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Protein profile and physicochemical properties of Liluva (soaking, boiling and sprouting water) from three food legumes

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A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Master of Science in Food Innovation

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Yanyu Zhang

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Science in Food Innovation.

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by

Yanyu Zhang

Legume waster water has been revealed to contain sufficent nutritional value as an industry by-product. In this study, physicochemical properties and protein profile of pea, chickpea and soybean soaking and cooking water were investigated. Among the three legumes isolates with their wastewater, all of the cooking wastewater exhibited the lowest content of free amino acid. In the yellow pea free amino acid assay, the soaking method contains the highest volume of Ser (6.00mg/g), His (6.01 mg/g), Phe (7.48 mg/g), Ile (4.12mg/g) Thr (4.27 mg/g) and Met (1.68mg/g). Chickpea soaking water has the highest volume of His (12.05mg/g) Phe (16.43mg/g), Ile (9.77mg/g), Thr (10.12mg/g) and Met (11.08mg/g). Pea cooking water Met to Lys is 0.8, which gave it the most elastic properties to form the sponge cake and more nutritional value. To identificate the emulsifying properties, oil-in-water emulsions containing 50% Canola oil were prepared using the legumes wastewater (pea, chickpea and soybeans). These emulsions were then stored at 4°C, and changes in particle size were monitored throughout storage. Chickpea cooking emulsions showed the smallest droplets distribution in the original emulsions. The mean emulsion droplet diameter (nm) evaluated differed significantly($p<0.05$) for all centrifugated legume wastewater-based emulsions. Different types of legumes affected emulsifier stability because of different protein content. In conclusion, soybean soaking and cooking water have the best emulsifier stability both at room temperature and the refrigerator temperature, pea cooking water was investigated to form the hardest sponge cake because of its highest protein content.

Keywords: Legume waste water, Total protein content, Petitedes, Free amino acid, emulsification

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Chapter 1 A mini-review

1.1 Introduction

Legumes are rich in carbohydrates, protein (20%-25%), amino acid, soluble and insoluble fibre, lipids, minerals and vitamins (Maphosa & Jideani, 2017). Peas (Pisum sativum L.), chickpeas (Cicer arietinum L.) and soybeans (Glycine max L.) are three typical dry legumes used in daily human diets worldwide. The demand for pulses in developing countries is expected to be increasingly growing because the population numbers are increasing that enabling access to available legume products. Legumes are also common due to their nutritional capacity for improving chronic noncommunicable diseases and competitive crop features such as durability and growing temperature flexibility (Luzardo-Ocampo, Cuellar-Nuñez, Oomah, & Loarca-Piña, 2020). However, legumes are processed with multiple processing procedures in the industry, including soaking, sprout, high-temperature cooking that may reduce plenty of macro-and micro-nutrients into the wastewater. This suggests that a variety of products can be produced taking into account technical tools available, creating an important quantity of waste used mostly for animal consumption or for processing high-fibre food products such as bread and meat products (Zhong et al., 2018). For instance, aquafaba is a well-known egg substitute in bakery products, which is the cooking water of chickpeas.

1.2 Legume nutritional significance

1.2.1 Phenolic compound

Pea, chickpea and soybeans are all investigated to contain the high nutritional effect on human health. Serious public health concerns have been globally risen, nowadays, in obesity and related co-morbidities including diabetes type 2 (T2D), cardiovascular disease (CVD) and cancer (van der Klaauw & Farooqi, 2015). In general, the utilization of high vegetable intake is positively implicated in the prevention of chronic diseases such as CVD. It is proposed that 150 g/day addition of cooked legumes into the diet is correlated with lower mortality in the population. Phenolics are dynamic and can induce oxidative reactions in free radicals and chelators (Ozcan, Akpinar-Bayizit, Yilmaz-Ersan, & Delikanli, 2014). The nutritional activities of phenolic compounds rely on their composition, mixture, solubility, absorption and interaction with other compounds (Singh, Singh, Kaur, & Singh, 2017). Because of their antioxidant, antihypertensive and anti-bacterial function, they influence several physiological processes. Free radicals scavenging, stabilization of cations, chelation and regulation of antioxidants enzymes are responsible for phenolic compound activities (Giusti, Capuano, Sagratini, & Pellegrini, 2019). They modulate various cell components, such as formation, proliferation or apoptosis, and play an important role in cellular functions (Crozier, Jaganath, & Clifford, 2009). Because of their health-protective effects, flavonoids, catechins and their derivatives are used for medicinal purposes.

Phenolics are found in soaking and cooking legume water when phenolic agents are disseminated from the food material (Giusti et al., 2019). However, some phenolics were present in the presence of water but were not found in the raw materials, such as rutin,

delphinidin-3-glucoside and quercetine. The hydrolysis of such glycosylated forms, which were present in the raw material, may be clarified, leading to the aglycone form such as rutin quercetin. Nevertheless, the only plausible explanation for this process is because these phenolics are tightly bound to cell wall material and cannot be isolated easily nor can be released into the water, such as rutin, chlorogene and dolphinidin-3-glucoside (Padayachee et al., 2012). Furthermore, the dissemination of phenolics by cooking legume water is definitely enabled by the high temperature and degradation of the legume tissue's internal structure during cooking method (Kebe, Renard, El Maâtaoui, Amani, & Maingonnat, 2015).

1.2.2 Antioxidant Activity

A variety of legume proteins could also be used as natural antioxidants to prevent lipid oxidation (Kiokias, Proestos, & Oreopoulou, 2020). Proteins can oxidize more quickly than unsaturated fatty acids in certain conditions, which will slow oxidation and rancidity (Berton et al., 2012; Elias et al., 2008). Protein favoured oxidation happens as it is more oxidizable than the system's fatty acids and chemically is closer to the free radicals or reactive oxygen species (ROS) than the lipids (Ajibola, Fashakin, Fagbemi, & Aluko, 2011). Proteins may also serve as portable antioxidants to prevent oxidation process through numerous pathways (Samaranayaka & Li-Chan, 2011). Proteins may affect the oxidation process by different pathways in emulsions. First, the non-adsorbed proteins bond heavy metals in order to avoid they enter the interfaces of the fat droplets; then the adsorbent surface proteins will restrict and carry them closer to the droplets. The positive charge protein may electrolytically resist the heavy metal ions. At last, the protein with antioxidant side can clean up free radicals so that

can influence lipid oxidation process (Berton-Carabin, Ropers, & Genot, 2014; McClements & Decker, 2000; Silvestre, Chaiyasit, Brannan, McClements, & Decker, 2000; Waraho, McClements, & Decker, 2011). Moreover, ABTS measurements are used to detect the bioactivity of legumes; it displayed that pea contains the largest lens-based antioxidant activity determined by ABTS sulphonic acid. In yellow peas, chickpeas and soybeans, free phytochemicals added more to overall antioxidant activity than binding phytochemicals. By decortizing, antioxidant activity was the reduction in lens content by 80% and in yellow peas by < 30%, by cooking is 16–41% and in the lens by cooking 22–42% (H. Han & Baik, 2008). Also, the cooking manner contributed to increased total protein content (TPC) and methanol extract antioxidants due to cell disruption and enhanced polyphenol isolation. Even if in legume cooking water, polyphenols were missing, the boiled legumes had reasonable content of bio-actives. The increased TPC and antioxidant ability of the extracts resulted from gastrointestinal digestion in vitro. The most bio-accessible polyphenols have been found in soybeans than peas and chickpeas. Phenolics in the legume cooking water were primarily obtained by the human body during the intestine process (Lafarga, Villaró, Bobo, Simó, & Aguiló-Aguayo, 2019).

1.3 Total protein, free amino acid and peptides in legume wastewater

Lunasin, a 43-amino acid peptide used primarily for lung, breast, and colon cancer, is one of the most commonly observed peptides for soybeans. In the last decade, lunasin has been determined to use its anti-cancer mechanism via multiple pathways, including cell and apoptosis regulation and epigenetic processes, as well as prevention of regular cell transformation. The information on the molecular pathways of lunasin has been extended

recently (Hsieh, Martínez-Villaluenga, de Lumen, & Hernández-Ledesma, 2018; Wan et al., 2017). The total protein, free amino acid content, general protein type, and peptide in legumes (pea, chickpea, and soybean) are presented in Table 4.1.

1.4 Legume protein emulsifying abilities

Protein structure, size, molecular distribution, surface hydrophobicity, solubility, stability and external conditions (pH and ionic strength) as well as insulation methods are important factors influencing the emulsifying characteristics of proteins (Liu, Ru, & Ding, 2012). Certain variables may be related. Lower molecular dimensions, larger surface hydrophobicity, surface filling, solubility and versatility guarantee strong emulsion characteristics (Karaca, Low, & Nickerson, 2015). Relatively small size, greater versatility, and solutions over a wide pH spectrum are a smarter option for animal proteins as emulsifiers (Nishinari, Fang, Guo, & Phillips, 2014).

