STRUCTURAL INTERACTIONS AND FERMENTATION OF LENTIL PROTEIN WITH OTHER PROTEINS TO IMPROVE THE FUNCTIONAL AND NUTRITIONAL PROPERTIES

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A230	Wavelength 230 nm
AAB	Acetic Acid Bacteria
AFM	Atomic Force Microscopy
ANS	8-anilino-1-naphthalenesulfonic Acid
BPPs	Blood Plasma Proteins
CD	Circular Dichroism
CFPs	Cod Fish Protein
CFU	colony-forming units
CPHs	Corn Protein Hydrolysates
CPs	Casein Proteins
EAI	Emulsion Activity Index
ESI	Electrospray Ionization
ESI	Emulsion Stability Index
ESI+	Electrospray Interface
FAO	Food and Agriculture Organisation
FTIR	Fourier-Transform Infrared Spectroscopy
HPLC	High-performance Liquid Chromatography
ITC	Isothermal Titration Calorimetry
LAB	Lactic Acid Bacteria
LFs	Lactoferrins
LPs	Lentil Proteins
MRM	Multiple Reaction-Monitoring
ND	Not Detection
OAC	Oil Absorption Capacity

pI	Isoelectric Point
PPI	Protein-Protein Interaction
PPs	Peanut Proteins
QPs	Quinoa Proteins
RGs	Rice Glutelin
RPs	Rice Proteins
SC	Sodium Caseinate
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
SF	Soy Flour
SGs	Soy Globulins
SL	Soybean Lecithin
SLS	Static Light Scattering
SPIs	Soy Protein Isolates
SPIs	Soy Protein Isolates
SRM	Super-Resolution Microscopy
TEM	Transmission Electron Microscopy
TPC	Total Phenolic Compounds
TPC	Total Phenolic Content
t_R	Retention Time
TSC	Total Saponins Content
TSS	Total Soluble Solids
VA	Vitamin A
WAC	Water Absorption Capacity
WGPs	Wheat Gluten Proteins

WHO	World Health Organisation
WKS	Water Kefir Seeds
WPIs	Whey Protein Isolates
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
β-lg	β-lactoglobulin
λF_{max}	Maximum Emission

INTERAKSI STRUKTUR DAN FERMENTASI PROTEIN LENTIL DENGAN PROTEIN LAIN UNTUK PENAMBAHBAIKAN SIFAT FUNGSIAN DAN NUTRISI

ABSTRAK

Dalam beberapa tahun kebelakangan ini, ada peningkatan minat terhadap protein lentil (LP) disebabkan oleh ketersediaannya yang baik, kos rendah, dan pengeluaran global. Faktor utama yang membatasi penggunaan LP dalam industri makanan adalah kelarutannya yang rendah, kekurangan pencernaan, dan jumlah saponin yang tinggi, yang menyebabkan kepahitan. Interaksi protein-protein (PPI) dan fermentasi telah digunakan pada masa lalu untuk mengatasi beberapa batasan ini dalam protein lentil. Pengkompleksan protein dan prosedur fermentasi WKS berjaya digabungkan untuk meningkatkan aspek fungsian dan pemakanan kompleks protein berasaskan LP. Tujuan kajian ini adalah untuk meningkatkan kelarutan dan pencernaan protein lentil, serta penurunan kandungan saponinnya melalui PPI dan fermentasi. Untuk mencapai tujuan ini, kajian ini dipisahkan kepada 3 fasa. Objektif Fasa 1 adalah untuk membandingkan struktur protein dan kelarutan dan sifat antara 4 jenis protein yang berlainan: LP, protein quinoa (QP), protein kasein (CP), dan protein whey (WP). Kelarutan WP, QP, dan CP lebih tinggi daripada LP, masing-masing pada 92.7, 80.1, 89.7, dan 58.0% (P<0.05). Menurut penemuan kami, WPI, QP, dan CP mempunyai struktur protein dan sifat antara muka yang lebih unik daripada LP. Manakala hidrofobisiti permukaan LP lebih tinggi daripada WP, CP dan QP, sekitar 618, 24, 236, dan 404 a.u. (P<0.05), masing-masing. Sebaliknya, WPI, CP dan QP mempunyai cas permukaan yang lebih tinggi lebih berbanding LP, masing-masing kira-kira -33.2, -30.9, -32.9, dan -22.9 (P<0.05). Menurut penemuan kami, kadar

struktur tertiari LP adalah lebih tinggi daripada WPI, CP, dan QP yang dikaji menggunakan perolehan spektrum. Untuk Fasa 2, LP dikomplekskan dengan WP, CP, atau QP pada pelbagai nisbah untuk mengkaji pengaruh nisbah dan jenis protein pada struktur, kelarutan molekul pengikatan air dan sifat antaramuka kompleks protein. Melalui nisbah terbaik untuk kompleks protein, kelarutan LP meningkat dengan ketara (P<0.05) hingga mencapai 92.7, 76.8, dan 86.5% setelah kompleks pada nisbah 1: 1.2 (untuk LP-WP), 1:1 (untuk LP-QP), dan 1:0.1 (untuk LP-CP), masing-masing. Berdasarkan keputusan kami melalui analisis morfologi, pendarfluor dan spektrum UV, dan FTIR, LP berinteraksi dengan WP, CP, atau QP untuk membentuk struktur protein baru. Akhirnya, dalam Fasa 3, LP, QP, WP, CP dan kompleks protein berasaskan LP yang dipilih diperam menggunakan biji kefir air selama 5 hari. Dengan menggunakan proses fermentasi ini, peningkatan yang ketara (P<0.05) dalam pencernaan kompleks protein LP, LP-QP-1:1, LP-WP-1:1.2, dan LP-CP-1:0.1 meningkat dari 76.42 ke 84.1%, dari 76.46 ke 87.2%, dari 81.69 ke 93.0%, dan dari 79.53 ke 86.7%, masing-masing, selama tempoh penapaian. Jumlah kandungan saponin (TSC) kompleks protein LP, LP-WP-1:1.2, LP-QP-1:1, dan LP-CP-1:0.1 dikurangkan dengan ketara (P<0.05) pada nisbah 24.0, 16.5, 18.8, 30.0, dan 19.6%, masing-masing, semasa tempoh penapaian. Kompleks protein berasaskan LP yang ditapai berkembang menjadi kompleks protein baharu dengan struktur tersusun semula, menghasilkan kebolehcernaan yang lebih tinggi dan menurunkan TSC. Kesimpulannya, gabungan pengkompleksan protein dan teknik penapaian WKS berjaya meningkatkan sifat fungsian dan pemakanan untuk LP, QP, dan kompleks protein berasaskan LP.

