressure increased more rapidly compared to the reference sample. While the conjugate size had a bearing on the adsorption rate, in all cases Maillard conjugation enhanced the surface activity of black cumin protein concentrates.

In order to further elucidate the adsorption characteristics, the surface elasticity of the samples were also investigated. Firstly the linear viscoelastic region (LVR) was established for the samples (data not shown), consequently by the alteration of bubble volume at a constant frequency (0.1 or 0.5 Hz), the surface elasticity of the samples were determined (Table 4.8). In the case of glucose, although the adsorption was the fastest, surface elasticity was moderate and was found to be frequency dependent. Decreasing surface elasticity with frequency indicated possible deformation of the glucose conjugates at the surface which could affect the foaming capacity and stability. Lactose samples demonstrated the highest surface elasticity values. Consequently since the adsorption kinetics were reasonably fast and the elasticity was pronounced, for delicate foam systems, it could be possible to utilize lactose conjugates. However, the rate of adsorption was slower than glucose counterparts. Finally maltodextrin conjugates demonstrated the slowest rate of adsorption and at neither frequency, the surface elasticity was ideal. Possibly the large hydrophilic groups of maltodextrin both decreased its rate of adsorption and due to the steric effects at the surface the packing

was limited, consequently these results could be utilized in the interpretation of foaming data.

Formation of protein-carbohydrate conjugates enhanced foaming activity, whereas the performance was highly dependent on molecular size of carbohydrates as well as processing conditions such as pH, heating temperature and duration. While conjugation enhanced surface characteristics; adsorption rate and surface elasticity was highly dependent on molecular size, which in turn will determine the surface activity and foaming characteristics of black cumin protein concentrates. TGase treatment was also studied to enrich foaming capacity of these proteins.

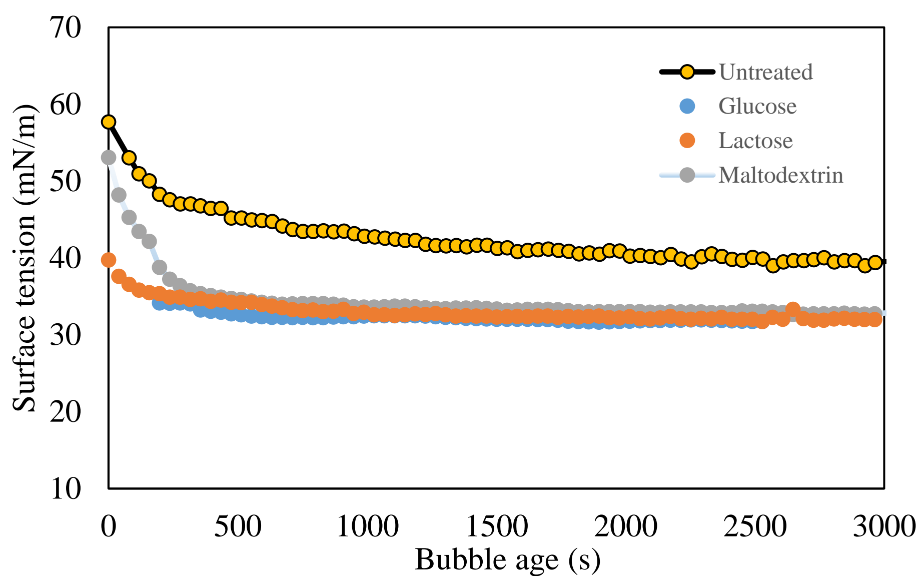


Figure 4.16. Dynamic surface tension of black cumin protein conjugates and the corresponding untreated sample as a function of time (i.e., bubble age) at the air-conjugate dispersion interface. Heat treatment was carried out at 100°C for 15 min at pH 7 and a protein:carbohydrate concentration ratio of 1:2. Drop shape tensiometry was utilized. Representative runs.

Table 4.8. Surface elasticity at the Maillard conjugate dispersion-air interface as a function of oscillatory frequency (Hz). Standar deviation was < 5% of the sample mean in all cases.

Conjugated Carbohydrate	Surface elasticity (mN/m)	
	Oscillatory Frequency (0.5 Hz)	Oscillatory Frequency (0.1 Hz)
Glucose	5.66	6.18
Lactose	18.28	7.71
Maltodextrin	9.90	4.70

.

4.4 Enhancement of foaming characteristics of current samples based on transglutaminase treatment

4.4.1 SDS-PAGE analysis

In the previous studies, the major bands were found to lay between 15-40 kDa for the untreated black cumin samples. The major bands were between 25-37 kDa, 20-25 kDa and finally around 10 kDa, for pumpkin seed protein concentrates. Finally, for the grape seed samples, although quite faint, the bands were located mostly between 20-40 kDa. After TGase treatment major bands lay between 25-75 kDa for black cumin protein concentrate. For the TGase treated pumpkin seed protein concentrate, major bands laid between 15-50 kDa and there were also some faint bands between 75-150 kDa. The major bands, although quite faint, located between 75-100 kDa (Figure 4.17). The extent of molecular weight increased with increased incubation with transglutaminase. The increased electrophoretic high molecular weight bands indicate the formation of biopolymers through intermolecular cross linking (Guillen et al., 2001).

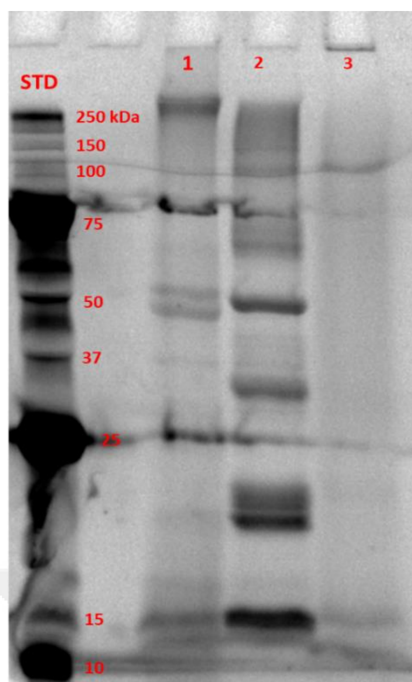


Figure 4.17. SDS-PAGE analysis of TGase treated black cumin, pumpkin seed and grape seed protein concentrates (Lane 1: black cumin, Lane 2: Pumpkin seed; Lane 3: grape seed protein concentrates).

4.4.2 Effect of crosslinking on foaming capacity and stability

Foaming capacity of TGase treated black cumin protein concentrate was found 70%, 72% and 64% for 2 hour, 4 hour and 18 hour treatment time respectively. TGase treated pumpkin seed protein concentrates foaming capacity was 18%, 20% and 50% for 2 hour, 4 hour and 18 hour treatment time respectively. Finally, foaming capacity of grape seed protein concentrate was found 8% for 18 hour treatment time and foam did not develop for 2 hour and 4 hour treatment time. According to these findings we concluded that optimum working time for black cumin, pumpkin seed and grape seed protein concentrates were 4 hour, 18 hour and 18 hour respectively (Figure 4.18).

In the previous studies we did not observed foam for untreated pumpkin seed and grape seed protein concentrates.Iit can be said that there has been a remarkable improvement especially for the pumpkin seeds protein concentrate. Also, foaming capacity of black cumin was developed for 11%. Anuradha and Prakash (2009) also reported that the transglutaminase treated β-Lg interacts and complex with 11S protein fraction of sesame and soybean with significant increase in foaming capacity. Transglutaminase catalyzed polymers cowpea proteins were also found to form better foam and emulsion forming ability than the native protein, results that were attributed to increase in strengthening of the interfacial protein film by the polymerized proteins (Aluko & Yada, 1995). Therefore, we concluded that foaming capacity could be developed by TGase treatment. This functional property is important especially in the bakery products. Foam formation is generally faster for more flexible random coiled structured proteins than for the tightly held structures (Halling, 1981; Damodaran, 1990). In addition increased hydrodynamic size (Damodaran, 1994), molecular weight (Mita et al., 1978) and elimination of charged amino groups of lysine residues by crosslinking could improve foaming capacity.

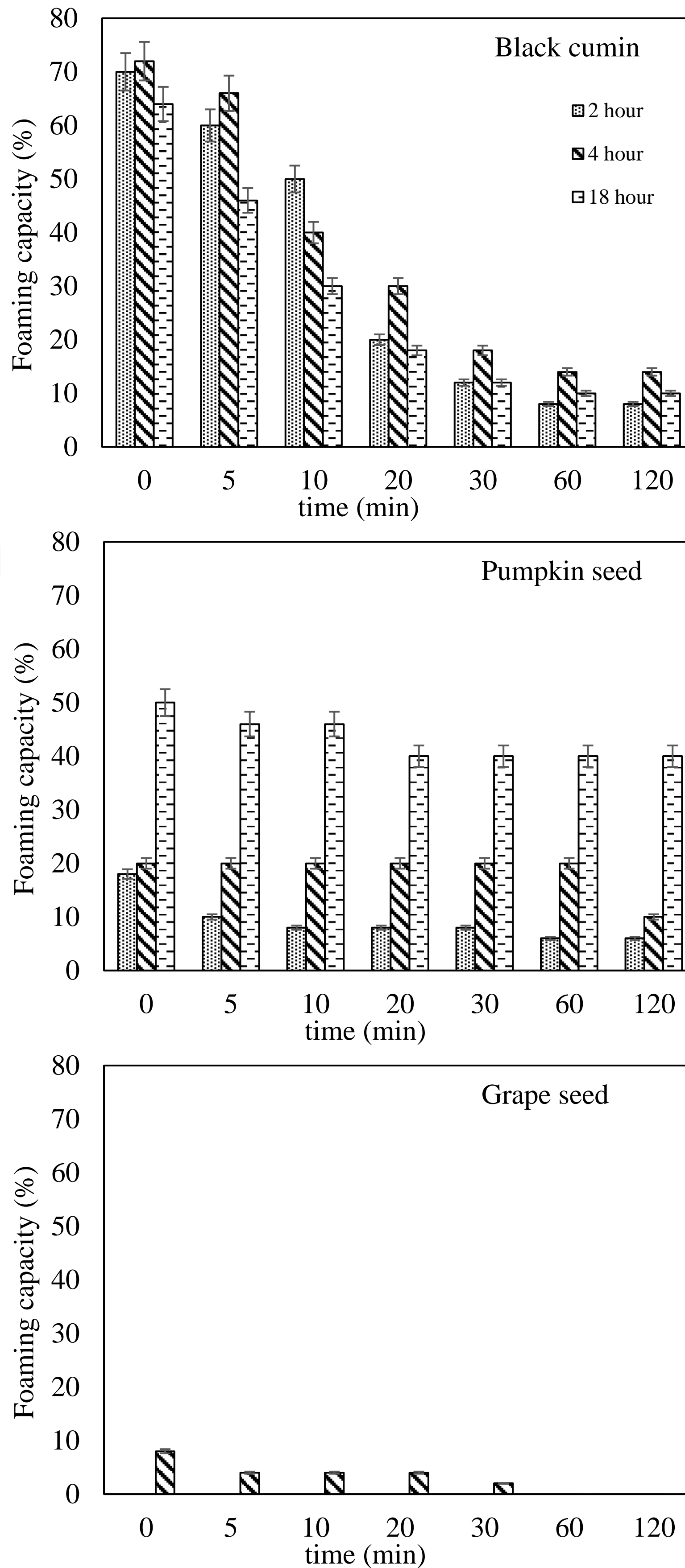


Figure 4.18. Foaming capacity and stability of TGase treated black cumin, pumpkin seed and grape seed protein concentrates.

4.5 Effect of black cumin, grape seed and pumpkin seed protein concentrates on the quality of wheat bread

4.5.1 Chemical composition

Protein (%), ash (%), moisture (%) and acidity (%) of the black cumin, grape seed and pumpkin seed enriched wheat flour and breads were given in Table 4.9. Protein contents of control, black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flour were 10.64, 11.64, 11.06, 10.67 (%), respectively. Also, protein contents (%) of control, black cumin, grape seed and pumpkin seed protein enriched breads were 8.00, 8.19, 8.31, 8.12 (%) respectively. According to this results; a slight increase was observed in the protein content of the enriched flour and bread. Ash contents of control, black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flour were 0.55, 0.72, 0.61 and 0.55 (%), respectively. Ash contents (%) of control and black cumin, grape seed and pumpkin seed protein concentrate enriched breads were 1.20, 1.32, 1.28 and 1.21 (%) respectively ($p<0.05$). In this context, ash content (%) increased for enriched samples. Osman et al. (2015) fortified flat bread with defatted black cumin and they reported protein and ash content (%) of control and 5% enriched flat bread were 15.10, 15.60 and 3.75, 3.87, respectively. El-Soukkary (2001) found protein contents of control and pumpkin seed protein concentrate enriched breads (protein level 19%) 13.10 and 19.13 (%), respectively. El-Adawy (1997) reported that addition of sesame seed protein concentrates to wheat flour increased both protein and ash content of the produced bread. Finally, Khan et al (1975) enriched bread with peanut protein concentrate and found 16.5 and 21.8 % protein content for control and fortified bread. Sabanis and Tzia (2009) reported that ash content of the rice, corn and soy- bread wheat flour blends increased due to the significantly higher mineral content of all the nonwheat flours compared to wheat flour. Our results were coherent with these investigators.

Table 4.9. Chemical composition of protein-fortified bread and flour. BCPC: black cumin protein concentrate enriched wheat flour/bread; GSPC: grape seed protein concentrate enriched wheat flour/bread; PSPC: pumpkin seed protein concentrate enriched wheat flour/bread. The data are the mean of the two measurements with the standard deviation ($p<0.05$).

	Samples	Protein (%)	Ash (%)	Moisture (%)	Acidity (%)
Wheat flour	Blank	10.64±0.0	0.55±0.0	12.24±0.0	3.24±0.3
	BCPC	11.64±0.0	0.72±0.0	12.18±0.0	3.80±0.2
	GSPC	11.06±0.1	0.61±0.0	11.81±0.0	4.10±0.1
	PSPC	10.67±0.2	0.55±0.0	11.90±0.0	3.71±0.3
Bread	Blank	8.00±0.0	1.20±0.0	46.18±0.2	2.20±0.3
	BCPC	8.19±0.1	1.32±0.0	45.18±0.0	3.09±0.3
	GSPC	8.31±0.0	1.28±0.0	44.38±0.1	3.71±0.1
	PSPC	8.12±0.0	1.21±0.0	45.15±0.0	3.19±0.2

4.5.2 Wheat flour analysis

Wet gluten, gluten index, sedimentation, delayed sedimentation, falling number and fungal falling number analysis of protein concentrate enriched wheat flours were shown in Table 4.10. Wet gluten value of control, black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flours were 25.2, 24.9, 27.9 and 25.1, respectively ($p<0.05$). Gluten index represents the ratio of strong gluten to total gluten (Mercier et al. 2012). Gluten index value of control, black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flours were 98.5, 97.5, 96 and 98, respectively ($p<0.05$). As expected, enrichment reduced the gluten content. Similar results were reported by Sabanis and Tzia (2009). They found lower gluten content since rice, corn, and soy flours were gluten-free and had significantly lower gluten content than wheat flour. Liu (1996) attributed similar results in breads fortified with chickpea or Northern bean flour to the decrease in gluten content.

