T.R.
SAKARYA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF ULTRASOUND AND HIGH-PRESSURE HOMOGENIZATION ON FUNCTIONAL PROPERTIES OF DATE SEED PROTEIN

PHD THESIS

Mohamed Ali Mahmoud Ahmed KELANY

Food Engineering Department

JANUARY 2023

T.R.
SAKARYA UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EFFECT OF ULTRASOUND AND HIGH-PRESSURE HOMOGENIZATION ON FUNCTIONAL PROPERTIES OF DATE SEED PROTEIN

PHD THESIS

Mohamed Ali Mahmoud Ahmed KELANY

Food Engineering Department

Thesis Advisor: Assoc. Prof. Oktay YEMİŞ

JANUARY 2023

The thesis work titled “EFFECT OF ULTRASOUND AND HIGH-PRESSURE HOMOGENIZATION ON FUNCTIONAL PROPERTIES OF DATE SEED PROTEIN” prepared by MOHAMED ALI MAHMOUD AHMED KELANY was accepted by the following jury on 28/01/2023 by unanimously of votes as a PhD THESIS in Sakarya University, Graduate School of Natural and Applied Sciences, Department of Food Engineering.

Thesis Jury

Head of Jury :	**Prof. Dr. Esra ÇAPANOĞLU GÜVEN** Istanbul Technical University
Jury Member :	**Assoc. Prof. Oktay YEMİŞ** Sakarya University
Jury Member :	**Assoc. Prof. Ayşe AVCI** Sakarya University
Jury Member :	**Assoc. Prof. Aslı CAN KARAÇA** Istanbul Technical University
Jury Member :	**Assis. Prof. Mustafa ÖZTÜRK** Sakarya University

STATEMENT OF COMPLIANCE WITH THE ETHICAL PRINCIPLES AND RULES

I declare that the thesis work titled " EFFECT OF ULTRASOUND AND HIGH-PRESSURE HOMOGENIZATION ON FUNCTIONAL PROPERTIES OF DATE SEED PROTEIN ", which I have prepared in accordance with Sakarya University Graduate School of Natural and Applied Sciences regulations and Higher Education Institutions Scientific Research and Publication Ethics Directive, belongs to me, is an original work, I have acted in accordance with the regulations and directives mentioned above at all stages of my study, I did not get the innovations and results contained in the thesis from anywhere else, I duly cited the references for the works I used in my thesis, I did not submit this thesis to another scientific committee for academic purposes and to obtain a title, in accordance with the articles 9/2 and 22/2 of the Sakarya University Graduate Education and Training Regulation published in the Official Gazette dated 20.04.2016, a report was received in accordance with the criteria determined by the graduate school using the plagiarism software program to which Sakarya University is a subscriber, I have received, I accept all kinds of legal responsibility that may arise in case of a situation contrary to this statement.

(28/01/2023)

Mohamed Ali Mahmoud Ahmed KELANY

To my family, children and everyone gives me energy during these hard times of my life.

ACKNOWLEDGMENTS

Initially, Praise and thanks be to Allah, Lord of the Worlds.

I wish to express my deepest appreciation and thanks to my supervisor Assoc. Prof. Oktay YEMİŞ, Department of Food Engineering, Faculty of Engineering, Sakarya University, for accepting me as a PhD student in his lab. He was my mentor, role model and friend since starting my PhD. His comments, advice, support and suggestions as well as his ideas and guidance have been of great help completion of this project. He was always available all the time to give the guidance that helped me from the initial stage, all the way through to the end of this thesis. My thanks go to my listen committee Prof. Dr Esra ÇAPANOĞLU GÜVEN and Assoc. Prof. Ayşe AVCI, for their support throughout this work. I am also grateful to thank Assoc. Prof. Doaa KABIL, University of Tanta, for her assistance and help to compelete this work.

Finally, thanks go to the sponsors (Egyptian high education ministry and Turkish scholarship) for funding this project.

.

Mohamed Ali Mahmoud Ahmed KELANY

TABLE OF CONTENTS

Sayfa

ABBREVIATIONS

DSPC : Date Seed Protein Concentrate
DSPC-N : Date Seed Protein Concentrates Native Form
DSPC-US : Date Seed Protein Concentrate Ultrasound Treated
DSPC-HPH : Date Seed Protein Concentrate High-Pressure Treated
HIUS : High İntensity Ultrasonic
HPH : High Pressure Homogenization
DPPH : 1,1-diphenyl-2-picrylhydrazyl
WBC : Water Binding Capacity
OBC : Oil Binding Capacity
EAI : Emulsion Activity İndex
ESI : Emulsion Stability İndex
FC : Foaming Capacity
FS : Foaming Stability
Td : Denaturation Temperature
ΔH : Enthalpy
SH : Free Sulfhydryl Group Content
H_0 : Surface Hydrophobicity

LIST OF TABLES

Page

LIST OF FIGURES

Page

EFFECT OF ULTRASOUND AND HIGH-PRESSURE HOMOGENIZATION ON FUNCTIONAL PROPERTIES OF DATE SEED PROTEIN

SUMMARY

Date kernel is a plant-derived byproduct that has the potential to be converted into a high-value-added food ingredient, such as protein concentrate, in the food industry. High-intensity ultrasound and high-pressure homogenization, which are alternative methods for improving the functional properties of food protein, are effective physical treatments for modifying protein functionality. Solubility is the main criterion that primarily influences other functional properties of protein concentrates such as emulsification, foaming, water, and oil binding. The aim of this study is to improve the techno-functional performance of date seed protein concentrate (DSPC) by maximizing solubility by high-intensity sonication (HIUS) and high-pressure homogenization (HPH). In the first stage of the study, the effect of ultrasonic homogenization at different amplitudes (40, 60, and 80%) and times (5, 10, and 15 min) on the functional properties of the DSPC was investigated using the response surface methodology (RSM). The face centered-central composite design (FC-CCD) showed that the optimal process conditions of HIUS were at 80% amplitude for 15 minutes.

In the second stage of the study, the influence of HPH at different pressures (50, 100, and 150 MPa) and protein concentrations (1, 2, and 3%) on the functional properties of the DSPC was analyzed using the Response Surface Methodology (RSM). A pressure of 150 MPa and a protein concentration of 1% were the optimum HPH conditions for maximizing the solubility. The optimum HIUS and HPH treatment conditions for physicochemical and functional properties of the ultrasonically treated date seed protein concentrate (DSPC-US) and the high pressure treated date seed protein concentrates (DSPC-HPH) were determined. The properties of DSPC-US and DSPC-HPH were compared to the native date seed protein concentrate (DSPC-N). The results revealed that the solubility of all DSPC samples treated by both HIUS and HPH was significantly ($p<0.05$) higher than native DSPC. In addition, emulsion activity/stability, foaming, activity/stability, antioxidant activity, and oil-binding capacity increased after HIUS and HPH homogenization treatments, while water-binding capacity decreased. These changes in the techno-functional properties of the DSPC-US and DSPC-HPH were explained by the modification of the physicochemical structure of the DSPC (particle size, zeta potential, SDS-PAGE, SEM, FTIR, DSC, free SH content, surface hydrophobicity, and intrinsic emission). This work demonstrated that HIUS and HPH could be effective treatments for enhancing the functional properties of date seed protein concentrate.

ULTRASON VE YÜKSEK BASINÇLI HOMOJENİZASYONUN HURMA TOHUM PROTEİNİNİN FONKSİYONEL ÖZELLİKLERİ

ÖZET

Hurma (*Phoenix dactylifera L.*), Aceraceae familyasında yer alan 200 cins ve 2000 türü olan dünyanın en eski meyvesidir (Zaid ve Wet, 2002). Hurma meyvesi, besleyici, sağlık özellikleri ve yüksek ekonomik değeri nedeniyle hem Arap yarımadasında hem de Ortadoğu'da en çok yetiştirilen meyvedir. Yağ, protein ve karbonhidratlar gibi temel besin maddelerinin zengin bir kaynağı olarak kabul edilir. Dünya genelinde hurma üretimi 9.45 milyon tondur ve Mısır 1.69 milyon ton hurma üretimi ile dünya çapındaki toplam hurma üretiminin %18'ini gerçekleştiren en büyük üretici ülke konumundadır (FAO, 2020). Hurma çekirdekleri, tüm meyve ağırlığının yaklaşık %11–18'ini oluşturmakta olup karbonhidrat, lif, yağ ve proteinden gibi birçok fonksiyonel gıda bileşenin yapısında barındırmaktadır. İşleme veya hasat sonrası açığa çıkan birçok tarımsal yan ürün, insan beslenmesi için gerekli bileşenleri içermekte olup yeni ve sürdürülebilir kaynak olarak görülmektedir. Hurma tohumu yeterince kullanılmayan bir yan ürün olup ve gıda endüstrisi için önemli bir sorun niteliğindedir. Dünya çapında yıllık hurma üretimi ve tohum verimi dikkate alındığında, yılda yaklaşık 1.0–1.7 milyon metrik ton (Mt) hurma tohumunun yan ürün olarak açığa çıktığı tahmin edilmektedir. Büyük miktarlarda elde edilebilen ve önemli miktarda protein içeren hurma taneleri, endüstriyel işleme yoluyla katma değerli ürünlere dönüştürülme potansiyeline sahiptir.

Bitkisel ve hayvansal kaynaklardan elde edilen proteinler, insan beslenmesi için kritik besin maddelerdir. Son zamanlarda, gıda formülasyonlarında alternatif bileşenler olarak kabul edilen yeni bitki bazlı protein içeriklerine yönelik artan bir talep olmuştur. Bu talep, dünya çapında vegan/vejetaryen eğilimin artmasından, bu gıdaların nispeten ucuz olmasından ve tüketicinin bitki bazlı proteinin hayvan bazlı proteinden daha sağlıklı olduğu algısı/farkındalığından kaynaklanmaktadır. Bu nedenlere ek olarak, bitki bazlı proteinlere olan talebin arkasındaki itici güç, söz konusu kullanılan materyallerin yenilenebilir ve sürdürülebilir kaynaklar olmasıdır. Tüm bu nedenlerden dolayı, gıda endüstrisi ucuz ve istenen fonksiyonel özelliklere sahip yeni protein kaynakları arayışındadır. Günümüzde baklagiller, tahıllar ve yağlı tohumlar, ticari bitki bazlı protein bileşenleri üretmek için protein kaynağı olarak kullanılmaktadır. Bitki kaynaklı proteinler, hem gıda hem de ilaç endüstrisinde emülsifiye etme, köpürme, enkapsülasyon ve jelleşme gibi çok önemli işlevlere sahiptir. Gıdadaki proteinin davranışını, kalitesini ve duyusal özelliklerini etkileyen proteinin tekno-fonksiyonel performansları, gıdalarda depolama ve işleme sırasında önemli bir rol oynar. Bitkisel proteinlerin potansiyel kullanımları, gıda sistemlerindeki işlevlerine bağlıdır. Bitkisel proteinlerin zayıf tekno-fonksiyonelliği, bir bileşen olarak endüstriyel uygulamalar için en göze çarpan dezavantajdır. Bugün, bir proteinin fonksiyonel özelliklerini değiştirmek için kimyasal, enzimatik ve fiziksel yöntemler kullanılmaktadır. Ultrases ve yüksek basınç homojenizasyon teknikleri, proteinlerin

modifikasyonunda kullanılabilecek sağlık açısından herhangi bir risk oluşturmayan güvenli ve çevre dostu fiziksel tekniklerdir.

Bu doktora tez çalışmasının amacı, yüksek yoğunluklu ultrases (HIUS) ve yüksek basınç homojenizasyon (HPH) teknikleri ile hurma çekirdeği protein konsantresinin (DSPC) tekno-fonksiyonel performansını artırmaktır. Bu çalışmada, Mısır'da en çok üretimi yapılan ticari hurma çeşitlerinden biri olan "Saidy" çeşidinin çekirdekleri kullanılmıştır. Çekirdeklerin pomolojik özellikleri belirlendikten sonra toz haline getirilmiş temel kimyasal kompozisyonunun yanı sıra fitokimyasal özellikleri (amino asit, mineral ve yağ asidi kompozisyonları ile toplam fenolik, antioksidan, antosiyanin, flavonoid ve saponin) belirlenmiştir. Hurma çekirdeklerinin, %6.17 protein içermesinin yanında, %9.56 yağ, %7.21 nem, %3.44 kül ve %73.62 karbonhidratlardan oluştuğu saptanmıştır. Kullanılan "Saidy" hurma çekirdeğinin amino asit profili, günlük alınması tavsiye edilen miktar açısından izolösin, lisin, metiyonin, treonin, valin ve histidin bakımından yeterli buna karşın, lösin ve fenilalanin bakımından yeterli olmadığı saptanmıştır. Çekirdeklerin mineral kompozisyonunda, en yüksek potasyum (807.1 mg/100g) içerdiği tespit edilmiş olup bunu sodyum (322.9 mg/100g), kalsiyum (263.1 mg/100g), magnezyum (95.67 mg/100g) ve demir (61.21 mg/100g) takip etmiştir. İz miktarda manganez (1.665mg/100g) ve bakır (0.940 mg/100g) tespit edilmiştir. Fitokimyasal olarak, 2.40 mg/g toplam fenolik ve 1.28 mg/g toplam flavonoid içerdiği tespit edilmiştir. Hurma çekirdeği ekstraktının GC-MS analizinin sonucu, laurik asidin %34.9 ile en yüksek konsantrasyon sahip bileşik olduğunu ortaya koymuştur. Bunu sırasıyla %15.45, %8.74 ve %7.98 ile palmitik asit, kaprik asit ve oleik asit izlemiştir.

Toz haline getirilmiş ve yağı giderilmiş örneklerden, klasik alkali ekstraksiyon ve ardından izoelektrik çökeltme yöntemi ile %70 protein içeriğine sahip protein konsantratı (DSPC) elde edilmiştir. Doğal formdaki bu konsantratın (DSPC-N), tüm fonksiyonel (çözünürlük, emülsifikasyon, köpük oluşturma, su ve yağ bağlama, antioksidan) ve fizikokimyasal (partikül boyutu, yüzey yükü, yüzey hidrofobisitesi, serbest sülfidril içeriği, protein profili, elektron mikroskobu ile mikro yapısı, floresans emisyonu) özellikleri belirlenmiştir. Elde edilen doğal haldeki protein konsantratın (DSPC-N) %14.1 çözünürlüğe, 2.76 g/g su bağlama, 1.73 g/g yağ bağlama, 11.92 m^2/g emülsiyon aktivitesi, 17.63 dakika emülsiyon stabilitesi, %11 köpük oluşturma ve %8 köpük stabilitesine sahip olduğu saptanmıştır. Elde edilen konsantratın parikül boyutu 123 nm, serbest sülfidril içeriği 1.58 µmol/g, denatürasyon sıcaklığı 87.7C, entalpisi 204 J/g ve zeta potansiyeli –28.73 mV olarak tespit edilmiştir.

Çalışmanın ikinci aşamasında, yanıt yüzey metodu ile ultrases homojenizasyonunun farklı genlik (%40, 60 ve %80) ve sürelerde (5, 10 ve 15 dakika) DSPC'nin fonksiyonel özellikleri üzerindeki etkisi incelenmiştir. Bu uygulamalarda fonksiyonel özelliklerden çözünürlük, emülsifiasyon ve antioksidan kapasitenin nasıl değiştiği göz önünde bulundurulmuştur. Elde edilen verilerden Design Expert istatistik programı ile matematiksel modeller üretilmiştir. Ancak üretilen bu modellerden yalnızca çözünürlük kriteri için geliştirilen modelin geçerli ve tamhinlemede güvenilir bir şekilde kullanılabileceği tespit edilmiştir. Uygulanan ultrases işlemin genlik ve süresinin artması ile çözünürlüğün arttığı tespit edilmiştir. Çözünürlük kriteri için önerilen kuadratik model, çözünürlüğü maksimum kılacak yüksek yoğunluklu ultrases homojenizasyonunun optimum işlem koşullarının 15 dakika ve %80 genlik olduğunu ortaya koymuştur. Daha sonra doğal haldeki hurma çekirdeği konsantratı (DSPC-N) optimum koşulları belirlenen bu işlem yoğunluğuna (60.56 W/cm^2) maruz bırakılmıştır. Optimum işlem koşulları ile modifiye edilmiş olan konsantratın (DSPC-

US) tüm fonksiyonel ve fizikokimyasal özellikleri doğal formdaki konsantratın özellikleri ile kıyaslanmıştır. Bu optimum koşullar altında temel kriter olarak göz önünde bulundurduğumuz çözünürlük değeri, %14.1'den %32.6'ya yükselmiştir. Buna karşın, fonksiyonel özelliklerden, su bağlama kapasitesi hariç diğer tüm fonksiyonel özellikleri (yağ bağlama, emülsiyon oluşturma aktivitesi ve stabilitesi, köpük oluşturma kapasitesi ve stabilitesi) önemli oranlarda artmıştır. Bu uygulama ile, partikül boyutunda (123 nm'den 100 nm'ye), denatürasyon sıcaklığında (Td, 87.7°C'den 61.9°C), denatürasyon entalpisinde (ΔH, 204 J/g'dan 191.5 J/g'a), yüzey hidrofobisitesinde ve floresan emisyonunda düşüşler gözlenirken, serbest süldifril içeriği ve yüzey yükünde artışlar saptanmıştır. Söz konusu uygulamanın protein profilinde hiçbir değişim yaratmadığı yalnızca bant yoğunluğunun azaldığı gözlenmiştir.

Çalışmanın son aşamasında ise, yine yüzey yanıt yöntemi ile yüksek basınç homojenizasyonunun farklı basınç seviyeleri (50, 100 ve 150 MPa) ve protein konsantrasyonları altında (%1, 2 ve %3) DSPC'nin nin fonksiyonel özellikleri üzerindeki etkisi incelenmiştir. Ultrases uygulamasında olduğu gibi, fonksiyonel özelliklerden çözünürlük, emülsifiasyon ve antioksidan kapasitenin değişimi incelenmiş ve elde edilen verilerden Design Expert istatistik programı ile matematiksel modeller üretilmiştir. Ancak üretilen bu modellerden yalnızca çözünürlük ve antioksidan kapasite kriteri için geliştirilen modellerin geçerli olduğu ve tamhinlemede güvenilir bir şekilde kullanılabileceği tespit edilmiştir. Uygulanan homojenizasyon işleminin basıncının artması ile çözünürlük ve antioksidan aktivitenin arttığı buna karşın, protein konsantrasyonun artması ile azaldığı saptanmıştır. Çözünürlük için linear bir model, antioksidan kapasite için kuadratik model önerilmiştir. Önerilen bu modellerden hem çözünürlük hem de antioksidan kapasiteyi maksimum kılacak şekilde, en uygun proses koşullarının, 150 MPa ve %1 protein konsantrasyonu olduğu saptanmıştır. Daha sonra doğal haldeki hurma çekirdeği konsantratı (DSPC-N) optimum koşulları belirlenen bu yüksek basınç homojenizasyon işlemine tabi tutulmuştur. Optimum işlem koşulları ile modifiye edilmiş olan konsantratın (DSPC-HPH) tüm fonksiyonel ve fizikokimyasal özellikleri doğal formdaki konsantratın özellikleri ile kıyaslanmıştır. Bu optimum koşullar altında temel kriter olarak göz önünde bulundurduğumuz çözünürlük değeri, %14.1'den %43.6'ya, antioksidan activite değeri 60.5 TE/g'dan 71.67 TE/g'a yükselmiştir. Buna karşın, fonksiyonel özelliklerden su bağlama kapasitesi 2.76 g/g'dan 1.38 g/g'a düşerken, yağ bağlama kapasitesi 1.73 g/g'dan 3.02 g/g'a yükselmiştir. Emülsiyon aktivitesi artarken stabilitesinde herhangi bir değişim gözlenmemiştir. Bu uygulama ile, partikül boyutunda (123 nm'den 65 nm'ye), denatürasyon sıcaklığında (Td, 87.7°C'den 64.3°C), yüzey hidrofobisitesinde ve floresan emisyonunda düşüşler gözlenirken, denatürasyon entalpisinde (ΔH, 204 J/g'dan 227.7 J/g'a), serbest süldifril içeriğinde (1.58 μmol/g'dan 2.65 μmol/g'a) ve yüzey yükünde (-28.73 mV'tan -32.73 mV'a) artışlar saptanmıştır. Söz konusu uygulamanın protein profilinde hiçbir değişim yaratmadığı yalnızca bant yoğunluğunun azaldığı gözlenmiştir.

Bu çalışma, yüksek yoğunluklu ultrases ve yüksek basınç homojenizasyonlarının, hurma çekirdeği proteini konsantrelerinin fonksiyonel özelliklerinin geliştirilmesinde etkili fiziksel yöntemler olabileceğini ortaya koymuştur. Elde edilen veriler göz önünde bulundurulduğunda, yüksek basınç homojenizasyonun (HPH), yüksek yoğunluklu ultrases homojenizasyonuna (HIUS) kıyasla çok daha etkin olduğu sonucuna ulaşılmıştır.

1. INTRODUCTION

Date palm (*Phoenix dactylifera L.*) is a fruit that belongs to the order Arecales family Aceraceae, which includes 200 genera and 2000 species. It is the oldest fruit in the world (Zaid and Wet, 2002). Date palm fruit has been cultivated and consumed by both humans and livestock because of its nutritional, health properties, and high economic value (Sulieman et al, 2012). It is considered a rich source of essential nutrients such as fat, protein, and carbohydrates. Additionally, waste seeds produced by food processing are rich sources of dietary fiber (Besbes et al, 2004). Date palm production around the world is 9.45 million tons, and the largest producer country was Egypt with 1.69 million tons of date palm, which was 18% of the worldwide total date production (FAO, 2020). Date pit is about 11-18% from whole fruit weight, consisting of carbohydrates, fiber, fat, and protein (Abdul Afiq et al, 2013).

Agricultural by-products have functional ingredients that are important for the utilization and development of novel sources to satisfy the protein demand for human diets (Granato et al, 2017). Changes and modifications of functional characteristics of protein have been related to their application in food systems (Asero et al, 2000; Ma et al, 2003; Thomas et al, 2007). Date seed is an underutilized by-product and a major issue for the food industry. It is predicted that approximately 1.0–1.7 million metric tons (Mt) of date seed is generated as a by-product per year, considering the annual production of date palm worldwide and the seed yield. Several studies previously reported that the protein content of date seed was about 5.56 and 5.17% (Besbes et al, 2004), 2.30-6.40% (Al-Farsi et al, 2007), 2.3-6.4 g/100 of date seed (Al-Farsi and Lee, 2011). Date kernels, which can be obtained in large quantities and contain considerable amounts of protein, have the potential to be converted into value-added products by industrial processing.

Proteins mainly come from plant and animal sources and are critical nutrients for human nutrition. Also, the quality of a protein differs significantly depending on its biological availability, digestion, amino acid content, purity, antinutritional considerations, and processing effects (Mattila et al, 2018). Recently, there has been a

growing demand for novel plant-based protein ingredients, which are considered alternative ingredients in food formulations. The growing trend of vegan/vegetarian diets worldwide is due to these foods being relatively cheap and the consumer perception/awareness of being healthier of plant-based protein than animal-based. In addition to these reasons, the driving force behind the demand for plant-based proteins is that the ingredients are produced from renewable and sustainable sources. For all these reasons, the food industry is seeking new protein sources that are inexpensive and have desirable techno-functional properties. Today, legumes, cereals, and pseudo cereals, oilseeds are used as protein sources to produce commercial plant-based protein ingredients (Ismail et al, 2020).

Plant-derived proteins have very important functions such as emulsifying, foamability, encapsulation, and gelation in both the food and pharmaceutical industries (Wen et al, 2019). The techno-functional performances of protein, which affect the behavior of protein, quality, and sensory properties in food, play an essential role in foods during storage and processing (Kinsella, 1976). The potential uses of plant-based proteins depend on their functionality in food systems. The poor techno-functional of plant-based proteins is the most noticeable drawback for industrial applications as an ingredient.

The functionality of a protein is related to its molecular structure (molecular weight, shape, amino acid sequence). Many techniques have been used to modify protein's functional properties, such as chemical, enzymatic, and physical processes for industrial applications (Mirmoghtadaie et al, 2016). But the modifications based on the chemical methods can affect the nutritional value of the foods, and cause health problems. The physical processes (extrusion, high-pressure, and ultrasound homogenization) are safe, and can be used to obtain attractive and multi-functional proteins for different food applications (Sim et al, 2022). High-intensity ultrasound (HIUS) homogenization is one of the most studied physical methods for modifying protein structure. The basic principle of HIUS is based on the microbubbles generated by sound waves, which create the shear stress and cavitation force with heat and turbulence. HIUS has many advantages such as wide waves frequency, simplicity, cost effective, energy saving, and environmentally friendly (Wen et al, 2018; Zhang et al, 2018). Recently, the effect of high-intensity sonication on the physicochemical properties of plant-based protein and, in parallel, its reflection on techno-functional

characteristics were intensively studied. Several authors investigated the effect of HIUS on the physicochemical and functional properties of soy (Jambrak et al, 2009; Hu et al, 2013), pea (Xiong et al, 2018), black bean (Jiang et al, 2014), sunflower (Malik et al, 2017), faba bean (Martinez et al, 2018), millet (Nazari et al, 2018), quinoa (Vera et al, 2019), peanut (Zhang et al, 2014), walnut (Zhu et al, 2018), rapeseed (Dong et al, 2011) and hempseed (Karabulut and Yemiş, 2022; Karabulut et al, 2022) protein isolate.

High-pressure homogenization (HPH) produces elevated shear forces, cavitation, and turbulence, conducive to protein structural changes. Also, the HPH technique is beneficial for food processing and preservation (Lakshmanan et al, 2007). HPH up to 350 MPa is a typical process for forming a pure and fixed emulsion in food, pharmaceuticals, and cosmetic products. The influences of HPH on the interface layers' structure and protein conformational change have been extensively studied (Bader et al, 2011). Recently, HPH has been used to modify the structure and functionality of several plant proteins (Song et al, 2013; Liu et al, 2016; Sun et al, 2014).

Although many studies have addressed the impact physical modification methods on the techno-functional performances of some plant-based protein isolates, there has been no study on the modification of techno-functional performances of date seed protein concentrates by HIUS or HPH. Limited studies on date seed proteins have essentially focused on the extraction methods and the physicochemical and functional properties of the protein concentrate obtained by these methods (Bouaziz et al, 2008; Al-Juhaimi et al, 2012; Akasha et al, 2012, 2016; Khatib et al, 2022).

1.1. Aim of the investigation

The purpose of the study was to enhance the techno-functional performance of date seed protein concentrate (DSPC) by high-intensity ultrasound (HIUS) and high-pressure homogenization (HPH) as two novel physical methods of protein modification.

In this study, DSPC was first prepared by a conventional method, which was alkaline extraction and then isoelectric precipitation. The effect of the independent process variables of HIUS and HPH on the solubility and antioxidant activity of DSPC was

investigated, and the optimal process conditions for maximizing the protein solubility were analyzed by the central composite design of the Response Surface Methodology (RSM). After processing at optimum conditions, the techno-functional performance (solubility, foaming, emulsifying, water/oil binding, and antioxidant capacity) of DSPC samples treated by optimal HIUS and HPH conditions was analyzed and compared to the native DSPC samples.

2. REVIEW OF LITERATURE

2.1. Importance of Protein in Human Nutrition

Protein is one of the main components of human nutrition, which is very important for human health as maintenance of muscle mass (Devries and Phillips, 2015), immune responses (Mowat, 1987), cell signaling (Scott and Pawson, 2009) and repair of damaged cells (Kastan et al, 1991). The value of a protein as a dietary element depends on the amount of essential amino acids that meet standard requirements and conform to the patterns required for a healthy diet (Boye et al, 2012; Marinangeli et al, 2017). Protein properties such as digestion, bioavailability, and amino acid content, especially the essential amino acids, have a positive effect on the protein value (Nenova and Drumeva, 2012). Dietary protein requirements are defined as the lowest amount of dietary protein required to offset nitrogen damage in the body and to maintain the protein mass that keeps the structure and function of the organism and grows, as reported by Berryman et al. (2018).

2.2. Natural Sources of Protein

It is common knowledge that meat and meat products, chicken and fish, and, dairy products have been defined as animal proteins. Likewise, different plant groups such as whole grains, legumes, nuts, fruits, vegetables, potatoes, and other plants have been used as plant protein sources (De Gavelle et al, 2018). Animal proteins are not available cheaply for all people worldwide (Hartmann and Siegrist, 2017). World production of meat and dairy products is projected to increase by more than 4.35 and 8.43 million tons respectively by 2050 for meat and dairy products as reported by Boland et al, (2013).

Although animal protein has several benefits, the accessibility of food and energy has a negative impact on the environment. Therefore, the lack of animal protein should be solved by improving alternative protein sources. Protein resources derived from plant by-products, which are important sources of protein, are often overlooked. By-products have functional ingredients that are important for the utilization and

development of novel protein resources to meet food and health needs (Granato et al, 2017).

2.3. Plant Protein

Many plants were extensively investigated and used as protein additives, for example legumes such as soybeans, peas, beans, chickpeas, lupins, field beans, cowpeas (Sun et al, 2012; Coda et al, 2017), cereal grains such as rice, wheat, millet, sorghum, corn and barley (Lopez et al, 2018), pseudocereal grains such as amaranth, quinoa and buckwheat (Lopez et al, 2018), some plant seeds such as chia, flaxseed, sesame, pumpkin (Conde et al, 2005) and sunflower (Mattila et al, 2018), almonds and nuts (Sousa et al, 2011). Although many studies have revealed that most of plant-derived protein provide necessary quantities of essential amino acids for nutrition (Sun-Waterhouse et al, 2014), plant protein is considered lacking and has lower nutritional value than animal protein (Hughes et al, 2011).

Plant proteins have essential properties in both the food and medical industries, such as in the areas of emulsions, foaming capacity, encapsulation ability and gel formation (Quintero et al, 2017). Although plant proteins are susceptible to dissolution during processing, this can result in protein breakdown, and affect its functionality. Mostly, this may affect their use in nutritional programs (Thomas et al, 2007; Ma et al, 2003). Therefore, it is crucial to improve the techno-functionality of protein by developing additional efficient processing methods.

2.3.1. Agro-Industrial By-products as plant protein source

Protein producing from agricultural production wastes, by-products and food processing residues is a viable option for minimizing wastes management, maximizing resources, and increase the commercial value of various new products. In addition, it promotes the improvement of diet products with a limited price, as reported by Salgado et al. (2012) and Sun-Waterhouse et al. (2014). According to El-Adawy and Taha (2001) the discarded pumpkin press cake contains 70% protein that could be extracted and used as a food additive. Production of protein hydrolysates, protein concentrates and protein isolate alternatives are a good way for the conversion of waste and by-products into high quality products with high protein bioavailability and functionality (Venuste et al, 2013).

In the production of vegetable oil, a large volume of defatted residues with a high protein content were disposed of as waste which can be used as targeted ingredients in human nutrition and add value to them through commercial goods, as noted by Mattila et al. (2018) and Pojic et al. (2018). Several studies have brought the issue of food by-products more into the focus of scientists and practitioners (Galanakis et al, 2013). Food by-products are the main source of environmental pollution, therefore recycling food waste contributes to environmental protection and ensures the sustainability of food systems (Halloran et al, 2014).

Food waste was reported to be rich in in terms of functional ingredients, which are antioxidants, fiber, protein, carbohydrates, and other phytochemicals (Lordan et al, 2011). Protein has recently received a lot of attention, many plant protein has been extensively evaluated, such as soybean, pea, wheat, etc. (Day, 2013), which have many benefits such as low-cost, greenhouse gas reductions and renewable (Dijkstra et al, 2003). In addition, plant protein is also rich in basic amino acids to achieve human health requirements (Day, 2013). Certainly, the plant based protein resources are green, novel, and fast-growing area of the food processing technology. But, their intricate structure and properties need an evaluation of the functional properties that may arise from isolated protein derived from by-products (Kinsella and Melachouris, 2012; Devries and Phillips, 2015).

Global consumption of natural sources such as plant protein is increasing (Amagliani et al, 2017) and the inclusion of seeds in the diet is a recent trend to improve diet quality (Anaya et al, 2015). Previous studies have indicated that some agricultural wastes were considered plentiful in protein (Aiking, 2011). Recently, according to benefits of plant protein such as higher nutritional value, religious concerns, low cost, excellent nutritional profile, especially essential amino acids, they are being widely used as a source of protein instead of animal protein in food formulations (Mir et al, 2019).

2.4. Functional Properties of Protein

Functional properties of proteins are classified as physical and chemical properties that play a critical role in the food system during storage, handling, preparation, and consumption and that influence the behavior, quality and organoleptic properties of proteins in food (Kinsella, 1982). There are two factors that impact on the functional

properties of a protein (internal and external factors). The internal factors could be specified as follows: molecular shape, particle size, amino acid profile, the negative charge distribution, the hydrophobic/hydrophilic ratio, the protein structure, and the protein's ability to react with other ingredients in the food environment (Damodaran, 1997). The external factors affecting the functional properties of proteins were pH, heating, humidity, chemical additives, mechanical handling, enzyme action, and ionic concentration (Kinsella, 1982). Vaclavik and Christian (2003) suggested that some proteins are related to specific functions in the food industry, such as egg protein with coagulation or soy protein gelation. Before using proteins in food systems and food products, it should be evaluated whether they are appropriate and applicable by characterizing the functional properties of proteins (Kinsella, 1982).

2.4.1. Solubility of protein

It is well known that the application of proteins in food systems depends on a protein's solubility, and other functional characterizations such as emulsification, foaming and gelation are important in this trend (Vaclavik and Christian, 2003). Solubility depends on the balance between hydrophilic and hydrophobic interactions. pH, temperature, and ionic strength are major factors affecting the solubility of a protein (Bolontrade et al, 2013). In addition, other critical factors directly affect protein solubility, such as freezing, heating, drying, and shearing (Vaclavik and Christian, 2003). On the other hand, insoluble protein is unfavorable for food applications and therefore it is important that the thermal energy induced by denaturation is controlled such that protein solubility remains unaffected (Raikos et al, 2007).

2.4.2. The protein emulsification

Emulsions are an important type of food and have been widely studied (Tan et al, 2017). Emulsions consist of two fluids that mix, one of the liquids being scattered in the other in the shape of tiny droplets within a continuous layer of the other formed by the presence of emulsifiers under mechanical shear. The system in which oil droplets propagate through a liquid phase is referred to as an oil-in-water (O/W) emulsion. Dickison and McClements (1995) explained that droplets in an emulsion are called the internal phase, since ambient fluid is referred to as a continuous or external phase. Once water and oil are mixed, they quickly separate into two layers, a top layer of high-density oil and a bottom layer of low-density water. This is called phase

separation and has to do with the droplets merging with neighboring droplets that are similar to them.

In general, the average width of the droplets in emulsion ranged from 0.1 to 100 nm as reported by Zhang et al. (2014). According to Tan et al. (2017), considering the main character, emulsion stability depends on the interaction between oil and water droplets. In addition, Celis et al. (2016) have shown that emulsion stability is also related to the emulsifiers, methods, and emulsion conditions such as pH and ionic concentration. Furthermore, Dickison and McClements (1995) showed that emulsifier is an active surface particle that allows two separate phases to mix without separation for extended periods of time. These active surface molecules were amphiphilic, and they have both hydrophobic and hydrophilic parts, allowing two liquids to mix.

2.4.3. Foaming ability and foam stability of protein

Foams are described which consist of a gas phase, a liquid phase and a surface-active agent and form foams by beating or shaking. Foods made from foam, such as whipped cream, meringues, ice cream, chiffon desserts, and angel cake (Yang and Baldwin, 1995). Foams are created by expanding and absorbing the protein at the air-water interface and by forming a film over the gas bubbles. Proteins have different talents for creating and stabilizing foams and as in the case of protein and their completely different emulsifying properties, this may be related to completely different physical properties of the proteins. Formation and stability of foams depend on protein types and their ability and emulsifying properties based on physical properties of proteins. Emulsification properties require that protein should also have a high solubility in liquid due to its ability to move quickly around the bubbles within the food media. The external factors affecting foam properties are pH, temperature, and ionic strength. The protein is expected to show limited denaturation at the surface to maintain viscosity and rigidity (Kinsella, 1982).

2.5. Modification of Protein

Protein substances are used extensively in the food processing for their dietary advantages as well as functional properties. The amphiphilic character of proteins forms them into flexible structural elements in food arrays thus they function as dynamic surfactants for protein–polysaccharide, protein–oil, protein–gas, protein–

water interactions (Osullivan et al, 2016a). Awareness of food security and the challenge of an ever-growing world population are driving the search for sustainable and environmentally responsible nutrient-dense foods, especially alternative protein sources, which require the development of techniques to increase digestibility and bioavailability (Adenekan et al, 2018; Sa et al, 2019).