Legume proteins do not require crosslinkers because of their sufficient hydrophobic nature (Elzoghby, Samy, & Elgindy, 2012). However, due to the hydrophilicity of these proteins and the requirement of glutaraldehyde for hardening, nanoparticles animal protein-based drug delivery systems cannot release drugs in aqueous medium sustainably. Plant protein use is relatively cost-effective and is an alternative to animal proteins in dairy, cosmetics and medical industry for the encapsulation of bio-actives ((Nesterenko, Alric, Silvestre, & Durrieu, 2013)). In the emergence of prion and bovine spongiform encephalopathy, animal proteins are often

thought to have a position as well as to inhibit their use in some religious and moral judgments of consumers (Karaca et al., 2015).

The same emulsifying abilities between peas and soybeans on β-lactoglobulin and Tween-20 were recorded in the study of Benjamin, Silcock, Beauchamp, Buettner, and Everett (2014). They investigated some physicochemical characteristics of plant proteins as a better choice than canola, cereals and flaxseed proteins (Benjamin et al., 2014). Besides, the interfacial layers of adsorbed wheat proteins, owing to the disparity in molecular size, volume and surface function, are thicker than the layers of dairy proteins (Wong et al., 2012). Protein activity at the O/W interface plays an important role in assessing physical and chemical emulsion capacity and stability (Jiang, Zhu, Liu, & Xiong, 2014). A research stated that the molecular stability of the emulsion increased due to structural changes in pea-protein arising from alkaline-pH-treatments. The method of legume protein insulation and the type of protein also have a tremendous influence on emulsifying properties and emulsion storage stability (Boye et al., 2010). The emulsifying abilities and foaming capacity of pea and soybean protein isolates have been contrasted (Aluko, Mofolasayo, & Watts, 2009). Pea protein has been found to have more efficient emulsifying activity with smaller emulsion droplet than soybean protein, and it's a better foaming agent than soybeans protein. As a result of its solubility in NaOH solution, water, NaCl solution and aqueous ethanol, four pea proteins' fractions functional properties were differentiated with each other (Adebiyi & Aluko, 2011). The most solid emulsion was the ethanol-soluble fraction of smaller droplets, while the water-soluble fraction was more soluble and more susceptible to foaming. Researchers used the process of isoelectric precipitation

to find that chickpea protein emulsifying properties are similar to soybean protein rather than peas. However, legume proteins have less emulsifying abilities isolated by the salt extraction process. Pea protein displayed the lowest emulsifying characteristics of all of the legumes analyzed due to lower solubility and surface electric charge.

The structure and composition of proteins, influencing their adsorption and tolerance to various conformational changes on the interface, are a significant effect on emission properties. The high content of β-sheet (~30-44%) and comparatively low α-helix (~12-20%) make legume proteins special and help them to establish a stronger conformational transition at the water-oil interface compared to cereals and animal proteins (~ 4-11% and α-helix: 4-16%), respectively (Carbonaro, Maselli, & Nucara, 2012). In order to understand the effect of the complex formation towards enhancement in rheological behaviour, researchers investigated a structural-functional relationship between visillns (phaseolus storage protein). They proposed that the simplicity of structural change of adsorbed-protein on the interface, instead of original adsorption or diffusion, is the key explanation for better emulsion (Liang & Tang, 2013). They suggested the emulsifying mechanism for native visillns to determine the emulsification behaviour index and the stability index in quaternary and tertiary functional studies (Tang & Sun, 2011).

Chapter 2 Introduction

2.1 Background

Human population has rapidly increased all around the world, even New Zealand's population increasing from 800,000 people in 1900 to five million people in 2020, reported by Stats NZ. The demand of legumes and crops are increasing with the rising population, while the yield of legumes is decreasing due to the climate change, soil salinization, water scarcity, air contamination and series of environmental calamity (Scheelbeek et al., 2018). In this situation, making full use of industrial wastewater becomes especially important. For instances, the wastewater generated from tofu processing industry has been proved to contain considerable soy wheat (Chua & Liu, 2019). Nevertheless, wastewater is generally limited reused for irrigation for agriculture purpose, greatly overlooking their nutritional value (Vij et al., 2021). Recently, the concept of *Liluva* was first proposed by Dr. Luca Serventi in 2020 (Serventi, 2020b). It refers to wastewater from legume industry after soaking, cooking or sprouting. The physicochemical properties of soaking, cooking and sprouting legume water were discussed by Serventi (2020b) in his book, including forming abilities, thickening, emulsifying capacity, etc. Particularly, *liluva* has been proved to contain considerable amounts of proteins that contributes to its emulsifying ability, so that it is promising to replace animal materials in the baking process as a good emulsion. For instance, aquafaba, the wastewater from chickpea cooking, is considered to be a good egg white substitute in food production (Buhl, Christensen, & Hammershøj, 2019). Meanwhile, the properties of milk-protein, oil-in-water emulsions are defined by the frame and surface-rheology of the adsorbent surface on the oil-water interfaces,

which is also found in legume proteins, such as chickpea, split yellow pea and soybean wastewater. This functionality brings benefits to vegans to find a substitute in bakery food products (Mustafa & Reaney, 2020). The compositions of legume water are mainly fibre, protein and phytochemical compounds, which can improve the physical properties of gluten-free bread (Huang et al., 2018). Based on existing research, both of soaking and cooking legume water including pea, chickpea and soybeans are all provided with various physicochemical properties (Serventi, Gao, Chen, & Chelikani, 2020), illustrated their nutritional consistency (Serventi, 2020a), but did not investigate the peptide and amino acid profile.

2.2 Protein profile of legume soaking and cooking water

Legumes constitute sufficient protein sources, refined carbohydrates and dietetic fibre. They produce around 30% equivalent or higher protein content than meat (around 25%). Pulse proteins are primarily consisting of globulins which are soluble in salt solutions and water-soluble albumins. Albumins account for just about 15% of the overall legume seed proteins. Although low molecular weights are defined, the amino acid composition is supposed to be the most nutritious proteins in legume soaking and cooking water (Alsalman & Ramaswamy, 2021).

Soaking and cooking are common legume processing in the industry can home diet. However, both the soaking and cooking process can result in the legume protein content decreasing. Studies of Abd El-Hady and Habiba (2003) showed that the average protein content loss in pea

and chickpea soaking phase, from 26.16 g to 25.98 per 100 grams. It means that during the soaking process, the water-soluble protein is passed in the beans to the solution. The reason could be the revealed endosperm is broken peas. Therefore, the division in half of the peas leading to high protein leaching when it was intoxicated is rational to predict. Yellow soybean cooking water (SCW) was stored 5.59 g/100 g dry matter, mostly composed of insoluble (2,46 g/100 g) and soluble (1,66 g/100 g) carbohydrates (Alsalman & Ramaswamy, 2021). The boiling process for legumes will result in significant losses of oligosaccharides. Boiling soybeans around 30 minutes can reduce the oligosaccharide content of soybean by about 85% (I. H. Han & Baik, 2006).

2.3 Nutrition and physicochemical properties of legume water

Split yellow peas contains nutrients, comparable to other peas, providing large amounts of proteins minerals, vitamins, fibre and carbohydrates (Maskus, Bourre, Fraser, Ashok, & Malcolmson, 2016). Soaking split yellow pea water contains the highest amount of soluble fibre, soluble carbohydrates and protein content than chickpea and soybeans (Serventi, 2020a). While with about 14.7 million tonnes yield, chickpea is worldwide the third largest produced grain legume following regular beans and peas. After Spain, Italy is Europe's second-largest producer of chickpeas.(Summo et al., 2019) In addition to the protein content, the dietary fibre, which is one of the best among split yellow peas and soybeans, is the main nutritious value of chickpeas (Jukanti, Gaur, Gowda, & Chibbar, 2012). In fact, as shown in a variety of studies, fibre-rich food intake is highly recommended. Diabetic fibres, in particular, decrease the risk

of coronary artery conditions, stroke, high blood pressure and type 2 diabetes. Furthermore, a fibre replacement may help increase the weight loss in obese people (Anderson et al., 2009). Asian diet cultures produce conventional foods by using soybeans, making soy milk, soy paste, tempeh, tofu, etc. Instead of only being cultivated in Asia, it is known worldwide for its oil and protein richness (Medic, Atkinson, & Hurburgh, 2014). Protein solubility of legume soaking water is nearly 100%, pH value from 4.7 to 5.7. The higher protein content they have, the more remarkable foaming capacity they show in a bakery product. Legume soaking water shows more affinity to oil than water (Serventi, 2020b). In terms of legume cooking water, protein solubility was ranging from 86% to 100%, pH value of legume cooking water ranging from 6.1 to 6.5, they also proved to have greatly foaming capacity including split yellow peas, chickpeas and soybeans (Serventi, Gao, et al., 2020).