The sedimentation value according to Zeleny (i.e., Zeleny value) method describes the degree of sedimentation of flour suspended in a lactic acid solution during a standard time interval and this is taken as a measure of the baking quality. Swelling of the gluten fraction of flour in lactic acid solution affects the rate of sedimentation of a flour suspension. Both a higher gluten content and a better gluten quality give rise to slower sedimentation and higher Zeleny test values. The sedimentation value of flour depends on the wheat protein composition and is mostly correlated to the protein content (Shewry and Tatham, 2000). Zeleny sedimentation and delayed sedimentation values of control, black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flours were 30.5, 33, 26, 31 and 37.5, 36.5, 32.5 and 37.5 respectively ($p<0.05$). According to results, addition of these protein concentrates to wheat flour reduced Zeleny sedimentation value. Similarly, Švec and Hrušková (2014) reported substitution of wheat flour with hemp seed flour caused fall in Zeleny sedimentation value.

The falling number method is a viscometric assay that involves the rapid gelatinization of a flour or meal suspension in water, by immersion in a boiling water bath, with subsequent measurement of the liquifaction of the starch by α-amylase (Mares and Mrva, 2008). Because α-amylase is an endo-acting enzyme, that inserts breaks in the interior of the very large starch molecules, small amounts of enzyme cause dramatic reductions in viscosity (Barnes and Blakeney, 1974). The quantity of

fungal α-amylase used in wheat flour must be controlled since an excess negatively affects the baking process. Since the classical falling number method does not quantify fungal alpha-amylase, a method must be used that does determine the amount present (Gutkoski et al., 2009). Falling number and fungal falling number values of control, black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flours were 378, 416.5, 399,389 and 824, 922, 912.5, 905.5, respectively ($p<0.05$). Falling and fungal falling number of the flours increased with the protein content of the flours increased (Table 4.10). Ayoub et al. (1994) similarly stated that a rise in the protein content was associated with an increase in Falling Number. Changes in the functional properties of the protein, like solubility and water absorption ability (Belitz et al. 2004) could be an explanation for this increase in falling number.

Table 4.10. Analytical quality parameters of protein-fortified flours. BCPC: black cumin protein concentrate enriched wheat flour; GSPC: grape seed protein concentrate enriched wheat flour; PSPC: pumpkin seed protein concentrate enriched wheat flour. The data are the mean of the two measurements with the standard deviation (p<0.05).

Samples	Wet gluten	Gluten Index	Normal Sedimentation	Delayed Sedimentation	Falling number	Fungal falling number
Wheat flour (blank)	25.2±0.1	98.5±0.0	30,5±0.0	37.5±0.7	378±5.6	824±5.6
BCPC	24.9±0.1	97.5±2.1	33±0.0	36.5±0.7	416.5±23.3	922±0.0
GSPC	27.9±0.2	96±1.4	26±0.0	32.5±0.7	399±7.0	912.5±4.9
PSPC	25.1±0.0	98±1.4	31±0.0	37.5±0.7	389±8.4	905.5±7.7

4.5.3 Dough rheological properties

The extensograph analysis measure the balance between viscous and elastic characteristics of flour dough. The curve specify a measure of the resistance to extension and the extensibility of the dough. The resulting curve heights from stretching the dough is related with dough's resistance-to-extension. Extensibility is the total length of the curve at the base line in centimetres which reflects the extent to which dough was stretched. The dough resistance, BU, is measured at the maximum curve height and reflects the applied maximum force and indicate dough resistance. In general, for good bread dough a balance of these two factors is desired. The procedure calls for stretching the dough after three resting times 45, 90 and 135 min. Extensograph test, can also evaluate the effects of baking ingredients and fermentation duration on dough viscoelasticity (Osman et al., 2015). Extensograph analysis of black cumin, grape seed and pumpkin seed protein concentrate enriched wheat flour were shown in Table 4.11. Resistance to extension (BU) value of control, black cumin, grape seed, pumpkin seed protein concentrate enriched wheat flours were 484, 540, 572 and 413 (BU) at 90 minute ($p<0.05$). Black cumin and grape seed protein concentrate enriched wheat flour were more resistant and less extensible than control sample (Table 4.11). Pumpkin seed protein concentrate enriched wheat flour was less resistant than control sample. Extension (mm) value of control, black cumin, grape seed, pumpkin seed protein concentrate enriched wheat flour were 129, 113, 116 and 128, respectively. Supplementation of black cumin, grape seed and pumpkin seed protein concentrates decreased the extensibility. Similar effects have been previously reported for lupin and soy flour (Doxastakis et al. 2002) and cowpea flour (Hallen et al. 2004).

Table 4.11. Extensograph parameters of protein-fortified flours. BCPC: black cumin protein concentrate enriched wheat flour; GSPC: grape seed protein concentrate enriched wheat flour; PSPC: pumpkin seed protein concentrate enriched wheat flour. The data are the mean of the two measurements with the standard deviation ($p<0.05$).

Dough resting time	Samples	Energy (cm²)	Resistance to extension (BU)	Extension (mm)	Maximum (BU)	Ratio Number	Ratio Number (max)
	Blank	70±3.2	302±5.9	134±4.2	375±10.0	2.3±0.2	2.8±0.1
	BCPC	72±4.1	344±6.3	128±11.3	411±5.9	2.7±0.1	3.2±0.5
	GSPC	85±5.6	331±7.2	143±3.2	448±8.9	2.3±0.5	3.1±0.7
45 minutes	PSPC	73±7.1	274±10.2	149±6.5	364±15.6	1.8±0.6	2.4±0.8
	Blank	100±4.5	484±9.5	129±4.5	554±25.3	3.7±0.1	4.3±0.9
	BCPC	91±5.3	540±4.8	113±1.2	620±30.2	4.8±0.4	5.5±0.6
	GSPC	101±6.2	572±3.6	116±10.3	692±24.8	4.9±0.3	6±0.3
90 minutes	PSPC	87±1.3	413±4.2	128±4.6	508±41.3	3.2±0.5	4±0.1
	Blank	86±2.6	473±7.9	117±5.3	542±12.3	4±0.2	4.6±0.8
	BCPC	100±6.1	544±8.1	121±8.2	637±26.3	4.6±0.1	5.3±0.7
	GSPC	93±3.2	576±11.6	111±9.6	662±45.6	5.2±0.2	6±0.2
135 minutes	PSPC	78±4.7	389±13.9	127±4.6	476±30.2	3.1±0.3	3.8±0.8

Farinograph stability time is correlated with flour strength. Long stability times are generally more suited for variety bread production and often require longer mixing times (Aydoğan et al., 2015). Dough development time and stability value are indicators of the flour strength, with higher values suggesting stronger doughs (Wang et al., 2002). Farinograph analyzes were carried out to determine the degree of softening of the wheat flours enriched with black cumin, grape seed and pumpkin seed protein concentrates. Farinograph was also used to determine the effect of black cumin, grape seed and pumpkin seed protein concentrate on the water absorption of the control flour (Table 4.12). According to current results, water absorption capacity (WAC) of the black cumin and pumpkin seed protein concentrate enriched flours increased by 0.6% and 0.4%, respectively ($p<0.05$). The results agreed with the findings of Mubarak (2001) who found that water absorption increased substantially by addition of lupin products at levels of 3, 6, 9 and 12%. WAC of grape seed protein concentrate enriched flour decreased 0.4%. The drop in water absorption due to grape seed protein concentrate could be due to low hydration rate of to grape seed protein concentrate relative to wheat flour. These findings agree with Karaoğlu et al. (2006) who reported drop in water absorption with the addition of Cephalaria flour. Dough development time was higher for all enriched wheat flour compared to the control (Table 4.12). Accordingly, dough development time of control, black cumin, grape seed and pumpkin seed protein concentrates were 1.5, 2, 1.7 and 1.5 (min), respectively ($p<0.05$). Generally, the increase in dough development time may be due to the differences in the physico-chemical properties of protein concentrates and that of wheat flour as previously detected and reported by Morad et al. (1980) for different protein sources. Dough stability is the difference in time (min) between the time when the top of the curve arrives at the 500 BU and the time where it departs. Dough stability of control, black cumin, grape seed and pumpkin seed protein concentrates enriched flours were 3, 7.2, 2.5 and 3.4 minutes, respectively ($p<0.05$). Grape seed protein concentrate addition decreased the dough stability (Figure 4.12). El-Adawy (1997) reported lower stability times with the addition of sesame products to wheat flour. Degree of softening decreased after 10 minutes from the experiments start. A possible reason is the weakening of the dough by the added nongluten proteins. The obtained weakening of dough resulting from the addition of nonwheat flours could be due to (a) the presence of sulphyhydryl groups in protein products, which causes the dough softening, (b) an effective decrease in wheat gluten content, and (c) competition

between proteins of nonwheat and wheat flour for water hydration (El-Adawy 1997). Fleming and Sosulski (1978) described the weakening of dough with supplemented proteins to the decomposition of the well-defined protein–starch complex in wheat flour dough by the supplemental proteins.

Table 4.12. Farinograph parameters of protein-fortified flours. BCPC: black cumin protein concentrate enriched wheat flour; GSPC: grape seed protein concentrate enriched wheat flour; PSPC: pumpkin seed protein concentrate enriched wheat flour. The data are the mean of the two measurements with the standard deviation ($p<0.05$).

Samples	Water absorption (%)	Development time (min)	Stability (min)	Degree of softening (FU)[a]	Degree of softening (FU)[b]
Blank	57.2±0.1	1.5±0.01	3±0.02	60±0.1	69±0.2
BCPC	57.8±0.2	2±0.03	7.2±0.05	42±0.2	58±0.3
GSPC	56.8±0.1	1.7±0.02	2.5±0.02	50±0.5	76±0.5
PSPC	57.6±0.1	1.6±0.01	3.4±0.01	48±0.2	66±0.2

a: 10 minutes after the experiment started; b: After 12 minutes maximum (ICC).

4.5.4 Bread characteristics

Images of baked black cumin, grape seed and pumpkin seed protein concentrate enriched breads with different fermentation times (80,110,140 minutes) were shown in Figure 4.19. Fermentation temperature was 35 °C and relative humidity was 90%. Visually, in the case of volume and shape of breads, black cumin protein concentrate enriched bread did not open after 80 min fermentation time. Based on the extensograph data, black cumin protein concentrate yielded resistance to dough and decrease the extensibility of dough. Again visually, there was a great differeance between 80 min fermented and 140 min fermented black cumin enriched bread, since there was no fermentation stability. According to extensograph values, the second resistant dough was grape seed protein concentrate enriched dough. It has been confirmed in the case of opening (Figure 4.19). Pumpkin seed protein concentrate enriched bread was similar to the control bread (Figure 4.19). These observations were in accordance to Ribotta et al. (2005). Doxastakis, et al. (2002), also reported a loss on bread crumb structure with increasing levels of lupin or soy flour and attributed this decrease to the dilution of the wheat gluten by the legume proteins. Blended breads retained less gas, hence providing a dense texture to bread, which was not desirable (Sabanis and Tzia, 2009). Iwuoha et al. (1997) also reported the deterioration in texture on supplementation of fenugreek flour in wheat flour. Lazo- Velez (2015) observed the slightly lower bread volume in soybean protein enriched breads is mainly attributed to the addition of a non-gluten forming protein and the loss of gluten interaction. It is also known that incorporation of single-cell protein can disrupt the elastic gluten structure, allowing losses of gas during proofing and baking (Fleming and Sosulski, 1978). Ribotta et al. (2005) showed that different soybean flours and proteins produced a gluten film which was more permeable to the CO2 generated by yeast. Fleming and Sosulski (1978) showed that the loss of gas during baking may be through small pores in the viscoelastic gluten film protein observed by scanning electron microscopy in breads supplemented with soybean protein concentrate. According to Mohammed et al. (2012) this decrease in bread crumb quality was justified by the combined effects of gluten dilution and mechanical disruption of the gluten network structure by the chickpea proteins.

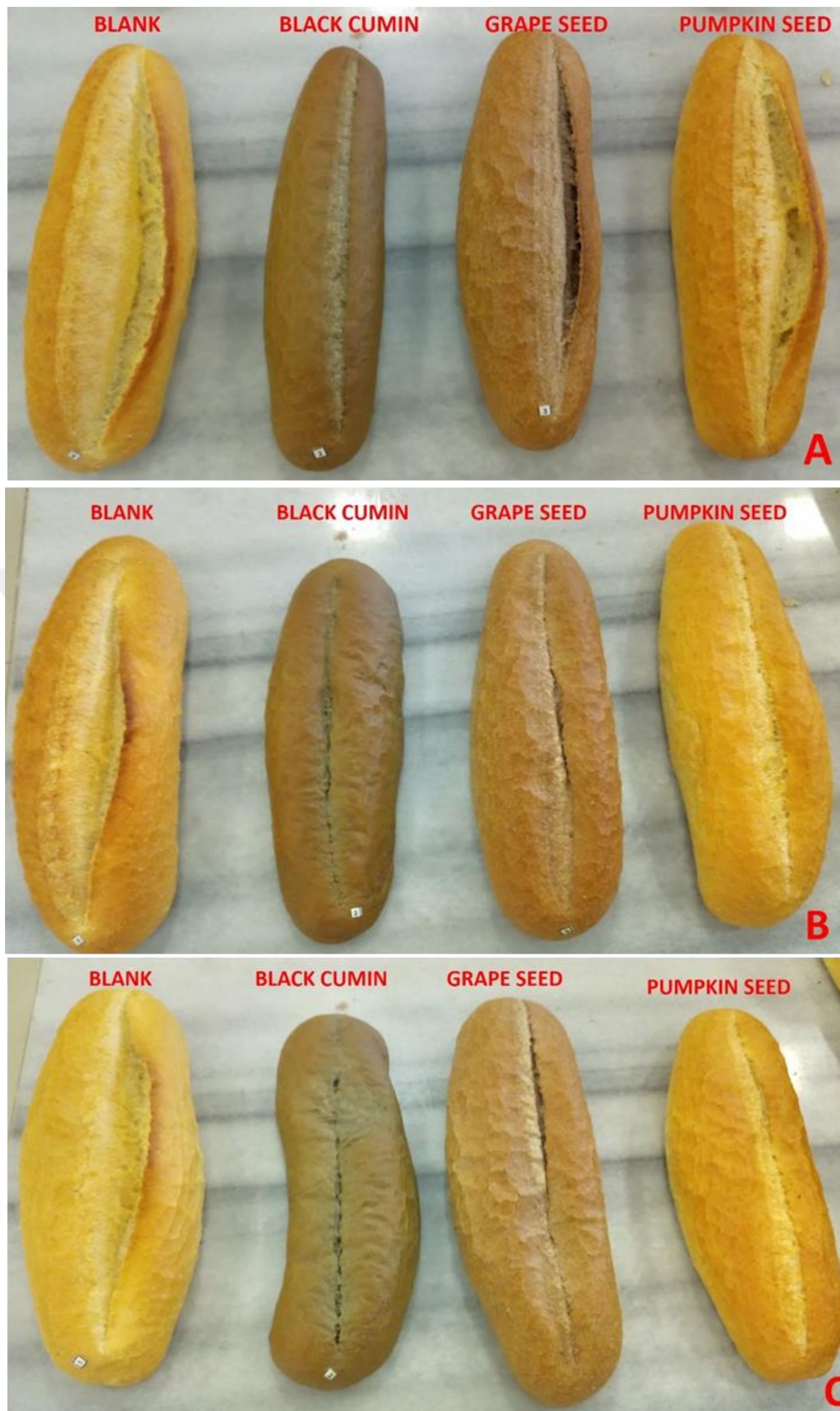
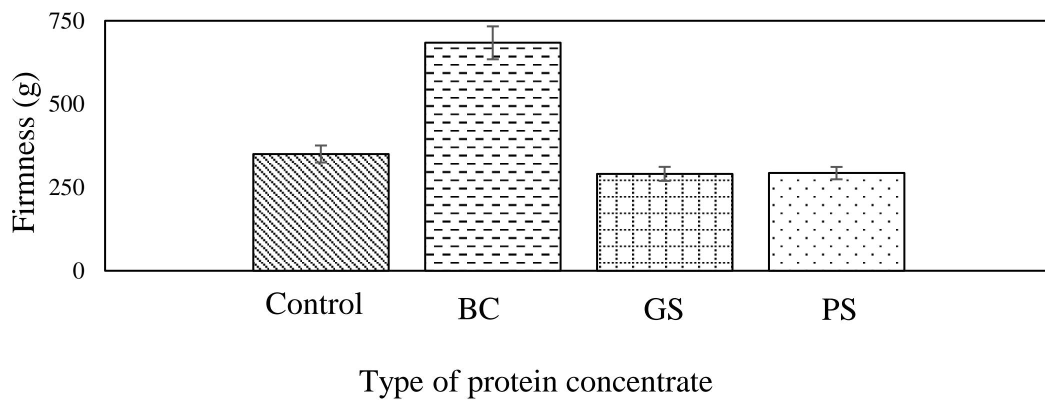


Figure 4.19. Images of control and enriched breads with different fermentation times after baking. Duration of fermentation; A: 80 min, B: 110 min, C: 140 min.