Interestingly, plant-based proteins are used as a cheap and flexible alternative source of animal protein in food and as functional components for product formulation (Alemayehu et al, 2015; Lopez et al, 2018). Most of the original protein does not have the desired functionality for use in many different industries. Protein functionality could be modified using physical and chemical methods. Due to the negative effects of chemical methods on human health, numerous physical modification strategies have received more attention (Singh et al, 2018).

2.6. Modification of protein using High Intensity Ultrasound Homogenization (HIUS)

Ultrasound is defined and widely used as a new, environmentally friendly, future-oriented, and durable technology. Sonication is a mechanical, non-ionizing, environmentally friendly energy option (Unver, 2016) that has potential applications in high-value food production. In addition, sonication had a positive effect on the functionality and physio-chemical properties of food (Terefe, 2016), improves their productivity and performance (Kentish and Feng, 2014). In addition, ultrasound is used to determine the composition of meat products, vegetables, cheese, oils, bread, and grains. In addition, it can be used to detect contamination in honey. Generation of HIUS can be achieved by an ultrasonic bath and/or portable, inexpensive ultrasound dipping probes for several purposes in food (Awad et al, 2012). These eco-innovation technologies can enable the reuse and reproduction of protein resources, provide food security, protect the environment, and confirm food system viability (Poji et al. 2018).

Many studies have been done on modifying proteins using ultrasound from several other sources such as whey protein by Jambrak et al. (2008), milk protein by Yanjun et al. (2014), soy protein by Hu et al. (2013a), wheat grain protein by Zhang et al. (2011), squid ovary by Singh et al. (2018) and other distinct proteins by Arzeni et al. (2012). Recently, sonication treatment has sparked interest in high-intensity ultrasound

due to its benefits for the modification of plant proteins (Zhang et al, 2014; Biswas and Sit, 2020).

2.6.1. Mechanism of ultrasound on protein modification

Sonication alters the functional properties of protein primarily through localized hydrodynamic shearing and heating of the protein molecules in solution, and consequently modifying their structure. Hydrodynamic shear is because of sonication power generated by the ultrasonic sonotrode. In addition, ultrasonic cavitation is characterized by rapid build-up and collapse of gas bubbles created by localized pressure differences in wave propagation over a short time. As shown in Figure 2.1., sonication decreases the particle size by hydrodynamic shearing, and exposes hydrophobic residues through localized heating (O'Sullivan et al, 2017).

Several theories have been suggested to explain the mechanism of ultrasonication. The frequency between 20 and 100 kHz with high intensity (10-1000 W/cm^2) used in high-energy ultrasound that causes mechanical changes due to the generation of high pressure (1000 atm) and high temperature (5000 K) during the cavitation phenomenon, were improved the physical or chemical/biochemical properties of proteins. In contrast, low-intensity frequency (1 W/cm^2) between 5 and 10 MHz has non-destructive effects and is used to ensure high quality and safety of food applications, as reported by Arzeni et al. (2012) and Hu et al. (2013).

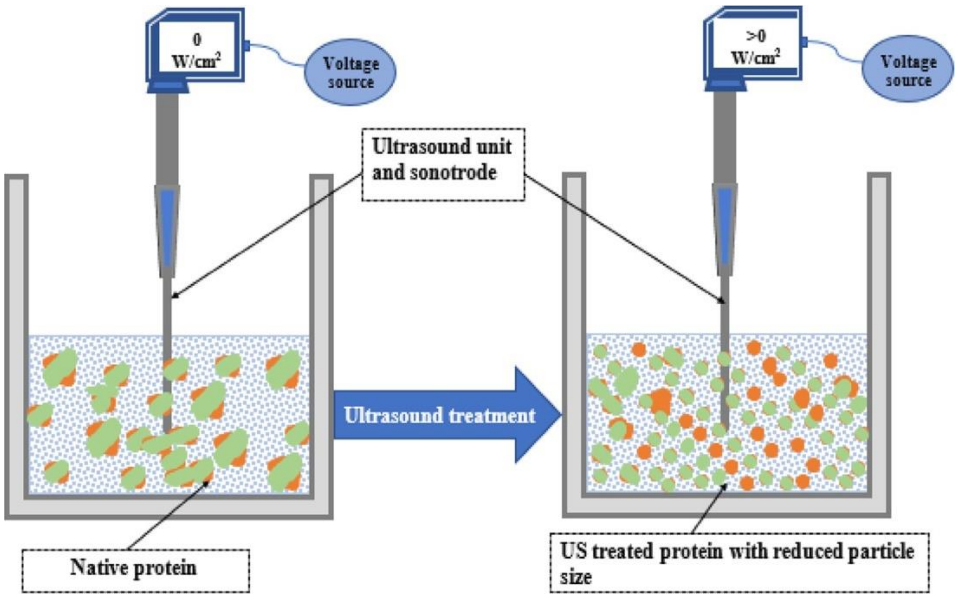


Figure 2.1. Ultrasound effect on protein solution before and after homogenization (Akharume et al., 2021).

2.6.2. Effects of HIUS on Proteins

According to Singh et al. (2018) physical and chemical methods were used to modify the protein to improve its functionality. However, chemical changes can affect the nutritional value of foods. Between different physical modification strategies, HIUS sonication has increased since its propagation in biological material induces the compression and decompression of bubbles that alter the physicochemical properties of the material and improve the quality of different systems, as reported by Higuera-Barraza et al. (2016). Sonication is one of the protein modification methods, defined as a sound wave with a frequency exceeding the hearing limit of the human ear (20 kHz), is simple, inexpensive, energy-saving and environmentally friendly technique (Zhang et al, 2014; Wen et al, 2018). Furthermore, other publications have shown that sonication can change the physicochemical and techno-functional properties of the protein (Malik et al, (2017).

Jiang et al. (2014) included low-frequency 20 kHz ultrasound treatment used at different powers and different time periods on techno functional properties of the isolates of black bean proteins. Resultant change in tertiary protein formation after sonication across the fluorescence emission spectrum in which the particle size had decreased approximately after 24 minutes of treatment. Furthermore, the soy protein extract yield and cost were designed to be significantly reduced by the sonication process, which generates high concentrations of free amino groups in short periods of time, compared to time-consuming heating processes (Mu et al, 2010). The ultrasonic homogenization technique deserves great attention due to its propagation through biomaterials causing compressions and decompressions. This is due to the significant modifications in the native structure of proteins that are desirable in terms of the functionality (Higuera-Barraza et al, 2017). Also, Zhu et al. (2018) carried out the probe sonication at different power levels of 200, 400, and 600 W for 15 and 30 minutes, respectively. They found that techno-functional properties such as protein solubility and emulsification had improved after sonication treatment.

O'Sullivan et al. (2016a) discovered that US treatment reduced the particle size of pea and soy protein isolates, which is related to hydrodynamic shear forces caused by ultrasound treatment. The functional properties of the investigated protein samples, such as solubility and emulsification, improved significantly after sonication. Previously, many studies have found that sonication impact on the structural properties

and improves the functionality of plant protein, such as protein isolate from grain by-products (Ren et al, 2015; Yang et al, 2017), legume by-product proteins (Jiang et al, 2014; Liu et al, 2016) and oilseed protein (Wang et al, 2016; Malik et al, 2017). Recently, Martnez-Velasco et al. (2018) revealed that HIUS is an effective instrument to improve foam formation and surface properties of faba bean. Flores-Jimnez et al. (2019) also examined the effect of HIUS on rapeseed protein isolates and found a general development of protein functionality.

Table 2.1. The studies on the effect on the effects of ultrasound treatment on the functional properties of some plant-based protein.

Protein	Protein Functional properties				References
	Solubility	Gel	Foam	Emulsion	
Wheat	_	_	↑	↑	Zhang et al. (2011)
Wheat Germ	↑	_	_	↑	Yang et al. (2017)
Soybean	_	_	_	↑	O'Sulivan et al. (2016)
Soybean	↑	_	_	↑	Chen et al. (2012)
Soybean	↑	_	_	_	Hu et al. (2013)
Black Bean	↑	_	_	_	Jiang (2014)
Mung bean	_	↑	_	_	Malik et al. (2017)
Faba Bean	↑	_	↑	_	Martinez-Velasco et al. (2018)
Sunflower	↑	_	↑	↑	Malik et al. (2017)
Rapeseed	↑	_	_	↑	Dong et al. (2011)
Peanut	_	_	_	↑	Zhang, et al. (2014)

2.6.3. Effects of HIUS on the functional properties of protein

Functional properties of proteins can be divided into three categories, the first group is hydration properties such as solubility, dispersibility and swelling. The second group is emulsifying properties such as emulsification, foaming and water/oil holding capacity. The third group is rheological properties such as gelation, and elasticity (Zayaz, 2012). The ultrasonic homogenization process improves the functionality of the protein extracted from plant by-products, including solubility, gelling, foaming, and emulsifying properties.

2.6.3.1. The effect of HIUS on protein solubility

Solubility is a favorable attribute for the use of protein isolates in combination with food and enhances protein denaturation due to exposure to thermal and chemical processes. In addition, sonication has shown a substantial enhancement in protein

solubility of tamarind seed protein isolates compared to the native ones. Arzeni et al. (2012) cleared that ultrasonic treatment can weaken compounds, break down the present hydrogen bonds in proteins and cause structural changes that could improve protein solubility. In addition, sonication could reduce aggregate particle size, improve protein-water interaction, and increase solubility.

In addition, the effect on protein solubility is primarily due to one or more of the following: The ultra-sonication can (a.) change the secondary and tertiary architecture of proteins, exposure of hydrophilic amino acid components; (b.) decrease the molecular weight of the protein; and (c.) rise the temperature of the reaction systems. The possible changes in contact of the protein surface with water and improve solubility (Jambark et al, 2008, 2009). O'Sullivan et al. (2016b) concluded that sonication has the potential to improve the solubility, emulsification ability, and stability of protein isolates that otherwise exhibit low functionality.

Hu et al. (2013a) and Jambrak et al. (2009) reported similar results for soybean isolate, which showed increasing solubility after sonication. Recently, Biswas and Sit (2020) found that sonication increased the water holding capacity (WHC) of isolated proteins from tamarind seeds, which could be attributed to unfolding of the aggregates due to sonication revealing hydrophilic groups. Furthermore, they reported that the hydrophilic/hydrophobic rate of amino acids in the protein molecules, and carbohydrates, lipids and tannins associated with protein, and several parameters such as particle size, shape and steric factors are also responsible for changing the WHC. Jiang et al. (2014) suggested that sonication improves the solubility of proteins. Increasing the intensity and treatment time of sonication also improves solubility, which is attributed to the partial dilatation of a protein molecule to promote protein-water particle interaction.

Sonication led to decrease the particle size of protein disturbances, facilitating solubility due to the larger surface area between the protein and the water molecule, as reported by Liu et al. (2017). Similar results recorded for millet protein concentrates showed increasing solubility with sonication by Nazari et al. (2018) and for walnut protein by Zhu et al. (2018) and soy protein by Arzeni et al. (2012). According to Tang et al. (2009) the increase in solubility after sonication could also be due to numerous factors such as breaking of internal hydrogen and hydrophobic bonds, exposing hydrophilic groups of amino acids to a polar environment. In addition, cavitation also

led to a decrease in the molecular mass of the protein, which was accompanied by an increased interaction of protein and water particles. But on the other hand, the reduced value of the solubility was also observed with a long sonication time of 35 minutes. This can be attributed to aggregations of denatured protein, leading to the formation of higher molecular weight complexes, which in turn reduces protein solubility, as reported by Malik et al. (2017).

2.6.3.2. The Effect of HIUS on emulsification of protein

The properties of the emulsion are important as one of the functionalities of proteins affecting the performance of food. Emulsification describes the ability of a protein to be adsorbed to the oil/water surface and is expressed in terms of the interfacial stability per unit mass of protein. Oil-in-water emulsions are volatile systems that are liquid and quickly separate in a short time. To improve the stability of food emulsions, the addition of a chemical emulsifier and/or a stabilizer is necessary in conjunction with intense mechanical strength as defined (homogenization treatment) to decrease the droplet size and thus retard the split phase kinetics, as defined by McClement's (1999).

Several studies are focused on using proteins and polysaccharides as natural food components as natural emulsifiers and stabilizers (Evans et al, 2013; Albano et al, 2019). In addition, Evans et al. (2013) reported that the adsorption of proteins at the oil/liquid interface depends on the amino acid content, since adsorption is driven by hydrophobic residues that can be used to interact with the oil phase.

Emulsifying activity and stability showed that emulsion activities and stability increased with sonication with time and intensity level. The highest emulsifying activity and stability of (79.41%) and (82.53%), respectively, were obtained for the tamarind seed protein isolates processed at an intensity of 200 W for 30 minutes, as recorded by Biswas and Sit (2020). The activities of the emulsions are attributed to solubility, surface charges, surface hydrophobicity, and molecular exbibits of the protein. Sonication can change the functionality of proteins by certain secondary structural shifts, as revealed by Kresic et al. (2008).

According to Evans et al. (2013) the stability of emulsions can be achieved using proteins and polysaccharides through different mechanisms, which can be conjugated to generate static electricity and enhance an individual property of each polymer under certain conditions. In addition, there are several factors that affect the formation of the

protein-carbohydrate complex, for example pH, heat, ionic strength, homogenization methods and the natural protein: saccharide ratio. On the other hand, the stability of emulsions could also take advantage of the interaction between protein and polysaccharide, thereby creating the appropriate amphiphilic forms. This is based on the essential character of individually macromolecule, for example flexibility, conform state, and charge density, which in turn are directly impacted by the physicochemical terms of the medium, like pH, ionic strength, temperature, and treatment conditions (Albano et al, 2019).

The cavitation phenomenon is the main influence on the liquid medium arising from the formation, growth and collapse of vaporized gas microbubbles resulting from the compression and expansion of medium-sized molecules (Arzeni et al, 2012). In addition, sonication creates a stable emulsion with a narrow small droplet size distribution. Changes in ultrasonic conditions result in significant changes in oil droplet size and emulsion constancy produced (Kaltsa et al, 2013). The results of Biswas and Sit (2020) agree with of Jambrak et al. (2009); Zhang et al. (2011); Yanjun et al. (2014) and Stefanovic et al. (2017) for sonicated soybean, gluten, milk, and egg albumin proteins respectively. Higher levels of solubility facilitate more rapid partitioning of the protein into oil-water chains at the interfacial stages to promote amplifiability, as reported by Deep Singh et al. (2008).

2.6.3.3. The effect of HIUS on the foam activity of protein

From a technical point of view, proteins serve various non-food purposes, such as building or stabilizing structures in food, such as foamability and stability. Foams are considered gas-in-liquid distributions and their stability depends on the used process in their manufacture. Therefore, conditions that modify the functional properties of proteins attract significant research attention, due to the preservation of the sensory properties of food depends on the properties of these large molecules (Higuera-Barraza et al, 2017; O'Sullivan et al, 2017).

Foamability and stability of the native and sonicated tamarind protein isolate reported by Biswas and Sit (2020) who found that foamability improved significantly for the sonicated isolates. The improvement in foamability by ultrasonic homogenization could be attributed to the protein denaturation and the unfolding of the polypeptide chains. It is believed that the denaturation and unfolding of polypeptide chains exposes

the hydrophobic regions at the air-liquid interfaces important for the adsorption of the molecules, as reported by Kresic et al. (2008). Similarly, Barac et al. (2010) indicated that foam strength increases with ultrasonic homogenization.

The application of sonication has been used to improve the foamability of various animal protein like egg albumin (Stefanovi et al, 2017), chicken products (Xue et al, 2018), beef products (Amiri et al, 2018) and plant proteins such as wheat (Jambrak et al, 2014) and soybeans (Morales et al, 2015). This technique resulted in conformational modifications in protein structure, leading to protein unfolding, which in turn exposed the hydrophilic areas to the liquid phase and the hydrophobic areas to the air phase (Singh et al, 2018). Recently, the sonication treatment presented an alternative method for enhancing the foaming property of giant squid mantle proteins (Arredondo-Parada et al, 2019).

2.6.4. The application ultrasonic homogenized protein in nutritional industries

Ultrasonic technology is known to be widely used and applied in nutritional systems including food production during extraction, dehydration, homogenization, emulsification, and protein modification as shown by Chemat and Khan (2011). In particular, the ultrasonic technique converts proteins into high-quality food because of the production of food products with high quality and high nutritional value. Gupta and Nayak (2015) explained that the use of ultrasonic processes in food production involves the following essential steps: (a) as nutrient additives to support the diet; (b) as additives in meat products, food extrusion and our product processing; (c) as an encapsulant for the preservation of biologically active materials; (d) as a raw material to produce bioactivity peptides. Interestingly, vegetable protein is widely used as a bulking agent to improve dietary and sensory properties in the food industry, which includes processing processed meats, supplements for extrusions, and the processing of our production, among others as reported by Lin et al. (2017). Previously, several studies have confirmed that adding modified proteins to surimi, meat sauces, and cream production resulted in significant improvements in texture, flavor, and quality (Luo et al, 2008).

Ultrasonic treatment promotes the interaction between protein and other nutrients, which increases the food nutritional value. Additionally, plant-based renewable protein from agricultural by-products could be reused: as scavengers to protect and

expand suppressed material from environmental degradation. Yadav et al. (2011) mentioned biocompatible and biodegradable substances are commonly used in drug delivery. Ultrasonic treatment could significantly improve the process of encapsulation, which, in addition to the easily compostable properties, has economic value requiring less time and low energy consumption (Tang and Li, 2013).

Due to recent problems with people's increased demand for performance products, more attention has been paid to the commercialization of protein products. Hydrolyzed biologically active proteins and sonicated peptides as functional components are expected to have great commercial and industrial potential. Various studies reported that ultrasonic treatment not only greatly promoted the production of biologically active peptides such as (antioxidants, ACE inhibitory activity, antibacterial and hypoglycemic peptides, etc.), but also improved the supply of peptide drugs (Kadam et al, 2015). Zhu et al. (2018) showed that sonicated protein has great opportunities and prospects in the food industry. Therefore, it should be scaled to expand trading applications.

2.7. High Pressure Homogenization (HPH)

High-pressure homogenization (HPH) comprises high shear forces, cavitation, and turbulence, leading to structural changes of proteins. Also, the HPH technique is extremely useful for food processing and preservation. Continuous operation is cost-effective and highly efficient. HPH influences the nutritional and sensory properties of processed foods. When liquid foods pass through the homogenization valve, physical factors such as shear force, turbulence, and cavitation contribute to the breakup of the droplets and alter the protein structure and physicochemical properties of the food (Donsi et al, 2009).

2.7.1. Mechanism of HPH

HPH is a homogenization technique that results in a change in the physical structures, microbial and enzymatic inactivation, and technological function of food. Yong et al. (2021) have summarized in Table (2.2) the main differences between HPP and HPH techniques recently used in food technology. Augusto et al. (2018) reported that HPP system is pressure dependent while HPH technology is shear stress distribution dependent.

Table 2.2. Brief description of the distinctive high-pressure homogenization and high-pressure processing. (Yong et al. 2021)

Description	HPH	HPP
Theory	Allocation of shearing stress around products and modify product structure	Equal sharing of pressure in all directions in products also non-absorbent food shall preserve the original form
Pressure rating	100–350 MPa	400–600 MPa
Rise in temperature	15–18°C/100 MPa Irreversible	Adjustable after unpacking
Product phase	Liquid	fluid and solid
Process type	Continuous	Batches and semi-constant
Industrial usage	limited	Lot

The typical application of the continuous HPH process is summarized in Figure 2.2. The main element of the HPH consists of a homogenization flap and a high-pressure surge. However, in the first phase of HPH technology deployment, a pressure of 34 MPa was seen as HPH, recently pressures of more than 350 MPa can be used (Diels and Michiels, 2006). Harte (2016) reported that more than 100 MPa pressure could be used for liquid food. In general, a pressure greater than 200 MPa is defined as ultra-HPH treatment as summarized by Marszaek et al. (2017).

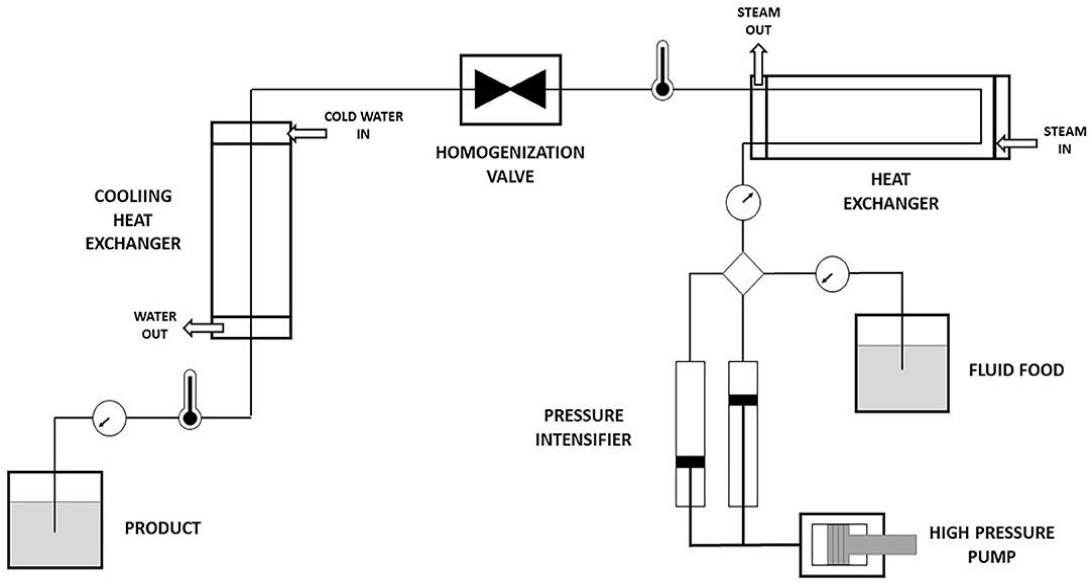


Figure 2.2. Schematic presentation of high-pressure homogenization technique (Yong et al, 2021).

2.7.2. The impact of HPH on proteins

HPH with pressures up to 350 MPa is a commercial process to form pure and stable emulsions in food, pharmaceuticals, and cosmetics. The influences of HPH on the structure of the interface layer and the conformational change of proteins have been extensively investigated using high hydrostatic pressure (Bader et al, 2011). Compared to hydrostatic pressure therapies, the dynamic pressure during high-pressure homogenization only lasts for a short time (~10^{-4} s) and at the same time various side effects such as shear stress, turbulence, cavitation, and temperature increase occur (Bouaouina et al, 2006).

Therefore, the effects of high hydrostatic pressure and HPH treatments on protein conformation and protein functionality cannot be associated. Some studies have reported on the effects of dynamically high pressures on the functional properties of globular proteins and reported that the functionality can be improved up to pressures of 150 to 200 MPa (Floury et al, 2002; Bouaouina et al, 2006). As reported by Balny and Masson (1993) it has previously been discussed how static high pressure affects protein structure and functionality as a function of protein structure, pressure level, and other external parameters such as pH, temperature, and solvent composition. In addition, the pressure disturbs the equilibrium of the molecule and the liquid-protein interaction, resulting in an altered three-dimensional architecture of a protein fragment.

2.7.3. The impact of HPH on the functional properties of protein

The HPH technique is widely used to stabilize dairy products by destroying milk fat globules (Diels et al, 2005). Also, HPH is a significant process to obtain fine emulsions, especially protein-stabilized emulsions, and solubility in soy protein (Keeratiet and Corredig, 2009), lupine protein isolate (Bader et al, 2011). Bouaouina et al. (2006) reported that HPH up to 300 MPa improves the foamability of whey protein isolates. Shen and Tang (2012) suggested that the exchange of sulfhydryl groups and disulfide bonds may be involved in the formation of soluble soy aggregates induced by HPH at 120 MPa. Keeratiet and Corredig (2009) reported that HPH at 65 MPa caused changes in the aggregation status of soy protein due to partial protein unfolding. Recent studies reported that HPH affects protein aggregation such as emulsion and foam interfacial activities (Floury et al, 2002).

However, further research is needed to understand how HPH affects protein accumulation and shape in solutions, and how such changes can affect protein solubility and functional properties (Bader et al, 2011). Furthermore, most of the previous studies has only focused on protein and enzymes of milk and dairy (Martinez et al, 2011). Functional characteristics identify the effectiveness of protein applications for the food industry. In general, natural protein has great functionality required for food processing. Non-thermal techniques are often used to improve techno functional properties of proteins. Recently, HPH has been used to modify the structure and functionality of several plant proteins (Liu et al, 2011).

2.7.3.1. The impact of HPH on solubility of protein

Sashikala et al. (2015) explained that good solubility for process handling, protein functional characteristics, consistency and sensory qualities are required to stabilize the food. Yang et al. (2018) reported that the solubility of 1% native faba bean proteins at pH 7 increased from 35% to (98 and 99%) after treatment with HPH at 15 and 30 kpsi, respectively. The increase in solubility value is a consequence of the separation of the large insoluble protein.

Recently, Wu et al. (2019) reported that increasing HPH up to 120 MPa improved the solubility of treated myofibrillar protein samples to 81.1% compared to 40.5% for untreated. This may be because shear forces generated by HPH treatment damaged hydrogen bonding and hydrophobic interactivity, contributing to protein aggregates. Additionally, the HPH reduced the particle size of the investigated protein samples. When the particle size is smaller, it has more surface areas, which promotes interaction in protein solutions (Bader et al, 2011).

2.7.3.2. The impact of HPH on emulsification of protein

According to previous studies focusing on the effect of HPH on protein aggregation and conformation, particularly at the interfaces to disrupt emulsifying properties, it has been suggested that this technology is also a useful method to prepare pure emulsions, especially protein-stabilized emulsions. Also, Keerati-u-rai and Corredig (2009) showed that HPH at 65 MPa modified soy proteins as proteins were partially unfolded. Emulsion capacity decreased from 27.0 m^2/g for untreated sample protein to 22.7 and 19.7 m2/g after HPH at 15 and 30 kpsi pressure, respectively.

In addition, the emulsification stability also decreased from 39.9 to 16.6 min when the pressure level was increased to 30 kpsi for the investigated field bean protein isolated by Yang et al. (2018). Also, Wu et al. (2019) reported that emulsifying capacity and stability had improved significantly after HPH up to 120 MPa for myofibrillar protein. On the other hand, Liu et al. (2011) revealed that the HPH of 160 MPa had negatively affected the emulsifying properties of whey protein due to the separation and reaggregation of the studied protein samples after the treatment. The emulsifying properties were increased by HPH up to 80 MPa for myofibrillar protein. Then, increasing the pressure by 120 MPa decreased the EAI while continuing to increase the ESI, as reported by Chen et al. (2017).

Recently, Wu et al. (2019) reported a reduction in droplet size of myofibrillar protein treated with HPH. Droplet reduction was also demonstrated by Yang et al. (2018) for HPH-treated soy protein isolates, which improved the emulsifying properties of the samples studied.

2.7.3.3. The impact of HPH on Foamability of protein

It has been reported that HPH up to 300 MPa improves the foaming properties of whey proteins, related to the dissociation of large protein aggregates (Bouaouina et al, 2006). Maresca et al. (2017) discovered that the foam ability of bovine treated at 100 MPa was increased due to protein conformational changes. Grácia-Juliá et al. (2008) discovered that HPH at 250 to 300 MPa stimulated the accumulation of isolated whey proteins through hydrophobic interaction. Another study by Shen and Tang (2012) suggested that the interactions of sulfhydryl groups and disulfide bond might participate in the form of soluble aggregates in soybeans caused by HPH at 120 MPa.

Yang et al. (2018) showed that foamability improved significantly from 91% to 259.6% by increasing the pressure level up to 30 kpsi HPH for 1% broad bean at pH 7. This increase in foam capacity could be related to diffusion and adsorption, which lowers surface tension, as suggested by Althouse et al. (1995). Wierenga et al. (2003) also elucidated that HPH can modify faba bean protein isolates to make the surface more hydrophobic, which reduces the energy of protein aggregates.

Tabilo-Munizaga et al. (2019) illustrated that the big protein aggregates were separated into soluble small molecule aggregates which migrated faster than the large aggregates. There was no significant difference in the stability of foams stabilized by

lentil proteins before and after HPH, both showing high foam stability. Conversely, HPH had a negative impact on faba bean protein foamability (Yang et al, 2018).

The opposite effects of HPH on the foaming and emulsifying properties have been observed in previous studies on whey protein (Baier et al, 2015). This could be partly related to a reduced viscoelasticity of the interface (Murray, 2011). On the other hand, this may also be related to occlusion effects caused by the soluble small molecular aggregates (Yang et al, 2018).

2.8. The Date Palm Fruit (*Phoenix dactylifera L.*)

The date palm tree (Figure 2.3) is regarded as the oldest tree in the world. It was grown in regions of North Africa and the Middle East around a thousand years ago, although the exact place where it was first cultivated is not known (Zaid and Wet, 2002). Several studies mentioned that date palms have been growing since almost 4000 BC in ancient Iraq (Popenoe, 1973) and in ancient Egypt around 2000-3000 BC (Manickavasagan et al, 2012). In addition, there are over 2000 different varieties of date palms worldwide, however few were used to produce a high-quality date fruit. Date fruits play an essential role in human society in foods and medicines used to treat many nutritional disorders such as obesity (Sulieman et al, 2012).

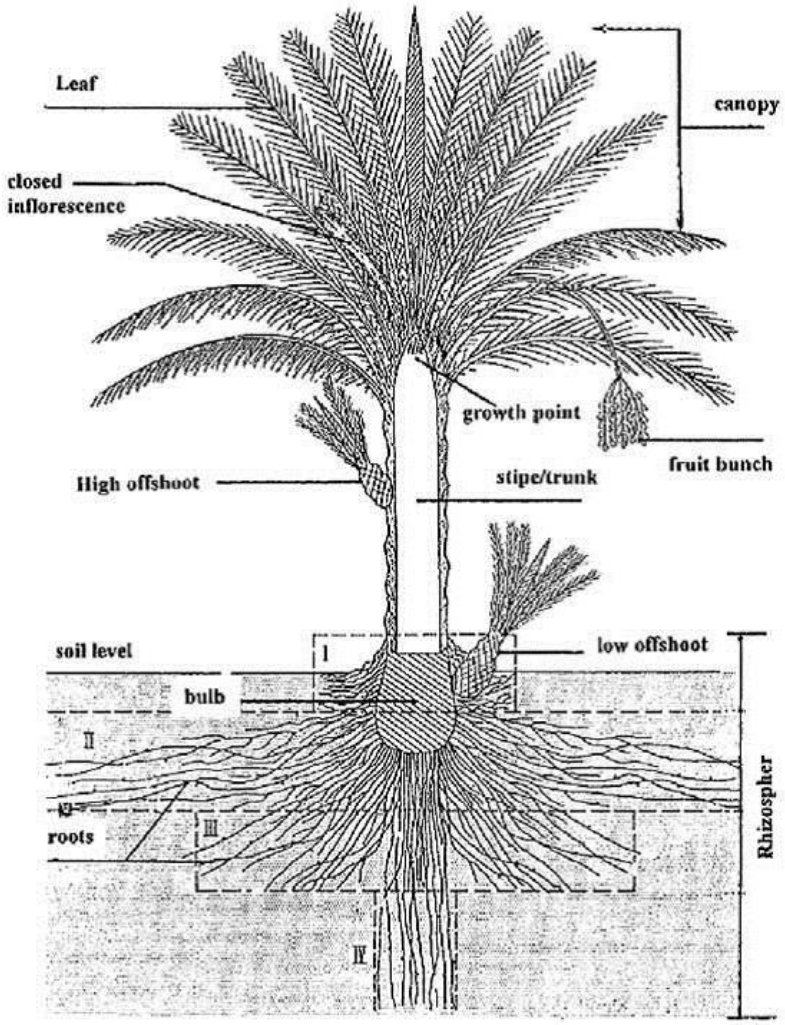


Figure 2.3. The cross section of date palm tree shows the distribution of roots, offshoots, leaves, and fruits according to Zaid and Wet (2002).

2.8.1. The worldwide distribute and fruit producing of date tree

In general, date fruit is a clear case of crops that are closely related to human behavior and can be grown worldwide. It is usually found in locations between 39 north and 20 south and specifically on five continents. The Middle East and North Africa are believed to be the main areas of date palm production, where about 90% of the world date fruit crop is produced (Manickavasagan et al, 2012).

Worldwide production, industrialization and use of dates has steadily increased. World production of date fruit had increased significantly to 7 million Mt in 2005, compared to 2 tons in 1960, as reported by Chao and Krueger (2007). After this higher global production of date palm fruit, about 800,000 tons of date palm seeds are currently unused. Also, the world production of date palms in (2012) was 7.5 million Mt.

Several reports showed that Egypt, Iran, Algeria, and Saudi Arabia are considered the most producers (Figure 2.4), with a high production volume of more than one million tons per country as annual production level (Abdul Qadir et al, 2011; FAO 2008; FAO, 2014), which accounts for about half of the world production of date fruit. According to the FAO (2020) around 75% of global date production takes place in the Arab world. In Saudi Arabia, production reached 1.5 million tons, increasing from 14.4% to 17.0% of world production.

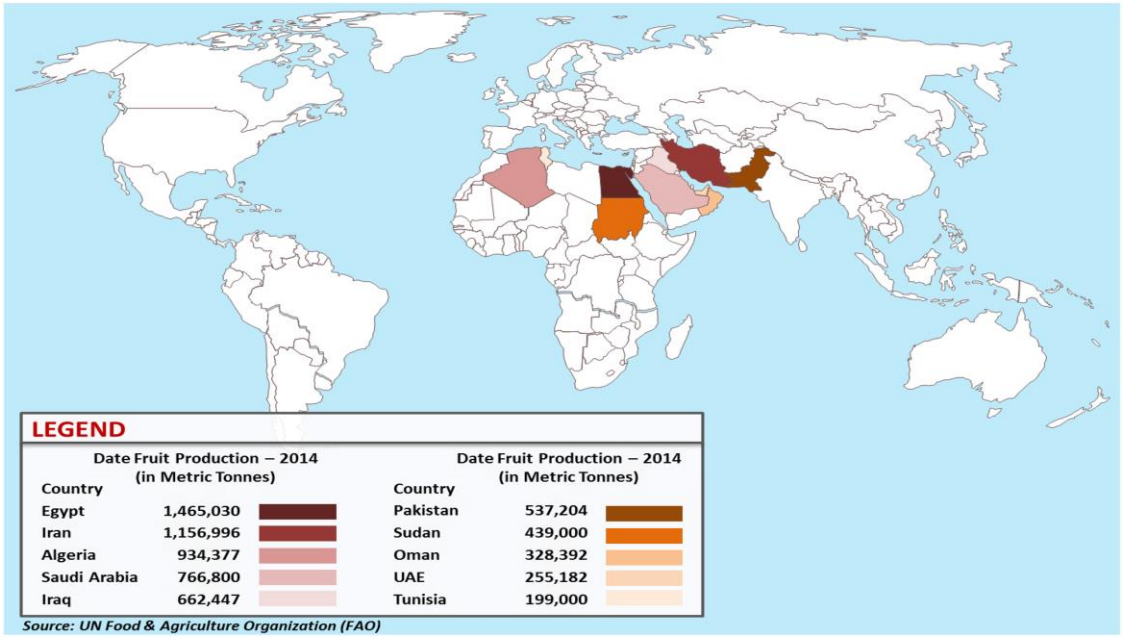


Figure 2.4. Global map of the 2014 worldwide top ten producers of date fruits. (Al-Alawi et al. 2017).

2.8.2. The stages of maturity in date fruit

After pollination, date fruits grow through five ripening periods as shown in Figure (2.5). Periods referred to as Hababouk Stage, Kimri Stage, Khalal Stage, Rutab Stage and Tamar Stage in order of age. Each stage can be identified by specific physical properties and chemical composition. Therefore, stages have usually been explained by accounting for flavor, color, texture, and chemical changes in fruit content, as explained by Tafti and Fooladi (2006).

