EFFECT OF PROTEIN AND CALCIUM CHLORIDE CONCENTRATION, pH, AND TEMPERATURE ON ALMOND PROTEIN GELATION

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ผลของความเข้มข้นของโปรตีนและแคลเซียม, พีเอช, และอุณหภูมิ ต่อการเกิดเจลของโปรตีนอัลมอนด์

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เมล็ดอัลมอนด์ (*Prunus dulcis*) เป็นแหล่งที่ดีของโปรตีน มีกรดไขมัน และใยอาหารที่ดีต่อสุขภาพ กระบวนการสร้างเจลของโปรตีนสามารถทำได้โดยการให้ความร้อน แต่ปัจจัยอื่นๆ อาจส่งผลต่อโครงสร้างเจลที่แตกต่างกันในการทำให้เกิดเจลโปรตีน อย่างไรก็ตาม งานวิจัยส่วนใหญ่มุ่งเน้นศึกษาการสร้างเจลด้วยความร้อนของโปรตีนนมถั่วเหลือง มีงานวิจัยที่ศึกษาคุณสมบัติทำให้เกิดเจลของโปรตีนนมอัลมอนด์อย่างจำกัด ดังนั้น งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของความเข้มข้นของโปรตีนและแคลเซียมคลอไรด์ ค่า pH และอุณหภูมิต่อการสร้างเจลโปรตีนอัลมอนด์ และวิเคราะห์คุณสมบัติทางเคมีกายภาพของเจลโปรตีนอัลมอนด์ ทำการศึกษาการเกิดเจลด้วยความร้อนของนมอัลมอนด์ โดยใช้อัตราส่วนต่างๆ ของเมล็ดอัลมอนด์ต่อน้ำกลั่น (1:2, 1:3, 1:4 และ 1:5 (w/v)) ความเข้มข้นของแคลเซียมคลอไรด์ (0.3, 0.5 และ 1%) , pH (3, 4, 5, 6 และ 7) และอุณหภูมิ (90, 95 และ100º C) ประเมินเจลนมอัลมอนด์ที่มีไขมันเต็ม (FFAM) โดยวิเคราะห์เนื้อสัมผัส Texture Profile Analysis (TPA), ความสามารถในการอุ้มน้ำ (WHC) และความแข็งแรงของเจล (gel strength) นอกจากนี้ยังศึกษาเจล FFAM ที่นำปัจจัยต่างๆ รวมกัน นำมาวิเคราะห์ TPA, WHC, ความแข็งแรงของเจล และ rheological measurement ผลการวิจัยพบว่าอัตราส่วนเจล FFAM ที่ 1:2 มีค่า hardness (43.03 ก.) ความแข็งแรงของเจล (0.18 นิวตัน) และ WHC (95.44 %) สูงสุด เมื่อเทียบกับเจล FFAM อัตราส่วนอื่นๆ เนื่องจากมีโปรตีน ไขมัน และปริมาณของแข็งรวมสูงสุด เจล FFAM (1:2) มีความแตกต่างอย่างมีนัยสำคัญ ($p<0.05$) กับเจล FFAM (1:4) ในด้านคุณสมบัติของเนื้อสัมผัส ความแข็งแรงของเจล และ WHC การเติมแคลเซียมคลอไรด์ 0.3% ในเจล FFAM 1:2 ทำให้เจลมีค่า hardness (44.76 ก.) ความแข็งแรง (0.22 นิวตัน) และ WHC (99.2%) สูงสุด แคลเซียมคลอไรด์ 0.3% มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ระหว่าง 0.5% และ 1% ของแคลเซียมคลอไรด์ในเจล FFAM แต่ละเจล ในความแข็งแรงของเจล ค่า hardness ความแข็งแรงของเจล และ WHC สูงสุด เมื่อเจล FFAM ปรับ pH 6 อย่างไรก็ตาม เจล FFAM (1:2) ที่มีความเข้มข้นของโปรตีนสูง (5.37%) สามารถเกิดเจลได้ที่ค่า pH ใดๆ เมื่อให้ความร้อน เมื่อ FFAM ในทุกอัตราส่วน ถูกให้ความร้อนด้วยอุณหภูมิ 90º C มีค่า hardness ความแข็งแรงของเจล และ WHC สูงสุด ของ FFAM เจล FFAM มีค่า hardness ความแข็งแรง และ WHC ของเจลลงลด เมื่อใช้อุณหภูมิที่สูงขึ้น (95 และ 100º C) การให้ความร้อนที่อุณหภูมิสูงของ FFAM ทำให้เกิดการเสียสภาพของโปรตีนที่สูงขึ้น ซึ่งไม่สามารถเกิดเป็นเจลได้ นอกจากนี้ เมื่อรวมปัจจัยต่างๆ เพื่อสร้างเจล FFAM (1:3+0.3% $CaCl_2$+pH 6+90ºC) ความแข็งแรงของเจลแตกต่างกันอย่างมีนัยสำคัญ ($P<0.05$) จากเจล FFAM สำหรับแต่ละปัจจัย และ storage modulus (G’-4359.4 Pa) ยังสูงกว่า loss modulus (G”-751 Pa) ซึ่งบ่งชี้ว่าเจลได้สร้างโครงสร้างเครือข่ายอย่างต่อเนื่อง ข้อมูลที่ได้จากการศึกษานี้เป็นข้อมูลพื้นฐานเกี่ยวกับปัจจัยและคุณสมบัติของเจลนมอัลมอนด์ และเป็นประโยชน์สำหรับการใช้เจลนมอัลมอนด์ในผลิตภัณฑ์อาหารมังสวิรัติ

สาขาวิชา วิทยาศาสตร์และเทคโนโลยีทางอาหาร
ปีการศึกษา 2563
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ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
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Thiri Hlaing : EFFECT OF PROTEIN AND CALCIUM CHLORIDE CONCENTRATION, pH, AND TEMPERATURE ON ALMOND PROTEIN GELATION. Advisor: Asst. Prof. SIRIMA PUANGPRAPHANT, Ph.D. Co-advisor: Asst. Prof. THANACHAN MAHAWANICH, Ph.D.

Almond (*Prunus dulcis*) seeds are good sources of protein that contain healthy fatty acids and dietary fiber. Protein gelation has been traditionally achieved by heating, but other factors can be affected in different gel structures in protein gelation. However, most studies were focused on the heat-induced gel formation of soymilk proteins, there was limited research on the gelling property of almond milk proteins. Therefore, this study aimed to investigate the effects of protein, and calcium chloride concentration, pH, and temperature on almond protein gel-forming and to analyze the physicochemical properties of the almond protein gel. Heat-induced gel formation of almond milk was studied using different ratios of almond seeds to distilled water (1:2, 1:3, 1:4, and 1:5 (w/v)), calcium chloride concentration (0.3, 0.5, and 1%), pH (3, 4, 5, 6, and 7) and temperature (90, 95, and 100º C). Textural profile analysis (TPA), water-holding capacity (WHC), and gel strength of full-fat almond milk (FFAM) gels were evaluated. In addition, the combined factors of FFAM gels were analyzed for the TPA, WHC, gel strength, and rheological measurement. The results showed that the FFAM gel ratio of 1:2 showed the highest hardness (43.03 g), gel strength (0.18 N), and WHC (95.44 %) than other ratios of FFAM gels due to the higher protein, fat, and total solid contents. FFAM (1:2) gel had a significantly different ($p<0.05$) with FFAM (1:4) gel on texture properties, gel strength, and WHC. The addition of 0.3% calcium chloride in 1:2 FFAM gels yields the highest gel hardness (44.76 g), strength (0.22 N), and WHC (99.2 %). The 0.3% calcium chloride was significantly different ($p<0.05$) between 0.5% and 1% calcium chloride in each FFAM gels on gel strength. The maximum hardness, gel strength, and WHC values obtained the pH 6 in each FFAM gels. However, only a high protein concentration (5.37%) of FFAM (1:2) could be obtained at any pH value when heating. The FFAM gels induced with a temperature of 90º C results in the maximum hardness, gel strength, and WHC in each ratio of FFAM. FFAM gels decreased in gel hardness, strength, and WHC when higher temperatures (95 and 100º C) were applied. High-temperature heating of FFAM leads to higher denaturation of protein which does not set into gels upon cooling. In addition, when the factors were combined to form FFAM gel (1:3+0.3% CaCl2+pH 6+90ºC), the gel strength was significantly different ($P<0.05$) from that of FFAM gels for each factor. Moreover, storage modulus (G’- 4359.4 Pa) was higher than loss modulus (G”- 751 Pa) indicating that the gel had formed a continuous network structure. The data obtained from this study provide basic information on the factors and properties of almond milk gel and useful for the application of almond milk gel in vegetarian products.

Field of Study:	Food Science and Technology	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....
		Co-advisor's Signature ....

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CHAPTER 1

INTRODUCTION

Plant proteins are become popular due to increasing vegetarian consumers, health awareness, and ecological issues. Multiple factors are influencing this growth, such as food safety concerns, rise in food intolerances, allergies and increased susceptibility of vegan foods, and acceptance of proactive approaches to health and wellbeing by consumers. Plant protein is a food source of protein that is from plants, e.g., pulses, tofu, soy, tempeh, nuts, seeds, certain grains, and peas. They are good sources of proteins and provide other nutrients such as fiber, vitamins, and minerals. These proteins tend to be lower in calories and fat than animal proteins but higher in fiber and essential nutrients. The nutritional quality of a protein source differs depending on its bioavailability, digestibility, amino acid profile, and processing effects (Sá et al., 2020). The most widely noticed and consumed plant proteins are soybean, beans, and peas. Soy protein has been used for a long time in non-vegan foods to improve the functional properties and nutritional value of these foods. Soy protein has good functionalities such as gelling, emulsifying, foaming, and water-oil holding capacity, thus has been highly used in the food industry such as in tofu products (Nishinari et al., 2014). However, soybean has its distinct “beany” flavor that may contribute to a reduced consumer acceptance and insufficient to meet the requirements from the markets. To satisfy the continuous increase in consuming plant-based protein foods, new products need to be developed with desirable protein content without adjusting taste and texture.

Almonds, the edible seeds of *Prunus dulcis*, are considered to be a great source of proteins (Gallier et al., 2012). Almond seeds contain 16-26% protein and rich in fats (44-61%) and vitamins, especially B and E (Devnani et al., 2020). Almonds also contain trace minerals, such as calcium and magnesium, and health-promoting unsaturated fatty acids (Barreca et al., 2020). They are also monosaturated and polyunsaturated fatty acids which possibly may lower LDL cholesterol (Gallier et al., 2012). Almond seeds are utilized in various food products, such as almond-based beverages, yogurt, snacks, and baking products. Almond milk is processed by grinding almond seeds with water and is used as an alternative source of plant-based protein products for vegetarian consumers. Depending on the functional property of proteins, heat-induced gelation of almond milk can be used to modify the protein structure (Devnani et al., 2020, Nicolai and Durand, 2013). The protein denaturation of almond milk and gelation results in a continuous protein network in which fat droplets are integrated (Devnani et al., 2020). These gel structures of almond proteins can potentially act as a nutritious base for vegetarian products. It could serve as an alternative for new vegan gelled products to soymilk protein which is commonly used as a protein source in plant-based foods.

One function of proteins in foods is the property of gelation which provides a structural matrix for holding water, flavor, sugar, and food ingredients in various food applications, as well as providing to obtain desirable sensory, textural structures and mechanical support in foods. Plant proteins including almond milk proteins can form different types of gel structures and thus extend their applications in a food-related field. Gelation refers to the transformation of proteins in the liquid state into a gel-like structure through physical or chemical means (Cong et al., 2018). It requires a driving force to unfold the native protein structure, followed by an aggregation maintaining a certain degree of order in the matrix formed by an association between protein standards (Totosaus et al., 2002). Gelation phenomenon of protein has been commonly attained by heating, but the development of protein network during gel formation and characteristics of the gel is dependent upon factors like protein concentration, degree of denaturation caused by pH, temperature, and ionic strength (Totosaus et al., 2002, Xiang and Susan, 2010). Accordingly, the effect of protein and calcium chloride concentration, pH, and temperature can be affected the thermal-induced aggregation and gelling property of almond milk protein.

However, most studies were focused on the heat-induced gel formation of soymilk proteins, there was limited research on the gelling property of almond milk proteins. So far, almond protein gels have been investigating the effect of thermal treatment and protein concentration in skim and full-fat almond milk (Devnani et al., 2020). Therefore, this study is focused on the factors affecting the gelling properties of almond milk protein by heat. This information is important to develop more choices for vegan gelled products.