4.5.5 Texture profile analysis

Texture profile analysis of black cumin, grape seed and pumpkin seed protein concentrate enriched bread, were shown in Figure 4.20. Firmness (N) of the bread crumb is considered as the maximum resistance to the penetration of the probe and calculated as the height of the first force peak (Graça et al., 2017). Springiness is the ability to regain original shape after pressing down the crumb with the middle finger. Springiness is associated with a fresh and elastic product; therefore, high quality bread will be related to high springiness values (Matos and Rosell, 2012). Low springiness value is indicative of brittleness and this reflects the tendency of the bread to crumble when is sliced (McCarthy et al. 2005). Firmness and springiness value of control, black cumin, grape seed and pumpkin seed protein concentrate enriched breads were 350.2, 683.8, 290.7, 293.04 g and 66.4, 60.5, 66.02 and 65.2 g, respectively ($p<0.05$). Sabanis and Tzia (2009) found that corn, rice and soy flour increased the firmness of wheat flour bread and as the substitution with rice, corn, or soy flour increased, the crust and crumb texture became harder. They thought that this might be due to the lower gluten content of those samples. Gluten plays an important role on bread staling by forming an extensible protein network that keeps the crumb structure soft by slowing the movement of water from crumb to crust (Roach and Hoseney 1995). Martin and Hoseney (1991) also reported that interactions exist between swollen starch granules and the gluten network, through hydrogen bonding, preventing the staling of bread. Mohammed et al. (2012) hypothesized that the chickpea flour suppressed the amount of steam generated, as a result of their high water absorption capacity, leading thus to reduced loaf volume and greater crumb firmness. Springiness of enriched breads were comparable with control. Serventi et al. (2017) enriched soy-wheat bread with chickpea protein concentrates and they reported springiness values were not different among the samples.



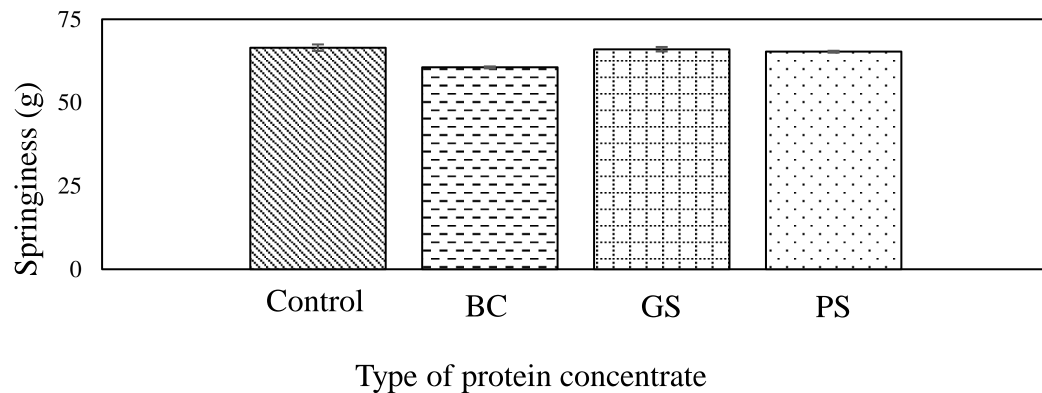


Figure 4.20. Textural parameters of protein-fortified breads. BC; black cumin protein concentrte enriched bread, GS: grape seed protein concentrate enriched bread, PS: pumpkin seed protein concentrate enriched bread. The data are the mean of the two measurements, and the error bars represent the standard deviation ($p<0.05$).

4.5.6 Color parameters

Crumb color analysis of protein concentrates enriched breads were shown in Table 4.13. L* value of control, black cumin, grape seed and pumpkin seed protein concentrate enriched breads were 55.6, 3.01, 32.01 and 55.75, respectively ($p<0.05$). This confirmed that, black cumin and grape seed enriched breads, had darker color than control (Figure 4.19). Pumpkin seed protein concentrate enriched bread had similar color with control sample (Table 4.13). Color evaluation of legume-fortified wheat products (noodles) studied by Hung et al. (1991) showed a decrease in brightness with increased levels of legume flour. Al-Hootietal.(2002) reported that the use of wheat germ at the level of 10 or 20% significantly affected the whiteness in bread samples. Control, black cumin, grape seed and pumpkin seed enriched breads had 2.6, 2.09, 8.03 and 2.01 a* value ($p<0.05$). Grape seed protein concentrate was the most red sample depends on color analysis (Table 4.13). Our results were in agreement with those of Charoenthaikij et al. (2010) for germinated brown rice.

Results show that supplementation of bread wheat flour with black cumin, grape seed and pumpkin seed protein concentrates at protein level of 1.5% produced dough with satisfactory rheological properties. However, enriched bread, beside pumpkin seed protein concentrate enriched bread, had lower volume, higher firmness and darker color. High water absorption capacity of these protein concentrates were probably responsible for these changes.

Table 4.13. Color parameters for crumb. BCPC; black cumin protein concentrte enriched bread, GSPC: grape seed protein concentrate enriched bread, PSPC: pumpkin seed protein concentrate enriched bread. The data are the mean of the two measurements with the standard deviation ($p<0.05$).

Samples	L*	a*	b*
Blank	55.6±0.9	2.6±0.5	24.09±0.10
BCPC	3.01±0.99	2.09±0.07	8.95±0.02
GSPC	32.01±0.93	8.03±0.4	13.65±0.5
PSPC	55.75±0.23	2.01±0.45	23.45±2.03

4.6 Effect of black cumin, grape seed and pumpkin seed protein concentrates on the gluten-free bread characteristics

4.6.1 Gluten-free bread making

In current studies, black cumin, grape and pumpkin seed protein concentrates had good water and oil holding capacity as well as emulsification activity, enrichment of gluten-free bread with these proteins resulted in improved water absorption and texture properties. Structure forming ability of individual protein types is related to their swelling ability and emulsification properties (Ziobro et al. 2013). In the previous studies it was also reported that lupine protein added to wheat flour at levels below 10% improve water absorption, texture and hardness of the final bread (Kohajdová et al., 2011). According to Kohajdová et al. (2011) lupine protein was highly soluble and had good water and lipid binding ability, as well as emulsification and stabilization activity.

It is generally accepted that the breadmaking quality of wheat is related to the presence of gluten proteins. The gliadin fraction has been reported to contribute to the viscous properties and dough extensibility of wheat dough (Pomeranz, 1988; Don et al., 2003a, 2003b). The glutenin fraction of wheat gluten has long been considered to have a prominent role in the elastic and strengthening of dough (MacRitchie, 1980; Xu et al., 2007). The relative proportions of gliadin and glutenin found in dough affect the physical properties of dough, with higher relative proportion of glutenin imparting greater dough strength (MacRitchie, 1987). Gluten-free breads have unique structural characteristics that are responsible for their gritty mouthfeel. Consequently, in the absence of gluten, and with a low hydration rate resulted in gritty/powdery mouth-feel. Due to this fact, there have been major efforts to improve the texture of these products by obtaining wheat bread-like structures (Gallagher et al., 2004). Storck et al. (2013) studied protein-enriched, rice-based, gluten-free product in the presence of TGase to improve crumb textural properties and they found better specific volume and crumb texture with the combination of TGase (1,35 U/g of protein), albumin (0.67 g/100 g) and casein (0.67 g/100 g).

In this study, gluten-free breads were baked with varying water levels. The water level was increased by 8% or 15% (i.e., by 24 ml or 45 ml). Enriched breads were shown in (Figure 4.21). Visually, black cumin protein enriched samples had the

lowest volume among all the original water content gluten-free breads.This finding could possibly be attributed to the water holding property of the proteins (Aprodu et al., 2016; Renzetti et al. 2008). Grape seed protein concentrate enriched gluten-free bread had higher volume than the black cumin counterpart but still had less volume than the control bread. It was observed that the volume of pumpkin seed protein concentrate enriched gluten-free bread had slightly increased volume compared to the control. Black cumin enriched bread had higher volume compared to original water content but still they had less volume than the control sample when the water level increased by %8 (i.e. 24 ml). Finally, grape and pumpkin seed protein concentrate enriched gluten-free breads had higher volume than control sample. In all cases, the volume of gluten-free breads that were enriched with black cumin, grape seed and pumpkin seed protein concentrate significantly improved when water level increased by 15%. Ribotta et al. (2004) reported that production of gluten-free flour using soybean flour resulted in a low bread volume when it was baked 200 °C. Black cumin and grape seed protein enriched gluten-free bread had larger pores than the pumpkin seed protein concentrate enriched and control gluten-free breads (Figure 4.21). Alvarez-Jubete et al. (2010) produced gluten-free bread with amaranth, quinoa and buckwheat and they reported quinoa, amaranth and buckwheat bread had bigger cell volume than control gluten-free bread. By considering the change in crumb cell volume fraction relative to the number of cells observed at the cut crumb surface, ingredients such as emulsifiers (i.e. proteins) tend to lower the number of missing cell walls created during processing (Scanlon and Zghal, 2001) and hence affect the physical texture in the resulting crumb.

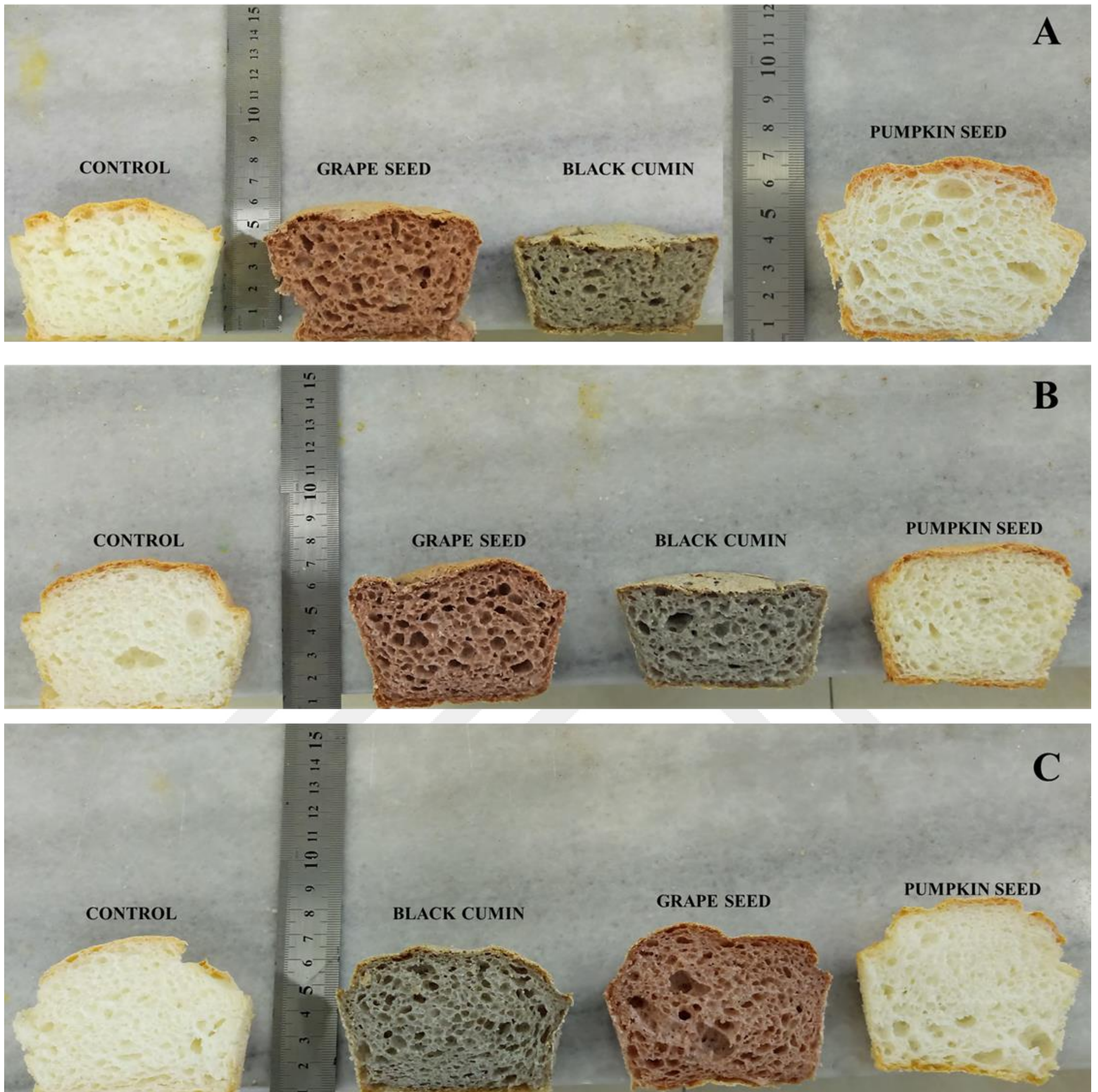


Figure 4.21. Baked gluten-free breads enriched with black cumin, grape seed and pumpkin seed protein concentrate with different water levels (A: Same water level; B: water level increased by 8%; C: water level increased by 15%).