STRUCTURAL INTERACTIONS AND FERMENTATION OF LENTIL PROTEIN WITH OTHER PROTEINS TO IMPROVE THE FUNCTIONAL AND NUTRITIONAL PROPERTIES

ABSTRACT

In recent years, there has been increasing interest in plant-based proteins such as lentil proteins (LPs) due to their good availability, low cost, and global production. The main factors limiting the use of lentil proteins in the food industry are its low water solubility, poor digestibility, and high amount of saponins, which leads to bitterness. Protein-protein interactions (PPI) and fermentation have been used in the past to address some of these limitations in plant-based proteins. Protein complexation and WKS fermentation procedures were combined successfully to improve the functional and nutritional aspects of LP-based protein complexes. The aim of this study is to enhance the solubility and digestibility of LPs, as well as the degradation of its saponins content through PPI and fermentation. In order to achieve this aim, this study is separated into 3 phases. For Phase 1, the objective was to compare the protein structural and solubility and interfacial properties of 4 different types of protein: LPs, quinoa proteins (QPs), casein proteins (CPs), and whey proteins (WPs). Solubility of WPs, QPs, and CPs was higher than that of LPs, at 92.7, 80.1, 89.7, and 58% (P<0.05), respectively. According to our findings, WPIs, QPs, and CPs have more different protein structures and interfacial properties than LPs. While the surface hydrophobicity of LPs was higher more than WPs, CPs and QPs, around 618, 24, 236, and 404 a.u. (P<0.05), respectively. On other hand, the WPIs, CPs, and QPs have higher surface charges than LPs, approximately -33.2, -30.9, -32.9, and -22.9 (P<0.05), respectively. According to our findings, the rate tertiary structure of LPs

was higher than that of WPIs, CPs, and QPs studied using spectral acquirements. For Phase 2, LPs were complexed with WPs, CPs, or QPs at various ratios in order to study the effect of ratio and types of protein on the structural, molecular binding water solubility and interfacial properties of the protein complexes. Through the best ratio of protein complexes, the solubility of protein complexes increased significantly (P<0.05) to reach 92.7, 76.8, and 86.5% after complexation at ratio 1:1.2 (for LP-WP), 1:1 (for LP-QP), and 1:0.1 (for LP-CP), respectively. Based on our findings using morphological analysis, fluorescence and UV spectra, and FTIR, the LPs interacted with WP, CP, or QP to form novel protein structures. Finally, in Phase 3, the LPs and selected LP-based protein complexes were fermented using water kefir seeds for 5 days. Using this fermentation process, a significant (P<0.05) increase in the digestibility of LPs, LP-QP-1:1, LP-WP-1:1.2, and LP-CP-1:0.1 protein complexes increased from 76.42 to 84.1%, from 76.46 to 87.2%, from 81.69 to 93.0%, and from 79.53 to 86.7%, respectively during the fermentation period. Total saponins content (TSC) of LPs, LP-WP-1:1.2, LP-QP-1:1, and LP-CP-1:0.1 protein complexes was reduced significantly (P<0.05) at ratio 24.0, 18.8, 30.0, and 19.6%, respectively, during the fermentation period. Fermented LP-based protein complexes developed into new protein complexes with reorganised structures, resulting in higher digestibility and decreased TSC. In conclusion, the combination of proteins complexation and WKS fermentation techniques were successful in improving the functional and nutritional properties of LPs, QPs, and LP-based protein complexes.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In the last decade, demands for high nutritional value-added food products and plant-based protein have increased in line with the growth of the human population and consumers' health awareness (IFT, 2020). Many efforts have been focused on replacing animal-based proteins with plant-based proteins (Sha & Xiong, 2020; Wild et al., 2014). Protein is an essential component of human nutrition because they are highly important to the recovery of damaged cells, immune response, cell signals, and the maintenance of muscle mass (Gombart et al., 2020) Plant- and animal-based proteins are the two important dietary sources of protein. However, a diet rich in animal-based proteins is also associated with higher saturated fat intake (Segovia-Siapco et al., 2020) and salt (Petit et al., 2019). Overconsumption of meat, especially processed meat, can lead to health problems (Bujnowski et al., 2011; Kafatos, 2009), coronary heart disease (Clifton, 2011; Richi et al., 2015), high blood pressure (Elliott et al., 2006; Richi et al., 2015; Tuso et al., 2013), and increased serum and urine uric acid (Tracy et al., 2014). On contrary, various studies have shown plant proteins have had several beneficial health effects, such as anti-cancer activities (Bingham, 1999; James et al., 2017) enhancing metabolic dysfunction due to obesity (Dinu et al., 2017; Tonstad et al., 2009; Tuso et al., 2013; Wanezaki et al., 2015), preventive effects against cardiovascular diseases (Kim et al., 2019; Kwok et al., 2014; Marsh et al., 2012; Tuso et al., 2013) and nutritional support for cirrhotic patients (Kim et al., 2019; Tuso et al., 2013; Bianchi et al., 1993). The global production of plant-based protein in 2013 was about 6,452 thousand tonnes, and in 2018 it was approximately 8,541.2

thousand tonnes, with the production of plant-based protein forecasted to be around 10,555 thousand tonnes by 2023 (Kerry, 2021). The demand for plant-based protein in the United States of America (USA) increased, whereby the USA consumers' appetite for plant-based protein e.g., lentil and quinoa, increased around 44 and 36% in 2020, respectively (IFT, 2020). Lentil (*Lens culinaris*) is an edible legume with a high amount of protein content, between 20.6 and 31.4 g/100 g (Jarpa-Parra et al., 2014), as well as possessing favourable ratios of leucine to isoleucine (1.24–1.98) and leucine to lysine (1.08–2.03) (Stevenson et al., 2007). It is also reported to be high in fibre and low in fat (Reif et al., 2021). However, lentil proteins (LPs) have poor solubility in the water, with their solubility reported to be approximately 55% (Jarpa-Parra et al., 2014).

