REPUBLIC OF TÜRKİYE
HARRAN UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DOCTORATE THESIS

DETERMINATION OF PHYSICOCHEMICAL PROPERTIES AND HYDROGELLING ABILITY OF AVOCADO SEED PROTEINS DRIED WITH DIFFERENT METHODS

BAKHTIYAR AZAD ABDULLAH ABDULLAH

DEPARTMENT OF FOOD ENGINEERING

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2024

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BAKHTIYAR AZAD ABDULLAH ABDULLAH

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ABSTRACT

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A significant proportion of the avocado (*Persea americana* Mill.) cultivated globally is used for consumption following various processing methods to create different products. During fruit processing, remove unwanted materials, such as stems, leaves, fruit peels, and seeds, from the final product. Seeds, which constitute a large part of the fruit and contain different macromolecules in their structure, have the potential for evaluation among these wastes. However, it was not yet conducted a commercial evaluation of avocado seed. Within the scope of this study, protein was extracted from avocado seed, and the extracted protein was evaluated based on the hydrogel system. This current study focused on extraction of proteins by using alkali isoelectric precipitation techniques from avocado seed and used extracted protein based on the natural fabricated hydrogel. Avocado seed protein was dried into powder by using various drying techniques including spray, freeze, vacuum, oven and ambient drying methods. Different dried avocado seed protein isolate (ASPI) powder was evaluated based on the comparing functional and physiochemical properties such as water activity, protein content, fluidity (carr index and hausner ratio), scanning electron microscopy, secondary structure, amino acid and nutritional parameters, thermal stability. The results demonstrated that drying techniques significantly altered the protein structure and conformation of ASPI, resulting in different physical, chemical, and functional characteristics. The spray drying technique showed the best functional characteristics, such as solubility, water holding capacity, oil holding capacity solubility, foaming capacity and foaming stability, and emulsion activity and stability. Moreover, the capacity of hydrogel models was determined by fabricating hydrogel in the presence of a mixture of protein and locust bean gum in order to assess the influence of different drying techniques. To achieve thus, the results clearly showed that spray dried techniques based on the hydrogel system had the highest protein leachability, swelling ratio and water holding capacity. According to their textural properties, hydrogel models made from freeze-dried and spray-dried proteins produced the best results. Hydrogel models displayed elastic characteristics instead of viscous features. Nevertheless, the degrees of elasticity differed. Incorporating spray-dried protein and locust bean gum into hydrogel models significantly improved the bioactive component delivery method's efficiency compared to using only protein models.

KEYWORDS: Avocado seed protein isolate; Drying techniques, Fabricate natural hydrogel; Release behavior

ÖZET

DOKTORA TEZİ

FARKLI YÖNTEMLERLE KURUTULMUŞ AVOKADO ÇEKİRDEĞİ PROTEİNLERİNİN FİZİKOKİMYASAL ÖZELLİKLERİNİN VE HİDROJEL OLUŞTURMA YETENEĞİNİN BELİRLENMESİ

BAKHTIYAR AZAD ABDULLAH ABDULLAH

HARRAN ÜNİVERSİTESİ
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Son yıllarda dünya genelinde gittikçe artan üretimi ve tüketimi ile bilinen avokado (*Persea americana mill*) büyük bir kısmı işlendikten sonra tüketiciye sunulmaktadır. Meyve işleme sırasında gıda sanayinde sap, yaprak, meyve kabukları ve tohumlar gibi istenmeyen malzemeleri nihai üründen çıkartılır. Meyvelerin büyük bir kısmını oluşturan ve yapısında farklı makromoleküller bulunduran tohumlar (çekirdek), bu atıklar arasında değerlendirilme potansiyeline sahiptir. Ancak avokado çekirdeğinin ticari değerlendirmesi henüz yapılmamıştır. Bu çalışma kapsamında avokado çekirdeğinden protein ekstrakte edilmiş ve elde edilen protein hidrojel sistemlerine dahil edilerek değerlendirilmiştir. Alkali-izoelektrik çökeltme yöntemleriyle avokado çekirdeğinden protein ekstraksiyonuna odaklanılmış. Avokado çekirdeğinden elde edilen proteinler, ilk olarak oda şartlarında kurutma, etüv kurutma, vakum etüv kurutma, dondurarak kurutma ve püskürterek kurutma gibi çeşitli kurutma teknikleri kullanılarak toz haline getirilmiştir. Farklı teknikler ile kurutulmuş avokado çekirdeği protein (ASPI) tozu, protein içeriği, su aktivitesi, akışkanlık (carr indeksi ve hausner oranı), SEM, FTIR, TGA, amino asit ve beslenme parametreleri gibi fonksiyonel ve fizyokimyasal özelliklerin karşılaştırılmasına dayalı olarak değerlendirilmiştir. Sonuçlar, ASPI'nın protein yapısının ve konformasyonunun kurutma tekniklerinden büyük ölçüde etkilendiğini, farklı fiziko-kimyasal ve fonksiyonel özelliklere yol açtığını göstermiştir. Püskürterek kurutma tekniği ile elde edilen avokado çekirdeği proteinleri, çözünürlük, su tutma kapasitesi, emülsiyon aktivitesi/stabilitesi ve köpük oluşturma kapasitesi/stabilitesi gibi en iyi fonksiyonel özellikleri göstermiştir. Ayrıca kurutma yöntemlerinin hidrojel üeritimi üzerindeki etkilerini belirlemek amacıyla avokado çekirdeği proteini keçiboynuzu zamkı varlığında doğal hidrojel sistemlerine dahil edilmiştir. Sonuçlar, püskürtmeli kurutma tekniği ile kurutulan avokado çekirdeği proteinin oluşturduğu hidrojellerin en yüksek su tutma kapasitesine, şişme oranına ve protein süzülebilirliğine sahip olduğunu açıkça göstermiştir. Püskürterek kurutma tekniği ve dondurularak kurutma tekniği kullanılarak elde edilen avokado çekirdeği proteinlerini içeren doğal hidrojel sistemleri, dokusal özelliklerine göre en iyi sonuçları sergilemiştir. Bu hidrojeller viskoz özellikler yerine elastik özellikler sergilemiştir. Ancak esneklik dereceleri arasında farklılıklar tespit edilmiştir. Püskürterek kurutma tekniği kullanılarak kurutulmuş avokado çekirdeği proteini, keçiboynuzu zamkı ile birlikte doğal hidrojel sistemlerine dahil edilmesi kullanılması, biyoaktif bileşik dağıtım sisteminde tek başına protein hidrojel sistemlerinin oluşturulmasından çok daha iyi sonuç vermiştir.

ANAHTAR KELİMELER: Avokado çekirdeği proteini; Kurutma yöntemleri, Doğal hidrojel sistemleri; Salınım davranışı

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ABBREVIATIONS

AD : Ambient drying

ASPI : Avocado seed protein isolate

cm : Centimeter

EA : Emulsion activity

ES : Emulsion stability

FAO : Food and Agriculture Organization

FC : Foaming capacity

FD : Freeze drying

FS : Foaming stability

FTIR : Fourier transform infrared

g : Grams

h : Hours

HCl : Hydrochloric acid

KBr : Potassium bromide

kg : kilogram

KHz : kilohertz

Kpa : kilopascal

l : Litter

M : Molarity

mg : Milligram

min : Minute

mL : Milliliter

mL : Milliliter

Na2HPO4 : Sodium hydrogen phosphate

NaCl : sodium chloride

NaH2PO4 : Sodium dihydrogen phosphate

NaOH : sodium hydroxide

nm : Nanometer

NY : New York

OD : Oven drying

OHC : Oil holding capacity

Pa : Pascal

pH : Potential of hydrogen

rpm : Revolutions per minute

SD : Spray drying

SDS : Sodium dodecyl sulfate

sec : Second

SEM : Scanning electronic microscopy

USA : United Stated of America

V : Volume

VD : Vacuum drying

WHC : Water holding capacity

WHO : World Health Organization

µL : Mikrolitre

1. INTRODUCTION

Due to the rising global population and a progressive decline in protein resources, there is a growing demand for alternative sources of protein. Proteins are crucial nutrients that are necessary on a daily basis to facilitate the body's development, growth, and repair, as well as to maintain overall health. In recent years, people have become more conscious of the value of eating foods that are healthier and more natural. This has resulted in a shift towards dietary preferences such as veganism and vegetarianism. The factors including the high cost and limited availability of animal protein for individuals with limited financial means, as well as the negative health effects associated with excessive consumption of animal protein leading to cardiovascular diseases, have prompted humanity to explore alternative protein source. Currently, plant proteins are considered the optimal source for research and consumption. Plant proteins are highly nutritious, cost-effective, readily available, environmentally sustainable, and possess numerous functional attributes. Consequently, they are widely regarded as a viable substitute for animal protein. An inexpensive and readily available protein source holds the potential to significantly address the issue of malnutrition.

Studies have demonstrated that fruit proteins possess diverse functional properties that make them suitable substitutes for additives commonly employed in food manufacturing. Previously, products such as fruit and vegetables, including seeds, peels, stems, and leaves, which are considered food waste, were typically discarded and allowed to naturally decompose, resulting in environmental contamination. Studies have demonstrated that the by-products generated in the food business include a significant amount of nutrition such as protein, fat, and minerals, and possess various functional qualities. As a result, there has been an increase in studies and research aimed at evaluating plant by-products and utilizing their constituents, such as protein, as primary materials and additives in the food industry.

Persea americana Mill., known as avocado, belongs to the Lauraceae family of dicotyledonous plants. It is a flowering plant native to Central America and Mexico. Avocado fruits are highly popular and extensively consumed worldwide (Bangar et al., 2022b). Avocado farming has experienced substantial growth in the last ten years, leading to a doubling of production from 4 to 8 million metric tons, as reported by the 2020 data from the Food and Agriculture Organization (FAOSAT, 2022). Avocado seeds are deemed a superfluous by-product and subsequently disposed of throughout the process of industrial manufacturing. Using these by-products may reduce or eliminate environmental contamination (Figueroa et al., 2018). Effective management of waste by-products has the potential to benefit both the environment and the economy. This is because the avocado seed contains a substantial portion (13 to 17 %) of whole fruit and contain a wide range of bioactive compounds including lipid, vitamins, proteins, polysaccharides, fiber and mineral (Melgar et al., 2018; Lightfoot et al., 2018). Protein is the most significant component of the many macromolecules found in the avocado seeds. Proteins are complex compounds composed of amino acids that perform essential functions in various biological processes such as growth, cell signaling, enzyme regulation, and catalyzing biochemical reactions (C Egbuonu, 2018). The increasing need for nutritionally superior food has sparked a heightened interest in plant-derived nutrients, including protein. As a result, researchers focused extensively on investigating sustainable alternatives for food sources that are rich in nutrients. The protein content in avocado seeds has been determined to be approximately 2.64 % (C Egbuonu, 2018), 7.75 % (Mahawan et al., 2015), 15.55 % (Ejiofor et al., 2018), 17.94 % (Arukwe et al., 2012) and 23 % (Ifesan et al., 2015).

On a global scale, the prevalence of protein insufficiency has increased significantly, affecting countries at various stages of development. The primary factors contributing to this issue are population expansion and the increasing need for protein in industrial applications, feeding production, and agriculture sectors. While animals, plants, and microbes can serve as sources of protein, their production capacities are insufficient to fulfill the growing protein demand (Qin et al., 2018). Therefore, it is considered a feasible, beneficial, cost-effective, and repeatable method to enhance protein availability by extracting protein from these waste materials or by-products. An essential obstacle in the extracting protein from the waste materials is effectively removing impurities while also improving protein output, lowering extraction time, and maintaining the functional characteristics of the proteins (Li et al., 2021).

In the present day, protein is obtained from various seeds by diverse extraction procedures and then dried using a variety of drying techniques. Proteins obtained from various plants have variations in their functional characteristics, such as emulsion activity and stability, solubility, water holding capacity, foam capacity and stability and oil absorption capacity. The heterogeneous structure and composition of proteins can be the cause of the variances. Protein extraction methods and drying techniques are the major factors that influence the functional quality of proteins. Shen et al., (2021) indicated that vacuum, oven, freeze, and spray drying are the most frequently employed technologies in drying protein. The fluctuations in temperature and drying duration may result in modifications to proteins' physicochemical attributes and functional attributes (Li et al., 2021). As a result, these alterations might lead to various functional characteristics in the final dried products. Prior research has indicated that the drying methods used for fenugreek (Feyzi et al., 2018), chia (Shen et al., 2021), quinoa (Shen et al., 2021), hempseed (Lin et al., 2021), and mung bean (Brishti et al., 2020) have a significant impact on their physiochemical and functional characteristics of protein.

Hydrogels have a distinct physical state that displays the properties of both liquids and solids, making them neither completely liquid nor completely solid. Hydrogels are both flexible and elastic, functioning as a middle ground between these two states. Hydrocolloids, like proteins and polysaccharides, play a crucial role in the production of these hydrogels (Nazir et al., 2017; Yang et al., 2021b). Foods in the form of hydrogels may be classified as soft, solid substances, where the water phase makes up more than 80 % of their total structure (Zuniga and Aguilera, 2008). Examples of hydrogels in this category include desserts, jam, quick-set gels, jelly and confectionery goods. Recently, there has been a noticeable trend toward using these distinct architectures. These characteristics can be linked to their high-water content, ability to decompose naturally, pleasant flavor, affordability, low calorie content, compatibility with living organisms, and ability to enhance feelings of fullness (Yang et al., 2021a; Cao and Mezzenga, 2020; Nazir et al., 2017).

The primary purpose of this thesis is to evaluate the suitable processes for protein extraction and production of dried avocado protein powder. The specific goals to be achieved are:

- Optimizing production conditions for the extraction of protein from avocado seeds.
- Drying avocado seed protein isolates (ASPI) into powder with different drying techniques, including spray drying (SD), freeze drying (FD), vacuum drying (VD), oven drying (OD) and ambient drying (AD).
- The functional characteristics of ASPI, including foaming capacity, solubility, foaming stability, water holding capacity, emulsion activity, oil holding capacity and emulsion stability were analyzed.
- This study also evaluated the physicochemical characteristics of ASPI,

including amino acid content, secondary structure, and morphology.

- Choosing suitable drying techniques will help the industry enhance the functional characteristics of protein for specific food uses.
- The second goal of this study is to assess the impact of drying protein by of various techniques on the hydrogel systems. For this purpose, hydrogel samples were prepared from the mixture of dried ASPI, including AD, OD, VD, FD and SD with locust bean gum (LBG).
- Evaluated the functional properties of hydrogel samples, including protein leachability, water holding capacity, and swelling ratio.
- Determined the physiochemical properties of hydrogel samples such as rheological behavior, scanning electronic microscope (SEM) images, texture profile and Fourier transform infrared spectroscopy (FTIR).
- Determine the efficacy of the hydrogel systems in terms of bioactive compound delivery.

2. PREVIOUS STUDIES

2.1. Avocado

The avocado (*Persea americana Mill.*) belongs to the Lauraceae family and the genus Persea. It is the only species in its genus that has commercial value. The fruit is tropical and subtropical, originally native to Mexico and Central America. However, it is currently cultivated in various places all over the world. Cultivating avocados requires a substantial amount of resources, as it takes approximately three to five years for an avocado tree to start producing fruit (Rodríguez-Martínez et al., 2022). The fruit comprises the peel for 11-15 %, the pulp for 65-73 %, and the seed for 16-20 %. The genotype determines the characteristics of the endocarp, including its size, structure, color, and phytochemical content (Calderón-Oliver et al., 2016).

The weight of an avocado can range from 120 g to 2 kg. The surface may be smooth or rough, the skin may be thin or thick, and the berry may be pyriform, obovate, or globose (Abraham et al., 2018). The avocado fruit exhibits significant heterogeneity and undergoes an extended period of immaturity, primarily attributed to the limited incidence of self-fertilization. Litz et al., (2005) classify avocado cultivars into two types, A and B, based on their blossoming patterns, where the blooms are either functionally male or female. Nevertheless, type A exhibits functional female bloom properties during the morning. On the other hand, type B exhibits female bloom properties throughout the afternoon. Avocado ripening differs from most other fruits because it occurs immediately after the fruit is harvested, not on the tree. Avocados may spend many months on the tree, undergoing physiological ripening before harvest. (Yahia and Woolf, 2011; Blakey et al., 2009).

The proportion of dry matter that governs the tree's maturation is inversely related to the proportion of moisture that governs the tree's maturation. This relationship has been found to strongly correspond with the avocado's ability to ripen after being harvested. Researchers Cowan and Wolstenholme, (2016) and Zafar and Sidhu, (2010) have shown that this method is universally accepted for assessing the ripeness of avocados and is employed on a global scale.

The avocado fruit is distributed throughout the world in tropical and subtropical climates. Avocado cultivars vary significantly over the globe, adapting to different climates and exhibiting diverse properties such as flavor, texture, color, form, and aroma (Litz et al., 2005).

2.1.1. Production, harvesting, and yield of avocados in various countries

In 2020, the world's avocado acreage totaled 807,469 hectares, growing at an average annual rate of 29 % from 2016 to 2020. Undoubtedly, the high global demand for avocados, as well as the favorable price for growers and marketing firms, is driving up avocado production. Global avocado output surpassed 8 million tons in 2020, a 41 % increase from the previous year. The primary producers of avocados, accounting for 71.2 % of global output, are Mexico, Colombia, the Dominican Republic, Peru, and Indonesia, with a value of 2,394,000, 877,000, 676,000, 660,000, and 609,000 tones, respectively (Rodríguez et al, 2023).

The avocado is a very adaptable crop that thrives in tropical and subtropical climates. This adaptability allows for successful production in locations with a typical Mediterranean environment. In these regions, water scarcity is common during the summer months, and there is a significant risk of excessive salt in irrigation water. Coastal communities often grapple with this challenge, which complicates agriculture in the face of climate change. In the Mediterranean basin, water scarcity is the primary constraint on agricultural output (Rodríguez et al, 2023).

Figure 2.1 displays avocado production in the USA, Asia, and the European Union from 2010 to 2022. In 2010, the USA had the highest production output of avocados, at about 2.6 million tones, followed by the EU and Asia, with values of about 1 million and 440 thousand tones, respectively. Between 2010 and 2022, avocado production in the USA increased from 2.6 million to 6.5 million tons. Also, from 2010 to 2022, avocado production in Asia rose from 440 thousand to 1 million tons. In contrast, the production of avocados in the EU had stabilized (FAOSTAT, 2023).

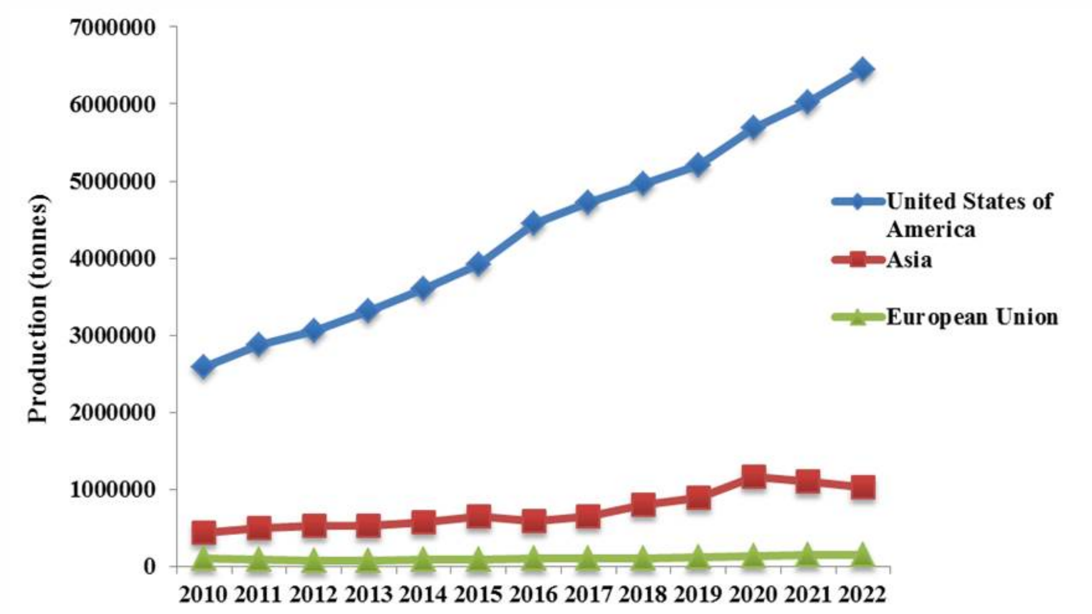

Figure 2.1. Avocado production in the USA, European Union and Asia from 2010-2022 (FAOSAT, 2023)

Figure 2.2 depicts the land area dedicated to avocado cultivation in the USA, Asia, and the EU. From 2010 to 2022, the USA achieved the highest production output and utilized more land for avocado production than any other country. Although avocado yield strongly correlates with production area, production in the USA is considered to have increased, whereas it fluctuates over the years in Asia and the EU (FAOSTAT, 2023). According to FAOSAT data, the area of avocado harvesting in the USA increased to double from 2010 to 2022, with a value of 285,104 hectares and 597,460 hectares, respectively. In addition, from 2015 to 2016, there was a favorable change in the area harvested, from 384915 hectares to 462609 hectares. In Asia and EU between 2010 and 2022, the area of harvesting avocados rose from 49685 to 87934 and 11384 to 25176, respectively.

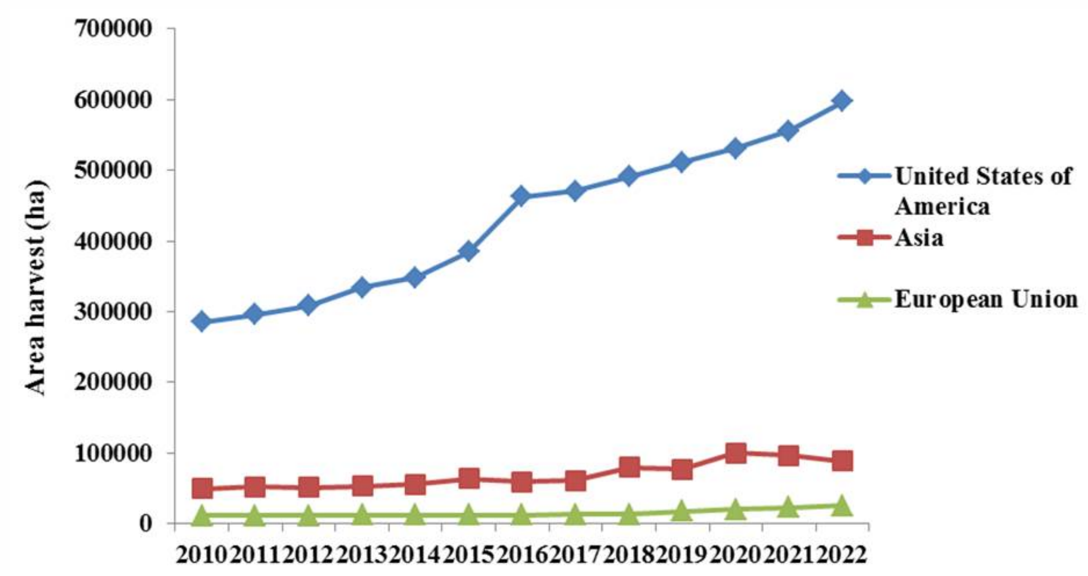

Figure 2.2. Avocado area harvesting in the USA, Asia, and European Union from 2010–2022. (FAOSAT, 2023).

Figure 2.3 depicts the amount of avocado produced per hectare of land. Throughout the years, the USA and Asia consistently achieved the best output per hectare, whereas the yield of avocados in Asia increased from 2019 to 2022. Conversely, avocado yields in the EU have decreased in recent years, from 2010 to 2022.

The yield of avocados in Asia fluctuated from 2010 to 2018. However, after 2019, the yield of avocados increased and then remained relatively unchanged until 2022. In the USA, the yield of avocados fluctuated from 2010 to 2019, after which it increased until 2022. In EU, the yield of avocados declined significantly from 2010 to 2013, with a value of 89415 to 26002 hg/ha. In contrast, the yield of avocados increased from 2014 to 2018, reaching a value of 68702 to 74205 hg/ha in 2019. However, in 2019, the yield of avocados declined for the second time until 2022 (FAOSTAT, 2023).

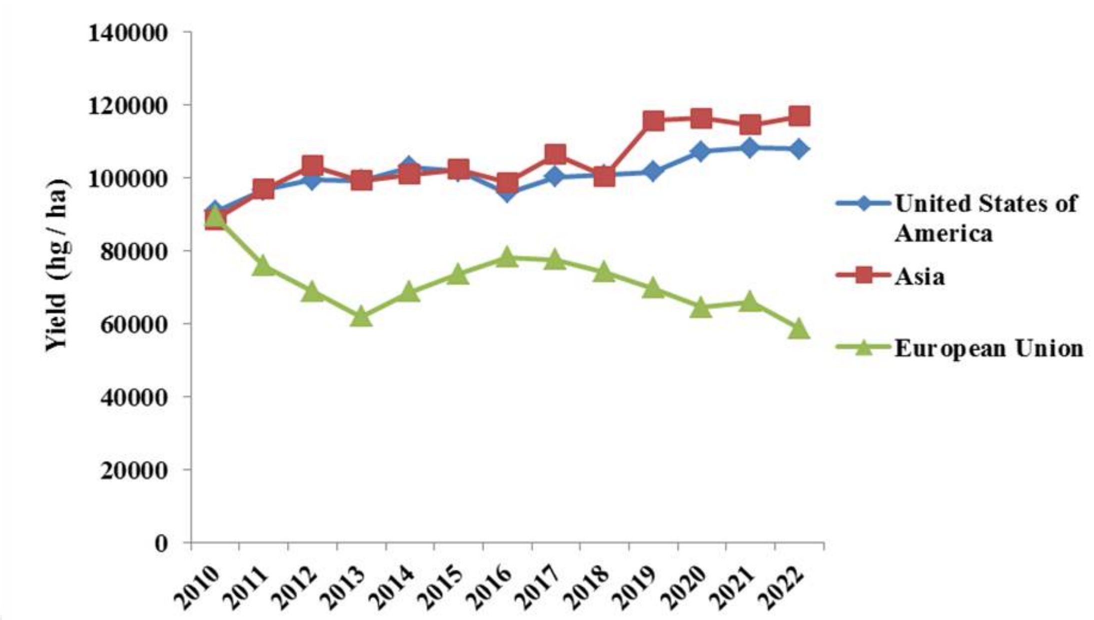

Figure 2.3. Yield of avocado in USA, Asia and European Union from 2010-2022 (FAOSAT, 2023).

2.1.2. Economically important avocado varieties

The weight, shape, and size of an avocado fruits may differ depending on factors such as climatic conditions, fruit varieties and agricultural practices used during growth (Cruz de la Cruz and Vera Ramirez, 2020, Cheikhyoussef and Cheikhyoussef, 2022). According to Yahia and Woolf (2011), around 500 different types of avocado have been recognized, but only a few are grown for commercial purposes as show in Table 2.1., such as Reed, Bacon, Fuerte, Pinkerton, Lula and Ettinger due to several issues. These issues include long production times, inadequate protein and fat levels, susceptibility to diseases, and susceptibility to damage during transport. Due to its extended shelf life and exceptional nutritional quality, the Hass cultivar is particularly notable for its dominant presence in the international market. The varieties of avocado are different from each other in terms of size, form, flavor, and weight, but the most noticeable distinction is the skin color as it ripens (Yahia and Woolf, 2011). Botanically, avocado can be divided into three groups, which are Persea americana var. americana recognized as a West Indian, Persea americana var. drymifolia recognized as a Mexican, and Persea nubigena var. guatemalensis recognized as a Guatemalan. Researchers Zafar and Sidhu (2010) reported that these names derive from the distinct origins, growth conditions, and qualities of the fruit. Guatemalan-Mexican and Guatemalan hybrid cultivars currently dominate the commercial avocado market. Additional, cultivars including Ettinger, Bacon, Reed, Pinkerton, Hass and Fuerte, are presently available for commercial distribution as illustrated in Table 2.1. (Dabas et al., 2013; Cowan and Wolstenholme, 2016).

Table 2.1. Varieties of avocado (Araújo et al., 2018)

Variety	Parentage	Seed size	Skin texture	Blossom Type	Fruit Shape	Skin Colour Unripe	Skin colour ripe	Skin Thickness	Average Fruit weight (oz)	Origin
Hass	Hybrid	Medium	Pebbly	A	Narrowly obovate to obovate	Green	Black	Medium	140–340	California
Bacon	Mexican	Large	Smooth	B	Obovate	Green	Green	Thin	170–510	California
Ettinger	Mexican	Large	Smooth	B	Narrowly obovate	Green	Green	Thin	255–570	Israel
Pinkerton	Hybrid	Small	Rough-pebbly	A	Pyriform	Green	Green	Medium	255–510	California
Reed	Guatemalan	Large	Medium	A	Spheroid	Green	Green	Medium	480–680	California
Fuerte	Hybrid	Large	Medium	B	Obovate	Green	Green	Medium	255–455	Puebla, Mexico
Lam Hass	Hybrid	Medium	Pebbly	A	Obovate	Black	Black	Medium	280–510	California
Williams	Guatemalan	Medium	Medium	A	High spheroid	Green	Black	Medium	225	California
Zutanno	Mexican	Medium	Smooth	B	Obovate	Green	Green	Thin	310–400	California
Stewart	Mexican	Small	Smooth	A	Obovate	Black	Black	Thin	170–370	California
McDonald	Guatemalan	Medium	Rough	B	Spheroid	Green/Black	Black	Thick	340–450	Hawaii
Lula	Hybrid	Large	Almost smooth	B	Pyriform	Green	Green	N/A	450–680	Florida
Gwen	Hybrid	Medium	Pebbly	A	Obovate	Green	Green	Medium	170–425	California
Nabal	Guatemalan	Large	Smooth	B	Spheroid	Green	Green	Medium	450–850	Guatemala
Mexicola	Mexican	Large	Smooth	A	Obovate	Black	Black	Thin	110–185	California
Mexicola grande	Mexican	Large	Smooth	A	Obovate	Black	Black	Thin	170–200	California
Dickinson	Guatemalan	Small	Pebbly	A	Narrowly obovate	Black	Black	Thick	170–340	California
General Bureau	West India	Large	Mostly smooth	A	Pyriform	Light green	Light green	Medium	200–510	Morocco
Gil	Hybrid	Large	Pebbly	A	Narrowly obovate	Green/Black	Black	Thick	255–400	Israel
Dickey	Guatemalan	Small	Rough	A	Pyriform	Green	Green	N/A	340–680	Mexico
Lewis	West India	Medium	Smooth	N/A	Pyriform	Green	Black	N/A	595	Hawaii
Puebla	Mexican	Large	Smooth	A	Obovate	Black	Black	Thick	170–450	Mexico

2.1.3. Nutritional composition of avocado fruit

The fruit's flesh, being the sole consumable constituent, garners attention from industries that employ it in condiment and oil manufacturing. Avocado is a rich source of fiber, proteins, minerals, carbohydrates, lipids, and vitamins C, E, and K as show in Table 2.2. Avocado fruit processing generates a significant amount of by-product, which consists of peel and seeds. The discarded avocado peel accounts for seventeen percent of the fruit's overall weight (Araujo et al., 2021).

Avocado has abundant nutritional value, and its health advantages are mainly due to its phytochemical content and fat soluble nutrients (Álvarez et al., 2020). Avocado pulp contains high amount of fiber, including 30 % soluble and 70 % insoluble, and, compared to other fruits, contains more proteins. Furthermore, avocado pulp contains sugars such as 7-carbon carbohydrates and sucrose, tannins, polyphenols, perseitol, phytoestrogens, and pigments (Zafar and Sidhu, 2011). Avocado pulp's nutritional composition includes ash (0.8-1.5 %), moisture (67-78 %), protein(1.0-3.0 %), fat (12-24 %), carbohydrate (0.8-4.8 %), and fiber (1.4-3.0 %) (Duarte et al., 2016; Cowan and Wolstenholme, 2016; Rodríguez-Carpena et al., 2011). Maturation stages, varieties, climate, soil composition, and fertilizers influence the composition of avocados and other fruits (Álvarez et al., 2020).

Avocados contain numerous minerals, including phosphorus, calcium, sodium, potassium, and magnesium. Avocados also contain small amounts of iron and zinc, with concentrations below 1 mg/g of fresh avocado. This product's high potassium and low salt levels provide benefits for people following low sodium diets and provide protection for cardiovascular ailments (Cowan and Wolstenholme, 2016; Álvarez et al., 2020).

Table 2.2. Avocado fruit nutritional value (USDA, 2011)

Nutrient/phytochemical	Unity	Value per 100 g	1 fruit 136 g	1 serving 30 g
Proximate				
Water	(g)	72.3	98.4	21.7
Energy	(kcal)	167	227	50
Energy (insoluble fiber adjusted)	(kcal)	148	201	44
Protein	(g)	1.96	2.67	0.59
Total lipid (fat)	(g)	15.4	21	4.62
Ash	(g)	1.66	2.26	0.5
Carbohydrate	(g)	8.64	11.8	2.59
Fiber	(g)	6.8	9.2	2
Sugars	(g)	0.3	0.41	0.09
Starch	(g)	0.11	0.15	0.03
Minerals				
Calcium	(mg)	13	18	4
Iron	(mg)	0.61	0.83	0.18
Magnesium	(mg)	29	39	9
Phosphorus	(mg)	54	73	16
Potassium	(mg)	507	690	152
Sodium	(mg)	8	11	2
Zinc	(mg)	0.68	0.92	0.2
Copper	(mg)	0.17	0.23	0.05
Manganese	(mg)	0.15	0.2	0.05
Selenium	(ug)	0.4	0.5	0.1
Vitamins and **Phytochemicals**				
Vitamin C	(mg)	8.8	12	2.6
Thiamine	(mg)	0.08	0.1	0.02
Riboflavin	(mg)	0.14	0.19	0.04
Niacin	(mg)	1.91	2.6	0.57
Pantothenic acid	(mg)	1.46	2	0.44
Vitamin B-6	(mg)	0.29	0.39	0.09
Folate food	(µg)	89	121	27
Choline total	(mg)	14.2	19.3	4.3
Betaine	(mg)	0.7	1	0.2
Vitamin B-12	(µg)	0	0	0
Vitamin A	(µg)	7	10	2
Carotene beta	(µg)	63	86	19
Carotene alpha	(µg)	24	33	7
Cryptoxanthin beta	(µg)	27	37	8
Lutein + zeaxanthin	(µg)	271	369	81
Vitamin E (alpha-tocopherol)	(mg)	1.97	2.68	0.59
Tocopherol beta	(mg)	0.04	0.05	0.01
Tocopherol gamma	(mg)	0.32	0.44	0.1
Tocopherol delta	(mg)	0.02	0.03	0.01
Vitamin k1 (phylloquinone)	(µg)	21	28.6	6.3

2.1.4. Avocado seed nutritional composition

Avocado processing companies frequently dispose of avocado seeds as waste by-products. The by-product has not been used to a large extent and may lead to contamination of the environment (Figueroa et al., 2018). Efficient waste by-product management would be advantageous when considering both environmental and economic factors (Araújo et al., 2020). Avocado seeds make up a significant proportion (13-17 %) of the whole fruit. The avocado seed contains a wide range of nutritious and functional substances, including lipids, carbohydrates, proteins, minerals, vitamins, various phytochemicals and fiber (Tremocoldi et al., 2018; Melgar et al., 2018).

The avocado seed contains a wide variety of bioactive compounds, including tannins, flavonoids, and phenolics, as shown in Table 2.3. The bioactivities of these extracts have been analyzed, including their anti-microbial (Villarreal-Lara et al., 2019, anti-oxidant (Soledad et al., 2021), anti-cancer (Lara-Marquez et al., 2020), anti-hyperglycemic (Tremocoldi et al., 2018), anti-inflammatory (Dabas et al., 2019) and anti-hypercholesterolemic (Uchenna, Shori, and Baba, 2017). Furthermore, these extracts have a long history of being used in traditional dermatological applications. Due to the absence of poisonous or hazardous substances, they serve as an excellent natural reservoir of biological activity components for the cosmetic, pharmaceutical, and food industries. Due to their significant antioxidant capacity, they effectively inhibit food oxidation, which is a breakdown process in which lipid, carbohydrate, proteins, and vitamins, react with nitrogen as well as oxygen compounds, resulting in alterations to the nutritional and sensory characteristics of food items (Tremocoldi et al., 2018; Saavedra et al., 2017).

The avocado seed contains a significant amount of protein, which is one of the main types of macromolecules (C Egbuonu, 2018). Comprising of amino acids, proteins are complex macromolecules that play a crucial role for development, enzyme control, growth, cell signaling and acting as biocatalysts. Researchers have extensively studied sustainable alternative food sources that are rich in nutrients.