The objectives of this research are to study the effects of protein, and calcium chloride concentration, pH, and temperature on almond protein gel-forming and to analyze the physicochemical properties of the almond protein gel. Knowledge of the behavior of almond protein gel in this study can be helpful in the development of the physical properties of new vegan gelled products.

CHAPTER 2

LITERATURE REVIEW

Plant proteins including plant-based milk are become popular due to the increase of vegetarian consumers, health awareness, and economical issues. Plant milk is a product derived from a plant source that similarity of the functional properties, nutritive value, and sensory characteristics of this milk is used as substitutes for animal milk (Alozie et al., 2015). Almond milk naturally contains high levels of monounsaturated and polyunsaturated fatty acids, protein, carbohydrates, and dietary fiber, as well as including vitamin E and several trace elements, all of which are essential nutrients for daily consumption (Yilmaz-Ersan and Topcuoglu, 2021).

2.1 Chemical composition of almond seeds

Almond seeds (*Prunus dulcis*) contain 16-26% protein and rich in fats (44-61%) and vitamins, especially B and E (Alozie et al., 2015). The compositions of some plant proteins are shown in Table 2.1.

Table 2.1 Proximate composition of plant proteins

Plant protein	**Protein (%)**	**Fat (%)**	**Moisture (%)**	**Carbohydrate (%)**	**References**
Almonds	20.39-29.37	56.76-62.25	3.00-5.05	2.88-12.73	Hasan (2012), Memon (2019)

Soybean	37.69	28.20	8.07	16.31	Ogbemudia et al. (2018)
Pea	21.09	2.01	14.19	60.29	Boye et al. (2010)
Walnut	31.17	63.37	2.50	1.46	Memon (2019)
Pistachio nut	17.85	60.82	4.50	14.03	Memon (2019)
Peanut	28.79	49.81	3.77	14.14	Memon (2019)
Pine nut	26.22	66.96	2.83	1.49	Memon (2019)

According to this information, almonds are a great choice in plant-based protein sources for low carbohydrate diets. Yada et al. (2011) described that carbohydrate contents within a range of about 2.5-13% in almonds (*Prunus dulcis*) on a dry weight basis. Researchers have also identified that almonds can be a beneficial plant source of lipids and protein in the human diet.

Almond seeds typically contain around 575 kcal per 100 g and about 50 % fat. Richardson et al. (2009) also reported that almond seeds contain a high level of monosaturated fatty acids, and the saturated fat content is the lowest of all nuts.

The lipids and proteins in almond seeds are present in the form of oil bodies and protein bodies (Devnani et al., 2020). A typical serving of almond seeds (28-30 g) provides about 14% of the daily fiber requirement (Richardson et al., 2009). Almond milk is a colloidal dispersion obtained by grinding almond seeds with water. The protein content in almond milk is typically much lower than in dairy milk (~ 0.7% vs ~ 3%, respectively) as shown in Table 2.2, although the nutritional composition is a function of the ratio of almonds and water used in the formulation (Vanga and Raghavan, 2018).

Table 2.2 Nutritional content of cow milk, soy milk, and almond milk

Composition	Cow milk (240 ml)	Almond milk (100 ml)	Soy milk (100 ml)
Carbohydrate (g)	11.50	1.32	5.00
Fibers	n.d.	0.64	0.96
Fats (g)	9.05	2.71	4.35
Saturated	5.64	0	0.64
MUFA	2.62	1.67	0.84
PUFA	0.35	0.67	2.40
Cholesterol (mg)	34.10	0	0
Protein (g)	8.11	1.67	8.71

n.d. refers to not detected.
Source: Vanga and Raghavan (2018)

Considering the nutritional profile of these kinds of milk, soybean, and almond milk can be the ideal raw material for replacing cow milk in the human diet. They are also rich in dietary fiber, monosaturated fats, and polyunsaturated fats which possibly may lower LDL cholesterol. The properties of almond protein extracts in almond milk depending on the method of extraction applied and the amount of water used. Heat stability is critical to the functionality of almond and other plant proteins and also assists in the development of food structures (Devnani et al., 2020).

2.2 Functional property of proteins

The functional properties of a protein can be seen as physical and chemical properties of proteins affecting their behavior in food systems. The functional properties of proteins can be divided into some general classes including hydration (e.g., solubility, dispersibility, water absorption, swelling, and thickening), organoleptic (e.g., color, flavor, odor, texture, and mouthfeel) and rheological (e.g., aggregation, stickiness, gelation, extrudability, and elasticity) properties (Wang et al., 2018). Among these properties, gelation is one of the most important functional properties of proteins, provides a structural matrix for holding water, flavor, sugar, and food ingredients in various food applications, as well as providing desirable sensory and textural structures in foods.

2.3 Mechanism of protein gelation

Gelation is the transformation of proteins in the liquid state into a gel-like structure through physical or chemical means. Protein gel formation is a result of protein-protein interaction and protein-solvent interaction that resulting in the three-dimensional network of protein fibers which develop high structural rigidity. A simple definition of protein gelation is an aggregation of denatured molecules with a certain degree of order, resulting in the formation of a continuous network (Peng et al., 2016). Figure 2.1 shows the mechanism of globular proteins in the liquid state to a gel-like structure.

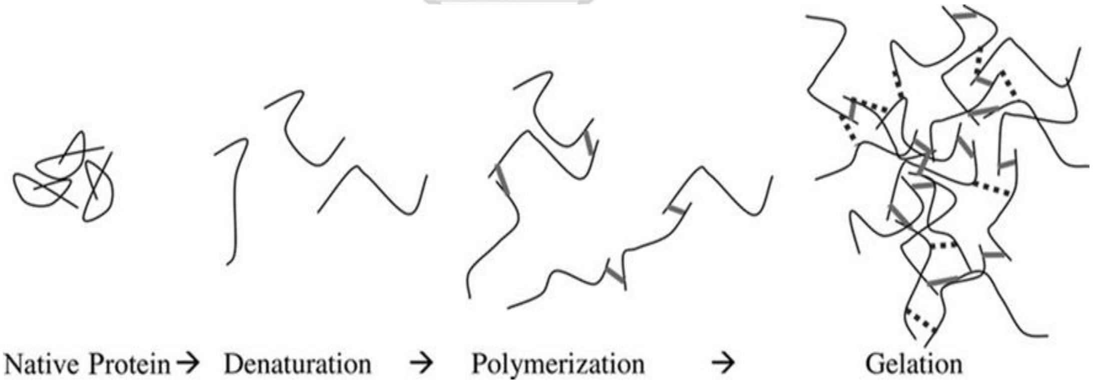


Figure 2.1 Illustration diagram of globular proteins in the liquid state to a gel-like structure. Solid short connections indicate chemical bonding (disulfide), while dotted connections indicate physical interactions (hydrogen bonding, hydrophobic interactions, and electrostatic interactions).
Source: Sullivan et al. (2009)

The gelation phenomenon requires a driving force to unfold the native protein structure, followed by an aggregation retaining a certain degree of order in the matrix formed by the association between protein standards. Gelation is involving three steps: (i) unfolding of the protein by denaturation to expose residues buried in the core, (ii) aggregate formation by the interaction of the exposed residues, and (iii) formation of a continuous network by an arrangement of the aggregates (Totosaus et al., 2002). Figure 2.2 shows the gelation mechanism of protein.

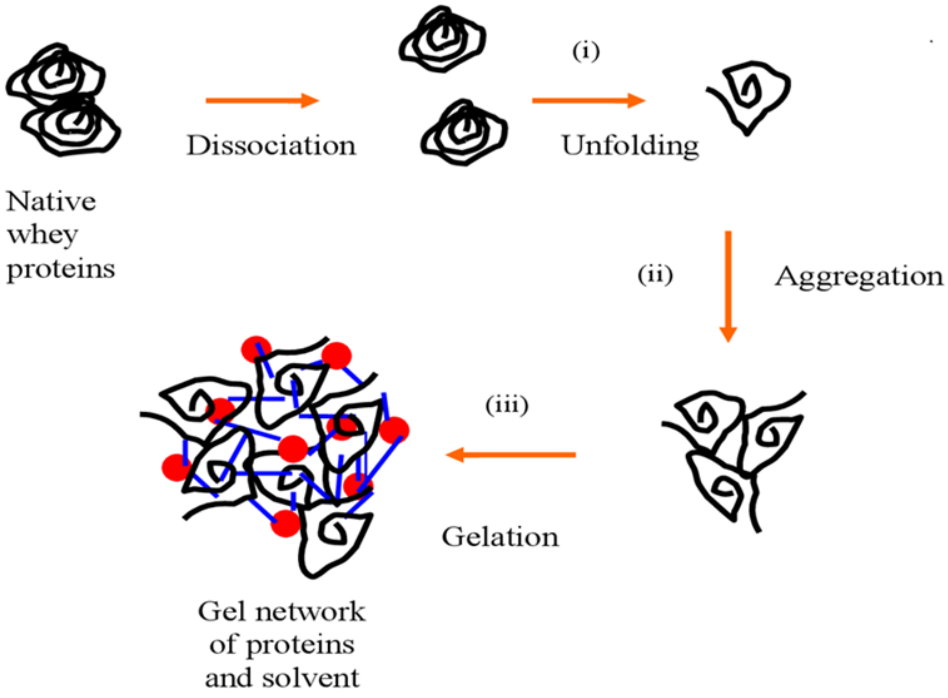


Figure 2.2 Schematic phenomenon of protein gelation.
Source: Alting et al. (2003)

The protein-protein interaction is generally formed via non-covalent cross-links such as electrostatic forces, hydrophobic interactions, and hydrogen bonding (Totosaus et al., 2002). This interaction normally occurs by heat-induced gel network formation. The partial unfolding of proteins with slight changes in secondary structure is required for gelation. Heat induction to facilitate gel formation has long been practiced and usually involves the unfolding of the protein and aggregate formation by the interaction of the exposed residues. The arrangement of the aggregation became the formation of a continuous network (Jacoba et al., 2000).

2.4 Factors affecting the protein network

Protein gelation has been traditionally achieved by heating, but some physical and chemical processes analogously form protein gels to heat -induction (Totosaus et al., 2002). Amandin, the major storage protein in almonds, belongs to the legumin class of seed proteins, which itself is a part of the globulin family. The storage protein amandin constitutes 65-70% of the total soluble proteins (Devnani et al., 2020). 95% of the total almond protein was consist of two major fractions, globulin (74%) and albumin (21%), although glutelin and prolamine represented only minor fractions, it is not glycoprotein (Yada et al., 2011). It has a hexametric structure and each of the six subunits is composed of acidic and basic polypeptides (α-chain of about 40 kDa and β-chain of about 20 kDa) linked by a disulfide bridge, with a molecular weight of approximately ~275-450 kDa (Devnani et al., 2020, Kshirsagar et al., 2011). The functional properties of a protein are affected by both intrinsic and extrinsic factors. The extrinsic factors that affect the functionality of proteins are pH, temperature, chemical additives, mechanical processing, enzymes, and ionic strength (Totosaus et al., 2002). These factors can be affected by the gelation properties of proteins in almond milk and impacted the strength of the gel. Accordingly, the effect of heat treatment, protein, and calcium chloride concentration in almond milk can be affected the thermal-induced aggregation and gelling property of almond protein when heating.

2.4.1 Protein concentration

The heat-induced gelling property of almond proteins in skim milk and full-fat almond milk was reported by Devnani et al. (2020). Heating causes proteins to unfold, exposing reactive groups from which intermolecular bonds can be formed with neighboring protein molecules. With sufficient intermolecular bonding, a three-dimensional network can be developed, forming a gel. It formed a self-supporting weakly flocculated particulate gel structure, resulting in gels with higher water holding capacity and strength like dairy gels. Besides, Devnani et al. (2020) showed that full-fat almond milk samples indicated stronger gels with a higher but more variable gel modulus, likely due to the greater porosity of the gel network and more heterogeneous distribution of fat. The presence of almond fat increased gel strength but led to a more heterogeneous microstructure, which may be improved by homogenization. The cross-linking of macromolecules is required for gelation and is proportional to the protein concentration (Totosaus et al., 2002). It cannot be formed a continuous three-dimensional structure in which the concentration of the protein itself, below the minimum. For example, the minimum concentration of heat-induced gelation for pea protein isolates was 16% (w/v) as described by Xiang and Susan (2010). The protein solution remains in the liquid state under conditions of low molecular weight and low protein concentration but if the molecular weight and concentration of protein are high, it can be changed into a gel structure when cooled (Totosaus et al., 2002).