Solubility is considered one of the most important functional properties on which other functional properties rely upon. Thus, considerable efforts have focused on improving the solubility of proteins by preparing soluble biopolymer composites (He et al., 2020b; Wang et al., 2018a). Whey protein isolates (WPIs) and casein proteins (CPs) have high solubility and nutritional value, they have been a popular use in food industries. Many researchers have revealed that WPIs and CPs can interact with other plant-based proteins, such as rice proteins (Lin et al., 2017; Wang et al., 2019a; Wang et al., 2018b). In addition, quinoa proteins (QPs) are a new trend in food industries because of their high solubility and an excellent source of amino acids, as well as having a high level of solvent-protein interaction capabilities (Dakhili et al., 2019; Elsohaimy et al., 2015; Suárez-Estrella et al., 2021). Hence, it will be interesting to investigate how the functional properties of LPs upon protein complexation with QPs. The interaction between animal- and plant-based proteins is being comprehensively investigated to develop balanced mixtures of protein complexes. The protein structure is a feature of the interaction of protein components (Mezzenga et al.,

2005; Ubbink et al., 2008). Molecular forces that are involved in protein-protein interaction (PPI) include non-covalent ones, such as electrostatic, hydrogen and hydrophobic forces (He et al., 2020a; Nicolai, 2019; Sun et al., 2018), van der Waals forces (Karplus & Šali, 1995; Howell, 1992; Barclay & Ottewill, 1970), steric and hydration repulsive forces (Howell, 1992), and covalent disulphide linkages (Lin et al., 2019; Wang et al., 2017). These forces have a major role in modifying the protein microstructure and functional properties, especially on the solubility of the protein (He et al., 2020a; Wang et al., 2019a).

PPI is considered one of the best techniques to enhance the functional properties, such as solubility, emulsion, and foaming, of plant protein (He et al., 2020b; Sun et al., 2018; Wang et al., 2019a; Wang et al., 2018a). The functional properties of a protein are influenced by surface hydrophobicity more than hydrophobicity because of the polymeric aspect of the protein (Chandrapala et al., 2011; Poncin-Epaillard et al., 2012). The hydrophobicity of the protein surface plays a major role in solubility, physical stability, adsorption behaviour, and the aggregating tendency of the protein (He et al., 2020a; Nicolai, 2019; Wang et al., 2019a). The surface hydrophobicity of a protein is determined by the length of the chain in the protein through hydrogen bonding (Narbutt, 2020). Whereby the PPI effect on the surface charge and surface hydrophobicity of protein via electrostatic and hydrophobic forces and hydrogen bonds (He et al., 2020a; Nicolai, 2019; Wang et al., 2019a).

The solubility of 7S and 11S globulins of LPs depend on the degree of dissociation, aggregation, denaturation, composition, and structure (Tezuka et al., 2004; Renkema et al., 2000; Puppo & Añón, 1999). The pH of an aqueous solution is also a major factor in the solubility of the protein. Non-covalent forces, such as hydrophobic and electrostatic interactions, govern the solubility of protein between

molecules. The solubility of protein is improved when the electrostatic repulsion between molecules is greater than hydrophobic interactions (Wang et al., 2019a; Mills et al., 2001; Utsumi, 1992). Hydrophobic interactions in an aqueous solution can also enhance PPI between LPs and WPIs, QPs, or CPs that could lead to an increase in the solubility of LPs after PPI.

Another key limitation of the use of plant protein in the food industry is its low digestibility resulting from its densely packed rigid system that is mainly due to hydrophobic interactions and disulphide bonds (Elsohaimy et al., 2015; Singh & Singh, 2016; Utsumi, 1992). Digestibility is often used as an indicator to evaluate the proteolysis capability and availability of a protein. Compared with proteins with low digestibility, proteins with high digestibility are considerably more suitable because of their high bioavailability, thus higher nutritional benefit. Fermentation is a suitable technology for biochemical changes, especially on the bio-accessibility and bioavailability of the food structure, as a result of acetic acid and lactic acid bacteria, such as *Streptococcus*, *Leuconostoc*, *Lactococcus*, and *Lactobacillus spp.*, as well as their enzymatic actions (Azi et al., 2020; Tu et al., 2019; Nkhata et al., 2018; Randazzo et al., 2016; Hotz & Gibson, 2007). Fermentation can lead to a decrease in non-nutritive compounds, including trypsin, and promotes the cross-linking of proteins, as well as promoting proteolysis and release of the proteins from the matrix, thus improving the digestibility of plant-based proteins (Chandra-Hioe et al., 2016; Reyes-Moreno et al., 2004; Shekib, 1994). Çabuk et al. (2018) investigated the effect of fermentation on the digestibility of pea protein and found out that the fermentation increased the protein digestibility from 80.0 to 83.2% after 11 h with *Lactobacillus plantarum* fermentation.

Furthermore, fermentation can also enhance the nutritional value (increased total phenolic content) and decrease anti-nutrients (compounds that inhibit digestibility, such as trypsin and phytate). Both studies by Azi et al. (2020) and Tu et al. (2019) showed that kefir fermentation decreases the trypsin and phytate of soybean and then improves the digestibility of soybean protein. Kefir has become a very popular microbial consortium because of its excellent composition of microorganisms with probiotic function (Fiorda et al., 2017). This consortium is composed mainly of lactic acid bacteria, acetic acid bacteria, and yeast (Azi et al., 2020). Recently, Yang et al. (2020) reported enhancement of the digestibility of pea protein after 24 h fermentation with *Aspergillus oryzae*, *Bacillus licheniformis*, *Bacillus subtilis*, *Lactobacillus casei*, and *Lactobacillus plantarum*. Furthermore, fermentation could also degrade saponins, which are responsible for the bitterness in plant-based proteins (Suárez-Estrella et al., 2021). According to Lai et al. (2013), a 24 h lactic acid fermentation can reduce the total saponins content in soy milk.