The goal of this study is to determine the protein content and composition of pea, chickpea and soybean soaking and cooking water, to demonstrate the principles of their physicochemical properties applied in bakery product development, and to provide ideas on legume wastewater recycling in future perspective.

Chapter 3 Material and Methods

3.1 Materials

The pea, chickpea and soybean soaking and cooking water samples in this study are obtained from dry legumes. Split yellow pea is a commercial product provided by McKenzie's, supplied from Australian farms. The dried chickpea raw materials are offered by America supplier, Kelley Bean Co, NE, USA. The soybean dried raw material is YZ NONGMO BEAN, supplied by Sunson Asian Food Market, New Zealand. The nutritional information is shown in Table 2.1. Oil for syneresis analysis is canola oil (Simply-oil, New Zealand). In bakery application step, the sponge cake recipe included plain wheat flour (Pams, New Zealand), baking powder (Pams, New Zealand), apple vinegar (DYC, New Zealand) and sugar powder (Chelsea, New Zealand).

Table 2.1 Nutritional information of three legumes

Ingredients	Split yellow pea	Chickpea	Soybean
Energy (kJ/100g)	1320	1581	1866
Protein(g/100g)	23	20	36
Fat	2	6	20
Carbohydrate	45	63	30
Dietary Fibre	10	12	9

**Data retrieved from https://www.mckenziesfoods.com.au/product/mckenzies-yellow-split-peas-2/,*

https://en.wikipedia.org/wiki/Chickpea#Nutrition

https://en.wikipedia.org/wiki/Soybean

3.2 Sample preparation

Split yellow peas, chickpeas and soybeans were soaked in tap water for 16 hours, with the ratio of water versus dried legume at 3.3:1, following the previous instructions (Huang et al., 2018). The soaking process achieved three samples, including Soaking Pea water (SP), Soaking Chickpea water (SC) and Soaking Soybean water (SS). After the soaking process, cooking

soaked legume for 90 minutes with 1:1.75 of dry versus water ratio. Cooking process gave other three samples, Cooking Pea water (CP), Cooking Chickpea water (CC) and Cooking Soybean water (CS). The soaking and cooking water was kept in fuse box at room temperature for one day to precipitate. The supernatant solutions were used for further experiment.

3.3 SDS-PAGE protein profile

Samples were elucidated in the form of CriterionTMTGX Strain-FreeTM from 4% to 20% pre-cast gels (Bio-Rad, Richmond, CA, United States) and carried out as the stated process of Laemmli (1970) with modifications, with the support of the sodium Dodecyl sulphate Poly-Acrylamide gel electrophoresis (SDS-PAGE). The sample water was combined with buffer (20 mm of Triss, 2% SDS, 20% glycerol, pyronin Y), and a decrease in disulphides bond was achieved with 0.2 M of dithioerythritol (DTE) in the water bath at 100 °C for 5 min, prior to the spinning to make samples homogenized. Next, 15 μL of all samples, including split yellow pea soaking and cooking water, chickpeas soaking and cooking water, soybean soaking and cooking water were loaded separately into the gel, and the electrophoresis was carried on a steady current of 200 V at a buffer (0.25 M Tris, 0.192 M glycine, 0.1%SDS) running for 45 minutes. Established 8 μL molecular weight (Mw) markers (Thermo ScientificTM, SpectraTM Multicolor Wide Range Protein Marker and SpectraMT, SpectraTM Multicolor) were used for estimating the molecular weights of protein bands. Both untouched and gels with Coomassie Dazzling Blue G-250 were prepared to imagine certain bands of protein visualisation. Technology data analysis estimated program of Wide Range Protein and Image Lab 5.2.1 supported the result figures (Bio-Rad, Richmond, CA, USA).

3.4 Free amino acid detection

An Agilent 1100 series (Agilent Technologies, Walbronn, Germany) autoanalyzer based on HPLC method was used to quantify the free amino acids, and 9-fluorenylmethyl chloroformate (FMOC) for secondary amino acid derivatization reagent, originated from Heems, 1998, (Carducci et al., 1996; Heems, Luck, Fraudeau, & Vérette, 1998) 3 um C-18 column (150m*4.6mm, Winlab, Scotland) separated the free amino acids at 40c; the machine equipped with a binary pump, auto-sampler with a thermostat. The free amino acids were detected at 4 °C. The Pre-column fluorescence derivatization performed at 440 nm and then switched detector to the second channel for detecting secondary amino acid proline and at 21min, with the emission of 315nm. The amino acid content in each sample was calculated by comparing the peak area of the detected amino acid with that of the standard, using o-phthaldialdehyde (OPA) for primary amino acid derivatization reagent, The reaction solution (A, B) was prepared, the column temperature was set at 40 °C, the flow rate of the reaction solution was 0.70 mL/min, and the gradient conditions of the reaction mixture solution are shown in Table 2.2. Six legume water sample solutions (12 μL) were successively injected into the automatic analyzer, and the peak area of each amino acid in the sample was obtained. We calculated the content of each amino acid using the Software EzChrom Elite (version 3.3.2).

Table 3.2 Pump gradient condition of the solution

Time (min)	B%
0	0
14	40
20	50
24	100
29	100
30	0
36	0

3.5 Stability analysis of emulsion

The size of emulsion droplet and their spread situation were measured by integrated light scattering with the Matersizer 2000 (Malvern Instruments Ltd, Malvern, U. K.). Emulsions with a ratio 1:2 (v:v) of 1% SDS and a mean diameter weighted by volumes were diluted, D 4,3 was achieved. A quadruplicate measurement of three technical replications per sample was carried out in this analysis. Each sample's droplet sizes and distribution were measured. 20 ml of canola oil was added into another 20 ml water samples. Six tubes of legume wastewater samples and oil mixture were homogenized at room temperate. The emulsion liquid was then measured at 25 ℃. Then stored samples at 4 ℃ for 24 hours and 4 days, and measurements will be taken at 24 hours and 4 days, respectively.

3.6 Syneresis Analysis

The procedure of S. Wang, Chelikani, and Serventi (2018) was used to assess oil absorption. In pre-weighted centrifugal tubes, 20ml water samples were mixed with 20 ml Canola Oil. In order to disperse the oil with legume water samples, the contents were homogenized at high speed for 1 min, and with a homogenizing system for 30 seconds. The tubes were centrifugated after 30 minutes at 20℃ for 10 minutes at 222 g. A scaled tube was then used to weigh segregated crude. The absorption potential of water and oil was expressed in percentage of sample water gram per gram oil.

3.7 Application

3.7.1 Sponge cake preparation

In this trial, experimenters made sponge cakes with CP, CC and CS water to substitute eggs in sponge cake receipt, following the instruction of Mustafa, He, Shim, and Reaney (2018). First, the foam was made with a Brabantia Kitchen Mixer. Add 110 ml of pea cooking water, chickpea cooking water and soy cooking water, with a tsp (3g) apple vinegar, beginning at a low speed before most legume wastewaters became foamy and no liquid was left. The pace was then raised, and the mixture was smoothed for seven minutes. In high-speed blending, the creamy blend was whipped in a stiff peak for 3 min, and 130 g of powdered sugar were applied. Plain wheat flour (130 g) and baking powder (7 g) were combined in a separate cup; then the dry ingredients were gently folded with a rubber spatula into a foam in three aliquots. Then the cake batter was placed into three baking pans (5 to 15 cm) and baked for 30 min at 180 °C (360 °F) in a preheated conventional oven. After heating, the pans were taken out of the oven and inverted in a cutting board with a higher mesh shelf in case of the soggy underneath caused by the heat. Cooling for 30 minutes until texture and colour analysis was undertaken at room temperature (25℃). Meanwhile, some cooled cakes were packaged and frozen in polypropylene bags for possible further inspection.

3.7.2 Profile study of instrumental Texture Analysis (TA)

A Texture Analyser (TA.XT2, Stable Microsystem, UK) interfacing with a device using Texture Labor Pro Program, version 1.13-002, calculated sponge cake texture profiles. Sliced sponge cake into three cubes. (50mm* 50mm*50 mm). It is set up to test the hardness and

adhesion of the sponge cakes as opposed to its control, using a load cell of 5,000 grams and a 3-point bend fixture. A circular support rig (A/CS) was applied for sampling (Serventi, Zhu, et al., 2020). Penetration measurements have, instead, been performed in the mid-area of the sample with a 5 mm SMS P/5 sample calibration with a speed of 1.5 mm/s and a speed of 10.0 mm/s after testing.