2.4.2 Temperature

Devnani et al. (2020) showed protein surface hydrophobicity and particle size increased and alpha-helical structure decreased at above 55^{o}

C, reducing the stability of skim or full-fat almond milk. At 65-75º C, fractal protein clusters have occurred, and more extensive denaturation and gelation were observed at higher temperatures of 85-95ºC. However, high thermal treatments being a more intense increase in the size of fat globules, due to the flocculation and coalescence phenomenon. Bernat et al. (2015) studied the effect of heat treatments and found that at high temperature (121º C/15 min) of almond milk caused an increment of particle size due to the formation of oil-droplet-protein body clusters, associated with protein concentration. They also found that low heat treatment (85º C/ 30 min) was mitigated by the greater stability of smaller fat globules which are less sensitive to the flocculation and coalescence phenomena than the higher heat treatment (Bernat et al., 2015). Excessive heating of the protein solution to a higher than needed for denaturation leads to a state which does not set into a gel upon cooling (Totosaus et al., 2002). There is no study on the effect of high temperature on almond milk gelation. However, Bernat et al. (2015) mentioned that thermal treatment at the lowest temperature (85ºC/30 min) in almond milk induced thermo-stability of protein denaturation, thus enhancing the aggregation process than high temperature (121ºC/15 min).

2.4.3 Calcium Chloride

The addition of salts, as an extrinsic factor, can influence the repulsion forces in the protein system through their own positive and negative charges, thus affecting the protein-protein and protein-solvent interactions. Salts and the denaturation of soy proteins form coagulates when metal ions such as Ca^{2+} can neutralize surface charges and form salt bridges with the negatively charged protein. Figure 2.3 shows the schematic diagram of soy protein aggregation by the addition of calcium chloride salt. This cross-link structure is due to the electrostatic interactions between the cations and soy proteins (Tay et al., 2006). The formation of salt bridges upon the addition of calcium chloride increases ions bonds, thus increasing gel firmness (Zhao et al., 2020)

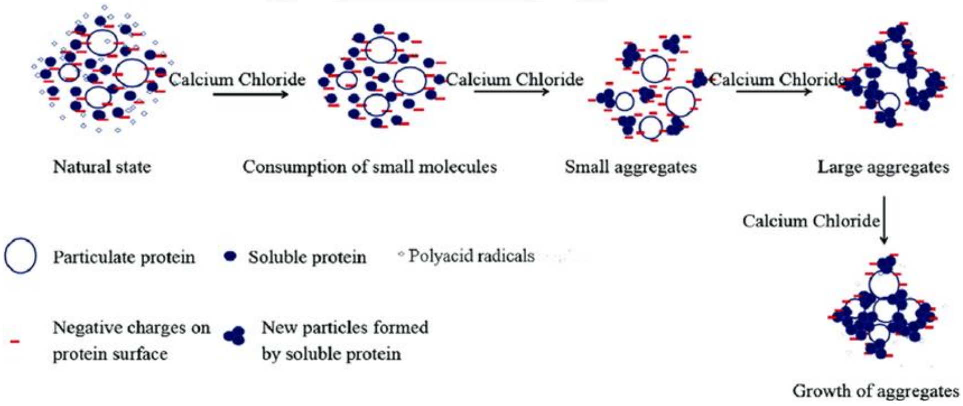


Figure 2.3 Schematic diagram of aggregation of soy protein by addition of calcium chloride. Source: Wang et al. (2015)

The different types of calcium improve the gelation rate to different degrees, in the order formate > acetate > chloride > lactate, and consequently, influence the gelation behavior of soy tofu (Zhang et al., 2013). The protein particles in soy milk with calcium chloride salts are more sensitive than non-salt protein particles as they more easily lose solubility at a low calcium chloride level. The higher content of protein particles with the lower level of calcium chloride in which soy milk forms precipitates and the harder texture of the tofu product (Wang et al., 2003). In addition, Zhao et al. (2020) studied that the addition of different salts such as 0.1 g/100 ml KCl, 0.05 g/100 ml $CaCl_2$, and 0.15 g/100 ml $CuSO_4$ can maximally improve the hardness of citric acid-induced soy tofu. Maltais et al. (2008) reported that soy protein cold-set gels were composed of homogeneous fine filamentous structures at 10 mM of $CaCl_2$, while gels formed at 20 mM of $CaCl_2$ were composed of random aggregated particulate structure. The use of low concentrations of $CaCl_2$ (0.3 and 0.5 %) in reconstituted soy milk provided a higher degree of product fluidity and increased the hardness of tofu (Leiva-Vega et al., 2011). In addition, the physical and chemical properties of tofu do not change completely with the addition of calcium chloride. Therefore, calcium chloride salts could be used as a coagulant agent for the quality improvement of protein gelation. However, there is no study on the effect of the concentration of salts on almond protein gelation and the properties of these gels.

2.4.4 pH

A balance of both protein-protein and protein-solvent interactions is essential for heat-induced gel network formation. The net charge of the protein molecule is modified by attractive and repulsion forces, affecting these interactions (Totosaus et al., 2002). The greater the net charge of protein molecules formed the greater the electrostatic repulsion between molecules. The net charge of protein at its isoelectric point is equivalent to zero. Protein gelation can be induced by lowering the electrostatic repulsion by lowering the pH forms a different structure compared with heat-induced gels, but depending upon the protein concentration (Mäkinen et al., 2015). Bengoechea et al. (2017) stated that carob protein isolate (isoelectric point - 4.5) produced self-supporting gels with a higher denaturation temperature and became more denatured at acid pH (pH2) than at alkaline pH (pH 10), but carob protein isolate gels can only be obtained at protein concentrations higher than 20%. Also, the isoelectric point (pI) of almond protein was in the range of 4.5-5.5 (Li and He, 2004), however, the effect of pH on almond protein gelation has not been studied in detail. Accordingly, pH and protein concentration had an impact on the strength of almond protein gels. Table 2.3 shows the conditions used to induce gelation of almond proteins from different sources.

Table 2.3 Conditions used to induce gelation of almond proteins

Preparation	Protein concentration (%) (w/v)	Temperature (ºC)	pH	References
FFAM	~ 4	≥ 85º C	6.2	Devnani et al. (2020)
Almond protein isolate	4	NA	8.2	Sharma et al. (2010)
Almond protein isolate	6	100	7	Sharma et al. (2010)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and reagents

Dried raw almond seeds were purchased from the local supermarket (Bangkok, Thailand). All chemicals were of analytical grade, used to conduct this experiment.

3.2 Chemicals

Reagent grade concentration sulphuric acid (Merck, Germany)

Anhydrous copper sulfate (Dae-Jung Chemicals & Metals, Korea)

Anhydrous potassium sulfate (Dae-Jung Chemicals & Metals, Korea)

Sodium hydroxide (Qrec, New Zealand)

Boric Acid (Qrec, New Zealand)

Hydrochloric acid (Merck, Germany)

Methyl red (Merck, Germany)

Methyl blue (Merck, Germany)

Petroleum ether (TTK Science Co; Ltd, Thailand)

Sodium Azide (TTK Science Co; Ltd, Thailand)

Calcium chloride.2 H_2O (KemAus, Australia)

95% Ethanol (A.R grade, Qrec, New Zealand)

3.3 Laboratory equipment

Drying oven (Memmert UN 30 plus, Germany)

Distillation unit (K-324) (Buchi, Switzerland)

Digestion unit (K-424) (Buchi, Swizerland)

Soxhlet extractor (Gerhardt, Germany)

Blender (Phillips HR- 2102, China)

Homogenizer (IKA®T25 Digital Ultra Turrax)

Seven compact pH meter (Mettler Toledo Co; Ltd, Victoria, Australia)

Water bath (SW 23, Germany)

TA. XT2i texture analyzer (Stable Micro Systems Co; Ltd, Godalming, UK)

Centrifuge (Hettich®Universal 320/320 R, Andreas Hettich GmbH & Co. KG., Tuttlingen, Germany)

3.4 Methodology

3.4.1 Proximate analysis of almond seeds

The moisture content and total solids were estimated following the AOAC method (AOAC, 1995). The protein content was quantified by nitrogen analysis following the Kjeldahl method (AOAC, 1995), where nitrogen to the protein conversion factor for almond seed was 5.18. The total fat was estimated using the Soxhlet method (AOAC, 1995). The fiber, ash, and carbohydrate content were estimated using by AOAC method (AOAC, 1995).

3.4.2 Preparation of full-fat almond milk (FFAM)

Raw almond seeds were soaked in water with a ratio of 1:3 (w/w) into a beaker with plastic wrap and stored at 4º C for 18-20 h (Devnani et al., 2020). After soaking was complete, this water was discarded. The almond seeds were deskinned by hand and mixed with water at a ratio of 1:2, 1:3, 1:4, and 1:5 (w/v) and then grounded with the blender (Philips HR-2102, China) at high speed for 3 min. These mixtures were blended using a homogenizer (IKA® T25 Digital Ultra Turrax) at 20,000 rpm for 10 min, followed by stirring with a homogenizer (IKA® T25 Digital Ultra Turrax) at 3000 rpm for 30 min. These resulting slurries were filtered through a double layer of filter cloth and a 180-µm sieve to obtain full-fat almond milk (FFAM). Sodium azide (0.02 % w/v) was added to the FFAM samples to prevent bacterial growth. The FFAM samples were stored at 4º C and analyzed within 3 days of production. All the samples were done in triplicates.

3.4.3 Determination of physicochemical properties of FFAM

The yield of FFAM was estimated as the percentage of milk obtained per unit of raw materials (dry almonds and water) on a w/w basis as described by Devnani et al. (2020). The pH of FFAM was measured using a Seven Compact pH meter (Mettler Toledo Co. Ltd., Victoria, Australia). The proximate analysis of FFAM was determined followed the AOAC method (AOAC, 1995). The protein content was quantified by nitrogen analysis following the Kjeldahl method (AOAC, 1995) where nitrogen to the protein conversion factor for almond seed was 5.18.

3.4.4 Effect of protein concentration on almond milk gelation

Protein concentration was varied from 2 to 5% with the soaked almond seeds mixing with distilled water at the ratio of 1:2, 1:3, 1:4, and 1:5 (w/v). FFAM samples were poured into a centrifuge tube (20 ml) and then were heated in a water bath with a temperature of 90º C for 30 min. After heating the samples were rapidly cooled using running tap water.

All samples were cooled and stored at 4º C until further analysis. The gel samples were analyzed within one week. The FFAM gels were analyzed for texture profile analysis (TPA), gel strength, and water holding capacity (WHC). The details were followed in the next section.

3.4.5 Physical properties of almond milk gelation

3.4.5.1 Texture Profile Analysis (TPA)

Texture profile analysis was performed as described by Yang and Li (Yang and Li, 2010). The texture profile analysis (TPA) test was performed by using a TA. XT2i texture analyzer (Stable Micro Systems Co. Ltd., Godalming, UK). An SMS P/0.5 probe was used to measure the TPA of the samples at room temperature, which was done in 3 repetitions. During the pre-test, compression, and relaxation of a sample, the test speed of the probe was 10 mm/sec, while the speed of obtaining the data was 200 points per second (pps). The thickness of the samples was 1.5 cm and 70 % of the original depth was compressed during the first stage.

3.4.5.2 Gel Strength (Firmness)

The almond milk gel samples were analyzed using a TA.XT2i texture analyzer (Stable Micro Systems Co. Ltd., Godalming, UK) (Tezel et al., 2019). A 1 kg load and aluminum cylindrical 36 mm probe were used to determine the gel strength. The test speed was 10 mm/sec during the measurements. Samples were measured in triplicate from all experiments.

3.4.5.3 Water Holding Capacity (WHC)

The almond milk gels were subjected to 5-min centrifuged with Hettich® Universal 320/320R (Andreas Hettich GmbH & Co. KG., Tuttlingen, Germany) at 2000 rpm (Devnani et al., 2020) and water was allowed to drain from the centrifuge tube. The water holding capacity of the samples were calculated using the following equation:

$$\text{WHC (\%)} = W_2 / W_1 \times 100 \qquad (1)$$

where: W_1 and W_2 were corresponded to the weight of the gel (g) before and after centrifugation, respectively.

3.4.6 Effects of temperature on almond milk gelation

To study the effect of temperatures, FFAM samples (1:2, 1:3, and 1:4) were poured in a centrifuge tube (20 ml) and heated at 90 °C, 95 °C, and 100 °C for 30 min. After heating, the samples were rapidly cooled using running tap water. All samples were stored at 4º C for 2 h to form a gel and gel samples were analyzed within one week. The resulting almond milk gels were analyzed for the texture profile analysis, gel strength, and water holding capacity.