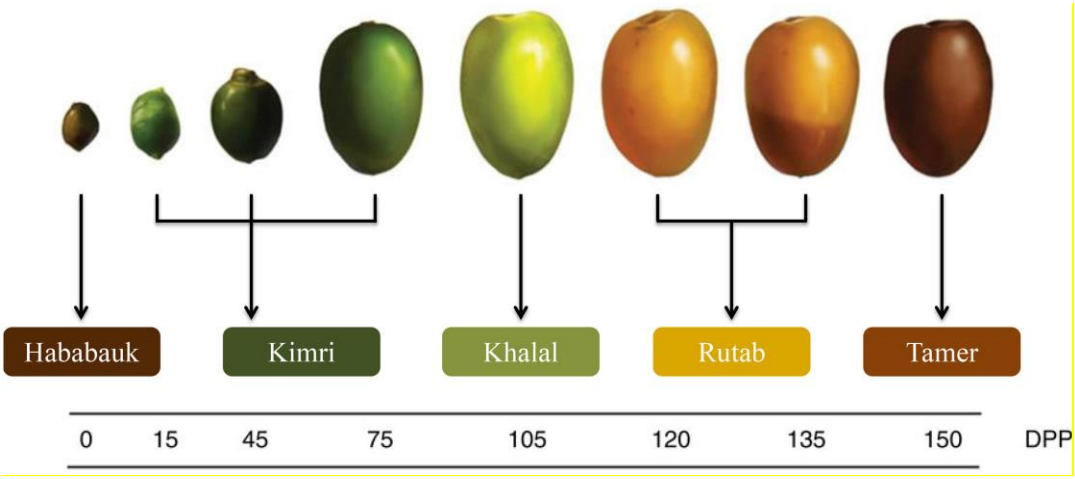


Figure 2.5. Stages of growth and maturity of date fruit after the pollination process (Al-Mssallem et al, 2013).

2.8.3. Dates fruit harvest and manufacturing

The harvest period of dates normally starts in summer and continues for about 2-3 months, it is not always possible to consume immediately. Thus, post-harvest processing places may get vast quantities of fresh dates in Tamar batches within short, which is over then fresh consumption needs. For this reason, producers should store this over amount for late period of marketing (Ismail et al, 2008).

Date fruit might be ready to eat at Khalal, Rutab or Tamar stages. Several different varieties were not permitted to get to the Tamar period, and they are available commercially as either a Khalal or Rutab stage (El-Hadrami and Al-Khayri, 2012). While, just at the fruits which in the complete maturity could be store to any farther process like eating and manufacturing (Al-Yahyai and Al-Kharusi, 2011).

2.8.4. Nutritional value and medical uses of date fruit

Dates have a fleshy sensation in the mouth and sweetness, which provide important nutrients and are the supposed advantages for human health (Manickavasagan et al, 2012). Date fruit by-products could be a valuable resource of dietary fiber and phytochemicals like phenolics which play an important role as natural antioxidant which might be food additive as reported by Al-Farsi et al. (2007).

Date fruits could have a potential health advantage against many different cancers as suggested by experimental evidence and phytochemical composition (Al-Alawi et al, 2017). It was used as a medicinal herb in many states and still proves valuable tradition or in new medicine (Krentz and Bailey, 2005). Date fruit is commonly used to treat a

sore throat, brunch wheezing, the common cold, infections, edema, cystitis, and alcoholism. Also use in treating abdomen and hepatic disorders as reported by Barh and Mazumdar (2008).

2.8.5. Benefits and uses of date palm tree parts

Every part of the date palm tree can be useful in human life. Various parts of palm tree were used as animal feed, pulp, and paper and as a building material (Anwar, 2006; Ata, 2011). From the leaf of palm tree many simple issues could be made for example manual fan, mat, hut, containers, bread plate, and some other traditional devices as reported by Ata (2011).

Date fruits were edible fresh or utilized food industries for products such as jams, jellies, fruit bars, date syrup, date pies, alcohol, date chocolate, salads, sauces, baked goods, breakfast cereals, and date confections. This was taken whole fruit or mixed with dairy products or Arabic coffee (Bessbes et al, 2009; Manickavasagan et al, 2012).

2.9. Date fruit (*Phoenix dactylifera L.*) seeds

The world production of date palm is 9.45 million tons, and the main producing country was Egypt with 1.69 million tons of date palm (FAO, 2020). About 11-18% of the weight of date fruit is the seed, which consists of carbohydrates, fiber, fat, ash, and protein (Abdul Afiq et al, 2013). Date seeds are typically discarded, used as an ingredient in animal feed, or processed into decaffeinated coffee by the Arabs. Date seeds could be one of the plant protein sources. Additionally, date seed dust is used in traditional drugs (Boukouado and Yousfi, 2009) particularly in Egypt (Duke, 1992). Date seeds have been shown to have nutritional and biological value due to their content of bioactive compounds such as phenolic content and dietary fiber (Hamada et al, 2002; Habib and Ibrahim, 2011) which play an important role in the treatment of hypercholesterolemia and hyperglycemia and treatment of chronic diseases such as diabetes and liver disease (Duke, 1992).

2.9.1. The date seed structure

The date is a pitted fruit and consists of pulp, skin, and seeds. Date kernels are elongated with a belly groove, the seed length is between 0.5 cm and 1.5 cm.

Endosperm of seed is composed nearly entirely of linear mannan molecules which give fibrillar consistence and stiffness protecting seed from the mechanical damage (Rodriguez-Gacio, 2012). The date seed has a small embryo located in the center of the seed surrounded by thick-walled endosperm (Figure 2.6). According to Buckeridge (2010) the storage polysaccharides exist in many seeds as the fluent types, mannan, and gluco- or galacto- mannans, while in date seed the abdominal is mannans. According to date palm composition, seed, bulb, roots, and tubers contain many types of mannans as storage polysaccharides (Hagglund, 2002).

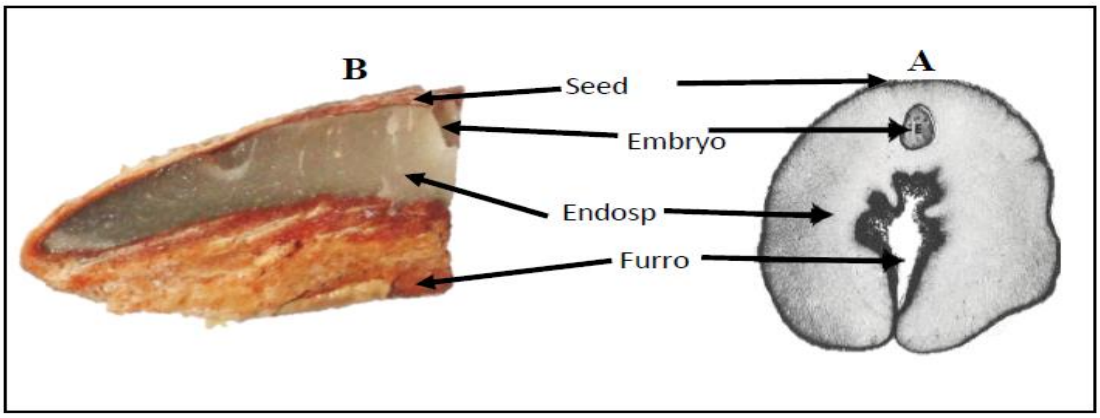


Figure 2.6. The date seed structural sections (Yousuf, 2018).

The main storage compound in date seed endosperm was lipid, composed of many tiny lipid organs, and protein, present as large peptide molecules. A wide range of proteins have been described in the endosperm of date seeds, which vary in size but appear to have a similar structure in each part of the seed (Demason et al, 1985).

2.9.2. Chemical composition of date seed

Date kernels contain a low moisture content while they contain a high amount of protein and lipids compared to date pulp. This describes the differences in metabolism that occur between the two parts of the date fruit. Date seeds have been reported to contain adequate amounts of fat, protein, and total carbohydrates (Bebes et al, 2004).

The seeds of date fruits also contain enough protein to provide an important study. The Sayer date variety had a high protein content (10.6%) compared to the other Iranian varieties studied. Similarly, EI-Shurafa et al. (1982) discovered that seeds from six date varieties growing in southern Libya have a maximum protein content of 6.42%.

Another study by Al-Showiman and Baosman (1992) reported that the proteins ranged from 4.79% to 7.50% of seven date varieties from different areas in Saudi Arabia. The carbohydrate content is different between date seeds and pulp. The carbohydrate in date flesh consists of mono- and disaccharides such as sucrose, fructose, and glucose. While date seeds contain a little number of simple sugars, the remaining carbohydrates are discovered as more complicated polysaccharides such as cellulose, hemicelluloses, and lignin (Manickavasagan et al, 2012).

Akasha, (2014) revealed that variation in the direct composition of date varieties was reported. According to several previous studies on date seed chemical composition, the approximate content ranged as (3.1-12.5 %) moisture, (5.1-12.5 %) fat, (2.3-6.9 %) protein, (0.84-1.2) ash, and (74-86.9 %) carbohydrate. On the same topic, Besbes et al. (2004) found that a date seed contains protein (5.56 and 5.17) %, fat (10.19 and 12.67) %, ash (1.15 and 1. 12%) and total carbohydrates (83.1 and 1.12%) contained 81.0) %. In addition, Al-Faris et al. (2007) showed that date seed consists of (3.10-7.10) % moisture, (2.3-6.40) % protein, (5.13-9.20) % fat. In addition, Bouhlali et al. (2015) reported that date seeds contained crude fiber (15.84–19.90); protein (4.31-6.144); lipid (566-6,97); ash (1.10-1.30); moisture (4.55-8.26); Total sugars (8.70-9.55) g/100g dw. Recently, Khatib et al. (2022) reported that the moisture content was very similar in all studied varieties, by a mean of 10 %, with exception of Barny variety, was 8%.

In terms of mineral content, date seed contains high levels of minerals; Potassium was the highest content and ranged between 2296 - 4153 mg/kg, magnesium 615 - 827 mg/kg, and calcium 395 - 626 mg/kg. On the other hand, sodium was lowest, ranging from 108.1 to 319.4 mg/kg. High concentrations of iron 27.7–70.3 mg/kg, manganese 5.5–11.0 mg/kg, and copper 4.8–0.3 mg/kg were measured as the micronutrient content found lowest micronutrient of dry weight (Bouhlali et al, 2015).

Most previous studies on the amino acid profile have focused on the pulp of the date fruit, however few studies have examined the contents of seeds. Hussein El-Zeid (1975) examined the seed of the Khalas variety of dates and reported that the amino acid profile contained 16 amino acids with significant amounts of essential types. Also, Bouaziz et al. (2008) found 17 amino acids in seeds of the date varieties Deglet Nur and Alleg. According to Al-Showiman and Baosman (1992) the essential amino acids, except for tryptophan, were found in five date varieties studied.

2.9.3. Phytochemical analysis of date palm seed

Since ancient times, the date palm has played an important role in the daily lives of people and their animals. Phytochemical studies have shown that the fruit contains multiple bioactive compounds such as anthocyanins, phenols, sterols, carotenoids, procyanidins, and flavonoids that have multiple beneficial effects on human health.

2.9.3.1. The phenolic content of date seed

Date seed was rich in phenolics (gallic, vanillic, caffeic, p-coumaric and syringic acids) in variable amounts (Al-Juhaimi et al, 2017). El-Hadrami and Al-Khayri, (2012) showed that many factors affect the phenol content in dates, such as growing conditions, geographical origin, varieties, stages of maturation, soil composition, time, manure, sampling methods and isolation and storage conditions.

According to Al-Farsi et al. (2007) the phenolic compounds of three Omani date variety seeds ranged between 3102-4430 (mg gallic eq.). In addition, Al-Farsi and Lee (2008) studied the phenolic content of the Mabseeli date seed variety. They reported that the main profile of phenolic acids is p-hydroxybenzoic acid, protocatechuic acid, m-coumaric acid, ferulic acid, p-coumaric acid, vanillic acid, o-coumaric acid, gallic acid and caffeic acid. Previously, a study by Mistrello et al. (2014) confirmed that the phenolic content of date seeds ranged between 2058 and 2983 mg GAE/100 g.

2.9.3.2. Flavonoid content of date seed

Several studies have shown that date seeds are a rich source of flavonoids, such as Bouhlali et al. (2015) who reported that the total flavonoid content of date seeds was recorded (1.659-1.844 mg RE/100g). Bouhlali et al. (2015) mentioned that date seeds contain high levels of flavonoids, which contain (1224-1844 mg RE/100g). These data agree with previous studies by Mistrello et al. (2014) who reported that the flavonoid content varied between (1271-1932 mg CE/100 g).

2.9.3.3. Antioxidant activity of date seeds

Dates have been used as a holiday food for many years due to their high polysaccharide content. It helps prevent disease with its biological activities such as antioxidant, anti-inflammatory and antibacterial properties (Taleb et al, 2016). Maqsood et al. (2015)

showed that the ability of date seed extract to scavenge DPPH radicals is related to the total content of phenols and flavonoids.

Date seeds improve immune system abilities and may also reduce the risk of cancer and cardiovascular disease because they contain high levels of phenolic components and nutrients such as fiber, fat, moisture, protein, ash, and vitamins (Bouhlali et al, 2015; Adeosun et al, 2016). Previous studies have shown that date components act as powerful antioxidants, anti-tumours and anti-inflammatory agents, providing a suitable alternative therapy in curing various diseases. It also has medicinal value, summarized in terms of therapeutic effects in disease control through antioxidant, anti-inflammatory, anti-tumor and anti-diabetic effects (Rahmani et al, 2014).

2.9.4. The Nutrition and medicine value of date seeds

Date fruits have a fleshy mouthfeel with a sweet taste and contain essential nutrients that play an important role in human health as reported by Manickavasagan et al. (2012). Furthermore, Al-Farsi et al. (2007) mentioned that date by-products and waste can be considered as a valuable source of dietary fiber, phenols, and natural antioxidants, since these date by-products, especially seeds, could serve as a functional food additive. Besbes et al. (2004) found that date seeds contain enough fat, protein, and total carbohydrate to meet some of the nutritional needs of animal feed. Additionally, the waste and seeds from date manufacturing could be a great source of food ingredients as they contain fiber and minerals with intriguing technical functionality. In traditional medicine, the powdered form of date seeds is used as an ingredient to relieve fever and toothache and used as a folk remedy to treat liver disease, diabetes, and gastrointestinal disorders. Also, Bouhlali et al. (2015) suggested that date seeds have the potential to be used as an ingredient in the food, cosmetic and pharmaceutical industries.

2.9.5. Benefits and applications of date seed

According to FAO (2010), more than 870,000 tons of date kernels were dismissed annually and disposed of as a by-product. The use of date seed by-products could be particularly important for growing date palms and improving revenues for the sector, as confirmed by Al-Farsi and Lee (2008). It could be notcied that a hugh amount of date seed are thrown away every season becouse of the non utilization. Almana and Mahmoud (1994) used 10% coarsely ground date powder to improve bread properties

compared to fine bran. They found that the bread produced was similar or better than the original bread, in addition, the finely ground date kernel fraction improved bread smell, taste, color, uniformity and overall acceptance.

The properties of the produced date fruits have always been valued (FAO, 1999; Al-Hooti et al, 2002). In the past, various value-added products such as date syrup, date candies, date desserts and candies were produced. Technological change in date crops and the production of value-added products that resulted in large quantities of date seeds (Yousif and Alghamdi, 1999).

Date fruit waste and by-products, especially seeds, can be an important source of technologically functional food ingredients because they contain a balance of fat, protein, minerals, and carbohydrates. Date gum as a caffeine-free substitute has recently been introduced to the market (Rahman et al, 2007), either as a pure powder or as a mixture of date gum with coffee powder (Al-Farsi et al, 2007).

3. MATERIALS AND METHODS

3.1. Materials and Chemicals

In this study, the seeds of the Saidy variety (Figure 3.1), which is one of the most widely grown commercial date varieties in Egypt, were used. All reagents and chemicals were obtained from Merck (Darmstadt, Germany) and Sigma (Steinheim, Germany) and were analytical grade. Distilled water used in all experiments was obtained from a water purification system (Elga, Purelab DV25, UK).

Figure 3.1. Date seeds (Saidy variety)

3.1.1. Proximate composition

The approximate chemical composition of date seed, defatted date seed meal, and DSPC for moisture, protein, fat, and ash was performed in triplicate using the methods described by AOAC (1990). The nitrogen was determined using the DUMAS method and the nitrogen content was converted into protein by multiplying by a factor of 6.25. Carbohydrates are calculated by the following equation (3.1). All measurements were conducted in triplicate and the values are given as percentages.

$$\boldsymbol{Carbohydrate}\ (\%) = 100\ - (Protein + Lipid + Ash + Moisture) \quad (3.1)$$

3.1.2. Determination of minerals content

Some minerals of date seeds powder (Ca, Fe, K, Mg, Mn, Na, Cu and Zn) were determined according to the methods of AOAC (2005).

3.1.3. Amino acid profile

From date seed powder, 150 mg was hydrolyzed with concentrated HCl (6N) at 110 °C for 24 h. Then, the hydrolysate was analyzed by HPLC method according to the method described by Benitez (1989).

3.1.4. Fatty acid content by gas chromatograph–mass spectrometry (GC-MS) analysis

The fatty acid content of date seeds was measured using a Trace GC 1310-ISQ mass spectrometer (Thermo-Scientific, Austin, TX, USA) with a TG-5MS direct capillary column (30 m 0.25 mm 0.25 m film thickness). The temperature of the column oven was initially held at 50°C and then increased from 7°C/min to 230°C, held for 2 min, increased to the final temperature of 300°C at 30°C/min, held for 2 min. The injector and MS transfer line temperatures were maintained at 270 and 260°C, respectively. Helium was used as the carrier gas at a constant flow rate of 1 m/min. The solvent delay was 3 min and diluted samples of 1 L were injected automatically using the AS1300 autosampler coupled with GC in split mode. The components were identified by comparing their retention times and mass spectra to those of the WILEY 09 and NIST 11 mass spectral database.

3.2. Phytochemical Analyses of Saidy Variety Date Seed

3.2.1. The total phenolic content determination

The total phenolic content of date seed powder (DSP) was determined by Folin-Ciocalteu method (Sengul et al, 2009). Aliquots (0.3 mL) of the diluted date seed methanol extract (0.02g/ml) were mixed with 1.5 mL of Folin-Ciocalteu solution, 1.2 mL of Na_2CO_3 solution was added after 5 min, then the mixtures were stored at room temperature in the dark for 2 h incubated. The absorbance was measured at 760 nm

against a blank as a reference. Total phenol content was calculated using a calibration curve for gallic acid. The results were expressed as mg gallic acid equivalents (GAE)/g.

3.2.2. The total flavonoids content determination

The total flavonoid content of date seed powder (DSP) was determined by a colorimetric assay according to Zhishen et al. (1999). DSP 100 µL of the diluted date seed methanol extract (0.02g/ml) was added to 4 ml of distilled water. Then 0.3 ml of 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminum chloride was added. In 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately the mixture was diluted by the addition of 3.3 mL of distilled water and mixed thoroughly. The absorbance was determined at 510 nm against a blank. Catechin was used as a standard for the calibration curve. The total flavonoid content of the extract was expressed as mg catechin equivalents per gram of sample (mg/g).

3.2.3. Total antioxidant capacity (TAC) assay

The total antioxidant capacity of date seed powder (DSP) was determined according to Prior et al. (2005). A 0.5 ml aliquot of DSP methanol extract (0.02g/ml) was combined with 4.5 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In the case of a blank, 0.5 ml of 45% ethanol was used in place of the sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each sample was measured at 695 nm against blank in a UV-2450 spectrophotometer (Shimadzu, Japan).

3.2.4. Saponin content assay

Saponin content of date seed powder (DSP) was determined according to Hiai et al. (1975). DSP (0.1 g) was extracted three times with 95% ethanol. The clear supernatants were collected and made up to volume (10 ml) and then 0.5 ml of this ethanol extract was mixed with 0.5 ml of 8% vanillin in ethanol. The mixture was placed in an ice bath and mixed with 5 ml of 72% sulfuric acid and heated in a water bath at 60°C for 10 min, followed by cooling in an ice-cold water bath. The absorbance was measured at 544 nm.

3.2.5. Total anthocyanin content of date seed assay

The anthocyanin content was determined using the method of Guista and Wrolstad (2001). DSP (1 g) was dissolved in 10 ml acidic methanol (HCl 1% v/v) for 5 min, then centrifuged (5000 rpm/20 min). Anthocyanin was quantified by measuring the difference in absorbance at 525 nm and 585 nm (A525 A585) in 10 ml/g.

3.3. Techno-Functional Performances of Date Seed Protein Concentrate (DSPC)

3.3.1. Protein solubility

Protein solubility of DSPC in water was determined using protocol described by Morr et al. (1985), with slight modifications. A ten mL of sonicated DSPC at concentration of 1% was stirred for 1h at room temperature after ultrasonic treatment. The suspension was centrifuged at 9000 rpm for 20 min at 25˚C and the protein content in clear supernatant was determined by Bradford (1976) method. The protein amount of DSPC (1%) in 0.1 *N* NaOH was accepted as total protein content in DSPC. Solubility (%) was calculated using Equation (3.4).

$$\boldsymbol{Protein\ solubility}(\%) = \frac{Protein\ content\ in\ supernatant}{Total\ protein\ content\ in\ sample} \times 100 \qquad (3.2)$$

3.3.2. Determination of emulsifying properties

The indices of emulsion activity (EAI) and stability (ESI) of DSPC, which are indicators of emulsifying properties, were measured using the Spectro-turbidimetric method of Pearce and Kinsella (1978) with some modifications. 3.25 ml of sunflower oil was added to 10 ml of sonicated DSPC suspension (1% in water, pH 7.0) and this mixture was homogenized at (18000 rpm for 2min) using a homogenizer (T18, Ultra-Turrax, IKA, Staufen, Germany). 200 L of the emulsion from the bottom of this homogenate was diluted with 25 mL of SDS solution (10 mg/mL). The absorbance (A_0) at 500 nm was determined to calculate the EAI with a UVVis spectrophotometer (Shimadzu, UV-1240, Kyoto, Japan). For ESI, 200 µl of the emulsion was again taken from the bottom of the homogenate after 10 min and diluted with 25 ml of SDS solution (10 mg/ml). The absorbance (A_{10}) was measured at 500 nm. Equation (3.5) and Equation (3.6) were used to calculate the EAI and ESI, respectively.

$$\text{EAI}\ (m^2/g) = \frac{2 \times \text{T} \times \text{A}_0 \times \text{dilution factor}}{\text{C} \times \varphi \times 1000} \quad (3.3)$$

$$\text{ESI (min)} = \frac{\text{A}_0}{\text{A}_0 - \text{A}_{10}} \times \Delta\text{t} \quad (3.4)$$

Where, T = 2.303, A_0 = Absorbance at zero-time, dilution factor = 100, C = the weight of protein per unit volume (g/mL), φ = the oil volumetric fraction (0.25), A_{10} = Absorbance after 10 min, Δt = 10 min.

3.3.3. Determination of foaming properties

Foaming capacity (FC) and stability (FS) were measured using the volumetric method described by Aydemir and Yemenicioglu (2013). 25 mL of a sonicated DSPC suspension (1%) was transferred to a 50 mL graduated conical Falcon tube. The suspension was homogenized with a homogenizer (T18, Ultra-Turrax, IKA, Staufen, Germany) at 20,000 rpm for 2 min. After homogenization, the total foam volume (V_0) was recorded at time 0 and FC was calculated using Equation (3.7). The total volume of the foam was measured again after 10 min and this value (V_{10}) was used for the calculation of FS (equation 3.8).

$$FC(\%) = \left(\frac{(V0-25)}{25}\right) \times 100 \quad (3.5)$$

$$FS(\%) = \left(\frac{(V10-25)}{(V0-25)}\right) \times 100 \quad (3.6)$$

where V_0 is the height of DSPC dispersion at 0 min after homogenization, V_{10} is the height after 10 min.

3.3.4. Determination of water/oil binding capacity

DSPC water-binding capacity (WBC) and oil-binding capacity (OBC) were determined using the gravimetric method described by Aydemir and Yemenicioglu (2013) with minor modifications. A 50 mg sample of DSPC was weighed into a 2 mL Eppendorf tube and 1 mL of water or sunflower oil was added to the tubes. The suspensions were vortexed for 30 s to obtain a homogeneous mixture. After 30 min incubation at room temperature, the tubes were centrifuged at 30000 rpm for 10 min at +5°C. The clear supernatants were carefully decanted without loss of material and the resulting pellets weighed accurately using an electronic balance (Ohaus, Explorer X224, Parsippany, USA). The water and oil binding capacity were determined from the weight difference of the samples.

3.3.5. Antioxidant capacity determination of DSPC

The free radical scavenging activity of DSPC was determined using the diphenyl picrylhydrazyl (DPPH) assay according to Sengul et al. (2009). The evaluation method is based on monitoring the decrease in absorbance of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl) in the presence of antioxidants. The reaction mixture contained 150 µL DSPC-HPH and 2850 µL methanolic DPPH (0.24 mM). The mixture was incubated at room temperature for 30 min and the absorbance at 515 nm was determined. The percentage of DPPH scavenged was calculated from the following equation (3.2). IC_{50}, which denotes the amount (mg) in 1 ml of solution required to reduce the initial concentration of DPPH radicals by 50%, was also determined as in equation (3.3).

$$\textbf{\textit{DPPH scavenging}}\ \% = (A_0 - A_s/A_0)\ X\ 100 \tag{3.7}$$

$$TE/g = IC50\ of\ Trolox\ (\mu g/l)\ /\ IC50\ of\ sample\ (\mu g/l) \tag{3.8}$$

Where A_0 is the absorbance of the blank, and A_S is the absorbance of sample at 515 nm.

3.4. Production of Date Seed Protein Concentrates (DSPC)

DSPC was produced by the conventional alkaline extraction-isoelectric precipitation method, with slight modification (Akasha et al. 2012). First, date seeds were ground through stone mill then sieving by 40 M sieve. Uniform flour was defatted by the Soxhlet extraction method for 6 h. Defatted date seed flour was air-dried at room temperature overnight and stored at 4°C until processing. Defatted date seed flour was dispersed in distilled water at a ratio of 1:10 (solid:liquid, w/v) and then the pH of suspension was adjusted to 10 by 0.1 *M* NaOH. The extraction of protein was carried out by a Heidolph heating magnetic stirrer (Schwabach, Germany) at 45˚C for 1h. Next, the resulting slurries were spun at 10000 rpm for 10 min at 4°C in a centrifuge (Beckman Coulter, Allegra 64R, California, USA). The clear supernatant containing soluble proteins were collected and the pH value was adjusted to the isoelectric point (pH 4.5) using 0.1 M HCl. The mixture was centrifuged at 10000 rpm for 10 min at 4 ˚C to obtain the protein pellet. The collected pellets were redissolved in alkaline solution (pH 10) and then reprecipitated to increase the purity of protein concentrate,

and then the collected pellets were suspended in minimal amount of water and adjusted to pH7. The neutralized protein solutions were lyophilized in a Labconco Freezone 6 Freeze dryer (Labconco Corporation, Kansas City, MO, USA) for 72 h to produce a powder of protein concentrate. DSPC was kept at –20˚C until next step. Method was summarized in Figure (3.2).

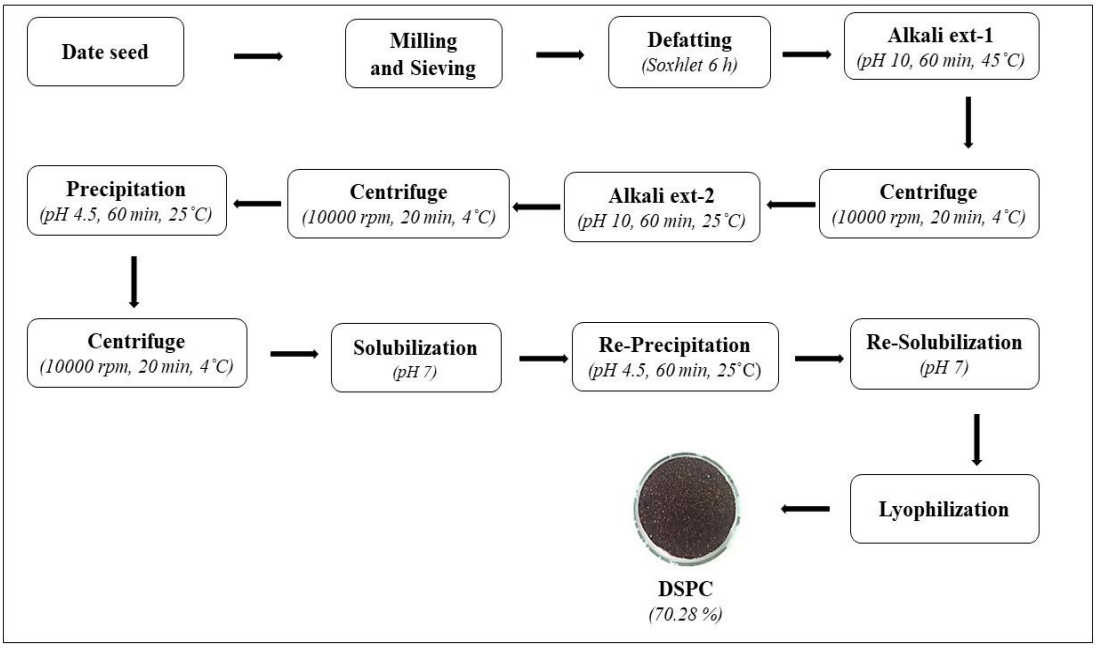


Figure 3.2. Extraction and purification of date seed protein concentrate (DSPC)

3.5. Physicochemical Properties of Date Seed Protein Concentrate (DSPC)

3.5.1. Scanning electron microscopy (SEM)

The morphology of dried DSPC was investigated using the scanning electron microscope of Carl Zeiss Gemini Supra 40VP field emission (Carl Zeiss SMT AG, Oberkochen, Germany) at voltage of 15 kV. Samples were coated with platin using an ion sputter (Quorum Q 150R-ES, Quorum Technologies, Laughton, UK), and was examined at the range of 250x-2000x magnification.

3.5.2. Particle size and zeta (ζ) potential determination

The average particle size and zeta potential of the DSPC were determined by dynamic light scattering (DLS) using a Malvern Zeta-sizer Nano ZS (Malvern, Worcestershire, UK). The DSPC samples were dissolved in distilled water (2 mg/ml) at room temperature by stirring for 1 hour. The protein suspensions were centrifuged at 15500

rpm for 10 min at +4°C. The supernatants were filtered through Whatman #1 filter paper to obtain a clear filtrate. A Zetasizer folded capillary cell (DTS 1070) and a disposable plastic cell (DTS 0012) were used for zeta potential and particle size analysis, respectively. The absorption and refractive index values were 0.001 and 1.330, respectively. All measurements were performed in triplicate and the mean values are presented.

3.5.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The effect of ultrasound on the molecular structure of DSPC was evaluated by SDS-PAGE analysis performed according to the method of Peng et al. (2017) with minor modification. The acrylamide concentrations of the separating and stacking gels were 12% and 5%, respectively. The lyophilized DSPC samples were diluted to 1 mg/ml in the SDS-PAGE buffer by the presence or absence of 2-mercaptoethanol (-ME). The samples were heated to 95°C for 5 min and then 12 µL of protein suspension was loaded into the gel lanes after the samples had cooled. A commercial pertained protein marker in the 10-180 kDa range (Thermo Fisher Scientific, Rockford, IL, USA) was used to identify the protein profile. Gels were stained with Coomassie Brilliant Blue R-250 and distained in 10% acetic acid.

3.5.4. Fourier-transform infrared (FTIR) spectroscopy

The infrared spectrums of freeze-dried DSPC samples were examined by a FTIR spectrometer (Perkin Elmer Spectrum Two, USA) with Attenuated Total Reflectance (ATR) unit. The samples were analyzed in the region of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} for 16 scans at ambient conditions.

3.5.5. Surface sulfhydryl groups (SH) determination

Free sulfhydryl group content was determined using Ellman's reagent (5,5-ithiobis-(2-nitrobenzoic acid), DTNB) method as described by Xiong et al., (2016). The DSPC samples were dissolved in Tris HCl buffer (containing 86mM Tris, 90mM glycine and 4mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) to obtain a 0.3% (w/v) protein solution and then 50 l DTNB was added to 5 ml DSPC solution. After incubation in a thermostatic shaking water bath (WitegWSB-30, Germany) for 1 h at 25°C, the tubes were centrifuged at 9000 rpm for 10 min at 4°C and the absorbance of the solutions at 412 nm using a UV-Vis -spectrophotometer (Shimadzu, UV-1240, Kyoto, Japan).

3.5.6. Intrinsic fluorescence emission

The intrinsic fluorescence emission spectra of DSPC (1 mg/ml in PBS buffer (10 mM, pH 7.0)) were measured at room temperature using a fluorescence spectrometer (Hitachi, F7000, Tokyo, Japan). The measurements were carried out at an excitation wavelength of 280 nm and the emission spectra were evaluated in the range from 300 nm to 450 nm with a constant slit of 10.0 nm.

3.5.7. Surface hydrophobicity (H_0) determination

The surface hydrophobicity of DSPC was measured by a fluorescence spectrum assay (Hitachi, F7000, Tokyo, Japan) using 8-anilino-1-naphthalenesulfonic acid (ANSA) as a fluorescent probe (Peng et al., 2017; Xiong et al., 2016). The 10 ml DSPC solution (1 mg/ml in 10 mM PBS, pH 7.4) was mixed with 0.1 ml ANSA solution (2.4 mM in 10 mM PBS, pH 7.4). The excitation wavelength was 390 nm. The emission and excitation gaps were 5 nm, and the emission spectrum was measured from 400 to 650 nm. The relative exposed hydrophobicity was calculated using equation (3.9).

$$\mathrm{H0} = S1 - S2 \tag{3.9}$$

where S_1 is the area of sample solution, S_2 is the area of solvent.

3.5.8. Differential Scanning Calorimetry (DSC)

The thermal stability of DSPC was analyzed using a differential scanning calorimeter (TA Instrument Q2000 Thermal Analysis System, New Castle, USA). The samples (approximately 2.5-3.0 mg) were weighed into aluminum pans and the pans then sealed. The sealed pans were heated from 20 to 200°C at a rate of 5°C/min with continuous dry nitrogen. An aluminum pan without a sample was used as a blank. TA Universal Analysis 2000 software (TA Instrument, New Castle, USA) was used to calculate the denaturation temperature (Td, °C) and thermal enthalpy of denaturation (ΔH, J/g protein) of DSPC samples.

3.6. Modification of Date Seed Protein Concentrate (DSPC)

3.6.1. High-intensity ultrasound homogenization (HIUS)

DSPC was dissolved in water (1% w/v) at pH7. The suspension was stirred at room temperature for 2 h and then at 4°C overnight at a speed of 500 rpm. 80 ml of DSPC suspension in a 250 ml reactor connected to a water circulator at 10°C (Scientz, DC2006, Ningbo, China) were processed by a HIUS processor (Sonics, VCX750, Newtown, USA) with a 1.3 cm diameter titanium probe ultrasonicated. Each ultrasonic treatment was performed at a constant frequency of 20 kHz under the process conditions given in Table (3.1) including amplitude (40-80%) and time (5-15 min). Treatment temperature was kept below about 45˚C during sonication. After the optimization study of the process conditions that maximized the solubility, the native DSPC was ultrasonicated under the optimum conditions.

Table 3.1. Two factors, three level face-centered central composite design (FC-CCD) of HIUS

Run	Process variables	
	Amplitude (%) A	Time (min) B
1	40 (-1)	5 (-1)
2	80 (+1)	15 (+1)
3	60 (0)	10 (0)
4	60 (0)	10 (0)
5	60 (0)	5 (-1)
6	60 (0)	10 (0)
7	80 (+1)	5 (-1)
8	40 (-1)	10 (0)
9	60 (0)	10 (0)
10	80 (+1)	10 (0)
11	40 (-1)	15 (+1)
12	60 (0)	15 (+1)

The processed DSPC under the optimal high-intensity ultrasound condition was defined as DSPC-HIUS. After HIUS treatment, the samples for the techno-functional properties (solubility, emulsification, foaming, water/oil binding) were directly analyzed while the samples for the other analyses (zeta potential, particle size, DSC, SDS-PAGE, SEM, FTIR, surface hydrophobicity, intrinsic fluorescence emission and free sulfhydryl group) were freeze-dried and stored +4°C until analysis.

3.6.1.1. Determination of acoustic energy

The transmitted sonic energy of the ultrasonic homogenizer probe was measured by a calorimetric method based on measuring the temperature rise in a liquid medium over time. The sound power (W) and the sound energy density (W/cm^2) were calculated as follows in equations (3.10) and (3.11) by calorimetrically tracking the change in suspension temperature during the sonication process (Taurozzi et al, 2012). The powers generated by the sonication were 9.24-10.41, 22.82-24.82, and 57.62-63.29 W/cm^2 at amplitudes of 40, 60, and 80%, respectively, for a 1% w/ v protein concentration.

$$P = m \times Cp \times (dT/dt) \tag{3.10}$$

$$Ia = P/A \tag{3.11}$$

where P is the acoustic power (W), m is the mass of suspension (g), Cp is the heat capacity of suspension (4.18 J/ gK) and dT/dt is the slope of the temperature change with time curve during the first 60 s, Ia is the acoustic power intensity (W/cm^2), A is the surface area of the HIUS probe (cm^2).