Microorganisms that produce enzymes responsible for the cleavage of ester bonds, resulting in the release of bound phenolics. Most of the non-nutritive compounds, such as phenolic compounds, build ether and ester linkages with lignin through their hydroxyl groups in the aromatic ring, and ester linkages with structural carbohydrates and proteins via their carboxylic group (Bhanja et al., 2009; Liyana-Pathirana & Shahidi, 2006; Bartolomé et al., 1997). The cleavage of these ester bonds leads to an increase in protein digestibility and releases of free-phenol compounds that are water-soluble.

Therefore, PPI may improve the solubility of LPs and can be used to prepare novel complex protein composites of LPs with WPIs, QPs or CPs. Subsequently, these protein complexes can be subjected to a fermentation process using water kefir seeds

to enhance the digestibility and reduce the bitterness of these complexes. Various protein-protein interactions were studied in recent years on plant-based proteins. He et al. (2020b) explored the use of mixed soy protein and wheat gluten proteins to increase protein solubility. Wang et al. (2019a) also found that a mixture of 1% rice proteins and whey protein isolates at a ratio of 1:1 increased the solubility to over 50% based on the synergistic interaction; hydrophobic, hydrogen, and ionic interactions, which may be taking place. These molecular forces may have collaboratively favoured the formation of the protein complexes. Yan et al. (2019) showed that the complexation of WPIs with lactoferrins can control the rheological properties of the emulsion gels of protein complexes by varying the pH through non-covalent bonds, such as electrostatic interactions between droplets. Wang et al. (2018b) reported that a mixture of 1% rice proteins and soy protein isolates, increased the solubility to over 80% through synergistic interaction.

LPs are a strong alternative to soy protein because they are not genetically engineered and do not cause allergic problems. Developing a design for generating fermented LPs and protein complexes of LPs with WPIs, CPs or QPs with improved nutritional properties is extremely desirable for food technology and industrial food production.

1.2. Rationale of Study

1.2.1 Aim and Objectives

The aim of the study is to produce LPs-based protein complexes with enhanced properties (e.g. water solubility, digestibility, and bitterness) via the aid of synergistic interaction between LPs and other proteins (WPIs, QPs, and CPs). In order to achieve this aim, the study was carried with the following objectives:

1. To investigate protein quality, microstructure, solubility in water, protein structure, secondary protein structure, and interfacial properties of LPs, QPs, WPIs, and CPs.

2. To determine the effects of ratio and types of proteins (WPIs, QPs, and CPs) on the microstructure, water-solubility, protein structures, molecular bindings, and interfacial properties of LPs-based protein complexes using PPI.

3. To determine the effect of water kefir seeds fermentation on the digestibility and saponins content of the LPs-based protein complexes.

1.2.2 Problem Statements

1. Lentil proteins have poor solubility in water. The solubility of lentil proteins were reported to be around 55% at pH 7.0, compared to 93, 90, and 80% for WPIs, CPs, and CPs, respectively.

2. Lentil protein has poor digestibility (approximately 77%) compared to dairy-based proteins, such as 93 and 82% for WPIs and CPs, respectively.

3. Saponins are found in many edible legumes. Lentils have saponins content, between 700–1,260 mg/kg, which is responsible for the bitterness in lentils.

1.2.3 Hypotheses

1 Study basic solubility and structure of lentil proteins and their application in food production.

2 Study the effect of ratio and types of different proteins on the structural and solubility of lentil protein based on protein complexation.

3 Identifying effect fermentation on complexes protein using water kefir grains, to increase digestibility and decrease the bitterness of lentil proteins.