3.4.7 Effects of calcium chloride on almond milk gelation

To study the effect of calcium chloride, calcium chloride in 3 concentrations (0.3%, 0.5% and 1%) were added into the FFAM samples (1:2, 1:3 and 1:4). These FFAM samples were poured in a centrifuge tube (20 ml) and heated at 90º C for 30 min. After heating, the samples were rapidly cooled using running tap water. All samples were stored at 4º C for 2 h to form a gel and gel samples were analyzed within one week. The resulting almond milk gels were analyzed for the texture profile analysis, gel strength, and water holding capacity.

3.4.8 Effects of pH on almond milk gelation

To study the effect of pH, different pH values (3, 4, 5, 6, and 7) were adjusted with 1 N HCl or 1 N NaOH by titration method into the FFAM samples (1:2, 1:3, and 1:4) (Bengoechea et al., 2017). These FFAM samples were poured in a centrifuge tube (20 ml) and heated at 90º C for 30 min. After heating, the samples were rapidly cooled using running tap water. All samples were stored at 4º C for 2 h to form a gel and gel samples were analyzed within one week. The resulting almond milk gels were analyzed for the texture profile analysis, gel strength, and water holding capacity.

3.4.9 Effect of combination factors on almond milk gelation

To understand the effect of the combination of these factors on almond milk gelation, I have been selected each factor according to hardness, gel strength, and WHC of FFAM gels.

FFAM was adjusted pH and added $CaCl_2$, and then poured in a centrifuge tube (20 ml) and heated at 90 ºC for 30 min. After heating, the samples were rapidly cooled using running tap water. Samples were stored at 4ºC for 2 h to form a gel and gel samples were analyzed within one week. The resulting almond milk gels were performed texture profile analysis, gel strength, and water holding capacity according to the same procedure as mentioned above and rheological measurement.

3.4.9.1 Rheological Measurement

The rheological properties of almond milk gelation were observed using the method of (Zhao et al., 2020) with slight modification. Frequency sweep test was performed using a Modular Compact Rheometer 102 (Anton Paar GmbH, Austria) with a parallel plate of 25 mm diameter and 2 mm gap. The temperature cycle was programmed at

80º C for 30 min and cooling to 25º C at a rate of 5º C min^{-1}. The cylindrical almond milk gels were cut into slices; storage modulus (G') and loss (G") modulus were recorded at a frequency sweep over 0.1-100 Hz and 0.5% strain.

3.4.10 Statistical analysis

All properties were measured in triplicate and the average values were compared between three independent batches of almond milk (n = 3 ± the standard deviation). Data were analyzed by one-way analysis of variance (ANOVA). Duncan's new multiple range tests were used to determine the difference among sample means at the significance level ($p < 0.05$) which was performed by SPSS version 22 for windows.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Determination of proximate analysis of almond seeds

The proximate analysis of almond seeds was performed using the AOAC method (AOAC, 1995). The result of the proximate analysis of almond seeds is shown in Table 4.1. Almond seeds contained 51.54 ± 0.0.5 % fat, 21.23 ± 3.7 % protein, 12.40 ± 4.2 % carbohydrate, and 4.77 ± 0.1 % moisture content. Our results of the protein content of almond seeds (21.23 %) were similar to Hasan (2012) who found the protein content of almond seeds was 20.39 %. The protein content of soybean (37.69%) (Ogbemudia, 2018) was higher than almond seeds (21.23 %) in this study. The fat content of almond seeds (51.54%) was higher than soybean (28.2%) as described by Ogbemudia et al. (2018). Almond seeds typically contain about 50 % fat, the predominant fatty acid composition is monounsaturated fatty acids, and unsaturated fat content is the lowest among all nuts (Richardson et al., 2009). The moisture content of almond seeds in this study was 4.77 ± 0.1 %, and this low moisture content of almond seeds is of advantage because it enables the seed to be stored for a long time. Generally, the results obtained in this study suggest that almond seeds can be a good source of protein, fat, and carbohydrate. The major protein of almond, amandin, is a globulin made up of at least two types of polypeptides (molecular weight range ~275-450 kDa) linked through disulfide bonds (Devnani et al., 2020). The lipids in almonds are composed predominantly of mono- and polyunsaturated fatty acids. Sugars, starches, and nonstarch polysaccharides are founds in almond seeds as carbohydrates. Only sugars, starch, and some sugar alcohols in almonds can be digested and absorbed by humans to provide a source of energy (Yada et al., 2011).

Table 4.1 Proximate analysis of almond seeds

Components	Percent
Fat	51.54 ± 0.5
Protein	21.23 ± 3.7
Carbohydrate	12.40 ± 4.2
Moisture Content	4.77 ± 0.1
Fiber	6.38 ± 0.4
Ash	3.25 ± 0.3

4.2 Physiochemical properties of full-fat almond milk (FFAM)

Full fat almond milk (FFAM) was obtained by grinding the almond seeds with water and the water-soluble extract was filtered to obtain a particle slurry. Table 4.2 shows the composition of almond milk prepared with different ratios of almond seeds and water in the extraction procedure.

Table 4.2 Composition of almond milk

Milk yield and characteristics	The ratio of almond seeds: water (w/v)			
	1:2	1:3	1:4	1:5
Total solids (%)	23.14±0.10^{a}	19.63±0.30^{b}	15.59±0.00^{c}	11.06±1.10^{d}
pH	5.93±0.07^{a}	5.94±0.01^{a}	5.96±0.02^{a}	5.96±0.02^{a}
Yield (%)	66.67±0.00^{c}	78.75±1.30^{b}	82±2.00^{a}	83.87±1.00^{a}
Proximate analysis (%)				
Moisture content	76.86±0.10^{d}	80.20±0.20^{c}	84.41±0.00^{b}	88.94±1.00^{a}
Protein	5.37±0.30^{a}	4.81±0.10^{b}	4.07±0.00^{c}	3.23±0.20^{d}
Fat	8.16±2.60^{a}	6.29±1.70^{ab}	3.40±1.40^{b}	2.92±1.10^{b}
Carbohydrate	5.55±2.40^{a}	5.16±1.90^{ab}	4.18±1.40^{ab}	2.09±0.30^{c}

$^{a\text{-}d}$ Row values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

The protein content of FFAMs varies from 5.37±0.3 to 3.23±0.2 %, with 5.37 % was the highest value of the FFAM ratio of 1:2 and FFAM ratio of 1:5 had the least value of 3.23 %. The protein content of FFAM was significantly ($P < 0.05$) different between the ratio of 1:2 and 1:5. The protein content of FFAM samples in this study is higher than that which was reported by Alozie et al. (2015) and Vanga and Raghavan (2018), but this protein content was within the range of that in FFAM as reported by Devnani et al. (2020). The protein content is varied due to differences in the extraction procedure and the ratio of almond seeds to water. The content of the protein of FFAM in this study was similar to the value of soy milk protein 4.5% reported by Soumitra Banerjee et al. (2019). These protein and total solid contents can be affected in the gelling property (Devnani et al., 2020). Also, the FFAM ratio of 1:2 had the highest value of protein, fat, total solids, and carbohydrate composition. The amount of water needed to make soymilk is important because it affects soymilk's solid content and protein content, which concerns soy tofu texture (Rekha and Vijayalakshmi, 2013). The moisture content of FFAM samples was significantly ($P< 0.05$) different from each other. The concentration of other nutrients is affected by moisture levels. The carbohydrate content of FFAM samples (1:2 and 1:3) in this study is higher than the value of almond milk (4.50%) reported by Alozie et al. (2015). The yield of all FFAM (1:2) was significantly ($P< 0.05$) different, depending on the solids level of milk. The pH of all samples was not significantly ($P\geq 0.05$) different.

The FFAM samples were then heated at 90º C, for 30 min and cooled at 4º C to form gels. Heat stability is important to the functional property of the protein. The FFAM (1:2, 1:3, and 1:4) samples were formed protein gelation as shown in Figure 4.1.

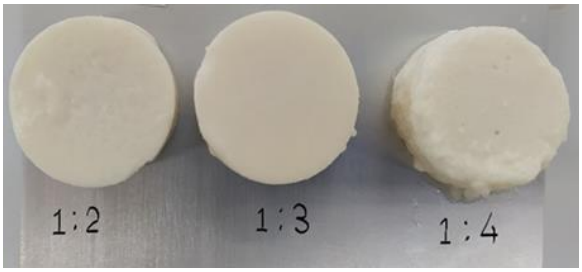


Figure 4.1 Gelation of full-fat almond milk: visual observation of the occurrence of gel formation

This study can be confirmed that almond milk can form a gel at high temperatures by protein denaturation. However, the lesser percentage of protein concentration in FFAM (1:5) indicated a low gel strength. Devnani et al. (2020) found that FFAM with the ratio of 1:4 preparations formed the visible aggregates of almond protein at temperatures higher than 85º C, after heating for 30 min followed by cooling to room temperature. Heat-induced gelation of plant protein is affected by protein composition and total solids content (Silva et al., 2019). In this study, FFAM with a ratio of 1:5 formed heat-induced gel with a protein concentration of 3.23%, but the gel strength (0.01 N) is too low. Therefore, it cannot be formed a gel structure and it was difficult to analyze other properties. Yilmaz-Ersan and Topcuoglu (2021) found almond yogurt with a higher ratio of reconstituted almond milk had a higher firmness due to its higher protein and total solids content.

Devnani et al. (2020) used an FFAM ratio of 1:4 gelled with a protein concentration of 3.6 %. Therefore, a higher protein concentration was increased protein-protein interactions and led to an increasing gel strength (Silva et al., 2019). Ningtyas et al. (2021) found that the suspension of fat globules acts as active or inactive filler particles depending on their interactions during the gelation of soy milk. To establish protein gelation in soy milk, protein-lipid coagulates gather through intermolecular interaction and massive random cross-linking occurs; thus, particle type of protein cluster is observed (Peng et al., 2016). Interestingly, FFAM gels formed a continuous protein network than skim almond milk gels, due to the more heterogeneous distribution of fat in full-fat almond milk (Devnani et al., 2020). Therefore, the gelation of protein particles depends on the structure, size, and interactions of protein aggregate with the suspension components, such as fat and carbohydrate. Interaction of the relative ratio of proteins, carbohydrates, fats, and total solids may also be attributed to the gel network formation in heat-induced denaturation of protein (Ningtyas et al., 2021).

4.3 Effect of protein concentration on almond milk gelation

The amount of water required to prepare almond milk is important because it affects the protein concentration and texture of these gels.

Higher water content results in lower protein concentration and other components in milk which may produce a soft texture in the gel. The texture of almond milk gels was evaluated on Texture Analyzer and data are presented in Table 4.3.

Table 4.3 Texture profile analysis of FFAM gels

Textural Properties	**FFAM gels (the ratio of almond seeds: water (w/v))**		
	1:2	**1:3**	**1:4**
Hardness (g)	43.03 ± 8.66^{a}	42.57 ± 7.18^{a}	34.11 ± 6.48^{b}
Cohesiveness	0.79 ± 0.018^{a}	0.73 ± 0.13^{a}	0.66 ± 0.08^{a}
Springiness (-)	0.33 ± 0.03^{a}	0.21 ± 0.07^{b}	0.23 ± 0.03^{b}
Gumminess (g)	34.01±7.39^{a}	30.33 ± 0.63^{ab}	22.92 ± 6.84^{b}
Adhesiveness	-5.67±0.99^{b}	-1.07 ± 0.72^{a}	-1.27 ± 0.77^{a}
Chewiness	11.01 ± 1.93^{a}	6.44 ± 2.01^{b}	5.21 ± 1.33^{b}

$^{a-b}$ Row values followed by the same superscript letter are not significantly different (P≥0.05). Means ± SD of triplicates.

The texture parameters of these gels were analyzed with hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness. Hardness is the force necessary to attain a given deformation. Greater hardness means harder and firmer. The hardness of the FFAM gels ranged from 43.03, 42.57, and 34.11 g in samples 1:2, 1:3, and 1:4, respectively. The formation of the gel network was mainly dependent on soy protein concentration and this protein concentration was directly influenced the hardness of soy tofu as described by Zhao et al. (2020). The higher protein, fat, and total solids content in almond milk caused an increase in the degree of cross-linkage of the gel network and resulted in a much denser and firmer gel structure in almond milk yogurt (Yilmaz-Ersan and Topcuoglu, 2021). Moreover, the lower solids content in soy milk resulted in higher moisture content, which may produce a soft texture in tofu and high solids content was increased the hardness of tofu as described by Rekha and Vijayalakshmi (2013). Canola protein isolate aggregation was growth and the formation of gel network was stronger at the higher protein concentration (5% to 9%) (Kim et al., 2016). Our results were the same with other studies; the FFAM gel ratio of 1:2 resulted in the greatest hardness, cohesiveness, springiness, gumminess, and chewiness of other ratios of FFAM gels due to the higher protein and solid contents.