Carbohydrates constitute a substantial amount (64.9 %) of the macromolecules present in avocado seeds. Avocado seeds have 91.2 % starch in their total carbohydrate content (Tesfaye et al., 2018). Bangar et al., (2022a) discovered that fractions of plant-based polysaccharides demonstrate diverse biological functions. Avocado seeds had high levels of two C7 sugars namely perseitol and D-mannoheptulose with the value 88.3 mg/g and 63.8 mg/g, respectively. The prevalence of these C7 sugars in avocado seed suggests that they have significance in these tissues. These kinds of sugars could potentially serve as a means of transporting and storing sugars in avocados (Liu et al., 2002). The composition of carbohydrate in avocado seed as follows: sucrose (18.5 mg/g), perseitol (88.3 mg/g), starch (246.1 mg/g), D-mannoheptulose (63.8 mg/g) and hexose (1.9 mg/g). The proportion of C7 sugars in the avocado seed's total sugar content was 36.3 % (Liu et al., (2002); Tesfay et al., (2012)).

In terms of nutrition and vitamins, avocado seeds include a diverse range of minerals, including potassium, iron, calcium, phosphorus, sodium, lead, copper, cobalt, and zinc, as well as vitamins including vitamin C, E, and A, thiamine, riboflavin, and niacin (B1, B2, and B3), respectively. In their study, Ifesan and Olorunsola (2015) determined the levels of different minerals, specifically calcium, potassium, zinc, and sodium, in the avocado seed. They found the concentrations to be 0.09, 4.16, 0.18, and 0.82 mg per 100 g, respectively. Avocado seeds include minerals that make them an excellent option for human nutrition, particularly for addressing micronutrient deficiencies.

Table 2.3. Nutritional value of avocado seed (Bangar et al. 2022b)

Group	Composition
Proximate analysis	
Moisture Content	13.09 %
Dry Matter	86.91 %
Crude Fibre	2.87 %
Ash	3.82 %
Sugar components (mg/g of DW)	
Hexose	1.9
Glucose	5.62
Fructose	12.93
Sucrose	7.86–18.5
d-Mannuheptulose	10.51–63.8
Perseitol	12.54–88.3
Carbohydrate (%)	64.9
Protein in different studies	**%**
Crude protein content (AOAC, 1990 method)	2.64
Protein content	23
Protein content	17.94
Protein content	7.75
Protein content	15.55
Lipid's profile	
Long-chain fatty acids	**(µg/g)**
Tetracosanoic acid	4.29
Nervonic acid	2.88
Behenic acid	3.63
Erucic acid	2.44
Arachidic acid	2.39
Stearic acid	5.06
Oleic acid	5.32
Linoleic acid	4.06
Palmitic acid	7.1
Myristic acid	2.49
Fatty acid derivatives (aliphatic acetogenins)	
Avocatins	32.28
Polyhydroxy fatty acids	24.26
Pahuatins	4.26
Persins	10.12
Minerals	**mg/100 g**
Calcium	0.82
Potassium	4.16
Phophorus	0.09
Zinc	0.18
Sodium	1.41
Iron	0.31
Copper	0.98
Vitamins	**mg/100 g**
Vitamin A	10
Thiamin	0.33
Riboflavin	0.29
Niacin	0.06
Ascorbic acid	97.8
Vitamin E	0.12

2.1.5. Recent studies on the avocado seed.

Avocado seeds contain abundant phytochemicals used in herbal medicine. This study is focused on investigating the extraction of phenolic, flavonoid, and antioxidant compounds from avocado seed extract. The extraction process from avocado seed was achieved by maceration and reflux with a 70 % ethanol solvent. Using a 70 % ethanol solvent, yield productivity was 43.07 % for maceration and 39.58 % for reflux. This study looked at the phytochemicals in avocado seeds by screening them for phytochemicals, testing their antioxidant activity, and doing a total phenolic analysis and a total flavonoid analysis. Phytochemical analysis revealed that avocado seeds include phenols, flavonoids, tannins, saponins, quinones, and alkaloids. The 70 % ethanol extract of avocado seeds exhibited an antioxidant activity of 77.298 g/mL through maceration and 98.626 g/mL through reflux. In comparison, the IC50 value of vitamin C was 12.883 g/mL. Phenolics and flavonoids were extracted from avocado seeds using a 70 % ethanol extract through maceration and reflux. Both extractions had phenolic values of 276.96 mg GAE/g and 294.96 mg GAE/g, as well as flavonoid values of 1.73 mg QE/g and 12.70 mg QE/g, respectively. The 70 % ethanolic extracts from the avocado seed produced during maceration and reflux exhibit strong antioxidant activity (Munthe et al., 2023).

Del-Castillo-Llamosas et al., (2023) discussed the use of avocado seed in a green biorefinery approach based on microwave-assisted autohydrolysis. This study analyzed the solids and liquids produced during the treatment, conducted at temperatures ranging from 150 to 230 °C for 5 min. At 220° C, the liquor exhibits optimal levels of phenolic and flavonoids with the value 42.15 mg GAE/g and 31.89 RE/g, respectively, in addition to 38.8 g/L of glucose and glucooligosaccharides. Using ethyl acetate for extraction facilitated bioactive component retrieval while preserving the polysaccharides in the solution. The extract included a high concentration of vanillin (99.02 mg/g AS) and also contained numerous flavonoids and phenolic acids. During the treatment, the solid phase contained glucose concentrations of 9.93 g/L, while the phenolic-free fluid had concentrations of 105 g/L. This study exhibits how microwave assist autohydrolysis in biorefinery process can convert avocado seeds into fermentable sugars and antioxidants known as phenolic compounds (Del-Castillo-Llamosas et al., 2023).

This study examined and contrasted the physicochemical, molecular, and digestibility properties of starches in three types of avocado seed, namely Hass, Fuerte, and Criolla. Researchers analyzed the thermal properties, size distribution, morphology, pasting properties, FTIR, and digestibility. The average size of starch extracted from Criolla was 24.55 µm, which was larger than the average sizes of Hass and Fuerte starches, which were 21.37 µm. Compared to gelation among the three types of avocado, starch extracted from fuerte had the superior 75.57 °C pasting temperature, 75.28 °C gelation temperature, and 8.55 J/g gelation enthalpy. When compared to the extracted starch from Fuerte and Criolla, the starches extracted from Hass avocado showed greater viscosities, with breakdown at 17.68 mPa.s., setback at 588.78 mPa.s., final at 1407.37 mPa.s., and maximum at 836.27 mPa.s.. Furthermore, the tested avocado starches showed a significant concentration of resistant starch ranging from 60.06 % to 68.90 %. Variations in avocado starch structure and chemical composition can have an impact on the digestion of unmodified starch (Salazar-Irrazabal et al., 2023).

Research has shown that avocado seeds contain beneficial bioactive components. This study examined how roasting impacts fermented avocado seeds' metabolites and anti-cancer properties. Fermenting avocado seeds at lower temperatures for shorter durations improved their anti-cancer properties. Suppression was most effective against Hep G2 cancer cells, with the MDA-MB-231 and MCF-7 cell lines following. Through untargeted metabolite profiling, gas chromatography-mass spectrometry identified 208 metabolites. PLS-R discovered a total of 41 metabolites with values greater than 1 that were associated with anticancer activity. Sugars and amino acids were more abundant at lower roasting temperatures and showed a favorable correlation with anticancer properties. The ideal roasting settings for maximum anticancer and antioxidant effects were found. Fermented avocado seed was show potential as a functional ingredient in food with favorable bioactive qualities (Zhao et al., 2023).

The avocado seed is a by-product rich in extractable polyphenols, which have garnered interest from the food and cosmetic industries for their strong antioxidant properties. This characteristic makes it a favorable option for the cost-effective and environmentally friendly extraction of these substances. The purpose of research was to extract the total polyphenols from avocado seed utilizing water as a sustainable solvent by ultrasonic power (ranging from 0 to 104 W) and temperature (ranging from 20 to 60 °C). Various mathematical models, including film theory, Peleg's, Fick's law, and empirical were employed to determine the most suitable model for describing the extraction kinetics. The experimental findings indicated that temperature and ultrasonic power had a notable impact on the polyphenol extracted from the avocado seed. Higher temperatures and ultrasonic power led to extracts with increased polyphenol content and antioxidant capacity. Moreover, there is a direct correlation between the total polyphenol and antioxidant capability. Mathematical models improved the understanding of the extraction process, which involved two stages: fast and slow. Ultrasound-assisted extractions were more effective than classical extractions. Avocado seeds could serve as an economical and sustainable source of polyphenols in industry through solid-liquid extraction (Segovia et al., 2016).

2.2. Protein Extraction from Plant Seeds

Generally, protein extraction from seeds is not a difficult process. However, in order to achieve success, the initial stage of proteomic analysis necessitates meticulous attention due to the fact that the seed is not a uniform tissue (Gadalkar et al., 2017). The composition and structure of seeds and tissues can significantly differ among different species. Various components in seeds can significantly reduce protein extraction or change the types of proteins, as observed during two-dimensional electrophoresis (2-DE). Polyphenols such as hemicellulose, arabinoxylans, and lignans often comprise the outer layers of the seeds. The cell wall substance in several species consists of arabinoxylans and arabinogalactans. The seed frequently serves as a storage site for many substances, with certain tissues containing vast amounts of polysaccharides, lipids, and several secondary metabolites.

The main proteins found in seeds are storage proteins, which are supplied by amino acids during seed germination. Based on the solubility, storage proteins can be categorized into four classes. The first class is albumins, which consist of water-soluble proteins. The second class is globulins, which are soluble in salt solutions. The third class is prolamin, which includes proteins that are soluble in hydroalcoholic solutions. The fourth and final class is glutelins, which are soluble in either alkali or acid solutions (Givonetti et al., 2021).

2.2.1. Protein extraction techniques

2.2.1.1. Alkaline isoelectric precipitation

It is the most widely used and important method for purifying and isolating proteins from complicated biological mixtures. This method utilizes the phenomenon of protein solubility at isoelectric point, which a protein has no overall charge, causing it to precipitate from the solution. The method commences with choosing a suitable buffer system to uphold a consistent pH environment. Next, the pH of solution is adjusted by salt (NaOH) or acid (HCl) to the acceptable level. The isoelectric precipitation extraction procedure effectively retrieves protein of excellent nutritional quality and functional qualities from challenging sources such as plant seed, poultry, fish, krill, and beef processing by-products (Sebii et al., 2021). Salcedo-Chávez et al., (2002) and Sethi et al., (2021) indicated that recent developments for extracting protein have concentrated on optimizing different factors, including temperature, pH, buffer composition, the presence of salt in the extraction medium, and duration, to improve the effectiveness and quantity of protein extraction.

2.2.1.2. Enzymatic extraction

Enzymes are spherical proteins found in individuals, organisms, plants, and animals. Enzymes are used extensively in manufacturing processes like bread, dairy products, and meat tenderization (Raveendran et al., 2018). Food-grade enzymes, including amylase, proteases, and lipases are currently easily accessible on the market (Ramos and Malcata, 2011). Cellulases and pectinases are examples of carbohydrates that break down the outer cell wall. Proteolytic enzymes, on the other hand, break down proteins inside the cytoplasm (Ravindran and Jaiswal, 2016).

The enzymatic extraction method is a noninvasive, environmentally friendly technology that uses enzymes instead of chemicals or physical conditions for extraction (Pojić et al., 2018; Rommi, 2016). Furthermore, it allows for the effective extraction of amino acids that are quickly degraded due to acid and alkali hydrolysis, such as asparagine and glutamine (Lowenson et al., 2016). Therefore, manufacturers often design the final goods for direct human consumption. Several factors influence the application of enzymatic extraction, including the specific temperature and pH, the ratios of substrate and enzyme, and the extraction duration. The type of enzyme used leads to an increase in crude protein production (Sair et al., 2013).

Any number of enzymes, whether single or multiple, can aid in the extraction of superior-quality proteins with excellent emulsification properties by breaking down cell walls using cellulases and alcalases. Consequently, the separated proteins exhibit low fluidity, strong resistance to heat, and low susceptibility to oxidative breakdown. Protein products undergo changes through enzyme extraction, leading to improved biological and functional qualities such as solubility, improved foaming activity and stability, and improved emulsification activity and stability. In conjunction with enzyme extraction, mechanical methods such as ultrasound, sonication, and microwave therapy can yield a greater quantity and superior quality of protein extract (Nadar et al., 2018).

Enzyme-based protein extraction provides a significant protein yield while causing no harm to the environment. However, it has some disadvantages, such as being time-consuming, costly to operate, challenging to scale up, resulting in uneven yields, and needing a procedure that consumes a significant amount of energy. The extraction procedure produces food items of the utmost quality that are appropriate for human consumption (Kumar et al., 2021).

2.2.1.3. Ultrasonic extraction

The application of ultrasonic extraction in food manufacturing technologies is intriguing due to its ability to optimize the separation of components from both plant and animal sources (Vilkhu et al., 2008). In 1982, researchers first used ultrasonication to enhance the extraction of proteins from soy flakes. However, it has recently garnered increased study interest over the past decade (Rahman and Lamsal, 2021). Ultrasound is a type of mechanical wave that exhibits pressure or density fluctuations at frequencies beyond human hearing, typically exceeding 18 kHz. Ultrasonic extraction can be categorized into two main types: high-intensity, which involves high power and low frequency, and low-intensity, which involves low power and high frequency. Low-intensity ultrasound is distinguished by the use of relatively small power levels, often below 1 W/cm2, and operates within a frequency range of 5-10 MHz. On the other hand, High-intensity ultrasonic extraction utilizes power levels that are notably higher, often ranging from 10 to 1000 W/cm2, and operates within a frequency range of 20-100 kHz. In ultrasonic extraction systems, a variety of factors influence the extraction of target compounds, such as proteins. These factors include the shape of the probe (stepped, linear taper, or exponential taper), its diameter, the depth at which it is immersed in the sample solution, and the position of the container holding the solution within the bath (Das et al., 2022).

2.2.1.4. Microwave extraction

Since its introduction in the late 1980s, microwave extraction has gained popularity due to its energy efficiency, time-saving capabilities, and ability to preserve the nutritional and sensory qualities of food (Kamal et al., 2021;Khan et al., 2011). Non-ionizing electromagnetic pulses with frequencies between 300 MHz and 300 GHz are used in microwave extraction (Gomez et al., 2020). The matrix sample receives these waves, which disrupt or modify the cell structure. Microwave-induced dipole rotation and ion migration break the cell wall, facilitating solvent penetration into the matrix. This results in the disruption of the cell membrane and an abrupt discharge of cellular constituents into the adjacent liquid. When microwave radiation is combined with enzymes, it accelerates the enzymatic degradation of proteins. This process expedites the degradation of cell membranes, decreases the extraction time from hours to minutes, and additionally aids in the release of minerals that are bound to the protein (Flórez et al., 2015).

2.2.2. Extraction of proteins from various plant seeds

Deng et al., (2019) showed that alkaline extraction and isoelectric precipitation could be utilized to separate a protein from Chinese quince seeds. Researchers studied the functional and physicochemical characteristics of protein isolate from Chinese quince seed (CPI). According to the WHO and FAO protein isolate from Chinese quince seed contains all necessary amino acids except for methionine, meeting the minimum requirements for adults. The examination using differential scanning calorimetry revealed that the denaturation protein isolate from Chinese quince seed was at temperature 103.4 °C. Researchers measured the protein isolate from Chinese quince seed surface hydrophobicity at 932.80. The results were shown that protein isolate from Chinese quince seed had the best emulsifying, water holding capacity, foaming stability and oil holding capacity.

In their study Yu et al., (2017) discussed the extracted proteins from two types of cultivation of Torreya grandis seeds: Shengzhou and Dazinaiyou. Protein extraction from both varieties had comparable protein levels and balanced amino acid profiles, with around 41 % essential amino acids. The seed protein fractions had molecular weights that ranged predominantly from 31 to 37 kDa and 20 to 21 kDa. Protein isolate from Shengzhou and Dazinaiyou seed exhibited comparable denaturation temperatures of at 93.7 °C, with small variations in disulfide bond and free sulfhydryl group. The protein isolated from Dazinaiyou exhibited a surface hydrophobicity of 982, which was considerably higher ($p < 0.05$) compared to the protein isolated from Shengzhou, which had a hydrophobicity of 649. Despite their low in vitro digestibility, both proteins displayed good solubility with favorable emulsifying, foaming, and oil-holding capacity characteristics. Thus, the seed proteins have the potential to be excellent sources of nutrition and functional additives in the food industry.

Gundogan and Can Karaca, (2020), extracted protein from five indigenous of

Turkish beans at pH 9.0 for extracting protein then pH 4.5 for precipitation. Protein extractes were examined for their amino acid content, physicochemical and functional properties. The results were show a little change in the composition of amino acid of the protein isolates from bean. The protein extractes had high levels of critical amino acids, including lysine, phenylalanine, and leucine, but the content of methionine was insufficient The temperature of the denaturation protein isolate varied from 90 to 152 °C, with the β-layer structure identified as the primary secondary structure. At pH 9.0, it was detected the highest solubility of over 97 %, with a net surface charge of approximately -23 mV. The bean protein isolate's emulsion capacity index with the value ranged from 402.7 to 468.5 g oil/g and emulsifying activity index ranged from 15.6 to 22 m2/g. The emulsifying and foaming properties of protein isolate with a higher solubility and net surface charge were better than those in the other studies.

Wu et al., (2021) investigated the impact of protein extraction at different pHs (7.0, 8.0, 9.0, 10.0, 11.0, 12.0, and 13.0) on tartary buckwheat. After the protein extraction, the study examined tartary buckwheat protein isolate (TBPI) and how varying extraction pH levels affected Osborne-type protein fractions, functional qualities, structural properties, and in vitro GI digestion. The findings indicated that alkaline extraction successfully boosted the yield of TBPI. As the extraction pH increased, the albumin level in TBPI reduced steadily, whereas the glutenin content increased. Extracting TBPIs at a pH over 12 dramatically reduced their solubility while significantly increasing emulsion stability, which was linked to a notable rise in surface hydrophobicity. TBPIs exhibited elevated digestion rates at pH 7 and 8. When the pH increased from 8 to 10, TBPI digestion rates decreased. TBPIs' compact structure exhibited higher amounts of α-helixes, more disulfide bonds, and lower intrinsic fluorescence intensity. As pH increased continuously, the digestion rate of TBPIs also increased. The TBPIs exhibited unfolded and denatured structures at pH levels equal to or greater than 12. This was demonstrated by the disruption of disulfide connections, reduced α-helix structure, a shift towards longer wavelengths in intrinsic fluorescence, and the separation of subunits. The pH during protein extraction has a significant impact on TPBIs.

This study examined the physical, chemical and functional characteristics of protein isolate from three various source of red lentil including the USA, Nepal, and Turkey. The red lentil protein isolate was separated using alkaline-isoelectric precipitation. The isolated protein from red lentils had a water holding capacity of 3.1 to 3.5 g/g and oil holding capacity of 5.8 to 7.3 g/g., and showed significant variations ($p < 0.05$). The red lentil protein isolate was contain vicilin and legumin, with a high glutamic acid and glutamine content with the value from 8.72 g/100 g to 10.55 g/100 g. At pH 5.2, solubility, emulsifying, the surface charge, and foaming capabilities of red lentil protein isolates were the the lowest quality. RLP's favorable functional qualities allow it to imitate several types of meat in both highly acidic and alkaline environments (Lee et al., 2021).

Das et al., (2021) found that extracting proteins at various pH levels and treating them with different pH levels changes the physical, chemical, and thermal properties of protein isolate from amaranth seed. Protein was isolated from amaranth seed at different pH values, including 9.0, 10.0, 11.0, and 12.0, namely P1, P2, P3, and P4, respectively. These four protein isolate samples underwent further treatment at pH levels ranging from 3 to 9. P1 samples exhibited increased total protein concentration and purity compared to the other samples. P1 had a particle size of 299.68 μm, which was substantially greater than the other samples with a p-value of < 0.05. The P1 sample treated at pH 9 exhibited improved emulsifying capacity and stability, solubility, water/oil binding capabilities and foaming qualities. Compared to the other three samples, the amaranth seed protein extracted at pH 9.0 exhibited superior thermal characteristics.

Stone et al., (2015) studied the protein extraction from three different cultivations of pea using three different extraction techniques, including salt extraction, alkalin-isoelectric precipitation, and micellar precipitation. Researchers evaluated the functional characteristics of protein isolate from pea, such as their ability to dissolve, emulsion stability, water/oil holding capacity, and foaming. They also examined the surface properties, such as charge and hydrophobicity. Salt extraction yielded the highest isolate, followed by alkali-isoelectric precipitation, and finally micellar precipitation. The surface charge was consistent among all samples, but hydrophobicity was highest in the protein samples extracted with alkali extraction/isoelectric precipitation. Overall, when using a specific extraction method, there were slight variations in functioning across different cultivars. The maximum protein solubility was determined by Salt-extracted method, while micellar precipitation method had the lowest. Moreover, the salt-extracted method exhibited the minimum water holding capacities, whereas, the superior oil holding capacities. The foaming qualities were influenced by the extraction process and cultivar. Generally, salt-extracted isolates exhibited superior foaming capacity, while extracted/isoelectric precipitation isolates generated more stable foams. Salt-extracted have a higher emulsion capacity compared to extracted/isoelectric precipitation isolates. All extraction methods showed a high level of emulsion stability.

2.3. Drying Techniques for Extracting Protein

2.3.1. Different drying techniques used for protein

Dehydration, or drying, is the process of extracting water from a moist substance or liquid to obtain a dry substance with a very little moisture content. Researchers have prioritized stabilizing plant proteins by decreasing water activity to a level below 0.6, as these proteins are highly vulnerable to stressors such as denaturation, aggregation, and proteolysis (Kapoor et al., 2024). Traditionally, researchers used dehydration to prolong the life of proteins, often resulting in the creation of powders. Eliminating water and reducing water activity greatly improves the stability of protein. This phenomenon occurs due to the reduction of protein mobility and the elimination of specialized breakdown mechanisms that depend on water (Vargas et al., 2022).

Proteins obtained from various seed plant and display differences in their functional attributes, such as emulsion stability, solubility, water holding capacity, foam stability, oil holding capacity. These differences result from the various protein structures and compositions. The functional characteristics of plant protein are largely responsible for its effective integration into food processing, which are highly effected by the protein extraction process and drying methods. Different drying processes applied to a protein obtained from the same source will result in proteins with distinct functional characteristics (Brishte et al., 2020).

The drying methods are essential in the process of preparing protein to obtained powder. Drying can improve a substance's storage stability, but it also causes the formation of irreversible and insoluble protein clumps due to partial denaturation of the proteins, which alters their functional capabilities (Rudra et al., 2016). The protein drying processes widely employed include oven, freeze and spray drying. The drying process by freeze drying is frequently employed to assess functionality of protein, whereas drying process by spray drying is generally applied for large quantity commercial protein manufacturing. One cost-effective method is oven drying, which allows for the regulation of the drying temperature below the point at which proteins denature. However, the drying process takes a longer time (Brishti et al., 2020)

The drying process's goal is to improve storage stability and protect the nutritional value of the protein. Various drying techniques will greatly influence the ultimate functional properties of those proteins. Vacuum, spray, and freeze drying are the most common and extensively employed methods for drying food proteins. However, during the drying process, changes in temperature and duration can alter the physicochemical characteristics of proteins, which in turn might affect the functional capabilities of the drying products (Li et al., 2022). For instance, different drying processes resulted in notable variations in the physical, chemical and functional qualities of protein isolate from quinoa (Shen et al., 2021), protein isolate from mung bean (Brishti et al., 2020), and protein isolate from hemp seed (Lin et al., 2021).

Alinejad et al. (2017) and Pratap Singh et al., (2020), reported that each drying technique has advantages and disadvantages. The most important reasons for using freeze drying techniques are that they inhibit the majority of protein degradation and reduce microbial activity, while also being a costlier and more time-consuming method of drying. Also, freeze drying can be used for materials that are heat-sensitive because the mechanism of freeze drying is ice sublimation under low temperature and pressure. Spray drying is a highly effective and widely utilized method in the food business. This is due to its ability to establish a continuous process with a large output, as well as its capacity to produce desirable powder by adjusting particle characteristics. However, this process has the potential to cause some degradation in the quality of protein. Vacuum drying is widely recognized as a straightforward and widely used method. On the other hand, it may be costly for large-scale manufacturing and may result in the degradation of certain heat-sensitive (Lin et al., 2020). According to Feyzi et al. (2018), oven drying is a cost-effective method that allows for control over the drying temperature below the point at which proteins lose their structure. However, this method requires a longer residence time.

2.3.2. Impact of drying methods on the functional and physicochemical characteristics protein isolate from plant seeds

Shen et al., (2021) isolated protein from quinoa and dried it into powder by various drying techniques, including vacuum, freeze, and spray drying. The primary aim of this research was to investigate how various drying methods impact the functional and physicochemical characteristics of quinoa proteins. This includes things like morphology, secondary structure, SDS-PAGE, amino acid, surface hydrophobicity, thermal stability, and sulfhydryl/disulfide content. The freeze drying showed superior emulsification capacity, stability, and oil-holding capability due to increase the surface hydrophobicity. In contrast, the spray drying demonstrated the maximum water holding capacity and solubility at pH 7.0. The freeze dried proteins had the maximum temperature of denaturation, but the minimum enthalpy due to its increased random coil content and decreased regular β-sheet and α-helix. Protein isolate from quinoa was dried into powder through various drying techniques and exhibited various functional characteristics between drying methods.

Researchers extracted protein from Antarctic krill using alkaline-isoelectric precipitation technique, followed by phosphorylation with sodium tripolyphosphate. The study examined the impact of freeze, spray, and hot air drying on the structural and functional characteristics of phosphorylation protein isolated from Antarctic krill. The phosphorylation protein isolate from Antarctic krill dehydrated using spray drying and exhibited superior sensory characteristics and had a much smaller particle size compared to dried protein by hot air and freeze drying. The scanning electron microscope (SEM) revealed a consistent particle size and even distribution of particles in the spray dried powder. X-ray diffraction examination indicated that the spray drying sample had more crystallinity compared to the freeze and hot air drying sample. A differential scanning calorimeter test showed that the spray-dried sample was the most thermally stable and had the least amount of protein denaturation ($\Delta H = 210.80$ J/g). This was followed by the freeze-dried sample ($\Delta H = 80.48$ J/g) and the air-dried sample ($\Delta H = 73.94$ J/g; $P < 0.05$). The FTIR analysis indicated that the spray dried sample had a higher concentration of protein secondary structure. Compared to the standard deviation, the phosphorylated group in freeze and air dried showed partial disruption of chemical bonds. The functional properties, including oil holding capacity, solubility, emulsifying, and foaming activities, of the spray dried were significantly higher than those of the freeze and air dried ($P < 0.05$). Freeze dried samples had the largest water holding capacity. However, samples made with air drying had the poorest functional performance. As a result, various drying techniques used to prepare P-AKP can have an impact on its physical and chemical properties, and spray drying may be a suitable technique and can used for a large scale production with improved functions (Lin et al., 2020).

Brishti et al., (2020) investigated that mung beans are a cost-effective and eco-friendly protein source. This study examined the impact of drying methods namely oven drying, spray drying, and freeze drying on the mung bean protein isolate. All samples exhibited intact protein subunits and demonstrated thermal stability. Based on morphological research, freeze drying produced a porous protein, while spray drying and oven drying produced wrinkled and compact crystals, respectively. Freeze drying demonstrated remarkable protein solubility and water/oil holding capacity. Spray drying showed the lowest particle size, a high emulsion activity and stability index with the value of 29.21 m2/g and 351.90 min, respectively, and the highest quantity of β-sheet at 37.61 %. The FTIR spectra of all samples displayed distinct peaks that aligned well with the secondary structure of legume proteins. The study discussed how different drying procedures can customize the varied final properties of mung bean protein isolate for various food systems. Researchers recommend freeze-drying for meat extenders, consider spray-drying suitable for meat emulsions, and find oven-drying effective for protein-based applications.

Timilsena et al., (2016a) studied on the extraction protein from Australian chia seed. The extracted protein is dried into powder using three different methods: spray, freeze, and vacuum drying. Following that, this study investigated how drying methods affect the physical, chemical, and functional properties of isolated protein derived from chia seeds. The proximate composition showed no significant difference. Vacuum dried chia seed protein isolate had the maximum oil holding capacity and bulk density, while spray dried chia seed protein isolate showed the superior water holding capacity and solubility, and minimum surface hydrophobicity. All drying methods increased protein solubility with higher temperatures and alkaline pH. The foaming capability and stability of chia seed protein isolate increased as the pH and protein concentration increased. Spray dried chia seed protein isolate exhibited the lowest level of denaturation, while vacuum drying chia seed protein isolate showed the highest level of denaturation, indicating the worse solubility and foaming characteristics of the latter. The results will help in choosing a drying method to produce chia seed protein with improved functionality.

Lin et al., (2021) studied on the extraction protein from by-products derived from hempseed. The goal was to analyze and assess the impact of drying methods on hempseed protein isolate. Oven, vacuum and freeze drying were used to dry hempseed protein isolates into powder. The hempseed protein isolate had a protein content above 70 % and contained essential amino acids at levels equal to or greater than the recommended values for adult humans. Osborne fractionation showed that glutelin was the predominant portion in hempseed proteins. Freeze dried Hempseed protein has a lower surface hydrophobicity and higher in vitro protein digestibility compared to oven drying and vacuum oven drying. Freeze dried Hempseed protein exhibited superior functional characteristics compared to those of dried in oven and vacuum oven. This study presents the initial findings on the characterization of hempseed protein, a by-product of hempseed processing. Hempseed protein has the potential to be a new dietary protein element that could lead to a greater use of hempseed.

Joshi et al., (2011) in their study, focused on the drying protein into powder by spray, freeze and vacuum drying methods. Protein isolate from lentils through alkaline extraction at pH 8 and isoelectric precipitation at pH 4.5. Researchers noted variations in color, protein component composition, particle size distribution, and surface morphology among the three drying techniques. Spray and freeze dried lentil protein isolate powders had greater solubility with the value 81 % and 78 %, respectively, in comparison to vacuum dried, which had a solubility rate of 50 %. The spray dried samples exhibited a lower water holding capacity with the value 0.43 g/g, in comparison to freeze dried with the value 0.48 g/g and vacuum dried with the value 0.47 g/g lentil protein isolate powders. Overall, this study noted that the drying procedures used to prepare lentil protein isolate can impact their physicochemical and functional properties

2.4. Hydrogel

2.4.1. Hydrogel and application in the food industry

In the field of food sciences, the word "hydrogel" refers to structures made up of cross-linked polymer networks. A polymer network may serve as an effective delivery method for a variety of active chemicals. Natural and/or synthetic polymers form hydrogels, intricate three-dimensional structures capable of absorbing significant quantities of water (Corkovic et al., 2021).

The term hydrogel was first used in the literature in 1894. Hydrogel advances throughout history may be categorized into three primary phases. The first phase involved creating a material with significant expansion and favorable mechanical characteristics while maintaining a simple composition. Subsequently, in the 1970s, a new hypothesis emerged: hydrogels demonstrated the ability to react to variations in environmental factors such as concentration, pH, or temperature by triggering the substance's polymerization process. The last phase of hydrogel advancements focused on exploring and creating intricate materials. The advancement in this field has sparked a growing fascination with the creation of "smart hydrogels," which are polymeric structures capable of effectively retaining, releasing, and loading fluids with diverse characteristics (Chirani et al., 2015).

According to Yang et al. (2021), consumers and academics increasingly prefer gels because of their high nutritional value, enjoyable flavor, ability to enhance satiety, low calorie content, and safe biological features. Yemenicioğlu et al. (2020) indicated that hydrogels are becoming more often used in the food industry to enhance the nutritional value of meals and provide health advantages. Many food products use them to enhance their nutritional content, safety, functional characteristics, and stability. Moreover, hydrogels derived from edible natural polymers have considerable promise in several food-related uses, including nutrition delivery systems for nutrition, satiety, freshness detection, food packaging, and safety monitoring in food products. Technology can help retain food contents during packaging and processing, which are essential for maintaining health and sustaining life. Additionally, delivery mechanisms safeguard these nutrients in various settings until they reach the specific organs in the body for their intended purpose (Galland 2013).

Hydrogels have extensive use throughout several industries, including agriculture, cosmetics, medicine, textiles, and, more recently, the food industry. Researchers have been exploring hydrogels for years because of their wide range of potential applications. The pharmaceutical and biomedical sectors mostly use hydrogels to deliver substances, support cell growth, and advance tissue engineering (Hila, Florowska and Wroniak, 2023). Nevertheless, the food industry has limitations when using hydrogels due to regulations that restrict the use of certain materials that must meet food-grade standards. Based on their source, food-grade biopolymers are categorized into polysaccharides and proteins. These biopolymers provide significant potential to effectively tackle current problems about consumer health and environmental sustainability. They can be renewable, cost-effective, compatible with living organisms, and capable of breaking down naturally. Additionally, they offer a diverse array of capabilities and methods for forming hydrogels (Cao et al., 2020).

Several food items are either in the form of hydrogels or play a crucial role as hydrogels in the food business (Yang et al., 2021). Food hydrogels may be a source of biocompatible, renewable, biodegradable, inexpensive, and edible biopolymers. These biopolymers have a wide range of nutritional activities, and their qualities are becoming more and more equivalent to, and sometimes even better than, natural foods (Cao and Mezzenga 2020). Hydrogels made from edible ingredients are crucial in a range of food-related uses, such as substituting fat, delivering nutrients, yoghurts, dairy hydrogels, packaging food, and creating customised foods (Krop et al. 2019; Devezeaux de Lavergne, van de Velde, and Stieger 2017; Capuano 2017).

Depending on their intended use, hydrogels can transform into various forms, such as emulsions, coatings, nanoparticles, films, and more. These suitable designs have the potential to optimize the nutritional value and quality of food.

2.4.2. Natural polymers utilized for the production of hydrogels in the food industry

Functionally uniform and too delicate to meet customer and market demands. In this context, hydrogels with many components become relevant. Proteins and polysaccharides, the most common binary food hydrogels, form food hydrogels. The interactions between these polymers determine the structure of the hydrogels in these mixes (Yang et al., 2021). The synthesis process divides the significant techniques for preparing food hydrogels into biological processes cross-linking, chemical cross-linking, and physical cross-linking.

Hydrogel production using proteins and polysaccharides is highly desirable because it has many benefits, including biocompatibility and human safety. Proteins are essential in the human diet due to their composition of bioactive peptide amino acids, which can also serve as a source of additional nutrients. Additionally, they possess functional groups that are capable of interacting with other molecules (Xie, Zhu and Liu 2017).

The unfolding of protein molecules, which expose hydrophilic and thiol groups, creates protein-based hydrogels. Factors such as pH change, or salt concentration adjustments can trigger the denaturation process, which involves the unfolding of the structure. Denaturation makes it easier for chains to connect via covalent contacts, such as hydrogen bond formation, electrostatic or hydrophobic interactions, and sometimes disulfide bond formation. The polymer chains aggregate and create a 3-D hydrogel structure due to the covalent contact between molecules (Klein et al., 2020; Zhu et al., 2021). Among the widely used plant proteins based on hydrogel are pea (Moreni et al., 2020), zein (Gagliardi et al., 2020), soya (Liu et al 2018), and wheat (Wang et al., 2019; Zhang et al., 2022).

The process of creating polysaccharides based on hydrogels is simpler than that of creating proteins based on hydrogels. Several techniques, including but not limited to pH change, temperature, salt concentration, as well as freeze-thaw cycles, and sucrose, can trigger the production of hydrogel-based polysaccharides (Manzoor et al., 2022). Commonly used polysaccharides with gelling properties in the food sector include gum Arabic (Makar et al., 2022), starch (Zeng et al., 2021), chitosan (Ma et al., 2022), gellan gum (Das and Giri 2020), carrageenan (Berton et al., 2020), alginate (Florowska et al., 2022), cellulose (Hu et al., 2021), and inulin (Michalska-Ciechanowska et al., 2020).