1.3 Framework of Study

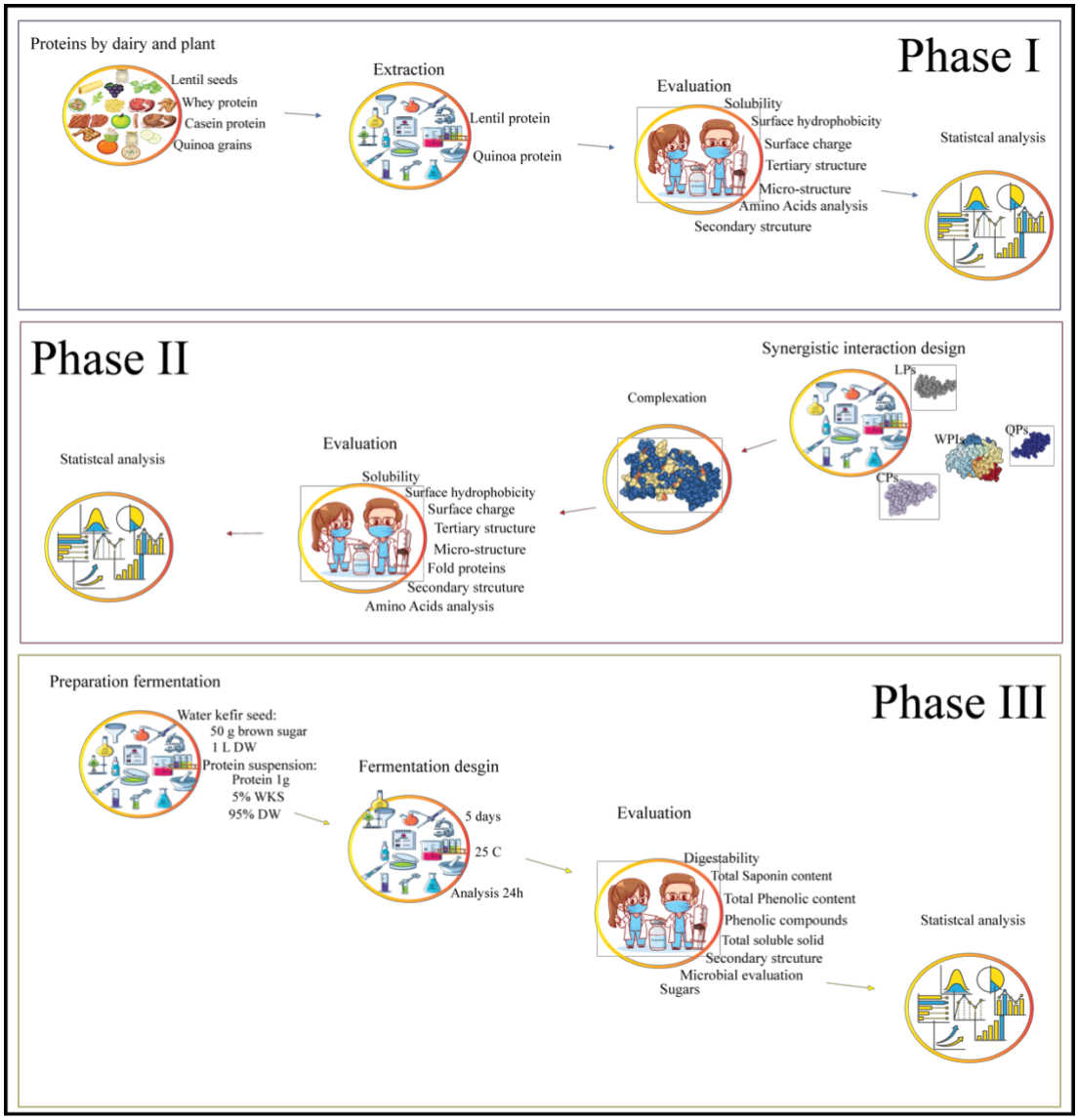

Figure 1.1. Schematic diagram of the framework of the study: Phase I, Phase II, and Phase III

LPs = lentil proteins; WPIs = whey protein isolates; CPs = casein proteins; QPs = quinoa proteins; WKS = water kefir seeds; DW = distilled water

CHAPTER 2

LITERATURE REVIEW

2.1 Plant-Based Proteins

With the increasing popularity of plant-based diets and growing environmental issues, there has been a growing interest in the use of alternative protein sources (Lin et al., 2019; Sun et al., 2018) and a decrease in animal-based protein diets. Thus, plant-based proteins (e.g. quinoa, soya, and lentil) as an alternative to animal-based proteins (e.g. whey protein isolates, caseins, and gelatines) have gained widespread interests in recent years due to their lowest possible environmental footprints as compared to those related to the development and consumption of animal-based proteins (Jędrejek et al., 2016; Koneswaran & Nierenberg, 2008; Zheng et al., 2020). As shown in Table 2.1, animals, plants, and fungi are the three main dietary sources of protein. Interactions between plant proteins with other types of protein undoubtedly occur in different ways. These interactions could have significant impacts on the structural and functional properties of plant proteins due to the molecular forces, e.g. hydrogen bonds and hydrophobic and electrostatic forces (He et al., 2020a).

Table 2.1 Summary of food proteins sources commonly used in the food industry

Sources of Protein			Types and Ratio of Proteins	References
Animal		Milk	Whey 15–20% [β-lactoglobulin (35–65%), α-lactalbumin (12–25%); immunoglobulins (8%], albumin (5%), and lactoferrin (1%)] Casein [80%) (αS1-casein (40%), αS2-casein (10%), κ-casein (15%), and β-casein (35%)]	(Camargo et al., 2018; Ma et al., 2017b) (Beliciu & Moraru, 2013)
		Egg	Ovoglobulin (G2 and G3, 8%), ovotransferrin (12%), lysozyme (3.5%), ovomucin (3.5%), ovalbumin (54%), and ovomucoid (11%)	(Abeyrathne et al., 2018)
		Blood	Bovine serum albumin, haemoglobin, and ferritin	(Alimam et al., 2018)
		Meat	Elastin, myosin, actin, and collagen	(Hurrell et al., 2006)
		Insect	Fibroin, resilin, chemosensory, Sf caspase-1, major royal jelly protein, melittin, myoblast city, doublesex, and transfer genes	(Ardell & Andersen, 2001; Angeli et al., 1999)
Plant	Cereals	Wheat	Albumin; globulin; glutelin (80%); prolamin/gliadin	(Shewry et al., 2009)
		Corn	Albumin; globulin; glutelin (18%); prolamin/zein (60%) (α-zein, β-zein, γ-zein, δ-zein)	(Hojilla-Evangelista, 2012; 2014; Muthukumarappan & Swamy, 2018; Zhuang et al., 2013)
		Barley	Albumin (22%); globulin; glutelin (47–27%); prolamin (35–55%)	(Kirkman et al., 1982)

Table 2-1. Continued

Sources of Protein			Types and Ratio of Proteins	References
		Oats	Albumin; globulin (75%); glutelin (4−14%); prolamin (10%)	(Friesen, 2017; Webster, 2016)
		Rice	Albumin (5–10%), globulin (7–17%), glutelin (75–81%) and prolamin (3–6%)	(Amagliani et al., 2017)
	Legumes and pulses	Soybeans	2S globulin (17%), 7S globulin (33%), 11S globulin (45%); 15S globulin (5%)	(Singh et al., 2010: Amagliani et al., 2017)
		Peas	2S globulin (25%), 7S globulin (43%), 11S globulin (28%); 15S globulin (4%)	(Amagliani et al., 2017)
		Mung bean	2S globulin (15%), 7S (79%) globulin; 11S globulin (6%)	(Amagliani et al., 2017)
		Lima bean	2S globulin (52%), 7S globulin (21%); 15S globulin (27%)	(El Fiel et al., 2002)
		Chickpea	Albumin (8.39–12.31%), globulin (53.44–60.29%) prolamin (3.12–6.89%); glutelin (19.38–24.40%).	(Portari et al., 2005)
		Lentil	Albumins (16.8%), globulins (11S/legumin (44.8%), 7S/vicilin (4.2%)), glutelins (11.2%); prolamins (3.5%).	(Boye et al., 2010)
	Tubers	Potato	Sporamin +A N-termina (80%), glycoprotein, arabinogalactan	(Wang et al., 2016)
	Oilseeds	Rapeseed, peanut, sunflower, hemp seed	Albumin + globulin (60%), gluten	(Bur et al., 2004)