Since the cells were large and not homogeneously distributed, these properties were improved by Maillard conjugation and TGase treated samples. This situation could be explained by their ability to bind CO_2 (Ziobro et al. 2013). The leavening agent generates gas (CO_2) within the liquid phase, which diffuses in solution to the nuclei due to a concentration gradient. As a result, the nuclei expand into gas cells and the density of the dough is reduced (Scanlon and Zghal, 2001). Poor foaming capacity tend to poor gas network formation (Moore et al., 2006). Ziobro et al. (2013) reported that high specific volume of the bread with albumin could be explained by their foaming capacity. Gluten-free breads produced with protein concentrates yielded improved properties (e.g., foam capacity) were shown in Figure 4.22. Accordingly, in gluten-free bread that were enriched with Maillard conjugates of black cumin had lower volume than the others but pores were more homogeneous and smaller (Figure 4.22A). In the case of TGase treated black cumin, grape seed and pumpkin seed protein concentrates enriched gluten-free breads, pumpkin seed protein concentrate enriched gluten-free bread was similar with the control sample and black cumin and grape seed protein concentrate enriched gluten-free bread had lower volume compared to control sample. The decreased in the volume produced by TGase might be due to the increase in the molecular weight and the loss of the flexibility protein chains produced by the crosslinking activity (Marco et al., 2007; 2008). However, black cumin enriched gluten-free bread pores were smaller and more homogen compared the control sample (Figure 4.22B). Similarly, a more compact and homogeneous protein network due to the protein crosslinking was reported by Bonet et al. (2006) in wheat doughs with soy flour when they were treated with TGase.

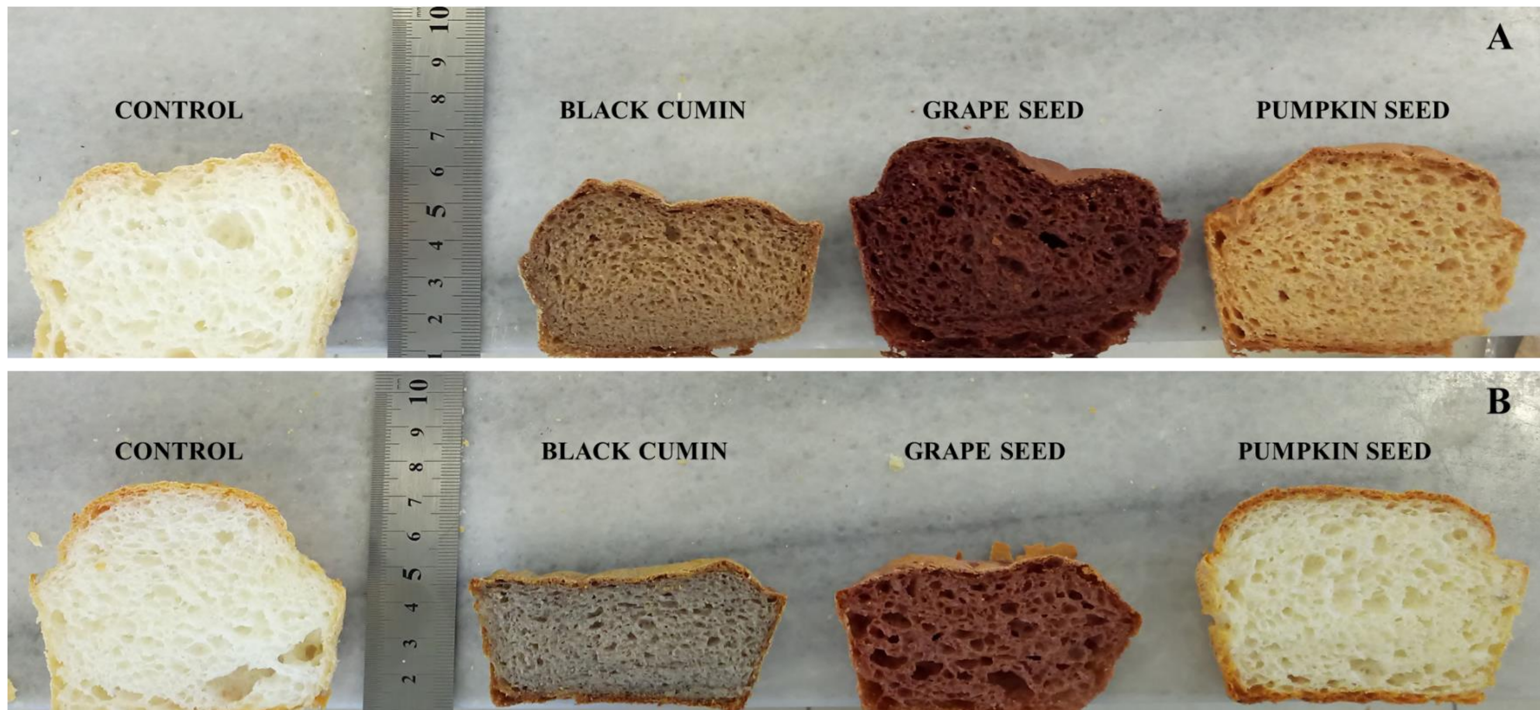


Figure 4.22. Baked gluten-free breads enriched with modified protein concentrate of black cumin, grape seed and pumpkin seed (A: Maillard conjugation treatment, B: TGase treatment).

4.6.2 Loaf volume analysis

The loaf volume of gluten-free bread enriched with protein concentrates of black cumin, grape seed and pumpkin seed was less than control sample at the equal water level. As the water level increased, the volume of the enriched gluten-free breads increased in all cases. (Table 4.14). For example, loaf volume of black cumin, grape seed and pumpkin seed protein enriched gluten-free breads were 380, 465 and 850 ml, respectively whereas loaf volume of control sample was 920 ml for the equal water level. However, loaf volume of black cumin, grape seed and pumpkin seed protein concentrate enriched gluten-free breads were 800, 1000 and 950 ml respectively when the water level increased by 15% ($p<0.05$). The specific volumes of gluten-free breads increased with the, primarily due to the water retention capacity of proteins (Sanchez et al. 2004). Ziobro et al. (2013) reported that the addition of pea protein had no significant influence on gluten-free bread volume The addition of soy protein decreased the volume while lupine increased the volume of gluten-free bread The key factor seems to be the presence of protein used for supplementation, which could swell and denature at high temperatures providing structural support to starch and hydrocolloids (Ziobro et al., 2013).

Maillard conjugates of black cumin, grape seed and pumpkin seed protein glucose conjugates enriched gluten-free breads's loaf volumes were 710, 880 and 905 ml, respectively. Loaf volume of the control sample was 1010 ml under similar conditions. TGase treated black cumin, grape seed and pumpkin seed protein concentrates enriched gluten-free breads had 660, 860 and 905 loaf volume, respectively. Again, loaf volume of the control sample was 940 ml in this conditions ($p<0.05$). Mohammadi et al. (2015) reported that TGase addition decreased significantly the specific volume of bread compared to the control, TGase activity leads to the formation of cross-link binding between glutamine and lysine which in turn inhibits the expansion of gas cells during the fermentation process (Mohammadi et al., 2015). Similar results were observed by Basman et al. (2002) and Renzetti et al. (2008) for breads made by brown rice and black wheat flour fortified with TGase. Our results were coherent with these researchers since addition of TGase treated black cumin, grape seed and pumpkin seed proteins decreased the bread volume. Similarly the addition of Maillard conjugates of black cumin, grape seed and pumpkin seed protein concentrates decreased the bread volume.

Table 4.14. Loaf volume analysis results for gluten-free breads (BC: black cumin; GS: grape seed; PS: pumpkin seed). The data represent the average of two independent experiments and ± standart deviation ($p<0.05$).

Sample	Equal water level	Water level increased by 8%	Water level increased by 15%	Maillard conjugation treatment	TGase treatment
Control	920±20.8	790±28.5	715±11.3	1010±22.4	940±25.0
BC	380±30.5	565±25.1	800±15.1	710±33.7	660±31.2
GS	465±16.2	850±34.0	1000±20.0	880±18.6	860±20.
PS	850±20.0	860±22.7	950±15.2	905±29.1	905±13.2

4.6.3 Texture parameters

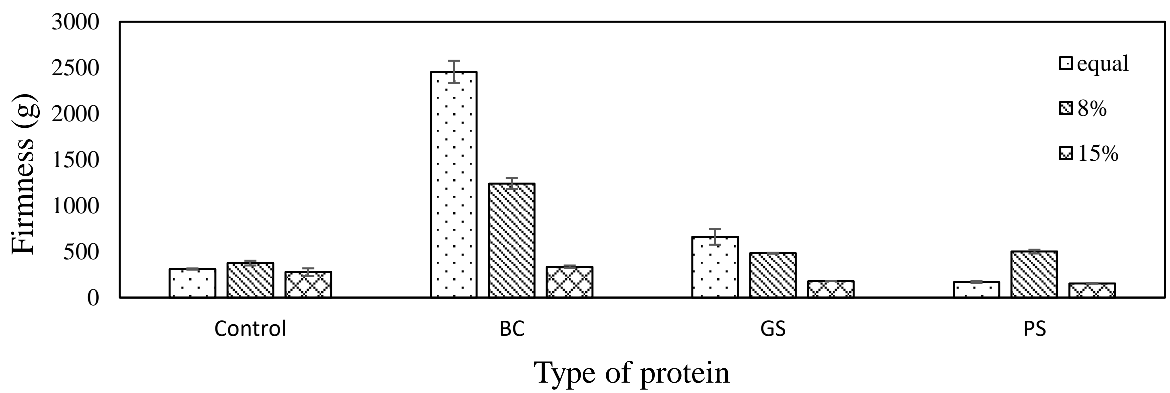
Firmness and springiness are important sensory attributes of gluten-free breads. Bread firmness is caused mainly by the formation of cross-links between partially solubilized starch and gluten proteins (He and Hoseney, 1990). Springiness is the ability to regain original shape after pressing down the crumb with the middle finger. Springiness is associated with a fresh and elastic product; therefore, high quality bread will be related to high springiness values (Matos and Rosell, 2012). Low springiness value is indicative of brittleness and this reflects the tendency of the bread to crumble when is sliced (McCarthy et al. 2005). Also the increased springiness in breads can be considered beneficial, as gluten-free breads are often characterised by a crumbly, brittle texture (Alvarez-Jubete, 2010).

Texture parameter such as firmness (g) and springiness (g) values were determined for the texture analysis of control, black cumin, grape and pumpkin seed protein enriched gluten-free bread samples (Figure 4.23). In this context, firmness and springiness values of control, black cumin, grape and pumpkin seed enriched gluten-free breads were 310.5, 2455, 659.7, 168.08 g and 65.5, 49.9, 55, 70.8 g for original water level respectively. In the case of water level increased by 8%, firmness and springiness value of control sample and black cumin, grape seed and pumpkin seed enriched gluten-free breads were 374, 1239.4, 482.7, 500 g and 61.5, 57.7, 56, 54.4 respectively. Finally, firmness and springiness value of control, black cumin, grape and pumpkin seed enriched gluten-free breads were 277.2, 335.9, 178.4, 154.3 g and 73.6, 72.4, 75.6, 75.8 when water level increased by 15% respectively ($p<0.05$).

Phongthai et al (2016) reported that addition of rice bran and egg protein albumin to the gluten-free breads increased firmness value and also, as the amount of protein increased, firmness value increased (Kittisuban et al. 2014). As the water level increased, firmness values decreased (Figure 4.23). Increase of crumb firmness upon enrichment of proteins in gluten-free breads might be attributed to the thickening of the gas cell walls within the bread crumb (Rodriguez Furlan et al. 2015). Ponte et al. (1962), who studied crumb firmness and firming rates of bread, found a strong relationship between compression force and specific volume of the bread crumb specimen as it varied across a loaf. Similar studies were made by Phimolsiripol et al. (2012), Schoenlechner et al. (2010) and Crockett et al. (2011) and all investigators were reported that addition of protein increased firmness values of gluten-free breads.

Also, Marco and Rossell (2008) enriched gluten-free bread with soybean protein concentrate and they found higher firmness value than control. Ziobro et al. (2013) analyzed soy enriched gluten-free bread and they reported springiness of the soy protein enriched gluten-free bread was smaller than the control sample. According to our results, in all cases the springiness values were lower than the control except for the gluten-free bread enriched with pumpkin seed protein concentrates. However, when the water level increased by 15%, the springiness values were once again found to be higher than control sample (Figure 4.23).

Firmness (g) and springiness (g) value of Maillard conjugation and TGase treated black cumin, grape seed and pumpkin seed enriched gluten-free breads were shown in Figure 4.24. In this context, firmness value of Maillard conjugate control, black cumin, grape seed and pumpkin seed enriched gluten-free breads were 152.6, 302.1, 345.9 and 282.8 g respectively and springiness value of control, black cumin, grape seed and pumpkin seed enriched gluten-free breads were 72.2, 68.2, 71.3 and 71 g respectively. Firmness and springiness value of TGase treated control, black cumin, grape seed and pumpkin seed enriched gluten-free breads were 159.2, 519.9, 707.1, 178.6 g and 70, 63, 71.3, 71.14 respectively ($p<0.05$). Basman et al. (2002) reported that 1.0 and 1.5% TGase addition levels, firmness values increased, probably due to the formation of overstrong dough after excessive crosslinking. Again, Marco and Rossell (2008) enriched gluten-free bread with soybean protein concentrate and TGase and they found higher firmness value than control sample. According to the our results, firmness results were increased especially in the case of black cumin and grape seed protein enriched gluten-free bread.



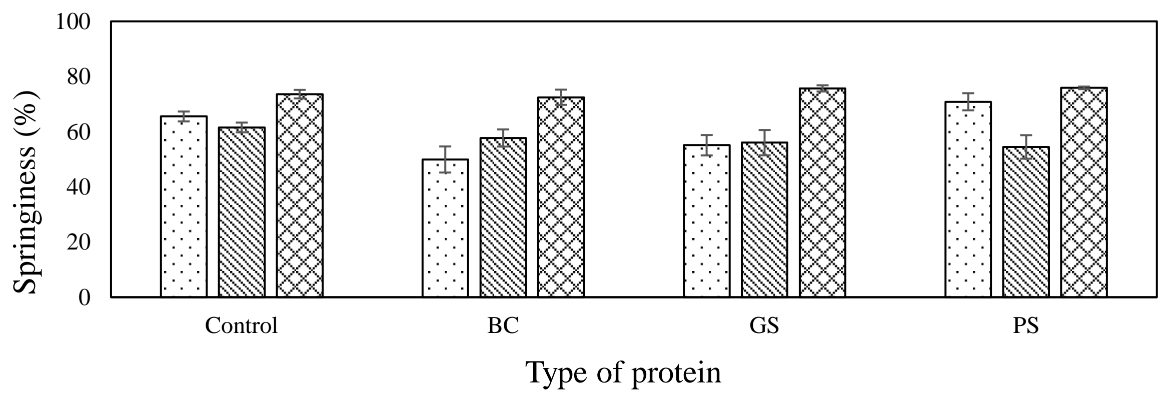


Figure 4.23. Texture parameters of control gluten-free bread and gluten-free breads enriched with proteins concentrates. BC: black cumin; GS: grape seed; PS: pumpkin seed protein concentrates. Presented data are mean values of two replications. Error bars mean standart deviations.