2.4.3. Classification of formation hydrogel

Hydrogels are classified according to their ionic charge, configuration, crosslinking, source, composition, and environmental stimulation. Polymer chains are cross-linked to generate hydrogels (Zhang et al., 2021; Li et al., 2015). Three different types of polymers can yield hydrogels: natural, synthetic, and semi-synthetic. Hydrogels are classified into three groups, including natural, synthetic, or semi-polymer, based on the source of the polymer. Natural hydrogels formed from natural sources include a variety of substances such as collagen, gelatin, chitosan, agarose, cellulose, fibrin, alginate, and hyaluronic acid (Benwood et al., 2021). Their mechanical strength and stability are very modest, but they possess intrinsic bioactivity, biocompatibility, and biodegradability. While most people may safely use natural hydrogels, there are a few rare instances when certain components in them might cause allergies (Wan et al., 2021; Eshraghi et al., 2021). Therefore, administering natural hydrogels to immunocompromised patients may raise immunological concerns. Synthetic hydrogels are formed using artificial polymers. Polymerization of a monomer, such as polyethylene glycol, polyacrylic acid, polyethylene oxide, polyacrylamide, polyvinyl alcohol, poly-N-isopropyl acrylamide, and poly-N-isopropyl acrylamide, creates these synthetic polymers (Zhang et al., 2021; Bashir et al., 2020). While certain materials, like polyacrylamide, are biocompatible, they also possess stability and mechanical strength. Chemically altered from their original form, semi-synthetic polymers are a blend of natural and synthetic polymers. They are used as materials to create semi-hydrogels. Chemically modified polymers from natural sources include acrylate-modified hyaluronic acid and methacryloyl-modified gelatin (Dienes et al., 2021). Furthermore, it may consist of both natural and manufactured polymers, such as albumin and gelatin or PEG-conjugated fibrinogen (Berkovitch and Seliktar, 2017). These hydrogels exhibit the bioactivity characteristics of natural hydrogen and allow for numerous adjustments through their diverse chemical parameters (Park and Park, 2016).

2.4.4. Various methodologies for crosslinking in the fabrication of hydrogels

Various methodologies are available for the fabrication of hydrogels. A distinct mechanism and set of circumstances distinguish each approach. The physical, chemical and biological characteristics of polymers are a crucial component in determining the suitability of particular procedures for creating hydrogels.

Hydrogels that are cross-linked typically exhibit a variety of cross-linking types. Multiple cross-linked networks, which combine biological, chemical, and physical cross-linking methods, make up the majority of modern food hydrogel networks. Each type of cross-linking contributes its own unique characteristics and improves different mechanisms for hydrogel food creation.

2.4.4.1. Crosslinking via physical

The majority of food hydrogels belong to the physical hydrogels category, where non-covalent bonds primarily drive the gelation process. Under certain conditions, intra- or intermolecular forces, such as electrostatic complication, hydrogen bonding, intermolecular entanglement, or ionic contact, create food hydrogels through a process known as "physical crosslinking". Physical activation of these cross-links creates a 3-D network structure between the molecules, eliminating the need for cross-linking chemicals or external pressures (Khalesi et al., 2021). This hydrogel has a delicate network structure because there is no cross-linking agent used in the gelling process. Instead, the food-based polymers only connect with each other at the molecular and intermolecular levels. This allows for a smooth transition from a liquid to a hydrogel state. Food hydrogels, especially those derived from gelatin and polysaccharides, stand out for their reversible nature (Yang et al., 2021). Cross-linking via physical can be divided into three groups:

Crosslinking via hydrogen bonds: Cross-linking with hydrogen bonds is used to make food hydrogels. The joining of hydrogen and oxygen atoms in the chemical groups of polymers creates hydrogels. Hydrogels for food preservation use a variety of polysaccharides, including picten, black sodium alginate, pullulan, and others (Yan et al., 2020; Fan et al., 2021). Bueno et al. (2021) and Rajesha Shetty and Lakshmeesha Rao (2022) reported that cross-linking with hydrogen bonds in polyvinyl alcohol for food packaging made the film work better.

Crosslinking via Ionic: The process involves matching the polymer molecules of cations and anions, or between metal cations and the polymers' anions. Usually, the process may start at ambient temperature, and the sodium alginate, which has a carboxyl group, can be ionically cross-linked with the calcium ions. It is a widely used technique due to its easy and safe preparation, as well as physiological pH (Li et al., 2021).

Crosslinking via hydrophobic association: According to Madaghiele et al. (2021), hydrophobic associations form hydrogels by clustering hydrophobic groups on the molecular chains of hydrophilic compounds due to hydrophobic interactions. This leads to the creation of food hydrogels through the bonding of macromolecular chains, either inside or between molecules. A hydrophobic interaction creates an example of a satiating hydrogel using carboxymethyl cellulose, unsubstituted block.

2.4.4.2. Crosslinking via chemical substance

According to Khalesi et al., (2021); Zha, Rao, and Chen, (2021), the formation of chemically cross-linked hydrogel foods requires the use of disulfide bonds between molecules, food-grade cross-linking chemicals, or Maillard reactions. In food hydrogels, the cross-linking method creates more stable molecular structures than physical cross-linking. Additionally, this approach allows for the fabrication of hydrogel meals with varying mechanical strengths and cross-linking densities. Also, because the cross-linking agent is safe for food, these chemically cross-linked gels don't need to have the reactants removed after they have formed. Chemical crosslinking can be classified into four groups:

Crosslinking via reactions: Polymers that are soluble in water can form hydrogels by inducing cross-linking processes between the molecules and using various chemical cross-linking agents. For instance, researchers Madaghiele et al., (2021) and Zhang, Meng, et al., (2021) found that citric acid can crosslink sodium, while carbodiimide can crosslink compounds with amide groups. However, during the preparation phase, this hydrogel must be cross-linked with either a food-grade or natural cross-linking agent.

Group reactions: Group reactions are used to synthesize hydrogels based on the covalent interactions among distinct functional groups of various polymers. For example, Schiff base reactions may be used to generate molecules containing amino groups into food hydrogels, and amide reactions between amines and carboxylic acids or their derivatives can produce food hydrogels (Li, Xu, et al., 2021; Thongsrikhem et al., 2022; Liu, Wang, et al., 2022).

Initiators polymerization reaction: One method for creating hydrogels is to use initiators to facilitate monomer polymerization. Free radical initiators are a group of substances that break down easily into free radicals, like primary radicals, when heated. They can also start the polymerization of alkene and diene monomers. These cross-linking processes often prevent the formation of food hydrogels due to concerns about initiator biosafety (Rezaeisadat, Bordbar, and Omidyan, 2021; Qin et al., 2021).

Crosslinking via radiation: High-energy radiation is mostly used in radiation crosslinking to make covalent or chemical connections between the chains of macromolecular. This creates a network of links between the chains. This approach typically operates at inert gas protection, pH that is physiological, and ambient temperature, eliminating the need for cross-linking agents (Sivaselvam et al., 2021). Assemand, Lacroix, Mateescu (2004) and Sommer et al. (2010) say that γ-irradiation may speed up the cross-linking reactions between nearby protein molecules by turning tyrosine and phenylalanine residues into free radicals. However, the radiation cross-linking process can create free radicals that could damage the food hydrogel's bioactive compounds.

2.4.4.3. Crosslinking via biological

The researchers Robert, Chenthamara, and Subramaniam (2022) reported that biological crosslinking involves the enzymatic catalysis of natural substances, resulting in the development of crosslinks. This process may occur between the molecules of protein and sugars and the molecules of protein, leading to the formation of a hydrogel. Enzymatic crosslinking is a gentle and precise method of crosslinking, making it very intriguing. The most commonly used enzymes for the production of food hydrogels are tyrosinase, horseradish peroxidase, transglutaminase, and laccase. Researchers favour these enzymes for their specificity, exceptional effectiveness, and ability to function under gentle reaction circumstances (Zhang, Fan, et al., 2015; Du et al., 2021; Chen et al., 2019). The enzymatic reaction occurs in conditions of moderate temperature, reasonable pH, and water presence. This eliminates the impact of initiators and crosslinkers, ensuring the biological functionality of food hydrogels.

2.4.5. Recent studies on the fabricated hydrogel

In their study He et al., (2021a) looked at how different amounts of pectin and degrees of esterification (DE) affect the formation of hydrogel from ginkgo seed protein isolate (GSPI) and pectin mixed together. The fabricated hydrogels were made by heating a mixture with 12 % GSPI and different amounts of pectin such as 0, 0.1, 0.5, and 1.0 % w/v) with DE values of 38 %, 60 %, and 76 % at 90 °C and pH 6.0 for 30 minutes. The addition of pectin resulted in an increase in the apparent thickness and electrical charge of the sols while simultaneously decreasing the size of the particles. Adding 0.1 % and 0.5 % pectin improved the rheological and textural properties of the hydrogels, while the addition of 1.0 % pectin resulted in a deterioration of these properties, leading to reduced water retention capacity. Adding pectin causes alterations in the gel microstructure. Hydrogen bonding was critical to the hydrogel's formation. Incorporating low-methoxyl pectin (DE = 38 %) with a high charge density improved the hydrogel characteristics. Managing the concentrations of pectin and DE can help to adjust the physical and textural characteristics of the fabricated hydrogel containing GSPI and pectin.

Researchers did an experiment to see what happened when different amounts of Lycium barbarum polysaccharides (LBP) were added to whey protein hydrogels that were made at different pH levels (3.0, 5.2, and 7.0). The LBP concentrations used were 0, 0.1, 0.5, and 1 % w/v. After forming the hydrogel, we evaluated its texture, flow properties, hydration characteristics, and microscopic structure. The study found that the composite hydrogel with 0.1 % (w/v) LBP had the highest functional value. However, when higher concentrations of LBP (1 % w/v) were used, hydrogels with more holes were made, which was confirmed by SEM. Additionally, whey protein created white particle hydrogels and transparent fibrillar hydrogels at pH levels close to and distant from the isoelectric point. Regarding hydration characteristics, there was no variation in water retention capacity and swelling ratio across all pH levels. This study replicated real-world manufacturing settings to establish a theoretical foundation for utilizing LBP in composite gels and creating customized hydrogel-type food products to cater to various consumer tastes (He et al., 2021b).

Demirkıran et al., (2022) said that their study looked into whether adding microbial transglutaminase (MTG) to sour cherry seed protein (SCSP) could make it better at its technological functions of hydrogel properties. In hydrogel models, MTG-SCSP was integrated. Three distinct contents were used to create the hydrogels: SCSP alone, a mixture of SCSP and pectin, and a mixture of microbial transglutaminase and pectin as control groups. Researchers used SEM pictures to analyze the microstructure of hydrogels. When MTG-SCSP and pectin were present together, they had a big effect on how well hydrogels worked, affecting things like their ability to hold water (WHC), their ability to swell, and their volume gel index

(VGI). The textural and rheological characteristics showed similar results.

Amino acids including histidine, lysine, and arginine were studied for their impact on the hydrogel characteristics of whey protein isolate hydrogels heated at different pH levels (2.0, 5.2, 7.59, 9.74, and 10.76). Simple amino acids alter whey protein isolate hydrogels based on pH and the specific amino acids present. Arginine improved the hardness and gumminess of the WPI gel at pH 7.59, while histidine boosted springiness at pHs 7.59 and 9.74, and lysine did so at pH 7.59 ($P < 0.05$). Whey protein isolate created a weak hydrogel at pH 2.0. At pH 2.0, lysine and arginine helped β-lactoglobulin cross-linking and stopped protein from leaking out of the hydrogel ($P < 0.05$). At a pH of 5.2, whey protein isolate created a particulate hydrogel with low water holding capacity (WHC). By changing the structure of the hydrogel network, lysine made the whey protein isolate gel better at holding water at pH 5.2. Basic amino acid added at pH levels other than 5.2 improved the hydrogel structure's consistency and permeability, as well as significantly enhanced its water retention ability ($P < 0.05$). Depending on the pH and desired characteristics of the product, researchers can use various basic amino acids as enhancers for whey protein isolate hydrogels (Wang et al., 2020c).

Başyiğit et al., (2024), in their study, aimed to produce pea protein-based natural hydrogels without a poly/cross linker via cryogenic application. The process consisted of three steps: cryogenic treatment was applied to the pea protein isolate before hydrogelation (PHB), then after hydrogel formation (PHA), and finally to both the pea protein and the formed hydrogel (PHD). Hydrogels with non-cryogenic treated pea protein alone as a negative control (NC) and a mixture of untreated protein and locust bean gum as a positive control (PC). In comparison to NC, the FTIR spectra showed losses and shortenings in bonds. SEM images showed more porous structures. The cryogenic process was resulted in a higher water-holding capacity and swelling ratio. The process also improved protein leachability. PHB and PHD hydrogels demonstrated elasticity, but elasticity levels varied. The study indicates that cryogenic applications, which are sustainable and environmentally friendly technologies, have great promise in this particular area.

3. MATERIALS AND METHODS

3.1. Material

Avocado seeds were sourced from local businesses in Mersin province, Turkey. Locust bean gum (CAS number: 9000-40-2) was purchased from Öz Yildiz, Chemical Industry Trade Ltd., Co., based in Istanbul, Turkey. Chemicals including sodium chloride (NaCl) (CAS number: 7647-14-5), sodium hydroxide (NaOH) (CAS number: 1310-73-2), hydrochloric acid (HCl) (37 % w/w, CAS number: 7647-01-0), sodium hydrogen phosphate (Na2HPO4) (CAS number: 7558-79-4), sodium dihydrogen phosphate (NaH2PO4) (CAS number: 7558-80-7), and hexane (CAS number: 110-54-3) were purchased from Sigma (St. Louis, MO, USA). All solvents and chemicals used were of high quality and met analytical testing standards.Avocado seeds were sourced from local businesses in Mersin province, Turkey. Locust bean gum (CAS number: 9000-40-2) was purchased from Öz Yildiz, Chemical Industry Trade Ltd., Co., based in Istanbul, Turkey. Chemicals including sodium chloride (NaCl) (CAS number: 7647-14-5), sodium hydroxide (NaOH) (CAS number: 1310-73-2), hydrochloric acid (HCl) (37 % w/w, CAS number: 7647-01-0), sodium hydrogen phosphate (Na2HPO4) (CAS number: 7558-79-4), sodium dihydrogen phosphate (NaH2PO4) (CAS number: 7558-80-7), and hexane (CAS number: 110-54-3) were purchased from Sigma (St. Louis, MO, USA). All solvents and chemicals used were of high quality and met analytical testing standards.

3.2. Methods

This thesis consists of five main stages. Figure 3.1. displays each individual stage. The initial phase of the study involved analyzing the amount of moisture, ash, fat, protein, and carbohydrate of avocado seed. Then, Soxhlet extraction was used to remove oil from the avocado seeds during the defatting process. After that, alkali-isoelectric precipitation method was used for extraction protein from defatted avocado seed powder and then dried into powder by using various drying techniques, namely spray, freeze, vacuum, oven, and ambient drying. Next, hydrogels were prepared from five different dried portions with locust bean gum. Finally, analysis was carried out for all the different dried protein extractions and hydrogels.

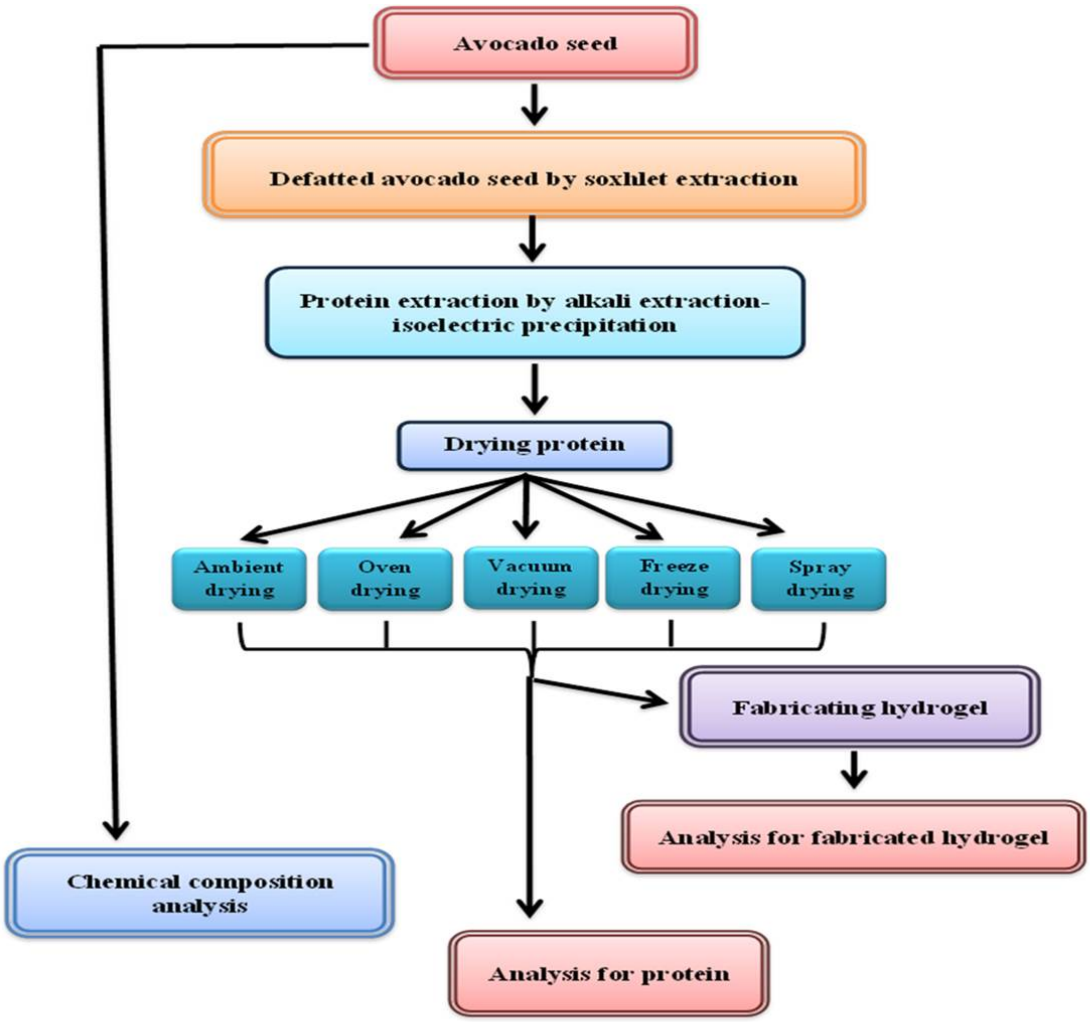

Figure 3.1. The flow chart of the operations carried out within the scope of the study

3.2.1. Defatting process of avocado seed

The technique of removing fat from avocado seed were carried out based on earlier investigations (Gan et al., 2020; Timilsena et al., 2016a) with some adjustments. First of all, a DMS 253 laboratory grinder (Demsan, Turkey) was used to crush the avocado seeds to make powder. Following that, avocado seed powders were dried for 24±2 h at 45 °C by using oven Nüve EN120 (Nüve, Turkey). The soxhlet extraction method was employed to remove the fat from avocado seed powders. For this purpose, 20 g of powder were added to 200 mL of hexane and then placed in the chamber of the extractor. To reach the solvent's boiling point, the temperature was raised to 65 °C, and the extraction process was maintained under reflux conditions for 2 h. Once the procedure was complete, hexane was removed by evaporation at a temperature of 110 °C. Then, the seed powders that had been defatted were gathered and placed oven at 50 °C for 60 min to ensure the complete removal of hexane. Finally, the defatted avocado seed powders were placed in a polyethylene bag with the dimensions (width × high × thickness (20 cm × 30 cm × 0.05 mm)). The sample was storage at 4 °C in the refrigerator to make analysis easier in the future.

3.2.2. Protein extraction by alkali extraction and isoelectric precipitation

The alkali extraction and isoelectric precipitation method was used to extract proteins from defatted avocado seed powders, following the methodology outlined in a prior study (Dia et al., 2019) with slide modifications. The process of protein extraction and drying into powder were illustrated in figure 3.2. Two hundred mL of 0.12 M NaOH was added to the 20 g of defatted avocado seed powder. The extraction process of protein was started by shaking the mixture at 250 rpm for 1 h at ambient temperature. At the end of extraction process, the mixture was centrifuged for 15 min at 4000 rpm under 4 °C by Nüve NF 615 (Ankara, Turkey) to separate the precipitation from supernatant. After discarding the precipitation, the supernatants were gathered and the pH adjusted by a Hanna Instruments (HI2202–02 pH meter, RI, USA) to 4.5 using 1 M HCl. Then, keep the supernatant in the refrigerator at 4 °C overnight. Finally, the precipitation of protein was centrifuged at 4000 rpm for 25 min at 4 °C, and the supernatant was removed. At the end of extraction protein process, the precipitated protein was dried into powder by using different drying techniques.

3.2.3. Drying of extracted protein

3.2.3.1. Ambient drying (AD)

After transferring the protein precipitate into a plastic plate with a depth of approximately 5 mm, the precipitated protein was left for 5 days at room temperature (~ 25 °C) in order to achieve protein drying.

3.2.3.2. Oven drying (OD)

After transferring the protein precipitate into a plastic plate with a thickness of around 5 mm, and then kept the sample into hot air oven for drying by using cabinet dryer EN120 (Nüve, Turkey) after adjusted temperature of 50 °C for 48 h (Lin et al., 2021).

3.2.3.3. Vacuum drying (VD)

After pouring protein precipitate into a plastic plate, it was placed in the vacuum drying oven (BINDER, New York, USA), and then adjusted the temperature and pressure to 50 °C under 85 Kpa (Feyzi et al., 2018).

3.2.3.4. Freeze drying (FD)

Initially, the protein precipitate was froze under -18 °C for duration 18 h. Subsequently, it was transferred to a freeze dryer (Coolormed, Turkey) and exposed to a compressor temperature of -55 °C for a period of 1 day while maintaining a vacuum pressure of 20 Pa (Agarwal et al., 2023).

3.2.3.5. Spray drying (SD)

The protein precipitate was mixed with deionized water in a ratio of 1:2 v/v. the mixture was mixed for 2 min at 10000 rpm. The diluted solutions were subsequently fed into a spray dryer utilizing the Unopex B15 model (Izmir, Turkey), with adjustments made to set the inlet temperature at 140 °C and the feeding rate at 6 mL/min. (Haque et al., 2015).

After drying avocado seed protein isolate into powder, all dried proteins were kept in the plastic bag with the dimension (width × high × thickness (20 cm × 30 cm × 0.05 mm)). To facilitate future analyses, stored the protein samples in the refrigerator at 4 °C.

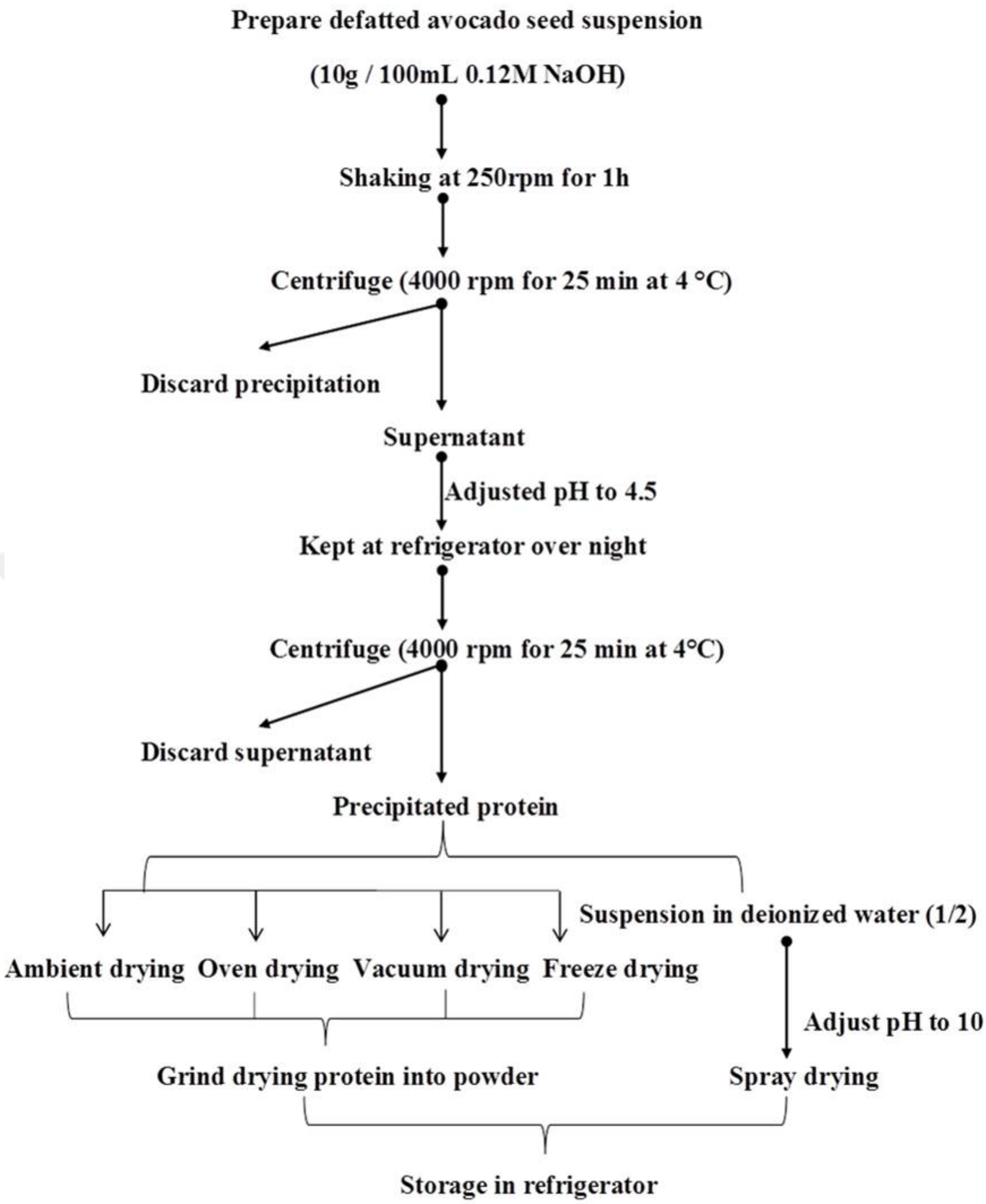

Figure 3.2. The production flow chart for extracting protein from avocado seeds and drying them into powder.

3.2.4. Hydrogel preparation

The hydrogel was synthesized using protein and locust bean gum, following the methodology outlined in the earlier study conducted by (He et al., 2021b), with slight adjustments. First of all, prepare two solutions: the first one was protein solution (24 % w/v) and the second one was locust bean solution (2 % w/v) by using distilled water and shaking them for 5 hours at 250 rpm at (~25 °C). Then, the solutions were maintained at 4 °C in the refrigerator overnight to provide completely hydration. Following that, protein and locust bean solutions were mixed together at concentration (1:1, v/v). The mixture was carried out in a shaker for 2 h at 150 rpm after adjusted the pH of final solution to 7.0. For preparing hydrogel heat-induced techniques was used, The mixture was immersed in a water bath (Nüve, Ankara, Turkey) and heated at 90°C for 50 min. Lastly, the hydrogel samples were allowed to cool to ambient temperature (~25 °C) and stored in the refrigerator for future analysis.

3.3. Analysis of Avocado Seed, Protein Isolated and Fabricated Hydrogel

The analysis was carried out for avocado seed, avocado seed protein isolate, and hydrogels prepared from different dried avocado seed protein isolate, as shown in the Table 3.1.

Table 3.1. Specific analyses conducted within the study's scope

Materials	Analysis
Avocado seed	• Total moisture content • Total mineral matter content • Total fat content • Total protein content • Total carbohydrate content
Avocado seed protein isolate	• Color analysis • Water activity (a_w) • Carr index and Hausner ratio • Amino acid analysis • Fourier Transforms infrared (FTIR) spectroscopy • Scanning electronic microscope (SEM) • Thermal behavior (TGA) • Solubility • Water holding capacity • Oil holding capacity • Emulsion activity and stability • Foaming capacity and stability
Hydrogel	• Color • Water holding capacity • Swelling ratio • Hydrogel leachability • Fourier Transforms infrared (FTIR) spectroscopy • Scanning electronic microscope (SEM) • Texture profile analysis • Rheological analysis • Release analysis

3.3.1. Chemical composition of avocado seed and peotein

3.3.1.1. Total moisture content

To measure the total amount of moisture content in the avocado seed, initially, the avocado seeds were ground to obtain a powder. A petri dish was used as a drying container. In order to obtain more accurate results, the petri dish was brought to a constant weight by placing it in oven for 3h at 105 °C. After cooling the petri dish in a desiccator, the containers were ready for the next step. One gram of powder sample was weighed into cooled petri dishes and placed in a hot air oven after the temperature was adjusted to 105 °C. After 24 h, the petri dishes containing the samples were cooled in the desiccator. The following formula was used to determine the moisture content

Total moisture content (%) = $(x - y) / w \times 100$

x = Weight of sample (g) + weight of petri dish (g)

y = Dehydrated sample (g) + weight of petri dish (g)

w = Weighted sample (g)

3.3.1.2. Total mineral matter content

To measure the amount of mineral matter in powder sample, the porcelain crucibles were initially brought to a constant weight by placing them in oven for 3 h at 105 °C. The porcelain crucibles were left in a desiccator until cooled. Tow grams of sample into the crucibles and then place in furnace for 2 h at 550 °C. After cooling the crucibles containing the sample in the desiccator, the total amount of mineral matter was determined by using the following equation and expressing the result as a percentage.

Total mineral matter content (%) = $(x - y) / w \times 100$

x = Final weighing (remaining sample amount by mass + crucible weight (g)

y = Crucible weight (g)

w = Mass weight of the weighed sample (g)

3.3.1.3. Total fat content

Ten grams of the sample were placed in a flat-bottomed flask, followed by the addition of 200 mL of hexane. The extraction process for fat was carried out for 8 hours at room temperature. Following that, a flat-bottomed flask was connected to the relevant part of the rotary evaporator to evaporate hexane. To remove the smallest amount of hexane, a flat-bottomed flask was kept in oven at 105 °C (AOAC, 1998). The following formula was used to get the total quantity of fat.

Total fat content = (x – y) / w × 100

x = Final weighing (weight of remaining oil+weight of flat bottomed flask(g))

y = Weigh of flat bottomed flask (g)

w = Initial weigh of sample (g)

3.3.1.4. Total protein content

The protein content of sample was measured by using an elemental analyzer (FlashSmart Elemental, ThermoFisher Scientific, USA). The protein content of the samples was calculated by multiplying the nitrogen value by 6.25. The device settings were adjusted manually based on Table 3.2 for protein analysis.

Table 3.2. Elemental analyzer operating conditions

Parameters	Devise condition
Temperature of reaction CHNS	950 °C
Temperature of GC/MS	65 °C
Flow of helium	145 ml/min
Flow references of helium	105 ml/min
Flow of oxygen	240 ml/min
Time of injection oxygen	5 sec
Delay in the sample	12 sec
Total working time	600 less than sec

3.3.1.5. Total carbohydrate content

Total carbohydrate content was calculated by subtracting the sum of protein, fat, moisture, ash, and fiber percentages from 100. The equation used is as follows

Total carbohydrate (%) = 100 – (total Ash+ total protein + total fat + total moisture)

3.3.2. Protein Analysis

3.3.2.1. Color

The protein samples' color was assessed by Hunter Lab device (Color Quest® XE, USA) based on the parameters L*, a*, and b*. The equipment was initially calibrated using three parameters: L* = 96.90, a* = 1.21, and b* = 1.93. L* was associated with lightness, and the value was recorded from black to white (0 to 100). The value of a* corresponds to green to red, and the value was recorded from (-100 to +100), while the value of b* corresponds to blue to yellow, and the value was recorded from (-100 to +100) (Duangmal et al., 2008).

3.3.2.2. Water activity (aw)

Water activity (aw) values of sample were determined using the Pre Aqua LAB (METER Group, Inc., USA). Approximately 3 g of sample were added to the device's sample container and allowed to sit at ambient temperature until the equilibrium moisture value was reached. The aw values that reached equilibrium were read from the digital display and recorded (Tatar et al., 2014).

3.3.2.3. Hausner ratio and carr index

Hausner ratio and carr index value of sample were measured by tapped density and bulk density. Five grams of the sample were added to a 25 mL cylinder, and the volume was noted. Bulk density was found by dividing the sample's weight by its volume. For tapped density determination, the graduated cylinder containing the sample was placed on a flat surface and tapped 200 times. After tapping, the volume of the sample was measured. To calculate the tap density, divide the weight of the sample by the volume measured after tapping 200 times (Turchiuli et al., 2005). Using the equation provided below, the hausner ratio and carr index were computed.

$$\text{Hausner ratio} = \text{Tapped density} / \text{Bulk density}$$

$$\text{Carr index} = (\text{Tapped density} - \text{Bulk density} / \text{Tapped density}) \times 100$$

3.3.2.4. Amino acid analysis

The amino acid content of the protein samples was determined by an acid hydrolysis method based on Zhao et al. (2018) with slight adjustments. Initially, 50 mg of samples were added into 10 mL of 6 M HCl and the mixture was maintained in oven for 1 day at 110 °C to facilitate acid hydrolysis. After cooling the mixtures to room temperature, they were centrifuged at 4500 rpm for 20 min. The supernatant was obtained by passing through membrane filters with 0.45 µm pores. The resulting filtrate was diluted with 100-time with ultra-distilled water. Then, 50 µL of the diluted samples, 50 µL of internal standard, and 700 µL of reagent were mixed in vials and then placed in the device injection block. The final solutions obtained were injected into the LCMS - 8040 device (Shimadzu, Japan) and the device was adjusted according to the Table 3.4 (Saglik et al., 2019). The protein efficiency rate of the samples was assessed using the following equations.

Protein efficiency rate -1 = – 0.684 + Leucine (0.456) – Proline (0.047)

Protein efficiency rate -2 = – 0.468 + Leucine (0.454) – Tryptophan (0.105)

Protein efficiency rate -3 = –1.816 + Methionine (0.435) + Leucine(0.780) + Histidine(0.211) –Tryptophan(0.944)

Table 3.3. LC-MS device operating conditions for amino acid analysis

Parameters	Device condition
Column temperature	30 °C
Mobile system	Gradient
Phase of mobile (A)	0.05% formic acid prepared with water
Phase mobile (B)	acetonitrile
Phase mobile (A) / (B)	30% / 70%
Flow rate	0.07 µL / min
Desolvation line	300 °C
Injection volume	40 µL
Heat	500 °C
Nebulizing gas	3 L / min
Drying gas	20 L / min

3.3.2.5. Fourier transform infrared spectra (FTIR)

An FTIR analysis was utilized to determine the secondary structure of protein using FTIR spectroscopy IRTracer100 (EN230V) (Shimadzu Corporation, Koyta, Japan). The spectrum scans were obtained between wavelength ranges of 400 to 4000 with the resolution 1 cm-1 at room temperature (López-Monterrubio et al., 2020).

3.3.2.6. Scanning electronic microscopy (SEM)

SEM images were utilized to examine the morphology and microstructure of protein samples. The scanning electronic microscope Zeiss Sigma300 (Oberkochen, Germany) was employed to capture SEM images. First, the samples were covered with gold-palladium as a coat material for high conductivity. The samples coated with a layer were positioned in the appropriate instrument components and subjected to a high vacuum, and images were taken by scanning electron microscopy at 30 kV at different sizes (Molina Ortiz et al., 2009).

3.3.2.7. Thermogravimetric analysis (TGA)

The protein's thermal characteristics were analyzed using the DTG-60H Thermogravimetric Analyzer from Shimadzu Corporation, based in Kyoto, Japan. The heating process consisted of applying 5 g of samples with a heating rate of 10 °C/min, ranging from 30 °C to 1000 °C in a nitrogen gas (N2) atmosphere (Piletti et al., 2019).

3.3.2.8. Solubility

The solubility of samples were analyzed by making some modifications on a method previously noted in the literature (Shevkani et al., 2015). Five milliliters of distilled water were used to dissolve 50 mg of protein sample, and the pH of the mixture was adjusted to 7.0 using 0.12 M HCl or NaOH. The mixtures were shaken at room tempreature at 200 rpm for 1 h and the pH remained constant during this period. After this period, centrifuge the mixtures for 15 min at 4500 rpm at 4 °C and then filtered through 0.45µm filter paper at 45° angle for 30 min. Finally, the solubility of the samples was assessed using the Bradford method (Bradford, 1976). The following formula was used to get the solubility percentage:

Solubility (%) = (Weight of solubile / Proteinweight of protein) × 100

3.3.2.9. Water holding capacity (WHC)

The WHC of protein samples were measured following the methods described by Aydemir and Yemenicioğlu (2013) with minor modifications. Five milliliters of distilled water was added to the 1 g of protein samples in 50 mL of centrifuged tube. The prepared mixtures were vortexed every 5 minutes for duration 10 sec and this process was repeated for 30 minutes at ambient temperature. After this period, centrifuge the mixtures for 15 min at 4500 rpm under 4 °C. The precipitation was separated from supernatant by placing the centrifuge tubes on the filter paper at a 45º for 40 min, and finally the weighted precipitation was recorded. The water holding capacities of proteins were evaluated as a percentage according to the following equations.