Table 2-1. Continued

Sources of Protein			Types and Ratio of Proteins	References
		Cottonseed	Globulins (2S, 7S/congossypin, 12S)	
	Edible seeds	Quinoa, buckwheat	albumin (35%), globulin (37%), prolamins (0.7−7%)	(Steffolani et al., 2016)
	Pseudocereals	Chia	Albumin (22.8%), globulin (22.4%), glutelin (48.9%) prolamin (5.8%)	(Attalla & El-Hussieny, 2017)
	Algae	Micromonas		(Simon et al., 2017)
Fungi		Mycoprotein		(Stoffel et al., 2019)

2.1.1 Lentil Proteins (LPs)

Legume seeds that are mature are usually rich in proteins although they produce these proteins during their growth (Bennetau-Pelissero, 2019). They are primarily processing proteins categorised into glutelins, albumins and globulins based on their solubility behaviour, with salt-soluble globulins constituting the majority of the fraction contained in the pulses (Kiosseoglou & Paraskevopoulou, 2011). Globulin is classified into two forms, vicilin or legumin, called fraction 7S or fraction 11S, respectively (Jarpa-Parra et al., 2017). Albumin has a solubility in water about 10−20% of the legume proteins. At the same time, glutelin has a solubility in acid and base solution of approximately 10−20% of legumin proteins (Shewry & Tatham, 1997). Lentil seeds (*Lens culinaris*) have a high amount of protein between 20.6 and 31.4 g/100 g (Jarpa-Parra et al., 2014), are low in fat, and are high in fibre (Urbano et al., 2007).

2.1.1(a) Chemistry and Structure of LPs

LPs are composed of four protein fractions; 70% (7S and 11S) globulins, 16% albumins, 11% glutelins, and 3% prolamins (Boye et al., 2010). Glutelins, albumins, and prolamins, which have polypeptides around 4, 13, and 10, respectively. These proteins have low molecular weights (MWs), approximately 17−46, 20, and 16−64 kDa, respectively. Globulins are divided into two fractions: legumin and vicilin. Legumin has a molecular weight of 320−380 kDa and is made up of 6 polypeptides. They consist of a 20 kDa basic dimer and 40kDa acidic dimer bound by a disulphide bond (Shewry & Tatham, 1997), whereas vicilin has MWs ranging between 50 and 60 kDa, made up of subunits of glycosylated, and is usually referred to as 7S fraction

(Aydemir & Yemenicioğlu, 2013; Bhatty, 1988; Argos et al., 1985). The glycosylated subunits contain carbohydrates, which are made up of around 2.8% (Barbana & Boye, 2011; Bamdad et al., 2009; Martinez & Urbano, 2007).

2.1.1(b) Quality and Health Benefits of LPs

Interest in LPs has recently increased because of their excellent nutritional benefits, which are suitable leucine/isoleucine and leucine/lysine ratios; 1.24–1.98 and 1.08–2.03 mg/g, respectively (Urbano et al., 2007). According to the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), the recommended ratios of essential amino acids of leucine/isoleucine and leucine/lysine are 1.3-1.6 and 1.3-1.6 mg/g, respectively (FAO/WHO, 1991). The dietary qualities of lentils are related to reduced cholesterol and lipid levels in humans and a low risk of colon cancer and type 2 diabetes (Roy et al., 2010). LPs have high contents of tannins, flavonoids, and phenolics, with values of 5.97 mg of catechin equivalents/g, 5.97 mg of catechin equivalents/g, and 6.56 mg of gallic acid equivalents/g, respectively (Jarpa-Parra et al., 2017).

2.1.1(c) Functional Properties of LPs

Techniques used in protein extraction and its conditions have a direct effect on the functional properties, physicochemical properties, and composition of proteins, as well as the protein structure (Farooq & Boye, 2011). In general, protein functionalities, such as solubility, emulsifying, foaming, and water- and oil-absorption capabilities have been extensively studied (Table 2.1).

Protein solubility in water is an essential functional property because it affects most other functional characteristics (Joshi et al., 2011). Therefore, the profile of protein solubility in various pH levels can be a major predictor of proteins in food applications and the degree of protein denaturation affected by extraction. Studies by Jarpa-Parra et al. (2014), Boye et al. (2010), and Bora (2002) reported that LPs have lower solubility in water between pH 4.0 and 6.0 but have higher solubility at lower and higher pH ranges. The solubility levels of LPs are 17−49% at pH 3.0 and 55−58% at pH 7.0 (Jarpa-Parra et al., 2014; Joshi et al., 2011). Therefore, LPs have poor solubility of approximately 55% in water (Jarpa-Parra et al., 2014). Solubility is an important functional property that affects its other functional properties. Thus, considerable efforts have focused on the solubility of proteins to prepare soluble biopolymer composites (Apichartsrangkoon et al., 1998; He et al., 2020b; Kong et al., 2007; Wang et al., 2019b). The solubility of 7S and 11S globulins depends on the degree of dissociation, aggregation, denaturation, composition, and structure (Tezuka et al., 2004; Renkema et al., 2000; Puppo & Añón, 1999).