3.6.1.2. Response surface methodology

In this study, the RSM was used to determine the optimal conditions of high-intensity ultrasound treatment by the statistical program Design-Expert (version 13.0, Stat-Ease Inc., Minneapolis, USA). The two-variable, three-level Face Centered Central Composite design was applied to study the influence of amplitude A: (40, 60, and 80%) and time B: (5, 10, and 15 min) on the solubility response of DSPC. The twelve treatments, including four midpoint replicates, were performed in random order (Table 3.1). The results were analyzed using a quadratic model as shown in equation (3.12).

$$Y = \beta 0 + \beta 1A + \beta 2B + \beta 3AB + \beta 4A^2 + \beta 5B^2 \tag{3.12}$$

where Y is the estimated response variable, β_0 is constant, β_1, β_2 linear, β_3, interaction, and β_4, β_5 are quadratic coefficients determined by the model, while A and B are the independent variables, respectively.

The significance of the proposed models and individual model coefficients were assessed by analysis of variance (ANOVA). The validation of the proposed solubility model was performed by comparing the actual experimental data with the predicted responses from the proposed mathematical equation. The relationships between dependent and independent variables were explored through the surface response plots

of polynomial regression equations. The optimal conditions based on solubility were determined using the Design Expert 13.0 software desirability function.

3.6.2. High-pressure homogenization (HPH)

In this work package titled "Optimization of high-pressure homogenization", optimization of high-pressure application of date seed protein isolate in terms of solubility, emulsion and antioxidant was carried out. For this purpose, the Design Expert 13 (Stat-Ease., Inc., MN) statistical package program was used. DSPC were prepared at 1%, 2%, 3% concentrations and the prepared dispersions (100 mL) were subjected to high pressure application. In the homogenization process, a Microfluidics brand LM-10 model high pressure homogenizer was used. It was exposed to the relevant pressure exactly four times in each trial. Temperature control was carried out for each trial, and it was ensured that remained below 40°C.

Determination of optimum conditions in high pressure application and the effects of these process factors on solubility, emulsion, and antioxidant activity of DSPC were determined by surface response method. Pressure (X_1) and protein concentration (X_2) were chosen as independent variables, while solubility, emulsion and antioxidant activity were selected as dependent variables.

Table 3.2. Two-factor-three-level experimental ranges in surface response method center composite design of HPH.

Independent variables	Coded and actual values			
	Unit	-1	0	+1
Pressure (X_1)	MPa	50	100	150
Protein concentration (X_2)	%	1	2	3

The working ranges of the independent variables were decided by a detailed literature review. For this purpose, two factor-three level experimental intervals were determined in the surface response method central composite design. Pressure (50, 100 and 150 MPa) and protein concentration of the dispersion (1, 2 and 3 g/100 mL) were investigated as the main factors within the scope of the surface response method (Table 3.2). At these levels, regression models were obtained by performing experiments in 12 different combinations. Significance analysis of models and coefficients analysis of variance (ANOVA) was performed. (Table 3.3)

Table 3.3. Two factors, three level face-centered central composite design (FC-CCD) of HPH.

Std	Run	Factor 1(X_1)	Factor 2(X_2)
		A: Pressure MPa	B: Protein Concentrate %
3	1	50(-1)	3(+1)
2	2	150(+1)	1(-1)
12	3	100(0)	2(0)
7	4	100(0)	1(-1)
11	5	100(0)	2(0)
9	6	100(0)	2(0)
6	7	150(+1)	2(0)
4	8	150(+1)	3(+1)
10	9	100(0)	2(0)
5	10	50(-1)	2(0)
1	11	50(-1)	1(-1)
8	12	100(0)	3(+1)

The results were statistically tested at the significance level of p-values < 0.05. The fit of the model was assessed using the ANOVA outputs, the lack of fit, the regression coefficient (R2), and Fisher's test value (F-value). To validate the mathematical models determined from RSM, protein solubility and particle size were measured under the predicted optimal variables. Optimal conditions are confirmed in the next step by tests under these conditions. The answers obtained are compared with the model prediction data by equation 3.11.

3.7. Statistical Analysis

All analyzes were performed in triplicate. The data obtained were given as mean plus stdev. Then compare with the independent samples by t-test. One-way variance was applied using the SPSS 20.0 package program (SPSS Inc., Chicago, USA) with a 95% confidence interval.

4. RESULTS AND DISCUSSION

4.1. Physical Characterization and Biochemical Composition of Date Seed

4.1.1. Physical properties of date seeds (DS)

In food systems, the quality control of date processing depends on important indicators such as the physical characteristics of the date palm fruit (Manickavasagan et al, 2012). Regarding to the obtained results in Table 4.1, for the physical properties of date seeds. It was noticed that the average weight of one seed was 1.37g. This result was higher than that those reported for pit weight (0.92-1.23g) of five Sudanese date varieties (Sulieman et al, 2012). Similarly, that value was higher than those demonstrated by Abd-Ellah (2009); Herchi et al. (2014) and Abdelkarim (2016) they found that, the weight of six varieties of date seeds ranged between 0.84 to 1.35g based on dry weight basis. The length date seed used in our study was 2.15 cm, which was lower than that reported by Herchi et al. (2014), while it was higher than that of Abd-Ellah (2009).

The number of seeds per kilogram was 730 seeds/kg. Width, volume, and density results were recorded 0.38 cm, 1.91 cm^3 and 0.79 g/cm^3, respectively. These results were consistent with previous studies, which suggested that thirteen date palm cultivars grown in Libya with a seed weight of 1.43 g are the highest for the Tediss cultivar, while 0.79 g for Deglet Nur cultivar (El-Alwani and El-Ammari, 1999).

On the other hand, Akasha et al. (2014) mentioned that the average weight of date palm seeds was 0.98 g. There were variations in date seed weight among different cultivars, which could be due to different seasons, agricultural treatments, and environmental conditions during the cultivation of the dates (Abdullah and Salah, 1999). Furthermore, our results agree with those of Bouhali et al. (2015) who found that date seed ranged in diameter from 0.77 to 1.51 cm and in length from 1.71 to 2.75 cm. The obtained density was higher than the data reported by Desai et al. (2019) which ranged from 0.491 to 0.477 cm^3.

Table 4.1. Physical properties of date seeds (DS)*.

Weight of pits (g/seed)	Number of (seeds/Kg)	Length (cm)	Width (cm)	Volume (cm^3)	Density (g/cm^3)
1.37±0.15	730±0.13	2.15±0.07	0.38±0.02	1.91±0.03	0.79±0.01

**Values represented as mean ± standard deviation*

4.1.2. Proximate chemical composition of date seed (DS), defatted date seed (DDS), and DSPC

Table 4.2 shows the main components (moisture, protein, fat, ash and total carbohydrates) of date seed, defatted date seed and DSPC. In terms of quantity, carbohydrates and fat are the largest part of the composition of the date kernels. The crude protein and fat content of the date seeds used in this study were 6.17% and 9.56%, respectively. There are many studies on the chemical composition of date seeds in the literature, it was reported that the protein and fat content ranged from 2.29-7.08% and 5.02-13.3%, respectively (Besbes et al, 2004; Akasha et al, 2012; Golshan Tafti et al, 2017). However, we cannot make any comparisons as there is no information available in the literature about the protein content of the Saidy variety.

Table 4.2. Principal components of date seed, defatted date seed, and DSPC as g/100g fresh weight

	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Date seed	7.21±0.25	6.17±0.28	9.56 ±0.14	3.44±0.01	73.62±0.34
Defatted date seed	6.74±0.09	8.81±0.21	0.49±0.12	4.22±0.10	79.74±0.26
DSPC	5.96±0.09	70.28±0.33	0.13±0.04	3.38±0.06	20.25±0.40

Results presented as the mean values ±standard deviation (n=3)

The obtained data agree with those of Besbes et al. (2004), who studied seeds of the varieties Deglet Nur and Allig grown in Tunisia at the Tamar stage of maturity. They reported that the protein content of these varieties was 5.56% and 5.17%, respectively. These considerable inter-cultivar differences in date seed chemistry could be related to many factors such as cultivar, fruit ripeness, agricultural practices, geographic and climatic conditions. The results showed that the Saidy variety used in this study was relatively high in protein and fat, so date seeds could become a potential source for converting a by-product into a value-added product.

The DSPC prepared by alkaline extraction-isoelectric precipitation had a protein content of 70.28%. Akasha et al. (2012) performed treatments to convert defatted date kernel flour into protein concentrates using five different methods. They reported that

the protein powder by alkaline extraction-isoelectric precipitation method had a single protein content of 11.79%, which was much lower than our result (70.28%) shown in this study. The conflicting result can be attributed to extraction conditions such as time, temperature, solid-to-liquid ratio, and centrifugation, which may have altered the protein content of concentrates.

4.1.3. Amino acid profile of date seed protein

The nutritional value of the protein depends on the amino acid composition, especially the content of essential amino acids that cannot be synthesized by humans. The amino acid profile of DSP is shown in Table 4.3 and Figure 4.1. Except for leucine and methionine, the amino acid profile of date seed protein showed a percentage higher than the daily recommended RDA for humans by WHO (2007) which is considered a food additive in the human diet. The detected amino acids in Saidy variety were isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, threonine, and valine with sufficient amount comparing with WHO recommendations. On the other hand, the studied variety contained a small amount of histidine and tryptophan.

Comparing Akasha's (2016) results for Deglet Nur, Saidy variety was higher in isolysine, lysine, valine, histidine, aspartic acid, glycine, and alanine, while lower in leucine, methionine, phenylalanine, threonine, glutamine, serine, and proline. While Saidy date seeds contained 22.9 mg/g of tryptophan, there was a deficiency of tryptophan in Deglet-Nur date seeds. Also, our results rvealed more phenylalanine than those reported by Ramadan (1995) in the same variety.

Al-Showiman and Baosman (1992) examined the amino acid content of five Saudi date seed varieties, namely Berhey, Sekkeri, Berni, Maktomey and Shaqra. They reported that sulphurous amino acids, (glycine and tyrosine) were agree with our results. Similarly, Akasha et al. (2016) reported that glutamic acid is the most abundant amino acid in full-fat date seeds, followed by arginine, aspartic acid, leucine, glycine, alanine, lysine, valine, and phenylalanine, which were consistent with the amino acid composition of date seed tested (variety Saidy). The presence of some phenolic acids like quinic and caffeic acid in the protein content causes them to interact with amino acids like tryptophan, arginine, and lysine while caffeic acid interacts with tyrosine, lysine, and tryptophan (Suryaprakash et al, 2000; Yada 2004).

Table 4.3. Amino acid composition (mg/g of protein) of Saidy date seed variety powder comparing with Deglet Nur and RDA

Amino acid (Mg/g)	Saidy	Deglet Nur (Akasha, 2014)	RDA*(WHO,2007)
IsoLysine (Lle)	79.6±0.21	33.5	30
Leucine (Leu)	39.2±0.11	74.1	59
Lysine (Lys)	185.8±0.31	54.7	45
Methionine (Met)	28.8±0.01	28.2	22
Phenylalanine (Phe)	26.8±0.17	40.6	38
Threonine (Thr)	23.4±0.13	33.5	23
Valin (Val)	72.3±0.35	54.7	39
Histidine (His)	56.6±0.67	14.1	15
Aspartic acid (Asp)	115.1±0.29	82.9	NE
Glutamic acid (Glu)	144.0±0.34	183.4	NE
Serine (Ser)	43.3±0.23	60.0	NE
Glycine (Gly)	82.8±0.27	68.8	NE
Alanine (Ala)	95.7±0.17	61.7	NE
Tryptophan (Tyr)	22.9±0.33	-	NE
Proline (Pro)	35.6±0.36	40.6	NE

**Mean protein requirement of 105mg protein/kg per day (0.66g protein/kg per day)*
NE Non-essential amino acid

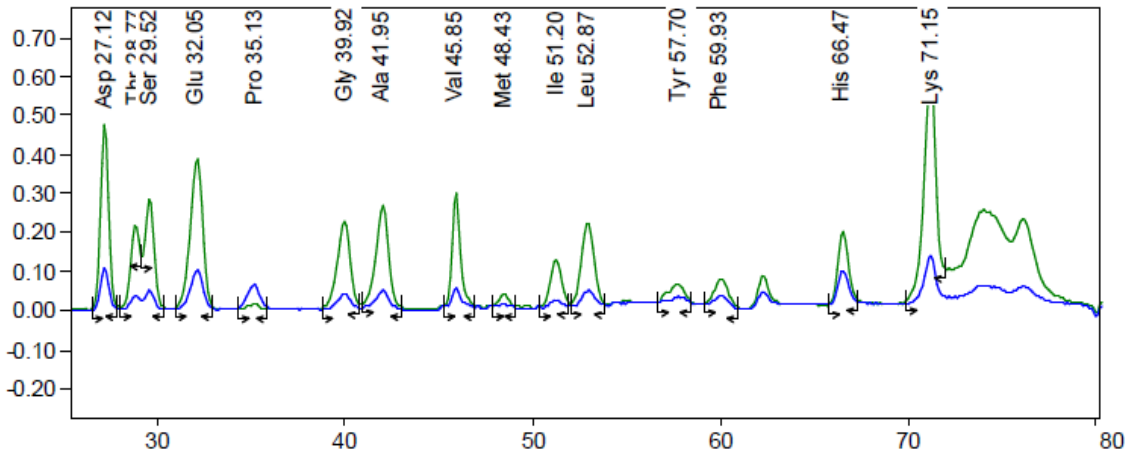


Figure 4.1. Amino acid profile of Saidy date seed powder

4.1.4. Minerals content of date seed powder (DSP)

The inductively coupled plasma (ICP) instrumental method is used to determine the various mineral contents of date seeds, given in Table 4.4 as mg/100g dry weight. Our results revealed that potassium was the predominant element, with potassium concentration being the highest (807.1 mg/100g), followed by sodium (322.9 mg/10g),

calcium (263.1 mg/100g), magnesium (95.67 mg/100g), iron (61.21 mg/100g) and manganese (1.665 mg/100g). The copper showed the lowest value (0.940 mg/100g).

The results obtained from the study agreed with previous studies by Reynes et al. (1994), who reported that date seed contains a large amount of minerals such as calcium and phosphorus. Chaira et al. (2007) confirmed that date stones are rich in minerals (potassium, calcium, sodium, iron, zinc, copper, and manganese). In addition, potassium was the abdomen mineral that was present in higher value. Also, Ali-Mohamed and Khamis (2004) studied the mineral composition of six varieties of date seed, and they reported that appropriate amounts of different types of minerals are required for human nutrition. Similarly, Besbes et al. (2004) reported the mineral content of two Tunisian varieties (Deglet Nur and Allig), the results of the two varieties mentioned in Table 4.4.

Table 4.4. Mineral content of the Saidy date seed variety compared to some other different varieties (mg/100g dw).

Mineral	Saidy cultivar	Bahraini cultivars*	Tunisian cultivars**
Potassium	807.1	486	229, 293
Magnesium	95.67	66	51.7, 58.4
Sodium	322.9	24	10.4, 10.25
Calcium	263.1	10	38.8, 28.9
Manganese	1.665	1.5	–
Zinc	–	1.2	–
Copper	0.940	0.5	–
Phosphorus	–	–	68.3, 83.6
Iron	61.21	–	2.30, 2.21

Mineral content for Average of six Bahraini cultivars Ali-Mohamed and Khamis (2004), Two Tunisian cultivars Besbeset al., (2004).*

Previous studies revealed that date seeds contain high levels of minerals; Potassium had the highest content and ranged between (22967.1 - 4153.3 mg/kg dw), magnesium (615.3 - 827.6 mg/kg dw) and calcium (395.0 - 626.71 mg/kg dw).). On the other hand, sodium was at the lowest level, ranging from (108.1 to 319.4 mg/kg dw). In terms of micronutrient content, iron was found at high levels, ranging between (27.7-70.3 mg/kg dw), manganese (5.5-11.0 mg/kg dw) and copper (4.8-0 .3 mg/kg TG) with the lowest micronutrient (Bouhlali et al, 2015).

4.1.5. Phytochemical screening of saidy date seeds (DS)

Date seed is a rich source of phenolic compounds and an industrial by-product with minimal human use (Sirisena et al, 2017). The results obtained in Table (4.5) showed that the Saidy date seed variety had a high content of phenolic compounds (2.40±0.23 mg/g). These results are consistent with previous studies by Mistrello et al. (2014) who confirmed that the total phenolic content of date seeds ranged between (2.58-2.83 mg GAE/100 g fresh weight).

Similarly, Hamada et al. 2002; Habib and Ibrahim 2011; Bouhlali et al. 2015 reported that date seeds contain a high number of phenols. Meanwhile, Al Juhaimi et al. (2012) found that the total phenolic content of date seed powder was 1.98 - 4.65 mg GAE/100 g DW, these differences may be due to the cultivars and growing conditions. Regarding the total flavonoid content, the results showed that the total flavonoid content was 1.280±0.05 mg/g DW.

Previously, several studies suggested that date seed is a rich source of flavonoids as reported by Bouhlali et al. (2015) who revealed that the total flavonoid content of date seeds was recorded 1.659-1.844 mg RE/100 g DW. Mistrello et al. (2014) reported that the flavonoid content was in the range of 1.27-1.93 mg CE/100 g FW. On the other hand, Maqsood et al. (2015) found that water date seed extract contained the lower flavonoid content. Our results of TAC, saponin and anthocyanin were 2.3±0.2, 274±2.5 and 277±0.14 (mg/100g DW), respectively.

It was proposed by Al-Farsi et al. (2007) that phenolic compounds from three date seed varieties (Mabseeli, Shahal and Um-Sellah) from Oman have total phenolic components ranging from 3.10 to 4.43 mg (GAE/100 g fresh weight). At the same time, the study by Mistrello et al. (2014) confirmed that the phenol content of date seeds ranged from 2.58 to 2.98 mg GAE/100 g fresh weight). Content of phenolic compounds was 2.69- 5.34 mg (GAE/100g DW). Al Juhaimi et al. (2012) reported that phenolic content of date seed was 1.98-4.65 mg (GAE/100 g DW), this variation may be due to the varieties and growing conditions. Growing conditions, geographic origin, cultivar, maturity, soil type, season, fertilizers, sampling, extraction conditions, and storage conditions are considered important factors affecting the phenolic content of date seeds (El-Hadrami and Al-Khayri, 2012).

Yada 2004 reported that phenolic compounds and carotenoids responsible for the dark colors and astringent taste of some fruits were found in date seeds. In addition, Sunder et al. (2017) mentioned that date seed contains higher numbers of tannins, saponins, phenols, alkaloids and sterols. Similarly, Maqsood et al. (2015) found that date seed extract contained the lower total phenol content. The scavenging activity of DPPH from date seed extract is related to the content of phenols and flavonoids. For our studies DSP the DPPH was 78.77±0.24, and the IC_{50} of DPPH was 0.64±0.04 mg/ml. The antioxidant activity of date seed extract could be attributed to the presence of phenolic compounds due to its ability to donate hydrogen for electron transfer. In addition, Al-Farsi and Lee (2008) reported that date seed extract has high antioxidant activity, about 27 times that of date fruit.

Table 4.5. Phytochemical analysis of Saidy date seed (DS)

Phytochemical analysis	Date seeds extract
DPPH scavenging activity (%)	78.77±0.24
IC_{50} of DPPH (mg/mL)	0.64±0.04
Total phenolic (mg/g)	2.40±0.23
Total flavonoids (mg/g)	1.28 ±0.05
TAC (mg/100g)	2.3 ±0.21
Saponin (mg/100g)	274±2.5
Anthocyanin (mg/100g)	227±0.14

4.1.6. Fatty acid fractions of saidy date seed

The obtained result of chromatograph–mass spectrometry (GC-MS) analysis of the date seed extract (Table 4.6), revealed that various fatty acids were found. Lauric acid is the highest concentration among the various compounds, which was 34.9% followed by the concentrations of palmitic acid, capric acid and oleic acid which were 15.45, 8.74 and 7.98%, respectively. In addition, the concentrations of diisooctyl phthalate, dodecanoic acid, 1,2-benzenedicarboxylic acid, diethyl ester were the lowest values, which were 1.04, 1.08, and 0.83%, respectively. Previous studies showed that the alcoholic compound (1-heptatriacotanol) had potent antibacterial activity. The biochemical compound 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z) has been reported to have anti-inflammatory and CNS depressant effects (Herchi et al, 2014).

The results obtained are also consistent with those reported by Akbari et al. (2012), who reported that the primary fatty acid of the three varieties was oleic acid with a percentage of 37.6%, 31.47% and 31.79% in kabkab, shekar and shahabi varieties, respectively. Other fatty acids found were lauric, myristic, palmitic, linoleic, and stearic acid. Similarly, date seed oil has been reported to contain oleic acid (44.92-48.38%), lauric acid (1.74-20.34%), myristic acid (10.23-12.28%), palmitic acid (9.82-10.91), linoleic acid (8.3-9.02%) and stearic acid (2.86-3.73%) which were more than 98% of the total oil as determined by Bouhlali et al. (2015). Recently, Harakat et al. (2022) confirmed that the major fatty acid was oleic acid (42.74-50.19%), followed by lauric acid (18.4-22.26%), myristic (8.83-10.17%), palmitic acid (9.11-10.37%), linoleic acid (6.58-8.12%) and stearin ((3.07-3.64%).

Table 4.6. The most abundant phytochemical compounds analyzed by GC-MS of the hydro alcohol date seed extract

No.	RT (min.)	IUPAC Name	Common Name	M.F	M.W	P.A %
1	11.95	1-dodecanamine, n, n-dimethyl-	Lauric acid	$C_{14}H_{31}N$	331	34.9
2	15.72	1-Tetradecanamine, N, N-dimethyl-	Palmitic acid	$C_{16}H_{35}N$	241	15.45
3	22.49	2-methylenebrexane	Capric acid	$C_{10}H_{14}$	134	8.74
4	23.00	Oleic Acid	Oleic acid	$C_{18}H_{34}O_2$	282	7.98
5	3.96	Benzyl chloride		C_7H_7Cl	126	6.58
6	20.25	Hexadecenoic		$C_{16}H_{32}O_2$	256	5.39
7	22.32	9-octadecenoic acid (z)-, methyl ester		$C_{19}H_{36}O_2$	296	2.79
8	25.53	2-methylenebrexane		$C_{10}H_{14}$	134	2.73
9	19.55	Hexadecenoic acid, methyl ester		$C_{17}H34O_2$	270	1.97
10	22.22	9,12-Octadecadienoic acid, methyl ester, (E, E)-		$C_{19}H_{34}O_2$	294	1.92
11	27.02	9,12-Octadecadienoylchloride, (Z,Z)-	Linoleic acid	$C_{18}H_{32}O_2$	298	1.26
12	23.34	9-octadecenoicacid	Elaidic acid	$C_{18}H_{34}O_2$	282	1.21
13	11.36	1-dodecanol		$C_{12}H_{26}O$	186	1.21
14	28.58	Diisooctylphthalate		$C_{24}H_{38}O_4$	390	1.08
15	13.69	1,2-benzenedicarboxylicacid, diethyl ester		$C_{12}H_{14}O_4$	222	1.04
16	13.23	Dodecanoic acid		$C_{12}H_{24}O_2$	200	0.83

4.2. Effect of High Intensity Ultrasonic Homogenization (HIUS) on Saidy Date Seed Protein Concentrate (DSPC)

High-intensity ultrasound (HIUS) homogenization is one of the most studied physical methods for modifying protein structure. The basic principle of HIUS is based on the microbubbles generated by sound waves creating the shear stress and cavitation force with heat and turbulence. HIUS has many advantages such as wide waves frequency, simple, cost effective, energy saving and environment friendly (Wen et al, 2018 and Zhang et al, 2018). Recently, the effect of high-intensity ultrasound on the physicochemical properties of plant-based proteins and, in parallel, its reflection on techno-functional properties have been intensively studied.

Several authors investigated that the effect of HIUS on the physicochemical and functional properties of soy (Jambrak et al, 2009 and Hu et al, 2013), pea (Xiong et al,

2018), black bean (Jiang et al, 2014), sunflower (Malik et al, 2017), faba bean (Martinez et al, 2018), millet (Nazari et al, 2018), quinoa (Vera et al, 2019), peanut (Zhang et al, 2014), walnut (Zhu et al, 2018) and hempseed (Karabulut and Yemiş, 2022; Karabulut et al, 2022) protein isolate. Although many studies have addressed the impact of HIUS on the techno-functional performances of various plant-based protein isolates, there has been no study on the modification of techno-functional performances of date seed protein concentrates by HIUS. Limited studies on date seed proteins have mostly focused on extraction methods and the physicochemical and functional properties of the protein concentrates obtained by these methods.

The purpose of the study was to enhance the techno-functional performance of DSPC by HIUS. In this study, DSPC was first produced by conventional method which was alkaline extraction and then isoelectric precipitation. The effect of the independent process variables on the solubility of DSPC was investigated and determination of optimum process conditions for maximizing the protein solubility using the central composite design of Response Surface Methodology (RSM). Amplitude and time of ultrasonic treatment were the independent process variables of HIUS. After treatment by ultrasound technique at optimal conditions, the techno-functional performance of DSPC-US was analyzed and compared to the native DSPC.

4.2.1. Response surface modelling

Table (4.7) shows the process variables (amplitude and time) and values of protein solubility obtained under different combinations of high-intensity ultrasound conditions by the face-centered central composite design (FC-CCD). The 'Fit Summary' statistics (*F*, *p* value, Lack-of-fit and R^2) produced by Design Expert software was used to decide the best-fit model for the protein solubility. The 'Fit Summary' statistics indicated that the quadratic model is highly significant for the protein solubility. Analysis of variance and regression coefficients of the suggested quadratic model is given in Table (4.8). The 'lack-of-fit' value of the suggested quadratic model for protein solubility was 0.1052, confirming the fitting of the data to the suggested model (0.1052 > 0.05). The probability (*p*) values lower than 0.05 was another criterion which shows the model and variables are significant. The ANOVA analysis showed a p-value of 0.034 for the quadratic model, confirming the significance of the model. Both amplitude and time had a positive effect on the protein solubility at the 0.01 level.

Table 4.7. Two factors, three level face-centered central composite design (FC-CCD) and the protein solubility of DSPC under the different conditions of high-intensity ultrasound

Run	Process variables			Response		
	Amplitude (%) A	Acoustic Power W/cm^2	Time (min) B	Solubility		
				Actual	Predicted	RD* (%)
1	40 (-1)	9.24	5 (-1)	20.32	21.37	-5.2
2	**80 (+1)**	**60.56**	**15 (+1)**	**32.56**	**32.87**	**-1.0**
3	60 (0)	24.82	10 (0)	22.04	22.83	-3.6
4	60 (0)	23.52	10 (0)	22.05	22.83	-3.6
5	60 (0)	22.82	5 (-1)	20.85	19.76	5.3
6	60 (0)	24.41	10 (0)	23.38	22.83	2.4
7	80 (+1)	63.29	5 (-1)	28.36	28.47	-0.4
8	40 (-1)	10.41	10 (0)	27.02	24.75	8.4
9	60 (0)	24.18	10 (0)	21.14	22.83	-8.0
10	80 (+1)	57.62	10 (0)	31.56	31.24	1.0
11	40 (-1)	9.46	15 (+1)	25.73	26.98	-4.9
12	60 (0)	24.62	15 (+1)	26.25	24.76	5.7

**RD: relative deviation. The obtained responses were compared with predicted using equation (3.11).*

The coefficient of determination (R^2) and adjusted-R^2 was considered to check the adequacy of the model. The R^2 value for the suggested quadratic model was 0.9173 which means that 91% of experimental data was compatible. Generally, the R^2 value for a good model adequacy and fitness should be greater than 0.8 (Joglekar and may, 1987). A high R^2 and low *p-value* revealed that the suggested quadratic model is sufficient to represent the relationship between variables and response. The best regression equation, which is an empirical relationship between the protein solubility and the process variables of high-intensity ultrasound, was presented in terms of codded values in equation (4.1). Each of the actual responses obtained from 12 different conditions were compared to the predicted values calculated from the equation (4.1) to check the adequacy of the suggested model (Table 4.8). The results revealed that the suggested model can be used to predict the optimal conditions for the protein solubility.

$$Y_{Solubility} = +22.81 + 3.23_A + 2.50_B - 0.30_{AB} + 5.16_{A^2} - 0.57_{B^2} \quad (4.1)$$

Where A is US amplitude (%) and B is US time (min)

Table 4.8. Regression coefficients and analysis of variance of the model for protein solubility of DSPC

Variables	Solubility		
	K	*F-value*	*p-value*
Intercept	+22.81		
A-Amplitude	+3.23	23.77	0.0028
B-Time	+2.50	23.77	0.0093
AB	-0.2896	14.23	0.7259
A^2	+5.16	0.1350	0.0020
B^2	-0.5738	0.3324	0.5852
Lack of Fit		5.17	0.1052
Model		13.31	0.0034
R^2		0.9173	
Adjusted R^2		0.8484	
C.V. %		6.47	
Main effects			
Amplitude			**
Time			**

**k, coefficients, R^2, coefficient of determination, C.V., coefficient of variance, NS, not significant.*
*** Significant at p <0.05. *** Significant at p <0.01. Lack of fit is non-significant at p >0.05.*

4.2.2. Optimization of high-intensity ultrasound variables based on protein solubility

Values of DSPC solubility after optimization trials of ultrasound treatment were presented in Table 4.7, which were in the range of 20.32-32.56%. The maximum solubility with 32.56% was reached by R2 treatment with an amplitude of 80% for 15 min (60.56 W/cm^2). The lowest solubility (20.32%) was observed at R1 treatment with an amplitude of 40% for 5 min (9.24 W/cm^2) which was higher than untreated DSPC solubility (14.1%). Both the amplitude (A) and time (B) had a positive influence on the solubility. It can be concluded that all ultrasound treatments positively affected the solubility of DSPC.

To show the main effect and interactive effect of the ultrasound variables on the protein solubility, 3D response surface plots produced from equation (2) were given Figure (4.2A). The solubility of DSPC increased linearly with increasing amplitude and time. The positive effect on the protein solubility could be attributed to the increasing transferred energy into reaction media depending on the amplitude and applied time of ultrasound. This may be explained by two possible scenarios. One possible consequence is that water molecules can interact more with protein by the partial

unfolding of protein molecules. Another possibility is that the ultrasound treatment can reduce the particle size of protein by the shear force and micro-streaming, which were produced by cavitation. The decreases in the particle size may result in an increase water-protein interaction by increasing the surface area of the protein, and therefore an enhanced in protein solubility. As for solubility according to surface response analysis, the results revealed that the interaction between amplitude and time (AB) was non-significant (p = 0.7259 > 0.05, Table 4.8).

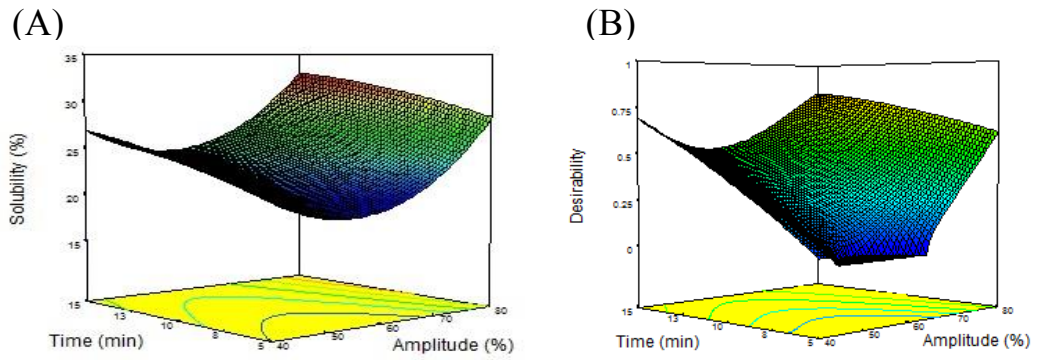


Figure 4.2. 3D response figures representing the effect of HIUS amplitude and HIUS time on solubility (A) and desirability (B).

Our findings agreed with the results in the literature which on the solubility of the protein isolates or concentrates by ultrasound treatment could be improved the solubility depending on the amplitude and time. Malik et al. (2017) reported that the ultrasound treatment at a fixed acoustic power intensity of 58-61 W/cm^2 produced a linear increase in the solubility of isolate produced from sunflower meal by the of time treatment up to 20 min. They reported that this linear trend disappeared after the treatment time of 20 min. Similarly, Karabulut and Yemiş (2022) studied changes of the functional properties of hemp seed protein isolate after treatment with ultrasound. They also observed a similar behavior of the isolate, which the solubility enhanced linearly by the treatment time up to 10 min. They determined the solubility decreased at ultrasound treatments at higher amplitudes of 65% and time of 10 min. This behavior was reported at many studies on the different plant protein isolates produced from millet (Nazari et al, 2014), black bean (Jiang et al, 2014), faba bean (Martínez-Velasco et al, 2018), chickpea (Wang et al, 2020) and perilla (Zhao et al, 2022). Decreasing in solubility of isolate because of critical energy density is related to conversion of protein molecules to higher molecular weight aggregates by non-covalent interactions.

However, in our study, we did not observe in decreases for the solubility of DSPC in the amplitude range of 40–80% and time range of 5–15 min. This contradictory result in our study may be explained by the fact that the intensity of energy transferred to the protein solution under the ultrasound conditions was not sufficient to cause the aggregation of date seed protein molecules. Hence, the optimization studies on the maximizing the solubility for determination of the optimal ultrasound conditions are critical.

Numerical optimization by Design Expert revealed that the optimal conditions of ultrasound treatment for maximum solubilization of DSPC were 80% amplitude for 15 min (60.56 W/cm^2) by the highest desirability value of 0.83 (Figure 4.2B). The predicted optimum conditions for the solubility were the same with the harshest conditions (80% amplitude and 5 min) within the studied ultrasound condition ranges. The predicted solubility of DSPC under these optimal conditions by the model was 32.87% while the actual experimental value was 32.56%, indicating that the predicted optimum value was valid. In further stage of our study, the optimal conditions consist of the amplitude of 80% and treatment time of 15 min were applied to the DSPC, and the techno-functional properties of DSPC-US were compared with DSPC-N.

4.2.3. Comparison of techno-functional properties of DSPC

4.2.3.1. Solubility and water/oil binding

Protein solubility is the most critical techno-functional attribute due to its impacts on the performances of ingredients in food systems, especially emulsification, gelling, and foaming. It is well known that the application of protein to the food system depends on the solubility of the protein in addition to the other functional properties of protein (Sim et al, 2021). This is the main reason the choosing solubility at the optimization of ultrasound variables, which is the main property for protein functionality. The samples of DSPC-US which applied the ultrasound treatment under the optimal conditions exhibited a 32.56% protein solubility while the untreated ultrasound samples (DSPC-N) had only 14.1% protein solubilization (Table 4.9). The level of solubility of DSPC was observed an increase of 131% after ultrasound treatment under the optimal conditions. Similarly, there are many studies in the literature that have examined the effects of ultrasound on the solubility of protein isolates and concentrates produced from various plant materials.

The higher solubility values for the protein isolates that were treated with ultrasound treatment have been previously declared for hemp seed (78%, Karabulut and Yemiş 2022), faba bean (77%, Martínez-Velasco et al., 2018) sunflower (27%, Malik et al. 2017), walnut (22%, Zhu et al. 2018), black bean (10%, Jiang et al. 2017), and millet (Nazari et al, 2018). Our finding for solubility differs from the studies in literature. Compared to these studies, the solubility of concentrate obtained from date seed was high for the ultrasound treatments. There are two plausible reasons for these differences in the protein solubility. The first one was that the use of different process conditions (amplitude, time, and protein concentration) for the ultrasound treatment, which means that transferred energy intensity into reaction media can differ depending on process conditions. Another possible reason was that different plant-derived proteins have different molecular structures.

The positive effect of ultrasound treatment on solubility rely on high temperature and pressure caused by acoustic cavitation, turbulent flow, shear force, micro-streaming, and shock waves which are responsible from chemical and physical modifications in protein molecules (Kadam et al, 2015; Shirsath et al, 2012). The observed increase in solubility of DSPC by ultrasound treatment could be attributed to the conformational and physical changes of protein. Similarly, Martínez-Velasco et al. (2018) studied the effect of high intensity ultrasound conditions on the surface, foaming and structural properties of faba bean protein by the surface response methodology. But the models were not important for solubility, so they could not determine the optimum conditions of ultrasound. Mir et al. (2019) showed that the solubility of album seed protein isolate improved by increasing residence time of up to 25 min at fixed amplitude of 25%. They concluded that the effect of ultrasound treatment on the solubility was due to the exposed the hydrophilic groups and increased electrical conductivity, which represent the conformational changes in protein molecules.