WHC (%) = ((W3 – W2) – W1 / W1) ×100

W1: Weight of protein powder sample (g)

W2: Centrifuge tube (g)

W3: Defined as final weighing (g)

3.3.2.10. Oil holding capacity (OHC)

The percentage of OHC was performed similarly to the WHC analysis. In 50 mL of centrifuged tube, 1000 mg of protein samples were dissolved in 5 mL of oil (sunflower). The prepared mixtures were vortexed every 5 minutes for duration 10 sec and this process was repeated for 30 minutes at ambient temperature. After this period, centrifuge the mixtures for 15 min at 4500 rpm under 4 °C. The precipitation was separated from supernatant by placing the centrifuge tubes on the filter paper at a 45º for 40 min , and finally the weighted precipitation was recorded (Cho et al., 2004). OHC of samples was calculated as a percentage using the following equations.

OHC (%) = ((W3 – W2) – W1 / W1) × 100

W1: Weight of protein powder sample (g)

W2: Centrifuge tube (g)

W3: Defined as final weighing (g)

3.3.2.11. Emulsion properties

Emulsion properties including emulsion activity and stability index were measured in a previously conducted study with some modifications (Karaca et al., 2011). To achieve this, fresh emulsions were prepared by mixing 500 mg of protein samples into 100 mL potassium phosphate buffer (10 mM, pH 7.0). A water-in-oil emulsion was formed by blending 25 mL of corn oil with 75 mL of protein dispersion and homogenizing the mixture with an Ultra-Turrax at 10,000 rpm for 2 min.

To determine emulsion activity (EA), 50 µL from fresh prepared emulsion and 5 mL SDS (1% w/v) were mixed. Subsequently, the absorbance was read by UV-Vis spectrophotoscopy (Model UV-1280, Shimadzu, Japan) at a wavelength 500 nm. In order to assess the emulsion stability (ES), the same procedure of EAI was applied to the mixture after keeping for 10 and 30 min at room tempreature (EAI10 and EAI30) and the absorbance was read. The formulae provided were used to calculate EAI and ESI (Lee et al., 2021).

$$EA\ (m^2g) = 2\times2.303\times A0\times D / FC\times\varphi\times\theta\times1000$$

$$ES\ (min) = (A0 / A0 - At) \times t$$

A0 : the absorbance of emulsion immediately after homogenization

DF: Dilution factor

C : Sample concentration

Φ : Path of Optical

Θ : Fraction volume of fat

At : the a bsorbance of fresh prepared emulsion after kept for 10 and 30 min after homogenization process

t = Time for absorbance reading after emulsion preparation

3.3.2.12. Foaming properties

The foaming properties, specifically foaming capacity (FC) and stability (FS), were assessed by preparing a 1% w/v protein solution. The solution was stirred with a magnetic stirrer for 5 min to dissolve the protein. Subsequently, the protein solution was homogenized at 12,000 rpm for 1 min using an Ultra-Turrax homogenizer (IKA-T18 Basic, Japan) (Jarpa-Parra et al., 2014). Based on the equations provided below, foaming capacity and stability were computed.

FC (%) = (V2 – V1 / V1) × 100

FS 10 min (%) = Vt / V2 × 100

V1: Initial volume of the solution prior to the homogenization process (mL)

V2: Volume of solution following the homogenization process (mL)

Vt: Volume of solution after kept for 10 and 30 min after homogenization process (mL).

3.3.3. Hydrogel analysis

3.3.3.1. Color

The color of the hydrogel samples was evaluated using a Hunter Lab device (Color Quest® XE, USA) based on the parameters L*, a*, and b*.The equipment was initially calibrated using three parameters: L* = 96.90, a* = 1.21, and b* = 1.93. L* was associated with lightness, and the value was recorded from black to white (0 to 100). The value of a* corresponds to green to red, and the value was recorded from (-100 to +100), while the value of b* corresponds to blue to yellow, and the value was recorded from (-100 to +100) (Duangmal et al., 2008).

3.3.3.2. Water holding capacity

The water holding capacity of the hydrogel samples was assessed following the method outlined by (Wu et al., 2009) with certain adjustments. In summary, one gram of hydrogel samples were deposited in a centrifuge tube. Following that, subjected to centrifugation for 15 min at 4500 rpm. After that, the centrifuged tube was inverted for 45 min at a 45° for 45 min to separate water, and the weight was measured and recorded. The provided equation quantifies the water-retaining capacity as a percentage.

WHC (%) = W / W1 × 100

W : Weigh of hydrogel sample after centrifugation

W1 : Initial weigh of hydrogel sample

3.3.3.3. Swelling ratio

The hydrogel sample's swelling ratio was determined by cutting it into a cubic shape with dimensions of 8 mm × 10 mm (diameter × length). After weighting, place the hydrogel samples in a 250 mL beaker. Subsequently, immerse a beaker contain a cube-shaped hydrogel sample in a water bath after set temperature to 50 °C for 30 min. A cubic hydrogel specimen was removed from a water bath and dried using filter paper. Finally, a hydrogel sample in the shape of a cube was measured in terms of its weight. The swelling ratio was quantified as a percentage using the given equation (Chang et al., 2020).

$$\text{Swelling ratio (\%)} = (W1 - W) / W \times 100$$

W1: Final weigh of a cubic shape hydrogel (swollen)

W : Weigh of a cubic hydrogel.

3.3.3.4. Protein leachability

The percentage of protein leaching from the hydrogels were determined as a measure of protein leachability, following the methodology reported in a previous study with minor adjustments (Wang et al., 2013). To do this, the hydrogel samples, weighing a total of 2 g were mixed with 8 mL (0.05M) sodium phosphate buffer solution (The buffer was prepared by mixed two mixtures including NaH_2PO4 and Na_2HPO4). The mixture was then kept at a pH of 7 and maintained at ambient temperature for duration of 3 h. After centrifuged the mixture for 15 min at 4500 rpm, the supernatant was collected. The ability of leaching proteins from hydrogels was assessed using Bradford techniques.

3.3.3.5. Fourier transforms infrared (FTIR)

An FTIR spectrum was employed to analyze the hydrogel's secondary structure using FTIR spectroscopy IRTracer100 (EN230V) (Shimadzu Corporation, Koyta, Japan). The spectrum scans were obtained between wavelength ranges of 500 to 4000 with the resolution 1 cm^{-1} at room temperature (López-Monterrubio et al., 2020).

3.3.3.6. Scanning electronic microscope (SEM)

SEM images were utilized to examine the morphology and microstructure of hydrogel samples. The scanning electronic microscope Zeiss Sigma300 (Oberkochen, Germany) was employed to capture SEM images. First, the samples were covered with gold-palladium as a coat material for high conductivity. The samples coated with a layer were positioned in the appropriate instrument components and subjected to a high vacuum, and images were taken by scanning electron microscopy at 30 kV at different sizes (Molina Ortiz et al., 2009).

3.3.3.7. Texture analysis

The TA-XT Plus texture analyzer (Surrey, UK) was used to access the texture of hydrogel samples. Initially, the hydrogel sample was prepared in a cylindrical container with a dimension of 35 mm × 30 mm (diameter × height). Following that placed the hydrogel samples in a relevant part of the instrument. The texture of hydrogel sample was measured under compression along a single axis with a metal compression plate with a diameter of 3.5 inches at a pre-test speed and compression speed with 1 mm/s and 2 mm/s, respectively, 7mm of compression distance, and 5g of trigger force. Five texture parameters for each hydrogel sample were determined including hardness, chewiness, gumminess, springiness and resilience (Cheng et al., 2019).

3.3.3.8. Rheological behavior

The rheology analysis was conducted using the rheometer Haake MARS II (Karlsruhe, Germany) to determine the further characterize the hydrogel sample. The rheometer was used for rheology investigation and assessment of the viscoelastic structure of the hydrogel sample. The rheometer used parallel plate geometry, including a 0.5 mm gap and a 20 mm diameter. An oscillatory test was conducted to measure the storage modulus (G′) and loss modulus (G″) of the hydrogel samples. The hydrogel sample underwent a heating and cooling cycle with a temperature increase at a constant rate of 5 °C/min, beginning at 20 °C and reaching 60 °C. It was then lowered at the same rate, going back from 60 °C to 20 °C. After completing the temperature cycle, the resulting hydrogel underwent at 20 °C of frequency sweep test and spanning frequencies starting from 1.00Hz (Demirkıran et al., 2022).

3.3.3.9. Release behavior

This study used ascorbic acid as an example of a bioactive compound to assess how well hydrogels made from avocado seed protein delivered a bioactive ingredient. The release behavior was conducted following the fabricated hydrogel from avocado seed protein isolate and dried by spray with locust bean, as described in a previous research (Liu et al., 2018) with some minor adjustments. To accomplish this, prepared a 1.5 mL ascorbic acid solution and injected 2 mL of ascorbic acid into the hydrogel sample. The specimen was moved into three distinct solutions with varying pH levels (2.0, 6.0, and 10.0), NaCl with three different environments (0.5, 1.0, and 1.5 M), and diverse temperatures (25 °C, 50 °C, and 75 °C). Subsequently, a pipette transferred 2 mL of the release solution every hour for a total of 9 hours. After each pipetting session, from the buffer add 2 mL to the release solution. Ultimately, the absorbance of solutions were read by UV-Vis spectrophotoscopy (Model UV-1280, Shimadzu, Japan) at a wavelength of 518 nm (Tabakoglu and Karaca, 2018).

3.4. Statistical Analysis

The thesis study carried out all production and analysis in three repetitions and two parallels. The results were reported as a mean values and standard deviations. The data obtained were analyzed using one-way analysis of variance ANOVA (Duncan's) ($p<0.05$), with the statistical package SPSS 22 for Windows (SPSS Inc., Chicago, IL, USA). All graphs were obtained with OriginPro 2021b (Origin Lab Inc.) and Microsoft Excel (Microsoft Excel 2010 Inc.).

4. FINDINGS

4.1. Chemical Composition of Avocado Seed

The total moisture, total ash, total fat, total carbohydrate and total protein amounts of avocado seed were determined and the results obtained are given in Table 4.1. The total moisture and total ash amounts of avocado seed were determined to be 13.57±0.6 % and 1.53±0.8 %, respectively. In terms of total carbohydrate and total fat in avocado seed, these values were measured as 58.74±0.3 % and 13.53±0.4 %, respectively. The amount of total protein in avocado seed that forms the basis of the thesis study was determined to be 12.63±0.2 %.

Table 4.1. Chemical composition of avocado seed powder

Components	Unite	Avocado Seed
Moisture	%	13.57±0.6
Ash	%	1.53±0.8
Fat	%	13.53±0.4
Protein	%	12.63±0.2
Carbohydrate	%	58.74±0.3

4.2. Avocado Seed Protein Isolate

4.2.1. Proximate composition of ASPI powder obtained by various drying techniques

The proximate composition of ASPI was prepared by various drying techniques: spray, freeze, vacuum, oven and ambient drying, as presented in Table 4.2. The percentage of protein content of various dried methods of avocado seed protein isolate was varied significantly ($p < 0.05$). The superior protein content was FD with a value of 67.3 % compared to the other methods with values of 65.9 %, 64.8 %, 62.3 %, and 62.1 % for SD, AD, OD and VD, respectively. The SD had the minimum moisture content (4.22 %) compared to the other techniques with values of 4.69, 4.88, 5.35 and 5.35 % for FD, VD, OD and AD, respectively. VD and OD of ASPI exhibited slightly lower ash and lipid content compared to SD, FD and AD.

Table 4.2. Proximate composition of ASPI powder was prepared by various dried methods.

	AD	OD	VD	FD	SD
Protein	64.8±0.17^{b}	62.3±0.26^{c}	62.1±0.53^{c}	67.3±0.61^{a}	65.9±0.17^{a}
Moisture	5.35±0.07^{a}	5.35±0.08^{a}	4.88±0.04^{b}	4.69±0.08^{c}	4.22±0.04^{d}
Lipid	2.4±0.14^{ab}	2.25±0.07^{b}	2.15±0.07^{b}	2.6±0.14^{a}	2.7±0.15^{a}
Ash	8.7±0.14^{a}	8.7±0.32^{a}	8.55±0.35^{a}	8.8±0.21^{a}	8.8±0.28^{a}
Carbohydrate	18.73±0.24^{b}	21.22±0.63^{a}	22.1±0.93^{a}	16.63±1.0^{c}	18.59±0.36^{b}

The results presented as a mean (n=3) (± Standard Division). The different latters $^{(a-d)}$ at the same line illustrated the significant different between samples ($p < 0.05$).
Avocado seed protein isolate (ASPI) dried in to powder by different drying techniques, namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.

4.2.2. Physicochemical properties of ASPI powder

The physicochemical properties of dried ASPI including SD, FD, VD, OD and AD were evaluate in terms of color, water activity (aw), Carr's index and Hausner ratio

The color properties of dried avocado seed protein isolate are presented in Table 4.3. The findings noted a significant variation ($p < 0.05$) among various methods of ASPI for the parameters L*, a*, and b*. Among the five dried techniques, SD had the maximum L* value of 55.57 and the minimum a* and b* values of 5.40 and 7.40, respectively, compared to the other dried methods. In contrast, OD and VD had the lowest L* values of 40.03 and 40.56, respectively, and the highest a* values of 9.22 and 8.91, and b* values of 15.57 and 15.13, respectively. In other words, VD and OD were the darkest methods of drying ASPI.

Water activity values of dried ASPI by various drying techniques, including AD, OD, VD, FD and SD were display in Table 4.4. The value of water activity of different dried ASPI was 0.38, 0.27, 0.35, 0.34 and 0.32 for AD, OD, VD, FD and SD, respectively. The results indicate a statistically significant difference ($p < 0.05$) out of the five techniques of drying, OD exhibited minimum water activity. On the other hand, AD had the highest value of water activity.

The findings indicated a notable difference ($p < 0.05$) in the Carr's index and Hausner ratio among various methods of dried avocado seed protein isolate, as shown in Table 4.4. Carr index value of different dried ASPI was with the value 9.71, 14.84, 15.9, 33.1 and 38.77 % for OD, AD, VD, FD and SD, respectively. Hausner ratio of different dried ASPI was with the range 1.11, 1.17, 1.18, 1.5 and 1.63 for OD, AD, VD, FD and SD, respectively.

Table 4.2. The color analysis of the dried ASPI powder.

	Color		
Protein samples	L*	a*	b*
AD	42.21±0.41[c]	7.23±0.75[b]	12.22±0.19[b]
OD	40.03±0.53[d]	9.22±0.08[a]	15.57±0.21[a]
VD	40.56±0.40[d]	8.91±0.32[a]	15.13±0.24[a]
FD	45.52±0.06[b]	6.38±0.09[bc]	9.74±0.65[c]
SD	55.57±0.43[a]	5.40±0.17[c]	7.40±0.75[d]

The results presented as a mean (n=3) (± Standard Division). The different latters [(a-d)] at the same line illustrated the significant different between samples ($p < 0.05$).
Avocado seed protein isolate (ASPI) dried in to powder by different drying techniques, namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.
The letter L* represents the attribute of lightness, whereas the letter a* represents the attribute of redness to greenness, and the letter b* represents the attribute of yellowness to blueness.

Table 4.2. Water activity (aw), Carr index and Hausner ratio of different dried techniques of ASPI powder.

	Water activity (a_w)	Carr index	Hausner ratio
AD	0.38±0.002[a]	14.84±0.78[c]	1.17±0.01[c]
OD	0.27±0.005[e]	9.71±0.89[d]	1.11±0.01[d]
VD	0.35±0.002[b]	15.9±0.73[c]	1.18±0.01[c]
FD	0.34±0.001[c]	33.1±0.33[b]	1.5±0.01[b]
SD	0.32±0.002[d]	38.77±0.89[a]	1.63±0.02[a]

The results presented as a mean (n=3) (± Standard Division). The different latters [(a-e)] at the same line illustrated the significant different between samples ($p < 0.05$). Avocado seed protein isolate (ASPI) dried in to powder by different drying techniques, namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.

4.2.3. Amino acid composition and nutrition parameter of ASPI powders

In this present study, the composition of amino acid profile of five various dried avocado seed protein isolate were determined as shown in Table 4.5. Nine crucial amino acids were identified in ASPI through different drying techniques including histidine, lysine, threonine, methionine, valine, isoleucine, phenylalanine, leucine, and Tryptophan. The dominant essential amino acids of ASPI are isoleucine (AD: 5.86 %, OD: 5.39 %, VD: 5.54 %, FD:5.59 % and SD: 5.47 %), leucine (AD: 6.24 %, OD: 6.33 %, VD: 6.39 % FD: 6.56 % and SD: 6.27 %), lysine (AD: 5.58 %, OD: 5.57 %, VD: 5.79 %, FD: 5.95 % and SD: 6.05 %), threonine (AD: 5.71 %, OD: 5.39 %, VD: 5.33 %, FD: 4.83 % and SD: 5.18 %) and valine (AD: 6.19 %, OD: 6.02 %, VD: 6.02 %, FD: 5.89 % and SD: 6.29 %). The total amino acid content in proteins and the essential amino acids in FD, SD, AD, VD and OD were 38.11 % and 37.81 %, 35.89 %, 34.99 %, 34.72 %, respectively. The PER-1 values for AD (1.8 %), OD (1.8 %), VD (1.9 %), FD (1.99 %), and SD (1.9 %). The value of PER-2 and PER-3 for FD (3.4 and 3.8 %), SD (3.2 and 3.5 %), VD (3.3 and 3.2 %), OD (3.2 and 2.8%) and AD (3.2 and 2.6 %), respectively.

Table 4.2. Composition of amino acid and protein efficiency ratio of ASPI powders (g/100 g)

Amino acid profile	AD (%)	OD (%)	VD (%)	FD (%)	SD (%)
Histidine	2.49	2.08	2.19	4.74	3.43
Isoleucine	5.86	5.39	5.54	5.59	5.47
Leucine	6.24	6.33	6.39	6.56	6.27
Lysine	5.58	5.57	5.79	5.95	6.05
Methionine	0.67	0.76	0.78	1.27	1.67
Phenylalanine	3.13	3.17	2.93	3.22	3.41
Threonine	5.71	5.39	5.33	4.83	5.18
Tryptophan	0.02	0.02	0.02	0.04	0.03
Valine	6.19	6.02	6.02	5.89	6.29
Total-EAA	35.89	34.72	34.99	38.1	37.8
Aspartic acid	11.97	11.58	12.31	10.40	11.36
Glutamic acid	10.96	11.61	12.10	11.61	11.13
Serine	8.74	8.78	8.70	8.90	8.78
Glycine	9.40	9.65	9.01	7.85	7.25
Cystine	0.3	0.3	0.3	0.4	0.4
Tyrosine	1.3	1.3	1.1	1.1	1.1
Arginine	6.2	6.9	7.1	6.8	7.5
Alanine	7.8	7.6	7.6	7.4	7.6
Proline	7.3	7.3	6.8	7.3	6.9
Asparagine	0.03	0.10	0.01	0.04	0.04
Glutamine	0.03	0.10	0.01	0.01	0.03
Total non-EAA	64.11	65.28	65.01	61.89	62.18
PER-1	1.8	1.8	1.9	1.99	1.9
PER-2	3.2	3.2	3.3	3.4	3.2
PER-3	2.6	2.8	3.2	3.8	3.5

SD, FD, VD, OD and AD for spray, freeze, vacuum, oven and ambient, respectively.

EAA : essential amino acid

Non- EAA : non-essential amino acid

PER : portion efficiency ratio

4.2.4. Fourier transform infrared spectra (FTIR) of ASPI powders

Special groups naturally found in the structures of isolation protein from avocado seed and dried into powder by various dried techniques, including AD, OD, VD, FD and SD were investigated at the wavelengths between 500 to 4000 cm^{-1} as shown in Figure 4.1. The amide I, II and III were identifying at (1600 -1700cm^{-1}), (1470-1570cm^{-1}) and (1250-1350cm^{-1}). Also, amide A and B were determined in the wavelength range of (3300-3500cm^{-1}) and (2850-2980 cm^{-1}).

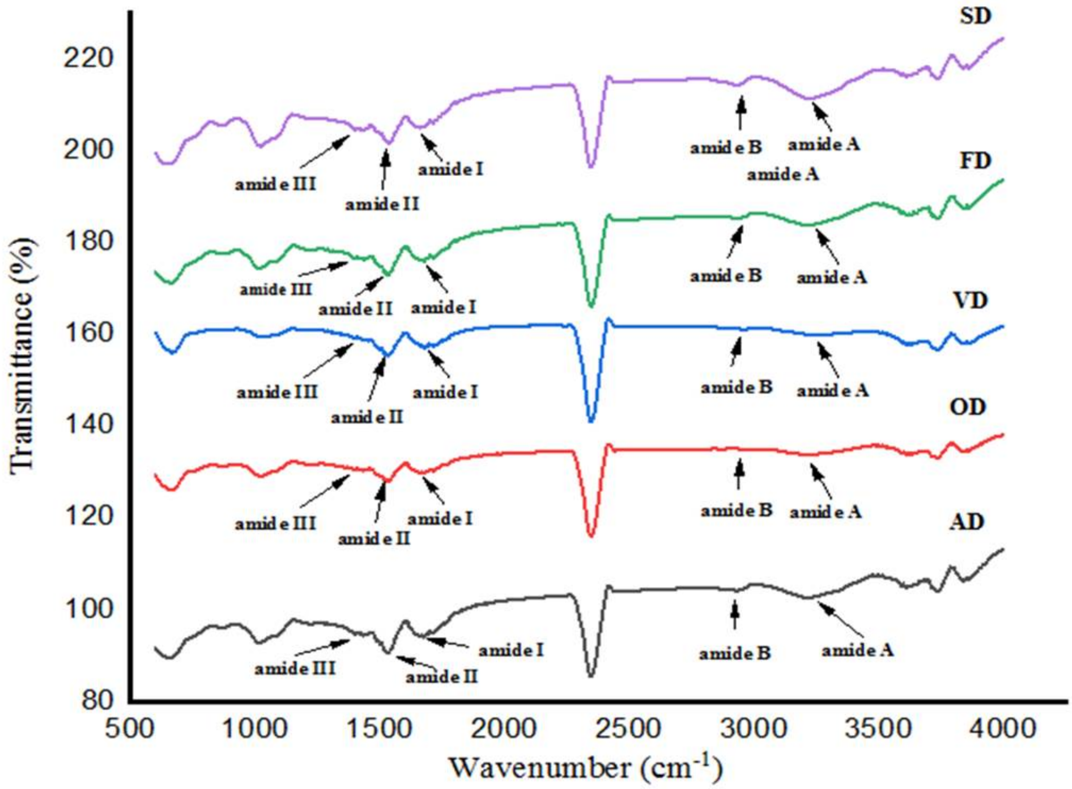

Figure 4.1. FTIR spectra analysis of dried avocado seed protein. AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.

4.2.5. Scanning electronic microscopy (SEM) images of ASPI powders

The surface morphologies of dried proteins were visualized using a scanning electron microscope, and corresponding images are provided in Figure 4.2. SEM analysis was employed to observe and assess the impact of drying methods on the surface pattern of avocado seed protein. The result shows that the microscopic morphology of all dried methods revealed distinct surface structures. When compared to other drying methods, SD protein had a uniform particle size distribution and round shape, and also the particles' surface had a smooth, spherical structure. Furthermore, some particles of SD protein appeared wrinkled or folded.

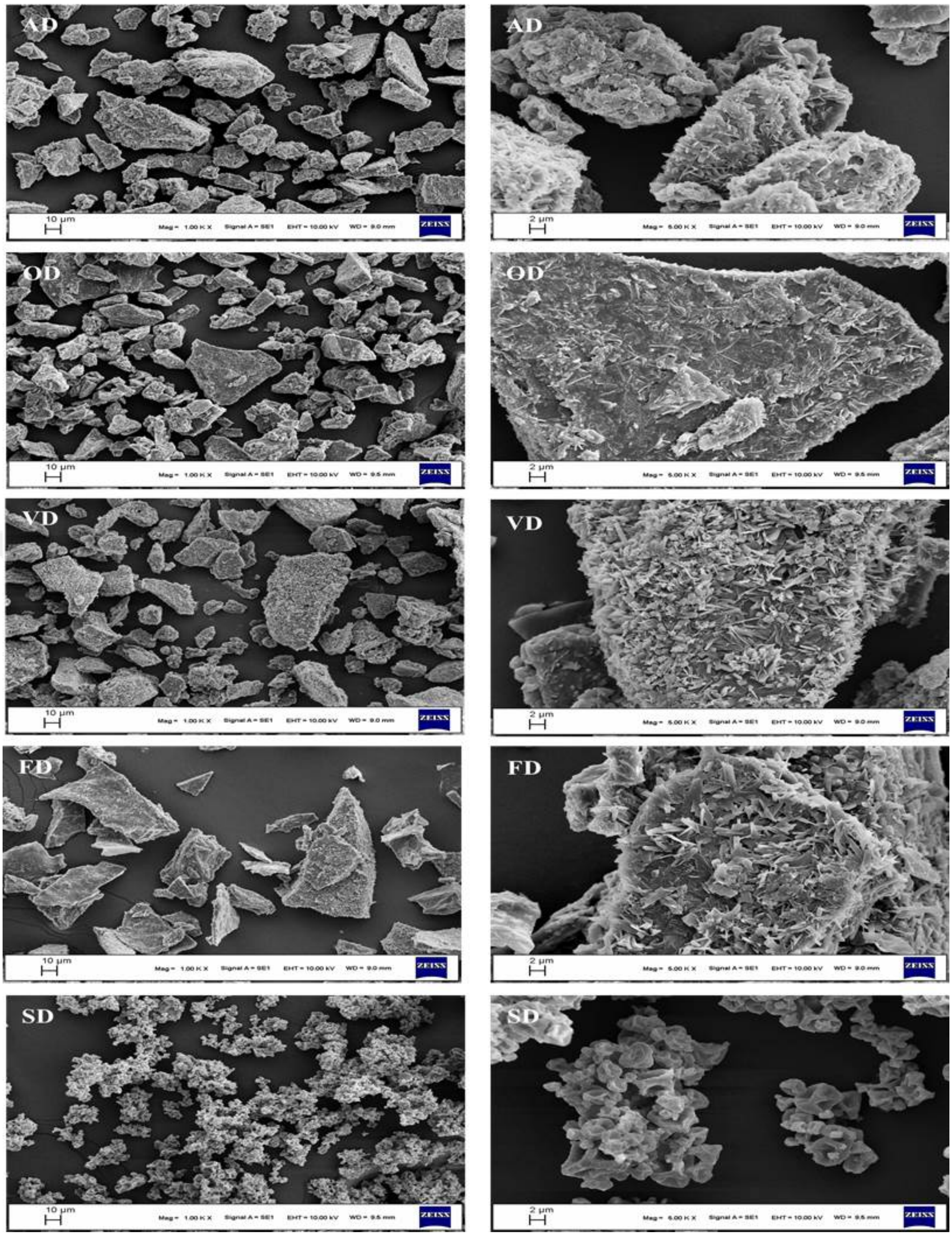

Figure 4.2. Scanning electronic microscope (SEM) of dried avocado seed protein. AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.

4.2.6. Thermogravimetric analysis (TGA) of ASPI powders

TGA indicates the maximum weight loss or gain experienced by the material with varying temperatures. Figure 4.3. shown the TGA weight loss curves for different drying methods of ASPI. Overall, all drying techniques for ASPI had comparable thermal characteristics, with the curves indicating a three-stage weight reduction. The initial weight loss occurred at a temperature lower than 200 °C. The evaporation of water may be the first heat event in polymeric materials that is associated with the evaporation of water. The maximum weight loss occurred within the temperature range of 170 to 500 °C. The following sequence shows the minimal weight reduction during this stage: SD, VD, AD, FD, and AD, with respective weight reductions of 39, 40, 42, 43, and 45 %.

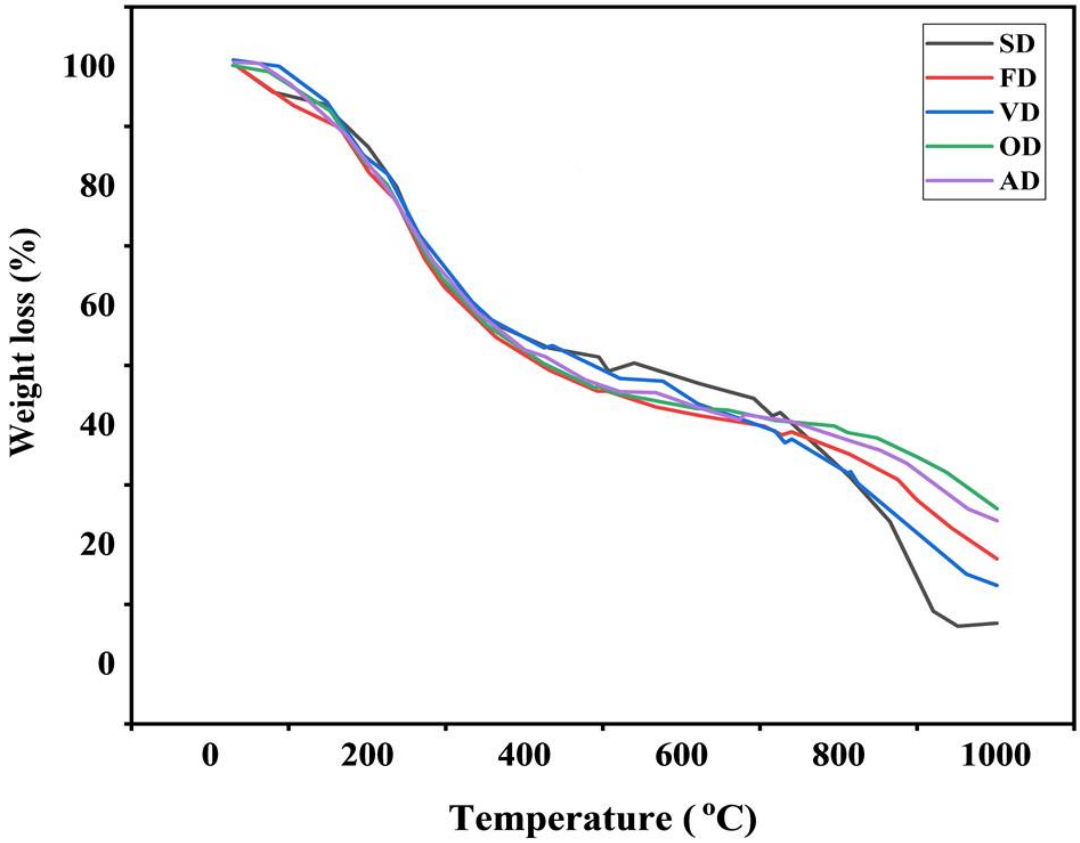

Figure 4.3. Thermogravimetric (TGA) analysis of dried avocado seed protein. AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.

4.2.7. Solubility of ASPI powder

This section was determined that how different drying techniques affected the solubility of ASPI powder, as presented in Figure 4.4. Drying techniques namely SD, FD, VD, OD and AD significantly ($p < 0.05$) affected the solubility of ASPI powder. The value of solubility of ASPI powder obtained by SD technique was the highest, with a value of 82.52 %. The solubility levels of protein powders obtained by FD, VD, AD, and OD techniques were 75.22 %, 68.77 %, 60.24 %, and 53.8 %, respectively. The powders obtained using SD exhibited superior solubility values that were consistent with literature findings.

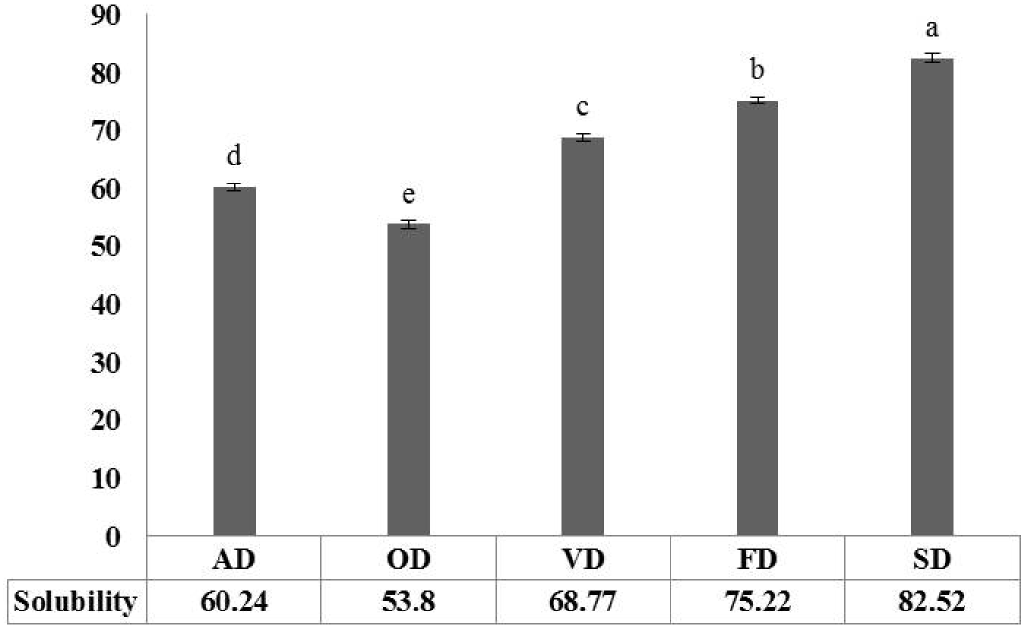

Figure 4.4. Solubility of avocado seed protein isolate, AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively. The results presented as a mean (n=3) (± Standard Division). The different latters (a-e) illustrated the significant different between samples ($p < 0.05$).

4.2.8. Water holding capacity (WHC) of ASPI powder

The data obtained in this study show that dried techniques including, SD, FD, VD, OD and VD significantly affected the WHC of ASPI powder ($p < 0.05$), as illustrated in figure 4.5. WHC of different dried ASPI powder were 393.33, 330.43, 201.21, 174.42, and 173.2 for SD, FD, AD, OD, and VD, respectively. SD protein was showed the maximum WHC among the other dried methods. While AD protein was given the lowest WHC compared to the other dried methods. In general, SD protein had superior WHC compared to proteins obtained using the other dried methods.

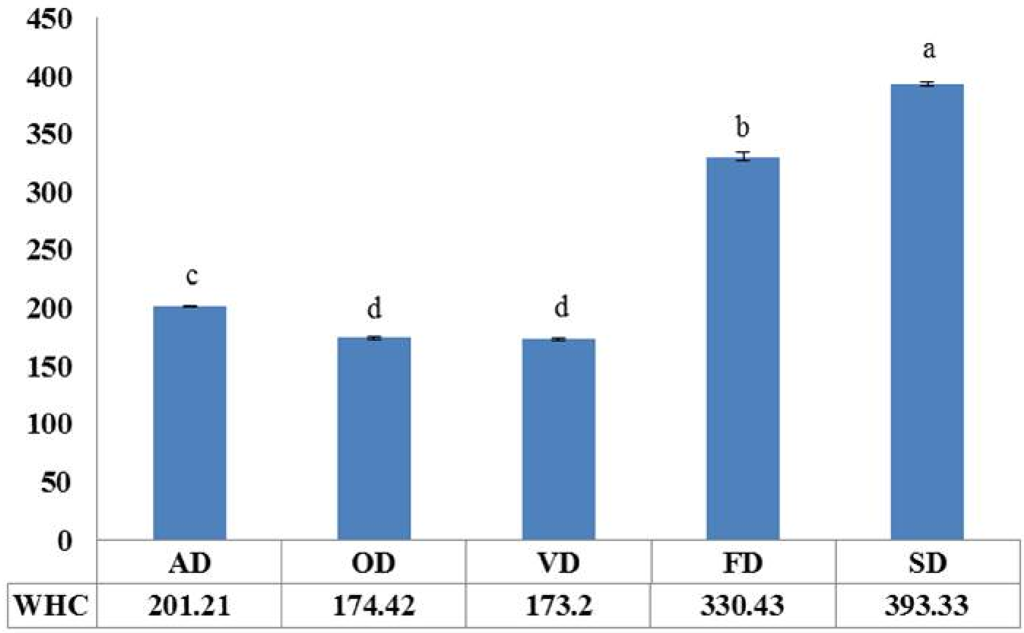

Figure 4.5. Water holding capacity (WHC) of avocado seed protein isolate, AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively. The results presented as a mean (n=3) (± Standard Division). The different latters (a-d) illustrated the significant different between samples ($p < 0.05$).