3.8 Statistical Analysis

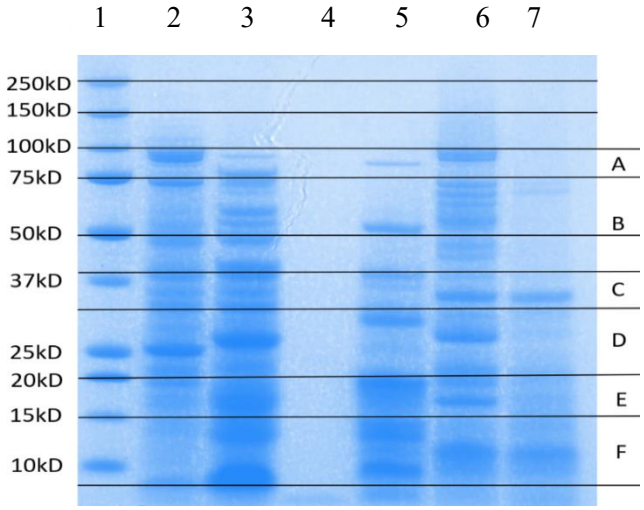
Means and standard deviations have been determined for all data by Microsoft Excel 2010 from Microsoft. The statistical analysis was conducted by Minitab version 18, analytical program (Minitab Inc, United Kingdom) Statistically significant was a minimum of 95% ($P < 0.05$). Set-ups were applied for one direction study (ANOVA). The honest Turkey main test of discrepancies was used to classify statistically relevant mean differences. In set-ups with two factors, droplet size and temperature, droplet size and time, and protein type and time, a two-way analysis (ANOVA) applied for two factors statistical analysis.

Chapter 4 Results

4.1. Protein profile

This study analysed the total protein content and free amino acid in legume wastewater. The assay recorded the free amino acid and total protein content of six legume wastewater. The SDS-PAGE results give an idea about which types of protein the legume water contains in this study. Assessment of centrifuge legume wastewater protein composition has shown as observable protein bands of proteins. (Figure. 4.1-a). The broad proteins were stained from 10kD to 250kD conditions as proteins persisted in the lane A to Lane F, except Chickpea.

(a)



(b)

Lane	Peptides	Accession No.
A	tRNA (Cytosine-5-)-methyltransferase	A0A163M953
	O-acyltransferase	A0A163IUM5
B	Legumin,	Q9SMJ4
	Provicilin	Q304D4
C	Oxidoreductase	A0A163HIE9
D	Dehydrin 1	Q8GUS4
E	Histone H2B, Histion H3,	A0A163DU27
	Late embryogenesis abundant protein2	O49817
F	Defensin,	Q2I2W0
	None-specific lipid-transfer protein	A0A076KXC0

Figure 4.1. (a) SDS-PAGE separation of six legume wastewater; Lane (1): Broad range molecular marker; Lane (2) pea soaking water; Lane (3) pea cooking water; Lane (4) chickpea soaking water; Lane (5) chickpea cooking water; Lane (6) soybean soaking water; Lane (7) soybean cooking water; (b) Protein identification by peptide mass fingerprint.

soaking water gel band (Figure.4.1-b). To unify the protein concentration, chickpea soaking water concentration was diluted. In lane A, pea soaking wastewater, as well as soybean soaking water, shown remarkable stains. In lane B, pea soaking water, pea cooking water and chickpea

cooking water, soybean soaking water contain the continuous band stain from 50kD to 75kD. Lane D only lack the stain from gel band of soybean cooking water and chickpea soaking water. The legume wastewater samples revealed a similar protein composition with bands at C, E and F lane, regardless of whether the soaking manner or cooking methods were used (Figure. 4.1-a).

Table 4.1. The free amino acid in legume wastewater

Legume water	Total Protein (%)	Free Amino Acid (μM)				
		Asp	**Ser**	**Gln**	**His**	**Thr**
Pea Soaking Water	0.60%	106.33± 5.21	342.56± 8.24	246.78± 9.60	128.58± 1.63	376.86± 10.36
Pea Cooking Water	1.30%	291.49± 4.33	197.30± 1.91	21.71± 7.57	127.60± 0.29	325.46± 7.05
Chickpea Soaking Water	0.10%	31.59± 7.67	84.93± 6.73	82.42± 6.88	32.65± 0.93	137.91± 4.27
Chickpea Cooking Water	1.00%	109.35± 2.46	159.64± 144.31	49.51± 1.05	85.26± 0.23	538.19± 9.82
Soy Soaking Water	0.03%	598.13± 0.95	334.80± 0.95	265.11± 0.00	87.27± 63.79	282.87± 37.40
Soy Cooking Water	0.70%	376.75± 2.24	2366± 2.24	72.27± 2.03	144.11± 1.83	204.12± 2.31
Legume water	**Ala**	**Tyr**	**Val**	**Met**	**Tryptophan**	**Phe**
Pea Soaking Water	389.20± 5.18	136.34± 1.79	218.58± 2.98	48.41± 0.84	49.39± 0.58	93.89± 1.03
Pea Cooking Water	392.26± 0.74	108.27± 0.51	136.62± 1.09	51.31± 0.20	63.31± 0.94	97.19± 0.04
Chickpea Soaking Water	54.87± 0.08	53.92± 0.68	86.42± 2.04	25.26± 0.66	54.24± 0.04	55.13± 0.08
Chickpea Cooking Water	154.79± 0.96	100.74± 0.43	116.07± 0.73	48.44± 0.37	327.13± 0.66	131.62± 0.61
Soy Soaking Water	440.69± 31.25	310.03± 3.78	268.80± 26.66	78.55± 23.47	269.48± 0.04	182.58± 11.33
Soy Cooking Water	184.61± 1.01	263.35± 1.69	227.42± 0.10	124.37± 0.10	297.15± 3.14	207.42± 0.20
Legume water	**Ile**	**Lys**	**Leu**	**Proline**		
Pea Soaking Water	97.44± 1.42	197.00± 0.72	123.54± 0.94	356.46± 7.78		
Pea Cooking Water	64.00± 0.26	171.85± 1.65	125.19± 0.32	208.06± 3.01		
Chickpea Soaking Water	44.07± 0.00	107.38± 1.66	54.07± 5.50	181.36± 12.97		
Chickpea Cooking Water	46.61± 0.33	242.24± 0.02	99.03± 0.76	345.27± 8.76		
Soy Soaking Water	146.33± 0.00	204.65± 2.20	199.40± 6.62	292.48± 1.47		
Soy Cooking Water	122.49±0.15	192.58±8.42	205.14± 2.02	218.64±4.33		

The free amino acid results are reported in **Table 4.1**. It revealed the contents of 20 free amino acids in six different legume wastewater. 9 amino acids are out of limit detection; the raw data with μM unit are shown in **Appendix I**. In this section, other results are displayed in **Table 4.1** with mg/g as the free amino acid unit for easier comparison with other studies. Results showed Phe as the most abundant amino acid in pea soaking

water (7.48mg/g), pea cooking water (2.98 mg/g) and chickpea soaking water, 16.43 mg/g) Met as a sufficient amino acid in chickpea cooking water (11.08mg/g) and soybean cooking water (18.35mg/g), Asp as the most content composition in soy soaking water(26.54mg/g), respectively.

Table 4.2. Comparison free amino acid profile of legume isolate with legume wastewater

Legume Amino Acid (mg g^{-1} protein)	Asp	Ser	His	Ala	Phe	Leu	Ile	Thr	Val	Met	Lys
Pea Isolate	11.4	4.7	2.7	4.3	4.9	7.7	3.8	4	4.9	1.2	7
Pea Soaking	2.36	6.00	6.01	3.33	7.48	5.78	4.12	4.27	1.20	1.68	2.58
Pea Cooking	2.98	1.59	0.24	1.52	2.98	2.69	1.51	1.23	0.59	0.99	1.23
Chickpea Isolate	57.00	9.00	5.00	9.00	14.00	73.00	2.00	7.00	5.00	5.00	18.00
Chickpea Soaking	4.21	8.93	12.05	5.07	16.43	4.89	9.77	10.12	3.77	11.08	9.11
Chickpea Cooking	1.46	1.68	0.72	1.32	6.41	1.38	1.83	1.36	0.72	6.68	2.17
Soybean Isolate	6.38	3.05	3.34	12.75	3.51	7.38	4.11	3.39	4.46	2.47	7.22
Soy Soaking	26.54	11.73	12.92	4.51	11.23	13.09	18.72	10.50	3.91	18.35	10.05
Soy Cooking	1.00	3.55	1.51	3.19	3.47	2.35	6.82	3.81	2.65	8.67	4.89

*Pea Isolate amino acid content data retrieved from the previous study of Millar, Gallagher, Burke, McCarthy, and Barry-Ryan (2019)

Chickpea Isolate amino acid content data retrieved from Cortés-Giraldo, Megías, Alaiz, Girón-Calle, and Vioque (2016)

Soybean Isolate amino acid content data retrieved from Chen, Wang, Liu, and Chen (2017)

Table 4.2 compared legume wastewater free amino acid with legume isolate. Among the three legumes with their wastewater, all of the cooking wastewater exhibited the lowest content of free amino acid. In the yellow pea free amino acid assay, the soaking method contains the highest

volume of Ser (6.00mg/g), His (6.01 mg/g), Phe (7.48 mg/g), Ile (4.12mg/g) Thr (4.27 mg/g) and Met (1.68mg/g). Chickpea soaking water has the highest volume of His (12.05mg/g) Phe (16.43mg/g), Ile (9.77mg/g), Thr (10.12mg/g) and Met (11.08mg/g). Soybean soaking water contains most volume than soybean isolate and soybean cooking water except Ala (4.51mg/g, less than 12.75mg/g soybean isolate) and Val (3.91mg/g) less than 4.46mg/g soybean isolate).