In various studies with different plant protein isolates, similar findings were revealed, and this is explained by the partial unfolding of protein molecules in which exposed of buried hydrophilic groups depending on the applied ultrasound intensity and time (Jiang et al, 2014; Wang et al, 2020; Nazari et al, 2018). In our study, the change in solubility of DSPC by ultrasound treatment was confirmed with conformational changes which were shown in FTIR (Figure 4.3), zeta potential results. The shifts in

amid I, and amid A regions by ultrasound treatment have revealed the conformational changes of date seed protein.

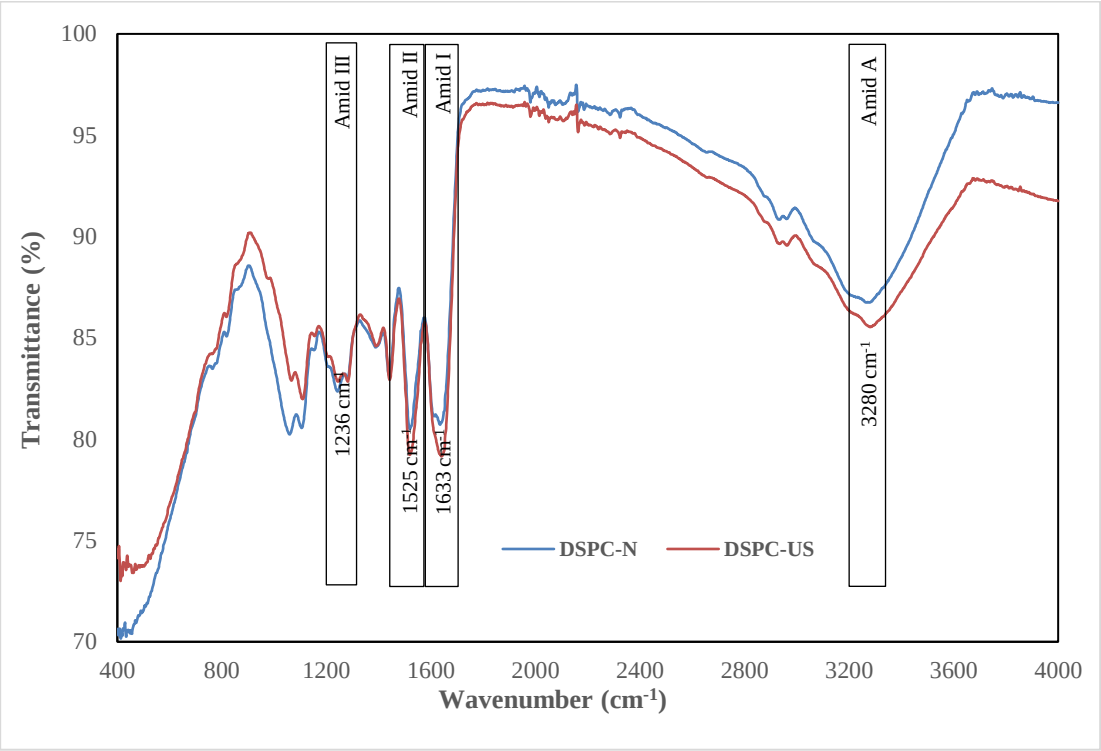


Figure 4.3. Fourier transform infrared spectra of native (DSPC-N) and ultrasound applied (DSPC-US) date seed protein concentrate.

Furthermore, the conformational changes upon ultrasound treatment were confirmed by zeta potential and DSC results. But this phenomenon was not observed at SDS-PAGE finding (Figure 4.4) that revealed no change in protein profile between DSPC-N and DSPC-US. Another explanation for the increase in the solubility was reduction in particle size by ultrasound treatment. It is well known that increase in surface area of protein lead to the interactions between protein and water (Arzeni et al, 2012). The observed increase in solubility of DSPC by ultrasound treatment agreed with the decrease in particle size which is from 123 nm to 100 nm in the present study (Table 4.9).

It has been reported by Du et al. (2018); Saha and Deka (2017) the water binding capacity (WBC) and oil binding capacity (OBC) indicate the interaction between water/oil and protein, that were related to conformational characteristics, amino acid composition, and hydrophilic and hydrophobic balance of protein. The ability of water binding is closely related to the protein solubility. As seen from Table (4.9), the WBC of DSPC lowered from (2.76 g/g) to (1.55 g/g) after ultrasound treatment while the

OBC enhanced from (1.73 g/g to 4.79 g/g). These changes indicate a 43.8% decrease in WBC and an increase of 176.8% in OBC, respectively. Similarly, Malik et al. (2017) declared a similar behavior in water and oil binding capacity of sunflower protein isolate by ultrasound treatment. They explained this behavior by the denaturation of proteins after ultrasound and the subsequent exposure of embedded hydrophobic groups.

Table 4.9. Techno-functional and physicochemical properties of DSPC-Native and DSPC-HIUS

Property	DSPC-N	DSPC-US
Solubility (%)	14.10±0.47[a]	32.56±0.31[b]
WBC (g/g)	2.76±0.18[a]	1.55±0.17[b]
OBC (g/g)	1.73±0.04[a]	4.79±0.26[b]
EAI (m^2/g)	11.92±0.20[a]	19.15±0.67[b]
ESI (min)	17.63±0.36[a]	23.88±0.53[b]
FC (mm)	11±0.82[a]	21±2.43[b]
FS (%)	8±0.82[a]	21±2.43[b]
Td (˚C)	87.7±0.83[a]	61.96±0.55[b]
ΔH (J/g)	204.0±2.21[a]	191.5±1.02[b]
Particle size (nm)	123.91±1.34[a]	100.87±1.96[b]
SH (μmol/g)	1.58±0.17[a]	3.06±0.24[b]
H_0	164.20±0.70[a]	147.30±0.10[b]
(ζ) potential (mV)	-28.73±1.34[a]	-37.83±0.47[b]

Data are presented as mean ± standard deviation (n=3). Mean values in each row with different lower case letter superscripts are significantly different ($p<0.05$). DSPC-N: date seed protein concentrates native form, DSPC-US: date seed protein concentrate ultrasound treated, WBO: water binding capacity, OBC: oil binding capacity, EAI: emulsion activity index, ESI: emulsion stability index, FC: foaming capacity, FS: foaming stability, Td: denaturation temperature, ΔH: enthalpy, SH: free sulfhydryl group content, H_0: surface hydrophobicity.

Recently, Karabulut and Yemis (2022) reported a similar increase of OBC by 11% for hemp seed protein isolate with slightly reduce in WHC after HIUS treatment. The improvement of OBC may be due to the release of hydrophobic groups on the surface which impact the hydrophilic-hydrophobic balance in the physicochemical solution (Malomo et al, 2014). But we observed that ultrasound treatment on the DSPC resulted in decrease surface hydrophobicity while free SH content increased under optimal conditions in our study (Table 4.9). It can be observed that the increase in free SH content of DSPC paralleled the increase in OBC. In contrast to our findings, Bouaziz et al. (2013) reported a WBC and OBC of approximately (4 g/g) and (6 g/g) for date seed protein powder produced from Allig and Deglet Nur varieties, respectively, which was much higher than the results of current study that were (2.76 g/g and 1.73 g/g).

4.2.3.2. Emulsifying ability and stability

Proteins are important ingredients used as an emulsifier at stabilization of food emulsions due to amphiphilic nature. Emulsifying property characterizes the ability of a protein to get absorbed into oil-water interface and expresses the interfacial area stabilized per unit weight of protein (Biswas and Sit 2020). Basically, emulsion stability is an important characteristic, depending on the interactions between the oil droplets (Tan et al, 2017). Emulsion ability index (EAI) and emulsion stability index (ESI) are used to determine emulsion performance of a protein (Boye et al, 2010).

Our study showed that the EAI value of DSPC ultrasound treated at optimal condition was 19.15 m^2/g while DSPC-N had an EAI of 11.92 m^2/g. A similar trend was observed for the emulsion stability of DSPC, which the ESI value increased from 17 min to 23 min after ultrasound treatment (Table 4.9). The results for emulsion performance of DSPC have been inconsistent with the results of Akasha et al. (2016), who revealed that the EAI and ESI of DSPC were approximately 50 m^2/g and 50 min, respectively.

The different results in terms of EAI and ESI could be indicated by the fact that the authors used the different protein extraction procedure and cultivar of date seed. Our findings indicated that the emulsifying performance of DSPC notably improved after ultrasound treatment at optimal conditions.

Also, Kresic, et al. (2008) demonstrated that ultrasonic homogenization can improve the functional properties of proteins with slight changes in secondary structure. Similar studies on the effect of ultrasonic homogenization on emulsion performance have been previously done for various plant-based protein isolates obtained from hemp seed (Karabulut and Yemiş, 2022), sunflower (Malik et al, 2017), peanut (Zhang et al, 2014), soy (Jambrak et al, 2009), millet (Nazari et al, 2018) chickpea (Wang et al, 2020) and albumin (Mir et al, 2019).

The emulsion performance of protein isolates/concentrates can be affected positively by ultrasound treatment that is associated with the exposure of inner hydrophobic groups of protein molecule in the literature. Meanwhile, our results of surface hydrophobicity (H_0) did not confirm the positive effect of ultrasound treatment, which the H_0 of DSPC-US was significantly lower than that of DSPC-N. Number of hydrophobic groups of protein surface could be measured by Hydrophobicity (H_0) that

exposed in an aqueous solution (Hu et al., 2013). The obtained results indicated that hydrophobicity of DSPC surface was reduced from (164.2 to 147.3) after sonication under optimum conditions as cleared in Table (4.9).

The inconsistent result with literature could be explained by the difference in the method of surface hydrophobicity and material used in our study. Another plausible explanation for decrease in surface hydrophobicity of DSPC during ultrasound treatment may be possible oxidative reactions on protein molecules of free radicals generated by ultrasound waves. It has been reported that free radicals occur the result of the decomposition of water molecule by sonochemical waves (Camargo-Perea et al, 2020). Wang et al. (2022) indicated that surface hydrophobicity of soy protein isolate treated by ultrasound initially reduced depending on treatment power at the range of 0–200 W, and then increased with increasing power.

This was explained by the oxidative aggregation that can occurs because of covalent and non-covalent cross-linking of protein molecules in the 0–200 W range of ultrasound application. Recently, Yan et al (2021) studied the effect of ultrasound treatment on flexibility and surface hydrophobicity on the emulsifying properties of soybean protein isolate. They reported that the correlation between the EAI/ESI and the flexibility was to be stronger than the correlation between the EAI/ESI and the surface hydrophobicity.

The increase in the emulsion performance of DSPC by ultrasound treatment can be associated with the reduction of particle size after treatment by the cavitation phenomenon, that caused by the shear force, micro-streaming, and shock waves. The increasing solubility together with decreasing of particle size of DSPC after ultrasound application can causes proteins to diffuse and rapidly absorbed to the oil-water interface, which results in a better emulsion performance. The particle size and solubility results in our study confirm this positive change in emulsion properties.

Similarly, it was declared that ultrasound treatment at 20% amplitude for 20 min emulsion conformation exhibited a small droplet size and higher interfacial protein concentration producing an improvement of stability against creaming (Sun et al, 2014). Also, Karabulut and Yemiş (2022) reported that EAI of hemp seed protein isolate increased from 21.45 m^2/g to 28.14 m^2/g by ultrasound treatment at an acoustic intensity of (37 $W/cm^{2)}$ for 7.8 min. Also, the obtained results are in accordance with the results of Biswas and Sit (2020), who revealed that emulsifying ability and stability

of tamarind seed protein isolates enhanced with ultrasonication by 79.41% and 82.53%, respectively.

4.2.3.3. Foaming capacity and stability

Foaming capacity (FC) and foam stability (FS) are used as indicators of the determination of the ability of foaming and permanency of the foam structure. The FC value of DSPC increased from 44% to 84% as ultrasound applied at optimal conditions, which means an increase of 91%. Similarly, the stability of the foam structure was 8% for the DSPC-N, while DSPC-US had a foam stability of 21% (Table 4.9). The obtained results revealed that the foaming performance of DSPC in terms of FC and FS was highly enhanced after the ultrasonic treatment under optimal conditions. The optimized condition of ultrasonic homogenization increased FC and FS by 91% and 162%, respectively. The positive effect ultrasound on the FC and FS was observed for protein isolates/concentrates obtained from sunflower (Malik et al, 2017), millet (Nazari et al, 2018), hemp seed (Karabulut and Yemiş, 2022), faba bean (Martinez et al, 2018), chickpea (Wang et al, 2020) and soy (Morales et al, 2015).

This improvement of ultrasound application on foaming capaciy of isolates/concentrates can be explained by the conformational and physical changes of protein (unfolding, exposing hydrophobic groups, flexibility, denaturation temperature, particle size), as observed in the emulsion performance. Our results for foaming properties of DSPC are confirmed by particle size, DSC, FTIR data. In our study, we observed that both FC and FS of DSPC improved after ultrasonication while the particle size value decreased from 123 nm to 100 nm.

Enhanced solubility by decreasing in particle size after ultrasonication can lead to a more adsorption of protein adsorption at air-water interface and stabile air bubbles. Similarly, FTIR (Figure 4.4) and DSC (Table 4.9) data revealed that the structural and thermal changes in DSPC occurred by ultrasound treatment. Recently, Karabulut and Yemiş (2022) studied the effect of high-intensity ultrasound treatment on modification of functional properties of hemp seed protein isolates. They reported that ultrasound treatment at optimal conditions revealed that the FC and FS values of 69% and 16% were improved, this effect might be related to increase in surface hydrophobicity and decreases in particle size.

4.2.4. Comparison of physicochemical properties of DSPC

Thermal stability of DSPC samples was analyzed by the differential scanning calorimetry (DSC), which indicates alterations in the structural and conformational of protein during thermal treatment. Td and ΔH values which are thermodynamic parameters measured by DSC analysis indicate the denaturation temperature of the protein and the amount of heat required for denaturation, respectively. The thermodynamic parameters (Td and ΔH) obtained from thermograms of DSPC samples were presented in Table (4.9). A single endothermic denaturation peak was observed for both DSPC-N and DSPC-US (data not shown). Denaturation temperature (Td) of DSPC decreased from 87.7°C to 61.9°C while the enthalpy value (ΔH) reduced from 204 to 191 J/g after ultrasound treatment.

Our results regarding of DSPC native are constant with results of Akasha et al. (2016), who studied on the Deglet Nur variety of date seeds (6% protein), and they found the denaturation temperature (Td) of 88.73˚C and the enthalpy (ΔH) of 235.6 J/g. The decreases in Td and ΔH values revealed that conformational changes in DSPC were occurred by ultrasound treatment, and ultrasound treated DSPC samples can be denatured by a lower energy. The observed change in the thermal stability of the DSPC samples could be attributed to the breaking intermolecular bonds of protein by shear force formed from the cavitation.

The zeta potential results of DSPC showed that ultrasound treatment performed under optimal conditions increased the negative charge of DSPC samples from –28.73 to –37.83 m/V (Table 4.9). This could be attributed to the ultrasound treatment resulting in more negatively charged amino acids exposed from the inner part of protein to the solvent. This change in zeta potential was confirmed by both FTIR and DSC data, which showed wavelength shifts at amid 1 region and decrease in Td and ΔH values. Similarly, Karabulut et al. (2022) mentioned that the negative ζ potential of hemp seed protein isolate increased from (–22.30 mV) to (–27.80 mV) after ultrasound treatment. They explained that ultrasonication might generate more negatively charged residues on display from inside part of the protein to the outside area because of unfolding protein structures. On the contorary, Xiong et al. (2018) stated that ultrasound treatment on pea protein isolate had lowered the negative charge of surface from (-43.1 to -37.9 m/V).

The particle size of the proteins has a crucial role that affects protein techno-functional properties (Hu et al, 2013b). The effectiveness of ultrasound treatment on particle size of DSPC was illustrated in Table (4.9), which cleared that the sonication treatment lowered the particle size of DSPC from 123.91 to 100.87 nm. Our results are in accordance with other studies on the plant-based protein isolates/concentrates, which are revealing that the sonication could decrease the particle size of protein. (Arzeni et al, 2012; Hu et al, 2013b; Hu Li-Chan et al, 2013; O'Sullivan et al, 2016b; Yanjun et al, 2014; Xiong et al, 2018). Also, Kadam et al. (2015) reported that treatment with ultrasound resulted in a reduction in particle size of DSPC that can be ascribed to the cavitation phenomenon, that caused the shear forces, micro-streaming, and shock waves.

SDS–PAGE electrophoresis was conducted to evaluate the effect treatment with ultrasound treatment on the protein profile of DSPC. Figure (4.4) shows a typical SDS–PAGE profile of DSPC samples under the reducing and non-reducing conditions. Non-reducing electrophoresis revealed that DSPC samples showed only one strong band at 55–65 kDa, which was a typical band for date seed protein (Akasha et al, 2016). However, the band was not observed in SDS-PAGE under reducing condition, indicating that the reduced fragments of protein by mercap to ethanol could be out of range of used protein marker (10-180 kDa).

Khoshroo et al. (2011) studied the protein profile of twelve different date seed varieties grown in Iran by SDS–PAGE technique. Their results indicated that one heavily stained band at around 65 kDa and minor bands ranged from 12 to 369 kDa. Similarly, Bouaziz et al. (2008) discovered 3 similar prominent protein bands at 32, 60 and 70 kDa in date seeds of Allig and Deglet Nur varieties. Variations in protein profile of date seed could be due to the extraction method and varieties used (Miernyk and Hajduch, 2011).

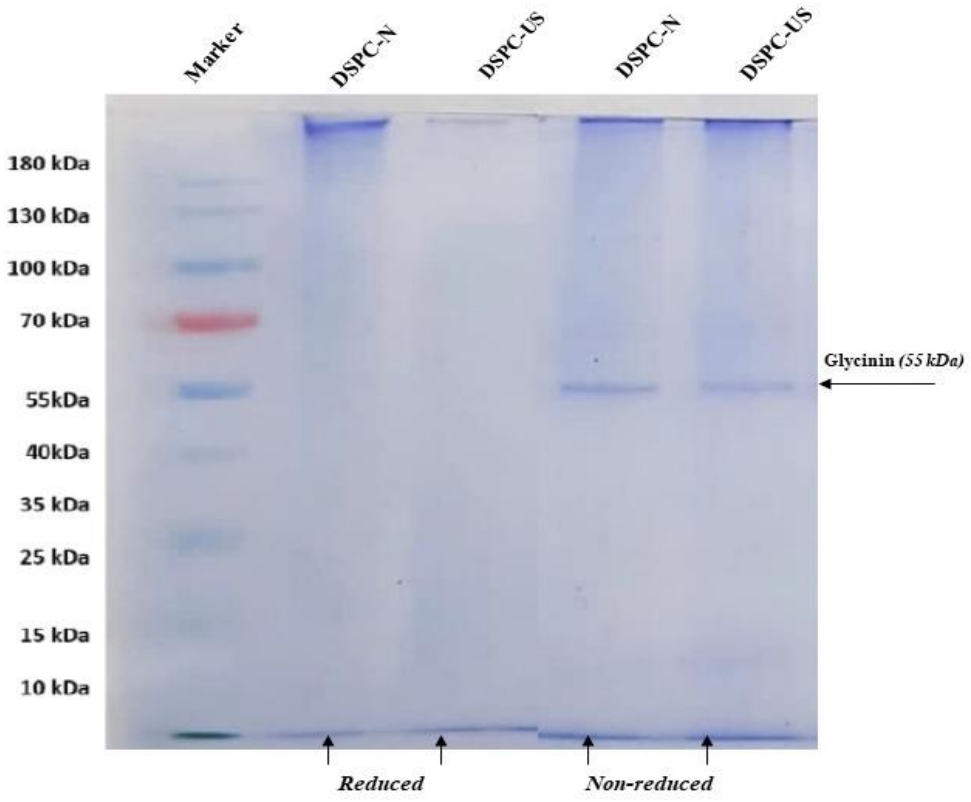


Figure 4.4. SDS-PAGE electrophoretic protein profiles of native (DSPC-N) and ultrasound applied (DSPC-US) date seed protein concentrate under reducing and non-reducing conditions

In our study, we didn't observe any change in the number of bands between native DSPC and ultrasound treated DSPC, but DSPC-US had a lesser band intensity than that of DSPC-N. Our results revealed that ultrasonication at the optimum conditions had no clear effect on the primary structure of date seed proteins. Similarly, several authors reported that ultrasound treatment did not change the protein profile of various plant-based protein isolates/concentrates such as chickpea (Wang et al, 2020), hempseed (Karabulut et al, 2022) and black bean (Jiang et al, 2014).

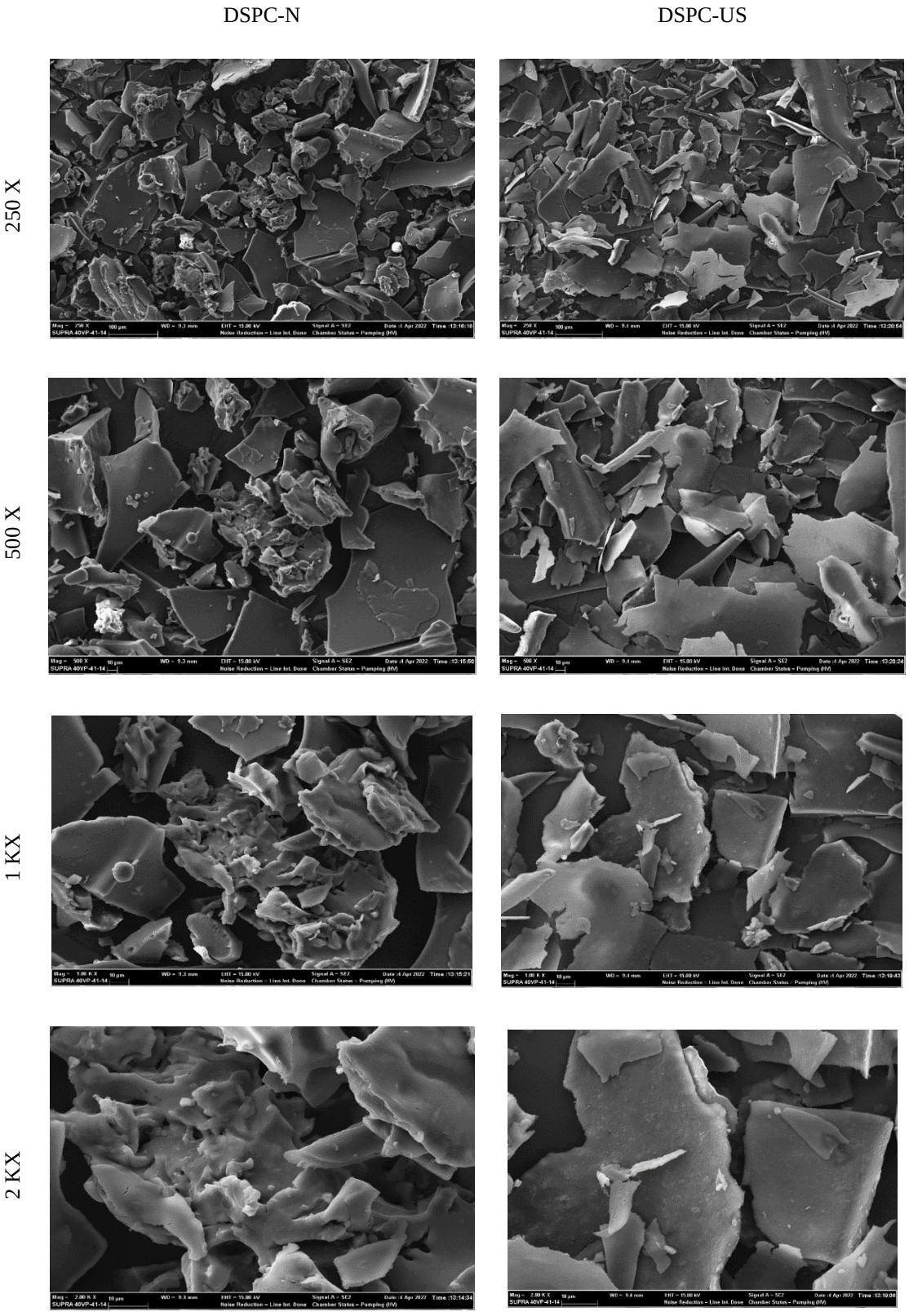


Figure 4.5. The morphology of native (DSPC-N) and ultrasound applied (DSPC-US) date seed protein concentrate determined by scanning electron microscopy

The effect of sonication on the microstructure of DSPC was illustrated in SEM images at 250x, 500x, 1000x, 2000x magnification (Figure 4.5). SEM images showed that ultrasound treated DSPC samples exhibited more regular fragments compared to the native form of DSPC samples, which are in the form of clumps. These uniform structures formed by the ultrasound treatment may be associated by the unfolding of proteins and increasing free SH content (Table 4.9), which caused the protein interaction (Resendiz-Vasquez et al, 2017).

FTIR, is considered a way for characterization of the secondary structure of proteins by the vibrational states of chemical bonds in proteins, has been used for investigation effects treatment with ultrasound on the structure of DSPC. (Ali et al, 2019). Figure (4.3) indicated the FTIR spectra of DSPC and US-DSPC in the range of (4000–400 cm^{-1}). DSPC samples had four typical peaks related to amid bonds, which were amid 1 (1700–1600 cm^{-1}), amid 2 (1580–1480 cm^{-1}), amid 3 (1400–1200 cm^{-1}), amid A (3500–3200 cm^{-1}). The peak of amid I increased from (1630 cm^{-1} to 1640 cm^{-1}) after ultrasound treatment, where is C=O stretching and C–N bending vibrations of protein linkages. The observed change in amid 1 band, which is the most sensitive to the secondary structure of the proteins, is associated with the β-sheet (1628–1642 cm^{-1}) structure (Sadat and Joye, 2020).

Moreover, we did not observe distinct shifts in amid II and amid III bands, which located at a wavelength of 1525 cm^{-1} and 1236 cm^{-1}, respectively. It was reported that (3000–3500 cm^{-1}) is the main peak of amide A produced by N–H bending and O–H stretching vibrations, that are related to hydrogen bonds on the main chain of polypeptide (Wang et al, 2021). The position of amid A shifted from 3280 cm^{-1} to 3285 cm^{-1} after ultrasonication in our study. The peaks range of amide A of DSPC and US-DSPC reported that sonication improved the hydrogen bond strength in the protein molecules of DSPC (Ali et al, 2019). A similar result was also revealed by Nazari et al. (2018), who showed that the peak spectra of millet protein isolate shifted from 3286 cm^{-1} to 3418 cm^{-1} after ultrasound treatment.

Treatment with HIUS resulted in significant increase in the content of sulfhydryl groups on the surface of DSPC from (1.58 μmol/g) to (3.06 μmol/g). (Table 4.9), which indicates exposure to the internal SH groups of the DSPC to the surface by the cavitation effect of ultrasound (Jin et al, 2016). Moreover, reduction in particle size may promote the release of buried sulfhydryl groups (Hu et al, 2013b). Similarly,

Karabulut et al. (2022) revealed that free SH group content of hemp seed protein isolate increased from 2.65 µmol/g to 4.22 µmol/g after ultrasonication under optimal conditions. Similar results were observed for pea protein isolate by Xiong et al. (2018), sunflower protein isolate by Malik et al. (2017) and chickpea protein isolate by Wang et al. (2020).

Tian et al. (2020) revealed that intrinsic fluorescence can indicate the specific characteristics of aromatic amino acids (Tyr and Trp) in proteins and describe tertiary structures of protein. It was reported that wavelength from (320 to 350 nm) plays an important role in fluorescence peak based on the emission intensity of the Tyr residues is weak (Ali et al, 2019). Figure (4.6) showed the intrinsic fluorescence spectra of DSPC-N and DSPC-US in a range of (300-450 nm). Our results showed that the maximum emission wavelength (λmax) increased from 331 nm to 339 nm while fluorescence intensity (FI) reduced from 765 to 165 Au.

This change observed in the fluorescence emission may be associated with the release of the chromophores in the proteins to the solvent as a result of the partial unfolding with ultrasound application. Liu et al. (2022) reported similar results for mung bean protein hydrolysate which decreased from 392.8 to 364.7 Au after ultrasonic treatment. Also, Xiong et al. (2016) reported that treatment with ultrasonic (amplitude 60% and 90%) reduced the intrinsic fluorescence intensity of ovalbumin reduced from (950 to 650 Au) that might lead to the change of tertiary structure and partial unfold of ovalbumin.

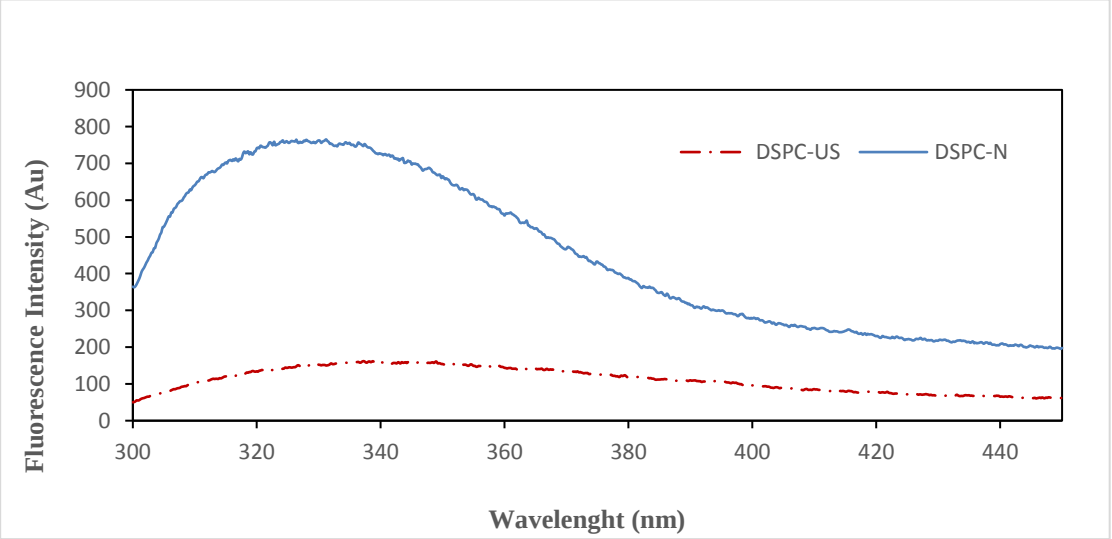


Figure 4.6. Fluorescence intensity of native (DSPC-N) and ultrasound applied (DSPC-US) date seed protein concentrate

4.3. Effect of High-Pressure Homogenization (HPH) on Saidy Date Seed Protein Concentrate (DSPC)

High pressure homogenization (HPH) is a technique that is widespread and used in many industries such as food, pharmaceutical and cosmetics (Galazka et al, 2000; Masson et al, 2001). More attention has been devoted to understanding how high-pressure treatment affects the structure and functions of biomacromolecules and other bioactive compounds (Paquin, 1999; Galazka et al, 2000). High-pressure homogenization (HPH) treatment led to structural modifications of proteins through high shear forces, cavitation, and turbulence (Lakshmanan et al, 2007). The treatment times of HPH for food industry applications can vary from 2 to 30 minutes depending on the food type. Treatment with HPH has little effect on the nutritional value and sensory properties of the treated food. These effects are related to the passage of liquid food through the homogenization valve, physical factors such as shear stress, turbulence and cavitation contribute to the breakup of the droplets, altering the protein structure and the physico-chemical properties of food (Paquin, 1999; Floury et al, 2003, 2004).

4.3.1. Suitability of models

The experimental design using the response surface method (RSM) as two factor three levels through the central composite design and the solubility, emulsion and antioxidant activity values obtained under 12 different conditions are in Table 4.10 specified. The Central Composite Design experiments with two variable and three different levels were chosen to investigate the influence of pressure (A: pressure 50-150 MPa) and time (B: protein concentrate 1-3%) on the solubility, emulsifying and antioxidant activities of DSPC. The design consisted of twelve runs, including four midpoint repetitions (Table 4.10). The results obtained were evaluated using a linear model for the solubility and a quadratic model for the emulsifying and antioxidant activities as shown in equation (4.1). The model was fitted as same as mentioned in paragraph (4.2.1) in this study.

4.3.2. Response surface modelling

The effects of pressure value (A) and protein concentration (B) on the reactions were studied using the Central Composite Design. Experiment and predicted responses

obtained from twelve different runs of HPH in terms of solubility, emulsifying and antioxidant activities from experimental design are presented in Table 4.10. Regression coefficients and analysis of variance of models for solubility, emulsifying and antioxidant activities of DSPC were shown in Table 4.11. To evaluate the fit of the models, the lack of fit, the F-test and the ANOVA test were considered.

The P values missing-fit were recorded as 0.1583, 0.0002, and 0.0699 for the linear and quadratic models of solubility, emulsifying, and antioxidant activities, respectively. The lack-of-fit was not significant for solubility and antioxidant activity (0.1583 > 0.05 and 0.0699 > 0.05), showing that the proposed central composite model was suitable for the experimental design. While the lack of fit for emulsifying responses (0.0002 < 0.05) was not significant, this means that the model was not suitable for emulsifying activity. The high F-values and low p-values indicated that the proposed models had a highly significant, indicating that the model was adequate to describe the solubility and antioxidant activity within the design. The results showed that the models could predict the solubility and antioxidant capacity properties of DSPC, while the design was not applicable to the emulsifying properties. To describe solubility and antioxidant capacity, the best regression equations were given by coded values as follows in Equations 4.2 and 4.3.

$$Y_{Solubility} = +35.05 + 4.32_A - 4.26_B \quad (4.2)$$

$$Y_{Antioxidant} = +62.26 + 2.91_A - 1.16_B - 0.3768_{AB} + 1.40_{A^2} + 3.92_{B^2} \quad (4.3)$$

$$Y_{Emulsifying} = +20.57 + 0.8440_A - 0.7579_{AB} - 0.4979_{A^2} + 0.6595_{B^2} \quad (4.4)$$

Where A is HP (MPa) and B is protein concentrate (%)

The equations can be used to predict the responses at each variable factor level for solubility and antioxidant activity. Usually, the increased values of the factors are coded as (+1), while the low values are coded as (-1). In an experimental model, the low p-value and high F-value for each independent factor variable indicated that the response was at a significant level.

Response surface analysis showed that the main effects of pressure level and protein concentration were statistically significant ($p<0.05$). The data in Table (4.11) showed

that the coefficient of determination (R^2) values for the quadratic regression models were 0.9273 for solubility and 0.9971 for antioxidant activity. This is still within the accepted range of R^2 less than 0.80 as suggested by Joglekar et al. (1987) an appropriate statistical design. The high R^2 indicated a high level of acceptability of patterns explaining the contrast between experienced and expected results.