Cohesiveness is related to the work required to overcome the internal bonding of the material. Cohesiveness ranged from 0.79, 0.73, and 0.66 in FFAM gels 1:2, 1:3, and 1:4, respectively, and were not significantly different ($P \geq 0.05$). The springiness is described as the rate at which a deformed material recovers to its undeformed conditions after the deforming force is removed. The chewiness is defined as the energy required to masticate a solid food product to a state of readiness for swallowing. Springiness ranged from 0.33, 0.21, and 0.23, and chewiness ranged from 11.01, 6.44, and 5.21 in FFAM gels 1:2, 1:3 and 1:4, respectively. The FFAM gel (1:2) resulted in greater chewiness and springiness than other ratios of FFAM gels. Gumminess ranged from 34.01, 30.33, and 22.92 in FFAM gels 1:2, 1:3, and 1:4, respectively. The FFAM gel (1:2) was significantly ($P < 0.05$) different from the FFAM gel (1:4). The adhesiveness value of FFAM gel (1:2) was also significantly ($P < 0.05$) different from FFAM gel (1:4).

4.4 Effect of composition of almond milk in gel strength and water holding capacity

Water holding capacity (WHC) indicates the protein-water interactions in a gel system. The WHC and gel strength of FFAM gels were shown in Table 4.4.

Table 4.4 Gel strength and WHC values of different FFAM gels

FFAM Gel (Almond seeds: water)	Gel Strength (N)	WHC (%)
FFAM (1:2)	0.18 ± 0.03^{a}	95.44 ± 2.64^{a}
FFAM (1:3)	0.04 ± 0.03^{b}	82.71 ± 2.03^{b}
FFAM (1:4)	0.03 ± 0.00^{b}	77.39 ± 1.22^{c}

$^{a\text{-}c}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

The WHC of 1:2, 1:3, and 1:4 FFAM gels were 95.44, 82.71, and 77.39 %, respectively. The higher protein concentration of FFAM gels led to strong protein-protein interactions and the formation of large aggregates, reinforcing the protein gel network with a higher WHC of

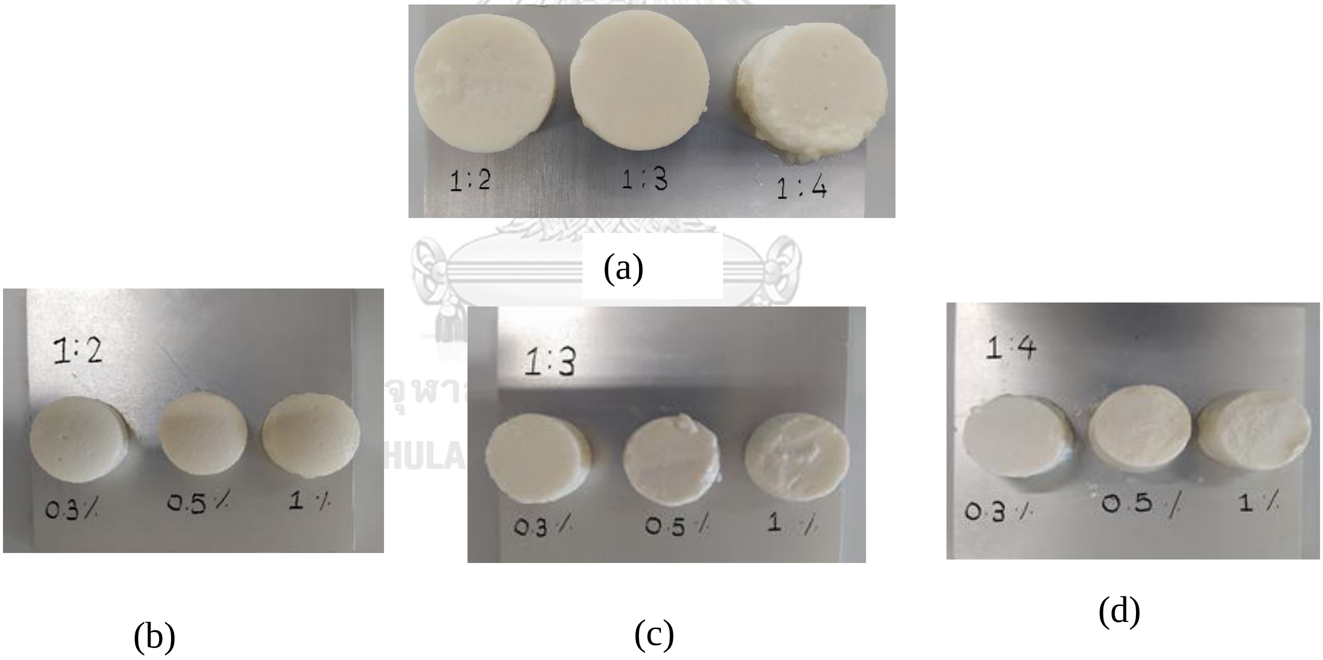
97% (Devnani et al., 2020). Besides, Devnani et al. (2020) found that the WHC of FFAM gels was higher than that of skim almond milk gels, consistent with fat and carbohydrate acting as filler particles.

The composition of almond milk is responsible for the gel strength and texture of the product. The gel strength of FFAM gels is present in Table 4.4. The firmness of FFAM gels increased from 0.03 to 0.18 (peak penetration force (N)) as protein concentration increased from FFAM 1:4, 1:3 to 1:2, respectively. On the other hand, the maximum gel strength value was obtained at a high concentration of protein from the 1:2 ratio of FFAM as seen in Table 4.4. The FFAM formed stronger gels at higher temperatures of 85-95º C inducing more extensive protein denaturation and resulting in gels with high WHC (Devnani et al., 2020). The presence of higher protein and fat contents of FFAM ratio of 1:2 resulted in a gel with increase gel strength and WHC. A higher WHC of 95.44 % was measured in FFAM gel (1:2) and it was also significantly ($P<0.05$) increasing the WHC of other FFAM gels (1:3) and (1:4).

For the study of combination factors, FFAM (1:3) has been selected due to the moderate amount of milk yield and composition of milk. Moreover, FFAM (1:3) can form a gel at a high temperature (100º C).

4.5 Effect of calcium chloride concentration on FFAM gels

The addition of salts, as an extrinsic factor, can influence the repulsion forces in the protein system through their own positive and negative charges, thus affecting the protein-protein and protein-solvent interactions. Calcium ions can neutralize surface charges and form salt bridges between proteins. The FFAM (1:2, 1:3, and 1:4 ratio) samples were formed protein gelation with different calcium chloride concentrations as shown in Figure 4.2.



(a)

(b) (c) (d)

Figure 4.2 Gelation of FFAM (a) FFAM gels (0% calcium chloride) and different calcium chloride concentration in (b) FFAM 1:2 (c) FFAM 1:3 (d) FFAM 1:4

The formation of salt bridges upon the addition of calcium chloride increases ions bonds, thus increasing gel firmness (Zhao et al., 2020). Differences in the degree of protein aggregations in whey protein isolate cold-set gelation formed at various calcium chloride concentrations as described by Totosaus et al. (2002). The addition of calcium increases the firmness of soy tofu due to the high contribution of soy protein particles (Leiva-Vega et al., 2011).

4.6 WHC and gel strength values of FFAM gels with calcium chloride

The WHC and gel strength of FFAM gels with different calcium chloride concentrations was shown in Table 4.5. and it compared with statistical within the same ratio of FFAM gels. The water holding capacity of proteins is the ability to retain water against gravity physiochemically. The addition of calcium chloride salts influences water binding by proteins because of their effects on electrostatic interactions (Zhao et al., 2020). The addition of 0.3% $CaCl_2$ increased the WHC values of other $CaCl_2$ concentrations in each ratio of FFAM gels. Minimum WHC values of 96.84%, 96.52% and 92.55% in each FFAM gels (1:2, 1:3 and 1:4, respectively) were obtained with the addition of 1% $CaCl_2$. At an appropriate gelation rate, there is sufficient time for structural and organization and homogeneous gel production, and the resulting enhancement of interactions between protein and water increases the WHC (Zhao et al., 2020). However, excessive calcium ions led to strong protein-protein interactions and the formation of large aggregates, which resulted in a reduction in WHC.

Table 4.5 Gel strength and WHC value of FFAM gels with calcium chloride concentration

FFAM Gels almond seeds: water	**% Calcium chloride added**	**Gel Strength (N)**	**WHC (%)**
1:2	0	0.18 ± 0.03^{b}	95.44 ± 2.64^{b}
	0.3	0.22 ± 0.03^{a}	99.20 ± 0.45^{a}
	0.5	0.18 ± 0.01^{b}	98.10 ± 0.85^{ab}
	1	0.12 ± 0.04^{c}	96.84 ± 2.22^{ab}
1:3	0	0.04 ± 0.03^{c}	82.71 ± 2.03^{b}
	0.3	0.15 ± 0.01^{a}	97.97 ± 1.81^{a}
	0.5	0.14 ± 0.00^{ab}	97.19 ± 0.38^{a}
	1	0.11 ± 0.00^{b}	96.52 ± 0.57^{a}
1:4	0	0.03 ± 0.00^{b}	77.39 ± 1.22^{c}
	0.3	0.07 ± 0.02^{a}	98.48 ± 0.61^{a}
	0.5	0.06 ± 0.01^{a}	98.06 ± 0.83^{a}
	1	0.04 ± 0.02^{ab}	92.55 ± 1.98^{b}

$^{a\text{-}b\text{-}c}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

The concentration of $CaCl_2$ is responsible for the gel strength and texture of the product. Lu et al. (2010) reported that gel strength increased with the addition of $CaCl_2$, while it decreased under excessive calcium ions. The gel strength of FFAM gels decreased in FFAM gels as calcium chloride concentration increased from 0.3% to 1%. However, the gel strength value of 0.3% $CaCl_2$ added in each FFAM is increased significantly ($P<0.05$) compared to FFAM without $CaCl_2$. The calcium chloride ions are added to weaken the repulsion forces of negative soy protein surface charges, and in addition, the formation of salt bridges results in the strengthening of ionic bonds in soy tofu (Zhang et al., 2013). Ion-induced gels are formed by the reduction of the electrostatic repulsion by the calcium chloride salt added during initial heating (Totosaus et al., 2002). However, the addition of $CaCl_2$ resulted in a coarse network with large pores, but the network structure was hardly maintained at high concentration, which is consistent with the larger aggregate size of soy tofu at high $CaCl_2$ salt concentration (Zhang et al., 2013). The whey protein of β-lactoglobulin gel strength increased at lower concentrations of $CaCl_2$ (20 mM), because of differences in the initial formation of protein aggregates mediated by calcium chloride salt (Totosaus et al., 2002). There is no other study on the effect of different calcium chloride concentrations on almond protein gelation mechanism, and how different affect the composition of protein, fat, and total solids of almond milk gelation. According to this study, the maximum gel strength value was obtained at 0.3% of $CaCl_2$ concentration in each FFAM gels as seen in Table 4.5.

4.7 Texture profile of FFAM gels added with calcium chloride

Table 4.6 shows the texture profile of FFAM gels added with calcium chloride. The addition of calcium chloride improved the textural properties of almond milk gels to varying degrees. Hardness is a measure of the resistance of a gel to the destructive force during compression (Zhao et al., 2020). Compare with control (0% calcium chloride), the addition of calcium chloride (0.3 and 0.5%) improved the hardness of each FFAM gels but decreased the hardness in the addition of 1% calcium chloride. The maximum hardness of 44.76, 43.29, and 39.41 g was obtained with the addition of 0.3% $CaCl_2$ in FFAM gels 1:2, 1:3, and 1:4, respectively. The addition of calcium chloride salts increased protein-protein and protein-solvent interactions, leading to the formation of a denser network, resulting in a stiffer texture. Divalent calcium ion-protein bridges contributed to a more compact gel structure and higher gel hardness as reported by Zhang et al. (2013). All FFAM gels in this study resulted that the minimum hardness of 42.49, 41.17, and 38.20 g was obtained with the addition of 1% $CaCl_2$ in FFAM gels 1:2, 1:3, and 1:4, respectively. Cohesiveness, springiness, gumminess, and chewiness of the product showed similar trends as hardness. Compared with the control (0% $CaCl_2$), FFAM gels prepared with 0.3% and 0.5% of $CaCl_2$ were not significantly ($P \geq 0.05$) different on each ratio of FFAM. According to the results, the texture profile of FFAM gel with the addition of 1% $CaCl_2$ was significantly ($P < 0.05$) decreased with FFAM gels with the addition of 0.3% and 0.5% of $CaCl_2$ in each ratio of FFAM.

According to the results, the highest texture profile and gel strength value of the addition of 0.3% $CaCl_2$ were taken for the combination study.