4.2 Emulsion ability

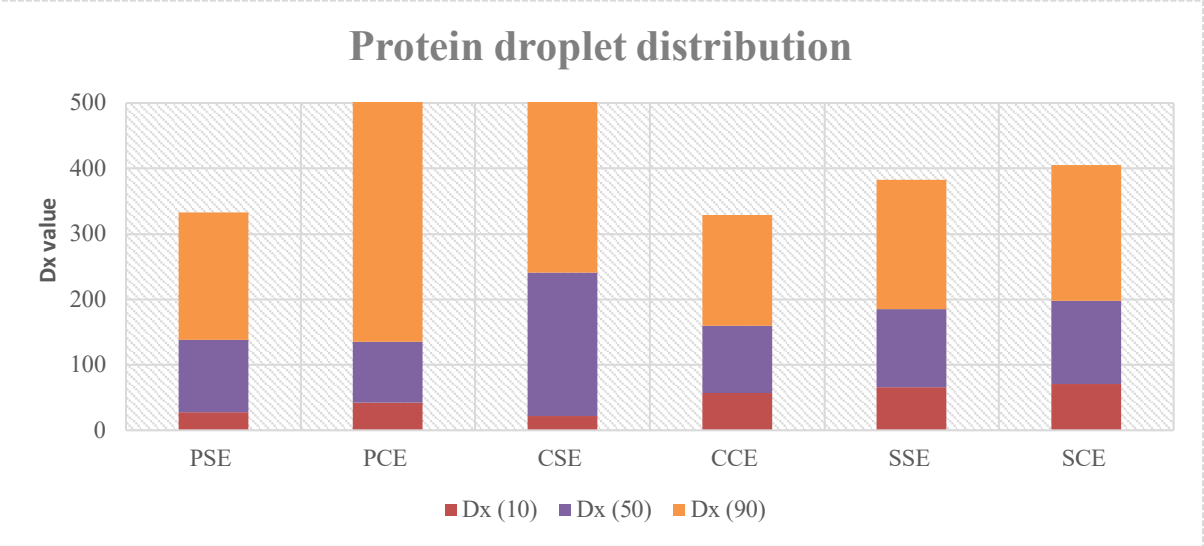
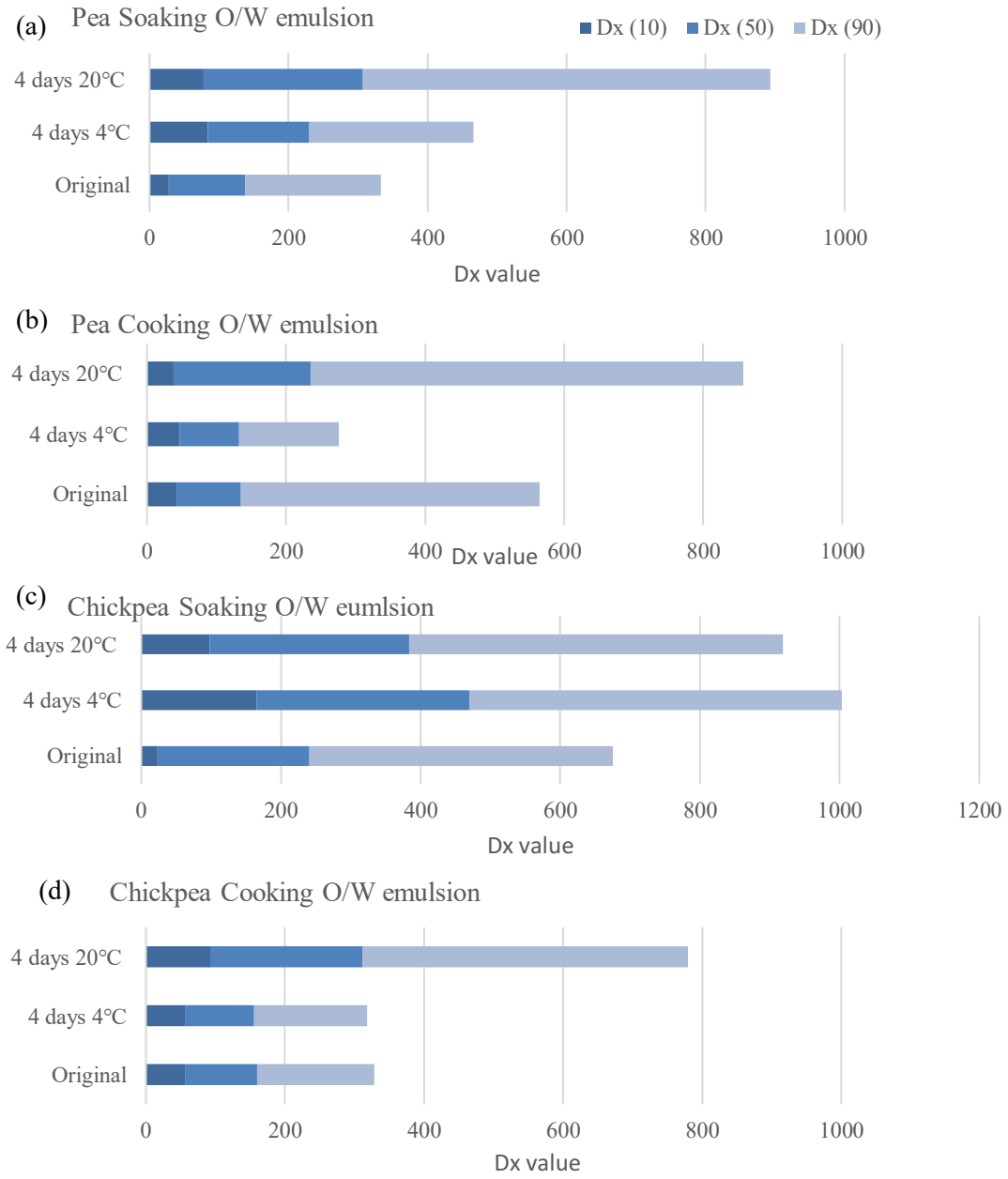


Figure 4.2. Droplet sizes distribution of legume wastewater O/W emulsion at 20°C. PSE: Pea Soaking water Emulsion; PCE: Pea Cooking water Emulsion; CSE: Chickpea Soaking water Emulsion; CCE: Chickpea Cooking water Emulsion; SSE: Soybean Soaking water Emulsion; SCE: Soybean Cooking water Emulsion. Dx (10): Size distribution up to and including 10% of the total volume of protein particles contain in the legume wastewater samples. Dx (50): Size distribution up to and including 50% of the total volume of protein particles in the legume wastewater samples. Dx (90) Size distribution up to and including 90% of the total volume of protein particles contain in the legume wastewater samples.

Oil-in-water emulsions containing 50% Canola oil were prepared using the legumes wastewater (pea, chickpea and soybeans). These emulsions were then stored at 4°C, and changes in particle size were monitored throughout storage. Initial experiments were carried out using emulsions formed directly by homogenization. Further study based on samples stored at 4°C for four days. Results of legume wastewater emulsions droplet size are exhibited in **Figure 4.2.** PCE and CSE droplet size distribution is largest among all legume emulsions at 20°C. CCE showed the smallest droplets distribution in the original emulsions. The mean emulsion droplet diameter (nm) evaluated differed significantly($p<0.05$) for all centrifugated legume wastewater-based emulsions at a different temperature, with increased time, the droplet diameters were increased at 20°C **(Figure. 4.3).** For the emulsion stored at 4°C after 4 days, PSE, CSE, SSE and SCE displayed droplets size increased. At the same time, PCE and CCE contained smaller droplets after 4 days in the fridge. The emulsion droplet size

distributions as volume density (%) are shown in Fig. 4. All of the wastewater emulsions had the highest volume density after 4 days in the fridge except PSE. **Figure 4.3** displayed the comparison of six legume wastewater emulsions after 4 days at a different temperature, both in the refrigerator and at room temperature. Soybean soaking and cooking water are good emulsifier options both at room temperature around 20°C and refrigerator temperature around 4°C. While PSE, PCE and CCE persist the good emulsifier properties at 4°C but lose their emulsifying ability at 20°C room temperature. Only CSE displayed bad emulsification, either room temperature or in the refrigerator.