Table 4.10. The coded and uncoded levels of HPH terms, actual, expected solubility (%), Emulsion Activity (EAI) (m^2/g) and Antioxidant Activity (TE/g) responses, and relative deviation (%) for DSPC with the central composite design

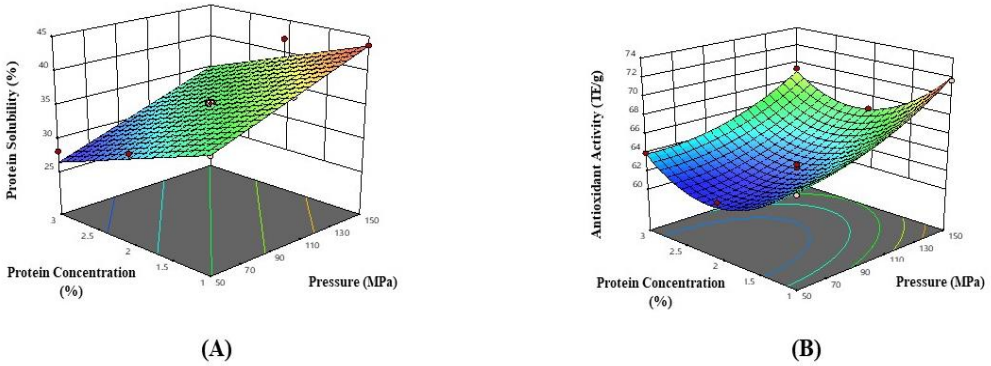
	Individual Values		Responses								
RUN	Factor 1(X_1) A: Pressure MPa	Factor 2(X_2) B: Protein Concentrate (%)	Response 1 (Y_1) Protein Solubility (%)			Response 2 (Y_2) Emulsion Activity (EAI) (m^2/g)			Response 3 (Y_3) Antioxidant Activity (TE/g)		
			Actual	Predicted	RD* (%)	Actual	Predicted	RD* (%)	Actual	Predicted	RD* (%)
1	50 (-1)	3 (+1)	28.12	34.99	-24.42	20.60	20.82	-1.05	63.96	63.89	0.11
2	150 (+1)	1 (-1)	43.69	43.63	0.14	22.29	22.17	0.54	71.67	72.03	-0.50
3	100 (0)	2 (0)	33.30	39.31	-18.04	20.53	20.57	-0.21	61.83	62.26	-0.70
4	100 (0)	1 (-1)	39.29	39.31	-0.05	21.23	21.06	0.78	68.30	67.34	1.41
5	100 (0)	2 (0)	34.03	39.31	-15.51	20.50	20.57	-0.36	62.71	62.26	0.72
6	100 (0)	2 (0)	35.32	39.31	-11.29	20.52	20.57	-0.26	61.56	62.26	-1.14
7	150 (+1)	2 (0)	42.11	43.63	-3.61	20.60	20.92	-1.56	66.62	66.58	0.07
8	150 (+1)	3 (+1)	34.59	43.63	-26.13	21.19	20.99	0.94	69.28	68.96	0.46
9	100 (0)	2 (0)	34.21	39.31	-14.90	20.55	20.57	-0.11	62.36	62.26	0.16
10	50 (-1)	2 (0)	31.46	34.99	-11.21	19.74	19.23	2.58	61.30	60.75	0.89
11	50 (-1)	1(-1)	34.89	34.99	-0.28	18.67	18.96	-1.57	64.84	65.45	-0.94
12	100 (0)	3 (+1)	29.62	39.31	-32.71	21.42	21.40	0.09	64.64	65.02	-0.59

**RD: relative deviation*

4.3.3. Influence of independent process variables on solubility and antioxidant activity of DSPC

The modification of food protein properties by high pressure homogenization (HPH) is receiving more research attention as reported by Huppertz et al. (2006). The results obtained from RSM, the solubility of DSPC after HPH ranged from 28.12 to 43.69% (Table 4.10 and Figure 4.7.A). The maximum solubility level was 43.69% after high pressure treatment, which took place in run R2 with a pressure of (150 MPa) and 1%

protein concentrate, compared to native DSPC that recorded (14.10%), this increase was almost (300%). It could be concluded that the pressure had a positive effect on DSPC solubility, while concentration had a negative effect on DSPC solubility. These results agree with Wu et al. (2019) who showed that the solubility of protein increased from 40.5% to 81.1% when the HPH pressure increased from 0 to 120 MPa. This effect can be attributed to shear forces generated by HPH-disrupted hydrogen bonds and hydrophobic interactions that have contributed to aggregate breakdown (Chen et al., 2016).



(A) (B)

Figure 4.7. 3D response figures representing the effect of Pressure and protein concentration on solubility (A) and antioxidant activity (B)

Antioxidant data showed that the initial antioxidant activity of DSPC before HPH treatment was (60.5 TE/g), while after the optimal HPH conditions at 150 MPa for a 1% protein concentration it increased to 71.67 TE/g at the conditions of (R2). According to the ANOVA results, pressure and protein concentration were considered statistically significant ($p<0.05$), with linear and quadratic effects of protein concentration being more dominant. When the images given in Figure (4.7. B) are examined, the antioxidant activity at high protein concentrations leads to higher results.

At the 50 MPa level, antioxidant activity was determined to be 61.3-64.84 TE/g; When the pressure was increased to 150 MPa, the antioxidant activity values varied between 66.62-71.67 TE/g. Therefore, protein concentration had a week effect at equal pressure on DSPC antioxidant activity.

The obtained results are consistent with the study of Puppo et al. (2004), who indicated that the solubility of SPI at pH 8.0 was increased after treatment with high pressure at 200-400 MPa. Similarly, Wang et al. (2008) confirmed that treatment with high

pressure homogenization of soy protein isolate (SPI) solutions at concentrations of 1, 3, and 5% at (200–600 MPa) affected positively on the solubility as compared to untreated samples. However, when the tested sample is treated at (600 MPa), dissociation of the globulin subunits can occur, leading to the formation of soluble complexes between the basic subunits of glycinin and some subunits of b-conglycinin, as reported by Molina et al. (2001). Chapleau and deLamballerie-Anton (2003) showed that the solubility of lupine globular proteins (1.5%, (w/v) had increased gradualy by increasing the pressure from 200 to 600 MPa because of the formation of small aggregates of the high molecular weight protein. Results revealed that the optimum condition of HPH for maximizing the solubility and antioxidant activity of DSPC was at 150 MPa and 1% concentration of protein.

Table 4.11. Regression coefficients and analysis of variance of the models for solubility and particle size of date seed protein concentrate (DSPC)

Variables	Solubility			Emulsifying			Antioxidant activity		
	K	F-value	p-value	K	F-value	p-value	K	F-value	p-value
Intercept	+35.05			+20.57			+62.26		
A-Pressure MPa	+4.32	58.26	< 0.0001	+0.8440	43.54	0.0006	+2.91	106.45	< 0.0001
B- Protein Concentrate %	-4.26	56.57	< 0.0001	+0.1684	1.73	0.2360	-1.16	16.81	0.0064
AB				-0.7579	23.40	0.0029	-0.3768	1.19	0.3174
A^2				-0.4979	6.73	0.0409	+1.40	10.98	0.0161
B^2				+0.6595	11.81	0.0139	+3.92	85.66	< 0.0001
Lack of Fit		3.64	0.1583		486.60	0.0002		2.55	0.2313
Model		57.41	< 0.0001		16.57	0.0019		51.23	< 0.0001
R^2		0.9273			0.9325			0.9971	
Adjusted R^2		0.9112			0.8762			0.9580	
C.V. %		3.96			1.52			1.06	
Main effects									
Pressure MPa			***			***			***
Protein Concentrate %			***			NS			**

***k,** coefficients, **R^2**, coefficient of determination, **C.V.**, coefficient of variance, **NS**, not significant.*
*** Significant at p <0.05. *** Significant at p <0.01. Lack of fit is non-significant at p >0.05.*

4.3.4. Functional properties of the DSPC treated by optimum HPH conditions

4.3.4.1. Emulsifying activity and emulsifying stability

Emulsion is an important food property that is getting more interest from researchers (Tan et al, 2017). The results presented indicated that the EAI and ESI values of untreated DSPC and DSPC-HPH treated samples were shown in Table 4.12. DSPC-HPH showed a significant EAI of 22.29 m^2/g under the optimal HPH conditions compared to the EAI of native DSPC which was recorded at 11.92 m^2/g. The emulsion

stability index decreased slightly from 17.63 to 16.15 min under the optimal conditions of HPH. The obtained results indicated that the HPH treatment stability at the optimal conditions has enhanced the emulsion ability, while it had no significant effect on the emulsion stability of DSPC.

The increase in EAI after HPH treatment could be caused by the liberation of more internal hydrophobic groups from protein molecules as suggested by Molina et al. (2001). These results are consistent with previous studies which showed that the EAI values of different SPI samples were significantly increased after treatment with HPH at 200 MPa, but pressure increase at 400 and 600 MPa had no effect on the EAI Value was SPI, as reported by Wang et al. (2008).

In addition, Puppo et al. (2005) stated that the high-pressure treatment with 200-400 MPa on the SPI (1%) resulted in a significant reduction in oil droplet size in emulsions abilty index compared to untreated control. In addition, treatment with high pressure homogenization produced a gradual and significant decrease in ESI. This effect could be caused by this reduction in molecular flexibility of proteins resulting from aggregation, as molecular flexibility is an important factor affecting emulsion stability (Kato and Nakai, 1980). Recently, Luo et al. (2022) indicated that high-pressure treatment of quinoa protein isolates at 50 MPa resulted in large protein aggregates destroyed and particle size reduced.

Properties such as solubility, emulsifying power and foam formation showed significant increases. The obtained results are also consistent with Keerati-u-rai and Corredig (2009) who showed that HPH at (65 MPa) caused changes in the aggregation status of soy protein due to the partial unfolding of the protein. Yang et al. (2018) reported that the emulsifying activity index was reduced from 27.0 m^2/g for untreated faba bean protein to 22.7 and 19.7 m^2/g for those treated at pressures of 15 and 30 kpsi, respectively. Also, the emulsion stability index decreased from 39.9 to 16.6 min when fava bean protein was treated under 30 kpsi HPH for studied faba bean protein.

4.3.4.2. Water-holding capacity and oil-binding capacity of DSPC and DSPC-HPH

The functional properties of the plant proteins used essentially depend on WHC and OBC. However, the modification of the functional properties of the plant protein was enhanced by the HPH treatment. As shown in Table 4.12, WHC was reduced from

2.76 to 1.38 g/g while OBC was increased from 1.73 to 3.02 g/g DSPC after HPH treatment under optimal conditions. The improvement in OBC was reported by He et al. (2014) for peanut protein isolate (PPI) which increased by 50.31% after 150 MPa for 5 min while reporting an increase in WHC under the same treatment more clearly than that of the native WHC. The results showed that the OBC of DSPC after treatment at 150 MPa was extremely higher than that of the commercial soy protein isolate (SPI). However, although the WBC of HPH treated PPI has significantly improved compared to natural PPI, the improvement was much less than that of commercial soybean proteins as revealed by (He et al, 2014).

Table 4.12. Techno-functional and physicochemical properties of DSPC-Native and DSPC-HPH

Properties	DSPC-N	DSPC-HPH
Solubility (%)	14.10±0.47^{a}	43.69±0.31^{b}
Antioxidant Activity (TE/g)	60.5±0.27^{a}	71.67±0.48^{b}
WHC (g/g)	2.76±0.18^{a}	1.38±0.06^{b}
OBC (g/g)	1.73±0.04^{a}	3.02±0.24^{b}
EAI (m^2/g)	11.92±0.20^{a}	22.29±0.65^{b}
ESI (min)	17.63±0.36	16.15±1.22
Td (˚C)	87.73^{a}	64.39^{b}
ΔH (J/g)	204.0^{a}	227.7^{b}
Particle size (d/nm)	123.91±1.34^{a}	65.63±0.14^{b}
SH (µmol/g)	1.58±0.17^{a}	2.65±0.28^{b}
H_0	164.20±0.70^{a}	111±0.07^{b}
(ζ) potential (m/V)	-28.73±1.34^{a}	-32.73±0.12^{b}

Data are presented as mean ± standard deviation (n=3). Mean values in each row with different lower case letter superscripts are significantly different (p<0.05). DSPC-N: date seed protein concentrates native form, DSPC-HPH: date seed protein concentrates high-pressure treated, WBO: water binding capacity, OBC: oil binding capacity, EAI: emulsion activity index, ESI: emulsion stability index, Td: denaturation temperature, ΔH: enthalpy, SH: free sulfhydryl group content, H_0: surface hydrophobicity.

4.3.4.3. Differential Scanning Calorimetry (DSC) of date seeds protein concentrate (DSPC)

Figure 4.8. shows the differential scanning calorimetry (DSC) of untreated and HPH-treated samples The results indicated that untreated samples had a peak starting at about 31.81°C and reaching a maximum at 87.73°C (corresponding to the denaturation temperature) and an ΔH of 204 J/g. After high pressure treatment (1% at 150 MPa), the peak reaches a maximum at 64.39 C and an ΔH of 227.7 J/g. These changes indicated that HPH treatment reduced the denaturation temperature for DSPC. The

results obtained were consistent with previous study that confirmed that treatment of protein with HPH resulted in unfolding and subsequent aggregation/denaturation of the protein (Wang et al., 2008).

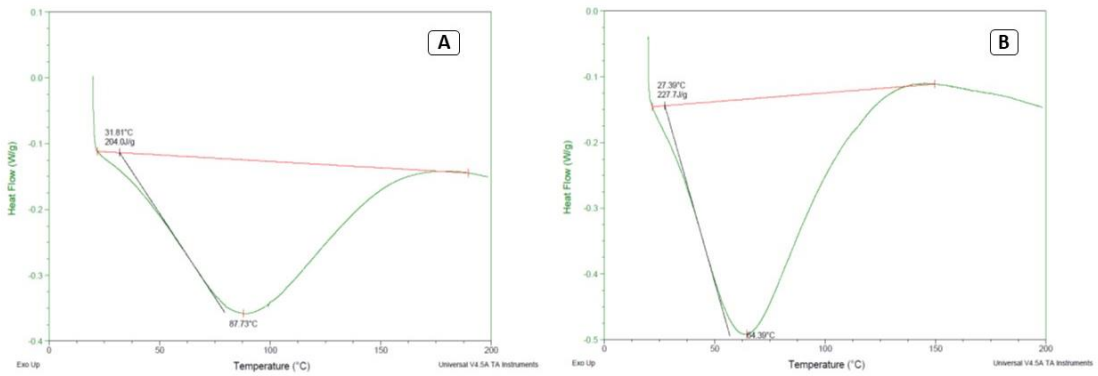


Figure 4.8. DSC Thermogram of 70.28% protein DSPC-N (A), DSPC-HPH (B)

Similarly Molina et al. (2001) investigated the thermal stability of glycinine and β-conglycinine fractions by HPH treatment but with more extensive denaturation as shown by the significant decreases in enthalpy. Pupo et al. (2004) showed that the Td of β-conglycinin in the SPI (at pH 8.0) could be reduced by HP treatment. These results indicated different effects of HP on the glycinine and β-conglycinine fractions. Changes in total enthalpy (ΔH) of glycinin and β-conglycinin fractions, which represent the proportion of undenatured protein in a sample or the content of ordered structure were significantly affected by HPH at 200 MPa caused a 69% H decrease at 1% SPI.

4.3.4.4. Surface charge (zeta potential) of DSPC and DSPC-HPH

DSPC zeta potential results showed that the negative charge increased from -28.73 to -32.73 m/V after HPH under optimal conditions (1% at 150 MPa). The potential value reflected on surface charge density of proteins. This increase in negative charge on the protein surface due to cationic amino groups of proteins converting them into anionic residues (Li et al., 2018), which is due to an increase in the zeta potential value of DSPC. The solution system had higher potential absolute values, leading to enhanced stability and surface particle dispersion (Shanmugam and Ashokkumar, 2014).

4.3.4.5. Surface hydrophobicity (H_0) determination of DSPC and DSPC-HPH

The fluorescence emission spectra of ANS (a polarity-sensitive fluorescent probe) were used to determine the index of H_0 for native DSPC and DSPC-HPH samples. Because of its exceptional sensitivity to environmental changes, ANS is also highly sensitive to conformational changes of protein molecules (Yin et al, 2010). Due to its exceptional sensitivity to changes in the environment when conformational changes of protein molecules have occurred, ANS is very sensitive to these changes (Yin et al, 2010). The HPH process reduced H_0 from 164.2 to 111 treatment under optimal conditions. The decrease in H_0 could be due to partial denaturation and subsequent aggregation of hydrophobic groups. The reduction of H_0 was also reported by Molina et al. (2001) for 10% SPI solutions.

Similarly, other studies examined 1% soybean protein isolate (SPI) for HPH at 200–600 MPa, and the results showed that H_0 has the maximum value in SPI treated at 400 MPa. The H_0 index of PPI showed a gradual decrease by treatment above 100 MPa as the structure of peanut protein isolates did not stabilize after pressure treatments. The unfolded protein was aggregated by increasing pressure. The conformational change of SPI could occur by high pressure treatment as reported by Puppo et al. (2004) and Wang et al. (2008). Therefore, the H_0 value was low due to differences in the polarity of the local environment, which was lower than that of the external water solution. However, treatment with high pressure at (50-200 MPa) led to denaturation of the protein. The increasing polarity of the environment increased the H_0 index (Yin et al, 2010).

4.3.4.6. Particle size determination of DSPC and DSPC-HPH

Particle size (d/nm) results showed a highly significant decrease by HPH treatment of DSPC sample comparable to the untreated sample from 123.91 to 65.63 d/nm. The data obtained agree with Chen et al. (2016) who showed that HPH reduced the particle size of the myofibrillar protein dispersion, the smaller particle size with larger surface area promoted the interaction between proteins and water. Treatment with HPH led to a significant reduction in the particle size of the myofibrillar protein dispersion, the interaction between proteins and water was promoted by smaller particle size with larger surface area. Recently, Luo et al. (2022) confirmed that HPH treatment reduced particle size (d/nm) at 50 MPa. The particle size of proteins plays an important role in the functional properties of proteins (He et al, 2013). Emulsion stability was determined by droplet size. It has been reported that smaller droplet size is associated with higher emulsifying ability. Recently, Wu et al. (2019) reported that increasing pressure leads to a reduction in droplet size. These results agree with the results of Yang et al. (2018) who reported that vortex cavitation modified soy protein isolate had smaller oil droplet size in emulsions.

4.3.4.7. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of DSPC and DSPC-HPH

SDS-PAGE electrophoresis for DSPC was performed to determine the changes in the molecular weight profile of the major proteins. DSPC-HPH was compared to native DSPC-N in Figure (4.9). Images of the SDS-PAGE gels are showed that the most abundant protein band was at 55-65 kDa, also some small bands are evident at the higher and lower molecular weights for HPH treated and untreated samples. These findings made it clear that high pressure homogenization had no real impact on DSPC molecular weights at optimal conditions. These native DSPC results were in full agreement with Akasha (2016). Khoshroo et al. (2011) found that similer results are based on an analysis of the seed protein of 12 cultivars of date palms grown in different regions of Iran. The researchers found a strongly stained band at around 65 kDa and smaller bands ranging from 12 to 369 kDa.

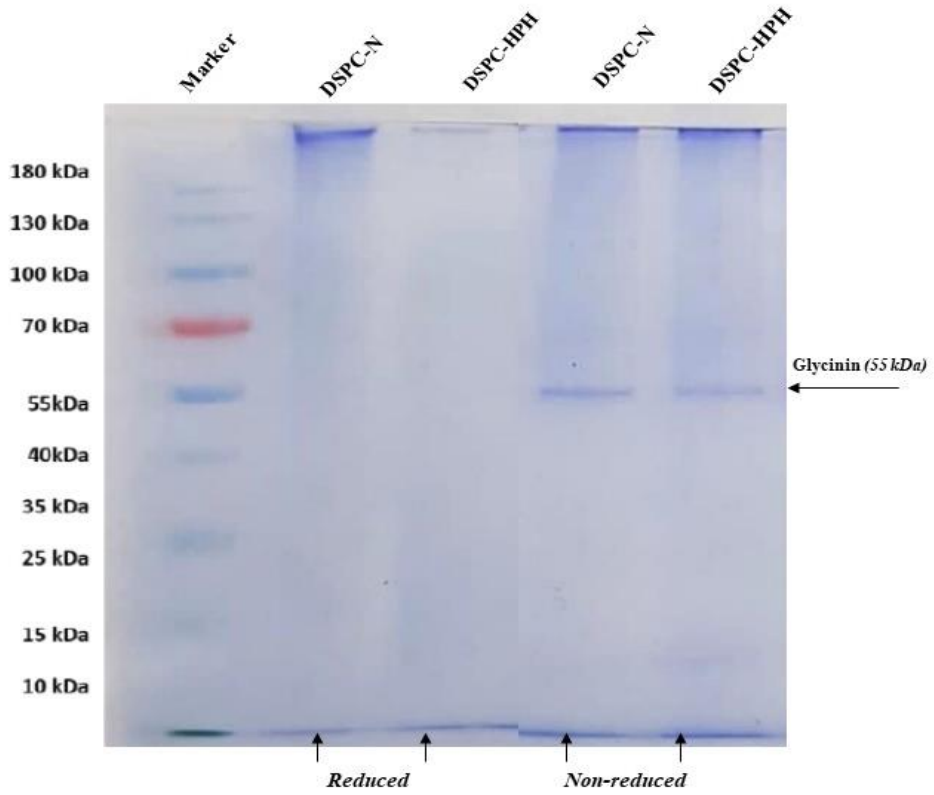


Figure 4.9. SDS-PAGE electrophoretic protein profiles DSPC-N) and DSPC-HPH under reducing and non-reducing conditions

Variations in protein profile between our results could be due to extraction method, variations between date cultivars, lipids, and genetic species types (Miernyk and Hajduch, 2011). The difference for the bands of the conarrachin I chaperone subunits in PPI was not significant and was observed between the HP-PPI and native samples, indicating that HP had no significant effect on the conarrachin I chaperone (Wang et al, 2008). SDS-PAGE analyzes of native and HP-SPI in the presence or absence of b-mercaptoethanol (a reducing agent for the SS bridges) confirmed the relative contribution of S-S bond formation to the aggregates formed after HP treatment (Wang et al, 2008). Recently, Chao et al. (2018) found that treatment with pressures of 200-600 MPa reduced the intensity of the 11S protein band for PPI, implying the formation of high molecular weight aggregates that were not resolved by the native PAGE extraction buffer.

4.3.4.8. Scanning electron microscopy (SEM) of DSPC and DSPC-HPH

The effect of HPH on the microstructure of DSPC was discovered by SEM which shown in Figure 4.10. SEM images announced that the proteins present in DSPC are

in the form of clumps (ball-shaped). Furthermore, the HPH treatment was broken down into smaller parts, which reflected that size of protein become small particle. Generally, SEM results implied that treatment with HPH modified the structure of DSPC proteins, which could increase the techno-functional properties of DSPC.

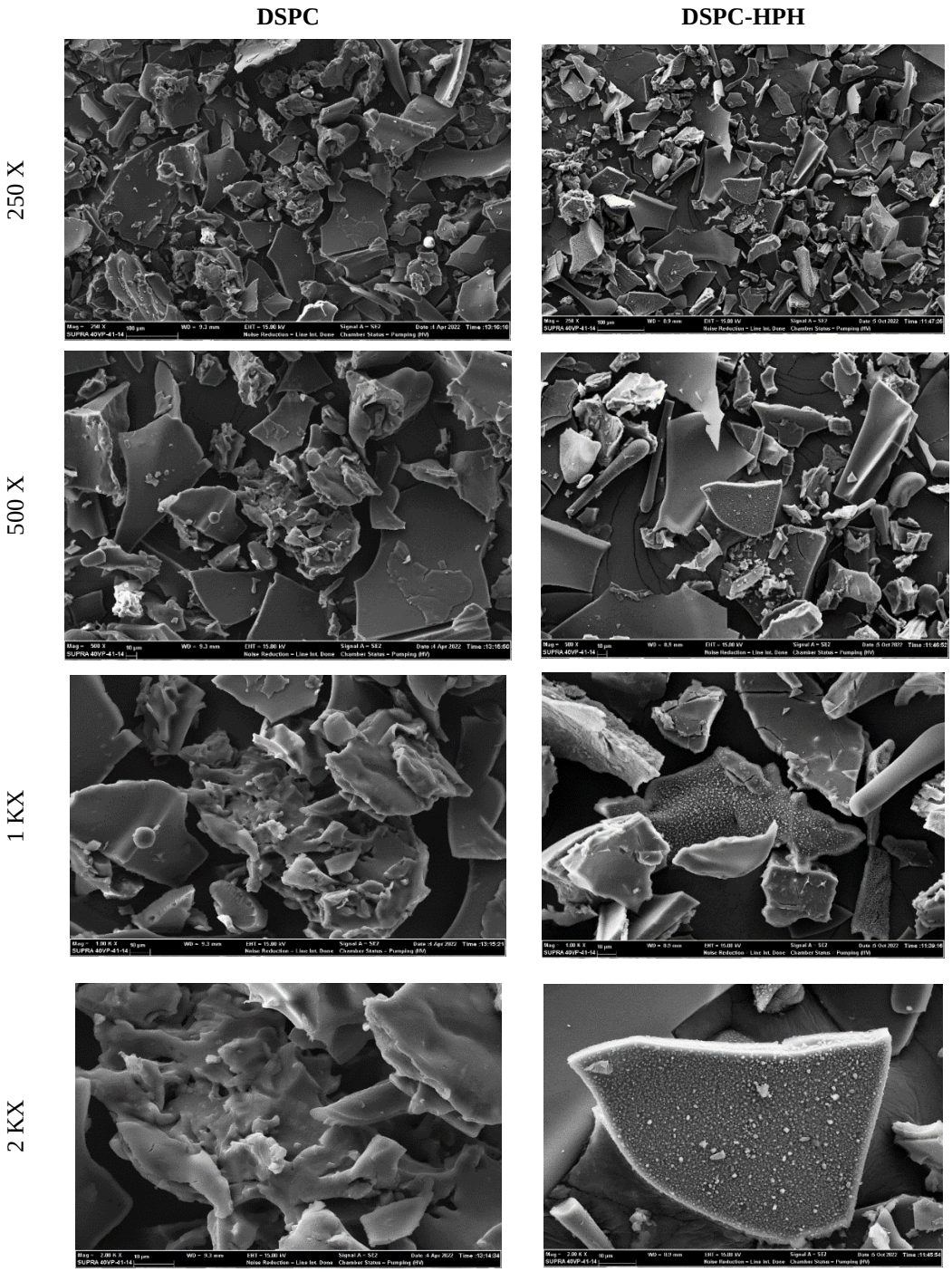


Figure 4.10. Morphology of native (DSPC-N) and high-pressure applied (DSPC-HPH) date seed protein concentrate determined by scanning electron microscopy

4.3.4.9. Fourier-transform infrared (FTIR) spectroscopy of DSPC and DSPC-HPH

FTIR, considered a way to characterization of the secondary structure of proteins by the vibrational states of chemical bonds in proteins, has been used to study the impact of HPH treatment on the secondary structure of DSPC (Ali et al, 2019). Figure (4.11) indicated the FTIR spectra of DSPC and DSPC-HPH in the range of (4000–400 cm^{-1}). DSPC samples showed four typical peaks related to amid bonds, which were amid 1 (1700–1600 cm^{-1}), amid 2 (1280–1380 cm^{-1}), amid 3 (1000–1100 cm^{-1}), amid A (3500–3200 cm^{-1}). The peak of amid I increased from (1625 cm-1 to 1636 cm^{-1}) after HPH treatment, where C=O stretching and C–N bending vibrations of protein linkages. The observed change in amid 1 band, which is the most sensitive to the secondary structure of the proteins, is associated with the β-sheet (1628–1642 cm^{-1}) structure (Sadat and Joye, 2020).

Moreover, we did not observe distinct shifts in amid II and amid III bands, which are located at a wavelength of 1525 cm^{-1} and 1236 cm-1, respectively. It was reported that (2900–3200 cm^{-1}) is the main peak of amide A produced by N–H bending and O–H stretching vibrations, which are related to hydrogen bonds on the main chain of the polypeptide (Wang et al, 2021).

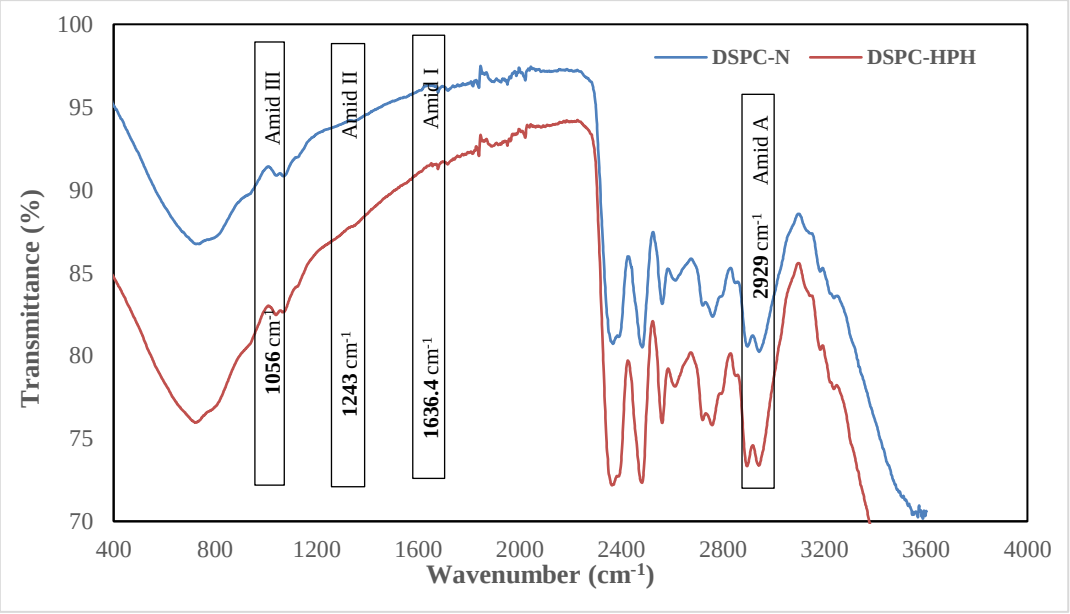


Figure 4.11. Fourier transform infrared spectra of native (DSPC-N) and high-pressure applied (DSPC-HPH) date seed protein concentrate

The position of amid A shifted from 2929 cm^{-1} to 2939 cm^{-1} after HPH in our investigation. The peaks range of amide A of DSPC and DSPC-HPH reported that high pressure enhanced the hydrogen bond strength in the protein molecules of DSPC (Ali et al, 2019). Treatment at 10000 psi shifted the peak for amide A slightly from 3293 cm-1 to a higher wavenumber of 3295 cm^{-1}, indicating HPH-induced changes in intra- and intermolecular interactions in WSMP-P. However, a slight frequency shift of amide A (N-H and O-H stretch) was observed following HPH treatment. Under HPH, the dissociation of myofibrils could be the cause of this. (Chen et al, 2016). C-H stretching was the cause of the faint peak at 2692 cm^{-1}. Amide I (80% C-O stretch, 10% C-N stretch) and amide II (60 percent N-H bend, 30% C-N stretch, and 10% C-C stretch) are associated with normal protein bands at 1655 and 1546 cm^{-1}, respectively. (Xu et al, 2016; Zhang et al, 2022).

4.3.4.10. Surface sulfhydryl groups (SH) of DSPC and DSPC-HPH

The HPH-treated sample had a value of 2.65 mol/g, while the native sample had a value of 1.58 mol/g, for free sulfhydryl (SH) content. According to Puppo et al. (2004), the effects of HPP treatment on free SH in -lactoglobulin at neutral pH and in 1% SPI at pH 3 and 8 were comparable. Zhang et al. (2003) reported that the free SH content of HPH-treated glycinine increased gradually from 0 to 600 MPa, but the concentration of the HPH-treated glycinine solution was not mentioned. The breaking of non-covalent bonds within protein molecules was the primary cause of the HPH treatment's significant increase in the free SH content of SPI samples.

Free SH content increased significantly after moderate HPH treatment at 200 MPa, while SH content decreased significantly and progressively with pressure above 200 MPa in all SPI samples. The findings also support the existence of HP-induced protein unfolding as well as the subsequent aggregation and reassembly of unfolded proteins. S-S bonds within the protein molecule, particularly those between acidic and basic glycinin polypeptides, may have been broken, as evidenced by the rise in the free SH content of SPI treated at 200 MPa. Free SH groups appear to be stable with relatively little aggregation at this pressure; however, at higher pressure levels, hydrophobic interactions would cause unfolded protein aggregation, enhancing S-S bond reformation (Wang et al, 2008).

4.3.4.11. Intrinsic fluorescence emission of DSPC and DSPC-HPH

The emission fluorescence spectra of native and HPH-treated DSPC samples were determined using the ANS as a fluorescent probe, as shown in Figure 4.12. These spectra are primarily attributed to the Trp, Tyr, and Phe residues, particularly the Trp residue. The fluorescence quantum yield of the residues decreased as the solvent exposure increased. (D'Alfonso et al. 2002).

SPI-1 and SPI-3 showed an increase in fluorescence peak intensity (460-480 nm) when subjected to pressures of 0.1 to 600 MPa. This suggests that exposed hydrophobic groups cover the protein surface, while SPI-5 showed a slight decrease in fluorescence intensity at 600 MPa, which may be related to the reassociation or aggregation of exposed hydrophobic groups to form a structure that is more stable. SPI-1 and SPI-3's fluorescence peak intensity (460-480 nm) gradually increased with pressure from 0.1 to 600 MPa, indicating that these hydrophobic groups gradually came into contact with the protein surface. However, the peak intensity of SPI-5 gradually increased up to 400 MPa, and the fluorescence intensity decreased slightly at 600 MPa, indicating that the exposed hydrophobic groups can reassociate or aggregate to form a structure that is more stable (Wang et al, 2008).

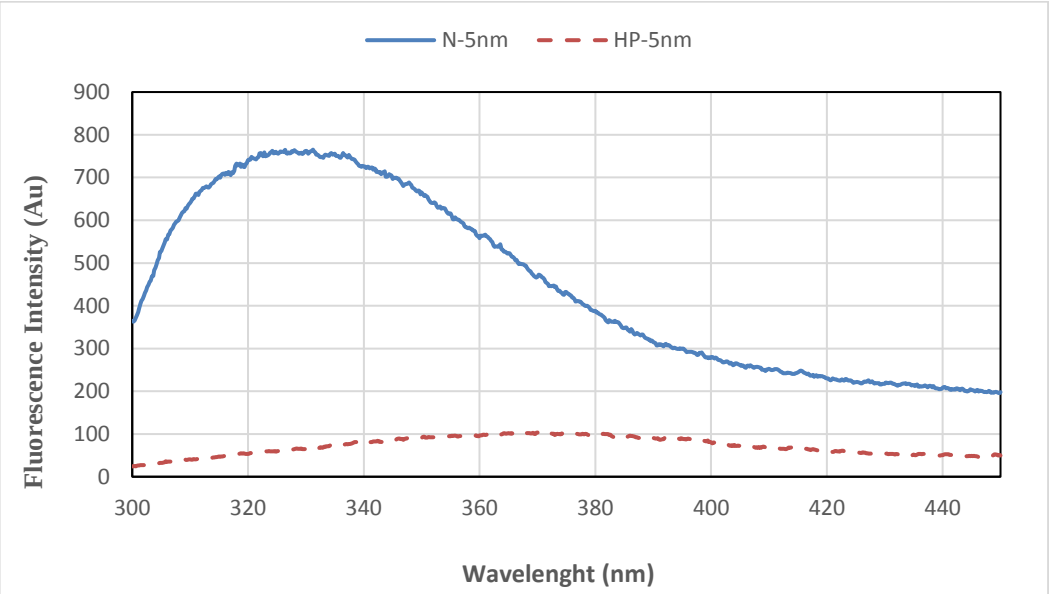


Figure 4.12. Fluorescence intensity of native (DSPC-N) and high-pressure applied (DSPC-HPH) date seed protein concentrate

5. SUMMARY AND CONCLUSION

Date fruit (*Phoenix dactylifera L.)* has been cultivated and consumed by both humans and livestock because of their nutritional, health properties and high economic value. Plant-derived -products that are important sources of protein that have often forgotten. By-products have functional ingredients that are important for utilization and development new sources of protein for human needs of nutrition and health The seeds of "Saidy" variety which is one of the most cultivated commercial date varieties in Egypt was used in this study.

High-intensity ultrasound (HIUS) and high-pressure homogenization (HPH), two novel physical methods of protein modification, were used in the study to improve the techno-functional performance of date seed protein concentrate (DSPC). The conventional process of alkaline extraction followed by isoelectric precipitation was the first step in the production of DSPC. Using the central composite design of Response Surface Methodology (RSM), the optimal process conditions of HIUS and HPH for maximizing protein solubility were determined by examining the effect of the independent process variables on solubility and antioxidant activity of DSPC.

The obtained results can be summarized as follow:

5.1. Physical characterization and chemical composition results

1. The physical properties of date seed revealed that one seed had an average weight of 1.37±0.15 g, a length of 2.15±0.07 cm, and a seed density of 730±0.13 seeds per kilogram. The results for the volume, density, and width were 0.38±0.02 cm, 1.91±0.03 cm^3, and 0.79±0.01 g/cm^3, respectively.
2. Results of chemical composition showed the higher content of moisture and fat were recorded in date seed (DS) sample in comparable to defatted date seed (DDS) and DSPC samples. The higher content of ash and carbohydrate were recorded in defatted date seed, while the highest content of protein was recorded in DSPC (70.28%).