4.2.9. Oil holding capacity (OHC) of ASPI powder

Figure 4.6 was shown the OHC of different dried ASPI powder. The data showed that statistically OHC of different dried ASPI powder including AD, OD, VD, FD and SD differed significantly ($p < 0.05$). The value of OHC of dried techniques was varied from 300.92 % to 85.92 %. Among the other dried techniques, SD technique had the superior OHC with the value 300.92 %. In contrast, the lowest OHC was noted in AD technique with the value 85.92 % following that OD and VD techniques with the value 89.89 % and 98.33 %, respectively.

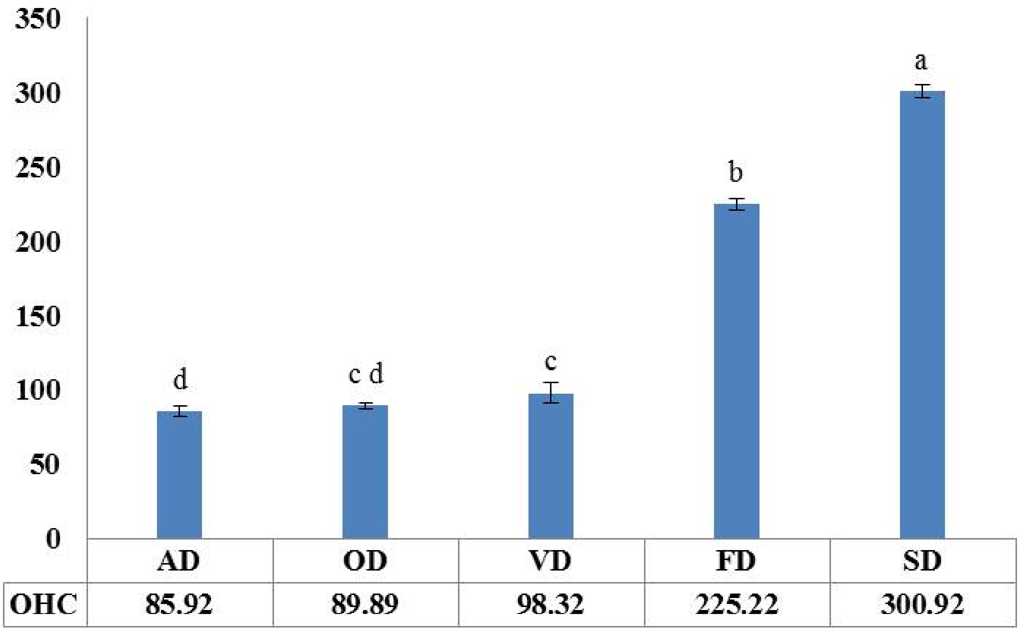

Figure 4.6. Oil holding capacity (OHC) of avocado seed protein isolate, AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively. The results presented as a mean (n=3) (± Standard Division). The different latters (a-d) illustrated the significant different between samples ($p < 0.05$).

4.2.10. Emulsion activity and stability of ASPI powder

The value of emulsion activity (EA) and stability (ES) of dried ASPI powder using SD, FD, VD, OD, and AD techniques are presented in Table 4.6. The EA values ranged from 20.4 m^2/g to 49.8 m^2/g. SD technique exhibited the superior EA value (49.8 m^2/g) compared to the other drying techniques. FD technique had a value of 37.2 m^2/g, AD technique had a value of 30.9 m^2/g, VD technique had a value of 26.8 m^2/g, and OD technique had a value of 20.4 m^2/g. Statistical evaluation determined that different drying techniques significantly affected the EA ($P < 0.05$). The ES indices after 10 min kept at room temperature were determined to be 71.6, 66.2, 50.3, 37.3, and 32.3 min for SD, FD, AD, VD, and OD techniques, respectively. Moreover, the SD technique used in dried ASPI into powder was showed a higher ES than other dried techniques. This section of the study measured the ES after maintaining it at ambient temperature for 30 min. The SD technique exhibited the maximum ES with a value of 112.7 min, surpassing the FD, AD, VD, and OD techniques, which had values of 102.8, 93.2, 85.3, and 63.1 min, respectively.

Table 4.4. Emulsion activity and stability of dried ASPI powder obtained by different dried techniques.

Functional properties	AD	OD	VD	FD	SD
EA	30.9±0.56 ^{c}	20.4±0.56 ^{e}	26.8±0.74 ^{d}	37.2±0.56 ^{b}	49.8±0.56 ^{a}
ES (10 min)	50.3±5.69 ^{b}	32.3±3.36 ^{c}	37.3±2.59 ^{c}	66.2±4.82 ^{a}	71.6±3.38 ^{a}
ES (30 min)	93.2±4.8 $^{b\,c}$	63.1±4.18 ^{d}	85.3±4.39 ^{c}	102.8±5.5 $^{a\,b}$	112.7±4.99 ^{a}

The results presented as a mean (n=3) (± Standard Division). The different latters $^{(a\text{-}e)}$ at the same line illustrated the significant different between samples ($p < 0.05$). Avocado seed protein isolate (ASPI) dried in to powder by different drying techniques, namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively.
EA: emulsion activity, ES: emulsion stability after kept for 10 and 30 min at ambient temperature.

4.2.11. Foaming capacity and stability of ASPI powder

The results of this study indicate that the drying methodsnamely, SD, FD, VD, OD, and VD had a significant impact on the foaming capacity (FC) of ASPI powder ($p < 0.05$), as demonstrated in Figure 4.7. The range was from 81.73 to 66 %. The super FC was FD and SD with the values of 81.73 % and 79.33 %, respectively, followed by AD, VD, and VD with the values of 72.66 %, 66.6 %, and 66 %, respectively. Among the various dried techniques of ASPI, freeze and spray dried had the best FS after 10 min at room temperature (46.49 % and 45.93 %), respectively, compared to the VD (35.87 %), OD (32.17 %), and AD (23.87 %). Additionally, the results were noted that among the dried techniques FS after 10 min of storage at room temperature show a significant variation ($p < 0.05$), as illustrated in Figure 4.8. Among the various dried techniques of ASPI there was statistically no difference ($p > 0.05$) in terms of FS after 30 min of storage at room temperature. Figure 4.9. showed the FS during 30 min, and the lowest value was AD (20.22 %), followed by OD, VD, SD, and FD, with values of 22.69 %, 24.03 %, 25.27 %, and 26.27 %, respectively

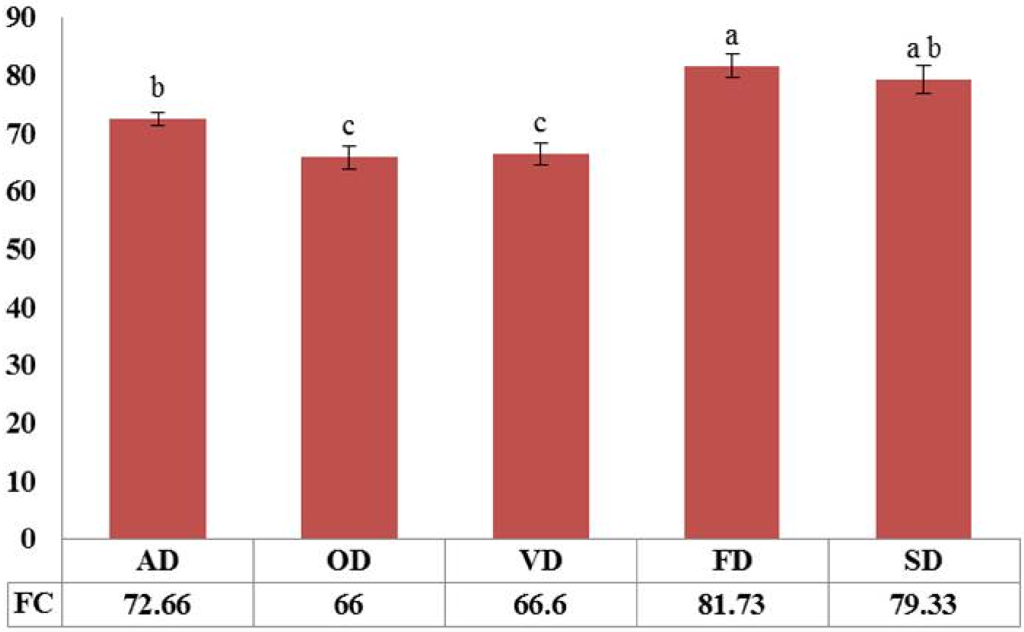

Figure 4.7. Foaming capacity (FC) of avocado seed protein isolate, AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively. The results presented as a mean (n=3) (± Standard Division). The different latters (a-c) illustrated the significant different between samples ($p < 0.05$).

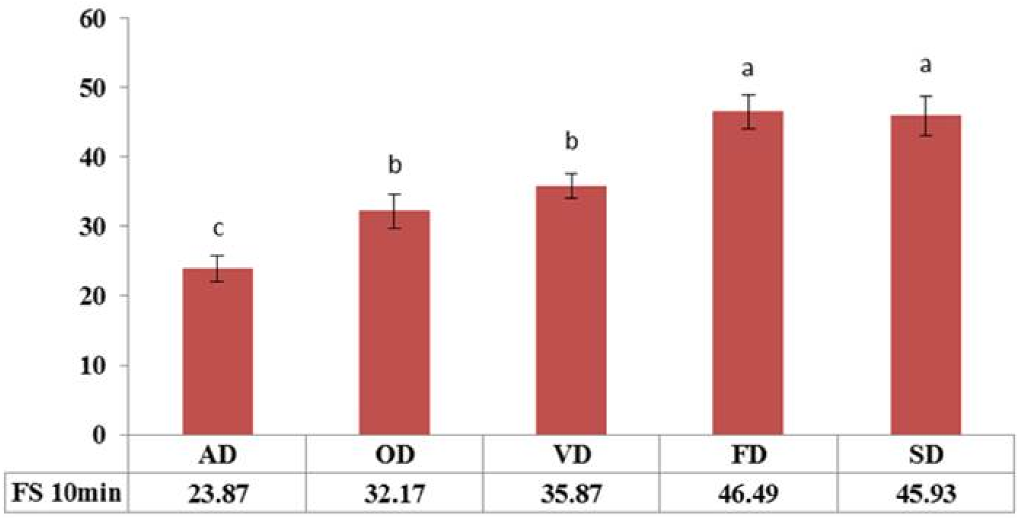

Figure 4.8. Foaming stability (FS) of avocado seed protein isolate, AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively. The results presented as a mean (n=3) (± Standard Division). The different latters (a-c) illustrated the significant different between samples ($p < 0.05$).

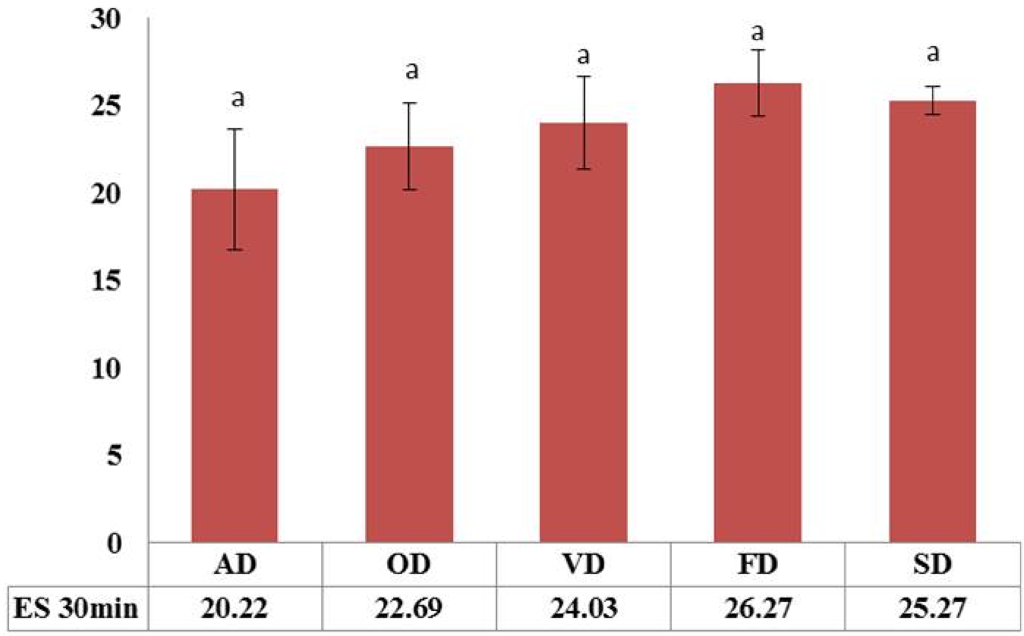

Figure 4.9. Foaming stability (FS) of avocado seed protein isolate, AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively. The results presented as a mean (n=3) (± Standard Division). The same latters (a) illustrated no significant different between samples ($p > 0.05$).

4.3. Hydrogel analysis

This section of research specifically examines the utilization of avocado seed protein isolation in hydrogel systems. Hydrogels were synthesized, including SD-ASPI+LBG, FD-ASPI+LBG, VD-ASPI+LBG, OV-ASPI+LBG and AD-ASPI+LBG by using a mixture of each dried technique of ASPI and locust bean gum (LBG) as the main component. This part of the study aimed to assess how various drying techniques affected the fabrication hydrogels. To achieve this objective, a comparative analysis was conducted to assess how different drying methods for avocado seed protein isolation affect hydrogel formation. The study investigated hydrogel's color, FTIR, SEM, texture, rheological behavior, and release behavior, as well as functional characteristics like swelling ratio, water holding capacity, and protein leachability.

4.3.1. Color analysis of hydrogels

The color characteristics of the hydrogel samples, namely SD-ASPI+LBG, FD-ASPI+LBG, VD-ASPI+LBG, OD-ASPI+LBG, and AD-ASPI+LBG, were determined based on the value of L*, a*, and b*. Table 4.7 displays the results of the analyses. All hydrogel samples exhibited significant variations ($p < 0.05$) in relation to the parameters L*, a*, and b*.

Table 4.6. Color analysis of fabricated hydrogel.

Hydrogel samples	Color		
	L*	a*	b*
AD-ASPI+LBG	29.09±0.05 b	2.17±0.22 b	1.34±0.13 b
OD-ASPI+LBG	26.62±0.23 c	2.63±0.31 b	1.88±0.02 b
VD-ASPI+LBG	28.12±0.33 b	2.61±0.1 b	1.57±0.02 b
FDASPI+LBG	26.77±0.99c	2.38±0.23 b	1.78±0.44 b
SD-ASPI+LBG	31.78±0.04 a	3.81±0.15 a	2.98±0.05 a

The results presented as a mean (n=3) (± Standard Division). The different latters (a-c) at the same line illustrated the significant different ($p < 0.05$).
Fabricated hydrogel samples were prepared from dried avocado seed protein isolation (ASPI), namely SD, FD, VD, OD and AD for spray, freeze, vacuum, oven and ambient drying, respectively, with locust bean gum (LBG)
The letter L* represents the attribute of lightness, whereas the letter a* represents the attribute of redness to greenness, and the letter b* represents the attribute of yellowness to blueness.

4.3.2. Functional properties of hydrogels

4.3.2.1. Water holding capacity of hydrogels

Table 4.8 provides the WHC values in ASPI-based hydrogel systems. The study showed that the WHC of the hydrogels made with different dried methods of ASPI with LBG namely, SD-ASPI+LBG, FD-ASPI+LBG, VD-ASPI+LBG, OD-ASPI+LBG, and AD-ASPI+LBG had significant differences ($p < 0.05$). In other words, drying techniques of ASPI had a significant impact on the hydrogel system by using the same amount of LBG. Hydrogel prepared from SD-ASPI+LBG demonstrated a superior water-holding capacity, with a value of 93.79 %. The hydrogel prepared from AD-ASPI+LBG exhibited the lowest WHC value, measuring at 64.8 %. The WHC of FD-ASPI+LBG had a value of 86.83 %. The WHC of VD-ASPI+LBG had a value of 76.17 %. The WHC of OD-ASPI+LBG had a value of 72.29 %.

4.3.2.2. Swelling ratio of hydrogels

This section specifically examined swelling characteristics of hydrogels, and the corresponding findings are presented in Table 4.8. AD-ASPI+ LBG demonstrated the lowest swelling behavior, with a value of 14.4 %. FD-ASPI+LBG and SD-ASPI+LBG exhibited the highest swelling ratios, with values of 34.2 % and 33.5 %, respectively, followed by VD-ASPI+LBG with a value of 23.1 % and OD-

ASPI+LBG with a value of 18.9 %. It is evident that the hydrogel's swelling ratio varies significantly ($p < 0.05$) depending on the dried methods of ASPI used by containing locust bean gum.

4.3.2.3. Protein leachability of hydrogels

The AD-ASPI+LBG had the highest percentage of protein diffusing into the surrounding fluid (sodium phosphate buffer), at 24 %. The SD-ASPI+LBG had the lowest percentage, at 7.99 %. VD-ASPI+LBG, OD-ASPI+LBG, and FD-ASPI+LBG released 19.2 %, 17.7 %, and 12.2 % of the protein from the hydrogel, respectively. The Various dried avocado seed protein significantly affected the protein leachability, similar to other functional properties ($p < 0.05$).

Table 4.8. Functional properties of hydrogel samples

	WHC (%)	Swelling ratio (%)	Hydrogel Leachability (%)
AD-ASP+LBG	64.8 ± 0.72^{e}	14.4 ± 0.15^{d}	24 ± 0.55^{e}
OD-ASP+LBG	72.29 ± 0.48^{d}	18.9 ± 0.21^{c}	17.7 ± 0.13^{c}
VD-ASP+LBG	76.17 ± 2.33^{c}	23.1 ± 0.11^{b}	19.2 ± 0.25^{d}
FD-ASP+ LBG	86.83 ± 0.18^{b}	34.2 ± 1.86^{a}	12.2 ± 0.13^{b}
SD-ASP+LBG	93.79 ± 0.96^{a}	33.5 ± 0.42^{a}	7.99 ± 0.67^{a}

The results presented as a mean (n=3) (± Standard Division). The different latters $^{(a-e)}$ at the same line illustrated the significant different ($p < 0.05$).

Fabricated hydrogel samples were prepared from dried avocado seed protein isolate (ASPI), namely SD, FD, VD, OD and ASD for spray, freeze, vacuum, oven and ambient drying, respectively, with locust bean gum (LBG)

4.3.3. Fourier transform infrared (FTIR) spectrum of hydrogels

FTIR analysis was carried out on fabricated hydrogels, including SD-ASPI+LBG, FD-ASPI+LBG, VD-ASPI+LBG, OD-ASPI+LBG, and AD-ASPI+LBG, after drying the hydrogel in a freeze dryer as shown in Figure 4.10. Overall, locust bean gum did not result in the appearance of new peaks in the spectrum of the different dried ASPI based on the hydrogel. It indicates that the composite hydrogels did not form any new covalent bonds, as indicated by the absence of new peaks in the spectrum of the different dried ASPI hydrogel samples.

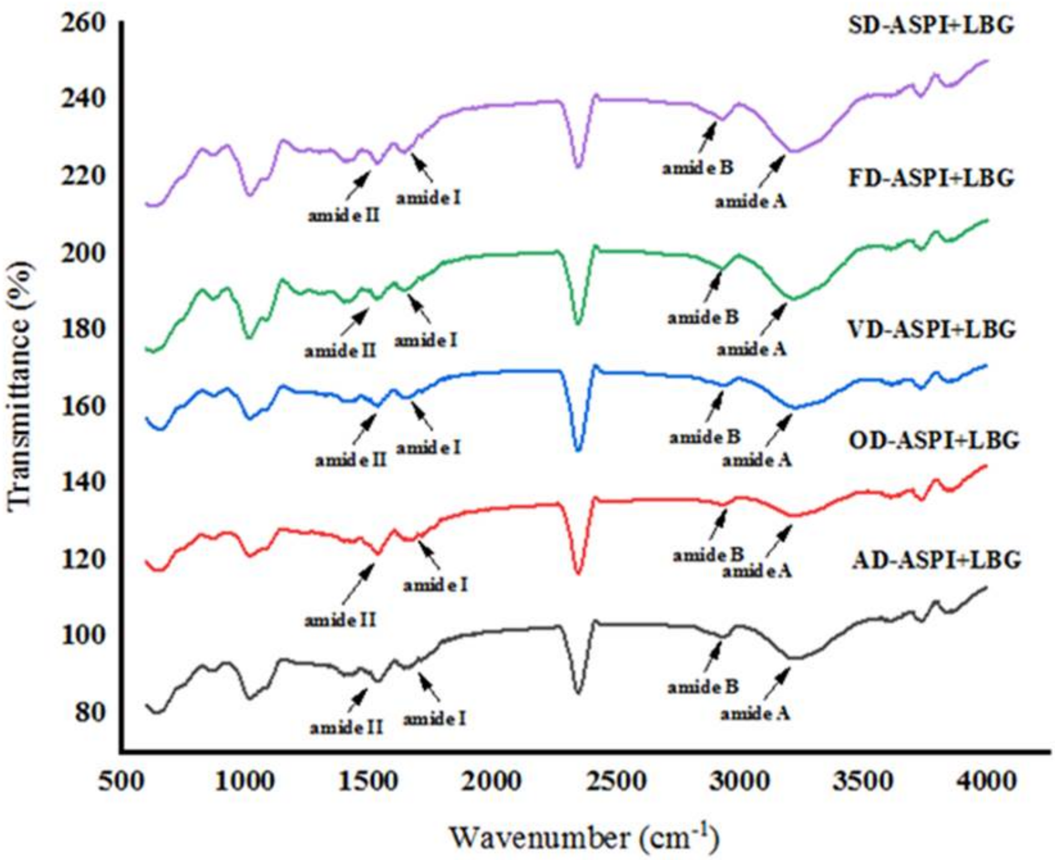

Figure 4.10. FTIR analysis for fabricating hydrogel from the mixture of dried avocado seed protein isolate (ASPI), including spray dried (SD), freeze dried (FD), vacuum dried (VD), oven dried (OD) and ambient dried (AD) with locust bean gum (LBG).

4.3.4. Scanning Electron Microscopy (SEM) images of hydrogel

The purpose of this section was to introduce the visual and structural features of hydrogel models. Figure 4.11 displayed the corresponding photographic and SEM images of fabricated hydrogel. The structures of VD-ASPI + LBG, OD-ASPI + LBG, and AD-ASPI + LBG displayed a non-homogeneous porous structure. Whereas, similar structures in SD-ASPI + LBG and FD-ASPI + LBG were disappear. The most important point in SEM analysis for hydrogel samples was to show the denser (more compact).

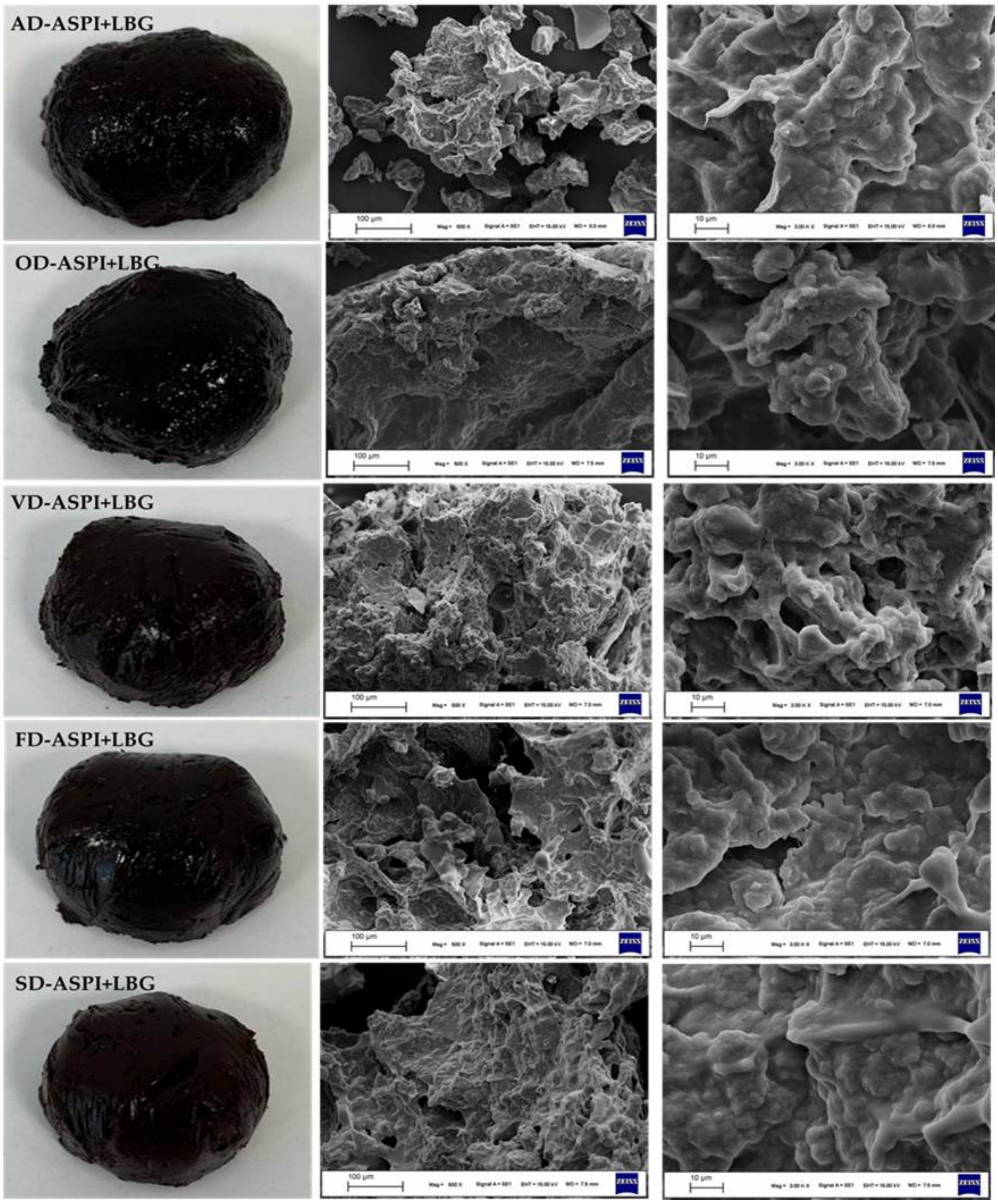

Figure 4.11. Scanning electronic microscopy (SEM) analysis for fabricating hydrogel prepared from the dried avocado seed protein isolate (ASPI) by various dried techniques namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively, with locust bean gum (LBG).

4.3.5. Texture analysis of hydrogels

Figure 4.12. presented the texture of fabricate hydrogel. The result of this study indicated that various dried methods of protein utilization in hydrogel formation have a significant impact on the textural qualities of hydrogels ($p < 0.05$). The hydrogel sample SD-ASPI + LBG had the highest hardness value, subsequently FD-ASPI + LBG, VD-ASPI + LBG, OD-ASPI + LBG, and AD-ASPI + LBG.

Hydrogels exhibit notable variations in hardness, as indicated by a statistically difference between hydrogel samples ($p < 0.05$).

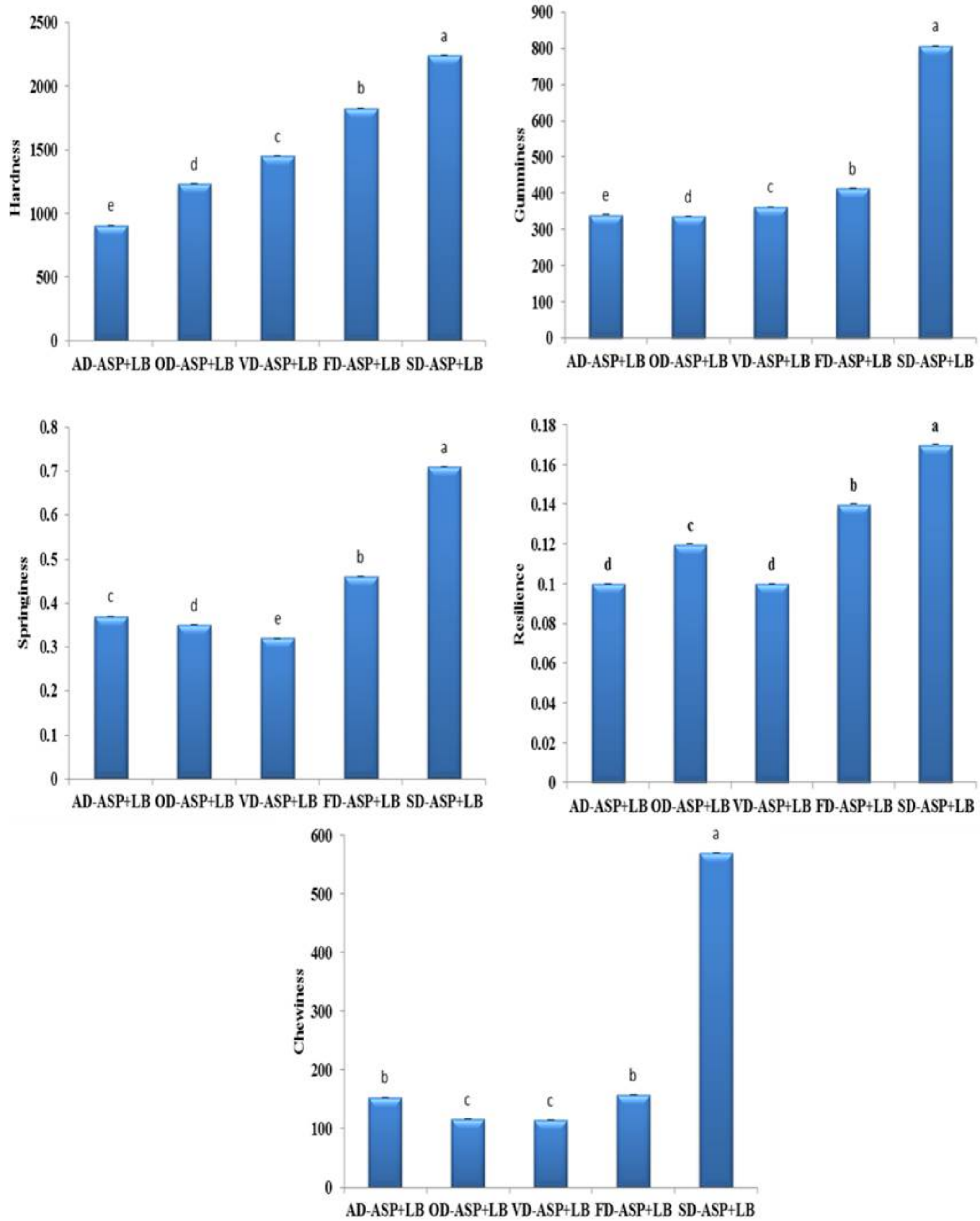

Figure 4.12. The textural behavior for fabricating hydrogel prepared from the dried avocado seed protein isolate (ASPI) by various dried techniques namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively, with locust bean gum (LBG).

4.3.6. Rheological behavior of hydrogels

The drying methods of ASPI significantly impacted the rheological behavior when measuring the values of G′ and G″ as illustrated in figure4.13. SD-ASPI+LBG exhibited the highest value of G′, followed by FD-ASPI+LBG, OD-ASPI+LBG, VD-ASPI+LBG, and AD-ASPI+LBG.

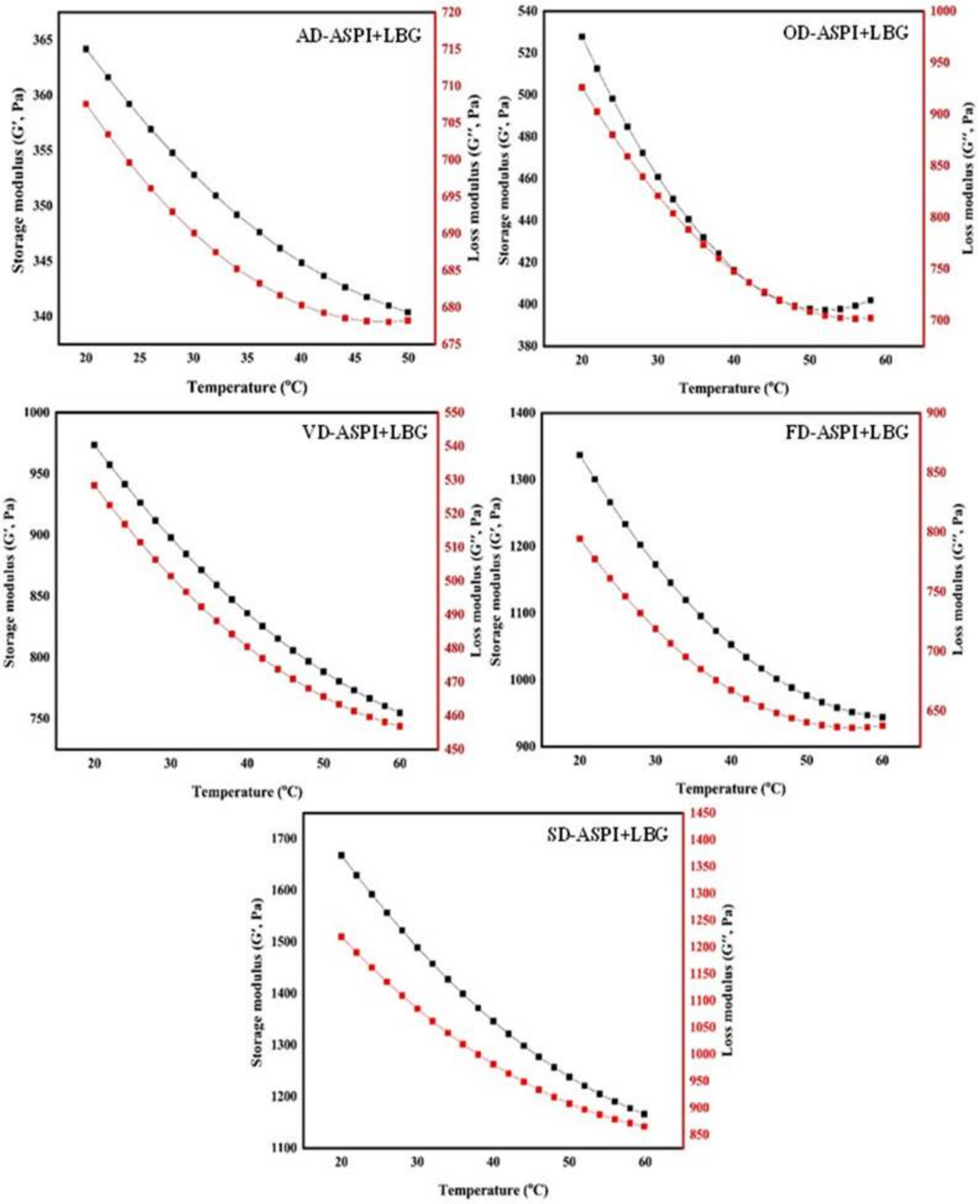

Figure 4.13. Rheological behavior for fabricating hydrogel were prepared from the dried avocado seed protein isolate (ASPI) by various dried techniques namely AD, OD, VD, FD and SD for ambient, oven, vacuum, freeze and spray drying, respectively, with locust bean gum (LBG).

4.3.7. Release behavior of hydrogels

Ascorbic acid was incorporated into a synthetic hydrogel composed of spray dried avocado seed protein isolate and locust bean gum. The research investigated how bioactive chemicals diffused from the hydrogel matrix into the surrounding environment over a period ranging from 60 to 540 minutes at room temperature. The surrounding environment included variations in pH value, molarity of NaCl, and temperature. The control hydrogel system contained only spray-dried ASPI. The result of leaching out bioactive compounds from the interior of a three-dimensional hydrogel system was presented in Figure 4.14. Based on the pH value shown in Figure 4.14 a and b, the least amount of ascorbic acid that leaked out of SD-ASPI+LBG at pH 6.0 was 1.1 %. This was compared to 5.96 % and 3.13 % of ascorbic acid that leaked out of the same hydrogel sample at pH 2.0 and 10.0, respectively. In contrast, during the first 60 min, the value of release of ascorbic acid for the control at pH 2.0, 6.0, and 10.0 was 12.69 %, 12.95 %, and 13.48 %, respectively. Furthermore, during the 120 to 240-min period, the release of ascorbic acid sharply increased in all pHs for both hydrogel samples. Consequently, the best pH for avoiding leaking out ascorbic acid in hydrogel models was 6.0. Figure 4.14 d and c, shown the bioactive compounds were released into the environment at different concentrations of NaCl. The value of released ascorbic acid in the hydrogel was 6.41 in 1.5 M NaCl, 6.14 in 1.5 M NaCl and 4.02 in 0.5 M NaCl. Compared to the control sample, the rate of diffusion of ascorbic acid in the 0.5, 1, and 1.5M NaCl was 12.86, 13.13, and 13.04 %, respectively, during the first 60 min. The maximum diffusion rate of ascorbic acid in both samples was between 180 and 300 min for all molarities of NaCl. It was noted that the best molarity for decreasing the rate of leaking out bioactive compounds was 0.5M NaCl. Figures 4.14 e and f clearly show that the lowest amount of ascorbic acid released inside the hydrogels was at 25 °C, followed by 50 °C and 75 °C for both hydrogel samples.