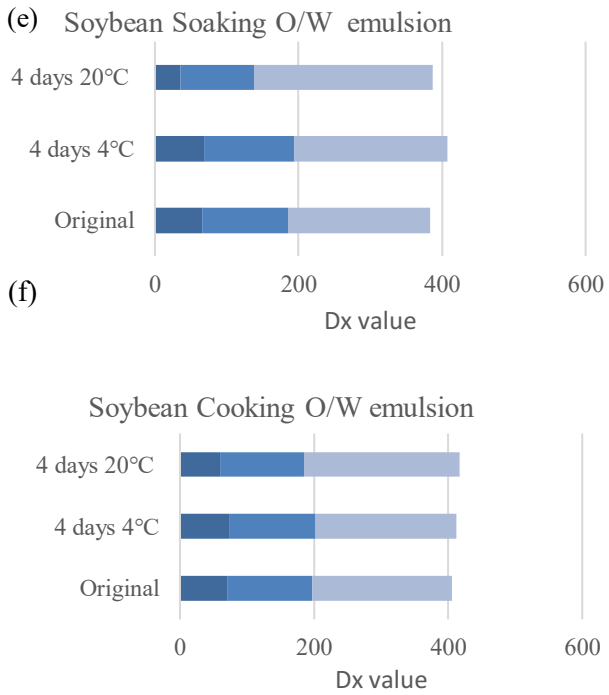


Figure 4.3. Legume wastewater Oil-in-Water emulsions droplet size initially tested at 20°C (Original) and after four days at 4°C and at 20°C. Dx (10): Size distribution up to and including 10% of the total volume of protein particles contain in the legume wastewater samples. Dx (50): Size distribution up to and including 50% of the total volume of protein particles in the legume wastewater samples. Dx (90) Size distribution up to and including 90% of the total volume of protein particles contain in the legume wastewater samples; (a) Pea soaking water O/W emulsions droplet size distribution four days after storage at 4°C and 20°C, respectively; (b) Pea cooking water O/W emulsions droplet size distribution four days after storage at 4°C and 20°C, respectively; (c) Chickpea soaking water O/W emulsions droplet size distribution four days after storage at 4°C and 20°C, respectively; (d) Chickpea cooking water O/W emulsions droplet size distribution four days after storage at 4°C and 20°C, respectively; (e) Soybean soaking water O/W emulsions droplet size distribution four days after storage at 4°C and 20°C, respectively; (f) Soybean cooking water O/W emulsions droplet size distribution four days after storage at 4°C and 20°C, respectively;

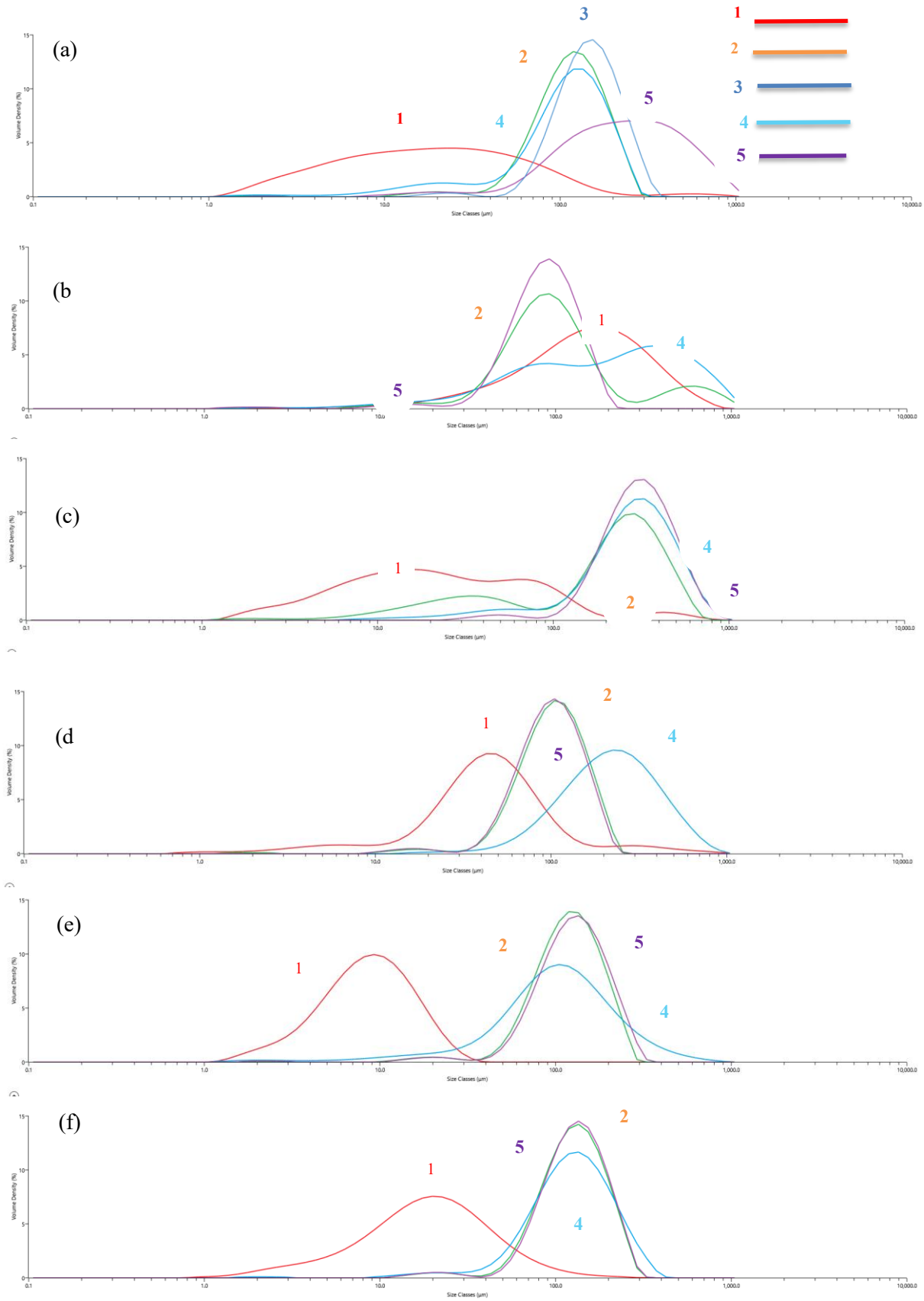


Figure 4.4. Legume wastewater and their O/W emulsion particle distribution, initially at 20°C, after 4 days at 4°C and 20°C respectively. (a-1): Pea soaking water particle distribution at 20°C; (a-2): Pea soaking water emulsion particle distribution at 20°C; (a-3): Pea soaking water emulsion particle distribution at 20°C after 1 hour; (a-4): Pea soaking water emulsion particle distribution stored at 20°C for 4 days; (a-5): Pea soaking water emulsion particle distribution stored at 4°C for 4 days. (b-1): Pea cooking water particle distribution at 20°C; (b-2): Pea cooking water emulsion particle distribution at 20°C; (b-4): Pea cooking water emulsion particle distribution stored at 20°C for 4 days; (b-5): Pea cooking water emulsion particle distribution stored at 4°C for 4 days. (c-1): Chickpea soaking water particle distribution at 20°C; (c-2): Chickpea soaking water emulsion particle distribution at 20°C; (c-4): Chickpea soaking water emulsion particle distribution stored at 20°C for 4 days; (c-5): Chickpea soaking water emulsion particle distribution stored at 4°C for 4 days. (d-1): Chickpea cooking water particle distribution at 20°C; (d-2): Chickpea cooking water emulsion particle distribution at 20°C; (d-4): Chickpea cooking water emulsion particle distribution stored at 20°C for 4 days; (d-5): Chickpea cooking water emulsion particle distribution stored at 4°C for 4 days. (e-1): Soybean

soaking water particle distribution at 20℃; (e-2): Soybean soaking water emulsion particle distribution at 20℃; (e-4): Soybean soaking water emulsion particle distribution stored at 20℃ for 4 days; (e-5): Soybean soaking water emulsion particle distribution stored at 4℃ for 4 days. (f-1): Soybean cooking water particle distribution at 20℃; (f-2): Soybean cooking water emulsion particle distribution at 20℃; (f-4): Soybean cooking water emulsion particle distribution stored at 20℃ for 4 days; (f-5): Soybean cooking water emulsion particle distribution stored at 4℃ for 4 days.