Table 4.6 Texture profile of FFAM gels added with different calcium chloride concentration

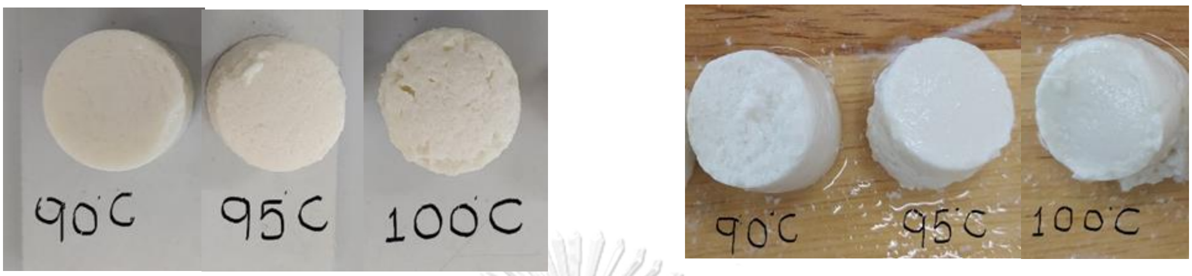
FFAM gels almond seeds: water	% calcium chloride concentration	Hardness (g)	Cohesiveness	Springiness (-)	Gumminess (g)	Adhesiveness	Chewiness
1:2	0	43.03 ± 8.66^{b}	0.79 ± 0.02^{a}	0.33 ± 0.03^{c}	34.01 ± 7.39^{a}	-5.67 ± 0.99^{a}	11.01 ± 1.93^{c}
	0.3	44.76 ± 1.04^{a}	0.78 ± 0.09^{a}	0.43 ± 0.02^{a}	35.12 ± 4.99^{a}	-3.53 ± 2.65^{a}	15.17 ± 3.03^{a}
	0.5	43.81 ± 1.60^{b}	0.68 ± 0.08^{b}	0.40 ± 0.02^{a}	31.37 ± 3.20^{b}	-2.42 ± 0.61^{a}	12.43 ± 1.57^{b}
	1	42.49 ± 0.61^{c}	0.63 ± 0.07^{b}	0.35 ± 0.04^{b}	30.76 ± 0.66^{c}	-5.56 ± 3.49^{a}	10.80 ± 1.40^{c}
1:3	0	42.57 ± 7.18^{b}	0.73 ± 0.13^{a}	0.21 ± 0.07^{c}	30.33 ± 0.63^{a}	-1.07 ± 0.72^{a}	6.44 ± 2.01^{c}
	0.3	43.29 ± 0.95^{a}	0.76 ± 0.06^{a}	0.36 ± 0.07^{a}	31.70 ± 1.42^{a}	-1.52 ± 0.94^{ab}	11.45 ± 2.49^{a}
	0.5	43.18 ± 0.58^{a}	0.70 ± 0.04^{a}	0.30 ± 0.01^{ab}	30.38 ± 2.08^{a}	-3.97 ±2.4^{b}	9.08 ± 0.67^{b}
	1	41.17 ± 1.37^{c}	0.66 ± 0.02^{b}	0.31 ± 0.02^{ab}	28.53 ± 2.21^{b}	-2.62 ± 1.04^{ab}	8.92 ± 0.94^{b}
1:4	0	34.11 ± 6.48^{b}	0.66 ± 0.08^{ab}	0.23 ± 0.03^{a}	22.92 ± 6.84^{b}	-1.27 ± 0.77^{a}	5.21 ± 1.33^{b}
	0.3	39.41 ± 2.11^{a}	0.69 ± 0.01^{a}	0.23 ± 0.06^{a}	27.17 ± 1.81^{a}	-1.59 ± 0.64^{a}	6.19 ± 1.73^{a}
	0.5	38.98 ± 1.74^{a}	0.60 ± 0.08^{ab}	0.21 ± 0.04^{a}	23.26 ± 2.08^{b}	-1.38 ± 0.27^{a}	4.88 ± 0.68^{b}
	1	38.20 ± 1.29^{a}	0.54 ± 0.09^{bc}	0.16 ± 0.03^{b}	20.42 ± 2.85^{c}	-1.44 ± 1.82^{a}	3.26 ± 0.18^{c}

$^{a\text{-}b\text{-}c}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

4.8 Effect of temperature on almond milk gelation

The FFAMs were heated at different temperatures of 90º C, 95º C, and 100º C for 30 min and stored at 4º C for 2 h to form a gel. Figure 4.3 shows heat stability and gelation of FFAM at different temperatures. According to Devnani et al. (2020), the higher temperatures of 85-95º C induced more extensive denaturation and gelation in almond milk. Purified amandin, which is the major protein of almond, has a denaturation temperature of 62.5º C and protein aggregation observed at temperatures up to 90º C (Albillos et al., 2009). High thermal treatments being a more intense increase in the size of fat globules, due to the flocculation and coalescence phenomenon. Heat treatment (121º C/15 min) of almond milk caused an increment of particle size due to the formation of oil-droplet-protein body clusters, associated with protein denaturation. However, heat treatment (85º C/30 min) was mitigated by the greater stability of smaller fat globules which are less sensitive to the flocculation and coalescence phenomena than the higher heat treatment (121º C/15 min) (Bernat et al., 2015). The excessive temperature heating (100º C) of almond milk protein for denaturation leads to a state which does not set into a gel upon cooling in FFAM (1:4). The protein and total solid contents of FFAM (1:4) are less than that of (1:2) and (1:3).

Therefore, FFAM (1:4) cannot form a gel at high temperatures but FFAM (1:2) and (1:3) can.



FFAM gel 1:2

FFAM gel 1:3

FFAM gel 1:4

Figure 4.3 Gelation of FFAM at different temperatures

4.9 WHC and gel strength values of FFAM gels treated with different temperatures

Table 4.7 shows WHC and gel strength values of FFAM gels treated with different temperatures. Labro (2016) described as protein concentration and temperature appear to have an impact on the strength of the soy protein gels. Traditional heating methods influence gel texture mainly by affecting the denaturation and aggregation of proteins. Major proteins present in almond milk, amandin, can induce gelation at high temperatures of 85-95° C, owing to extensive protein denaturation (Devnani et al., 2020). The high temperature would make disulfide breakdown, and some unfolding residual protein structure generates, that would influence the properties of protein gels (Lu et al., 2010). The results indicated that FFAM gel (90° C) yields higher gel strength of 0.15, 0.04, and 0.03 N with high WHC of 95.58, 82.71, and 77.39 % in each ratio of FFAM. Therefore, FFAM gel at 100° C is the minimum gel strength value in other temperatures, which is not significantly (P≥ 0.05) different from the FFAM gels treated with 90° C and 95° C.

Table 4.7 Gel strength and WHC value of FFAM gels with different temperatures

FFAM gels (almond seeds: water)	**Temperature**	**Gel Strength (N)**	**WHC (%)**
1:2	90° C	0.15±0.04^{a}	95.58 ± 4.55^{a}
	95° C	0.14±0.04^{a}	95.48 ± 2.29^{a}
	100° C	0.11±0.04^{a}	90.27 ± 1.72^{b}
1:3	90° C	0.04 ± 0.03^{a}	82.71 ± 2.03^{a}
	95° C	0.03 ± 0.03^{a}	81.60 ±1.45 ^{a}
	100° C	0.02 ± 0.02^{a}	80.71 ± 0.34^{a}
1:4	90° C	0.03±0.00^{a}	77.39±1.22^{c}
	95° C	0.01 ± 0.01^{b}	75.46 ± 2.24^{a}

$^{a-b-c}$ Column values followed by the same superscript letter are not significantly different (P≥ 0.05). Means ± SD of triplicates.

No study on the effect of different temperatures on almond milk protein gelation. Heat treatments at temperatures above 80º C induced glycinin denaturation, which resulted in protein aggregation and network formation in soy proteins with protein concentration above 7.5 % (Beliciu and Moraru, 2011). The cross-linking of the protein network is required for protein gelation and is dependent on the protein concentration. Therefore, gel strength is extremely dependent on the concentration of protein. According to the results, the gel strength value of FFAM gel (1:3 and 1:4) was less than that value of FFAM gel (1:2) and significantly ($P < 0.05$) different.

Table 4.8 Texture profile analysis of FFAM gels treated with different temperatures

FFAM gels almond seeds: water	Temperature	Hardness (g)	Cohesiveness	Springiness (-)	Gumminess (g)	Adhesiveness	Chewiness
1:2	90° C	43.03 ± 8.66^{a}	0.79 ± 0.02^{a}	0.33 ± 0.03^{a}	34.01 ± 7.39^{a}	-5.67 ± 0.99^{b}	11.01 ± 1.93^{a}
	95° C	41.21 ± 0.96^{b}	0.64 ± 0.03^{b}	0.19 ± 0.1^{b}	26.33 ± 0.74^{ab}	-0.72 ± 0.56^{a}	4.96 ± 2.65^{b}
	100° C	36.21 ± 6.80^{c}	0.54 ± 0.03^{c}	0.14 ± 0.03^{b}	19.36 ± 2.71^{b}	-0.96 ± 0.54^{a}	2.69 ± 0.96^{b}
1:3	90° C	42.57 ± 7.18^{a}	0.73 ± 0.13^{a}	0.21 ± 0.07^{a}	30.33 ± 0.63^{a}	-1.07 ± 0.72^{a}	6.44 ± 2.01^{a}
	95° C	27.04 ± 1.76^{b}	0.64 ± 0.03^{b}	0.11 ± 0.03^{b}	17.32 ± 1.63^{b}	-0.25 ± 0.14^{a}	1.81 ± 0.42^{b}
	100° C	25.52 ± 1.30^{b}	0.60 ± 0.01^{c}	0.10 ± 0.04^{b}	15.20 ± 0.93^{b}	-0.20 ± 0.06^{a}	1.54 ± 0.55^{b}
1:4	90° C	34.11 ± 6.48^{a}	0.66 ± 0.08^{a}	0.23 ± 0.03^{a}	22.92 ± 6.84^{a}	-1.27 ± 0.77^{b}	5.21 ± 1.33^{a}
	95° C	21.72 ± 1.79^{b}	0.53 ± 0.04^{b}	0.10 ± 0.03^{b}	11.45 ± 0.40^{b}	-0.60 ± 0.31^{ab}	1.19 ± 0.37^{b}

$^{a\text{-}b\text{-}c}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

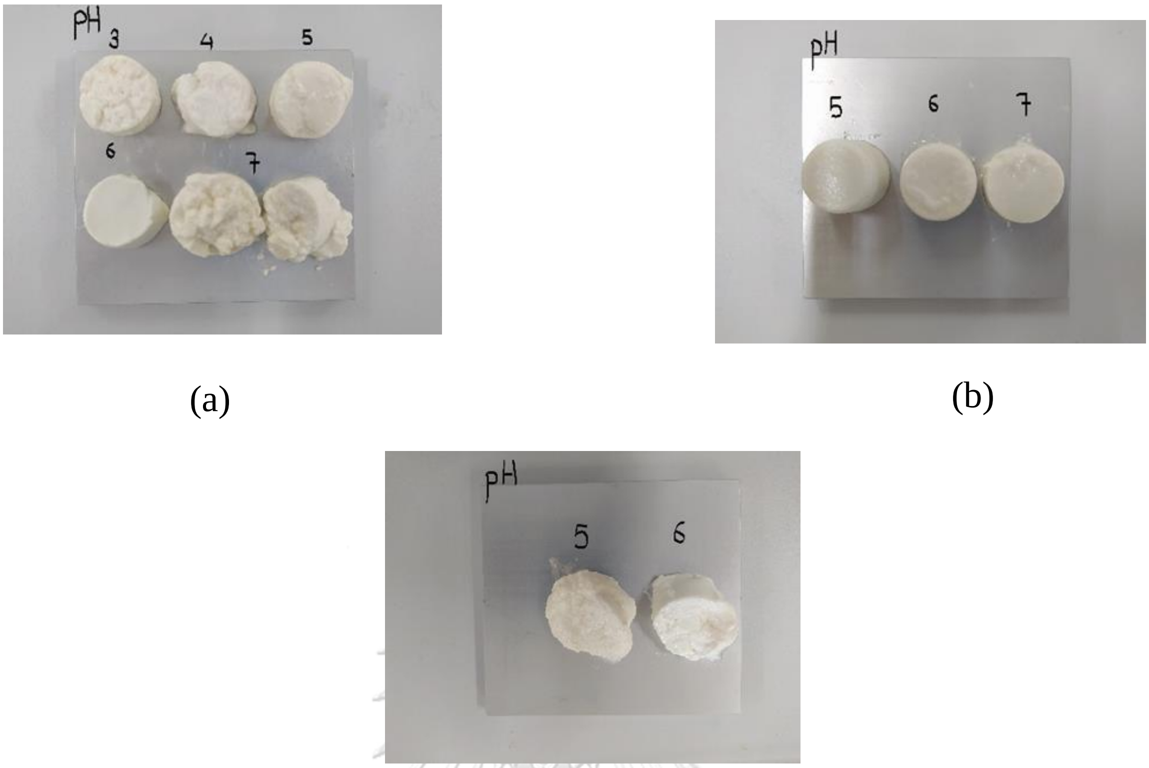
4.10 Texture profile of FFAM gels treated with different temperatures

Thermal treatment affects the unfolding and appears to influence the gel formation of amandin protein. Excessive temperature heating (100º C) of almond milk protein for denaturation leads to a state which does not set into a gel upon cooling in FFAM (1:4). Table 4.8 shows the texture profile analysis of FFAM gels treated with different temperatures. FFAM gel ratio at 90º C resulted in a greater hardness, cohesiveness, and gumminess than other ratios of FFAM gels at 95 and 100º C. According to the results, FFAM gels decreased in hardness when the high temperature was applied. The changes in the texture profile of FFAM gels occurring at high temperatures of 95º C and 100º C due to higher that needed for denaturation and may be related to the elimination of disulfide bonds and scission of peptide bonds.