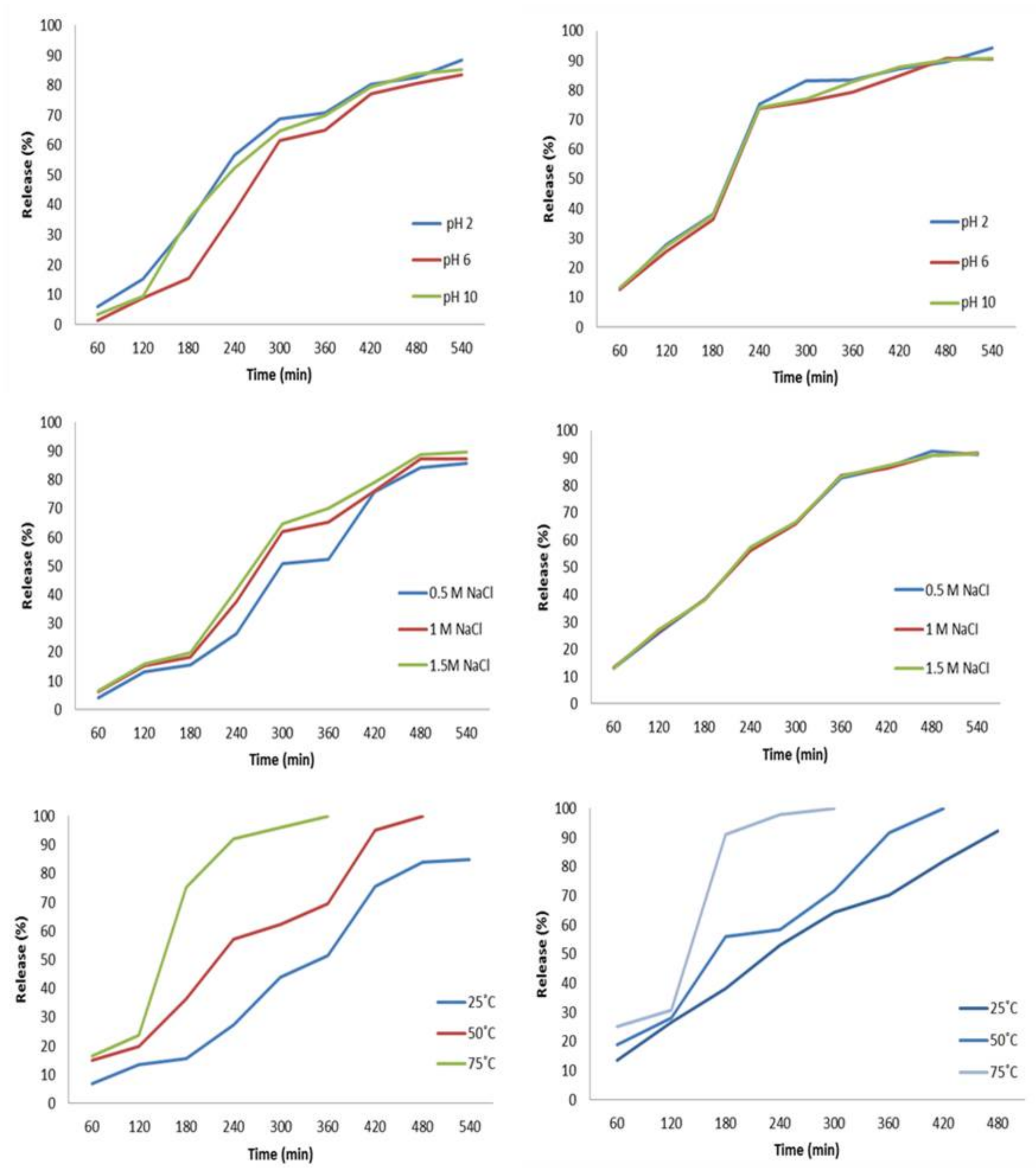

Figure 4.14. Release behavior of fabricating hydrogel from spray dried avocado seed protein isolate with locust bean gum (a, c, e) and fabricated hydrogel from spray dried avocado seed protein isolate alone (b, d, f). The rate of diffusion ascorbic acid from the hydrogel matrix into the surrounding habitats over a time period of 60 to 540 min at room temperature. The surrounding environment included variations in pH value (a, b), molarity of NaCl (c, d), and temperature (e, f).

5. DISCUSSION

This amount of protein content in avocado seed constitutes the answer to the question of why avocado seed should be considered the source of these macrostructures. According to the previous study, researchers determined the amounts of protein contents in different fruit seeds including apricot seed (23.60-26.20 %) (Sharma, Tilakratne and Gupta, 2010), apple seed (27.50-28 %) (Yu et al., 2007), orange seed (16.29 %) (Hussain et al., 2023), pomegranate seed (13.60 %) (Talekar et al., 2018), wheat flour (11.02 %) (Hussain et al., 2023). During fruit processing, all by-products are removed and considered waste materials. Upon comparing the obtained results with market-available equivalents from various studies, it becomes evident that evaluating avocado seed as a protein source is a positive approach

Li et al., (2022) and Brishti et al., (2020) reported that the heating during the drying process is responsible for the differences in protein content between different protein drying techniques. Freeze drying techniques are used to dry protein under cold conditions at -50 °C and pressure without any heat treatment. On the other hand, different drying techniques like spray, vacuum, and oven use different heating conditions to dry protein, with the degree of heating varying depending on the technique. Akpan and Umoh, (2004) found that heat exposure in the presence of moisture during AD, OD, VD and SD facilitated the process of thermal denaturation of proteins. This occurred because water molecules caused the protein to expand, enhancing its ability to move and eventually leading to the formation of combinations that were no longer similar to molecules based on nitrogen. These changes made it difficult to detect the protein using Kjeldahl analysis. AD, VD and OD of avocado seed protein isolate slightly had the lowest protein content. When proteins are heated for an extended period, amino acids and carbohydrates undergo a Maillard reaction, which decreases the protein content (Agoreyo et al., 2011). Furthermore, Bawa et al., (2020) say that lower protein content might be because tannins and proteins combine when proteins are dried out by heating processes. VD and OD of ASPI exhibited slightly lower ash and lipid content compared to SD, FD and AD due to the use of different drying techniques at varying temperatures and durations. Furthermore, compared to freeze drying, Ghribi et al., (2015) investigated that drying protein from chickpeas at 40 °C for 60 h and 50 °C for 48 h reduced the amount of ash and oil. This is due to the long drying process.

According to the L* values, the SD had the highest lightness, whereas the OD and VD had the lowest lightness. This finding was consistent with a prior investigation of chia seed protein, quinoa protein, lentil protein and antarctic krill protein (Timilsena et al., 2016a; Shen et al., 2021; Joshi et al., 2011; Lin et al., 2020), respectively. The darkness of the dried sample suggests that the protein was likely exposed to increased oxygen throughout the ambient drying process, causing certain components to undergo oxidation, resulting in discoloration and an unsatisfactory visual appearance. The findings indicate that the colors of the ASPI can be effected by various factors, including the drying process, drying temperature, and oxidation (Lin et al., 2020). According to Ghribi et al., (2015) the formation of dark pigments, specifically melanoidins, can be attributed to the creation of these compounds through the Maillard reaction when the drying process occurs at high temperatures and long drying process in the OD and VD. According to Shaviklo et al., (2010) before dried protein by the spray drying method, high-speed homogenizing is used for mixing protein solutions, which may destroy the protein tissue and remove some protein pigments. The color qualities were dictated by the inherent attributes of proteins, the degree of protein purity, the presence of pigment contamination, particle sizes of protein, so on (Li et al., 2022).

Water activity (aw) is measured of the amount of available water that is involved in the chemical processes that occur in foods (Quek et al., 2007). A high water activity number indicates a significant amount of free water that is accessible for metabolic activities, which in turn impacts the duration for which foods can be stored without spoiling (Vardin and Yasar, 2011). Generally, all drying techniques had a favorable aw range of 0.27 % to 0.38 % and none of dried methods of avocado seed protein was sensitive to biochemical reaction and microbial growth.

Carr's index and the Hausner ratio typically evaluate the flow characteristics of dry protein by measuring the powder's compressibility as a percentage. The threshold at which a substance transitions from being able to flow freely as granules to being non free flowing as a powder is approximately 20-25 % compressibility (Goyal et al., 2015). The Hausner ratio provides information on the adhesive properties of powder samples, whilst the Carr index value allows for observations on the unrestricted flow characteristics of powder samples (Karrar et al., 2021). In the literature, powder products are categorized based on their stickiness and flow characteristics using specific parameters. This classification assigns the following Hausner ratio ranges to different levels of quality: 1.00-1.11 is considered excellent, 1.12-1.18 is good, 1.19-1.25 is moderate, 1.26-1.34 is valid, 1.35-1.45 is weak, 1.46-1.59 is very weak, and above 1.60 is very poor. Similarly, Carr index values are categorized as 0-10 for excellent, 11-15 for good, 16-20 for moderate, 21-25 for valid, 26-31 for weak, 32-37 for very weak, and above 38 for very poor (Turchiuli et al., 2005). Powder flow is a complex process affected by the physical and chemical properties of the system as well as the inherent characteristics of the powder itself. The flowability of the powder depends on its bulk properties, such as particle size distribution, moisture content, composition, density, and shape. These properties can change due to factors like handling, variations in storage conditions, temperature shifts, and relative humidity. Although there has been considerable research in this field, it is important to note that the reported flowability values are highly dependent on the specific properties of the powder, the experimental conditions used, and the particular methods and equipment employed. In addition to factors like particle size powder, powder composition, and moisture content, flowability is also affected by surface and material properties of powder, as well as the shape, roughness, and hardness of the particles powder (Juliano and Barbosa-Cánovas, 2010; Ganesan et al,2008; Suhag et al. 2024). However, the effect of various dried protein isolates on the characteristics of protein powder flow has not been extensively studied.

An amino acid is the fundamental constituent of proteins and serves as a foundational component for protein synthesis. The amino acid content directly influences the protein's quality. Since the nutritional value of proteins is closely linked to their essential amino acid profile, it is crucial to investigate whether different drying techniques affect this profile. As seen in the result, the composition of amino acid of various dried ASPI powder was the same but different in the content. This can be linked to the presence of free amino acids in the ASPI, which leads to variations in the amino acid composition across different drying techniques. Furthermore, while undergoing the drying process, the protein may experience different levels of denaturation, leading to uneven levels of amino acids. The FAO suggested that essential amino acids should constitute more than 36% of the total amino acid content in proteins and the essential amino acids in FD and SD-ASPI were 38.11 % and 37.81 %, respectively, exceeding the 36 % threshold. According to the FAO, ASPI is the superior source of essential amino acids because its content surpasses the recommended threshold of 36 %. Out of all amino acids, lysine is sensitive to heat and often considered an indicator of the Maillard reaction. The analysis showed that OD, AD and VD-ASPI had the least amount of lysine, with 5.57 %, 5.58 % and 5.79 %, respectively. This suggests that the heating process in the OD, AD and VD methods started the Maillard reaction (Bhandari et al., 2013; Feyzi et al., 2018). Aspartic acid ranked first among the non-essential amino acids in all different dried ASPI in terms of ratio, followed by glutamic acid, glycine, serine, alanine, arginine, and proline. The protein efficiency ratio (PER) is the primary metric for assessing the nutritional value of protein in a diet. It quantifies the amount of weight gained per gram of protein. The quantity of PER-1, PER-2 and PER-3 are determined by the levels of leucine and proline, leucine and tyrosine, and methionine, leucine, histidine, and tyrosine, respectively. Typically, a PER number below 1.5 is seen as substandard. Conversely, a PER number over two is said to be of superior quality. ASPI was considered a dependable protein source with excellent nutritional value. The results shown that all ASPI drying techniques yielded higher PER values, with the FD method exhibiting the highest PER value in comparison to other drying techniques (Li et al., 2022; Shahidi et al., 1992). Among all the drying methods, freeze-dried (FD) samples retained the highest levels of essential amino acids, while oven-dried (OD) and vacuum-dried (VD) samples had the lowest amounts. This difference may be attributed to the dehydration stress that causes amino acids to be released during protein solubilization, which in turn promotes the Maillard reaction and the formation of carbonyl compounds at high temperatures. The Maillard reaction particularly affects essential amino acids, which are key reactants, resulting in their reduced presence in ambient-dried (AD) and spray-dried (SD) samples. The Maillard reaction likely also contributes to the increased loss of free amino acids. Additionally, OD and VD methods resulted in the lowest total amino acid content, likely due to extended heating times that promote the decomposition of heat-sensitive essential amino acids. Additionally, the majority of amino acids are found in the outer layer of the pollen membrane. This layer is rich in proteins and lipids, so it may undergo lipolysis, proteolysis, or enzymatic oxidation when exposed to heat, oxygen, or enzymes. This can trigger Maillard reactions and Strecker degradation, leading to the development of unique flavor compounds. Proteases break down proteins into large peptides, which further degrade into smaller peptides and free amino acids.

Secondary structure of protein is widely determined by FTIR spectrum, as it is sensitive to variations in the peptide chain's conformation. FTIR spectroscopy generates a spectrum characterized by changes in the vibrational motion of molecules caused by infrared radiation is absorbed by bonds within chemical functional groups, causing them to vibrate by bending and contracting (Du et al., 2018). This section analyzed the bands in the amide A, B, I, II, and III, areas to identify the unique characteristics of proteins. Carbonaro et al., (2008) found that the wavelength range of 1600-1700 cm-1 is associated with the amide I region. Approximately 80 % of the amide I region is composed of C-O strain vibrations, with the remaining 20 % attributed to N-H bending in the peptide bond. The Amide I band of proteins is a complex structure composed of various elements such as α-helix, β-sheet, random coil, or β-turn (Liu et al., 2009). The studies conducted by Kudre et al. (2013) and Liu et al. (2009) have demonstrated the presence of the following secondary structures in the amide I region: α-helix, which can be seen at around 1648-1658 cm-1; β-sheet, which can be seen at 1610-1640 cm-1; random coil, which can be seen at 1640-1648 cm-1; and β-turn, which can be seen at 1660-1688 cm-1. Generally, all drying techniques of ASPI were shown to have FTIR spectra at the wavenumbers 1656.41 cm-1, which corresponds to amide I (1700-1600 cm-1). These reports indicate that the α-helix structure is important in all dried methods of ASPI. The amide II band of ASPI prepared by different dried techniques exhibited a peak at around 1527.89 cm-1. The amide II region represents N-H bending and C-N stretching vibrations with values of 40-60 % and 18-40 %, respectively, occurring within the wavelength range of 1500-1550 cm-1. The major peaks that indicate the presence of proteins in the structure are referred to as the amide I and II bands (Withana-Gamage et al., 2011). The amide III band is indicated in the wavelength between 1220 and 1330 cm-1, exhibited peaks at a wavelength of approximately 1313 cm-1 for all different dried methods of ASPI. Examining the Amide III region allows for the detection of protein interactions with other macromolecules like carbohydrates (de la Rosa Millán et al., 2018). According to the Feyzi et al. (2018), the bands in the spectrum from 3200 to 3500 cm-1 are known as the amide A area, and they represent the O-H bond stretching vibration in proteins or nearby water molecules. The occurrence of a peak at approximately 3221 cm-1 in the FTIR spectrum indicates an interaction between protein and water molecules, associated with the stretching vibration of the O-H bond. The amide B area is observed at wavelengths (2850-2980 cm-1) and it is associated with the asymmetric stretching vibration of C-H, influenced by the presence of proteins, neutral lipids and carbohydrates. The detected wavelength of approximately 2932 cm-1 in the FTIR spectrum corresponds to the amide B area. Pietrzak and Miller, (2005) attributed the peaks detected at a wavelength of 1000-1200 cm-1 to the stretching vibration of C-H bonds, which arise from carbohydrates like cellulose and starch. The study examined the carbohydrate content of different dried methods of ASPI, which varied from 16.63 to 22.1 grams per 100 grams, as shown in Table 4.2. The FTIR spectrum exhibited peaks around 1030 cm-1, indicating the presence of carbohydrates in the protein isolate. According to the study conducted by Rahman et al. (2019), several factors can lead to the denaturation of the secondary structure of proteins. These factors include exposure to high temperatures, prolonged drying periods, and utilization of organic solvents. The proteins underwent denaturation due to the sustained elevated temperature employed in the oven drying techniques. Lin et al.

(2020) found that freeze drying can cause proteins to unfold and aggregate, leading to a decrease in their secondary structures. Rahman et al. (2019) noted that slight shifts in wavenumbers during the extraction protein could be influenced by pre-processing factors such as extraction oil by hexane, cold-press extraction oil and supercritical CO2 pressure. Generally, the sample dried by SD demonstrated higher intensity at the amide I, II, and III bands compared to other drying methods of ASPI. Additionally, proteins dried via spray drying exhibited a higher degree of phosphorylated chemical linkages.

The scanning electron microscope (SEM) is commonly employed for analyzing the microstructure of proteins. According to Lin et al., (2020)), SEM has many benefits, including the need for only a small amount of protein for analysis, high tool resolution, and good visualization of the shape of rough protein surfaces. The composition of this structure primarily consists of protein powders that have been water soluble. The asymmetrical spherical shape could potentially be attributed to the uneven dispersion of protein and water (Osthoff et al., 2010). The dried methods, including FD, VD, FD and AD had large particle sizes. The surface morphology of various drying methods applied to ASPI was in line with prior research conducted by Brishti et al. (2020) and Joshi et al. (2011). These studies explored drying methods such as oven, spray, and freeze drying for mung bean protein, as well as vacuum, freeze and spray drying for lentil protein. Wang et al., (2020a) reported that when a protein solution is dried using a spray drying, it is dispersed into tiny droplets and loses moisture rapidly. This causes the particles to take on a spherical shape. Additionally, the water evaporates unevenly throughout the drying process, resulting in wrinkled particles. The freeze dried avocado seed protein exhibited larger particle size and featured a thin layer with a flake-like structure when compared to SD avocado seed protein. The sublimation process in FD may result in bigger particles and a flake like structure his is because of the low temperature and the lack of disruptive forces that would typically fragment the frozen liquid into droplets (Lin et al., 2020). In contrast, an extended period of drying at an elevated temperature of 50 °C for 24 h in OD and VD causes water molecules to migrate from within the sample and evaporate quickly from its surface, resulting in the presence of a crystalline structure (Rudra et al., 2016). These findings indicate that various drying techniques can yield distinct microstructures in protein samples.

The thermal properties of ASPI obtained by using five various dried techniques, including SD, FD, VD, OD and AD were analyzed based on the TGA curve. This curve illustrates the thermal characteristics of the material. The initial weight loss occurred at a temperature lower than 200 °C. The evaporation of water may be the first heat event in polymeric materials that is associated with the evaporation of water. With rising temperature, the water contained within the proteins evaporates, causing a reduction in weight (da Costa Neto et al., 2019). The degradation or decomposition temperature of the protein's polymer chains begins after the full elimination of water and reaches 165 °C (Başyiğit et al., 2020). The weight loss continued until around 500 °C, at which point a noticeable and swift mass decline signaled the start of the second stage. This phase, characterized by protein decomposition, results in the most substantial decrease in weight. The results were similar to the previous studied by (Başyiğit et al., 2020), they investigated that he maximum weight loss occurred within the temperature range of 170 to 500 °C. The following sequence shows the minimal weight reduction during this stage. The first noticeable degradation and the highest rate of decomposition occurred around 170 °C. Polysaccharides' characteristic behavior aligns with this thermal degradation profile. Depolymerization is the process of breaking down macromolecules by heat breakdown, resulting in their eventual volatilization.

The solubility of proteins is an essential characteristic in the food system, as it directly influences the other functional qualities like emulsions and foaming. Protein solubility is dependent on the denaturation process and the specific arrangement of amino acids inside the proteins. Moreover, a significant degree of solubility in proteins acts as a dependable indicator of a native protein (dos Santos et al., 2009; Ye et al., 2024). Proteins must be in a water-soluble state, just like any other powdered substance. High resolution also enables easy integration of proteins into various applications while adding functional properties to them. Researchers have conducted various studies to enhance protein solubility and identify the factors influencing solubility. Studies have emphasized that proteins dissolve better in alkaline conditions when investigating the effect of pH change on protein solubility (Ghribi et al., 2015; Li et al., 2022). Moreover, these investigations noted that solubility increased as proteins moved farther away from their isoelectric point. This thesis study investigated how various drying methods affect the solubility of protein extracted from avocado seed using alkaline extraction followed by isoelectric precipitation techniques. Joshi et al., (2011) converted the protein extraction obtained from lentils into powder by using vacuum, spray and freeze drying techniques and compared the solubility behavior and the result show that the solubility of dried protein by spray was 81.19 %. The solubility value of dried protein obtained by the freeze was noted at 78.39 %. However, this study on lentil protein isolate found that the vacuum dried technique had no reasonable solubility for obtaining protein powder (50.34 %). In another study, Ghribi et al., (2015) found that the FD technique gave better results than the ventilated dryer technique 40 °C and 50 °C in terms of the solubility of protein concentrates extracted from chickpeas. According to Hu et al., (2009) obtaining protein from soy and dried into powder by spray drying was more suitable in terms of solubility than FD and VD techniques. Moreover, they were determined that the solubility of protein from soy and dried into powder at pH 7 was 70.20 % for SD, 40.80 % for FD, and 37.60 % for VD. The superior solubility behavior of dried powders by the SD techniques can be attributed to their small and consistent particle size distribution, as well as the minimal protein denaturation occurring during the process. The reason why the solubility of proteins obtained by FD is lower than that obtained by SD can be explained by the drying time. Similarly, in the other two drying processes, the long processing time and the application of heat in the OD may have caused a decrease in the solubility (Joshi et al., 2011; Ghribi et al., 2015). The drying process may result in reduced protein solubility as a moisture-resistant film forms. Proteins unfold at the particle-air interface to form this film (Brishti et al., 2020). The factors influencing the alteration in protein solubility include repulsion, increased charge, decreased hydrophobic residues, electrostatic and as well as ionic hydration at varying pH levels influence the change in protein solubility (Ye et al., 2024). Considering the data collected and information from the literature, it can be concluded that drying ASPI powder with the SD technique is a more reasonable approach compared to other drying methods for achieving the desired targets in terms of solubility within the scope of this study.

The presence of additives in food alters the sensory and physical aspects of the final product, including its viscosity and characteristic structure, due to the water they retain. While this is a favorable quality for development in certain foods, it may also be an undesirable situation in other foods (Ghribi et al., 2015). In the dough, soup, milk dessert, and pastry products industries, water retention capacity is a desirable functional characteristic, especially when high viscosity values are required. When there is a need to retain water, it is necessary to have protein structures that can retain water without dissolving. This increases the thickness of the liquid and gives the finished product a thick and sticky consistency. The ability of proteins to retain water is a fundamental functional characteristic in the food production sector (Brishti et al., 2020). The protein's ability to retain water depends on its structure, size, and interactions between hydrophobic and hydrophilic components. The presence of fat and carbohydrates in the environment, as well as the quality of amino acids in the environment, also affect WHC. These factors could explain the superior WHC of SD protein, including its smaller particle size and increased surface area. The results of this present study correspond with those reported in the previous research conducted by Shen et al., (2021), who founded that protein was extracted from quinoa and dried into powder using SD, FD, and VD. SD quinoa protein had significantly different WHC values between freeze and vacuum drying, with values of 2.76, 1.84, and 1.46 g/g, respectively. However, Timilsena et al. (2016a) found that statistically, the WHC of chia seed protein powder obtained through spray and vacuum drying methods showed no significant difference. Some factors including the sources of protein isolated, protein structure, pH changes, and the presence of polysaccharides in proteins, can influence the WHC (Steffolani et al., 2016).

OHC is the interactions between fat and protein. OHC is the most crucial factors that impact the sensory and physical characteristics of food products. The nature of these interactions can be impacted by several factors including temperature, pH and other environmental conditions. Research on proteins has revealed that protein molecules exhibiting elevated hydrophobicity has a greater capability for binding oil. Research has shown that protein powder forms with low density and small particle size have a greater capacity to bind oil compared to forms with high density (Brishti et al., 2020). Our findings, similar to those of Lin et al., (2020), revealed that SD technique of Antarctic krill protein had the maximum OHC with the value 2.65 mL/g compared to the FD and OD techniques with the value 1.71 and 1.49 mL/g, respectively. Brishti et al., (2020) reported that various factors affect the OHC, including sources of extracted protein, the size and concentration of the protein, the quantity of nonpolar amino acids, manner of processing, and interactions between protein and lipids.

The emulsion activity (EA) refers to the protein molecule's capacity to adhere to the interface during the formation of an emulsion containing two or more immiscible liquids, such as oil and water. The emulsion stability (ES) serves as a measure of the long-lasting nature of the emulsion. The term refers to the duration for which the produced emulsion maintains its stability without experiencing any phase separation. The emulsion activity and stability index quantifies proteins' emulsifying properties. The emulsion activity refers to the proteins' capability to create emulsions and their capacity to absorb oil and water in the interface region. Also, the stability of emulsions is determined by their ability to remain stable over a specified period and the term describes how long an emulsion remains stable without separating into phases (Karaca et al., 2011; Ye et al., 2024). The emulsion properties of proteins are primarily identified by several factors including the concentration of protein and surface hydrophobicity. Apart from these factors, several other criteria such as the sources of protein isolated, concentration, solubility of protein, temperature, environmental pH, and the method and equipment used also influence the ability of proteins to form emulsions (Wang et al., 2022). Studies on the emulsion activity index of protein molecules have concluded that it is parallel to solubility, conformation stability, and hydrophobicity. When it comes to the emulsion stability index, proteins with more surface charge and better solubility do better. On the other hand, when surface charges interact with hydrophobicity, it makes emulsion stability worse (Li et al., 2021). Similar phenomenon was also observed with Lin et al., (2020), Brishti et al., (2020) and Feyzi et al., (2018), they were extracted protein from Antarctic krill, mung bean and fenugreek, and drying isolated protein into powder by spray, freeze and oven drying. Moreover, the EA and ES of spray drying method were superior to freeze and oven drying methods.

Research indicates that there is a direct relationship between emulsion properties and solubility (Zhao et al., 2012). This approach showed that the SD technique's emulsion properties were superior to those of the other dried techniques, depending on the solubility as shown in figure 4.5 SD methods had the best solubility. The thermal effects of spray drying, where the outlet temperature reaches 90 °C, contribute to the elevated EA and ES values of SD, as found by (Brishti et al., 2020). This heat causes the globular proteins to undergo partial unfolding, which leads to hydrophobic amino acid residues being exposed to the surrounding environment. Consequently, this exposure enhances the adsorption at the oil in water interface. However, emulsifying activities decreased as a result of prolonged and extensive heating during the AD, OD, and VD methods. This is because heat-induced aggregation reduces protein solubility and surface hydrophobicity (Lin et al., 2020).

Foaming is a system where air cells are separated from the liquid layer by a thin film. This system is not very stable. Swiftly lowering the tension between the air and water phases enhances the stability of the foam layer. Surfactants improve the stability of the foam layer by quickly reducing the tension between the air and water phases. Protein hydrophobicity is the most essential physicochemical property of protein, along with its interfacial property and foam retention ability (Dakhili et al., 2019). The main factor for foaming capacity is the rapid reduction of the tension at the interface between water and air during the whisking or air injection process (Amagliani et al., 2021). Protein type, preparation method, solubility, composition, and concentration, as well as the pH of the environment, hydrophobic interactions and the presence of salt in the environment, affect foam capacity and stability. At the same time, some reactions and changes in molecules that occur with different applications affect foam capacity. There are also things like net charge, molecular flexibility, charge distribution, surface hydrophobicity, and hydrodynamic properties that affect foam capacity and stability (Zhao et al., 2021). When proteins are soluble and have a positive charge on the surface and there is an ideal balance of hydrophobic amino acids and hydrophilic, they can make more foam (Shevkani et al., 2015; Lafarga et al., 2018).

The increase in foaming stability depends on the dissolution of proteins that can be dissolved in the continuous liquid phase and their surface-active properties. An increase in protein interaction in the food leads to higher viscosity and improved foam stability of the film layer formed at the interface (Ye et al., 2024).

Li et al. (2022) discovered that dried protein isolate from Rana chensinensis ovum into powder by spray drying resulted in enhanced foaming capacity, attributed to its smaller particle size. Also, the rapid absorption of tiny particles into the gas-liquid interface during the stirring phase leads to an increase in foam volume. The investigation yielded consistent results, indicating that the gelatin derived from goat skin using the SD drying process exhibited superior foaming capabilities compared to other drying methods (Mad-Ali et al., 2016). According to Shavkani et al., (2015), several factors affect a substance's ability to foam and its stability. These include the protein's high solubility, tendency to become denaturated, and tendency to make the surface less water-repellent. The reason vacuum, oven, and ambient drying methods don't work as well at foaming and staying stable is because their surfaces are less hydrophobic and don't dissolve as easily.

According to Caballero and Davidov-Pardo, (2021), the color properties of food products are among their most crucial characteristics. Consumers typically first accept food products based on their color and appearance. The L* parameter is related to lightness with the values from 0 to 100, black to white, respectively. Regarding L* values, the SD-ASPI+LBG sample was lighter with a value of 31.78 than AD-ASPI+LBG, VD-ASPI+LBG, FD-ASPI+LBG, and OD-ASPI+LBG with values of 29.09, 28.12, 26.77, and 26.62, respectively. In terms of a* parameter, it was found that the value of a* was between 2.17 and 3.81, and the minimum a* value was AD-ASPI+LBG (2.17), while the maximum was SD-ASPI+LBG with the value of 3.81. Protein drying methods influenced the variation of the b* parameter. SD-ASPI+LBG had the superior b* with the value of 2.98, following OV-ASPI+LBG, FD-ASPI+LBG, VD-ASPI+LBG, and AD-ASPI+LBG with the values of 1.88, 1.78, 1.57, and 1.34, respectively. Moreover, the a* and b* parameters are represented reddish to yellowish, and the value of yellowness of SD-ASPI +LBG was the highest compared to other hydrogel samples. The findings indicate that the methods used to dry proteins significantly affect the color of the hydrogel system. SD-ASPI+LB exhibited higher values for all of these parameters. The hydrogel samples had a low L* value, indicating a pale black color. The observed behaviors are attributed to the Maillard reaction, which takes place between protein and carbohydrates during hydrogel formation at elevated temperatures.

A crucial functional feature of three-dimensional networks is their water holding capacity (WHC), which indicates the hydrogel's ability to retain water (Wang et al., 2019). Lu et al. (2020) reported that three-dimensional networks possess a greater capacity for water absorption. These findings were consistent with hydrogels' mechanical characteristics and SEM images. SD-ASPI+LBG produced the highest quality hydrogel among the various drying methods used to fabricate the hydrogel networks, followed by FD-ASPI+LBG, VD-ASPI+LBG, OD-ASPI+LBG, and AD-ASPI+LBG. These results were noted after the morphological structure was analyzed for each hydrogel sample. The study findings indicate that ASPI, when dried using spray and freeze dryer methods, exhibited a higher level of tolerance to centrifugal force (Rahman et al., 2019). The rise in the quantity of intermolecular linkages inside the hydrogel may contribute to the increase WHC in the hydrogel (Cui et al., 2020). Furthermore, According to the earlier research, hardness and WHC are positively correlated (Caballero and Davidov-Pardo, 2021). As mentioned earlier, SD-ASPI+LBG and AD-ASPI+LBG exhibited the superior and minimum hardness values, respectively. Additionally, WHC can serve as a reliable indicator of stability and strength in hydrogels. An increase in WHC suggests that hydrogels are likely to exhibit greater stability and strength (Yang et al., 2021a).

One of the most essential functional properties of hydrogel is its swelling ratio. This is because swelling properties display the cell adhesion of hydrogel, as adhesion plays a vital role in the oxygenation and uptake of nutrients by cells (Zhang et al., 2021). Gunasekaran et al., (2005) define the swelling ratio in other words; the swelling ratio is the most useful functional property of hydrogels, especially when it is utilized as a delivery system for bioactive compounds. The structure of the hydrogel samples (FD-ASPI+LBG and SD-ASPI+LBG) was more compact, as seen in the SEM image. This made the swelling ratio higher (Khubber et al., 2021). Gunasekaran et al., (2007) found that the swelling ratio is balanced by two conflicting forces: the solvent attracts polar and ionic groups, causing expansion of the hydrogel matrix, while the cross-linked hydrogel structure system exerts a contraction force. According to earlier studies, various factors influence the swelling properties. The incorporation of both protein and carbohydrates in the preparation of hydrogel results in a higher swelling ratio compared to using protein alone (Zhang et al., 2021). Another possible cause for the swelling ratio could be the electrostatic repulsive force between two polymers (Maltais et al., 2009). The final aspect might involve the presence of numerous hydrophilic groups within the hydrogel (Netanel Liberman et al., 2021). The AD techniques of ASPI based on the fabrication hydrogel had a much lower swelling value than the other hydrogel samples because they partially broke down inside them (Maltais et al., 2009).

The term "protein leachability" describes the movement or release of proteins from a hydrogel's three-dimensional structure (He et al., 2021b). The mechanism of hydrogel proteins' release significantly impacts their stability, functionality, and capacity to interact with their surroundings (Başyiğit et al., 2024). The research discovered that protein leachability means the ability of proteins to move from hydrogel systems into surrounding environment (pH 7.0, 0.05M) sodium phosphate buffer. High-quality hydrogels minimize the rate of protein leaching from hydrogels. Various dried avocado seed proteins in the presence of locust bean gum are used to make hydrogel system, and this section investigates the proportion of protein leached from these hydrogels. Table 4.8. showed the results of protein leachability in hydrogel samples. Our study's findings on protein leachability closely resembled those reported in a prior study by (He et al., 2021b). That study also looked at whey protein isolate hydrogels with different amounts of Lycium barbarum polysaccharides and found similar levels of protein leachability. Also, Demirkıran et al., (2022) used microbial transglutaminase modification to check how easily the proteins could be leached from hydrogels made by adding pectin to the protein from sour cherry seeds. As stated in the literature, this modification enhances the leachability of proteins. Rising the number of covalent bonds were formed in the presence of polysaccharides may contribute to the low leachability observed in the SD-ASPI+LBG hydrogel system. This results in proteins being bound more firmly to the structure (Wang et al., 2020c).

The FTIR spectrum was used to find out how the chemicals in each hydrogel made from different dried methods of ASPI and locust bean gum (LBG) interacted with each other. Furthermore, The transmission range of 4000-500 cm-1 utilized to examine the FTIR spectra of the ASPI samples based on the hydrogel (Rahman et al., 2019). FTIR spectra for various dried techniques of ASPI powder were discussed in the earlier part of the study. The primary absorption peaks of the hydrogel sample are observed at 3215 cm-1 and 2921 cm-1 corresponding to the stretching vibration O-H bonds and C-H bonds, respectively. The absorption peaks seen at 1639, 1529, and 1377 cm-1 were identified as the amide I, II, and III bands of CH, respectively (Ebrahimi et al., 2016). When compared the wavelength of amide A between hydrogel samples, the bands of SD-ASPI+LBG had the maximum wavelength (3215 cm-1) following by FD-ASPI+LBG (3220 cm-1), VD-ASPI+LBG (3234 cm-1), OD-ASPI+LBG (3228 cm-1), and AD-ASPI+LBG (3230 cm-1). Barth, (2007) and Wang et al., (2020b) reported that upon the addition of carbohydrate, it is important to observe that the absorption band (~3300 cm-1), which is related to the -OH stretching vibrations and changed to a lower wavenumber. This shift indicates a strengthening of the interactions between the hydroxyl groups of the protein and carbohydrate molecules. This finding is consistent with our hypothesis that the formation of hydrogel is significantly influenced by the enhanced hydrogen bonds between locust bean gum and protein molecules. He et al. (2021) reported that adding pectin to the ginkgo seed protein did not create new peaks in the spectrum of hydrogel. Also, Başyiğit et al., (2023) studied the fabricate hydrogel from soy protein and different carbohydrates such as gum arabic, maltodextrin, pectin, and locust bean gum, and they found that the composition of the hydrogels did not display any new covalent bands which means that did not create a new peak in the spectrum. These findings indicated the existence of electrostatic interactions among the carboxyl groups in different drying methods of ASPI and LBG. One more thing is that the peak area of O-H and N-H in SD-ASPI+LBG was the lowest compared to the other hydrogel samples. This means that hydrogen bonding probably played a part in making SD-ASPI+LBG hydrogels. Lastly, locust bean had no influence on the amide I, II, or III regions, according to the FTIR analysis of the dried hydrogel. Extracted protein was dried into powder by different methods of drying; particularly, the spray drying method raised awareness about the functional properties, mechanical properties, and delivery of bioactive substances in comparison to other drying methods.