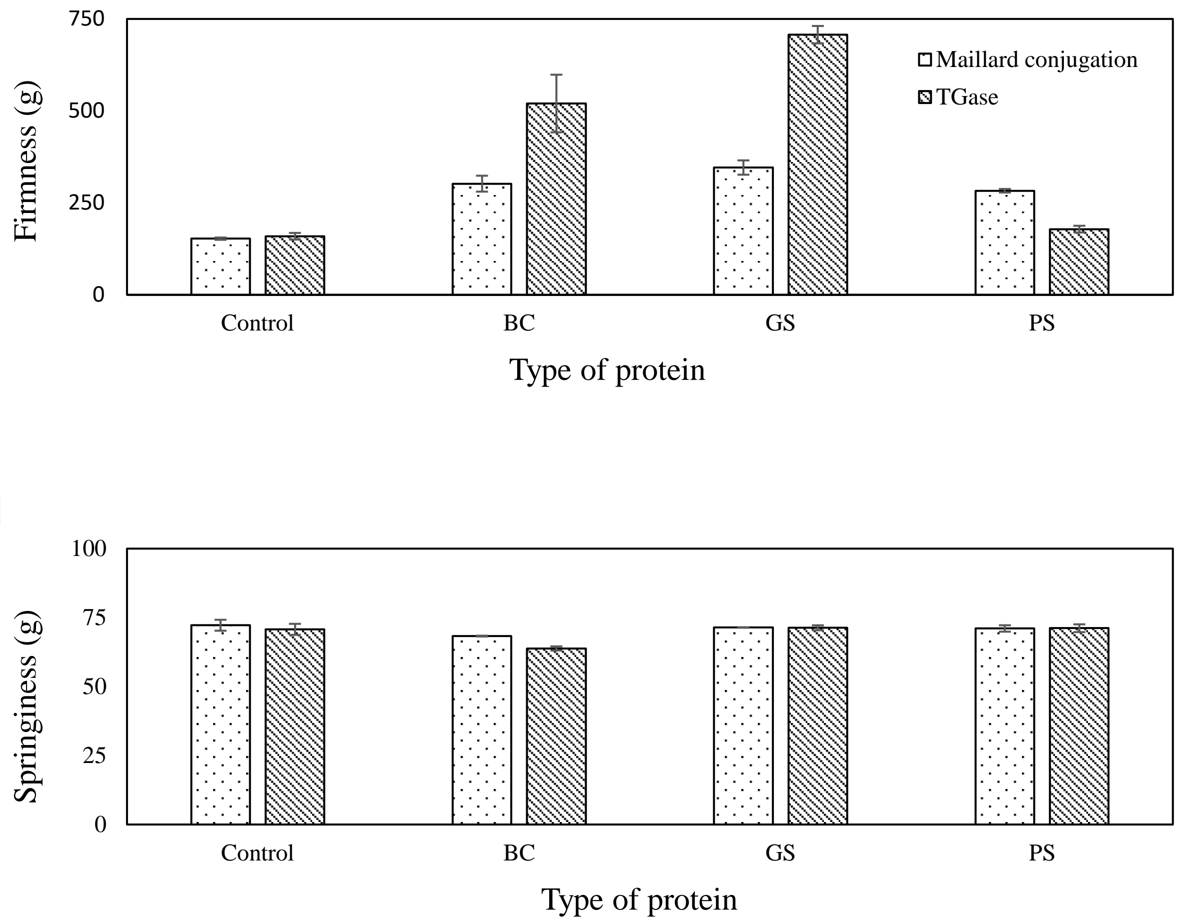


Figure 4.24. Texture parameters of control gluten-free bread and gluten-free breads enriched with modified protein concentrates. BC: black cumin; GS: grape seed; PS: pumpkin seed protein dispersion added. The data are the mean of the two measurements, and the error bars represent the standard deviation.

4.6.4 Color parameters

Protein concentrates enriched gluten-free breads color parameters were shown in Table 4.15. Based on the L* value of black cumin protein concentrate enriched gluten-free bread was the evident that the color of the sample was significantly darker (2.68). Grape seed protein concentrate enriched gluten-free bread (30.6) was less darker than black cumin protein concentrate enriched gluten-free bread but still darker than the control sample (54.59). a* value of grape seed protein concentrate enriched gluten-free bread (7.68) was more red than the other samples. a* value of control sample was 2.43. Black cumin and pumpkin seed protein concentrate enriched gluten-free breads had 2.05 and 1.71 a* value, respectively.

The color analysis results of gluten-free breads enriched with protein concentrates with improved properties were shown in Table 4.15. Maillard conjugates of black cumin (L* value, 39.8) and grape seed (L* value, 24.9) protein concentrates enriched gluten-free breads were darker than the control sample (L* value, 76.84). Pumpkin seed protein concentrate enriched gluten-free bread was also darker than the control sample (53.5), but it was stated that it was less darker than the other samples. L* value of TGase treated control, black cumin, grape seed and pumpkin seed protein concentrates were 78.5, 46.9, 35.5 and 76.6 respectively ($p<0.05$). As a result, black cumin and grape seed enriched gluten-free breads were darker than control bread and pumpkin seed protein concentrate enriched gluten-free bread was similar with control bread.

Ziobro et al. (2013) stated that addition of pea protein, collagen and lupine protein significantly lower the L* value which means that they were characterized by darker color. However, addition of soy protein did not significantly affect this value. Aguilar et al. (2015) analyzed chicpea and tigernut enriched gluten-free flour and they were reported that addition of these flour to the gluten-free breads resulted in darker crust due to the amino acids and sugars provided by chickpea and tiger nut flours respectively, which contributed to Maillard reaction. Phongthai et al. (2016) reported that addition of rice bran protein concentrate to the gluten-free bread decreased the L* value. Furthermore, enrichment with 2% increased a* value. It was stated that both protein and reducing sugars affect color due to the Maillard reaction during baking (Phongthai et al. 2016).

Supplementation of gluten-free bread with black cumin, grape seed and pumpkin seed protein concentrate resulted lower loaf volume of breads. However as the water level increased up to 15% loaf volume also increased. Enrichment also affected color values. Gluten-free breads had darker color beside pumpkin seed protein concentrate enriched gluten-free bread. The results of firmness and springiness showed that enriched bread had higher firmness and lower springiness value. However, as the water level increased up to 15% firmness value decreased and springiness value increased. Supplementation of protein requires individual optimization of blends because of the significant differences in the water binding capacity.

Table 4.15. Color parameters of gluten-free bread crumb (BC: black cumin protein concentrates fortified gluten-free bread; GS: grape seed protein concentrates fortified gluten-free bread; PS: pumpkin seed protein concentrates fortified gluten-free bread). The data represent the average of two independent experiments with ± standart deviation ($p<0.05$).

	Non-modified protein concentrates			Maillard conjugation treated protein concentrates			TGase treated protein concentrates		
Sample	L*	a*	b*	L*	a*	b*	L*	a*	b*
Control	54.59±0.8	2.43±0.1	23.34±0.1	76.84±0.4	-1.47±0.0	10±0.0	78.59±0.6	-1.43±0.1	9.89±0.1
BC	2.68±0.9	2.05±0.0	8.73±0.0	39.89±0.2	7.68±0.6	22.55±0.7	46.9±0.1	3.56±0.6	11.69±0.4
GS	30.60±1.2	7.68±0.3	12.03±0.4	24.90±0.1	18.85±0.2	17.26±0.0	35.5±0.7	18.77±0.7	16.89±0.9
PS	53.93±0.5	1.71±0.2	22.62±1.9	53.57±0.0	11.08±0.7	32.10±0.7	76.6±0.5	-1.10±0.1	15.65±0.9

5. CONCLUSION

In this study, black cumin, pumpkin seed, grape seed and pomegranate seed protein concentrates were utilized from cold pres meals of these samples. Multiple aqueous protein isolation methodologies with or without the application of an organic extraction step. The physicochemical and functional properties of the protein concentrates were investigated, since technical functionality studies on these protein systems are relatively scarce in this field. The simple methodologies utilized here are applicable to industrial settings and appropriate for the utilization of industrial by-product streams in order to reduce costs in the cold press processing valuable oils. In most cases, weak foam forming capacities were observed. Consequently, Mailard conjugation and TGase treatment were also studied to enhance foaming capacity. A special focus should be given to the fortification of food products that require improved functionality on water absorption and foaming capacity such as confectionary, bakery, meats, whipped topping, angel cake, ice cream, protein shakes. The oil-holding capacity of the protein concentrates were higher than soy protein isolate. This feature can provide advantages such as improvement of mouthfeel and aroma in bakery products such as cakes and biscuits. In addition, they reduce the loss of water and fat when used in meat products, thus extending the shelf life of the product and improving its flavor.

Celiac disease is a disorder associated with impaired intestinal digestive function occurring under the influence of gluten proteins found in food. Gluten must be eliminated from the diet of celiac sufferers, because its ingestion causes serious intestinal damage. Protein supplements are often used not only to increase the nutritional value but also to improve sensory characteristics and sensory acceptance of gluten-free bread. Therefore, in this study, gluten-free and wheat bread manufactured. The influence of protein enrichment on the textural properties of the gluten-free and wheat bread was investigated as well as the final bread quality.

The global protein demand is constantly increasing which in turn requires a sustainable supply. Cold press deoiled meals represent a viable and economic source of plant protein manufacture. Here, it was shown that based on simple methodologies, a variety of concentrates from deoiled black cumin, pumpkin seed, grape seed and

pomegranate meals could be generated. Hexane extraction clearly enhanced the functional characteristics in most cases, either due to the enhancement of protein-solvent interactions in the absence of oil or the partial denaturation of the proteins. Although the water holding capacities of these proteins were relatively weak, oil holding and emulsion formation characteristics were significant which could be instrumental especially in the production of foods and other industrial products.

Since plant proteins are natural ingredients and their Maillard conjugates can be manufactured in the absence of toxic chemicals or organic solvents. Formation of protein-carbohydrate conjugates enhanced foaming activity, whereas the performance was highly dependent on molecular size of carbohydrates as well as processing conditions such as pH, temperature and duration. Commercial TGase treatment also enriched the foaming capacity. After the development of the foaming performance of protein concentrates, untreated and functionality improved protein samples were used to manufacture gluten-free bread. Supplementation of protein required individual optimization of blends because of the significant differences in the water binding capacity. However as the water level increased up to 15% loaf volume and texture of gluten-free bread also increased.

It is highly desirable due to nutritional reasons to enrich wheat flour with other nongluten, high protein concentrates to produce an alternative product satisfying consumer demand. Therefore, we enriched wheat bread with black cumin, pumpkin seed and grape seed protein concentrates. Results show that supplementation of bread wheat flour with black cumin, grape seed and pumpkin seed protein concentrates at 1.5% levels produced dough with satisfactory rheological properties. Future bread analysis might elucidate its microstructure and shelf life as affected by the black cumin, grape seed and pumpkin seed protein concentrates.

All the results obtained from this study show that black cumin, pumpkin seed, grape seed and pomegranate seed are valuable residues that need for the valoriztion to be evaluated. This study should be supported by prospective investigation including protein characterization (separation of proteinin fractions) and identification of nutritional properties (amino acid content, antibacterial factors, biology value, digestibility) and testing of use in various food products.

REFERENCES

Abdel-Aal, E.M., Shehata, A.A., El-Mahdy, A.R., & Youssef, M.M. (1986). Extractability and functional properties of some legume proteins concentrated by three different methods. *Journal of the Science of Food and Agriculture, 37*, 553–559.

Acevedo-Pacheco, L. and Sema-Saldivar, S.O., 2016. In vivo protein quality of selected cereal-based staple foods enriched with soybean proteins. *Food & Nutrition Research,* 60: 1-9.

Adebowale, K. O., & Lawal, O. S. (2003). Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrate. *Food Chemistry*, *83*, 237–246.

Adebowale, Y.A., Schwarzenbolz, U., & Henle, T. (2011). Protein concentrate from Bambara groundnut (*Voandzeia subterranean* L.): chemical characterization and functional properties. *International Journal of Food Properties, 14*, 758–775.

Aguilar N., Albanel E. Minarro B., Capellas M. 2015. Chickpea and tiger nut flours as alternatives to emulsifier and shortening in gluten-free bread. *LWT - Food Science and Technology* 62 (2015) 225-232.

Aiking H., de Boer J., Vereijken, J.M. (Eds). Sustainable protein production and consumption: Pigs or peas? Environment & Policy Vol. 45, ISBN 1-4020-4062-8, Springer, Dordrecht, The Netherlands, 2006.

Akintayo, E. T., Oshodi, A. A., & Esuoso, K. O. (1999). Effect of ionic strength and pH on the foaming and gelation of pigeon pea *(Cajanus cajan*) protein concentrates. *Food Chemistry*, *66*, 51–56.

Al-Hooti S.N., Sidhu J.S., Al-saqer J.M. and Al-Othman A. 2002. Effect of raw wheat germ addition on the physical texture and objective color of a designer food. (pan bread). *Nahrung/Food* 46, 68–72.

Alvarez-Jubete L., Auty M., Arendt E.K., Gallagher E. (2010). Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations. *Eur Food Res Technol* (2010) 230:437–445.

Aluko, R. E., & Yada, R. Y. (1995). Some functional properties of a cowpea (Vigna unguiculata) globulin concentrate treated with transglutaminase. *Bioscience Biotechnology Biochemistry*, 59, 2298–2299.

American Association of Cereal Chemists International (AACCI), 2000. Approved methods of the AACCI (10th Ed.). Methods 54.21 and 54.10. AACCI, St. Paul, MN, USA.

Andersson, M., 2015. Protein enriched foods and healthy ageing: Effects of almond flour, soy flour and whey protein fortification on muffin characteristics, SP Technical Research Institute of Sweden, SP Rapport:02.

Anon. (2014). Proposal for new work on Codex Standart for Brown/Black Cumin (Whole and Ground), (prepared by India). Joint FAO/WHO Food Standards Programme.

Anuradha S.N., Prakash V., 2009. Altering functional attributes of proteins through cross linking by transglutaminase – A case study with whey and seed proteins. *Food Research International* 42 (2009) 1259–1265.

AOAC (2003). Official methods of analysis of AOAC International (17th ed.). Gaithersburg, MD, USA: Association of Official Analytical Chemists Inc (Revision 2).

Aprodu I., BAdiu E.A., Banu I. 2016. Influence of Protein and Water Addition on Gluten-Free Dough Properties and Bread Quality. *Int. J. Food Eng*. 2016; 12(4): 355–363.

Ash, M., & Dohlman, E. (2006). Oil crops situation and outlook yearbook. Electronic outlook report from the Economic Research Service, USDA.

Aydoğan S., Şahin M., Akçacık A.G., Hamzaoğlu S., Taner S., 2015. Relationships between Farinograph Parameters and Bread Volume, Physicochemical Traits in Bread Wheat Flours. *Journal of Bahri Dagdas Crop Research*, 3 (1):14-18, 2015.