For combination factors, the heating temperature of 90º C has been selected because that temperature can form a gel in each ratio of FFAM gels.

4.11 Effect of pH on FFAM gels

The FFAM (1:2, 1:3, and 1:4) samples with different pH were formed protein gelation as shown in Figure 4.4.



(a)

(b)

(c)

Figure 4.4 FFAM gels with different pH conditions (a) FFAM ratio 1:2 (b) FFAM ratio 1:3 (c) FFAM gel 1:4

The gelling properties of the protein, i.e., the type and number of protein cross-links during gelation are influenced by the pH. The major storage globulin, similar to the 11-12S legumin, in almonds is known as amandin (Kshirsagar et al., 2011). Amandin is the major protein present in almond milk, which shares a hexametric structure, and constitutes 70% of the total soluble proteins (Albillos et al., 2009). Almond protein has an isoelectric pH (pI) range of 4.5-5.5 (Li and He, 2004). A lower concentration of protein containing 10 % of the carob protein isolate did not produce any gel structure after thermal treatment at any pH value as described by Bengoechea et al. (2017). It was also specified that carob proteins presented a higher denaturation temperature and became more denatured at acid pH (pH 2) than at alkaline pH (pH 10). The ability of soy protein concentrations to form a gel when heating is generally considered an important functional property (Jacoba et al., 2000). A condition during gel formation in food products varies greatly due to variations in pH that can be affected the properties of the formed gel.

According to this study, FFAM 1:4 cannot form a gel at pH 3,4, and 7 due to the composition of almond milk. FFAM 1:3 can form a gel at pH 5, 6, and 7. Sharma et al. (2010) indicated that almond protein isolate (protein concentration 6%) induced gelation of almond proteins at pH 7 with high temperature. In this study, only for FFAM (1:2) thermally treated gels can be obtained at any pH (3, 4, 5, 6, and 7). Chen et al. (2016) studied that soy protein isolate (94 % protein) increasing aggregation and gelation rate at pH 5.9 with increasing protein concentration. Therefore, the different compositions of almond milk and protein concentrations can directly influence the properties of those gels formed. Charge distribution among the amino acid side chains is altered by pH and the ionic strength of the protein environment. The isoelectric point of the almond major protein, amandin, is in the range of 4.5-5.5. Gels formed at the isoelectric pH of proteins are less hydrated and less firm because of the lack of repulsive forces (Bengoechea et al., 2017). The type and stability of the gel structure are significantly affected by the net charge of the protein. The structure of gels will change from an aggregated to an ordered strand structure as the result of increased repulsive forces between molecules and suppression of the random aggregation (Totosaus et al., 2002).

4.12 WHC and gel strength values of FFAM gels with different pH values

The water holding capacity (WHC) of the gels formed by FFAM in this study, as measured by centrifugation. As shown in Table 4.9, FFAM gel (1:2) at pH 6 has a significantly ($P < 0.05$) higher WHC (99.43± 0.43 %) value than other pH values of FFAM gels, indicating that gel of pH 6 had a stronger binding ability to water. FFAM (1:3) gels at pH 5, 6, and 7 showed values for WHC of 95.12 ± 0.51, 98.29 ± 0.52, and 93.65 ± 4.53 %, respectively. FFAM (1:4) gel at pH 6 value was higher in WHC value (96.54 ± 0.93%) than pH 5. Thus, a much stronger gel was formed, and more water was trapped in the gel. The higher values found at pH 6 might be related to crosslinking reactions that take place when disulfide bonds are formed (Bengoechea et al., 2017). In addition, it was found that

FFAM (1:3 and 1:4) did not form gels at pH (3, 4, and 7). This may be because the composition of almond milk and protein concentrations were larger than that of other pH values (Li et al., 2020). A relatively rough gel structure was formed after heating, thus less water was trapped and the WHC was lowered (Li et al., 2020). The maximum WHC values of FFAM gels obtained at pH 6 in each ratio of FFAM, but the WHC values of FFAM gels (without adjusted pH) were significantly ($P<0.05$) increased with each ratio of FFAM gels in pH 6.

Table 4.9 Gel strength and WHC value of FFAM gels with different pH conditions

FFAM gels almond seeds: water	pH	Gel Strength (N)	WHC (%)
1:2	Without adjusted pH	0.18 ± 0.03^{a}	95.44 ± 2.64^{b}
	3	0.09 ± 0.00^{b}	94.16 ± 0.91^{b}
	4	0.05 ± 0.01^{c}	97.85 ± 1.91^{a}
	5	0.18 ± 0.02^{a}	99.19 ± 0.63^{a}
	6	0.18 ± 0.01^{a}	99.43 ± 0.43^{a}
	7	0.04 ± 0.02^{c}	91.91 ± 2.62^{c}
1:3	Without adjusted pH	0.04 ± 0.03^{b}	82.71 ± 2.03^{c}
	5	0.02 ± 0.02^{c}	95.12 ± 0.51^{a}
	6	0.08 ± 0.01^{a}	98.29 ± 0.52^{a}
	7	0.02 ± 0.00^{c}	93.65 ± 4.53^{b}
1:4	Without adjusted pH	0.03 ± 0.00^{a}	77.39 ± 1.22^{b}
	5	0.01 ± 0.01^{b}	95.57 ± 2.38^{a}
	6	0.01 ± 0.01^{b}	96.54 ± 0.93^{a}

$^{a\text{-}b\text{-}c}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

Table 4.9 shows the values of gel strength in FFAM gels treated with different pH values. The net charge of protein at its isoelectric point is equal to zero. However, the greater the net charge on the protein molecule, the greater the electrostatic repulsion between molecules, preventing the interactions required to form a gel (Totosaus et al., 2002). In acid conditions, pH reduction forms cluster aggregation in a sufficient protein-ordered structure. However, the cross-linking of protein molecules, of initial size distribution, is required for gelation and is proportional to the protein concentration. The results demonstrated that FFAM (1:2) can only obtain the gels at each pH value (3, 4, 5, 6, and 7). Therefore, gel strength and deformability of protein are highly dependent upon protein concentration. The maximum gel strength value was obtained at pH 6 in each FFAM, which may be related to crosslinking reactions that take place when disulfide bonds are formed. This performance may be involved to its proximity to the protein isoelectric point, at which protein molecules are lightly more aggregated. Compared with the FFAM gels (without adjusted pH), the gel strength value of FFAM gel (1:2) is the same (0.18) value in pH 6, and that value of FFAM gel (1:3) was significantly ($P<0.05$) increased. However, FFAM gel (1:4) (without adjusted pH) resulted in a greater gel strength value than pH 6. Based on the results, FFAM appears to form a gel without pH adjustment.

Table 4.10 Texture profile analysis of FFAM gels with different pH conditions

FFAM gels almond seeds: water	pH	Hardness (g)	Cohesiveness	Springiness (-)	Gumminess (g)	Adhesiveness	Chewiness
1:2	Without adjusted pH	43.03 ± 8.66^{a}	0.79 ± 0.02^{b}	0.33 ± 0.03^{a}	34.01 ± 7.39^{a}	-5.67 ± 0.99^{c}	11.01 ± 1.93^{a}
	3	23.92 ± 4.40^{c}	0.61 ± 0.14^{c}	0.21 ± 0.04^{b}	14.43 ± 3.64^{d}	-1.87 ± 0.63^{b}	3.14 ± 1.22^{c}
	4	18.74 ± 1.14^{d}	0.45 ± 0.05^{d}	0.09 ± 0.04^{c}	8.46 ± 0.92^{e}	-0.92 ± 0.67^{a}	0.75 ± 0.35^{de}
	5	22.12 ± 3.68^{c}	0.86 ± 0.06^{a}	0.09 ± 0.02^{c}	19.08 ± 3.79^{c}	-0.45 ± 0.22^{a}	1.72 ± 0.56^{d}
	6	37.51 ± 9.40^{b}	0.88 ± 0.10^{a}	0.20 ± 0.03^{b}	23.55 ± 5.12^{b}	-0.96 ± 0.41^{a}	4.77 ± 1.01^{b}
	7	15.48 ± 1.40^{d}	0.63 ± 0.01^{c}	0.12 ± 0.02^{c}	9.72 ± 0.79^{e}	-0.87 ± 0.38^{a}	1.13 ± 0.19^{d}
1:3	Without adjusted pH	42.57 ± 7.18^{a}	0.73 ± 0.13^{a}	0.21 ± 0.07^{a}	30.33 ± 0.63^{a}	-1.07 ± 0.72^{b}	6.44 ± 2.01^{a}
	5	18.82 ± 2.84^{c}	0.73 ± 0.13^{a}	0.10 ± 0.04^{ab}	13.54 ± 1.21^{c}	-0.44 ± 0.12^{a}	1.09 ± 0.16^{c}
	6	33.17 ± 5.11^{b}	0.74 ± 0.11^{a}	0.11 ± 0.03^{ab}	24.27 ± 3.95^{b}	-1.03 ± 0.66^{b}	2.52 ± 0.90^{b}
	7	13.37 ± 1.92^{c}	0.68 ± 0.24^{b}	0.05 ± 0.01^{b}	8.80 ± 1.95^{d}	-0.63 ± 0.52^{a}	0.42 ± 0.2^{c}
1:4	Without adjusted pH	34.11 ± 6.48^{a}	0.66 ± 0.08^{a}	0.23 ± 0.03^{a}	22.92 ± 6.84^{a}	-1.27 ± 0.77^{b}	5.21 ± 1.33^{a}
	5	14.54 ± 0.70^{b}	0.57 ± 0.22^{a}	0.04 ± 0.02^{b}	8.37 ± 3.49^{b}	-0.35 ± 0.28^{a}	0.37 ± 0.28^{b}
	6	17.16 ± 1.47^{b}	0.63 ± 0.26^{a}	0.07 ± 0.02^{b}	10.83 ± 4.95^{b}	-0.30 ± 0.13^{a}	0.80 ± 0.60^{b}

$^{a-b-c-d-e}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

4.13 Texture profile analysis of FFAM gels with different pH conditions

Texture parameters of the FFAM gels studied were found to be dependent on pH as shown in Table 4.10. According to the results, the greatest values of hardness for FFAM gels in each ratio were observed at pH 6. There is no research study on the effect of calcium and pH on almond protein gelation, including the composition of protein, fat and total solids resulting from the gel formation on different pH values. At pH 6, protein is electrostatically charged, which would favor the unfolding of the protein chains, due to the electrostatic repulsions (Bengoechea et al., 2017). Further, carob gels obtained at pH 6 displayed higher cohesiveness than those obtained at extreme pH values as described by Bengoechea et al. (2017). Each ratio of FFAM gels at pH 6 showed the highest values of hardness, cohesiveness, springiness, gumminess, and chewiness in this study.

Each FFAM ratio can form a gel structure at pH 6, therefore, pH 6 was chosen for the study of combination factors.

4.14 The study of combination factors on FFAM gel

The combination of factors (protein and calcium concentration, pH, and temperature) resulted in a better understanding of the gelation mechanism and how different affect the final gel product. The use of more than one gelation mode is another important process possibility that can be applied in the food industry. Figure 4.5 shows the FFAM gel with combination factors of FFAM (1:3) with 0.3% $CaCl_2$, pH 6, and temperature of 90° C.