The hydrogel-based fabrication process demonstrated a three-dimensional polymer network for all drying techniques. Nevertheless, the degree of contact between polymer chains varied while analyzing SEM image. Significant alterations were noted in the physical forms of different dried ASPI based on the hydrogels system, contingent upon the particular methods of drying employed. The observed behaviors can be ascribed to an increase in hydrogen bonding and stronger interactions electrostatic between carbohydrate and proteins inside the hydrogel

(Başyiğit et al., 2023). The presence of additional bonds of hydrogen and increased electrostatic interactions inside fabricated hydrogel result in the creation of larger and more rigid structures (Başyiğit et al., 2023). To achieve a suitable three-dimensional structure, it is crucial for the polymers in the hydrogel system to have strong interactions (Slavutsky and Bertuzzi, 2019). According to Demirkıran et al., (2022), the hydrogel system has a strong structure that has superior functional characteristics. Reviewing all the findings from the current investigation, including the data in Table 4.8 and Figure 4.10, further confirmed this phenomenon. The results of various fabricated hydrogels, including functional properties, textural profile, and rheological behavior for SD-ASPI + LBG and FD-ASPI + LBG, were in line with what was seen when pictures and scanning electron microscopy images were looked at.

The hydrogel's texture profile was evaluated based on characteristics like hardness, chewiness, gumminess, springiness and resilience, and hydrogel samples were prepared from various dried methods of ASPI with LBG, namely SD-ASPI+LBG, FD-ASPI+LBG, VD-ASPI+LBG, OD-ASPI+LBG and AD-ASPI+LBG. The hardness of the hydrogel was assessed by measuring its resistance to compression from the initial stage to the point of maximum force. The measurement of the springiness of hydrogel samples was calculated by the ratio of the distance between the input force during the second compression and the initial compression. Resilience is measured by the ratio of the upward force during the compression phase to the downward force during the decompression phase (Wang et al., 2019). Gumminess can be employed as an indicator to demonstrate the condition of a semisolid sample, and it is determined through the computation of hardness multiplied by cohesiveness (Wang et al., 2017). Chewiness is a characteristic that may be used to evaluate the state of a solid sample. The value is derived by the multiplication of hardness, cohesiveness, and springiness, (Yuan et al., 2016). Ikeda and Morris (2002), found that the structure of the protein network is mostly set by the balance between the forces that pull and push the molecules of proteins together, and this is based on the hydrogel that was made by heating the proteins. Zhang et al., (2021) discovered that there are two main factors that might increase the hydrogel's hardness. The first is that increasing the pH value of hydrogel beyond its isoelectric point enhances its hardness. Furthermore, increasing the quantity of protein or carbohydrate during hydrogel fabrication can enhance its solid content. There are many studies that supported the texture result of this study. According to Zhou et al., (2014); Guo et al., (2015); Lei et al., (2016); Lei et al., (2017) the researchers investigate the impact of basic amino acids on hydrogel. They observe that these amino acids form strong bonds with protein-charged residues via electrostatic interactions, resulting in alterations to the thermal properties and structure of proteins. The attributes of springiness, chewiness, gumminess, and resilience exhibited similar trends with hardness. Furthermore, FD-ASPI + LBG and SD-ASPI + LBG exhibit superior values for gumminess, chewiness, resilience, and springiness compared to other hydrogel ($p < 0.05$). The dataset supports all the observations made about the appearance, functionality, SEM image, and rheological of hydrogels.

The rheological properties associated with the mechanical characteristics were examined by assessing the oscillation moduli at different temperatures, ranging from approximately 20 to 60 °C. The rheological properties of the fabricated hydrogel were determined by measuring the storage modulus (G′) and loss modulus (G″) values. These values give information about the elastic and viscous properties, respectively. The curves had similar patterns in terms of the values G′ and G″, and as the temperature went up, the G′ and G″ values of plant protein-based natural hydrogels slowly went down. This demonstrated how the hydrogel's three-dimensional structure gradually changes from a solid-like gel to a liquid-like solution during the carving process. However, the rheological behavior of hydrogels was tremendously dependent on the various drying techniques utilized in fabricated hydrogel. It seems that using different dried forms of ASPI, such as AD, OD, VD, FD, and SD, to the hydrogel matrices caused the rheological features to rise, but they was motion at vary level (Demirkıran et al., 2022; Başyiğit et al., 2024). The results confirmed that the spray drying technique was the most rigid method for constructing the internal structure of three-dimensional networks. The amount increased of electrostatic complexes and H-bonding between the drying protein by the spray dryer and LBG may explain the presence of more junction zones, as mentioned in the section FTIR analysis. Moreover, According to the results, G′ exceeds G″ for all hydrogel samples, suggesting that hydrogels with a three-dimensional structure are successfully forming (Netanel Liberman et al., 2021). In addition, the differences in the values of G′ and G″ indicated the presence of hydrogel models characterized by dominance of elasticity rather than viscosity. The aim of this test in the current investigation was to examine whether different protein drying methods had a notable effect on the creation of a three-dimensional hydrogel involving carbohydrate involvement. The findings indicated that spray drying and freeze drying were effective methods for protein drying and hydrogel formation, as shown by the elastic modulus G' and the viscous modulus G". In addition, these drying techniques including spray and freeze outperformed other dryers in terms of hydrogel formation. Ruiz et al. (2016) found that proteins with high solubility could form well-structured hydrogels. In this context, SD techniques for avocado seed protein isolate demonstrated superior solubility compared to other drying methods, including ambient drying, oven drying, vacuum drying, and freeze drying, as detailed earlier. The processing of the SD-ASPI+LBG hydrogel samples was less denatured than those made with AD-ASPI+LBG, OD-ASPI+LBG, VD-ASPI+LBG, and FD-ASPI+LBG. So, it kept most of its natural properties and was easier to dissolve, which improved protein interactions and aggregation when heated, making it easier for hydrogel networks to form. This finding aligns with the observations of Joshi et al. (2011) and Shen et al. (2021), who reported that lentil proteins dried by vacuum and freeze drying could hardly form hydrogels as quickly as spray-dried lentil proteins due to poorer solubility, thus requiring higher concentrations or longer heating times to achieve hydrogel formation.

Ascorbic acid, also referred to as vitamin C, is a molecule that readily dissolves in water. This substance is an organic compound that possesses a crystalline structure and a white color. Fresh fruit can be the best source for extracting this structure. Ascorbic acid, a bioactive substance, exerts beneficial effects on human health. Its primary purpose in the human body is to stimulate several enzymes in the liver that are involved in hormone regulation, nervous system function, and drug detoxification. Moreover, this chemical demonstrates both anti-inflammatory and antioxidant properties and it plays a vital role in the treatment of a variety of illnesses, including cancer, neurological disorders, and cardiovascular diseases (Figueroa et al., 2018). Before being used in the described systems, it is necessary to encapsulate ascorbic acid into different matrices in order to enhance its stability and bioavailability. Achieving this condition involves integrating these substances into the hydrogel matrix through either physical entrapment or chemical cross-linking techniques, which are carried out during the formation of three-dimensional polymeric networks (Ciolacu et al., 2012). Bioactive molecules are released rapidly and cumulatively from the hydrogel via a burst release mechanism. This approach may result in a rapid exhaustion of the molecule and a subsequent decline in effectiveness over time, rendering it unfavorable for use in delivery systems (Chen et al., 2023). Consequently, these systems can be avoided in several applications, including cosmetics, medications, and food preservation (Lupu et al., 2023). Out of the five various dried methods used to dry avocado seed protein, the spray drying method was selected to study release behavior. This choice was based on the observed correlation between the size of pores (swelling ratio) and the rate of release. Ascorbic acid solution added to a sample with a low swelling ratio caused the hydrogel matrix to break down, which physically disrupted the three-dimensional hydrogel network. This was found by Başyiğit et al., (2024) the textural and rheological findings of ASPI-based natural hydrogels also supported this approach. Actually, it is possible to make hydrogels from plant-based proteins and LBG works better in bioactive compound delivery applications compared to ASPI alone. To put it another way, the datasets made it clear that drying ASPI in a spray dryer with LBG was good for the hydrogels. Consequently, these hydrogel matrices have the potential to enable precise administration of drugs to specific targets.

6. CONCLUSION

In recent years, consumer interest in functional and ready-to-eat foods has been increasing. As ready-made food consumption increases, waste in food processing stages has also begun to increase. Recently, there has been a significant increase in the importance of utilizing these resources, known as waste but possessing high functional value as biotransformation. Bioconversion not only reduces environmental pollution caused by waste but also makes significant contributions to companies and the country's economy.

The avocado seed protein isolate was subjected to several drying techniques, including spray, freeze, vacuum, oven and ambient drying. The investigations identified alterations in many functional, chemical, and physical characteristics of different dried avocado seed proteins powder and established the most effective drying methods. The results indicate that the drying techniques can enhance and maintain the functional characteristics of avocado seed protein. The data indicate that protein extracted from plant seeds has the potential to improve the quality and stability of food products during production stages. The study's findings are described below based on the collected data.

The physicochemical characteristic of the ASPI powder obtained by various drying techniques were determined by analyzing protein content, moisture determination, water activity, and fluidity (Carr index and Hausner ratio). The FD technique had the highest protein content of 67.3 % among the various drying techniques used for ASPI, followed by the SD, AD, OD, and VD techniques with protein contents of 65.9 %, 64.8 %, 62.3 %, and 62.1 %, respectively. In the moisture content of dried ASPI powder, the SD technique had a value of 4.22 %, which is the highest value compared to the other dried techniques. The lowest value of water activity of dried ASPI powder was 0.27 for the OD technique, while the highest value was 0.38 for the AD technique. SD techniques exhibited the highest carr index with the value 38.77 and hausner ratio with the value1.63 for dried ASPI powder. It can be said that the fluidity is lower than in other dried techniques.

The amino acid composition of different dried avocado seed protein powders contains total essential amino acids with the value 38.11, 37.81, 35.89, 34.99, 34.72 % for freeze, spray, ambient, vacuum and oven dried, respectively. The values of essential amino acids in spray-dried and freeze-dried avocado seed protein powder were similar isoleucine (SD: 5.47 % and FD: 5.59 %), leucine (SD: 6.27 % and FD:

6.56 %), lysine (SD: 6.05 % and FD: 5.95 %), threonine (SD: 5.18 % and FD: 4.83 %), and valine (SD: 6.29 % and FD: 5.89 %). The total amount of non-essential amino acids for spray, freeze, vacuum, oven and ambient dried were 62.18, 61.89, 65.01, 65.28 and 64.11%, respectively.

The physical and quality properties of different dried avocado seed proteins were determined by scanning electron microscopy (SEM). The protein that underwent spray drying had a consistent distribution of particle sizes and a circular shape. Additionally, the particles had a smooth surface and a spherical structure. FTIR analysis indicate that all drying methods can be used for drying proteins, as they clearly show the presence of amide groups with minor variations. The thermal properties of various dried ASPI powders were analyzed according to TGA curves. All dried techniques exhibited similar thermogravimetric stability. These values are considered reasonable levels in light of the sterilization processes applied in the food industry.

The functional properties of dried ASPI powders were determined among five drying techniques. The solubility of the different dried ASPI powders was examined; the maximum solubility was observed as (82.52 %) in the SD technique, while the lowest was observed as (53.8 %) in the OD technique. The solubility values in FD, VD, and AD techniques were 75.22 %, 68.77 %, and 60.24 %, respectively.

The water holding capacity of the SD dried techniques of ASPI was determined to be 393.33 %, and it is noted that the maximum WHC among the other dried techniques of ASPI. The SD method exhibited the highest oil holding capacity among the various drying techniques used for ASPI, with values of 300.92 % (FD: 225.22 %), (VD: 98.32 %), (OD: 89.89 %), and (AD: 85.95 %). The drying techniques significantly affected the water and oil holding capacity.

The emulsion activity index of the SD technique of ASPI was the highest, with values of 49.84 m2/g compared to the other dried techniques. The emulsion stability index of the SD method of ASPI was 71.62 and 112.67 after 10 and 30 min, respectively. The emulsion activity and stability values for dried ASPI were the highest with the SD method than with the FD, VD, OD, and AD methods.

Foam capacity was measured for all dried techniques of ASPI powder. In general, comparing the results, it was concluded that the foaming capacity and foaming stability of the FD and SD techniques of ASPI powder were better than the

AD, OD, and VD techniques. The values of the foaming capacity of FD and SD were 81.73 % and 79.33 %, respectively, while after 10 and 30 min kept at ambient temperature, the foaming stability for FD was 46.49 % and 26.27 %, respectively, and for SD it was 45.93 % and 25.27 %, respectively.

In the second section of this study, various dried ASPI were used based on the hydrogel system. To achieve this, hydrogel was fabricated by combining different dried ASPI powders and LBG. The study looked at the function properties of fabricated hydrogel samples and found that spray dried protein based on a hydrogel system had the best WHC (93.79 %), swelling ratio (33.51 %), and protein leachability (7.99 %), in that order.

The FTIR spectra of SD-ASPI+LBG among the other hydrogel samples indicate a strengthening of the interactions between the hydroxyl groups of the protein and carbohydrate molecules, as evidenced by the shift to a lower wavenumber. Our hypothesis suggests that the enhanced hydrogen bonds between locust bean gum and protein molecules significantly influence the formation of hydrogel, which is consistent with this finding. The physical properties of hydrogel samples were analyzed by SEM; the structures of SD-ASPI + LBG and FD-ASPI + LBG were found to be denser (more compact). While other hydrogel samples showed a non-homogeneous porous structure. Based on the texture, SD-ASPI+LBG had the spurious properties of texture in terms of hardness, chewiness, gumminess, springiness and resilience.

The drying methods of ASPI significantly impacted the rheological behavior when measuring the values of G′ and G″. SD-ASPI+LBG exhibited the highest value of G′, followed by FD-ASPI+LBG, OD-ASPI+LBG, VD-ASPI+LBG, and AD-ASPI+LBG. The findings indicate that all hydrogel samples form hydrogels with a satisfactory three-dimensional arrangement, as the magnitude of G′ is greater than G″.

For release analysis, spray dried ASPI powder alone (as a control) and spray dried ASPI powder with LBG was used based on the hydrogel system. Ascorbic acid was administered into the hydrogel system by using injection. Over a period of 60 to 540 min at ambient temperature, the diffusion rate of bioactive chemicals from the hydrogel matrix into the surrounding habitats was measured. The surrounding environment included variations in pH value, molarity of NaCl, and temperature. The findings show that plant-based proteins and LBG enable the production of hydrogels that deliver bioactive compounds more effectively in various environments compared to ASPI alone.

7. RECOMMENDATIONS

In this thesis study, protein was extracted from avocado seeds and dried into powder using different drying methods. The results show that drying techniques significantly affect the properties of proteins. Experts believe that the new products obtained will lead to the development of new employment areas in the food industry and nutrition. Experts expect that waste utilization will greatly contribute to the country's economy.

In the next stages,

- Researchers can evaluate the functional properties of drying avocado seed protein in a spray dryer by varying the temperatures, flow rates, and feed rates.
- It is possible to see how the functional and physicochemical characteristics of avocado seed protein improve by using different modification methods, such as ultrasonic and microwave pretreatments.
- Researchers can combine avocado seed protein with different oils to create a coating material for microencapsulation applications.
- Adding modified proteins to food products allows for the investigation of their functional properties.
- Research can be conducted to enhance the properties of avocado seed isolate and avocado seed protein based on hydrogels by various applications.
- Avocado seed protein can be blended with other proteins, and microencapsulation methods can be employed for applications like coating materials or various types of oil coatings.

REFERENCES

Abraham, J. D., Abraham, J., & Takrama, J. F. (2018). Morphological characteristics of avocado (Persea americana Mill.) in Ghana. *African Journal of Plant Science*, *12*(4), 88-97.

Agarwal, D., Kim, E. H. J., Feng, L., Wade, C., Moggré, G. J., Morgenstern, M. P., & Hedderley, D. I. (2023). Microstructure, rheological and water mobility behaviour of plant-based protein isolates (pea and quinoa) and locust bean gum mixtures. *Food Research International*, *164*, 112311.

Agoreyo, B. O., Akpiroroh, O., Orukpe, O. A., Osaweren, O. R., & Owabor, C. N. (2011). The effects of various drying methods on the nutritional composition of Musa paradisiaca, Dioscorea rotundata and Colocasia esculenta.

Akpan, E. J., & Umoh, I. B. (2004). Effect of heat and tetracycline treatments on the food quality and acridity factors in cocoyam [Xanthosoma sagittifolium (L) Schott]. *Pakistan Journal of Nutrition*, *3*(4), 240-243.

Ali, W. N. S. W., & Tarmidzi, N. A. A. (2021). A Rare Case of Contact Allergy towards Impression Compound Material. *European journal of dentistry*, *15* (04), 798-801.

Alinejad, M., Motamedzadegan, A., Rezaei, M., & Regenstein, J. M. (2017). The impact of drying method on the functional and antioxidant properties of whitecheek shark (Carcharhinus dussumieri) protein hydrolysates. *Journal of Food Processing and Preservation*, *41*(1), e12972.

Amagliani, L., Silva, J. V., Saffon, M., & Dombrowski, J. (2021). On the foaming properties of plant proteins: Current status and future opportunities. *Trends in Food Science & Technology*, *118*, 261-272.

Araújo, R. G., Rodríguez-Jasso, R. M., Ruiz, H. A., Govea-Salas, M., Rosas-Flores, W., Aguilar-González, M. A., & Aguilar, C. N. (2020). Hydrothermal–microwave processing for starch extraction from Mexican avocado seeds: operational conditions and characterization. *Processes*, *8*(7), 759.

Ćorković, I., Pichler, A., Šimunović, J., & Kopjar, M. (2021). Hydrogels: Characteristics and application as delivery systems of phenolic and aroma compounds. *Foods*, *10*(6), 1252.

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science & Technology*, *80*, 51-60.

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., & Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science & Technology*, *80*, 51-60.

Araujo, R. G., Rodríguez-Jasso, R. M., Ruíz, H. A., Govea-Salas, M., Pintado, M., & Aguilar, C. N. (2021). Recovery of bioactive components from avocado peels using microwave-assisted extraction. *Food and Bioproducts Processing*, *127*,

152-161.

Arukwe, U., Amadi, B. A., Duru, M. K. C., Agomuo, E. N., Adindu, E. A., Odika, P. C., & Anudike, J. (2012). Chemical composition of Persea americana leaf, fruit and seed.

Assemand, E., Lacroix, M., & Mateescu, M. A. (2004). Protective role of l-tyrosine in the sterilization of Ceruloplasmin therapeutic protein by gamma-irradiation. *Radiation Physics and Chemistry*, *71*(1-2), 405-409.

Aydemir, L. Y., & Yemenicioğlu, 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.

Álvarez, G. E. G., Gutiérrez, A. M. L., Valencia, K. Á., Mossos, P. S., Rozo, D. L. S., & Hurtado, N. C. (2020). Genetic and chemical characterization of avocado commercial cultivars avocado of Risaralda Colombia. *Revista Brasileira de Fruticultura*, *42*, e-593.

Bangar, S. P., Ashogbon, A. O., Lorenzo, J. M., Phimolsiripol, Y., & Chaudhary, V. (2022). Recent advancements in properties, modifications, and applications of legume starches. *Journal of Food Processing and Preservation*, *46*(11), e16997.

Bangar, S. P., Dunno, K., Dhull, S. B., Siroha, A. K., Changan, S., Maqsood, S., & Rusu, A. V. (2022). Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications. *Food Chemistry: X*, *16*, 100507.

Barth, A. (2007). Infrared spectroscopy of proteins. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1767*(9), 1073-1101.

Bashir, S., Hina, M., Iqbal, J., Rajpar, A. H., Mujtaba, M. A., Alghamdi, N. A., ... & Ramesh, S. (2020). Fundamental concepts of hydrogels: Synthesis, properties, and their applications. *Polymers*, *12*(11), 2702.

Başyiğit, B., Altun, G., Özaslan, Z. T., & Karaaslan, M. (2024). Synthesizing mechanically robust natural pea protein hydrogels via deep cryogenic treatment: State of the art in bioactive compound delivery system. *Food Hydrocolloids*, *146*, 109202.

Başyiğit, B., Altun, G., Yücetepe, M., Karaaslan, A., & Karaaslan, M. (2023). Locust bean gum provides excellent mechanical and release attributes to soy protein-based natural hydrogels. *International Journal of Biological Macromolecules*, *231*, 123352.

Başyiğit, B., & Çam, M. (2017). Püskürtmeli kurutucu ile nane (Mentha piperita ve Mentha spicata) esansiyel yağı mikroenkapsülasyonu. *Harran Tarım ve Gıda Bilimleri Dergisi*, *21*(1), 24-34.

Başyiğit, B., Sağlam, H., Köroğlu, K., & Karaaslan, M. (2020). Compositional analysis, biological activity, and food protecting ability of ethanolic extract of

Quercus infectoria gall. *Journal of Food Processing and Preservation*, *44*(9), e14692.

Batista, R. A., Espitia, P. J., Vergne, D. M., Vicente, A. A., Pereira, P. A., Cerqueira, M. A., & Cardoso, J. C. (2020). Development and evaluation of superabsorbent hydrogels based on natural polymers. *Polymers*, *12*(10), 2173.

Bawa, M., Songsermpong, S., Kaewtapee, C., & Chanput, W. (2020). Effects of microwave and hot air oven drying on the nutritional, microbiological load, and color parameters of the house crickets (Acheta domesticus). *Journal of Food Processing and Preservation*, *44*(5), e14407.

Benwood, C., Chrenek, J., Kirsch, R. L., Masri, N. Z., Richards, H., Teetzen, K., & Willerth, S. M. (2021). Natural biomaterials and their use as bioinks for printing tissues. *Bioengineering*, *8*(2), 27.

Berkovitch, Y., & Seliktar, D. (2017). Semi-synthetic hydrogel composition and stiffness regulate neuronal morphogenesis. *International journal of pharmaceutics*, *523*(2), 545-555.

Bhandari, B., Bansal, N., Zhang, M., & Schuck, P. (Eds.). (2023). *Handbook of food powders: Processes and properties*. Elsevier.

Blakey, R. J., Bower, J. P., & Bertling, I. (2009). Influence of water and ABA supply on the ripening pattern of avocado (Persea americana Mill.) fruit and the prediction of water content using Near Infrared Spectroscopy. *Postharvest Biology and Technology*, *53*(1-2), 72-76.

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

Brishti, F. H., Chay, S. Y., Muhammad, K., Ismail-Fitry, M. R., Zarei, M., Karthikeyan, S., & Saari, N. (2020). Effects of drying techniques on the physicochemical, functional, thermal, structural and rheological properties of mung bean (Vigna radiata) protein isolate powder. *Food Research International*, *138*, 109783.

Bueno, J. N., Corradini, E., de Souza, P. R., Marques, V. D. S., Radovanovic, E., & Muniz, E. C. (2021). Films based on mixtures of zein, chitosan, and PVA: Development with perspectives for food packaging application. *Polymer Testing*, *101*, 107279.

Caballero, S., & Davidov-Pardo, G. (2021). Comparison of legume and dairy proteins for the impact of Maillard conjugation on nanoemulsion formation, stability, and lutein color retention. *Food chemistry*, *338*, 128083.

Calderón-Oliver, M., Escalona-Buendía, H. B., Medina-Campos, O. N., Pedraza-Chaverri, J., Pedroza-Islas, R., & Ponce-Alquicira, E. (2016). Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts. *LWT-Food Science and Technology*, *65*, 46-52.

Cao, Y., & Mezzenga, R. (2020). Design principles of food gels. *Nature Food*, *1*(2),

106-118.

Capuano, E. (2017). The behavior of dietary fiber in the gastrointestinal tract determines its physiological effect. *Critical reviews in food science and nutrition*, *57*(16), 3543-3564.

Carbonaro, M., Maselli, P., Dore, P., & Nucara, A. (2008). Application of Fourier transform infrared spectroscopy to legume seed flour analysis. *Food Chemistry*, *108*(1), 361-368.

Chang, Z., Chen, Y., Tang, S., Yang, J., Chen, Y., Chen, S., ... & Yang, Z. (2020). Construction of chitosan/polyacrylate/graphene oxide composite physical hydrogel by semi-dissolution/acidification/sol-gel transition method and its simultaneous cationic and anionic dye adsorption properties. *Carbohydrate polymers*, *229*, 115431.

Cheikhyoussef, N., & Cheikhyoussef, A. (2022). Avocado (Persea Americana) wastes: Chemical composition, biological activities and industrial applications. In *Mediterranean fruits bio-wastes: Chemistry, functionality and technological applications* (pp. 699-719). Cham: Springer International Publishing.

Chen, H., Gan, J., Ji, A., Song, S., & Yin, L. (2019). Development of double network gels based on soy protein isolate and sugar beet pectin induced by thermal treatment and laccase catalysis. *Food Chemistry*, *292*, 188-196.

Chen, H., Qiu, X., Xia, T., Li, Q., Wen, Z., Huang, B., & Li, Y. (2023). Mesoporous materials make hydrogels more powerful in biomedicine. *Gels*, *9*(3), 207.

Cheng, Y., Donkor, P. O., Ren, X., Wu, J., Agyemang, K., Ayim, I., & Ma, H. (2019). Effect of ultrasound pretreatment with mono-frequency and simultaneous dual frequency on the mechanical properties and microstructure of whey protein emulsion gels. *Food Hydrocolloids*, *89*, 434-442.

Chirani, N., Yahia, L. H., Gritsch, L., Motta, F. L., Chirani, S., & Farè, S. (2015). History and applications of hydrogels. *Journal of biomedical sciences*, *4*(02), 1-23.

Cho, S. M., Kwak, K. S., Park, D. C., Gu, Y. S., Ji, C. I., Jang, D. H., & Kim, S. (2004). Processing optimization and functional properties of gelatin from shark (Isurus oxyrinchus) cartilage. *Food hydrocolloids*, *18*(4), 573-579.

Ciolacu, D., Oprea, A. M., Anghel, N., Cazacu, G., & Cazacu, M. (2012). New cellulose–lignin hydrogels and their application in controlled release of polyphenols. *Materials Science and Engineering: C*, *32*(3), 452-463.

Cowan, A. K. and Wolstenholme, B. N., (2016). Avocado. Encyclopedia of Food and Health.

Cui, Q., Wang, G., Gao, D., Wang, L., Zhang, A., Wang, X., & Jiang, L. (2020). Improving the gel properties of transgenic microbial transglutaminase cross-linked soybean-whey mixed protein by ultrasonic pretreatment. *Process Biochemistry*, *91*, 104-112.

da Costa Neto, J. J., Gomes, T. L., Justo, T. F., Pereira, K. S., Amaral, P. F., Leão, M. H. R., & Sant'Ana, G. C. F. (2019). Microencapsulation of tiger nut milk by lyophilization: Morphological characteristics, shelf life and microbiological stability. *Food chemistry*, *284*, 133-139.

Dabas, D., Elias, R. J., Ziegler, G. R., & Lambert, J. D. (2019). In vitro antioxidant and cancer inhibitory activity of a colored avocado seed extract. *International Journal of Food Science*, *2019*(1), 6509421.

Dabas, D., M Shegog, R., R Ziegler, G., & D Lambert, J. (2013). Avocado (Persea americana) seed as a source of bioactive phytochemicals. *Current pharmaceutical design*, *19*(34), 6133-6140.

Dakhili, S., Abdolalizadeh, L., Hosseini, S. M., Shojaee-Aliabadi, S., & Mirmoghtadaie, L. (2019). Quinoa protein: Composition, structure and functional properties. *Food chemistry*, *299*, 125161.

Das, D., Mir, N. A., Chandla, N. K., & Singh, S. (2021). Combined effect of pH treatment and the extraction pH on the physicochemical, functional and rheological characteristics of amaranth (Amaranthus hypochondriacus) seed protein isolates. *Food Chemistry*, *353*, 129466.

Das, M., & Giri, T. K. (2020). Hydrogels based on gellan gum in cell delivery and drug delivery. *Journal of Drug Delivery Science and Technology*, *56*, 101586.

Das, R. S., Tiwari, B. K., Chemat, F., & Garcia-Vaquero, M. (2022). Impact of ultrasound processing on alternative protein systems: Protein extraction, nutritional effects and associated challenges. *Ultrasonics Sonochemistry*, *91*, 106234.

de la Cruz, J. E. C., & Ramirez, O. J. V. (2020, September). Convolutional neural networks for the Hass avocado classification using LabVIEW in an agro-industrial plant. In *2020 IEEE XXVII International Conference on Electronics, Electrical Engineering and Computing (INTERCON)* (pp. 1-4). IEEE.

de la Rosa-Millán, J., Orona-Padilla, J. L., Flores-Moreno, V. M., & Serna-Saldívar, S. O. (2018). Physicochemical, functional and ATR-FTIR molecular analysis of protein extracts derived from starchy pulses. *International Journal of Food Science & Technology*, *53*(6), 1414-1424.

de Lavergne, M. D., van de Velde, F., & Stieger, M. (2017). Bolus matters: The influence of food oral breakdown on dynamic texture perception. *Food & Function*, *8*(2), 464-480.

Demirkıran, E., Başyi̇ğit, B., Altun, G., Yücetepe, M., Sağlam, H., & Karaaslan, M. (2022). Facile construction of fruit protein based natural hydrogel via intra/inter molecular cross-linking. *Food Hydrocolloids*, *133*, 107899.

Deng, Y., Huang, L., Zhang, C., Xie, P., Cheng, J., Wang, X., & Li, S. (2019). Physicochemical and functional properties of Chinese quince seed protein isolate. *Food Chemistry*, *283*, 539-548.

Dia, V. P., Wang, Z., Lin, Y., & Pangloli, P. (2019). Comparative Biological Activities Determination of Aqueous Extracts of Hempseed Oil and Hempseed Protein Isolate Production Coproducts. *Journal of the American Oil Chemists' Society*, *96*(11), 1265-1274.

Dienes, J., Browne, S., Farjun, B., Amaral Passipieri, J., Mintz, E. L., Killian, G., ... & Christ, G. J. (2021). Semisynthetic hyaluronic acid-based hydrogel promotes recovery of the injured tibialis anterior skeletal muscle form and function. *ACS Biomaterials Science & Engineering*, *7*(4), 1587-1599.

dos Santos, S. D. A., Martins, V. G., Salas-Mellado, M., & Prentice, C. (2011). Evaluation of functional properties in protein hydrolysates from bluewing searobin (Prionotus punctatus) obtained with different microbial enzymes. *Food and bioprocess technology*, *4*, 1399-1406.

Du, M., Lu, W., Zhang, Y., Mata, A., & Fang, Y. (2021). Natural polymer-sourced interpenetrating network hydrogels: Fabrication, properties, mechanism and food applications. *Trends in Food Science & Technology*, *116*, 342-356.

Duarte, P. F., Chaves, M. A., Borges, C. D., & Mendonça, C. R. B. (2016). Avocado: characteristics, health benefits and uses. *Ciência rural*, *46*(4), 747-754.

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

Duangmal, K., Saicheua, B., & Sueeprasan, S. (2008). Colour evaluation of freeze-dried roselle extract as a natural food colorant in a model system of a drink. *LWT-Food Science and Technology*, *41*(8), 1437-1445.

Ebrahimi, S. E., Koocheki, A., Milani, E., & Mohebbi, M. (2016). Interactions between Lepidium perfoliatum seed gum–Grass pea (Lathyrus sativus) protein isolate in composite biodegradable film. *Food Hydrocolloids*, *54*, 302-314.

Egbuonu, A. C. C., Opara, I. C., Onyeabo, C., & Uchenna, N. O. (2018). Proximate, functional, antinutrient and antimicrobial properties of avocado pear (Persea americana) Seeds. *Journal of Nutritional Health and Food Engineering*, *8*(2), 00260.

Ejiofor, N. C., Ezeagu, I. E., Ayoola, M., & Umera, E. A. (2018). Determination of the chemical composition of avocado (Persea americana) seed. *Adv Food Technol Nutr Sci Open J*, *2*, 51-55.

Eshraghi, B., Shaygan, P., Lajevardi, N., & Fazel, M. (2021). Nicolau syndrome, a rare though important complication of hyaluronic acid injection. *International Journal of Dermatology*, *60*(7).

Fan, Y., Yang, J., Duan, A., & Li, X. (2021). Pectin/sodium alginate/xanthan gum edible composite films as the fresh-cut package. *International Journal of Biological Macromolecules*, *181*, 1003-1009.

FAO (2023). FAOSTAT statistics division, food and agriculture organization of the

United Nations (available at: www.faostat.org).

Feyzi, S., Milani, E., & Golimovahhed, Q. A. (2018). Grass pea (Lathyrus sativus L.) protein isolate: The effect of extraction optimization and drying methods on the structure and functional properties. *Food Hydrocolloids*, *74*, 187-196.

Feyzi, S., Varidi, M., Zare, F., & Varidi, M. J. (2018). Effect of drying methods on the structure, thermo and functional properties of fenugreek (Trigonella foenum graecum) protein isolate. *Journal of the Science of Food and Agriculture*, *98*(5), 1880-1888.

Figueroa, J. G., Borrás-Linares, I., Lozano-Sánchez, J., Quirantes-Piné, R., & Segura-Carretero, A. (2018). Optimization of drying process and pressurized liquid extraction for recovery of bioactive compounds from avocado peel by-product. *Electrophoresis*, *39*(15), 1908-1916.

Flórez, N., Conde, E., & Domínguez, H. (2015). Microwave assisted water extraction of plant compounds. *Journal of Chemical Technology & Biotechnology*, *90* (4), 590-607.

Florowska, A., Hilal, A., Florowski, T., Mrozek, P., & Wroniak, M. (2022). Sodium alginate and chitosan as components modifying the properties of inulin hydrogels. *Gels*, *8*(1), 63.

Gadalkar, S. M., Gogate, P. R., & Rathod, V. K. (2017). Recovery of proteins from rice mill industry waste (rice bran) using alkaline or NaCl-assisted alkaline extraction processes. *Journal of Food Process Engineering*, *40*(3), e12430.

Gagliardi, A., Voci, S., Paolino, D., Fresta, M., & Cosco, D. (2020). Influence of various model compounds on the rheological properties of Zein-based gels. *Molecules*, *25*(14), 3174.

Galland, L. (2013). Functional foods: Health effects and clinical applications.

Gan, Y., Xu, D., Zhang, J., Wang, Z., Wang, S., Guo, H., & Wang, Y. (2020). Rana chensinensis ovum oil based on CO2 supercritical fluid extraction: Response surface methodology optimization and unsaturated fatty acid ingredient analysis. *Molecules*, *25*(18), 4170.

Ganesan, V., Rosentrater, K. A., & Muthukumarappan, K. (2008). Flowability and handling characteristics of bulk solids and powders–a review with implications for DDGS. *biosystems engineering*, *101*(4), 425-435.

Ghribi, A. M., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H., & Besbes, S. (2015). Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. *Journal of Food Engineering*, *165*, 179-188.

Givonetti, A., Cattaneo, C., & Cavaletto, M. (2021). What you extract is what you get: Different methods of protein extraction from hemp seeds. *Separations*, *8* (12), 231.

Gomez, L., Tiwari, B., & Garcia-Vaquero, M. (2020). Emerging extraction techniques: Microwave-assisted extraction. In *Sustainable seaweed* *technologies* (pp. 207-224). Elsevier.

Goyal, A., Sharma, V., Sihag, M. K., Tomar, S. K., Arora, S., Sabikhi, L., & Singh, A. K. (2015). Development and physico-chemical characterization of microencapsulated flaxseed oil powder: A functional ingredient for omega-3 fortification. *Powder Technology*, *286*, 527-537.

Gunasekaran, S., Ko, S., & Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of food engineering*, *83*(1), 31-40.

Gunasekaran, S., Xiao, L., & Ould Eleya, M. M. (2006). Whey protein concentrate hydrogels as bioactive carriers. *Journal of Applied Polymer Science*, *99*(5), 2470-2476.

Gundogan, R., & Karaca, A. C. (2020). Physicochemical and functional properties of proteins isolated from local beans of Turkey. *Lwt*, *130*, 109609.

Guo, X. Y., Peng, Z. Q., Zhang, Y. W., Liu, B., & Cui, Y. Q. (2015). The solubility and conformational characteristics of porcine myosin as affected by the presence of L-lysine and L-histidine. *Food chemistry*, *170*, 212-217.