4.3 Syneresis

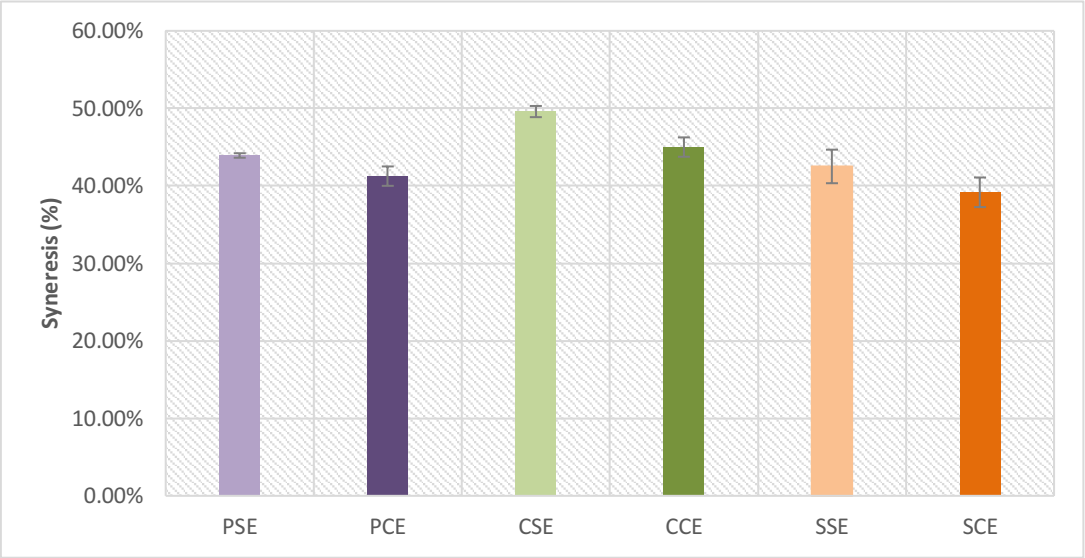


Figure 4.5. Syneresis (%) of centrifuged oil in legume wastewater emulsion.

Results of syneresis (%) analysis are displayed in **Figure 5**. Overall, three legumes showed no significant diffetence($p>0.05$) of syneresis ability. CSE indicated the highest amount of syneresis percentage while PCE has the lowest amount of syneresis percentage. In the view of legume types, chickpea contained the highest syneresis ability than pea and soybeans. In the view of process methods, soaking legume wastewater has better syneresis ability than cooking legume wastewater.

4.4 Instrumental analysis

Table 4.3. Texture analysis of legume wastewater sponge cake

	Hardness	Adhesiveness	Time difference%
Pea cooking water sponge cake	475.76^{a}±71.17	-0.858 ^{a} ±0.432	80.9% ^{a} ±0.524
Soy cooking water sponge cake	402.60^{ab}±36.99	-42.771 ^{a} ±67.65	85.3% ^{a} ±1.005
Chickpea cooking water sponge cake	321.60^{b}±59.19	-4.677 ^{a} ±3.72	83.5% ^{a} ±0.31

There is a big difference ($p>0.05$) of sponge cake hardness between pea cooking water sponge cake, soybean cooking water sponge cake and chickpea cooking water sponge cake. Sponge cake with Pea cooking water shown the highest hardness and adhesiveness, while chickpea cooking water sponge cake was tested to be softer than soybean cooking water and pea cooking water.

Chapter 5 Discussion

5.1 Protein profile

5.1.1 SDS-PAGE

Cytosine residue methylation plays a major function in nucleic acid control. Cytosine-5 RNA methylation has been observed in tRNA, rRNA, and mRNA among several specific RNA modifications (Rozenski, Crain, & McCloskey, 1999). DNA methylation of cytosine- 5 is an important epigenetic alteration that has been proved to be critical for the growth and aetiology of human disease, as well as for the control of genetic expression in eukaryotes (Jones & Baylin, 2007; Klose & Bird, 2006). Cytosine-5 methyltransferases are strongly retained in development and are examined in many species on biological function.(Schaefer, Steringer, & Lyko, 2008) For these nutritional value, pea soaking water, soybean soaking water are good sources of cytosine-5 methyltransferases which may have a promising significance for cancer study in the future. However, one of the specific characteristics of the enzyme is heat intolerance. It explains cooking, which is a typical heating process, legume to processed legume wastewater contains no cytosine-5 methyltransferase. (**Figure 4.1-a**). In lane B (legumin), only soybean wastewater showed no stain in the gel band, because the pea gel network is formed by pea legumin, while soybean contains extremely few legumin(van Boxtel, van den Broek, Koppelman, & Gruppen, 2008) Soybean proteins contain glycinin. Glycinin (20kDa, Lane E) is a legumin – like protein that offers the soybean gel formation ability instead of legumin. Some of the biologically active peptides and proteins present in soybean are lunasin, lectin and conglycinin. Also, lunasin (7~8kDa, Lane F) is a novel peptide in soybean recovered by researchers recent years, (W. Wang, Dia, Vasconez, De Mejia, & Nelson, 2008) In vitro and in vivo experiments have demonstrated that certain biologically advantageous peptides may have some beneficial roles in the body, including antihypertensive, early cancer detection, hypocholesterolemic activity, antifungal abuse and immunomodulation.(de Lumen, 2005)

5.1.2 Amino Acid

During the soaking method of each legume in water, changes in free amino acid contents happened with variation depending on types of the free amino acids. Most amino acids increased during soaking legume process, these amino acid accumulations and declines are obviously because of adding water triggered most of the enzyme actions. As shown in **Table 4.2**, the most variation in free amino acid content was pea soaking water. The previous study showed that enzymes are responsible for the degradation or the formation of free amino acids that contribute to the discriminate results between legume soaking water and legume isolate. (Saikusa, Horino, & Mori, 1994) Lysine is decreased primarily due to the methods of soaking processing, soaking peas and chickpeas causing a large rise in isoleucine and phenylalanine, while soaking soybean water has an increased lysine content. The cooking and soaking lead to the decrease of valine. Unlike the pea and chickpea soaking and cooking process, soybeans essential amino acids except valine increased in soaking. Methionine and leucine have risen through the raw chickpea and soybeans cooked process. Methionine to Lysine ratio illustrates nutritional significance. The ratio of Met to Lys for pea isolates, pea soaking water and pea cooking water are 0.17, 0.65 and 0.80, respectively; for chickpea isolates, chickpea soaking and cooking water are 0.28, 1.22 and 3.08, respectively; for soybean isolates, soybean soaking and cooking water are 0.34, 1.83 and 1.77, respectively. Recent research has shown that balancing essential amino acids will enhance the synthesis of milk protein (Haque, Guinard-Flament, Lamberton, Mustiere, & Lemosquet, 2015). Methionine and lysine is the most restrictive amino acid for milk protein synthesis, and a 1:3 ratio for Methionine to Lysine has been identified to be optimal for in vitro protein synthesis through changes in phosphorylation pathways of MTOR and abundance of mRNA (Dong et al., 2018; Nan et al., 2014) Previous findings also showed that Ile, Leu, Val and Thr could restrict optimum protein synthesis. (Doelman, Kim, Carson, Metcalf, & Cant, 2015) In diet, the study by Xia et al. (2019)observed that the Met / Lys dietary gestation ratio had major quadratic effects on the placental vascular density ($P <$

0,05), and the Met / Lys dietary ratio had a quadratic curved relation to birth weights and placental angiogenesis.(Xia et al., 2019) Consequently, the soaking and the cooking process have raised the ratio of Met to Lys, which draw a similar conclusion with the previous study by Mariam, Ahmed, and Osman (2020).

5.2 Emulsion

Legume wastewater O/W emulsions with lipid droplets of different particle sizes formed by the different types of proteins **showed in Figure 4.4**. The soaking and cooking process has also contributed to pea particle size variations after 4 days stored both at 25℃ and 4℃ (**Figure 4-a-4, 5; 4-b-4, 5**). Moreover, the chickpea cooking wastewater (aquafaba) O/W emulsions are better than chickpea soaking water O/W emulsion. The stability of protein emulsions and their associated products depending largely on the method of preparation. In other words, the emulsifying properties can be influenced by the heat treatment of the emulsion.(Qamar, Bhandari, & Prakash, 2019) Both maintain the protein emulsion either by reducing, altering or destabilizing the size of fat globules or by aggregating, denaturing and gelating proteins (Durand, Franks, & Hosken, 2003).