(a) FFAM gels (combination factors)

(b) FFAM gel (1:3)

(c) FFAM gel (1:3 with $CaCl_2$ 0.3%)

(d) FFAM gel (1:3 with pH 6)

Figure 4.5 FFAM gel with (a) combination factors, (b) FFAM gel 1:3, (c) FFAM gel 1:3 with $CaCl_2$ 0.3%, and (d) FFAM gel 1:3 with pH 6

4.15 Texture profile analysis of FFAM gel with combination factors

Table 4.11 shows the TPA results of FFAM gel with combination factors and FFAM gel (1:3) with other factors. Compared with the control, FFAM gel (combination factors and 1:3 + 0.3% $CaCl_2$) were significantly ($P< 0.05$) improved the hardness of the gel. The maximum hardness of 43.21 g and 43.29 g in FFAM gel were obtained with the combination factors and addition of $CaCl_2$ (0.3%), respectively, while that of control was 42.57 g. The cohesiveness and gumminess values showed similar trends as hardness. According to the results, FFAM gel (1:3+0.3% $CaCl_2$) resulted in the maximum value of hardness, due to the formation of salt bridges increases ion bonds, thus increasing the gel hardness. FFAM gel (pH 6) obtained the lowest value in hardness, therefore, full-fat almond proteins may offer gel structure without adjusting the pH. In comparison with FFAM gels (control, 1:3+0.3% $CaCl_2$, and combination factors), FFAM (1:3+0.3% $CaCl_2$) form a gel with a maximum hardness, and the addition of calcium chloride salts could improve the texture profile and set up the possibility for quality improvement.

Table 4.11 Texture profile analysis of FFAM gels with combination factors

FFAM gel	Hardness (g)	Cohesiveness	Springiness (-)	Gumminess (g)	Adhesiveness	Chewiness
Ratio 1:3(Control)	42.57±7.18^{b}	0.73±0.13^{b}	0.21±0.07^{b}	30.34±0.63^{b}	-1.07±0.72^{a}	6.44±2.01^{b}
Ratio 1:3 + 0.3% $CaCl_2$	43.29±0.95^{a}	0.76±0.06^{b}	0.36±0.07^{a}	31.70±1.42^{b}	-1.52±0.94^{a}	11.45±2.49^{a}
Ratio 1:3 + pH 6	33.17±5.11^{c}	0.74±0.11^{b}	0.11±0.03^{c}	24.27±3.95^{c}	-1.03±0.66^{a}	2.52±0.90^{d}
Ratio 1:3 + 0.3% $CaCl_2$ + pH6 (Combination)	43.21±1.83^{a}	0.91±0.06^{a}	0.12±0.00^{c}	39.42±3.63^{a}	-0.29±0.05^{a}	4.71±0.50^{bc}

$^{a-b-c-d}$ Column values followed by the same superscript letter are not significantly different (P≥0.05).

Means ± SD of triplicates.

4.16 Gel strength and WHC values of FFAM gel with combination factors

Table 4.12 shows the gel strength and WHC values of FFAM gel with combination factors and FFAM gel (1:3) with other factors. The maximum gel strength value of 0.19 N was obtained with the FFAM gel with combination factors. The FFAM gels (combination factors and 0.3% $CaCl_2$) were significantly ($P< 0.05$) increased the gel strength of the gel with the control and pH 6 FFAM gels.

Maximum WHC of 98.29%, 97.97%, and 96.45% were obtained with the FFAM gels (pH 6, addition of 0.3% $CaCl_2$, and combination factors), respectively, while the WHC of the control was 82.71%. This result suggested that FFAM gel (control) has a fewer network to trap water molecules, due to softer gel structure with minimum gel strength value. FFAM gels with other factors resulted in protein gels with homogenous fine structures that gave high water holding capacity.

Table 4.12 Gel strength and WHC values of FFAM gel with combination factors

FFAM Gel	Gel Strength (N)	WHC (%)
Ratio 1:3 (Control)	0.04 ± 0.03^b	82.71 ± 2.03^b
Ratio 1:3 + 0.3% $CaCl_2$	0.15 ± 0.01^a	97.97 ± 1.81^a
Ratio 1:3 + pH 6	0.08 ± 0.01^b	98.29 ± 0.52^a
Ratio 1:3 + 0.3% $CaCl_2$ + pH6 (Combination)	0.19 ± 0.02^a	96.45 ± 0.51^a

$^{a-b}$ Column values followed by the same superscript letter are not significantly different ($P \geq 0.05$). Means ± SD of triplicates.

4.17 Rheological property of FFAM combination gel

Figure 4.6 illustrates the result of the rheological behavior of almond milk gelation in combination factors.

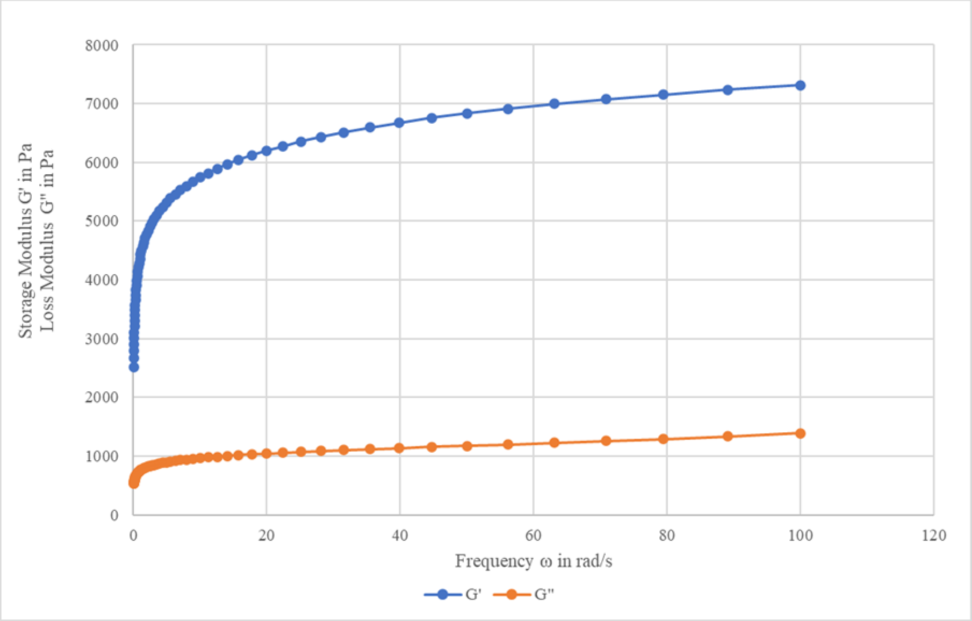


Figure 4.6 Mechanical spectra for FFAM gel at combination factors (ratio 1:3, pH 6, 0.3 % $CaCl_2$ and 90°C)

Storage modulus, G’, was higher than the loss modulus, G”, either before or after the thermal treatment, displaying the same tendency in the frequency range that was studied (0.1-100 rad/s). Frequency sweep can be used to indicate the gel type (Zhao et al., 2020). Figure 4.6 shows that G’ and G” slightly increased with the increasing frequency, indicating the formation of a continuous network and typical physical gel structure. In this study, FFAM gel prepared with combination factors showed a high G’ value (4359.4 Pa) at 1 Hz frequency and (7312.8 Pa) at 100 Hz frequency. Zhao et al. (2020) reported that acid-induced tofu (protein concentration 6.06 % and pH 6.6) with the addition of 0.05 g/100 ml $CaCl_2$ reached 3195.90 Pa at 1 Hz frequency and control was 2233.54 Pa. Therefore, the addition of calcium chloride salts can modify the water structure and arrange charge and polar groups, thus affecting electrostatic and hydrophobic interactions. The high G’ value in FFAM gel with combination factors induced in the presence of calcium chloride salt might have resulted from an increase in these chemical interactions in the almond milk protein system.

CHAPTER 5

CONCLUSION

5.1 Summary and conclusion

This study shows that protein, and calcium chloride concentration, pH, and temperature can affect the texture property, gel strength, and WHC on heat-induced almond (*Prunus dulcis*) milk gelation. Firstly, the gelling properties of heat-induced FFAM were investigated over the ratio of almond seeds and water (1:2, 1:3, 1:4, and 1:5 (w/v)) and processing temperatures (90º C). FFAM gels showed a lower texture profile and gel strength values at a lower protein concentration (4.07%) and composition of FFAM (1:4). However, at a high protein concentration of FFAM (1:2), texture profile and gel strength significantly improved due to their high composition of total solids. The results indicate that the higher protein concentration, fat, and total solid contents in full-fat almond milk caused an increased degree of cross-linkage of the heat-induced FFAM gel network formation. Interactions of protein aggregate with the other components, such as fat, carbohydrate, and total solids in full-fat almond milk act as a filler particle in the protein matrix in gel formation due to the heterogeneous distribution of fat.

The addition of calcium chloride salts into FFAM also increased protein-protein and protein-solvent interactions, leading to the formation of a denser network. However, the addition of $CaCl_2$ (1%) decreased hardness and gel strength in each ratio of FFAM gels. FFAM gels with the addition of $CaCl_2$ (0.3%) resulted in maximum hardness and gel strength value with high water holding capacity because the excessive calcium chloride ions led to strong protein-protein interactions and the formation of large aggregates, which can be resulted in the reduction of texture profile and gel strength in FFAM gels. A change in different pH values was investigated the heat-induced gelling property of FFAM. The isoelectric point of almond protein is in the range of 4.5-5.5 and the pH of almond milk is 5.9. Only for a high protein concentration of FFAM (1:2) thermally treated gels could be obtained at any pH (3, 4, 5, 6, and 7) values. Therefore, the minimum protein concentration of almond milk (~5%) is the major factor in the formation of almond milk gels obtained by heat. In addition, FFAM gels formation is favored at pH 6 in each ratio of FFAM (1:2, 1:3, and 1:4). It could be stated that almond proteins show up to be possible hard-set gels without pH adjustment. The adjustment of pH to alkali was needed to form a gel for a lower protein concentration of almond milk protein.

Heat-induced protein denaturation is also an important factor for protein-protein associations during the gelation so that temperature shows potential relation to the gel strength and hardness in FFAM gels. Protein gelation has been traditionally achieved by heating, but the changes in texture profile and gel strength of almond milk gel occurring at high temperatures of 90ºC, 95ºC, and 100ºC in each ratio of FFAM. High-temperature heating (100º C) of the FFAM (1:4) leads to a state that does not set into a gel upon cooling that higher needed denaturation. On the other hand, FFAM (1:4) cannot set up a gel structure with a low concentration of protein. In contrast, heat-induced gelation of almond milk protein is highly dependent upon protein concentration and composition of almond milk.

According to this study, the effect of protein and calcium chloride concentration, pH, and temperature can be influenced the gelling property of almond milk proteins. The combination of these factors (1:3+ 0.3% $CaCl_2$+ pH 6+ 90ºC) resulted differently the gelling property of full-fat almond milk and this understanding can be applied to plant-based proteins in the food industry in the future.

5.2 Suggestion for future work

This information is the fundamental understanding of almond milk protein gelation for the next step, for plant-based product food application development. It needed to more investigate the gelling mechanism of heat-induced almond protein and explore the lower protein concentration of FFAM (1:4+0.3% $CaCl_2$) at the alkali pH value. Consequently, statistical analysis should be conducted to study combined factors by using response surface methodology (RSM). More research is required on the effect of commercialized pasteurization conditions on almond milk protein for applying commercial products.

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Appendix A
Chemical Analysis Method

1. Preparation of calcium chloride solution

1.1 $CaCl_2.2H_2O$ (0.3 %) solution

23.5 mM/100 ml of almond milk

34.5 g of $CaCl_2.2H_2O$ was dissolved in 100 ml of distilled water and then, 10 ml of the solution to 100 ml of FFAM milk

1.2 $CaCl_2.2H_2O$ (0.5 %) solution

35.4 mM/100 ml of almond milk

52.0 g of $CaCl_2.2H_2O$ was dissolved in 100 ml of distilled water and then, 10 ml of the solution to 100 ml of FFAM milk

1.3 $CaCl_2.2H_2O$ (1 %) solution

70.7 mM/100 ml of almond milk

104.0 g of $CaCl_2.2H_2O$ was dissolved in 100 ml of distilled water and then, 10 ml of the solution to 100 ml of FFAM milk

2. Preparation of 1 N HCl solution

83 ml of concentrated hydrochloric acid mixed with 700 ml of distilled water and allowed to cool it. And made up the volume of 1000 ml with distilled water.

3. Preparation of 1N NaOH solution

40 gram of sodium hydroxide dissolved with 700 ml of distilled water and allowed to cool at room temperature. And made up the volume of 1000 ml with distilled water.

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