3. The Saidy variety of date seeds has a sufficient amount of isoleucine, leucine, lysine, S-containing amino acids (methionine), and valine, according to the amino acid profile results. The phenylalanine and threonine were found in insufficient amounts, which might make date seed protein important for PKU patients in terms of nutritional perspective.
4. According to the mineral content of the date seed, the highest concentration was potassium (807.1 mg/100g), followed by sodium (322.9 mg/100g), calcium (263.1 mg/100g), magnesium (95.67 mg/100g), iron (61.21 mg/100g), and manganese (1.665 mg/100g). Copper, on the other hand, had the lowest dry weight value (0.940 mg/100g).
5. According to the phytochemical screening, the dry weight of the date seed powder contained 2.39±0.2 mg/g of phenolic compounds, 0.280±0.05 mg/g of flavonoids, 274±2.5 mg/100g of saponin, and 227±0.14 mg/100g of anthocyanin. Date seed powder had an IC_{50} of DPPH of 0.6390.04 mg/ml, DPPH scavenging activity of 78.77±0.24%, and total antioxidant activity of 2.3±0.21mg/100g.
6. The date seed oil was subjected to GC-MS analysis, which revealed that of the various compounds found, lauric acid was the highest (34.9%). After that, the concentrations of palmitic, capric, and oleic acids were 15.45, 8.74, and 7.98, respectively. Additionally, the concentrations of dioctyl-phthalate, dodecanoic acid, 1,2-benzene dicarboxylic acid, and diethyl ester, which measured 1.04, 1.08, and 0.83, were all extremely low.

5.2. Results of high intensity ultrasound HIUS modification

1. Values of DSPC solubility after optimization trials of ultrasound treatment were in the range of 20.32-32.56%. The maximum solubility of 32.56% was reached by the treatment with an amplitude of 80% for 15 min (60.56 W/cm^2). The lowest solubility (20.32%) was observed at the treatment with an amplitude of 40% for 5 min (9.24 W/cm^2) which was higher than untreated DSPC solubility (14.1%). Both the amplitude (A) and time (B) had a positive influence on the solubility. It can be concluded that all ultrasound treatments positively affected the solubility of DSPC.

2. The predicted optimum conditions for the solubility were the same with the harshest conditions (80% amplitude and 15 min) within the studied ultrasound condition ranges. The predicted solubility of DSPC under these optimal conditions by the model was 32.87% while the actual experimental value was 32.56%, indicating that the predicted optimum value was valid.

3. The level of solubility of DSPC was observed an increase of 131% after ultrasound treatment under the optimal conditions. The values of water bending capacity (WBC) of DSPC lowered from 2.76 g/g to 1.55 g/g after ultrasound treatment while the oil bending capacity (OBC) enhanced from 1.73 g/g to 4.79 g/g. These changes indicate a 43.8% decrease in WBC and an increase of 176.8% in OBC, respectively.

4. The emulsion activity index (EAI) value of DSPC ultrasound treated at optimal condition was 19.15 m^2/g while DSPC-N had an EAI of 11.92 m^2/g. A similar trend was observed for the emulsion stability of DSPC, which the emulsion stability index (ESI) value increased from 17 min to 23 min after ultrasound treatment.

5. The foaming capacity (FC) value of DSPC increased from 44% to 84% as ultrasound applied at optimal condition, which means an increase of 95.45%. Similarly, (FS) the stability of foam was 8% for the DSPC-N, while DSPC-US had a foam stability of 21%. The obtained data showed that the foaming productivity of DSPC in terms of FC and FS has been highly increased because of treatment with ultrasonic at optimal conditions. The optimized condition of ultrasonic homogenization increased FC and FS by 84% and 21%, respectively.

6. The zeta potential results of DSPC showed that ultrasound treatment performed under optimal conditions increased the negative charge of DSPC samples from –28.73 to –37.83 m/V. The sonication treatment lowered the particle size of DSPC from 123 to 100 nm.

7. The FTIR results revealed that DSPC samples had four typical peaks related to amid bonds, which were amid I (1700–1600 cm^{-1}), amid 2 (1580–1480 cm^{-1}), amid 3 (1400–1200 cm^{-1}), amid A (3500–3200 cm^{-1}). The peak of amid I increased from 1630 cm^{-1} to 1640 cm^{-1} after ultrasound treatment, where is C=O stretching and C–N bending vibrations of protein linkages, while there

were not observe distinct shifts in amid II and amid III bands, which located at a wavelength of 1525 cm^{-1} and 1236 cm^{-1}, respectively.

8. SEM images showed that ultrasound treated DSPC samples exhibited more regular fragments compared to the native form of DSPC samples, which are in the form of clumps. These uniform structures formed by the ultrasound treatment may be associated by the unfolding of proteins and increasing free SH content which caused the protein interaction.
9. SDS–PAGE electrophoresis showed a typical SDS–PAGE profile of DSPC samples under the reducing and non-reducing conditions. Non-reducing electrophoresis revealed that DSPC samples showed only one strong band at 55–65 kDa. The findings indicate that the primary structure of date seed protein was unaffected by HIUS treatment.
10. Treatment with HIUS resulted in significant increase in the content of sulfhydryl groups on the surface of DSPC from 1.58 μmol/g to 3.06 μmol/g. which indicate to expose the internal SH groups of the DSPC to the surface by the cavitation effect of ultrasound.
11. The maximum emission wavelength (λmax) increased from 331 nm to 339 nm while fluorescence intensity (FI) reduced from 765 to 165 Au. This change observed in the fluorescence emission may be associated with the release of the chromophores in the proteins to the solvent because of the partial unfolding with ultrasound application.

5.3. Results of high-pressure homogenization (HPH) modification

1. The solubility of DSPC following HPH treatment ranged from 28.12 to 43.69%. When compared to the native DSPC that was observed (14.10%), the maximum level of solubility was 43.69% at 150 MPa and 1% protein concentrate. This represents a nearly 300% increase. Solubility was positively affected by samples treated with high-pressure homogenization, while solubility was negatively affected by protein concentrate. The ideal conditions for HPH were 150 MPa and 1% protein.
2. Under the best HPH conditions, the EAI values of DSPC increased from 18.56 m^2/g to 22.29 m^2/g. Emulsion formation was improved by HPH treatment

under ideal conditions, while emulsion stabiltiy had not affected. According to the findings. Emancipating more inner hydrophobic groups of protein molecules could be the cause of the increase in EAI following HPH treatment.

3. DSPC's antioxidant activity was 60.5 TE/g prior to HPH treatment, but it increased to 71.67 TE/g following the optimal HPH conditions of 150 MPa and 1% protein. Antioxidant activity ranged from 61.3 to 64.84 M TE/g at the lowest pressure (50 MPa), while antioxidant activity values ranged from 66.62 to 71.67 MTE/g at 150 MPa. At the same pressure level, protein concentration had no significant impact on DSPC's antioxidant activity.
4. After the optimal conditions of HPH treatment, WHC results decreased from 2.76 to 1.38 g/g, while OBC results increased from 1.73 to 3.02 g/g of DSPC.
5. The findings demonstrated that untreated DSPC had ΔH of 204J/g and a single peak with a maximum temperature of 87.73 °C (the denaturation temperature). The peak reaches its maximum at 64.39 °C and ΔH of 227.7 J/g following high-pressure treatment (1% at 150 MPa). These changes suggested that the DSPC denaturation temperature was lower after HPH treatment. It could be concluded that date seed protein becomes more susceptible after the HPH treatment.
6. The zeta potential of DSPC revealed that, under ideal conditions, HPH increased the negative charge from -28.73 to -32.73 m/V (1% and 150 MPa).
7. Under the ideal conditions of DSPC, the HPH process decreased the surface hydrophobicity (H_0) from 164.2 to 111. The partial denaturation and subsequent aggregation of hydrophobic groups could be the cause of the decrease in H_0.
8. When compared to the untreated sample, the results of the particle size measurements revealed that the treatment with HPH of DSPC resulted in a highly significant decrease from 123 nm to 65 nm.
9. The most abundant protein band was between 55 and 65 kDa on the SDS-PAGE gels, and there were also small bands with higher and lower molecular weights for HPH-treated and untreated samples. The findings indicate that the primary structure of date seed protein was unaffected by HPH treatment.

10. The DSPC was observed in clumps (ball-shaped) on SEM images. In addition, the HPH treatment was broken down into smaller pieces, indicating that the protein had reduced in size to a small particle. SEM findings generally suggested that HPH treatment altered the structure of DSPC proteins, potentially increasing their techno-functional properties.
11. The HPH process had little effect on the entire FTIR profiles of DSPC samples, indicating that the secondary structure remained unchanged. However, after HPH treatment, Amide A (N-H and O-H stretching vibration) showed a slight frequency shift, indicating that HPH altered the intramolecular and intermolecular interactions in WSMP-P.
12. The HPH-treated sample had a higher sulfhydryl (SH) content (mol/g) of 2.65 mol/g compared to the native sample's value of 1.58 mol/g.

5.4. Conclusion

According to our findings, date seed could be used as a novel and alternative source of plant-based protein. Using the HIUS and HPH treatments, the DSPC could be modified to improve its techno-functional properties. The main propose of the present invistigation was to maximize the functionality of DSPC based on solubility, which is a major barrier to using DSPC. The optimum HIUS treatment conditions based on the maximum solubility were 80% amplitude and 15 min (20 kHz, 60.56 W/cm^2), which increased the solubility by 131% compared to DSPC-N. Also, The optimum HPH treatment conditions were 150 MPa pressure and 1% protein concentration, which increased the solubility by 300% compared to DSPC-N. The predicted responses agreed with the experimental responses in the used model design for HIUS and HPH treatments. It could clearly noted that HPH modification more efftive on DSPC functional properties than HIUS. Even though the HPH method improved the techno-functional performance of date seeds protein, it is still more expensive and complicated than HIUS. It is evident that HPH modification has a greater impact on the functional properties of the DSPC than HIUS does. Despite the fact that the HPH method enhanced the techno-functional performance of date seeds protein, it is still more expensive and complicated than HIUS.

After the HIUS and HPH treatments under the optimal conditions, DSPC exhibited a higher techno-functional performance, including emulsion activity/stability index, foaming capacity/stability, antioxidant activity, and oil binding capacity, except for water binding capacity. The improved techno-functional properties of DSPC by using HIUS and HPH treatments have been explained with the physicochemical changes, which are particle size, zeta potential, SDS-PAGE, SEM, FTIR, DSC, free SH content, surface hydrophobicity, and intrinsic emission. It can be concluded that the response surface methodology can be used to optimize the process conditions of HIUS and HPH instead of the traditional one-factor-one-time approach. We can suggest that the optimization technique could be applied to maximize each techno-functional criteria of DSPC for specific food applications. The other physical methods of protein modification ought to be the primary focus of DSPC's future work. Additionally, additional analyses ought to be carried out to identify additional parameters that may have an impact on the DSPC.

REFERENCES

Abdelkarim, Naglaa A., (2016). *Chemical, Nutritional and Technological Studies on Date By-Products.* PhD Thesis Sohag University, Egypt.

Abd-Ellah, A. (2009). *Chemical and Technological Studies on Some Sudanese Date Cultivars.* PhD Thesis Nile Valley University, Sudan.

Abdul Afiq, M. J., Abdul Rahman, R., Che Man, Y. B., AlKahtani, H. A., & Mansor, T. S. T. (2013). Date seed and date seed oil. *International Food Research Journal* 20, 2035-2043.

Abdullah, M. E., & Salah, S. E. (1999). Tree Fruit Characteristics of Date Palm Cultivars Grown in Three Libyan Oases, *University of Garyounis,* Benghazi, Libya.

Abdul-Qadir, I. M., Garba, I. D., Eseigbe, E., & Omofonmwan, E. I. (2011). Nutritional components of date palm and its production status in Nigeria. *International Journal of Agricultural Economics and Rural Development*, 2, 83–89.

Adenekan, M. K., Fadimu, G. J., Odunmbaku, L. A., & Oke, E. K. (2018). Effect of isolation techniques on the characteristics of pigeon pea (Cajanus cajan) protein isolates. *Food Science and Nutrition*, 6(1), 146–152.

Adeosun, A. M., Oni, S. O., Ighodaro, O. M., Durosinlorun O. (2016). Phytochemical, minerals, and free radical scavenging profiles of *Phoenix dactilyfera L.* seed extract. *Journal of Taibah University Medical Sciences,*11, 1-6.

Akasha, I. A. (2014). *Extraction and characterization of protein fraction from date palm (Phoenix dactylifera L.) Seeds.* PhD Thesis Heriot-Watt University.

Akasha, I. A., Campbell, L., & Euston, S. R. (2012). Extraction and characterization of protein fraction from date palm fruit seeds. *World Academy of Science, Engineering and Technology*, 6, 10–25.

Akasha, I. A., Campbell, L., & Euston, S. R. (2016). The major proteins of the seed of the fruit of the date palm *(Phoenix dactylifera L.)*: Characterization and emulsifying properties. *Food Chemistry*, *197*, 799–806.

Akbari, M., Razavizadeh, R., Mohebbi, G., & Barmak, A. (2012). Oil characteristics and fatty acid profile of seeds from three varieties of date palm (Phoenix dactylifera) cultivars in Bushehr-Iran. *African Journal of Biotechnology*, (11), 12088–12093.

Akharume, F. U., Aluko, R. E., & Adedeji, A. A. (2021). Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 198–224. https://doi.org/10.1111/1541-4337.12688.

Al Juhaimi, F., Ghafoor, K., & Ozcan, M. (2012). Physical and chemical properties, antioxidant activity, total phenol, and mineral profile of seeds of seven different date fruit (*Phoenix dactylifera*) varieties. *International Journal of Food Sciences and Nutrition,* 63, 84 –89.

Al-Alawi, R. A., Jawhara H., & Al-Mashiqri, (2007). Date Palm Tree (Phoenix dactylifera L.): Natural Products and Therapeutic Options. *Front. Plant Science*: 117-122.

Albano K. M., Cavallieri Â. L. F., Nicoletti V. R. (2019). Electrostatic interaction between proteins and polysaccharides: physicochemical aspects and applications in emulsion stabilization. *Food Reviews International*, 35, 54–89.

Alemayehu, F. R., Bendevis, M. A., & Jacobsen, S. E. (2015). The potential for utilizing the seed crop amaranth (Amaranthus spp.) in East Africa as an alternative crop to support food security and climate change mitigation. *Journal of Agronomy and Crop Science,* 201(5), 321–329.

Al-Farsi, M. & Lee, C. Y. (2011). Usage of Date (*Phoenix dactylifera L.*) seeds in human health and animal feed. In nuts and seed in health and disease prevention; preedy, V.R., Watson, R.R., Patel, V.B., Eds.; Elsevier: Amsterdam, the Netherlands, chapter 53, 447-452.

Al-Farsi, M. A. & Lee, C. Y. (2008). Optimization of phenolic and dietary fibre extraction from date seeds. *Food chemistry,* 108, 977–985.

Al-Farsi, M., Alasalvar, C., Al–Abid, M., Al–Shoaily, K., Al–Amry, M., & Al–Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups and their by–products. *Food Chemistry,* 104:943–94

Al-Hooti, S., Sidhu, J. S., & Qabazard, H. (1998). Chemical composition of seeds of date fruit cultivars of United Arab Emirates. *Journal of Food Science and Technology*, 35, 44-46.

Ali, M. A., & Khamis, A. S. (2004). Mineral ion content of the seeds of six cultivars of Bahraini date palm (*Phoenix dactylifera L*). *Journal of Agricultural and Food Chemistry*, 52, 6522–6525.

Ali. M., Tang, T., Zhou, L., Ling, Z., Guo, S., & Jiang, A. (2019). Effects of different proteases on the emulsifying capacity, rheological and structure characteristics of preserved egg white hydrolysates, *Food Hydrocolloids*, 87, 933–942.

Almana, H. A., & Mahmoud, R. M. (1994). Palm date seed as an alternative source of dietary fibre in Saudi bread. *Ecology of Food and Nutrition,* 32, 261–270.

Al-Mssallem, I. S., Hu, S., Zhang, X., Lin, Q., Liu, W., & Tan, J., et al. (2013). Genome sequence of the date palm *Phoenix dactylifera L. Nature Communications* 4:2274. doi: 10.1038/ncomms3274.

Al-Showiman, S. S., & Baosman, A. A. (1992). Protein and amino acid contents of some Saudi Arabian date palm seeds (*Phenix dactylifera L.*). *Arab Gulf Journal of Scientific Research,* 10, 1-9.

Althouse, P. J., Dinakar, P., & Kilara, A. (1995). Screening of proteolytic enzymes to enhance foaming of whey protein isolates. *Journal of Food Science,* 60 (5), 1110–1112.

Al-Yahyai, R., & Al-Kharusi, L. (2011). Physical and chemical quality characteristics of freeze stored dates. *International Journal of Agricultural and Biology,* 14, 97–100.

Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017). The composition, extraction, functionality, and applications of rice proteins: A review. *Trends in Food Science and Technology,* 64, 1–12.

Amiri, A., Sharifian, P., & Soltanizadeh, N. (2018). Application of ultrasound treatment for improving the physicochemical, functional, and rheological properties of myofibrillar proteins. *International Journal of Biological Macromolecules,* 111, 139–147.

Anwar, M. A. (2006). Phoenix dactylifera L.: A bibliometric study of the literature on date palm. *Malaysian Journal of Library and Information Science,* 11, 41–60.

AOAC. (1990). Official method for analysis (15th ed.). Washington, DC: Association of Official Analytical Chemists.

AOAC. (2005). Official method of analysis,18thedn. (Association of Official Analytical Chemists, Washington.

Arredondo-Parada, I., Torres-Arreola, W., Suárez-Jiménez, G. M., Ramírez-Suárez, J. C.,Juárez-Onofre, J. E., Rodríguez-Félix, F., & Marquez-Rios, E. (2019). Effect of ultrasound on physicochemical and foaming properties of a protein concentrate from giant squid (Dosidicus gigas) mantle. *LWT-Food Science and Technology,* S0023-6438(19)31296-4.

Arzeni, C., Martinez, K., Zema, P., Arias, A., Perez, O. E., & Pilosof, A. M. R. (2012). Comparative study of high-intensity ultrasound effects on food proteins functionality. *Journal of Food Engineering,* 108 (3), 463–472.

Asero, R., Mistrello, G., Roncarolo, D., de Vries, S. C., Gautier, M. F., Ciurana, C. L. Verbeek, E., Mohammadi, T. Knul-Brettlova, V., & Akkerdaas, J. H. (2000). Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *International Archives of Allergy and Immunology,* 122, 20-32.

Ata, S. (2011). *A study of date palm market chain and its role in food security and livelihoods of farmers in the South Punjab.* PhD Thesis University of Agriculture, Faisalabad, Pakistan.

Augusto, P. D., Tribst, A., & Cristianini, M. (2018). High hydrostatic pressure and high-pressure homogenization processing of fruit juices, in Fruit Juices, eds G. Rajauria and B. K. Tiwari *San Diego, CA: Academic Press,* 393–421.

Awad, T., Moharram, H., Shaltout, O., Asker, D., & Youssef, M. (2012). Applications of ultrasound in analysis, processing and quality control of food: a review, *Food Research International,* 48(2), 410–427.

Aydemir, L. Y., & Yemenicioglu, A. (2013). Potential of Turkish Kabuli type chickpea and green and red lentil cultivars as source of soy and animal origin functional protein alternatives. *LWT-Food Science and Technology, 50*(2), 686–694. https://doi.org/ 10.1016/j.lwt.2012.07.023

Bader, S., Bez, J., & Eisner, P. (2011). Can protein functionalities be enhanced by high-pressure homogenization? - A study on functional properties of lupin proteins. *Procedia Food Science,* 1, 1359–1366.

Baier, D.; Schmitt, C. & Knorr, D. (2015). Changes in functionality of whey protein and micellar casein after high pressure-low temperature treatments. *Food Hydrocolloids,* 44, 416–423.

Balny, C., & Masson, P. (1993). Effects of high pressure on proteins. *Food Reviews International,* 9(4), 611–628.

Barac, M., Cabrilo, S., Pesic, M., Stanojevic, S., Zilic, S., Macej, O., & Ristic, N. (2010). Profile and functional properties of seed proteins from six pea (Pisum sativum) genotypes. *International journal of molecular sciences*, *11*(12), 4973–4990. https://doi.org/10.3390/ijms11124973

Barh, D., & Mazumdar, B. C. (2008). Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (phoenix sylvestris roxb.) sap in treatment of anemia. *Research Journal of Medicine and Medical Sciences,* 3, 173–176.

Benitez, L. V. (1989); Amino Acid and fatty acid profilesin aquaculture nutrition studies, p. 23- 35. In S.S. DeSilva (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. *Asian fish Society Special Publication.* 4, 166 p.

Berryman, C. E., Lieberman, H. R., Fulgoni, V. L., & Pasiakos, S. M. (2018). Protein intake trends and conformity with the Dietary Reference Intakes in the United States: analysis of the National Health and Nutrition Examination Survey, 2001–2014. *The American Journal of Clinical Nutrition*, 108, 1–9. 472 https://doi.org/10.1093/ajcn/nqy088

Besbes, S., Blecker, C., Deroanne, C., Drira, N. & Attia, H. (2004). Date seeds: chemical composition and characteristic profiles of the lipid fraction. *Food Chemistry,* 84, 577–584.

Besbes, S., Drira, L., Blecker, C., Deroanne, C. & Attia, H. (2009). Adding value to hard date (*Phoenix dactylifera L.*): compositional, functional, and sensory characteristics of date jam. *Food chemistry,* 112, 406–411.

Biswas B., & Sit, N. (2020). Effect of ultrasonication on functional properties of tamarind seed protein isolates. *Journal of Food Science and Technology*. https://doi.org/10.1007/s13197-020-04241-8

Bobbio, F.O., & Bobbio, P.A. (2003). Manual de laboratório de química de alimentos, 2nd edn. (Varela, São Paulo, 2003), p. 63

Boland, M. J., Rae, N. A., Vereijken, J. M., Meuwissen, M. P., Fischer, A. R., van Boekel, M. A., Rutherfurd, S. M., Gruppen, H., Moughan, O. J., & Hendriks, W. H. (2013). The future supply of animal-derived protein for human consumption, *Trends in Food Science and Technology,* 29, 62–73.

Bolontrade, A. J., Scilingo, A. A., & Añón, M. C. (2013). Amaranth Proteins Foaming Properties: Adsorption Kinetics and Foam formation Part 1. *Colloids and Surfaces B: Biointerfaces,* 105, 319–327.

Bouaouina, H., Desrumaux, A., Loisel, C., & Legrand, J. (2006). Functional properties of whey proteins as affected by dynamic high-pressure treatment. *International Dairy Journal,* 16(4), 275–284.

Bouaziz, M. A., Besbes, S., Blecker, C., & Attia, H. (2013). Chemical composition and some functional properties of soluble fibro-protein extracts from Tunisian date palm seeds. *African Journal of Biotechnology*, 12(10), 1121-1131. http://dx.doi.org/10.5897/AJB10.1877. Article No. 1BAB65922707.

Bouaziz, M., Besbes, S., Blecker, C., Wathelet, B., Deroanne, C., & Hamadi, A. (2008). Protein and amino acid profiles of Tunisian Deglet Nour and Allig date palm fruit seeds. *Fruits,* 63, 37–43.

Bouhlali, E. D. T., Alem, C., Ennassir, J., Benlyas, M., Mbark, A. N., & Zegzouti, Y. F. (2015). Phytochemical compositions and antioxidant capacity of three date (Phoenix dactylifera L.) seeds varieties grown in the Southeast Morocco. *Journal of the Saudi Society of Agricultural Sciences,* 16, 350–357.

Boukouada, M., & Yousfi, M. (2009). Phytochemical study of date seeds lipids of three fruits (Phoenix Dactylifera L.) produced in Ouargla region. *Annals of the Faculty of Sciences and Engineering Sciences,* 1, 66–74.

Boye, J., Wijesinha-Bettoni, R., & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition,* 108, 183–211. https://doi.org/10.1017/S0007114512002309.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International, 43*(2), 414–431.

Bradford M. M. (1976). A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-Dye Binding. *Anal Biochem*istry, 72:248-254

Buckeridge, M. S. (2010). Seed cell wall storage polysaccharides: Models to understand cell wall biosynthesis and degradation. *Plant Physiology,* 154, 1017–1023.

Camargo-Peream A, Rubio-Clemente, A & Peñuela, G. (2020). Use of Ultrasound as an Advanced Oxidation Process for the Degradation of Emerging Pollutants in Water. Review. *Water,* 12, 1068. doi:10.3390/w12041068

Celis, M., Contreras, B., Forgiarini, A., Rosenzweig, L. P., & Garcia- Rubio, L. H. (2016). Effect of emulsifier type on the characterization of o/w emulsions using a spectroscopy technique. *Journal of Dispersion Science and Technology,* 37, 512–518. https://doi.org/10. 1080/01932691.2015.1048459.

Chaira, N., Ferchichi A. Marbet, A., & Sghairoun, M. (2007). Chemical composition of the flesh andthe pit of date palm fruit and radical scavenging activity of their extracts. *Pakistan Journal of Biological Sciences,* 13, 2202-07.

Chao, C. T., & Krueger, R. R. (2007). The Date Palm (Phoenix Dactylifera L.): Overview of Biology, Uses, and Cultivation. *Horticulture Sciences,* 42, 1077–1082.

Chao, D., Jung, S., & Aluko, R. E. (2018). Physicochemical and functional properties of high pressure-treated isolated pea protein. *Innov. Food Sci. Emerg. Technol.* 45, 179–185.

Chapleau, N. & de Lamballerie-Anton, M. (2003). Improvement of emulsifying properties of lupin proteins by high pressure induced aggregation. *Food Hydrocolloids,* 17(3), 273–280.

Chemat, F., & Khan, M. (2011). Applications of ultrasound in food technology: processing, preservation, and extraction, *Ultrasonic Sonochemistry,* 18, 813–835.

Chen, K. I., Erh, M. H., Su, N. W., Liu, W. H., Chou, C. C., & Cheng, K. C. (2012). Soy foods and soybean products: From traditional use to modern applications. Applied Microbiology and Biotechnology, 96(1) 9-22.

Chen, X.; Zhou, R.; Xu, X.; Zhou, G. & Liu, D. (2016). Structural modification by high-pressure homogenization 348 for improved functional properties of freeze-dried myofibrillar proteins powder. *Food Research International,* 100(1), 193.

Coda, R., Varis, J., Verni, M., Rizzello, C. G., & Katina, K. (2017). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *LWT-Food Science and Technology,* 82, 296–302. https://doi.org/10.1016/j.lwt.2017.04.062.

Conde, J. M., Escobar, M. Y., Jiménez, J. J. P., Rodríguez, F. M., & Patino, J. M. R. (2005). Effect of enzymatic treatment of extracted sunflower proteins on solubility, amino acid composition, and surface activity. *Journal of Agricultural and Food Chemistry,* 53(20), 8038–8045. https://doi.org/10.1021/jf051026i.

D'Alfonso, L., Collini, M., & Baldini, G. (2002). Does b-lactoglobulin denaturation occur via an intermediate state? *Biochemistry,* 41, 326–333.

Damodaran, S. (1997). Food proteins: An overview. Damodaran, S. & Paraf, A. Mercel (eds.) Food Proteins and Their Applications (p. 22).

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

De Gavelle E, Huneau J. F., & Mariotti F. (2018). Patterns of protein food intake are associated with nutrient adequacy in the general French adult population. *Nutrients,* 10-226.

Deep Singh, G., Wani, A. A., Kaur, D., & Sogi, D. S. (2008). Characterization and functional properties of proteins of some Indian chickpea (*Cicer arietinum*) cultivars. *Journal of the Science of Food and Agriculture,* 88,778-786.

Demason, D. A., Sexton, R., Gorman, M., & Reid, J. S. G. (1985). Structure and Biochemistry of Endosperm Breakdown in Date Palm (Phoenix dactylifera L.) Seeds. *Protoplasma,* 126, 159–167.

Desai, N. N., Modi, M. V., Saxena, G. K., Chaudhari, R. H., Jaipal, M. K., Gohil, K. H., & Gora, A. (2019). Physical Properties of Dates Fruits. *International Journal of Current Microbiology and Applied Sciences,* 8(4), 1243-1249. doi: https://doi.org/10.20546/ijcmas.2019.804.143

Devries, M. C., & Phillips, S. M. (2015). Supplemental protein in support of muscle mass and health: advantage whey, *Journal of Food Sciences,* 80, A8-A15.

Dickinson, E., & McClements, D.J. 1995. *Advances in Food Colloids*. Blackie Academic and Professional, Glasgow.

Diels, A. M. J., & Michiels, C. W. (2006). High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. *Critical Reviews in Food Science and Nutrition,* 32, 201–216. doi: 10.1080/10408410601023516.

Diels, A. M. J., Callewaert, L., Wuytack, E. Y., Masschalck, B., & Michiels, C. W. (2005). Inactivation of Escherichia coli by high-pressure homogenization is influenced by fluid viscosity but not by water activity and product composition. *International Journal of Food Microbiology,* 101(3), 281–291.

Dijkstra, D. S., Linnemann, A. R., vanBoekel, & T. A. (2003). Towards sustainable production of protein-rich foods: appraisal of eight crops for Western Europe. Part II: analysis of the technological aspects of the production chain, *Critical Reviews in Food Science and Nutrition,* 43, 481-506.

Dong, X. Y., Guo, L. L., Wei, F., Li, J. F., Jiang, M. L., Li, G. M., . . .Chen, H. (2011). Some characteristics and functional properties of rapeseed protein prepared by ultrasonication, ultrafiltration and isoelectric precipitation. *Journal of the Science of Food and Agri- culture,* 91(8), 1488–1498. https://doi.org/10.1002/jsfa.4339.

Donsì, F., Annunziata, M., & Ferrari, G. (2013). Microbial inactivation by high pressure homogenization: Effect of the disruption valve geometry Journal of Food Engineering 115 (2013) 362–370.

Du, M. Xie, J., Gong, B., Xu, X., Tang, W., Li, X., Li, C., & Xie, M. (2018). Extraction, physicochemical characteristics and functional properties of mung bean protein. *Food Hydrocolloids,* 76,131–140.

Duke, J.A., (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants. CRC Press, Boca Raton.

El-Adawy, T. A., & Taha, K. M. (2001). Characteristics and composition of different seed oils and flours. *Food Chemistry,* 74(1), 47–54. https://doi.org/10.1016/S0308-8146(00)00337-X

EL-Hadrami, A., & AL-Khayri, J. M. (2012). Socioeconomic and traditional importance of date palm. *Emirates Journal of Food and Agriculture,* 24, 371–385.

El-Shurafa, M.Y., Ahmed, H. S., & Abou-Naji, S. E. (1982). Organic and inorganic constituents of date palm pit (seed). *Date Palm Journal,* 1, 275–284.

Evans, M., Ratcliffe, I., & Williams, P.A. (2013). Emulsion stabilization using polysaccharide–protein complexes. *Current Opinion in Colloid & Interface Science,* 18(4), 272–282.

FAO (2014). FAOSTAT database. Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/faostat/en/#data/QC.

FAO, The State of Food Security and Nutrition in the World (SOFI)2021. FAOSTAT. FAO. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO, 2020. FAOSTAT. FAO. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO/IAEA/WHO (1999). International conference on ensuring the safety and quality of food through radiation processing. Food and Environmental Protection Newsletter, 2, 11.

FAOSTAT (The FAO Statistical Database) (2008). Agro-Statistics Database. Rome: Food and Agriculture Organization of the United Nations.

Flores-Jiménez, N. T., Ulloa, J. A., Silvas, J. E. U., Ramírez, J. C. R., Ulloa, P. R., Rosales, P. U. B., Carrillo, Y. S., & Leyva, R. G. (2019). Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (*Brassica napus L.*) protein isolate. *Food Research International,* 121, 947-956.

Floury, J., Bellettre, J., Legrand, J., & Desrumaux, A. (2004). Analysis of a new type of high-pressure homogenizer. A study of the flow pattern. *Chemical Engineering Science,* 59 (4), 843–853.

Floury, J., Desrumaux, A., & Legrand, J. (2002). Effect of ultra-high-pressure homogenization on structure and on rheological properties of soy protein-stabilized emulsions. *Journal of Food Science,* 67 (9), 3388–3395.

Floury, J., Desrumaux, A., Axelos, M., & Legrand, J. (2003). Effect of high-pressure homogenization on methylcellulose as food emulsifier. *Journal of Food Engineering,* 58 (3), 227–238.

Galanakis, C. M. (2013). Emerging technologies for the production of nutraceuticals from agricultural by-products: a viewpoint of opportunities and challenges, *Food Bioproducts Process.* 91, 575-579.

Galazka, V. B., Dickinson, E., & Ledward, D. A. (2000). Influence of high-pressure processing on protein solutions and emulsions. *Current Opinion in Colloid & Interface Science,* 5(3), 182–187.

Gharibzahedi, S. M. T., & Smith, B. (2021). Effects of high hydrostatic pressure on the quality and functionality of protein isolates, con-centrates, and hydrolysates derived from pulse legumes: A review. *Trends in Food Science and Technology,* 107, 466–479. https://doi.org/10.1016/j.tifs.2020.11.016

Giusti, M. M., & Wrolstad, R. E. (2001). Unit F1.2. In: Wrolstad RE, Schwartz SJ (eds) Anthocyanins. Characterization and measurement with UV–visible spectroscopy. *Current protocols in food analytical chemistry*. Wiley, New York, pp 1–13.

Golshan, T. A., Solaimani, D. N., & Yasini, A. S. (2017). Physicochemical properties and applications of date seed and its oil. *International Food Research Journal,* 24(4): 1399-1406.

Grácia-Juliá, A., René, M., Cortés-Muñoz, M., Picart, L., López-Pedemonte, T., & Chevalier, D., (2008). Effect of dynamic high pressure on whey protein aggregation: A comparison with the effect of continuous short-time thermal treatments. *Food Hydrocolloids,* 22(6), 1014–1032.

Granato, D., Nunes, D. S., & Barba, F. J. (2017). An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: a proposal. *Trends In Food Science & Technology,* 62, 13-22. http://dx.doi.org/10.1016/j.tifs.2016.12.010.

Gupta, P., & Nayak, K. (2015). Characteristics of protein-based biopolymer and its application, *Polymer Engineering and Science*, 55, 485–498.

Habib, H., & Ibrahim, W. (2011b). Nutritional quality of 18 date fruit varieties. *International Journal of Food Sciences and Nutrition,* 62(5), 544-551.

Hagglund, P. (2002). *Mannan-hydrolysis by hemicellulases Enzyme polysaccharide interaction of a modular β-mannanase.* PhD Thesis, Department of Biochemistry, Lund University, Sweden.

Halloran, A., J. Clement, N.Kornum,C. Bucatariu, J.Magid, (2014). Addressing food waste reduction in Denmark, Food Policy 49, 294-301.

Hamada, J. S., Hashim, I. B., &Sharif, F. A. (2002). Preliminary analysis and potential uses of date pits in foods. *Food Chemistry,* 76, 135–137.

Harkat, H., Bousba, R., Benincasa, C., Atrouz, K., Gültekin-Özgüven, M., Altunta¸, Ü., Demircan, E., Zahran, H., & Özçelik, B. (2022). Assessment of Biochemical Composition and Antioxidant Properties of Algerian Date Palm (*Phoenix dactylifera L.)* Seed Oil. *Plants,* 11,381. https://doi.org/ 10.3390/plants11030381.

Harte, F. (2016). Food processing by high-pressure homogenization, in High Pressure Processing of Food: Principles, Technology and Applications, eds V. M. Balasubramaniam, G. V. Barbosa- Canovas, and H. L.M. Lelieveld (New York, NY: Springer), 123–141.

Hartmann, C., & Siegrist, M. (2017). Consumer perception and behavior regarding sustainable protein consumption: A systematic review. *Trends in Food Science and Technology*. 61, 11-25. https://doi.org/10.1016/j.tifs.2016.12.006.

He, X. H., Liu, H. Z., Liu, L., Zhao, G. L., Wang, Q., & Chen, Q. L. (2014). Effects of high pressure on the physicochemical and functional properties of peanut protein isolates. *Food hydrolysis*, 36, 123-129.

Herchi, W., Kallel, H., & Boukhchina, S. (2014). Physicochemical properties and antioxidantactivity of Tunisian date palm (Phoenix dactylifera L.) oil as affected by different extractionmethods. Food Science and Technology, 34, 464–470.

Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some sapogenins with vanillin and sulfuric acid. *Planta Medica*, 29,116–22

Higuera-Barraza, O. A., Del Toro-Sánchez, C. L., Ruiz-Cruz, S., & Márquez-Ríos, E. (2016). Effects of high-energy ultrasound on the functional properties of proteins. *Ultrasonics Sonochemistry,* 31, 558–562.

Higuera-Barraza, O. A., Torres-Arreola, W., Ezquerra-Brauer, J. M., Cinco-Moroyoqui, F. J., Rodríguez-Figueroa, J. C., & Marquez-Ríos, E. (2017). Effect of pulsed ultrasound on the physicochemical characteristics and emulsifying properties of squid (Dosidicus gigas) mantle proteins. *Ultrasonics Sonochemistry*, 38, 829–834. http://dx.doi.org/10.1016/j.ultsonch.2017.01.008

Hosseini, S., Djomeh, Z., Razavi, S., Moosavi, M.vahedi, A. A., Saboury, A. A., Atri, M. S., & Meeren, P. V. (2013). β-Lactoglobulin–sodium alginate interaction as affected by polysaccharide depolymerization using high intensity ultrasound. *Food Hydrocolloids*, 32(2), 235 –244.