However, pea cooking water emulsion got even smaller droplet size than original emulsion after 4 days at refrigerator temperature (**Figure 4.3-b**). It may be explained by the pea contains the most abundant volume of antioxidants and phenolics, which gives pea cooking water a slower oxidization process than chickpeas cooking water (Damian, Huo, & Serventi, 2018; Osborn & Akoh, 2004). In general, the particle size effects can be quite complicated and depend on several variables including the surface exposed, the concentrations present in the framework of pro-oxidants and antioxidant and the fraction of the adsorbing and non-adsorbed emulsifier. For instance, the lipid oxidation rate could be higher with a reduced droplet size since the lipid zone will be revealed to the aqueous phase. (Maryam Kargar, Fotios Spyropoulos, & Ian T Norton, 2011; Maryam Kargar, Fotis Spyropoulos, & Ian T Norton, 2011) However, research showed

that the particles in water oil-in-water emulsions do not influence the lipid oxidation rate (Osborn & Akoh, 2004). Lipid oxidation generally takes place on the surfaces of the droplets in emulsion systems where unsaturated fatty acids and hydroperoxides from the oil phase interact with transitioning metals from the liquid phase (Berton‐Carabin et al., 2014). Therefore, researchers predicted that the rate of lipid oxidation would rely on the emulsifier quality of lipids such as thickness, loading, hydrophobicity or antioxidant capability present on a lipid droplet. In particular, the physicochemical stability of legume protein emulsifiers was distinguished from the latest report attributed to their interfacial properties. (Gumus, Decker, & McClements, 2017)

In our study, the soybean soaking and cooking water emulsions contained the smallest droplet sizes. (**Figure 4.3-e, 4.3-f**) Soybeans have been studied to contain the greatest capacity to scavenge free radicals, restrain lipid peroxidation and chelate metals among three legumes.(H. Han & Baik, 2008) Other studies have explored the stability of emulsions and their underlying mechanism: different types of legumes have different emulsified property because of the structure of the protein, such as legumin and vicilin, oil fraction, solubility, viscosity and pH. (Qamar et al., 2019) Overall, soybean soaking and cooking water are recommended as an excellent emulsifier regardless on the market shelf or in the fridge, while pea soaking and cooking water and aquafaba can only be a favourable emulsifier at 4℃.

5.3 Syneresis

Generally, water is divided in a polymer into three forms, free water that connected to syneresis at the normal freezing point; intermediate water that froze at a temperature below the usual freezing point and freezing bound water not congealed at the usual freezing point (Roccia, Ribotta, Pérez, & León, 2009) In a colloidal solution (canola oil), water volume is mechanically trapped inside. The division of this liquid triggers syneresis because of contraction, according to L. Wang et al. (2010), syneresis occurred at a low-temperature index of the Degree of starch

retrogradation. In principle, the concentration of starch pastes influences the Degree of syneresis. Many researchers have been using syneresis degree to predict how much legume starches are retrograding. In both the food and certain non-food industry, there is an unwanted property for the use of starch. In this study, the syneresis of legume wastewater is from 40% to 50%, which is much higher than 20%, a previous study data by comparative syneresis of starch gels at 8% concentration of various beans. (Hoover & Sosulski, 1985) In this study, the pea cooking water resulted in the lowest syneresis percentage; it may be explained by the lowest starch content of pea cooking water among all the three types of legumes. According to Serventi (2020b), no starch was found in any cooking and soaking legume wastewater, because most of the starch is stored in the endosperm, soaking and cooking methods can hardly break the endosperm. Starches of the legumes are more synergized than starches of the cereals, mostly due to the higher amylose content. It is recognized that amylose is unstable in water and high temperature and rapidly accelerates as the cooling or ageing mechanism starts to increase rigidity.(Wani et al., 2016) Overall, it can be the reason for soaking legume water syneresis being higher than high temperature cooking legume wastewater, and three types of legume wastewater syneresis ability are similar in this assay.

5.4 Instrumental Analysis

According to the above assay results, chickpea cooking emulsion (CCE) and soybean cooking emulsion (SCE) is the best emulsifiers among other legume wastewater emulsions. **(Figure4.2.)** While pea cooking water contains the most volume of total protein content (%) (**Table 4.1**.). Instrumental analysis has assessed the texture profile of three legume water to compare the emulsifier ability in a bakery product. It is expected that pea cooking water would give sponge cake the highest hardness, which agreed with the Texture analysis result. (**Table 4.3**) Met to Lys ratio of pea cooking water, soybean cooking water and aquafaba is 0.80, 1.77 and 3.08, respectively. The increasing ratio of Met to Lys gives the sponge cake a more elastic properties, which can explain the pea cooking sponge cake has the highest hardness and lowest

adhesiveness. Also, chickpea cooking water (aquafaba) has been a commercial emulsifier for its nutritional significance and physicochemical properties, called EVANESSE. (Ingredion-USA) As a result, the existence of high capacity water-binding protein content will lower the free water and hence the softness of the sponge cake. Starch gel influences a crumb firmness in Wilderjans, Pareyt, Goesaert, Brijs, and Delcour (2008)and a springiness-related accumulation of the protein in cake crumb. During dough creation and baking, McWatters, Ouedraogo, Resurreccion, Hung, and Phillips (2003) related the softer texture of the cookies because of the increase of protein content and component interaction.

6 Conclusion

Legume wastewater has reasonable nutritional significance for their sufficient protein content. In comparison all of the cooking wastewater showed the lowest content of free amino acid. In the yellow pea free amino acid assay, the soaking method contains the highest volume of Ser (6.00mg/g), His (6.01 mg/g), Phe (7.48 mg/g), Ile (4.12mg/g) Thr (4.27 mg/g) and Met (1.68mg/g). Chickpea soaking water has the highest volume of His (12.05mg/g) Phe (16.43mg/g), Ile (9.77mg/g), Thr (10.12mg/g) and Met (11.08mg/g). Pea cooking water Met to Lys ratio gave it the most elastic properties to form the sponge cake and more nutritional value. Physochemical properties of legume wastewater also have significant study value. Different types of legumes affected emulsifier stability because of different protein content. Chickpea cooking emulsions showed the smallest droplets distribution in the original emulsions. Consequently, soybean soaking and cooking water exhibited to be the best emulsifier stability both at room temperature and the refrigerator temperature, pea cooking water formed the hardest sponge cake because of its highest protein content.

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Appendix I

Table 1. Free Amino Acid Raw data

Unit: μM	Chickpea Soaking water	Soybean soaking water	Chickpea Cooking water	Pea Soaking water	Pea Cooking water	Soybean Cooking water
Asp	31.59	598.13	109.35	106.33	291.49	376.75
Glu	398.19	801.12	*	*	*	520.97
Cystein	230.12	*	*	*	*	*
Asn	874.14	1005.64	2233.09	*	4428.08	807.41
Ser	84.93	334.80	159.64	342.56	197.30	236.46
Gln	82.42	265.11	49.51	246.78	21.71	72.27
His	32.65	87.27	85.26	128.58	127.60	144.11
Gly	184.88	360.64	229.59	*	288.65	237.36
Thr	137.91	282.87	538.19	376.86	325.46	204.12
Arg	353.42	*	*	*	*	*
Ala	54.87	440.69	154.79	389.20	392.26	184.61
Tau	*	*	*	*	609.93	*
Tyr	53.92	310.03	100.74	136.34	108.27	263.35
Val	86.42	268.80	116.07	218.58	136.62	227.42
Met	25.26	78.55	48.44	48.41	51.31	124.37
Tryptophan	54.24	269.48	327.13	49.39	63.31	297.15
Phe	55.13	182.58	131.62	93.89	97.19	207.42
Ile	44.07	146.33	46.61	97.44	64.00	122.49
Lys	107.38	204.65	242.24	197.00	171.85	192.58
Leu	54.07	199.40	99.03	123.54	125.19	205.14
Proline	181.36	292.48	345.27	356.46	208.06	218.64

*means the result exceeded detecting limit

Table 2. Syneresis results data

	Sample 1	Sample 2	Sample 3	AVERAGE	Percentage% (/40ml)
PSE	17.5^{b}	17.5^{b}	17.7^{b}	17.57^{b}	$43.92\%^{b}$
PCE	16^{bc}	16.5^{bc}	17bc	16.50^{bc}	$41.25\%^{bc}$
CSE	20^{a}	19.5^{a}	20^{a}	19.83^{a}	$49.58\%^{a}$
CCE	17.5^{b}	18^{b}	18.5^{b}	18.00^{b}	$45.00\%^{b}$
SSE	16^{bc}	17.5^{bc}	17.5^{bc}	17.00^{bc}	$42.50\%^{bc}$
SCE	15^{c}	15.5^{c}	16.5^{c}	15.67^{c}	$39.17\%^{c}$