Table 2.2 Summary outcomes of functional properties of lentil proteins (LPs), quinoa proteins (QPs), whey protein isolates (WPIs), and casein proteins (CPs)

Protein Type	Functional Properties	Description	Outcomes	References
LPs	Solubility	LPs extract at pH 8.5 Protein solution (LPs 15 mg/mL)	15 to 35% at pH 2.0 55% at pH 7.0; 5% at pH 3.5; 98% at pH 8.0; 95% at pH2.5	(Bora, 2002)
		Different genotypes of LPs	30% at pH3.0 and 57% at pH7.0 31% at pH3.0 and 56% at pH 7.0	(Ma et al., 2011)
		LPs extract at pH 9.0 Protein solution (0.5%)	50% at pH7.0	(Joshi et al., 2011)
		LPs extract at pH 10	97% at pH 10.0; 96% at pH 1.0; 5% at pH 5.0	(Jarpa-Parra et al., 2014)
		LPs extract at pH 9.0	5% at pH 5.0; 82% at pH 1.0; 97% at pH 10.0	
		LPs extract at pH 8.0	5% at pH 5.0; 96% at pH 10; 78% at pH 2.0	
	WAC	LPs extract at pH 8.5 Protein solution (LPs 15 mg/mL)	1.1 mL/g of protein	(Bora, 2002)
		LPs extract at pH 9.0	3.8 mL/g of protein	(Boye et al., 2010)
			0.4 g/g of protein at pH 7.0	(Joshi et al., 2011)

Table 2-2. Continued

Protein Type	Functional Properties	Description	Outcomes	References
	OAC	LPs extract at pH 8.5 Protein solution (LPs 15 mg/mL)	2.6 mL/g of protein	(Bora, 2002)
		LPs extract at pH 9.0	120%	(Boye et al., 2010)
	EAI	LPs extract at pH 8.5 Protein solution (LPs 15 mg/mL)	54%	(Bora, 2002)
		LPs extract at pH 8.0	41% to 47 at pH 7.0	(Lee et al., 2007)
		LPs extract at pH 9.0	5.0–5.9 m^2/g of protein at pH 7.0	(Boye et al., 2010)
		LPs extract at pH 10.0	44 m^2/g of protein at pH 7.0	(Karaca et al., 2011)
		Prepared by isoelectric precipitation	90 m^2 g^{-1} protein at pH 7.0	(Joshi et al., 2012)
	ESI	LPs extract at pH 8.5 Protein solution (LPs 15 mg/mL)	52%	(Bora, 2002)
		LPs 15 mg/mL	83 to 89% at pH 7.0	(Lee et al., 2007)
		LPs extract at pH 9.0	18 min at pH 7.0	(Boye et al., 2010)
		LPs 10 mg/mL	101 h at pH 7.0; 92 h at pH 6.0; 27h at pH 5.0, 245 h at pH 3.0	(Joshi et al., 2012)

Table 2-2. Continued

Protein Type	Functional Properties	Description	Outcomes	References
QPs	Solubility	QPs extracted at pH 9.0	30% at 3.0 pH solution, 60% at pH 8.0 and 75% at pH 10	(Elsohaimy et al., 2015)
		QPs extracted at	100% at pH 10.	(Paredes-López et al., 1993)
			76% at pH 7.0	(Shen et al., 2021)
	WAC	QPs extracted at pH 9.0 protein solution (3%)	3.94 mL/g protein	(Elsohaimy et al., 2015)
	OAC	QPs extracted at pH 9.0 Protein solution (3%)	1.88 mL/g protein	(Elsohaimy et al., 2015)
	EAI	QPs extracted at pH 9. Protein solution (3%)0	3.38 ±0.31 m^2/g of protein	(Elsohaimy et al., 2015)
		QPs extracted at pH 9.5 Protein solution (3%)	40.60% at pH 5; 44.72% at pH 6; 56.63%at pH 7; 61.01% at pH 8	(Shen et al., 2021)
		Protein solution (1%)	85.45%.	(Sánchez-Reséndiz et al., 2019
		QPs extracted at pH 9.0	30.4 and 46.34	(Elsohaimy et al., 2015)
			30.18 at pH 5; 42.04 at pH 6; 51.90 at pH 7; 57.98 at pH 8	(Shen et al., 2021)

Table 2-2. Continued

Protein Type	Functional Properties	Description	Outcomes	References
WPIs	Solubility	ND	95% at pH 7.0	(Floris et al., 2008)
			90% at pH 7.0	(Zembyla et al., 2021)
			95% at pH 7.0	(Gani et al., 2015)
	EAI	ND	30 min at pH 7.0	(Xu et al., 2019a)
			45 30 min at pH 7.0	(Ping-Ping et al., 2020)
			7.07 m^2/g	(Chen et al., 2019)
			66.05%.	(Chen et al., 2019)
			35%	(Ping-Ping et al., 2020)
CPs	Solubility	ND	95% at pH %	(Gani et al., 2015)
		pH 7.0	55%	(Augusta et al., 2007)
	ESI	ND	45.13 min.mL oil/g protein	(Gani et al., 2015)
		pH 7.0	1885 ± 34.20 nm at pH 7.0	(Li & Zhao, 2019)
		pH 7.0	43 min.mL oil/g protein	(Augusta et al., 2007)
		concentration 1%	0.08g/mg	(Augusta et al., 2007)
	EAI	pH 7.0	103 m^2/g	(Augusta et al., 2007)
	WAC	ND	18.48 mL/100g.	(Gani et al., 2015)

WAC= water absorption capacity; OAC= oil absorption capacity; EAI= emulsion activity index; ESI= emulsion stability index; ND= not determined; NM= pH value not mentioned

The concepts oil absorption capacity (OAC) and water absorption capacity (WAC) are usually used to refer to the amount of oil and liquid which could be absorbed per gram of sample, respectively. WAC values are good indicators of a protein's ability to resist liquid release from food during processing, transportation or storage (Farooq & Boye, 2011; Kiosseoglou & Paraskevopoulou, 2011). The OAC and WAC rates of LPs are commonly used to describe the outcomes. For instance, both Boye et al. (2010) and Bora (2002) reported that WAC level at the range between 1.1 and 4.2 mL/g of protein. Meanwhile, Lee et al. (2007) found that WAC of LPs at the range between 2.1 to 3.8% (w/w), while Joshi et al. (2011) reported that WAC at the range between 0.43 to 0.49 mL/g. For OAC, Bora (2002) found that the OAC for LPs to be between 2.0 to 2.6 mL/g. Meanwhile, according to Boye et al. (2010), the OAC of LPs ranges from 120 to 225%, depending on the technique used in the protein extraction. There has not been a great deal of research recently on the mechanisms and compounds involved in both OAC and WAC.