Ayoub M, Guertin S, Fregeau RL, Smith DL (1994). Nitrogen fertilizer effect on breadmaking quality of hard red spring wheat in eastern Canada. *Crop Sci* 34:1346–1352.

Babayan, V.K., Kootungal, D., Halaby, G.A. (1978). Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* L. seeds. *Journal of Food Science*, 43(4): 1314-15.

Barnes, W.C., Blakeney, A.B., 1974. Determination of cereal a-amylase using a commercially available dye-labelled substrate. Starke 26, 193–197.

Basman A., Köksel H., Ng P.K.W. (2002). Effects of increasing levels of transglutaminase on the rheological properties and bread quality characteristics of two wheat flours. *Eur. Food Res. Technol*, 215 (2002) 419–424.

Baydar, H. (2009). Science and technology of medicinal and aromatic plants (Expanded 3rd Ed.). Süleyman Demirel University, Faculty of Agriculture, Isparta, Turkey, Publ., No. 51: 227-228.

Baytop, T. (1999). Treatment with plants in Turkey. Past and present (Additional Second Edition). Nobel Tıp Kitapevleri, İstanbul, Turkey.

Belitz HD, Grosch W, Schieberle P (2004). Cereals and cereal products. 3rd edn. Springer, Heidelberg.

Benjamins J., Cagna A., Lucassen-Reynders E. H. 1996. Viscoelastic properties of triacylglycerol/water interfaces covered by proteins. Colloid Surf A. 114, 245-254, (1996).

Beuschel, B.C., Culbertson, J.D., Partridge, J.A., & Smith, D.M. (1992). Gelation and emulsification properties of partially insolubilized whey protein concentrates. *Journal of Food Science,* 57, 604- 609, 634.

Bildstein, M., Lohmann, M., Hennings, C., Krause, A., Hilz, H., 2008. An enzyme-based extraction process for the purification and enrichment of vegeTable proteins to be applied in bakery products, *European Food Research and Technology*, 228: 177-186.

Bonet A., Blaszczak W., Rosell C.M. (2006). Formation of homopolymer s and heteropolymers between wheat flour and several protein sources by transglutaminase-catalyzed cross-linking. *Cereal Chem* 83(6):655–662.

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

Bucko S.D., Katona J.M., Popoviç L.M., Vastag Z.G., Petroviç L.B. 2015. Functional properties of pumpkin (*Cucurbita pepo*) seed protein concentrate and hydrolysate. *J. Serb. Chem. Soc. 81* (1) 35–46 (2016) *JSCS–4825.*

Bucko S., Katona J., Popovic L., Vastag Z., Petrovic L., Vucinic-Vasic M., 2015. Investigation on solubility, interfacial and emulsifying properties of pumpkin (Cucurbita pepo) seed protein concentrate, *LWT - Food Science and Technology* 64 (2015) 609-615.

Caili F.U., Huan S.H., Quanhong L.I., (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods for Human Nutritions*, 61(2), 70–77.

Campbell G.M. 1999. Bubbles in food. Eagen Press, St. Paul, MN, USA (1999).

Castriotta, G., & Canella, M. (1978). Protein classification and nitrogen extractability of grape seed meal. *Journal of Agricultural and Food Chemistry, 26*(3), 763-765.

Chardigny, J.M., & Walrand, S. (2016). Plant protein for food: opportunities and bottlenecks. *Oilseeds & Fats Crops and Lipids,* 23(4), D404.

Charoenthaikij P., Jangchud K., Jangchud A., Prinyawiwatkul W., No, H.K., King, J.M. (2010). Physicochemical properties and consumer acceptance of wheat germinated brown rice bread during storage time. *Journal of Food Science*, 75 (6), S333–S339.

Colman P., Suzuki E., van Donkelaar A. (1980). The structure of cucurbitin: subunit symmetry and organization in situ. *European Journal of Biochemistry*, 103, 585-588.

Commission of the European Communities, 2002, The Agricultural situation in the European Union, 2000 Report, Brussels, 11.02.2002 COM (2002) 67 final.

Crockett, R., Ie, P., Vodovotz, Y., 2011. Effects of soy protein concentrate and egg white solids on the physicochemical properties of gluten-free bread. Food Chem. 129, 84-91.

Damodaran, S. (1990). Interfaces protein films and foams. *Advances in Food and Nutrition Research*, 34, 1–79.

Damodaran, S. (1994). Structure–function relationship of food proteins. In N. S. Hettiarachchy & G. R. Ziegler (Eds.), Protein functionality (pp. 1–37). New York: Marcel Dekker, Inc.f.

Damodaran, S. (1997). Food proteins: An overview. In S. Damodaran & A. Paraf (Eds.), Food proteins and their applications, New York: Marcel Dekker, pp 1-21.

Day L. (2013). Proteins from land plants e potential resources for human nutrition and food security. *Trends in Food Science & Technology*, 32 (2013) 25-42.

Delcour, J.A. and Hoseney, C., 2010. Principles of cereal science and technology, *AACC International*, 3rd edition, Minnesota, USA.

Delcour, J.A., Van Haesendonck, I.P., Cleemput, G., Rogers, D.E., Hoseney, R.C., 1998. Partial purification of a water-extracTable rye (*Secale cereale*) protein capable of improving the quality of wheat bread, *Cereal Chemistry Journal*, 75(4): 403-407.

Demirci, M., 2009. Beslenme. Yenilenmiş 4.baskı, Tekirdağ.

Deng J., Sun T., Cao W., Fan D., Chen N., Wang B., Gao H. and Yang H. (2013). Extraction, optimization and functional properties of proteins from kiwi fruit (Actinidia chinensis Planch.) seeds. *International Journal of Food Properties,* 17:1612–1625, 2014.

de Nigris F., Williams-Ignarro S Sica V., Lerman L.O., D'Armiento F.P., Byrns R.E., et al. (2007). Effects of a pomegranate fruit extract rich in punicalagin on oxidationsensitive genes and eNOS activity at sites of perturbed shear stress and atherogenesis. *Cardiovascular Research* 73(2): 414–423.

Dickinson E. 1995. Emulsion stabilization by polysaccharides and protein-polysaccharide complexes. In A. M. Stephen (Ed.), Food polysaccharides and their applications (pp. 501–515). New York: Marcel Dekker, (1995).

Dickinson E., 2012. Emulsion gels: the structuring of soft solids with protein-stabilized emulsions. *Food Hydrocoll*. 28, 224-241 (2012).

Dickinson E., Izgi E. 1996. Foam stabilization by protein-polysaccharide complexes. *Colloid Surf A* 113, 191-201, (1996).

Don, C., Lichtendonk, W. J., Pfijter, J. J., and Hamer, R. J. (2003a). Glutenin macropolymer: a gel formed by glutenin particles. J. Cereal Sci. 37, 1–7.

Don, C., Lichtendonk, W. J., Plijter, J. J., and Hamer, R. J. (2003b). Understanding the link between GMP and dough: from glutenin particles in flour towards developed dough. J. Cereal Sci. 38, 157–165.

Doxastakis, G., Zafiriadis, I., Irakli, M., Malani, H., & Tananaki, C. (2002). Lupin, soy and triticale addition to wheat flour dough and their effect on rheological properties. *Food Chemistry*, 77, 219–227.

Dua, S., Mahajan, A., & Mahajan, A. (1996). Improvement of functional properties of rapeseed (Brassica campestris Var. Toria) preparations by chemical modification. Journal of Agricultural and Food Chemistry, 44(3), 706–710.

Durani, F.R., Chand N., Zaka K., Sultan A., Khattak F.M., & Durrani Z. (2007). Effect of different levels of feed added black seed on the performance of broiler chicks. *Pakistan Journal of Biological Sciences, 10*(22):4164–67.

El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A., & Sobihah, T. Y.(2000). Effect of soaking on nutritional quality and protein solubility of some legume seeds. Nahrung, 44(5), 339–343.

El-Adawy, T.A. and Taha, K.M., 2001, Characteristics and composition of different seed oils and flours. *Food Chemistry*, 74: 47–54.

El-Soukkary, F.A.H., 2001. Evaluation of pumpkin seed products for bread fortification. *Plant Foods for Human Nutrition*, 56: 365–384.

Fang E.F., Wong J.H., Lin P., Ng T.B., (2010). Biochemical characterization of the RNA-hydrolytic activity of a pumpkin 2S albumin. *FEBS Letters*, 584, 4089-4096.

Fantozzi, P., & Betschart, A.A. (1979). Development of grapeseed protein. *Journal of the American Oil Chemists' Society*, *56*(3):457-459.

Fantozzi, P. (1981). Grape seed: a potential source of protein. *Journal of the American Oil Chemists' Society, 58*(12), 1027-31.

FAOSTAT: Agricultural production domain online. (2011). Rome, Italy: Food and Agricultural Organization of the United Nations. Available from: http://faostat.fao.org/.

Fazio, G., Gattuso, A., Cilluffo, V. & Arcoleo, G. (1983). Preparation and characterization of protein materials from grapeseed meals. *Rivista della Societa Italiana di Scienza dell'Alimentazione*, 6, 469–478.

Fleming, S.E., Sosulski, F.W., 1978. Microscopic evaluation of bread fortified whit concentrated plant proteins. *Cereal Chem.* 55 (3), 373-382.

Folk, J. E., & Finlayson, J. S. (1977). The ε-(ɤ-Glutamyl) lysine cross-link and the catalytic role of transglutaminase. Advances in Protein Chemistry, 31, 11–133.

Föste, M., Nordlohne, S.D., Elgeti, D., Linden, M.H., Heinz, V., Jekle, M., Becker, T., 2014. Impact of quinoa bran on gluten-free dough and bread characteristics, *European Food Research and Technology*, 239: 767-775.

Friedman M., 1996. Nutritional value of proteins from different food sources. A Review. *J. Agric. Food. Chem.* 44:6.

Fuhrmeister, H., & Meuser, F. (2003). Impact of processing on functional properties of protein products from wrinkled peas. *Journal of Food Engineering*, 56, 119–129.

Gallagher, E., Gormley, T. R., & Arendt, E. K. (2004). Recent advances in the formulation of gluten-free cereal-based products. *Trends in Food Science and Technology*, 15, 143-152.

Gambus, H., Gibinski, M., Pastuszka, D., Mickowska, B., Ziobro, R., Witkowicz, R., 2011. The application of residual oats flour in bread production in order to improve its quality and biological value of protein, *Acta Scientiarum Polonorum Technologia Alimentaria*, 10(3): 313-325.

Gazzola, D., Vincenzi S., Gastaldon L., Tolin S., Pasini G., Curioni A. (2014). The proteins of the grape (*Vitis vinifera* L.) seed endosperm: fractionation and identification of the major components. *Food Chemistry*, 155, 132–139.

Giami, S.Y. and Barber, L.I., 2004, Utilization of protein concentrates from ungerminated and germinated fluted pumpkin (*Telfairia occidentalis* Hook) seeds in cookie formulations. *Journal of the Science of Food and Agriculture*, 84: 1901–1907.

Gianazza, E., Tedesco, G., Villa, P., Scienza, A., Cargnello, G., & Righetti, P. (1989). Characterization of the major proteins from *Vitis vinifera* seeds. *Plant Science*, *62*, 73-81.

Gölükçü M., Tokgöz H., Kıralan M. 2008. Ülkemizde yetiştirilen önemli nar (*punica granatum*) çeşitlerine ait çekirdeklerin özellikleri, *GIDA* (2008) 33 (6) : 281-290.

Graça, C., Fradinho, P., Sousa, I., Raymundo, A., Impact of Chlorella vulgaris on the rheology of wheat flour dough and bread texture, *LWT - Food Science and Technology* (2017), doi: 10.1016/j.lwt.2017.11.024.

Graham, D. E., & Philips, M. C. (1976). The conformation of proteins at the air–water interface and their role in stabilizing foams. *In* R. J. Akers (Ed.). Foams, New York: NY, USA, Academic Press.

Graham, D.E., Phillips, M.C. (1979). Proteins at liquid interfaces: I. Kinetics of adsorption and surface denaturation. *Journal of Colloid & Interface Science,* 70: 403-414.

Gruener, L., & Ismond, M. A. H. (1997). Effects of acetylation and succinylation on the functional properties of the canola 12S globulin. Food Chemistry, 60(4), 513–520.

Gu F., Kim J. M., Abbas S., Zhang X., Xia S. and Chen Z. (2010). Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose. *Food Chem.* 120:505–511.

Guan Y. G., Lin H., Han Z., Wang J., Yu S.J., Zeng X.A., Liu Y.Y., Xu C. H., Sun W.W. 2010. Effects of pulsed electric field treatment on a bovine serum albumin–dextran model system, a means of promoting the Maillard reaction. *Food Chem.* 123(2):275–280, (2010).

Guillen, C. G. M., Ana, I. S., Teresa Solas, M., & Montero, P. (2001). Effect of microbial transglutaminase on the functional properties of megrim (Lepidodorhombus boscii) skin gelatin. *Journal of Science Food and Agriculture*, 81, 665–673.

Gujral, H.S., Rosell, C.M., 2004. Functionality of rice flour modified with a microbial transglutaminase. *Journal of Cereal Science* 39, 225–230.

Gutkoski L.C., Durigon A., Mazzutti S., Pinto V.Z., Colla L.M., Bertolin T.E., 2009. Use of the fungal falling number method in the determination of alpha-amylase activity in wheat flour. *Brazilian Journal of Food Technology* 2009 Vol.12 No.1/4 pp.295-302 ref.32.

Gülseren, İ., Güzey, D., Bruce, B. D., & Weiss, J. (2007). Structural and functional changes in ultrasonicated bovine serum albumin solutions. *Ultrasonics Sonochemistry*, *14*(2), 173-183.

Hallen, E., Ibanoglu, S., & Ainsworth, P. (2004). Effect of fermented cowpea flour addition on the rheological and baking properties of wheat flour. *Journal of Food Engineering*, 63, 177–184.

Halling, P. J. (1981). Protein stabilized foams and emulsions. *CRC Critical Reviews in Food Science and Nutrition*, 21, 155–203.

Hamid A.A., Laun Y.S. 2000. Functional properties of dietary fibre prepared from defatted rice bran. Food Chemistry 68 (2000) 15-19.

Haq A., Remo N., Al-Sedairy S.T. (1996). Fractionation of black seed (*Nigella sativa* Linn) proteins by using rotofor. *Journal of Liquid Chromatography & Related Technologies*, *19*(4), 593-599.

Harborne, J.B., & Van Sumere, C.F. (1975). The chemistry and biochemistry of plant proteins. Academic Press, New York, NY, USA.

Hasenhuettl G.L. 2008. Overview of food emulsifiers. In G. L. Hasenhuettl, & R. W. Hartel (Eds.), Food emulsifiers and their applications, 2nd ed. (pp. 1-9). New York: Springer (2008).

Haynes C.A., Norde W. 1995. Structures and stabilities of adsorbed proteins. *J. Colloid Interface Sci.* 169, 313-328 (1995).

He H., Hoseney R.C. 1990. Changes in Bread Firmness and Moisture During Long-Term Storage. *Cereal Chem.* 67(6):603-605.