Haque, M. A., Chen, J., Aldred, P., & Adhikari, B. (2015). Denaturation and physical characteristics of spray-dried whey protein isolate powders produced in the presence and absence of lactose, trehalose, and polysorbate-80. *Drying Technology*, *33*(10), 1243-1254.

He, Z., Liu, C., Zhao, J., Li, W., & Wang, Y. (2021). Physicochemical properties of a ginkgo seed protein-pectin composite gel. *Food Hydrocolloids*, *118*, 106781.

He, Z., Ma, T., Zhang, W., Su, E., Cao, F., Huang, M., & Wang, Y. (2021). Heat-induced gel formation by whey protein isolate-Lycium barbarum polysaccharides at varying pHs. *Food Hydrocolloids*, *115*, 106607.

Hilal, A., Florowska, A., & Wroniak, M. (2023). Binary hydrogels: Induction methods and recent application progress as food matrices for bioactive compounds delivery—A bibliometric review. *Gels*, *9*(1), 68.

Hu, X., Cheng, Y., Fan, J., Lu, Z., Yamaki, K. & Li, L., (2009). Effects of Drying Method on Physicochemical and Functional Properties of Soy Protein Isolates. *Journal of Food Processing and Preservation,* 34: 520-540.

Hu, Y., Zhang, M., Qin, C., Qian, X., Zhang, L., Zhou, J., & Lu, A. (2021). Transparent, conductive cellulose hydrogel for flexible sensor and triboelectric nanogenerator at subzero temperature. *Carbohydrate Polymers*, *265*, 118078.

Hussain, A., Laaraj, S., Kausar, T., Tikent, A., Azzouzi, H., Kauser, S., ... & Elfazazi, K. (2023). Food application of orange seed powder through incorporation in wheat flour to boost vitamin and mineral profiles of formulated biscuits. *International Journal of Food Science*, *2023*(1), 6654250.

Ifesan, B. O. T., Olorunsola, B. O., & Ifesan, B. T. (2015). Nutritional composition and acceptability of candy from avocado seed (Persea americana).

Ikeda, S., & Morris, V. J. (2002). Fine-stranded and particulate aggregates of heat-denatured whey proteins visualized by atomic force microscopy. *Biomacromolecules*, *3*(2), 382-389.

Jarpa-Parra, M., Bamdad, F., Wang, Y., Tian, Z., Temelli, F., Han, J., & Chen, L. (2014). Optimization of lentil protein extraction and the influence of process pH on protein structure and functionality. *LWT-Food Science and Technology*, *57*(2), 461-469.

Joshi, M., Adhikari, B., Aldred, P., Panozzo, J. F., & Kasapis, S. (2011). Physicochemical and functional properties of lentil protein isolates prepared by different drying methods. *Food chemistry*, *129*(4), 1513-1522.

Juliano, P., & Barbosa-Cánovas, G. V. (2010). Food powders flowability characterization: theory, methods, and applications. *Annual review of food science and technology*, *1*(1), 211-239.

Kamal, H., Le, C. F., Salter, A. M., & Ali, A. (2021). Extraction of protein from food waste: An overview of current status and opportunities. *Comprehensive Reviews in Food Science and Food Safety*, *20*(3), 2455-2475.

Kapoor, R., Karabulut, G., Mundada, V., & Feng, H. (2024). Unraveling the potential of non-thermal ultrasonic contact drying for enhanced functional and structural attributes of pea protein isolates: A comparative study with spray and freeze-drying methods. *Food Chemistry*, *439*, 138137.

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food research international*, *44*(9), 2742-2750.

Karrar, E., Mahdi, A. A., Sheth, S., Ahmed, I. A. M., Manzoor, M. F., Wei, W., & Wang, X. (2021). Effect of maltodextrin combination with gum arabic and whey protein isolate on the microencapsulation of gurum seed oil using a spray-drying method. *International Journal of Biological Macromolecules*, *171*, 208-216.

Khalesi, H., Lu, W., Nishinari, K., & Fang, Y. (2021). Fundamentals of composites containing fibrous materials and hydrogels: A review on design and development for food applications. *Food Chemistry*, *364*, 130329.

Khan, S. H., Butt, M. S., Sharif, M. K., Sameen, A., Mumtaz, S., & Sultan, M. T. (2011). Functional properties of protein isolates extracted from stabilized rice bran by microwave, dry heat, and parboiling. *Journal of Agricultural and Food Chemistry*, *59*(6), 2416-2420.

Khubber, S., Chaturvedi, K., Thakur, N., Sharma, N., & Yadav, S. K. (2021). Low-methoxyl pectin stabilizes low-fat set yoghurt and improves their physicochemical properties, rheology, microstructure and sensory liking.

Food Hydrocolloids, *111*, 106240.

Klein, M., & Poverenov, E. (2020). Natural biopolymer-based hydrogels for use in food and agriculture. *Journal of the Science of Food and Agriculture*, *100*(6), 2337-2347.

Krop, E. M., Hetherington, M. M., Miquel, S., & Sarkar, A. (2019). The influence of oral lubrication on food intake: a proof-of-concept study. *Food Quality and Preference*, *74*, 118-124.

Kudre, T. G., Benjakul, S., & Kishimura, H. (2013). Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture*, *93* (10), 2429-2436.

Kumar, M., Tomar, M., Potkule, J., Verma, R., Punia, S., Mahapatra, A., ... & Kennedy, J. F. (2021). Advances in the plant protein extraction: Mechanism and recommendations. *Food Hydrocolloids*, *115*, 106595.

Lafarga, T., Álvarez, C., Bobo, G., & Aguiló-Aguayo, I. (2018). Characterization of functional properties of proteins from Ganxet beans (Phaseolus vulgaris L. var. Ganxet) isolated using an ultrasound-assisted methodology. *Lwt*, *98*, 106-112.

Lara-Márquez, M., Baez-Magana, M., Raymundo-Ramos, C., Spagnuolo, P. A., Macías-Rodríguez, L., Salgado-Garciglia, R., ... & Lopez-Meza, J. E. (2020). Lipid-rich extract from Mexican avocado (Persea americana var. drymifolia) induces apoptosis and modulates the inflammatory response in Caco-2 human colon cancer cells. *Journal of Functional Foods*, *64*, 103658.

López-Monterrubio, D. I., Lobato-Calleros, C., Alvarez-Ramirez, J., & Vernon-Carter, E. J. (2020). Huauzontle (Chenopodium nuttalliae Saff.) protein: Composition, structure, physicochemical and functional properties. *Food Hydrocolloids*, *108*, 106043.

Lee, H. W., Lu, Y., Zhang, Y., Fu, C., & Huang, D. (2021). Physicochemical and functional properties of red lentil protein isolates from three origins at different pH. *Food Chemistry*, *358*, 129749.

Lei, Z., Fu, Y., Xu, P., Zheng, Y., & Zhou, C. (2016). Effects of L-arginine on the physicochemical and gel properties of chicken actomyosin. *International journal of biological macromolecules*, *92*, 1258-1265.

Lei, Z., Fu, Y., Zheng, Y., Xu, P., & Zhou, C. (2017). Effects of l-lysine on thermal gelation properties of chicken breast actomyosin. *Food Science and Biotechnology*, *26*, 549-556.

Li, N., Wang, Y., Gan, Y., Wang, S., Wang, Z., Zhang, C., & Wang, Z. (2022). Physicochemical and functional properties of protein isolate recovered from Rana chensinensis ovum based on different drying techniques. *Food Chemistry*, *396*, 133632.

Li, Q., Xu, J., Wang, J., Ge, Y., Li, J., & Sun, T. (2021). Composite coatings based on konjac glucomannan and sodium alginate modified with allicin and in situ SiO x for ginger rhizomes preservation. *Journal of Food Safety*, *41*(1), e12876.

Li, X., Shi, J., Scanlon, M., Xue, S. J., & Lu, J. (2021). Effects of pretreatments on physicochemical and structural properties of proteins isolated from canola seeds after oil extraction by supercritical-CO2 process. *Lwt*, *137*, 110415.

Li, Y., Zhou, C., Xu, L., Yao, F., Cen, L., & Fu, G. D. (2015). Stimuli-responsive hydrogels prepared by simultaneous "click chemistry" and metal–ligand coordination. *RSC Advances*, *5*(24), 18242-18251.

Liberman, G. N., Ochbaum, G., Bitton, R., & Arad, S. M. (2021). Antimicrobial hydrogels composed of chitosan and sulfated polysaccharides of red microalgae. *Polymer*, *215*, 123353.

Lin, N., Liu, B., Liu, Z., & Qi, T. (2020). Effects of different drying methods on the structures and functional properties of phosphorylated Antarctic krill protein. *Journal of food science*, *85*(11), 3690-3699.

Lin, Y., Pangloli, P., & Dia, V. P. (2021). Physicochemical, functional and bioactive properties of hempseed (Cannabis sativa L.) meal, a co-product of hempseed oil and protein production, as affected by drying process. *Food chemistry*, *350*, 129188.

Litz, R. E., Efendi, D., Raharjo, S. H. T., & Suarez, I. (2005). Genetic transformation of avocado (Persea americana Mill.): a coordinated strategy for crop improvement. *Acta Horticulturae*, *692*, 131.

Liu, G., Li, J., Shi, K., Wang, S., Chen, J., Liu, Y., & Huang, Q. (2009). Composition, secondary structure, and self-assembly of oat protein isolate. *Journal of Agricultural and Food Chemistry*, *57*(11), 4552-4558.

Liu, J., Li, Z., Lin, Q., Jiang, X., Yao, J., Yang, Y., ... & Chen, X. (2018). A robust, resilient, and multi-functional soy protein-based hydrogel. *ACS Sustainable Chemistry & Engineering*, *6*(11), 13730-13738.

Liu, X., Sievert, J., Arpaia, M. L., & Madore, M. A. (2002). Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American society for Horticultural Science*, *127*(1), 108-114.

Liu, Y., Wang, R., Wang, D., Sun, Z., Liu, F., Zhang, D., & Wang, D. (2022). Development of a food packaging antibacterial hydrogel based on gelatin, chitosan, and 3-phenyllactic acid for the shelf-life extension of chilled chicken. *Food Hydrocolloids*, *127*, 107546.

Lowenson, J. D., Shmanai, V. V., Shklyaruck, D., Clarke, S. G., & Shchepinov, M. S. (2016). Deuteration protects asparagine residues against racemization. *Amino Acids*, *48*, 2189-2196.

Lu, Y., Mao, L., Zheng, H., Chen, H., & Gao, Y. (2020). Characterization of β-carotene loaded emulsion gels containing denatured and native whey protein. *Food Hydrocolloids*, *102*, 105600.

Lupu, A., Gradinaru, L. M., Gradinaru, V. R., & Bercea, M. (2023). Diversity of bioinspired hydrogels: From structure to applications. *Gels*, *9*(5), 376.

Ma, L., Tan, Y., Chen, X., Ran, Y., Tong, Q., Tang, L., & Li, X. (2022). Injectable oxidized alginate/carboxylmethyl chitosan hydrogels functionalized with nanoparticles for wound repair. *Carbohydrate Polymers*, *293*, 119733.

Madaghiele, M., Demitri, C., Surano, I., Silvestri, A., Vitale, M., Panteca, E., & Sannino, A. (2021). Biomimetic cellulose-based superabsorbent hydrogels for treating obesity. *Scientific Reports*, *11*(1), 21394.

Mad-Ali, S., Benjakul, S., Prodpran, T., & Maqsood, S. (2016). Interfacial properties of gelatin from goat skin as influenced by drying methods. *LWT*, *73*, 102-107.

Mahawan, M. A., Tenorio, M. F. N., Gomez, J. A., & Bronce, R. A. (2015). Characterization of flour from avocado seed kernel. *Asia Pacific Journal of Multidisciplinary Research*, *3*(4), 34-40.

Makar, L. E., Nady, N., Abd El-Fattah, A., Shawky, N., & Kandil, S. H. (2022). Unmodified gum Arabic/chitosan/nanohydroxyapatite nanocomposite hydrogels as potential scaffolds for bone regeneration. *Polymers*, *14*(15), 3052.

Maltais, A., Remondetto, G. E., & Subirade, M. (2009). Soy protein cold-set hydrogels as controlled delivery devices for nutraceutical compounds. *Food hydrocolloids*, *23*(7), 1647-1653.

Manzoor, A., Dar, A. H., Pandey, V. K., Shams, R., Khan, S., Panesar, P. S., & Khan, S. A. (2022). Recent insights into polysaccharide-based hydrogels and their potential applications in food sector: A review. *International Journal of Biological Macromolecules*, *213*, 987-1006.

Melgar, B., Dias, M. I., Ciric, A., Sokovic, M., Garcia-Castello, E. M., Rodriguez-Lopez, A. D., ... & Ferreira, I. C. (2018). Bioactive characterization of Persea americana Mill. by-products: A rich source of inherent antioxidants. *Industrial Crops and Products*, *111*, 212-218.

Michalska-Ciechanowska, A., Majerska, J., Brzezowska, J., Wojdyło, A., & Figiel, A. (2020). The influence of maltodextrin and inulin on the physico-chemical properties of cranberry juice powders. *ChemEngineering*, *4*(1), 12.

Munthe, S. W. N., Riskianto, R., Juvi, D., & Novia, J. (2023). Antioxidant, Total Phenolic, and Total Flavonoid of 70% Ethanol Extract of Avocado Seeds (Persea americana Mill.). *Pharmacognosy Journal*, *15*(4).

Nadar, S. S., Rao, P., & Rathod, V. K. (2018). Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. *Food Research International*, *108*, 309-330.

Nazir, A., Asghar, A., & Maan, A. A. (2017). Food gels: Gelling process and new applications. In *Advances in food rheology and its applications* (pp. 335-353). Woodhead Publishing.

Ortiz, S. E. M., Mauri, A., Monterrey-Quintero, E. S., Trindade, M. A., Santana, A. S., & Favaro-Trindade, C. S. (2009). Production and properties of casein hydrolysate microencapsulated by spray drying with soybean protein isolate. *LWT-Food Science and Technology*, *42*(5), 919-923.

Osthoff, G., Hugo, A., Van Wyk, P., De Wit, M., & Meyer, S. (2010). Characterization of a spray-dried soymilk powder and changes observed during storage. *Food science and technology international*, *16*(2), 169-178.

Park, S., & Park, K. M. (2016). Engineered polymeric hydrogels for 3D tissue models. *Polymers*, *8*(1), 23.

Pietrzak, L. N., & Miller, S. S. (2005). Microchemical structure of soybean seeds revealed in situ by ultraspatially resolved synchrotron Fourier transformed infrared microspectroscopy. *Journal of agricultural and food chemistry*, *53* (24), 9304-9311.

Piletti, R., Zanetti, M., Jung, G., de Mello, J. M. M., Dalcanton, F., Soares, C., & Fiori, M. A. (2019). Microencapsulation of garlic oil by β-cyclodextrin as a thermal protection method for antibacterial action. *Materials Science and Engineering: C*, *94*, 139-149.

Pojić, M., Mišan, 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 & Technology*, *75*, 93-104.

Pratap Singh, A., Mandal, R., Shojaei, M., Singh, A., Kowalczewski, P. Ł., Ligaj, M., & Jarzębski, M. (2020). Novel drying methods for sustainable upcycling of brewers' spent grains as a plant protein source. *Sustainability*, *12*(9), 3660.

Qin, F., Johansen, A. Z., & Mussatto, S. I. (2018). Evaluation of different pretreatment strategies for protein extraction from brewer's spent grains. *Industrial crops and products*, *125*, 443-453.

Qin, Z., Zhang, R., Xu, Y., Cao, Y., & Xiao, L. (2021). A one-pot synthesis of thermosensitive PNIPAAM interpenetration polymer networks (IPN) hydrogels. *JCIS Open*, *1*, 100002.

Quek, S. Y., Chok, N. K., & Swedlund, P. (2007). The physicochemical properties of spray-dried watermelon powders. *Chemical Engineering and Processing: Process Intensification*, *46*(5), 386-392.

Rahman, M. M., & Lamsal, B. P. (2021). Ultrasound-assisted extraction and modification of plant-based proteins: Impact on physicochemical, functional, and nutritional properties. *Comprehensive Reviews in Food Science and Food Safety*, *20*(2), 1457-1480.

Rahman, M. S., Go, G. W., Seo, J. K., Gul, K., Choi, S. G., & Yang, H. S. (2019). Thiol concentration, structural characteristics and gelling properties of bovine heart protein concentrates. *LWT*, *111*, 175-181.

Ramos, O. S., & Malcata, F. X. (2011). 3.48—Food-Grade Enzymes. *Comprehensive Biotechnology, 2nd ed.; Moo-Young, M., Ed*, 555-569.

Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A. K., Madhavan, A., & Pandey, A. (2018). Applications of microbial enzymes in food industry. *Food technology and biotechnology*, *56*(1), 16.

Ravindran, R., & Jaiswal, A. K. (2016). Microbial enzyme production using lignocellulosic food industry wastes as feedstock: a review. *Bioengineering*, *3*(4), 30.

Rezaeisadat, M., Bordbar, A. K., & Omidyan, R. (2021). Molecular dynamics simulation study of curcumin interaction with nano-micelle of PNIPAAm-b-PEG co-polymer as a smart efficient drug delivery system. *Journal of Molecular Liquids*, *332*, 115862.

Robert, B., Chenthamara, D., & Subramaniam, S. (2022). Fabrication and biomedical applications of Arabinoxylan, Pectin, Chitosan, soy protein, and silk fibroin hydrogels via laccase-Ferulic acid redox chemistry. *International Journal of Biological Macromolecules*, *201*, 539-556.

Rodríguez-Carpena, J. G., Morcuende, D., Andrade, M. J., Kylli, P., & Estévez, M. (2011). Avocado (Persea americana Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of agricultural and food chemistry*, *59*(10), 5625-5635.

Rodríguez-Martínez, B., Ferreira-Santos, P., Alfonso, I. M., Martínez, S., Genisheva, Z., & Gullón, B. (2022). Deep eutectic solvents as a green tool for the extraction of bioactive phenolic compounds from avocado peels. *Molecules*, *27*(19), 6646.

Rommi, K. (2016). Enzyme-aided recovery of protein and protein hydrolyzates from rapeseed cold-press cake.

Rudra, S. G., Sethi, S., Jha, S. K., & Kumar, R. (2016). Physico-chemical and functional properties of cowpea protein isolate as affected by the dehydration technique. *Legume Research-An International Journal*, *39*(3), 370-378.

Rudra, S. G., Sethi, S., Jha, S. K., & Kumar, R. (2016). Physico-chemical and functional properties of cowpea protein isolate as affected by the dehydration technique. *Legume Research-An International Journal*, *39*(3), 370-378.

Ruiz, G. A., Xiao, W., van Boekel, M., Minor, M., & Stieger, M. (2016). Effect of extraction pH on heat-induced aggregation, gelation and microstructure of protein isolate from quinoa (Chenopodium quinoa Willd). *Food Chemistry*, *209*, 203-210.

Saavedra, J., Córdova, A., Navarro, R., Díaz-Calderón, P., Fuentealba, C., Astudillo-Castro, C., ... & Galvez, L. (2017). Industrial avocado waste: Functional compounds preservation by convective drying process. *Journal of Food Engineering*, *198*, 81-90.

Saglik, A., Koyuncu, I., Gonel, A., Yalcin, H., Adibelli, F. M., & Toptan, M. (2019). Metabolomics analysis in pterygium tissue. *International ophthalmology*, *39*,

2325-2333.

Salazar-Irrazabal, M. D., Ramirez-Tixe, E. E., Velasquez-Barreto, F. F., & Bello-Pérez, L. A. (2023). Avocado seed starch: effect of the variety on molecular, physicochemical, and digestibility characteristics. *International Journal of Biological Macromolecules*, *247*, 125746.

Salcedo-Chávez, B., Osuna-Castro, J. A., Guevara-Lara, F., Domínguez-Domínguez, J., & Paredes-López, O. (2002). Optimization of the isoelectric precipitation method to obtain protein isolates from amaranth (Amaranthus cruentus) seeds. *Journal of Agricultural and Food Chemistry*, *50*(22), 6515-6520.

Sari, Y. W., Bruins, M. E., & Sanders, J. P. (2013). Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals. *Industrial Crops and Products*, *43*, 78-83.

Sebii, H., Karra, S., Makhloof, I. G., Danthine, S., Blecker, C., Attia, H., & Bchir, B. (2021). Effect of isoelectric precipitation and ultrafiltration treatment on extraction and physicochemical properties of proteins from date palm pollen.

Segovia, F. J., Corral-Pérez, J. J., & Almajano, M. P. (2016). Avocado seed: Modeling extraction of bioactive compounds. *Industrial Crops and Products*, *85*, 213-220.

Sethi, S., Yadav, D. N., Snigdha, S., & Gupta, A. (2021). Optimization of process parameters for extraction of protein isolates from Khesari dhal (Lathyrus sativus L). *LWT*, *137*, 110368.

Shahidi, F., Naczk, M., Hall, D., & Synowiecki, J. (1992). Insensitivity of the amino acids of canola and rapeseed to methanol-ammonia extraction and commercial processing. *Food chemistry*, *44*(4), 283-285.
Shahidi, F., Naczk, M., Hall, D., & Synowiecki, J. (1992). Insensitivity of the amino acids of canola and rapeseed to methanol-ammonia extraction and commercial processing. *Food chemistry*, *44*(4), 283-285.
Sharma, P. C., Tilakratne, B. M. K. S., & Gupta, A. (2010). Utilization of wild apricot kernel press cake for extraction of protein isolate. *Journal of food science and technology*, *47*, 682-685.

Shaviklo, G. R., Thorkelsson, G., Arason, S., Kristinsson, H. G., & Sveinsdottir, K. (2010). The influence of additives and drying methods on quality attributes of fish protein powder made from saithe (Pollachius virens). *Journal of the Science of Food and Agriculture*, *90*(12), 2133-2143.

Shen, Y., Tang, X., & Li, Y. (2021). Drying methods affect physicochemical and functional properties of quinoa protein isolate. *Food Chemistry*, *339*, 127823.

Shetty, G. R., & Rao, B. L. (2022). Preparation and characterization of silk fibroin-polyvinyl alcohol (PVA) blend films for food packaging materials. *Materials Today: Proceedings*, *55*, 194-200.

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. *Food Hydrocolloids*, *43*, 679-689.

Shevkani, K., Singh, N., Kaur, A., & Rana, J. C. (2015). Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study. *Food Hydrocolloids*, *43*, 679-689.

Sivaselvam, S., Selvakumar, R., Viswanathan, C., & Ponpandian, N. (2021). Rapid one-pot synthesis of PAM-GO-Ag nanocomposite hydrogel by gamma-ray irradiation for remediation of environment pollutants and pathogen inactivation. *Chemosphere*, *275*, 130061.

Slavutsky, A. M., & Bertuzzi, M. A. (2019). Formulation and characterization of hydrogel based on pectin and brea gum. *International journal of biological macromolecules*, *123*, 784-791.

Soledad, C. P. T., Paola, H. C., Enrique, O. V. C., Israel, R. L. I., GuadalupeVirginia, N. M., & Raúl, Á. S. (2021). Avocado seeds (Persea americana cv. Criollo sp.): Lipophilic compounds profile and biological activities. *Saudi journal of biological sciences*, *28*(6), 3384-3390.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2010). Effect of gamma-irradiation on flavour 5′-nucleotides, tyrosine, and phenylalanine in mushrooms (Agaricus bisporus). *Food Chemistry*, *123*(1), 171-174.

Steffolani, M. E., Villacorta, P., Morales-Soriano, E. R., Repo-Carrasco, R., León, A. E., & Pérez, G. T. (2016). Physicochemical and functional characterization of protein isolated from different quinoa varieties (Chenopodium quinoa Willd.). *Cereal Chemistry*, *93*(3), 275-281.

Stone, A. K., Karalash, A., Tyler, R. T., Warkentin, T. D., & Nickerson, M. T. (2015). Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food research international*, *76*, 31-38.

Suhag, R., Kellil, A., & Razem, M. (2024). Factors Influencing Food Powder Flowability. *Powders*, *3*(1), 65-76.

Tabakoglu, N., & Karaca, H. (2018). Effects of ozone-enriched storage atmosphere on postharvest quality of black mulberry fruits (Morus nigra L.). *LWT*, *92*, 276-281.

Talekar, S., Patti, A. F., Singh, R., Vijayraghavan, R., & Arora, A. (2018). From waste to wealth: High recovery of nutraceuticals from pomegranate seed waste using a green extraction process. *Industrial Crops and Products*, *112*, 790-802.

Tatar, F., Tunç, M. T., Dervisoglu, M., Cekmecelioglu, D., & Kahyaoglu, T. (2014). Evaluation of hemicellulose as a coating material with gum arabic for food microencapsulation. *Food research international*, *57*, 168-175.

Tesfay, S. Z., Bertling, I., Bower, J. P., & Lovatt, C. (2012). The quest for the function of 'Hass' avocado carbohydrates: clues from fruit and seed development as well as seed germination. *Australian Journal of Botany*, *60*

(1), 79-86.

Tesfaye, T., Gibril, M., Sithole, B., Ramjugernath, D., Chavan, R., Chunilall, V., & Gounden, N. (2018). Valorisation of avocado seeds: extraction and characterisation of starch for textile applications. *Clean Technologies and Environmental Policy*, *20*, 2135-2154.

Thongsrikhem, N., Taokaew, S., Sriariyanun, M., & Kirdponpattara, S. (2022). Antibacterial activity in gelatin-bacterial cellulose composite film by thermally crosslinking with cinnamaldehyde towards food packaging application. *Food Packaging and Shelf Life*, *31*, 100766.

Timilsena, Y. P., Adhikari, R., Barrow, C. J., & Adhikari, B. (2016). Physicochemical and functional properties of protein isolate produced from Australian chia seeds. *Food chemistry*, *212*, 648-656.

Timilsena, Y. P., Wang, B., Adhikari, R., & Adhikari, B. (2016). Preparation and characterization of chia seed protein isolate–chia seed gum complex coacervates. *Food hydrocolloids*, *52*, 554-563.

Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A. P., Denny, C., Daiuto, É. R., ... & Alencar, S. M. D. (2018). Exploration of avocado by-products as natural sources of bioactive compounds. *PloS one*, *13*(2), e0192577.

Tremocoldi, M. A., Rosalen, P. L., Franchin, M., Massarioli, A. P., Denny, C., Daiuto, É. R., ... & Alencar, S. M. D. (2018). Exploration of avocado by-products as natural sources of bioactive compounds. *PloS one*, *13*(2), e0192577.

Turchiuli, C., Eloualia, Z., El Mansouri, N., & Dumoulin, E. (2005). Fluidised bed agglomeration: Agglomerates shape and end-use properties. *Powder Technology*, *157*(1-3), 168-175.

Uchenna, U. E., Shori, A. B., & Salihin Baba, A. (2017). Inclusion of avocado (Persea americana) seeds in the diet to improve carbohydrate and lipid metabolism in rats. *Revista argentina de endocrinología y metabolismo*, *54* (3), 140-148.

Vardin, H., & Yasar, M. (2012). Optimisation of pomegranate (Punica Granatum L.) juice spray-drying as affected by temperature and maltodextrin content. *International Journal of Food Science & Technology*, *47*(1), 167-176.

Vargas, L., Kapoor, R., Nemzer, B., & Feng, H. (2022). Application of different drying methods for evaluation of phytochemical content and physical properties of broccoli, kale, and spinach. *LWT*, *155*, 112892.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161-169.

Villarreal-Lara, R., Rodríguez-Sánchez, D. G., Díaz De La Garza, R. I., García-Cruz, M. I., Castillo, A., Pacheco, A., & Hernández-Brenes, C. (2019). Purified avocado seed acetogenins: Antimicrobial spectrum and complete inhibition of Listeria monocytogenes in a refrigerated food matrix. *CyTA-Journal of Food*, *17*(1), 228-239.

Wang, B., Liu, F., Luo, S., Li, P., Mu, D., Zhao, Y., ... & Zheng, Z. (2019). Effects of high hydrostatic pressure on the properties of heat-induced wheat gluten gels. *Food and Bioprocess Technology*, *12*, 220-227.

Wang, H., Tong, X., Yuan, Y., Peng, X., Zhang, Q., Zhang, S., & Li, Y. (2020a). Effect of spray-drying and freeze-drying on the properties of soybean hydrolysates. *Journal of Chemistry*, *2020*(1), 9201457.

Wang, S., Niu, L., Zhou, B., Peng, Y., Yang, X., Shen, Y., & Li, S. (2022). Drying methods affect physicochemical and functional characteristics of Clanis Bilineata Tingtauica Mell protein. *Frontiers in Nutrition*, *9*, 1053422.

Wang, S., Yang, J., Shao, G., Qu, D., Zhao, H., Yang, L., & Zhu, D. (2020b). Soy protein isolated-soy hull polysaccharides stabilized O/W emulsion: Effect of polysaccharides concentration on the storage stability and interfacial rheological properties. *Food Hydrocolloids*, *101*, 105490.

Wang, W., Zhang, X., Teng, A., & Liu, A. (2017). Mechanical reinforcement of gelatin hydrogel with nanofiber cellulose as a function of percolation concentration. *International journal of biological macromolecules*, *103*, 226-233.

Wang, Y., Xiong, Y. L., Rentfrow, G. K., & Newman, M. C. (2013). Oxidation promotes cross-linking but impairs film-forming properties of whey proteins. *Journal of Food Engineering*, *115*(1), 11-19.

Wang, Y., Zhao, J., Liu, C., & Li, W. (2019). Influence of γ-aminobutyric acid on gelling properties of heat-induced whey protein gels. *Food Hydrocolloids*, *94*, 287-293.

Wang, Y., Zhao, J., Zhang, W., Liu, C., Jauregi, P., & Huang, M. (2020). Modification of heat-induced whey protein gels by basic amino acids. *Food Hydrocolloids*, *100*, 105397.

Withana-Gamage, T. S., Wanasundara, J. P., Pietrasik, Z., & Shand, P. J. (2011). Physicochemical, thermal and functional characterisation of protein isolates from Kabuli and Desi chickpea (Cicer arietinum L.): A comparative study with soy (Glycine max) and pea (Pisum sativum L.). *Journal of the Science of Food and Agriculture*, *91*(6), 1022-1031.

Wu, L., Li, J., Wu, W., Wang, L., Qin, F., & Xie, W. (2021). Effect of extraction pH on functional properties, structural properties, and in vitro gastrointestinal digestion of tartary buckwheat protein isolates. *Journal of Cereal Science*, *101*, 103314.

Wu, M., Xiong, Y. L., Chen, J., Tang, X., & Zhou, G. (2009). Rheological and microstructural properties of porcine myofibrillar protein–lipid emulsion composite gels. *Journal of food science*, *74*(4), E207-E217.

Xie, A., Zhu, C., & Hua, L. (2017). Food gum based hydrogel polymers. *MOJ Food Process. Technol*, *4*, 192-196.

Yahia, E. M. (Ed.). (2011). *Postharvest biology and technology of tropical and subtropical fruits: fundamental issues*. Elsevier.

Yan, Y., Duan, S., Zhang, H., Liu, Y., Li, C., Hu, B., & Wu, W. (2020). Preparation and characterization of Konjac glucomannan and pullulan composite films for strawberry preservation. *Carbohydrate Polymers*, *243*, 116446.

Yang, J., Shen, M., Luo, Y., Wu, T., Wen, H., & Xie, J. (2021). Construction and characterization of Mesona chinensis polysaccharide-chitosan hydrogels, role of chitosan deacetylation degree. *Carbohydrate Polymers*, *257*, 117608.

Yang, X., Li, A., Li, D., Guo, Y., & Sun, L. (2021). Applications of mixed polysaccharide-protein systems in fabricating multi-structures of binary food gels—A review. *Trends in Food Science & Technology*, *109*, 197-210.

Yang, X., Li, A., Li, D., Guo, Y., & Sun, L. (2021). Applications of mixed polysaccharide-protein systems in fabricating multi-structures of binary food gels—A review. *Trends in Food Science & Technology*, *109*, 197-210.

Ye, M., Wang, Z., Yan, X., Zeng, Z., Peng, T., Xia, J., & Yu, P. (2024). Effects of Drying Methods on the Physicochemical and Functional Properties of Cinnamomum camphora Seed Kernel Protein Isolate. *Foods*, *13*(6), 968.

Yemenicioğlu, A., Farris, S., Turkyilmaz, M., & Gulec, S. (2020). A review of current and future food applications of natural hydrocolloids. *International Journal of Food Science & Technology*, *55*(4), 1389-1406.

Yu, X., Van De Voort, F. R., Li, Z., & Yue, T. (2007). Proximate composition of the apple seed and characterization of its oil. *International Journal of Food Engineering*, *3*(5).

Yuan, C., Du, L., Zhang, G., Jin, Z., & Liu, H. (2016). Influence of cyclodextrins on texture behavior and freeze-thaw stability of kappa-carrageenan gel. *Food Chemistry*, *210*, 600-605.

Yu, M., Zeng, M., Qin, F., He, Z., & Chen, J. (2017). Physicochemical and functional properties of protein extracts from Torreya grandis seeds. *Food Chemistry*, *227*, 453-460.

Zafar, T., & Sidhu, J. S. (2011). Avocado: production, quality, and major processed products. *Handbook of vegetables and vegetable processing*, *1871*, 525-543.

Zúñiga, R. N., & Aguilera, J. M. (2008). Aerated food gels: fabrication and potential applications. *Trends in food science & technology*, *19*(4), 176-187.

Zeng, S., Zhang, J., Zu, G., & Huang, J. (2021). Transparent, flexible, and multifunctional starch-based double-network hydrogels as high-performance wearable electronics. *Carbohydrate Polymers*, *267*, 118198.

ZHA, F., J. RAO, and B. CHEN., 2021. Plant-based food hydrogels: Constitutive characteristics, formation, and modulation. Current Opinion in Colloid &

Interface Science 56:101505.

Zha, F., Rao, J., & Chen, B. (2021). Plant-based food hydrogels: Constitutive characteristics, formation, and modulation. *Current Opinion in Colloid & Interface Science*, *56*, 101505.

Zhang, B., Meng, R., Li, X. L., Liu, W. J., Cheng, J. S., & Wang, W. (2021). Preparation of Pickering emulsion gels based on κ-carrageenan and covalent crosslinking with EDC: Gelation mechanism and bioaccessibility of curcumin. *Food Chemistry*, *357*, 129726.

Zhang, J., Ma, N., Ma, G., Zhong, L., Pei, F., Hu, Q., & Xu, J. (2021). Characterization of soy protein isolate/Flammulina velutipes polysaccharide hydrogel and its immunostimulatory effects on RAW264. 7 cells. *Food and Chemical Toxicology*, *151*, 112126.

Zhang, Y., & Huang, Y. (2021). Rational design of smart hydrogels for biomedical applications. *Frontiers in Chemistry*, *8*, 615665.

Zhang, Y., Fan, Z., Xu, C., Fang, S., Liu, X., & Li, X. (2015). Tough biohydrogels with interpenetrating network structure by bienzymatic crosslinking approach. *European Polymer Journal*, *72*, 717-725.

Zhang, Z., Chen, X., Liu, X., Liu, W., Liu, Q., Huang, J., & Hu, H. (2022). Effect of salt ions on mixed gels of wheat gluten protein and potato isolate protein. *Lwt*, *154*, 112564.

Zhao, Q., Wang, L., Hong, X., Liu, Y., & Li, J. (2021). Structural and functional properties of perilla protein isolate extracted from oilseed residues and its utilization in Pickering emulsions. *Food Hydrocolloids*, *113*, 106412.

Zhao, Q., Xiong, H., Selomulya, C., Chen, X. D., Huang, S., Ruan, X., & Sun, W. (2013). Effects of spray drying and freeze drying on the properties of protein isolate from rice dreg protein. *Food and Bioprocess Technology*, *6*, 1759-1769.

Zhao, X., Zhang, X., Liu, H., Zhang, G., & Ao, Q. (2018). Functional, nutritional and flavor characteristic of soybean proteins obtained through reverse micelles. *Food Hydrocolloids*, *74*, 358-366.

Zhao, Z., Hamid, N., Gutierrez-Maddox, N., Kam, R., Kantono, K., Wang, K., & Lu, J. (2023). Metabolite profiling identifies chemical markers associated with the cytotoxic properties of roasted fermented avocado seeds. *Food Research International*, *172*, 113131.

Zhou, C., Li, J., & Tan, S. (2014). Effect of L-lysine on the physicochemical properties of pork sausage. *Food Science and Biotechnology*, *23*, 775-780.

Zhu, P., Huang, W., Guo, X., & Chen, L. (2021). Strong and elastic pea protein hydrogels formed through pH-shifting method. *Food Hydrocolloids*, *117*, 106705.