The emulsification capacity of a protein is its capacity to aid in the preparation of an emulsion with oil droplets of a relatively small size via creating a film around them. These droplets need to be distributed in the liquid suspension to avoid droplet accumulation that may lead to phase separation (Karaca et al., 2011). Protein emulsification characteristics are typically defined by two parameters: emulsifying activity index (EAI) and emulsifying stability index (ESI). EAI is the highest area produced from each unit of protein and this is typically defined with high-dilute emulsion samples through the application of turbidimetry. ESI evaluates the emulsion's ability to maintain its shape over even a given time period, and it is measured by monitoring turbidity decreases with diluted emulsion during storage (Kiosseoglou & Paraskevopoulou, 2011). Studies on the emulsifying properties of LPs

show a lot of variation depending on the processing and protein extraction conditions (Table 2.1). Therefore, the comparison of the findings becomes extremely difficult. Generally, the emulsifying properties of LPs are close to those of other pulses, varying from 5 to 90 m^2/g for EAI (Joshi et al., 2012; Karaca et al., 2011; Boye et al., 2010). While the ESI of LPs are varying from 19 min to several days depending on the conditions of the method used. Except for Joshi et al. (2012), who measured both EAI and ESI in the pH range of 3.0 to 7.0, the majority of the studies on the EAI and ESI of LPs were conducted at pH 7.0.

2.1.2 Quinoa Proteins (QPs)

Quinoa seeds (*Chenopodium quinoa* Wild under the family Chenopodiaceae) are a great nutrient source as recognised by the FAO (FAO, 2011). Quinoa seeds are healthy ingredients and are characterised as food that reduces the risk of several diseases and has health-promoting characteristics (Repo-Carrasco et al., 2003). The FAO (2017) reported that quinoa seeds contribute to agricultural productivity in the 21st century because of their tolerance to salinity and stress conditions and potential to grow in marginal areas. Scientists have attempted to use quinoa seeds as a replacement for animal-based proteins (Ruiz et al., 2016; Miranda et al., 2010; Paśko et al., 2009; Drzewiecki et al., 2003). The seed contains 12−20% proteins (Toapanta et al., 2016; Elsohaimy et al., 2015; Abugoch et al., 2008).

2.1.2(a) Chemistry and Structure of QPs

The main fractions of quinoa protein are prolamins (0.5–7.0%), albumins (35%), and globulins (37%) (Kaspchak et al., 2017; Abugoch et al., 2008). The

solubility of QPs depends on the pH of the solution (Elsohaimy et al., 2015). The globular chenopedin has a hexamer structure and consists of six pairs of basic and acidic polypeptides with MWs of 22–23 and 32–39 kDa, respectively, linked by a single disulphide bond (Dakhili et al., 2019; Ruiz et al., 2016).

2.1.2(b) Quality and Health Benefits of QPs

QPs have higher amounts of essential amino acids than proteins in other cereals, such as rice, maize wheat, and barley (Zhu, 2020; Montaño et al., 2006). QPs have 0.4−1.0% methionine and 5.1−6.4% lysine (Bhargava et al., 2007). Quinoa seeds also have higher concentrations of methionine, lysine, and cysteine than other legumes and cereals and are thus a good replacement for these crops. Quinoa seeds have high nutritional value because of their high amounts of minerals, such as potassium, copper, magnesium, iron, and calcium, as well as vitamins, such as vitamin B, E, and C (Konishi et al., 2004). In addition, quinoa seeds have high levels of fibre and antioxidants, such as phenolic compounds (Repo-Carrasco-Valencia & Serna, 2011; Hirose et al., 2010). Hence, quinoa seed may be a good potential to be included in the development of designer foods since it may help to reduce the risk of common diseases and promote human health (Shen et al., 2021).

2.1.2(c) Functional Properties of QPs

The solubility levels of QPs are about 30% at 3.0 pH solution, 60% at pH 8.0 and 75% at pH 10 (Elsohaimy et al., 2015; Dench, 1982; Kumar & Venkataraman, 1980). The balance of hydrophilic and hydrophobic components affects the solubility of QPs, which then can interact with solvent. At alkaline pH, the ionisation of carboxyl

groups and the deprotonation of amine groups produces negatively charged species, which improve the protein–solvent interaction and increase the protein solubility (Adebowale & Lawal, 2004; Pearce & Kinsella, 1978). General the QPs have low solubility at pH range 4−6, and its solubility increased in an alkaline environment (Ruiz et al., 2016; Steffolani et al., 2016; Elsohaimy et al., 2015; Bejarano-Luján et al., 2010; Tömösközi et al., 2008). Generally, QPs demonstrates low solubility at acidic pH (around 5% at pH 4–6), which is close to the isoelectric point (pI) of the 11S quinoa globulin (Paredes-López et al., 1993). The maximum solubility of QPs approximately 100% at pH 10.0. Both Shen et al. (2021) and López-Castejón et al. (2020) investigated the solubility of QPs at different pH values and reported that the solubility of QPs around 76% at pH 7.0. OAC and WAC are very important between functional properties of food because it enhances the retention of flavour and mouthfeel (Jarpa-Parra et al., 2017). Both Elsohaimy et al. (2015) and Ashraf et al. (2012) studied the WAC and OAC of QPs and found them to be around 3.94 ± 0.06 and 1.88 ± 0.02 mL/g protein, respectively. Recently, Sánchez-Reséndiz et al. (2019) studied the OAC of QPs and discovered that QPs have an OAC of about 2.66 mL/g.

Emulsion properties are essential functional properties of proteins that influence food product behaviour. EAI and ESI of QPs were listed in Table 2.2. Elsohaimy et al. (2015) investigated the EAI and ESI of QPs and discovered that the EAI of QPs at a 3% (w/v) ratio is about 3.38 ±0.31 m^2/g and the ESI is between 30.4- and 46.34-min. Sánchez-Reséndiz et al. (2019) studied the EAI of QPs and reported it to be around 85.45%. A recent study by Shen et al. (2021) found that the EAIs of QPs were 40.60, 44.72, 56.63, and 61.0 1% at different pH values; pH 5, 6, 7, and 8, respectively. Meanwhile, the ESI of QPs were reported to be 30.18, 42.04, 51.90 and 57.98% at pH 5, pH 6, pH 7 and pH 8, respectively.