Hettiarachchy, N.S., & Ziegler, G.R. (1994). Structure–function relationship of food protein. *In* N. S. Hettiarachchy & G. R. Ziegler (Eds.), Protein functionality in food systems, New York, NY, USA: Marcel Dekker.

Hung T., Nithianandan V. and Black R.G. 1991. Preparation and evaluation of pasta supplemented with chickpea and lupin flours. In 'Cereals International.'Brisbane, Qld (D.W. Martin and C.W. Wrigley, eds.) pp. 87–91, Cereal Chemistry Division, Royal Australian Chemical Institute, NSW, Australia.

ICC Standard No. 116/1: Determination of the sedimentation value (according to Zeleny) as an approximate measure of baking quality.

Igartuburu, J.M., Del Río, R.M., Massanet, G.M., Montiel, J.A., Pando, E., & Luis, F.R., (1991). Study of agricultural by-products. Extractability and amino acid composition of grapeseed (*Vitis vinifera*) proteins. *Journal of the Science of Food and Agriculture*, *54*, 489–493.

International Association for Cereal Science and Technology (ICC) (2000) The Standard Methods of the ICC, 2000 ed. ICC Standard No. 137⁄1, Approved 1982 and Revised 1994. ICC, Vienna, Austria.

International Association for Cereal Science and Technology (ICC) (1968) The Standard Methods of the ICC, 1968 ed. ICC Standard No. 107⁄1, Approved 1968 and Revised 1995. ICC, Vienna, Austria.

Jafari M., Koocheki A., Milani E., 2017. Physicochemical and sensory properties of extruded sorghum–wheat composite bread. *Food Measure* DOI 10.1007/s11694-017-9649-4.

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

Kato A. 2002. Industrial applications of Maillard-type protein–polysaccharide conjugates. *Food Sci. Tech. Res.* 8, 193–199.

Kato Y., Matsuda T., Kato N., Nakamura, R. 1988. Browning and protein polymerization induced by amino-carbonyl reaction of ovalbumin with glucose and lactose. *J. Agric.Food Chem.* 36, 806–809.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochimica et Biophysica Acta, 624(1), 13–20.

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

Khalil, M., Ragab, M., & Hassanien, F. R. (1985). Some functional properties of oilseed proteins. Nahrung, 29(3), 275–282.

Khan MN, Rhee KC, Rooney LW, Cater CM (1975) Bread baking properties of aqueous processed peanut protein concentrate. *J Food Sci* 40: 580–583.

Kimberly A.E. and Roberts M.G., (1905). A method for the direct determination of organic nitrogen by the Kjeldahl process. *Public Health Papers and Reports* 31(Pt 2): 109–122.

Kittisuban, P., Ritthiruangdej, P., Suphantharika, M., 2014. Optimization of hydroxyl propylmethylcellulose, yeast, b-glucan, and whey protein levels based on physical properties of gluten-free rice bread using response surface methodology. *LWT - Food Sci. Technol.* 57, 738-748.

Kohajdová, Z., Karovicová, J., & Schmidt, S. (2011). Lupin composition and possible use in bakery e a review. *Czech Journal of Food Science*, 29(3), 203-211.

Korus, J., Witczak, M., Ziobro, R., Juszczak, L., 2015. The influence of acorn flour on rheological properties of gluten-free dough and physical characteristics of the bread, *European Food Research and Technology*, 240: 1135-1143.

Kozhevnikor, G.O., Danilenko, A.N., Braudo E.E., & Schwenker, K.D. (2001). Comparative studies on thermodynamic characteristics of pea legumin and legumin-T thermal denaturation. *International Journal of Biological Macromolecules, 29*, 225-236.

Kuntz, I. D., Jr., & Kauzmann, W. (1974). Hydration of proteins and polypeptides. *Advances in Protein Chemistry*, 28, 239–345.

Kuraishi, C., Yamazaki, K., Susa, Y., 2001. Transglutaminase: its utilization in the food industry. Food Reviews International 17, 221–246.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*(5259), 680-685.

Lampart-Szczapa, E. (1996). Preparation of protein from lupin seeds. *Nahrung*, *40*, 71–74.

Lawal, O.S. (2004). Functionality of African locust bean (*Parkia biglobossa*) protein concentrate: effects of pH, ionic strength and various protein concentrations. *Food Chemistry*, *86*, 345–355.

Lazos E.S. 1991. Composition and oil characteristics of apricot, peach and cherry kernel. *Grasas y Aceites*, Vol. 42 Fase. 2 (1991), 127-131.

Lertittikul W., Benjakul S., Tanaka M. 2007. Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH. *Food Chem.* 100(2), 669–677, (2007).

Li Y., Lu F., Luo C., Chen Z., Mao J., Shoemaker C., Zhong F. 2009. Functional properties of the maillard reaction products of rice protein with sugar. *Food Chem*. 117, 69-74 (2009).

Li Y., Zhong F., Ji W., Yokoyama W., Shoemaker C. F., Zhu S,. Xia W. 2013. Functional properties of Maillard reaction products of rice protein hydrolysates with mono-, oligo and polysaccharides. Food Hydrocoll. 30, 53–60 (2013).

Liadakis, G. N., Floridis, A., Tzia, C., Oreopoulou, V., 1993, Protein concentrates with reduced gossypol content from screw-pressed cottonseed meal. *J. Agric. Food. Chem.* 41:918.

Liu, L. H. (1996). Chickpea proteins for food applications. Doctoral dissertation, Melbourne Australia: Victoria University of Technology, Melbourne, (p 52-53).

Liu, S.H., Low N.H., & Nickerson, M.T. (2009). Effect of pH, salt, and biopolymer ratio on the formation of pea protein concentrate-gum Arabic complexes. *Journal of Agricultural and Food Chemistry*, 57, 1521–26.

MacRitchie, F. (1978). Proteins at interfaces. *Advances in Protein Chem*istry, *32*, 283-326.

MacRitchie, F. (1980). Studies of gluten protein from wheat flours. Cereal Foods World 25, 382–385.

MacRitchie, F. (1987). Evaluation of contributions from wheat protein fractions to dough mixing and breadmaking. J. Cereal Sci. 6, 257–268.

Madenci, A.B. and Bilgicli, N., 2014. Effect of whey protein concentrate and buttermilk powders on rheological properties of dough and bread quality, *Journal of Food Quality*, 37: 117-124.

Maier, T., Schieber, A., Kammerer, D.R., & Reinhold, C. (2009). Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. *Food Chemistry*, 112, 551–559.

Marco, C. and Rosell, C.M., 2008a. Functional and rheological properties of protein enriched gluten free composite flours, *Journal of Food Engineering*, 88: 94-103.

Marco C., Rossell C.M., (2008b). Breadmaking performance of protein enriched, gluten-free breads. *Eur Food Res Technol* (2008) 227:1205–1213.7.

Marcone, F. M., Kakuda, Y., Yada, R. Y. (1997). Salt-soluble seed globulins of various dicotyledonous and monocotyledonous plants – I. Isolation–Purification and Characterization. *Food Chemistry*, 62, 27–47.

Mares D., Mrva K. 2008. Late-maturity a-amylase: Low falling number in wheat in the absence of preharvest sprouting. *Journal of Cereal Science* 47 (2008) 6–17.

Markiewicz K. 2010. The economics of meeting future protein demand (2010). Retrieved from edepot.wur.nl, August 8, 2017.

Marti, A., Bock, J.E., Pagani, M.A., Ismail, B., Seetharaman, K., 2016. Structural characterization of proteins in wheat flour doughs enriched with intermediate wheatgrass (*Thinopyrum intermedium*) flour, *Food Chemistry*, 194: 994-1002.

Martin A.H., Grolle K., Bos M.A., Cohen Stuart M.A., van Vliet, T. 2002. Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. *J. Colloid Interface Sci*. 254, 175-183 (2002).

Martin, M. L., & Hoseney, R. C. (1991). A mechanism of bread firming. II. Role of starch hydrolyzing enzymes. *Cereal Chemistry*, 68, 503–510.

Matos M.E., Rosell C.M. 2012. Relationship between instrumental parameters and sensory characteristics in gluten-free breads. *Eur Food Res Technol* (2012) 235:107–117.

McCarthy D.F., Gallagher E., Gormley T.R., Schober T.J., Arendt E.K. (2005) Application of response surface methodology in the development of gluten-free bread. *Cereal Chem* 82:609–615.

Mercier S., Villeneuve S., Mondor M., Drolet H., Ippersiel D., Lamarche F., et al., (2012). Mixing properties and gluten yield of dough enriched with pea protein concentrates. *Journal of Food Research*, 1(1), 13–23.

Miquelim J.N., Lannes S.C.S., Mazenga R. 2010. pH Influence on the stability of foams with protein–polysaccharide complexes at their interfaces. *Food Hydrocoll*, 24, 398–405 (2010).

Mirmiran P., Fazeli M.R., Asghari G., Shafiee A., Azizi F. (2010). Effect of pomegranate seed oil on hyperlipidaemic subjects: a double-blind placebo-controlled clinical trial. *British Journal of Nutrition* 104(3): 402–406.

Mita, T., Ishida, E., & Matsumoto, H. (1978). Physicochemical studies on wheat protein foams II. Relationship between bubble size and stability of foams prepared with gluten and gluten components. *Colloids Interface Science*, 64, 143–146.

Mohammed, I., Ahmed, A. R., & Senge, B. (2012). Dough rheology and bread quality of wheat-chickpea flour blends. *Industrial crops and products*, 36, 196-202.

Mohammadi M., Azizi M.H., Neyestani T.R., Hosseini H., Mortazavian A.M. (2015). Development of gluten-free bread using guar gum and transglutaminase. *Journal of Industrial and Engineering Chemistry* 21 (2015) 1398–1402.

Molina, E., Papadopoulou, A., Defaye, A., & Ledward, D. A. (2002). Functional properties of soy proteins as influenced by high pressure: Emulsifying. In Progress in Biotechnology, 19 (Trends in High Pressure Bioscience and Biotechnology), pp. 557–562.

Moore, M. M., Heinbockel, M., Dockery, P., Ulmer, H. M., & Arendt, E. K. (2006). Network formation in gluten-free bread with application of transglutaminase. *Cereal Chemistry*, 83, 28-36.

Morad, M. M., S. B. El-Magoli and S. A. Afifi, J. Macaroni supplemented with lupin and defatted soybean flours. *Food Sci.* 45 (1980) 404–405.

Motoki, M., Okiyama, A., Nonaka, M., Tanaka, H., Uchio, R., Matsuura, A. et al., (1992). Transglutaminse. US Patent US 5156956.

Moure, A., Sineiro, J., Dominguez, H., & Parajo, J.C. (2006). Functionality of oilseed protein products: A review. *Food Research International, 39,* 945–963.

Mubarak A.E. 2001. Chemical, nutritional and sensory properties of bread supplemented with lupin seed (Lupinus albus) products. *Nahrung/Food* 45 (2001) No. 4, pp. 241–245.

Nkosi, C. Z., Opoku, A. R., Terblanche, S.E. (2006). Antioxidative effects of pumpkin seed (Cucurbita pepo) protein concentrate in CCl4-induced liver injury in low-protein fed rats. *Phytotherapy Research*, 20(11), 935–940.

NMKL 960 (1968). Nordic committee on food analysis.

Nowshehri, J.A., Bhat, Z.A., & Shah, M.Y. (2015). Blessings in disguise: bio-functional benefits of grape seed extracts. *Food Research International*, 77, 333–348.

Oliete, B., Gomez, M., Pando, V., Fernandez, E., Caballero, P.A., Ronda, F., 2008. Effect of nut paste enrichment on physical characteristics and consumer acceptability of bread, *Food Science and Technology*, 14(3): 259-269.

Oreopoulou V. and Tzia C. 2006. 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, Vol.3, Eds: V. Oreopoulou and W. Russ, 2007 Springer Science-Business Media, LLC, USA, pp. 216-223.

Osman M.A., Alamri M.S., Mohamed A.A., Hussain S., Gassem M.A., Rahman I.E.A. 2014. Black cumin-fortified flat bread: Formulation, processing, and quality. *Quality Assurance and Safety of Crops & Foods*, 2015; 7 (2): 233-238.

Özdemir Ö., Çakır B., Gülseren İ. Functional and proteomic characterization of protein products from defatted cold press meals. AOCS Annual Meeting, Orlando, FL, USA, (2017).

Paciulli, M., Rinaldi, M., Cirlini, M., Scazzina, F., Chiavaro, E., 2016. Chestnut flour addition in commercial gluten-free bread: A shelf-life study, *LWT-Food Science and Technology*, 70: 88-95

Paredes-Lopez, O., Ordorica-Falomir, C., & Olivares-Vazquez, M.R. (1991). Chickpea protein concentrate: Physicochemical, functional and nutritional characterization. *Journal of Food Science*, 56, 726–729.

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

Pericin D., Popovic Lj.R., Vastag Z., Popoviç S.M., Trivic S., (2009). Enzymatic hydrolysis of protein concentrate from hull-less pumpkin oil cake: Application of response surface methodology. *Food Chemistry* 115 (2009) 753–757.

Perten Instruments (1995). Fungal Falling Number, method description—enclosure 2, A modified FN method for determination of alpha-amylase activity in flours supplemented with fungal enzymes. Pp. 1–5. Huddinge, Sweden: Perten Instruments AB.

Pesavento, I.C., Bertazzo, A., Flamini, R., & Vedova, A.D. (2008). Differentiation of *Vitis vinifera* varieties by MALDI-MS analysis of the grape seed proteins. *Journal of Mass Spectrometry*, *43*, 234- 241.

Phimolsiripol, Y., Mukprasirt, A., Schoenlechner, R., 2012. Quality improvement of rice-based gluten-free bread using different dietary fibre fractions of rice bran. *Cereal Sci*. 56, 389-395.

Phongthai S., D'Amico S., Schoenlechner R., Rawdkuen S. (2016). Comparative study of rice bran protein concentrate and egg albumin on gluten-free bread properties. *Journal of Cereal Science* 72 (2016) 38-45.

Pomeranz, Y. (1988). Composition and functionality of wheat flour components. In: Pomeranz, Y. ed. Wheat Chemistry and Technology II, 3rd edn. St. Paul, MN: American Association of Cereal Chemists, pp. 219–370.

Ponte, J. G. Jr., Titcomb, S. T., & Cotton, R. H. (1962). Flour as a factor in bread firming. *Cereal Chemistry* , 39, 437–444.

Potter N., & Hotchkiss J. (1995). Food science (5th ed.). *New York, NY, USA: Springer Sciences Business Media* (Chapter 17).

Promprom W., Kupittayanant P., Indrapichate K., Wray S., Kupittayanant S. (2010). The effects of pomegranate seed extract and beta-sitosterol on rat uterine contractions. *Reproductive Sciences* 17(3): 288–296.

Qi J., Liao J., Yin S., Zhu J., Yang X.: Formation of acid-precipitated soy protein-dextran conjugates by maillard reaction in liquid systems. *Int. J. Food Sci. Technol.* 45, 2573-80 (2010).

Quanhong L., Caili F., (2005). Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. *Food Chemistry*, 92 (2005) 701–706.