Hu, H., Wu, J., Li-Chan, E. C., Zhu, L., Zhang, F., Xu, X., … Pan, S. (2013a). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. *Food Hydrocolloids*, 30(2), 647–655.

Hu, H., Wu, J., Li-Chan, E., Zhu, L., Zhang, F., Xu, X., Fan, G., Wang, L., Huang, X., & Pan, S. (2013b). Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. *Food Hydrocolloids*, 30(2):647–655.

Hughes, G. J., Ryan, D. J., Mukherjea, R., & Schasteen, C. S. (2011). Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: Criteria for evaluation. *Journal of Agricultural and Food Chemistry*, 59(23), 12707–12712. https://doi.org/10.1021/jf203220v

Huppertz, T., Smiddy, M. A., Upadhyay, V. K., & Kelly, A. L. (2006). High-pressure-induced changes in bovine milk: A review. *International Journal of Dairy Technology, 59, 58–66.*

Hussein, F., & El-Zeid, A. A. (1975). Chemical composition of 'Khalas' dates grown in Saudi Arabia. *Egyptian Journal of Horticulture*, 2, 209–214.

Ismail, B., Haffar, I., Baalbaki, R., & Henry, J. (2008). Physico-Chemical characteristics and sensory quality of two date varieties under commercial and industrial storage conditions. *LWT-Food Science and Technology*, 41, 896–904.

Ismail, B., Senaratne-Lenagala, L., Stube, A., & Brackenridge, A. (2020). Protein demand: review of plant and animal proteins used in alternative protein product development and production. *Animal Frontiers*, 10(4), 53-63.

Jambrak A., Lelas, V., Mason, J., Krešić, G., & Badanjak, M. (2009). Physical properties of ultrasound treated soy proteins, *Journal of Food Engineering*, 93, 386–393.

Jambrak A., Mason, T., Lelas, V., Herceg, Z., & Herceg, I. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions, *Journal of Food Engineering*, 86, 281–287.

Jambrak, A., Mason, T. Lelas, V., Paniwnyk, L., & Herceg, S. (2014). Effect of ultrasound treatment on particle size and molecular weight of whey proteins. *Journal of Food Engineering*, 121, 15–23. 432.

Jiang, L., Wang, J., Li, Y., Wang, Z., Liang, J., Wang, R., Chen, Y., Ma, W., Qi, B., & Zhang, M. (2014). Effects of ultrasound on the structure and physical properties of black bean protein isolates. *Food Research International*, 62, 595–601.

Jiang, Z., Yuan, X., Yao, K., Li, X., Zhang, X., Mu, Z., . . . Hou, J. (2017). Laccase-aided modification: Effects on structure, gel properties and antioxidant activities of α-lactalbumin. *LWT-Food Science and Technology*, 80, 355-363.

Jin, J., Ma, H., Wang, W., Luo, M., Wang, B., Qu, W., He, R., Owusu, J., & Li, Y. (2016). Effects and mechanism of ultrasound pretreatment on rapeseed protein enzymolysis. *Journal of Science and Food Agriculture, 96*, 1159–1166.

Joglekar, A. M., May, A. T., Graf, E., & Saguy, I. (1987). Product excellence through experimental design. In E. Graf, & I. Saguy (Eds.), *Food product development: From concept to the marketplace* (pp. 211–230). Chapman and Hall.

Kadam, S. U., Tiwari, B. K., Alvarez, C., & O'Donnell, C. P. (2015). Ultrasound treatments for the extraction, identification and delivery of food proteins and bioactive peptides. *Trends in Food Science and Technology*, 46(1), 60–67.

Karabulut G., & Yemis O. (2022*).* Modification of hemp seed protein isolate (Cannabis sativa L.) by high-intensity ultrasound treatment. Part 1: Functional properties. *Food Chemistry*, (375) 131843. https://doi.org/10.1016/j.foodchem.2021.131843

Karabulut G., Feng, H., & Yemis O. (2022). Physicochemical and Antioxidant Properties of Industrial Hemp Seed Protein Isolate Treated by High‐Intensity Ultrasound. *Plant Foods for Human Nutrition*, https://doi.org/10.1007/s11130-022-01017-7

Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., & Craig, R. W. (1991). Participation of p53 protein in the cellular response to DNA damage. *Cancer Research*, *51*(23 Pt 1), 6304–6311.

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

Keeratiurai, M., & Corredig, M. (2009). Effect of dynamic high-pressure homogenization on the aggregation state of soy protein. *Journal of Agricultural and Food Chemistry*, 57(9), 3556–3562.

Kentish, S., & Feng, H. (2014). Applications of power ultrasound in food processing. *Annual Review of Food Science and Technology*, 5(1), 263–284.

Khatib, M., Al-Tamimi, A., Cecchi, L., Adessi, A., Innocenti, M., Balli, D., & Mulinacci, N. (2022). Phenolic compounds and polysaccharides in the date fruit (Phoenix dactylifera L.): Comparative study on five widely consumed Arabian varieties. *Food chemistry*, 395, 133591. https://doi.org/10.1016/j.foodchem.2022.133591

Khoshroo, S. M. R., Khavarinjad, R., Fahimi, H., & Mohammadi, Z. N. (2011). Seed storage protein electrophoretic profiles in some Iranian date palm (*Phoenix dactylifera L.*) cultivars. *African Journal of Biotechnology*, 10, 17793–17804.

Kinsella, J. E. (1982). Relationships between structure and functional properties of food proteins. Food proteins. Edited by: Fox P.F. & Condon J.J. Applied science publishers LTD, England. (pp. 51-58).

Kinsella, J. E., & Melachouris, N. (1976). Functional properties of proteins in foods: a survey, *Critical Reviews in Food Science and Nutrition*, 7, 219–280.

Krentz, A. J., & Bailey, C. J. (2005). Oral antidiabetic agent: current role in type 2 diabetes mellitus. *Drugs*, 65, 385–411.

Kresic G., Lelas V., Jambrak, A. R., Herceg, Z., & Brncic, S. R. (2008). Influence of novel food processing technologies on the rheological and thermophysical properties of whey proteins. *Journal of Food Engineering,* 87(1), 64–73.

Lakshmanan, R., Parkinson, J. A., & Piggott, J. R. (2007). High-pressure processing and water-holding capacity of fresh and cold-smokedsalmon (Salmon salar). *LWT Food Science and Technology,* 40, 544–551.

Lee, H., Yildiz, G., Dos Santos, L. C., Jiang, S., Andrade, J., Engeseth, N. C., & Feng, H. (2016). Soy protein nanoaggregates with improved functional properties prepared by sequential pH treatment and ultrasonication. *Food Hydrocolloids,* 55, 200-209.

Li, Z., Wu, H., Yang, M., Xu, D., Chen, J., Feng, H., & Kang, W. (2018). Stability mechanism of O/W Pickering emulsions stabilized with regenerated cellulose. *Carbohydrate Polymers,* 181, 224-233.

Lin, D., Lu, W., Kelly, A. L., Zhang, L., Zheng, B., & Miao, S. Interactions of Vegetable Proteins with Other Polymers: Structure-Function Relationships and Applications in the Food Industry. *Trends in Food Science and Technology,* 17(68), 130–144.

Liu, C. M., Zhong, J. Z., Liu, W., Tu, Z. C., Wan, J., Cai, X. F., & Song, X. Y. (2011). Relationship between functional properties and aggregation changes of whey protein induced by high pressure micro fluidization. *Journal of Food Science,* 76(4), 341–347.

Liu, S., Liu, Y., Huang, X., Yang, W., Hu, W., & Pan, S., (2017). Effect of ultrasonic processing on the change's inactivity, aggregation and the secondary and tertiary structure of polyphenol oxidase in oriental sweetmel on (Cucumismelovar. makuwa Makino). *Journal of the Science of Food and Agriculture,* 97(4),1326–1334.

Liu, X., Wang, M., Xue, F., & Adhikari, B. (2022). Application of ultrasound treatment to improve the techno functional properties of hemp protein isolate. *Future Food,* 6(22), 100176.

López, D. N., Galante, M., Robson, M., Boeris, V., & Spelzini, D. (2018). Amaranth, quinoa and chia protein isolates: Physicochemical and structural properties. *International Journal of Biological Macromolecules,* 109, 152–159. https://doi.org/10.1016/j.ijbiomac.2017.12.080

Lordan, S., Ross, R. P., & Stanton, C. (2011). Marine bioactive as functional food ingredients: potential to reduce the incidence of chronic diseases, *Marine Drugs,* 9(6), 1056-1100. https://doi.org/10.3390%2Fmd9061056

Luo, L., Cheng, L., Zhang, R., & Yang, Z. (2022). Impact of high-pressure homogenization on physico-chemical, structural, and rheological properties of quinoa protein isolates. *Food Structure,* 32, 100265.

Luo, Y., Shen, H., Pan, D., & Bu, G. (2008). Gel properties of surimi from silver carp (*Hypophthalmichthys molitrix*) as affected by heat treatment and soy protein isolate, *Food Hydrocolloids*, 22, 1513–1519.

Ma, J. K., Drake, P. M., & Christou, B. (2003). Genetic modification: the production of recombinant pharmaceutical proteins in plants, Nat. Rev. Genet. 4, 794.

Malik, M. A., Sharma, H. K., & Saini, C. S. (2017). High intensity ultrasound treatment of protein isolate extracted from de-phenolized sunflower meal: effect on physicochemical and functional properties, *Ultrasonics Sonochemistry*, 39, 511–519.

Malomo, S. A., He, R., & Aluko, R. E. (2014). Structural and functional properties of hemp seed protein products. *Journal of Food Science,* 79 (8), 1512-21

Manickavasagan, A., Essa, M.M., & Sukumar, E. (Eds.). (2012). Dates: Production, Processing, Food, and Medicinal Values (1st ed.). *CRC Press.* https://doi.org/10.1201/b11874

Maqsood, S., Kittiphattanabawon, P., Benjakul, S., Sumpavapol, P., & Abushelaibi, A. (2015). Antioxidant activity of date (*Phoenix dactylifera* var. Khalas) seed and its preventive effect on lipid oxidation in model systems. *International Food Research Journal*, 22 (3).

Maresca, P., Ferrari, G., Júnior, B. R., Zanphorlin, L. M., Ribeiro, L. R. & Murakami, M. (2017). Effect of dynamic high pressure on functional and structural properties of bovine serum albumin. *Food Research International*, 99, 748–754.

Marinangeli, C. P. F., & House, J. D. (2017). Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. *Nutrition Reviews*, 75(8), 658–606. https://doi.org/10.1093/nutrit/nux025

Marszałek, K., Woźniak, Ł., Kruszewski, B., & Skąpska, S. (2017). The Effect of High-Pressure Techniques on the Stability of Anthocyanins in Fruit and Vegetables. *International journal of molecular sciences*, *18*(2), 277.

Martinez, K. D., Ganesan, V., Pilosof, A. M. R., & Harte, F. M. (2011). Effect of dynamic high-pressure treatment on the interfacial and foaming properties of soy protein isolate-hydroxypropyl methylcelluloses systems. *Food Hydrocolloids*, 25(6), 1640–1645.

Martínez-Velasco, A., Lobato-Calleros, C., Hernández-Rodríguez, B. E., Román-Guerrero, A., Alvarez-Ramirez, J., Vernon-Carter, E. J. (2018). High intensity ultrasound treatment of faba bean (*Vicia faba L.*) protein: Effect on surface properties, foaming ability and structural changes. *Ultrasonics Sonochemistry*, 44, 97–105.

Masson, P.; Tonello, C., & Balny, C. (2001). High-pressure biotechnology in medicine and pharmaceutical science. *BioMed Research International*, 1(2), 85–88.

Mattila, P., Mäkinen, S., Eurola, M., Jalava, T., Pihlava, J. M., Hellström, J., & Pihlanto, A. (2018). Nutritional value of commercial protein-rich plant products. *Plant Foods for Human Nutrition*, 73(2), 108–115. https://doi.org/10.1007/s11130-018-0660-7.

McClements, D. J. (1999). Food Emulsions. Boca Raton: *CRC Press*, 111-24.

Miernyk, J., & Hajduch, J. M. (2011). Seed Proteomics. *Journal of Proteomics*. 978-1-62703-630-6. 389 p. 10.1016/j.jprot.2010.12.004.

Miliauskas, G., Venskutonis, P. R., & van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, 85(2), 231–237

Mir, N. A., Riar, C. S., & Singh, S. (2019). Effect of pH and holding time on the characteristics of protein isolates from Chenopodium seeds and study of their amino acid profile and scoring. *Food Chemistry,* 272, 165–173.

Mirmoghtadaie, L., Shojaee Aliabadi, S., & Hosseini, S. M. (2016). Recent approaches in physical modification of protein functionality. *Food chemistry*, *199*, 619–627. https://doi.org/10.1016/j.foodchem.2015.12.067

Mistrello, J., Sirisena, S. D., Ghavami, A., Marshall, R. J., & Krish-namoorthy, S., (2014). Determination of the antioxidant capacity, total phenolic and flavonoid contents of seeds from three commercial varieties of culinary dates. *International Journal of Food Studies,* 3, 34-44.

Molina, E., Papadopoulou, A., & Ledward, D. A. (2001). Emulsifying properties of high pressure treated soy protein isolate and 7S and 11S globulins. *Food Hydrocolloids,* 15(3), 263–269.

Morales, R., Martínez, K. D., Ruiz-Henestrosa, V. M. P., & Pilosof, A. M. (2015). Modification of foaming properties of soy protein isolate by high ultrasound intensity: particle size effect. *Ultrasonics Sonochemistry*, 26, 48–55.

Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Buren, J. P. V., Kilara, A., … Mangino, M. E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *Journal of Food Science*, 50(6), 1715–1718. https://doi.org/ 10.1111/j.1365-2621. 1985.tb10572.x

Mowat A. M. (1987). The regulation of immune responses to dietary protein antigens. *Immunology Today*, *8*(3), 93–98.

Mu, L., Zhao, M., Yang, B., Zhao, H., Cui, C., & Zhao, Q. (2010). Effect of ultrasonic treatment on the graft reaction between soy protein isolate and gum acacia and on the physicochemical properties of conjugates. *Journal of Agriculture and Food Chemistry*, 58,4494–4499

Murray, B. S. (2011). Rheological properties of protein films. *Current Opinion in Colloid and Interface Science,* 16(1), 27–35.

Nazari, B., Mohammadifar, M. A., Shojaee-Aliabadi, S., Feizollahi, E., & Mirmoghtadaie, L. (2018). Effect of ultrasound treatments on functional properties and structure of millet protein concentrate. *Ultrasonics Sonochemistry*, 41,382–388.

Nenova, N., & Drumeva, M. (2012). Investigation on protein content and amino acid composition in the kernels of some sunflower lines. *Helia,* 35(56), 41–46. https://doi.org/10.2298/HEL1256041N.

O'Sullivan, J. J., Park, M., Beevers, J., Greenwood, R. W., & Norton, I. T. (2017). Applications of ultrasound for the functional modification of proteins and nano emulsion formation: A review. *Food Hydrocolloids,* 71, 299–310.

O'Sullivan, J., Murray, B., Flynn, C., & Norton, I. (2016a). The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocolloids,* 53,141–154.

O'Sullivan, J., Park, M., & Beevers, J. (2016b). The effect of ultrasound upon the physicochemical and emulsifying properties of wheat andsoy protein isolates. *Journal of Cereal Science,* 69, 77–84.

Paquin, P. (1999). Technological properties of high-pressure homogenizers: The effect of fat globules, milk proteins and polysaccharides. *International Dairy Journal,* 9, 329-335.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*(3), 716–723. https://doi.org/10.1021/jf60217a041

Peng, D., Jin, W., Li, J., Xiong, W., Pei, Y., Wang, Y., ... Li, B. (2017). Adsorption and distribution of edible gliadin nanoparticles at the air/water interface. Journal of Agricultural and *Food Chemistry,* 65(11), 2454–2460.

Pojić, M., Misan, A., & Tiwari, B. (2018). Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science and Technology,* 75, 93–104.

Popenoe, P. B. (1973). The Date Palm. Edited by Henry Field. Field Research Projects, Coconut Grove, *Miami, FL.*

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized Methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agriculture and Food Chemistry,* 53, 4290-4302.

Puppo, C., Chapleau, N., Speroni, F., de Lamballerie-Anton, M., Michel, F., An˜o´n, C., et al. (2004). Physicochemical modifications of high-pressure-treated soybean protein isolates. *Journal of Agricultural and Food Chemistry,* 52, 1564–1571.

Quintero, J., A. Naranajo, G. Ciro, J. Rojas, (2017). Vegetable proteins: non-sensitizing encapsulation agents for bioactive compounds, Allergen, InTech,

Rahman, M. S., Kasapis, S., Al-Kharusi, N. S. Z., Al-Marhubi, I. M., & Khan, A. J. (2007). Composition characterisation and thermal transition of date pits powders. *Journal of Food Engineering,* 80, 1–10.

Rahmani, A. H., Aly, S. M., Ali, H., Babiker, A. Y., Srikar, S., & Khan, A. A. (2014). Therapeutic effects of date fruits (*Phoenix dactylifera*) in the prevention of diseases via modulation of anti-inflammatory, antioxidant, and antitumor activity. *International Journal of Clinical and Experimental Medicine,* 7(3), 483–491.

Raikos, V., Campbell, L. & Euston, S. R. (2007). Rheology and Texture of Hen's Egg Protein Heat-set Gels as Affected by pH and the Addition of Sugar And/or Salt. *Food Hydrocolloids,* 21, 237–244.

Ramadan, B. R. (1995). *Biotechnological, nutritional, and technological studies on dates.* PhD Thesis Assiut University, Egypt.

Ren, X. Wei, X. Ma, H. Zhou, H. Guo, J. Mao, S., & Hu, A. (2015). Effects of a dual-frequency frequency-sweeping ultrasound treatment on the properties and structure of the zein protein, *Cereal Chemistry*, 92, 193–197.

Resendiz-Vazquez, J. A., Ulloa, J. A., Urías-Silvas, J. E., Bautista-Rosales, P. U., Ramírez- Ramírez, J. C., Rosas-Ulloa, P., & Gonzalez-Torres, L. (2017). Effect of high-intensity ultrasound on the techno functional properties and structure of jackfruit (*Artocarpus heterophyllus*) seed protein isolate. *Ultrasonics Sonochemistry*, 37, 436-444.

Reynes, M., Bouabidi, H., Piompo, G. & Risterucci, A.M. (1994). Characterization des principales varie´te´s de dattes cultive´es dans lare´gion du Dje´rid en Tunisie. *Fruits*, 49, 289–298.

Rodrıguez-Gacio, M. Del C., Iglesias-Fernandez, R., Carbonero, P., & Matilla, A. J. (2012). Softening-up mannan-rich cell walls, review paper. *Journal of Experimental Botany*, Oxford University Press, 1–14.

Sa, A. G. A., Moreno, Y. M. F., & Carciof, B. A. M. (2019). Food processing for improvement of plant proteins digestibility. *Critical Reviews in Food Science Nutrition*, 1–20. https://doi.org/10.1080/10408398.2019.1688249

Sadat A., & Joye I. J. (2020). Peak Fitting Applied to Fourier Transform Infrared and Raman Spectroscopic Analysis of Proteins. *Applied Sciences*. 10(17), 5918. https://doi.org/10.3390/app10175918

Saha, J., & Deka, S. C. (2017). Functional properties of sonicated and non-sonicated extracted leaf protein concentrate from Diplazium esculentum. *International Journal of Food Properties*, 20(5), 1051- 1061.

Salgado, P. R., Drago, S. R., Molina Ortiz, S. E., & Petruccelli, S. (2012). Production and characterization of sunflower (*Helianthus annuus L.*) protein-enriched products obtained at pilot plant scale. *LWT-Food Science and Technology*, 45(1), 65–72. https://doi.org/10.1016/j.lwt.2011.07.021

Sashikala, V. B., Sreerama, Y. N., Pratape, V. M., & Narasimha, H. V. (2015). Effect of thermal processing on protein solubility of green gram (*Phaseolus aureus*) legume cultivars. *Journal of Food Science and Technology*, 52(3), 1552–1560.

Scott, J. D., & Pawson, T. (2009). Cell signaling in space and time: where proteins come together and when they're apart. *Science*, 326, 1220-1224.

Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z., & Ercisli, S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pakistan journal of pharmaceutical sciences*, *22*(1), 102–106.

Shanmugam, A., & Ashokkumar, M. (2014). Ultrasonic preparation of stable flax seed oil emulsions in dairy systems-Physicochemical characterization. *Food Hydrocolloids*, 39, 151–162.

Shen, L., & Tang, C. H. (2012). Micro fluidization as a potential technique to modify surface properties of soy protein isolate. *Food Research International*, 48(1), 108–118.

Shirsath, S. R., Sonawane, S. H., & Gogate, P. R. (2012). Intensification of extraction of natural products using ultrasonic irradiations-A review of current status.

Chemical Engineering and Processing: Process Intensification, 53, 10–23. https://doi.org/ 10.1016/j.cep.2012.01.003

Singh, A., Benjakul S., & Kijroongrojana K. (2018). Effect of ultrasonication on physicochemical and foaming properties of squid ovary powder. *Food Hydrocolloids*, 77,286–296

Sim, S. Y. J., Srv, A., Chiang, J. H., & Henry, C. J. (2021). Plant Proteins for Future Foods: A Roadmap. *Foods, 10,* 1967. https://doi.org/10.3390/foods10081967

Sirisena, S., Dimitrios Zabaras, D., Ng, K., & Ajlouni, S. (2017). Characterization of Date (Deglet Nour) Seed Free and Bound Polyphenols by High-Performance Liquid Chromatography-Mass Spectrometry. *Food science,* 82(2), 333-340. https://doi.org/10.1111/1750-3841.13625

Sousa, A. G. O., Fernandes, D. C., Alves, A. M., Freitas, J. B., & Naves, M. M. V. (2011). Nutritional quality and protein value of exotic almonds and nut from the Brazilian Savanna compared to peanut. *Food Research International,* 44(7), 2319–2325. https://doi.org/10.1016/j.foodres.2011.02.013

Stefanović, A. B., Jovanović, J. R., Dojčinović, M. B., Lević, S. M., Nedović, V. A., Bugarski, B. M., & Knežević-Jugović, Z. D. (2017). Effect of the controlled high- intensity ultrasound on improving functionality and structural changes of egg white proteins. *Food Bioprocess and Technology*, 10, 1224–1239.

Sulieman, A. E., Abd Elhafise, I. A., & Abdelrahim A. M. (2012). Comparative Study on Five Sudanese Date (Phoenix dactylifera L.) Fruit Cultivars. *Food and Nutrition Sciences*, 3, 1245–1251.

Sun, M., Mu, T., Zhang, M., & Arogundade, L. A. (2012). Nutritional assessment and effects of heat processing on digestibility of Chinese sweet potato protein. *Journal of Food Composition and Analysis*, 26(1–2), 104–110.

Sundar, R. D. V., Segaran, G., Shankar, S., Settu, S., & Ravi, L., (2017). Bioactivity of Phoenixdactylifera seed and its phytochemical analysis. *International Journal of Green Pharmacy*, 11 (2), S292–S297.

Sun-Waterhouse, D., Zhao, M., & Waterhouse, G. I. N. (2014). Protein modification during ingredientpreparation and food processing: Approaches to improve food processability and nutrition. *Food and Bioprocess Technology,* 7(7), 1853–1893. https://doi.org/10.1007/s11947-014-1326-6

Suryaprakash, P., Kumar, R. P., & Prakash, V. (2010). Thermodynamics of interaction of caffeic acid and quinic acid with multi-subunit proteins. *International Journal of Biological Macromolecules*, 27(3), 219-228.

Tabilo-Munizaga, G., Villalobos-Carvajal, R., Herrera-Lavados, C., Moreno-Osorio, L., Jarpa-Parra, M. & Pérez-Won, M. (2019). Physic-ochemical properties of high-pressure treated lentil protein-based nano emulsions. LWT-Food Science and Technology, 101, 590–598.

Tafti, A. G., & Fooladi, M.H. (2006). A Study on the Physico–Chemical Properties of Iranian Shamsaei Date at Different Stages of Maturity. *World Journal of Dairy & Food Sciences,* 1, 28–32.

Tan, Y., Deng, X., Liu, T., Yang, B., Zhao, M., & Zhao, Q. (2017). Influence of NaCl on the oil/water interfacial and emulsifying properties of walnut protein-xanthan gum. *Food Hydrocolloids,* 72, 73– 80.

Tang, C. H., & Li, X. R. (2013). Microencapsulation properties of soy protein isolate and storage stability of the correspondingly spray-dried emulsions, *Food Research International,* 52, 419–428.

Tang, C. H., Wang, X. Y., Yang, X. Q., & Li, L. (2009). Formation of soluble aggregates from insoluble commercial soy protein isolate by means of ultrasonic treatment and their gelling properties. *Journal of Food Engineering,* 92(4),432–437.

Taurozzi, J. S., Hackley, V. A., & Wiesner, M. R. (2012). Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment-issues and recommendations. *Nanotoxicology,* 5, 711–729.

Terefe, N. S., Sikes, A. L., & Juliano, P. (2016). Ultrasound for structural modification of food products, in Innovative Food Processing Technologies: Extraction, Separation, Component Modification and Process Intensification, K. Knoerzer, P. Juliano, and G. W. Smithers, Eds., p. 510p, *Woodhead Publishing,* Cambridge, UK.

Thomas, K., Herouet-Guicheney, C., Ladics, G., Bannon, G., Cockburn, A., Crevel, R., Fitzpatrick, J., Mills, C., Privalle, L., & Vieths, S. (2007). Evaluating the effect of food processing on the potential human allergenicity of novel proteins: international workshop report. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*, *45*(7), 1116–1122.

Tian, R., Feng, J., Huang, G., Tian, B., Zhang, Y., Jiang, L., & Sui, X. (2020). Ultrasound driven conformational and physicochemical changes of soy protein hydrolysates. *Ultrasonics Sonochemistry*, 68, 105202.

Unver, A. (2016). Applications of ultrasound in food processing. Green Chemical and Technological Letters, 2(3), 121-126.

Vaclavik, V., & Christian, E. (2003). Essentials of Food Science. Second edition New York: Kluwer Academic/Plenum publishers, p. 142.

Venuste, M., Zhang, X., Shoemaker, C. F., Karangwa, E., Abbas, S., & Kamdem, P. E. (2013). Influence of enzymatic hydrolysis and enzyme type on the nutritional and antioxidant properties of pumpkin meal hydrolysates. *Food and Function,* 4(5), 811–820. https://doi.org/10.1039/c3fo30347k

Vera, A., Valenzuela, M. A., Yazdani-Pedram, M., Tapia, C., & Abugoch, L. (2019). Conformational and physicochemical properties of quinoa proteins affected by different conditions of high-intensity ultrasound treatments. *Ultrasonics Sonochemistry*, 51, 186–196.

Wang, B., Meng, T., Ma, H., Zhang, Y., Li, Y. Jin, J., & Ye, X. (2016). Mechanism study of dual frequency ultrasound assisted enzymolysis on rapeseed protein by immobilized Alcalase, *Ultrasonics Sonochemistry*, 32, 307–313.

Wang, T., Wang, N., Li, N., Ji, X. Zhang, H., Yu, D., & Wang, L. (2022). Effect of high-intensity ultrasound on the physicochemical properties, microstructure, and stability of soy protein isolate-pectin emulsion. *Ultrasonics Sonochemistry*, 8(22), 105871.

Wang, X. S., Tang, C. H., Li, B. S., Yang, B. Q., Li, L., & Ma, C. Y. (2008). Effects of high- pressure treatment on some physicochemical and functional properties of soy *protein isolates. Food Hydrocolloids,* 22, 560-567.

Wang, Y., Wang, Y., Li, K., Bai, Y., Li, B., & Xu, W. (2020). Effect of high intensity ultrasound on physicochemical, interfacial and gel properties of chickpea protein isolate. *LWT-Food Science and Technology*, *129,* 109563.

Wang, Y.-Y., Yan, J.-K., Ding, Y., Rashid, M. T., & Ma, H. (2021). Effect of sweep frequency ultrasound and fixed frequency ultrasound thawing on gelling properties of myofibrillar protein from quick-frozen small yellow croaker and its possible mechanisms. *LWT-Food Science and Technology*, 150, 111922.

Wen, C., Zhang, J., Zhang, H., Dzah, C. S., Zandile, M., Duan, Y., Ma, H., & Luo, X. (2018). Advances in ultrasound assisted extraction of bioactive compounds from cash crops - A review. *Ultrasonics Sonochemistry, 48,* 538–549.

Wen, Q. H., Tu, Z. C., Zhang, L., Wang, H., & Chang, H. X. (2017). Effect of high intensity ultrasound on the gel and structural properties of Ctenopharyngodon idellus myofibrillar protein. Journal of Food Bio- chemistry, 41(1), e12288.

WHO/FAO/UNU Expert Consultation. (2007). Protein and amino acid requirements in human nutrition. *World Health Organization Technical Report Series,* (935), 1–265. https://doi.org/ISBN 92 4 120935 6

Wierenga, P. A., Meinders, M. B. J., Egmond, M. R., Voragen, F. A. G. J., & deJongh, H. H. J. (2003). Protein exposed hydrophobicity reduces the kinetic barrier for adsorption of ovalbumin to the air-water interface. *Langmuir,* 19(21), 8964–8970.

Wu, F., Shi1, X., Zou, H., Zhang, T., Dong, X., Zhu, R., & Yu, C. (2019). Effects of high-pressure homogenization on physicochemical, rheological and emulsifying properties of myofibrillar protein. *Journal of Food Engineering, 263,* 272-279.

Xiong, T., Xiong, W., Ge, M., Xia, J., Li, B., & Chen, Y. (2018). Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. *Food Research International,* 109, 260–267.

Xiong, W., Wang, Y., Zhang, C., Wan, J., Shah, B. R., Pei, Y., ... Li, B. (2016). High intensity ultrasound modified ovalbumin: Structure, interface and gelation properties. *Ultrasonics Sonochemistry,* 31, 302–309.

Xu, X., Zhong, J., Chen, J., Liu, C., Luo, L., Luo, S., … McClements, D. J. (2016). Effectiveness of partially hydrolyzed rice glutelin as a food emulsifier: Comparison to whey protein. *Food Chemistry,* 213, 700–707.

Yada, R. Y. (2004). Proteins in food processing. Woodhead Publishing Limited, *Cambridge,* England & CRC Press LLC, USA.

Yadav, S. C., Kumari, A., & Yadav, R. (2011). Development of peptide and protein nanotherapeutics by nanoencapsulation and nano bioconjugation. *Peptides*, *32*(1), 173–187. https://doi.org/10.1016/j.peptides.2010.10.003

Yan, S., Xu, J., Zhang, S., & Li, Y. (2021). Effects of flexibility and surface hydrophobicity on emulsifying properties: Ultrasound-treated soybean protein isolate. *LWT*, 142, 110881. doi: 10.1016/j.lwt.2021.110881

Yang, J., Liua, G., Hongbo, Z. H. & Chena, L. (2018). Effects of high-pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. *Food Hydrocolloids*, 82, 275-286.

Yang, S. C., & Baldwin, R. E. (1995). Functional properties of eggs in foods. In: Egg science and technology. Fourth edition. Stadelman, W.J. & Cotterill O.J. (eds.) Binghamton: *Food Products Press;* Haworth Press, p. 405.

Yang, X., Li, Y., Li, S., Oladejo, A. O., Ruan, S., Wang, Y, Huang, S., & Ma, H. (2017). Effects of ultrasound pretreatment with different frequencies and working modes on the enzymolysis and the structure characterization of rice protein, *Ultrasonics Sonochemistry*, 38, 19–28.

Yanjun, S., Jianhang, C, Shuwen, Z., Hongjuan, L., Jing, L., Lu, L., Uluko, H., Yanling, S., Wenming, C., Wupeng, G., & Jiaping, L. (2014). Effect of power ultrasound pre-treatment on the physical and functional properties of reconstituted milk protein concentrate. *Journal of Food Engineering*, 124,11–18.

Yin, Y. X., Xiang, B. Q., & Tong, L. (2010). The application of studying fluorescence spectroscopy on protein. *Experimental Technology and Management*, 27(2), 34-38.

Yong, S. X. M., Song, C. P. & Choo, W. S. (2021). Impact of High-Pressure Homogenization on the Extractability and Stability of Phytochemicals. *Frontiers in Sustainable Food Systems*, 4:593259.

Yousif, A. K. & Alghamdi, A.S. (1999). Suitability of some date cultivars for jelly making. *Journal of Food Science and Technology*, 36(6), 515- 518.

Yousuf, R. G. (2018). *Novel polyhydroxy butyrate (PHB) production using a waste date seed feedstock.* PhD Thesis. The University of Manchester. England.

Zaid, A., & de Wet, P. F. (2002) Pollination and Bunch Management. In: Zaid, A., Ed., Date Palm Cultivation, Chapter 8, FAO, Plant Production and Protection, 156. Rev. 1. Rome, Italy.

Zayas J. F., (2012). Functionality of Proteins in Food, *Springer Science and Business Media*. 373 pp.

Zhang, H. K., Li, L. T., Tatsumi, E., & Kotwal, S. (2003). Influence of high-pressure on conformational changes of soybean glycinin. *Innovative Food Science and Emerging Technology, 4, 269-275.*

Zhang, H., Claver, I. P., Zhu, K. X., & Zhou, H. (2011). The effect of ultrasound on the functional properties of wheat gluten. *Molecules*, 16(5),4231–4240.

Zhang, Q. T., Tu, Z. C., Xiao, H., Wang, H., Huang, X. Q., Liu, G. X., Liu, C. M., Shi, Y., Fan, L. L., & Lin, D. R. (2014). Influence of ultrasonic treatment on the

structure and emulsifying properties of peanut protein isolate. *Food and Bioproducts Processing,* 92(1), 30–37.

Zhang, Y., Zhang, X., Zhang, Z., Chen, Z., Jing, X., & Wang, X. (2022). Effect of high hydrostatic pressure treatment on the structure and physicochemical properties of millet gliadin. *LWT - Food Science and Technology*, 154, 112755.

Zhao, Q., Xie, T., Hong, X., Zhou, Y., Fan, L., Liu, Y., & Li, J. (2022). Modification of functional properties of perilla protein isolate by high-intensity ultrasonic treatment and stability of o/w emulsion. *Food Chemistry, 368*, 130848.

Zhishen J., Mengcheng T., & Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64, 555-559.

Zhu, Z., Zhu, W., Yi, J., Liu, N., Cao, Y., Lu, J., ... McClements, D. J. (2018). Effects of sonication on the physicochemical and functional properties of walnut protein isolate. *Food Research International,* 106, 853–861.

CURRICULUM VITAE

Name Surname : Mohamed Ali Mahmoud Ahmed KELANY

EDUCATION:

- **Undergraduate** : 2004, South valley University, Faculty Agriculture, Food and Dairy Sciences Department
- **Graduate** : 2013, Assuit University, Food Science and Technology Department, Food Science and Nutrition Program

PROFESSIONAL EXPERIENCE AND AWARDS:

- He worked as an assistant lecturer at Sohag University between 2006-2014.
- He attended the UNICEF Training Course, Programming for Infant and Young Child Feeding, (Cornell University, 2012.

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

- Kelany, M.; Yemiş, O. Improving the Functional Performance of Date Seed Protein Concentrate by High-Intensity Ultrasonic Treatment. Molecules 2023, 28, 209. https://doi.org/10.3390/molecules28010209

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS::

- Kelany, M and Orabi, M. Effect of dairy products intake and some physical activities in body mass index and bone mineral density: A survey at Sohag university. J Obes Weight Loss Ther 2018, Volulme: 8DOI: 10.4172/2165-7904-C2-061
- **Kelany M. A.;** Ramadan B.R. & Sorour M.A. **(2015).** Utilization of Cereal Grain Fractions to Improve Oxidative Stability of Beef Burgers under Chilled Storage. Turkish Natural Nutrition and Lifelong Health Summit. May 21 – 23, NG Gural Wellness and Convention Hotel, Sapanca, Turkey.
- **Kelany M. A.;** Ramadan B.R. & Sorour M.A. **(2013).** Cereal grains by-products as source of natural antioxidants. LAB Lambert publishing. Pp 129.
- Ramadan B.R.; Sorour M.A. & **Kelany M. A. (2013).** Effect of cereal grain by-products addition on oxidative stability of chicken burgers during storage. Wudpecker Journal of Food Technology, Vol. 1(6), pp. 104 – 107.