Quanhong L., Ze T., Tongyi C., (2003). Study on the hypoglycemicaction of pumpkin extract in diabetic rats. *Acta Nutrimenta Sinica*, 25(1), 34–36.

Radha, C., Kumar, P.R., Prakash V. (2007). Preparation and characterization of a protein hydrolysate from an oilseed flour mixture. *Food Chemistry, 106*, 1166-1174.

Renzetti S., Dal Bello F., Arendt E.K. (2008). Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase. *J. Cereal Sci*. 48 (2008) 33–45.

Rezig, L., Chibani, F., Chouaibi, M., Dalgalarrondo, M., Hessini, K., Gueguen, J., et al. (2013). Pumpkin (Cucurbita maxima) seed proteins: sequential extraction processing and fraction characterization. *Journal of Agricultural and Food Chemistry*, 61, 7715-7721.

Ribotta, P.D., Arnulphi, S.A., Leon, A.E., Anon, M., 2005. Effect of soybean addition on the rheological properties and bread making quality of wheat flour. *J. Sci. Food Agric.* 85 (11), 1889-1896.

Ribotta P.D., Ausar S.F., Morcillo M.H., Perez G.T., Beltramo D.M., Leon A.E. 2004. Production of gluten-free bread using soybean flour. *J Sci Food Agric* 84:1969–1974 (online: 2004).

Roach, R. R., & Hoseney, R. C. (1995). Effects of certain surfactants on the starch in bread. *Cereal Chemistry*, 72(6), 578–582.

Rodriguez Furlan, L.T., Padilla, A.P., Campderros, M.E., 2015. Improvement of gluten free bread properties by the incorporation of bovine plasma proteins and different saccharides into the matrix. *Food Chem*. 170, 257-264.

Sabanis D., Tzia C. 2009. Effect of Rice, Corn and Soy Flour Addition on Characteristics of Bread Produced from Different Wheat Cultivars. *Food Bioprocess Technol* (2009) 2:68–79.

Sánchez H.D., Osella C.A., de la Torre M.A. (2004). Use of Response Surface Methodology to Optimize Gluten-free Bread Fortified with Soy Flour and Dry Milk. *Food Sci Technol Int* 10(1):5–9.

Sarabhai, S., Indrani, D., Vijaykrishnaraj, M., Kumar, V.A., Prabhasankar, P., 2015. Effect of protein concentrates, emulsifiers on textural and sensory characteristics of gluten free cookies and its immunochemical validation, *Journal of Food Science and Technology*, 52(6): 3763-3772.

Sathe, S.K., Deshpande, S.S., & Salunkhe, D.K. (1982a). Functional properties of winged bean (*Psophocarpus tetragonolobus* L. DC) proteins. *Journal of Food Science*, *47*, 503–509.

Sathe, S.K., Deshpande, S.S., & Salunkhe, D.K. (1982b). Functional properties of lupin seeds (Lupinus mutabilis) proteins and protein concentrates. *Journal of Food Science*, *47*, 491–497, 499–500.

Sathe, S.K., & Salunkhe D.K. (1981). Functional properties of the Great Northern Bean (*Phaseolus vulgaris* L.) proteins: emulsion, foaming, viscosity, and gelation properties. *Journal of Food Science*, *46*, 71-74.

Scanlon MG, Zghal MC (2001) Bread properties and crumb structure. *Food Res Int* 34:841–864.

Schoenlechner, R., Mandala, I., Kiskini, A., Kostaropoulos, A., Berghofer, E., 2010. Effect of water, albumen, and fat on the quality of gluten-free bread containing amaranth. *Int. J. Food Sci. Technol*. 45, 661-669.

Schieber, A., Stintzing, F.C., Carle, R., 2001. By-products of plant food processing as a source of functional compounds- recent developments. *Trends Food Sci. Technol.* 12, 401–413.

Schubert S.Y., Lansky E.P. and Neeman I., (1999). Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *Journal of Ethnopharmacology*, 66(1): 11–17.

Shewry P.R., Tatham A.S. (2000): Wheat. The Royal Society of Chemistry. Cambridge CB4 OWF, UK:335–339.

Siebert, K.J. (2003). Modeling protein functional properties from amino acid composition. *Journal of Agricultural and Food Chemistry*, *51*(26),7792–97.

Sosulski, F.W., McCurdy A.R. (1987). Functionality of flours, protein fractions and concentrates from field peas and faba bean. *Journal of Food Science*, 52 (4) (1987), pp. 1010–1014.

Stelten, S., Dekker, I.M., Ronday, E.M., Thijs, A., Boelsma, E., Peppelenbos, H.W., Van Der Schueren, M.A.E., 2014. Protein-enriched 'regular products' and their effect on protein intake in acute hospitalized older adults; a randomized controlled trial, *Clinical Nutrition*, 34: 409-414.

Stone, A.K., Karalash, A., Tyler, R.T., Warkentin, T.D., Nickerson, M.T. (2015). Functional attributes of pea protein concentrate prepared using different extraction methods and cultivars. *Food Research International*, *76*, 31–38.

Storck C.R., Zavareze E.R., Gularte M.A., Elias M.C., Rosell C.M., Dias A.R.G., 2013. Protein enrichment and its effects on gluten-free bread characteristics. *LWT - Food Science and Technology* 53 (2013) 346-354.

Sumner, A.K., Nielson, M.A., & Youngs, C.G. (1981). Production and evaluation of pea protein concentrate. *Journal of Food Science*, *46*, 364–372.

Sun, X.D., & Arntfield, S.D. (2010). Gelation properties of salt-extracted pea protein induced by heat treatment. *Food Research International*, *43*, 509–515.

Suzuki S., Tsumura N. (1972). Isomerization of glucose to fructose. *Japan Agricultural Research Quarterly, JARQ*, 6(4), 245-248.

Švec, I., Hrušková, M., The Mixolab parameters of composite wheat/hemp flour and their relation to quality features, *LWT - Food Science and Technology* (2014), doi: 10.1016/ j.lwt.2014.07.034.

Şahin, M., Göçmen Akçacık, A., Aydoğan, S., Demir, B., Önmez, H., Taner, S., 2013. Ekmeklik Buğday Ununda Ekmek Hacmi ile Bazı Fizikokimyasal ve Reolojik Özellikler Arasındaki İlişkilerin Tespiti, *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, 22(1):13-19.

Tekeli, A. (2014). Nutritional value of black cumin (*Nigella sativa*) meal as an alternative protein source in poultry nutritıon. *Journal of Animal Science Advances, 1*(4), 797-806.

Tomar P.P.S., Nikhil K., Singh A., Selvakumar P., Roy P., Sharma A.K., (2014). Characterization of anticancer, DNase and antifungal activity of pumpkin 2S albümin. *Biochemical and Biophysical Research Communications* 448 (2014) 349–354.

Tsaliki, E., Pegiadou, S., & Doxastakis G. (2004). Evaluation of the emulsifying properties of cottonseed protein concentrate. *Food Hydrocolloids,* 18, 631-637.

Tsoukala, A., Papalamprou, E., Makri, E., Doxastakis, G., & Braudo, E.E. (2006). Adsorption at the air–water interface and emulsification properties of grain legume protein derivatives from pea and broad bean. *Colloids and Surfaces B: Biointerfaces*, 53, 203–208.

Vermaut S., De Coninck K., Flo G., Cokelaere M., Onagbesan M., Decuypere E. 1997. Effect of deoiled jojoba meal on feed intake in chickens: Satiating or taste effect. *J Agric Food Chem*, 45(8), 3158-63, (1997).

Vijaykrishnaraj, M., Roopa, B.S. and Prabhasankar, P., 2016. Preparation of gluten free bread enriched with green mussel (*Perna canaliculus*) protein hyrolysates and characterization of peptides responsible for mussel flavour, *Food Chemistry*, 211: 715-725.

Visser A., Thomas A. 1987. Review: Soya protein products—Their processing, functionality, and application aspects. *Food Rev. Int.* 3:1.

Wang, J., Rosell, C. M., Benedito, B. C. (2002). Effect of the addition of different fibres on wheat dough performance and bread quality. *Food Chemistry.* 79 (2002) 221–226.

Wassermann, L. (1979). Relationship between structure and rheological properties of bread crumb. In P. Sherman (Ed.), Food texture and rheology (pp. 283–289). London: Academic Press

Wolf R.B., Cavins J.B., Kleiman R., Black L.T. 1982. Effect of Temperature on Soybean Seed Constituents: Oil, Protein, Moisture, Fatty Acids, Amino Acids and Sugars. *JAOCS*, vol. 59, no. 5 (May 1982).

Xu, J., Bietz, J. A., and Carriere, C. J. (2007). Viscoelastic properties of wheat gliadin and glutenin suspensions. Food Chem. 101, 1025–1030.

Yadav M., Jain S., Tomar R., Prasad G.B.K.S., Yadav H., 2010. Medicinal and biological potential of pumpkin: an updated review. *Nutrition Research Reviews*, 23, 184–190.

Yang H., Li M.,Qi X., Lv C., Deng J. and Zhao G., (2011). Identification of seven water-soluble non-storage proteins from pomegranate (Punica granatum Linn.) seeds. *Food Science and Technology International*, 18(4) 329–338.

Zeeb, B., Beicht, J., Eisele, T., Gibis, M., Fischer, L., Weiss, J. 2013. “Transglutaminase-induced crosslinking of sodium caseinate stabilized oil droplets in oil-in-water emulsions”, Food Research International, 54, 1712-21.

Zeng H. Y., Cai L. H., Cai X. L., Wang,Y. J., and Li Y. Q. (2013). Amino acid profiles and quality from lotus seed proteins. Journal of the Science of Food and Agriculture, 93(5), 1070-1075.

Zhou, T., Li, Q., Zhang, J., Bai, Y., & Zhao G. (2010). Purification and characterization of a new 11S globulin-like protein from grape (*Vitis vinifera* L.) seeds. *European Food Research and Technology*, *230*, 693–699.

Zhou, T., Zhang, T., Liu, W., & Zhao G. (2011). Physicochemical characteristics and functional properties of grape (*Vitis vinifera* L.) seeds protein. *International Journal of Food Science & Technology*, *46*, 635–641.

Zhu D., Damodaran S., Lucey J.A. 2008. Formation of whey protein concentrate (WPI)-dextran conjugates in aqueous solutions. *J. Agric. Food Chem.* 56, 7113-18 (2008).

Ziobro R., Witcak T., Juszczak L., Korus J. 2013. Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic. *Food Hydrocolloids* 32 (2013) 213-220.

ACKNOWLEDGEMENTS

This study was funded by a grant from TÜBİTAK 3501 Programme (Grant No. 115O569; The Scientific and Technological Research Council of Turkey).

The authors would like to also express their gratitude to Neva Foods Ltd. (İstanbul, Turkey) for the donation of the deoiled plant meals.

Anton Paar, Turkey is also acknowledged for their support in rheological analysis.

Polen Food, Turkey is also acknowledged for their support in bakery products manufacture.

CURRICULUM VITAE

ÖZGENUR ÖZDEMİR COŞKUN

ADDRES: Atakent Neighborhood 234 Street Manolya Site B-16 Flat Number:48 Number:32 Kucukcekmece/ISTANBUL

Mobile Phone : **+90 (544) 5891993**

e-mail : **ozgenurozdemir.93@gmail.com**

PERSONAL INFORMATION

Date of birth	:	17.02.1993
Place of birth	:	Bakirkoy-ISTANBUL
Nationality	:	Turkish
Marial status	:	Married
Driving License:		B class (issue date: 05.01.2012)

SUMMARY

Food Engineer, advanced English knowledge (YDS score; 76.25), studied abroad, research and product development skills, English or Turkish reporting skill, experienced in project management and organization for 2 years, ability to analyze and use analytical devices, MS Office and SAP knowledge.

JOB EXPERIENCES

TUBITAK 3501 R&D CAREER	:	Full-time Researcher
DEVELOPMENT PROGRAMME		15.10.2015- 15.06.2017
TAGEM R&D SUPPORT	:	Researcher
PROGRAMME		15.10.2015- 01.09.2016
ISTANBUL HALK EKMEK	:	Internship
		April 2015- April 2015

POLEN FOOD	**:**	Internship
		July 2014- August 2014
EKOM ADVERTISEMENT	**:**	Advertisement Hostess (Parttime)
		November 2012- June 2013
EGEVİZYON ADVERTISEMENT	**:**	Advertisement Hostess (Parttime)
		March 2012- June 2012

EDUCATION INFORMATION

Master Degree	:	**ISTANBUL SABAHATTIN ZAIM UNIVERSITY**
2015-2018	:	Institute of Science and Technology
		Food Engineering Programme
		Grade Point Avarage: 3.80
Bachelor Degree	:	**ISTANBUL SABAHATTIN ZAIM UNIVERSITY**
2011 – 2015	**:**	Faculty of Engineering and Natural Science
		%100 Scholarship Food Engineering Programme
		Grade Point Avarage: 3.25
Bachelor Degree	:	**WARSAW UNIVERSITY OF LIFE SCIENCE**
2014 Spring Semester:		Faculty of Food Science
		Erasmus Student Exchange Programme

LANGUAGE INFORMATION : English
2017 Spring YOKDIL- 78,75
2016 Autumn YDS- 76,25

COMPUTER SKILLS : MS Office, SAP

SEMINARS AND COURSES

AOCS Annual Meeting 2017	**:**	31.04.2017-03.05.2017
		(40 Hours)
ISIS Brigthon UK General English Course:		22.07.2013-13.09.2013
		(200 Hours)

PROJECTS

Production of protein concentrates and concentrates from oilseed and oil fruit meals and their utilization (Oct. 2015 – June 2017; TUBITAK 3501 Career Development Programme)

Encapsulation of cherry laurel polyphenols in sunflower pectin gels and the determination of their bioactivities (Oct. 2015 – Sep. 2016; TAGEM).

SCHOLARSHIPS

International : Erasmus Student Exchange Programme, 2014, Warsaw University of Life Science, Poland (5 months).

National : Tubitak Researcher Scholarship, Tubitak 115O369 (15.10.2015- 15.06.2017).

CERTIFICATES

1. ISIS UK Education Certificate of participation - 8 Weeks (25 Hour/Week) General English Course. Intermediate (B1) (22.07.2013-13.09.2013).
2. TS EN ISO 22000 Food Safety Management System Basic Education Participation Certificate (24 Hour).
3. TS 18001 Occupational Health and Safety Management System Basic Education Participation Certificate (18 Hour).
4. Basic Food Hygiene Training Participation Certificate (24 Hour).
5. TS EN ISO 9001 Quality Management System Basic Education Participation Certificate (16 Hour).

HOBBIES

I like to read boks that are related to feminism and women's rights. I enjoy cooking especially bakery products such as cake, cupcake, muffin, macaron, cookies.

REFERENCES

Asos. Prof. İbrahim GÜLSEREN- İstanbul Sabahattin Zaim University- Insructor

Phone number: (+90) 536 235 99 24 e-mail: ibrahim.gulseren@izu.edu.tr

Bilal ÇAKIR- İstanbul Sabahattin Zaim University Laboratory Manager

Phone number: (+90) 536 781 70 41 e-mail: bilal.cakir@izu.edu.tr