Research Article

Effects of Enzyme Treatment and Carrier Agents on Chemical and Physical Properties of Almond Protein-Based Product

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Abstract
There has been an emerging trend towards the research of plant-based proteins over the past few years; however, there has been limited conductive observation of plant-based proteins from almond by-products, as well as the potential of using Flavourzyme and different carrier agents to obtain an instant protein-based powder. The purpose of this study was to investigate the effects of Flavourzyme at various concentrations (0, 0.5, 1, 1.5, 2, and 2.5%) and incubation times (30, 60, 90, 120, and 180 min), as well as carrier agents at different ratios with a 20% total concentration on the physicochemical properties of almond protein-based products using freeze-drying (FD). The results showed a higher protein content (p-value ≤ 0.05) using 1.5% Flavourzyme for 120 min compared to the other levels. There was no significant difference (p-value > 0.05) in the protein retention rate after freeze-drying of the samples. However, the addition of Maltodextrin (MA), Gum Arabic (GA), and Inulin (IN) encapsulants improved the physical and functional characteristics of freeze-dried almond protein-based powder (FDAP). However, the solubility of the powder is moderate. Water-holding-capacity (WHC) and Oil-Holding Capacity (OHC) are inversely proportional, where GA-coated powder is the most hydrophobic (17.72 ± 0.87 mL oil/g) and IN-encapsulated powder is the most hydrophilic (15.00 ± 0.87 mL water/g). In conclusion, IN could be a potential encapsulant for almond protein-based powder, because powder produced from IN or MA: IN is acceptable in terms of physical parameters, and IN can also enhance fiber content in the final product.

Keywords: Almond (Prunus *Amygdalus*), Encapsulation, Enzymatic treatment, Flavourzyme, Freeze-drying

1 Introduction

The almond belonged to the group of genus Prunus and subgenus *Amygdalus* (*Rosaceae*, subfamily *Prunoideae*). Being originated in West Asia, almond is being commercially cultivated in many countries by the wellness they brings [1], [2] Almond seeds which can be called nuts contain a myriad of valuable components from lipids, proteins, and even minerals. Papademetriou and Herath [3] found the protein content in almonds to be 19–21%, the fat content of 44–46% mostly unsaturated fat, carbohydrates to be 23–25% and other considerable amounts of minerals. The potential of almonds also lies in their richness in unsaturated fatty acids, mostly linoleic and oleic acids [4]. MUFA accounts for the majority of 67% [5]. The importance of unsaturated fatty acids in mitigating cardiovascular disease, cancer, and postprandial glycemia has been proven [6], [7]. Therefore, almonds were extracted to obtain lipids from the inner kernel. Subsequently, a large number of almonds will go unused when the extraction process is completed. As demand increases, it is worth mentioning the parallel establishment of almond waste can be potentially harmful to the environment if its management is improperly regulated [8]. Therefore, waste management should be carefully considered so that the leftovers are still rich in other macronutrients, such as protein and fiber, since the extraction of oil only focuses on lipid content. In other words, protein can be obtained as a

by-product and transferred into a nutritional drink. In recent years, the demand for protein has increased even more when the world population is increasing and there is a preference for eating protein in a diet [9]. In addition, clinical trials have shown positive results in treating obesity and cardiovascular diseases by designing meals containing almond protein for panelists [10]. Other experiments have found the same result when providing panelists with nuts; the chances of having diabetes lowered by approximately 12% [11], and 23% for cancer [12]. Therefore, it is important to extract almond protein from oil waste in order to obtain the protein value of this nut.

Flavorzymes are one of the most novel enzymes that have received great attention in the scientific field. This enzyme is obtained from *Aspergillus oryzae*, a peptidase that is currently used in the food industry [13]. Flavourzyme is widely used to hydrolyze many types of proteins and has been proven to be significant [14]. This type of enzyme contains mostly exopeptidases with a minority of endopeptidases, and the pH range may vary from pH 4.0 to 8.0 with an optimum temperature range of 30–65 °C (Novozymes. (2001). Product sheet Flavourzyme). Recently, the plant-based protein has been widely recognized for its value; Flavourzyme has received great attention more than ever to aid the processing of this protein. Flavourzyme plays a significant role in hydrolyzing large polypeptides into amino acids in many plant proteins, such as oats [15], soybeans [16], and chia seeds [17]. Hence, Flavourzyme could be used to enhance the proteolysis of another plant protein, almonds.

Drying is another method of increasing the stability of proteins after being liberated by enzymolysis [18]. There are several drying methods, such as spray drying, sun drying, oven drying, and freeze-drying [19]. Although the cost of manufacturing freeze-drying is higher than that of other dehydration methods [20], it is said to save most of the nutrients from plant-based materials after drying because the loss rate of freeze-drying is low because there is no exposure to heat for dehydration [21]. For this reason, freeze-drying is widely used to protect and encapsulate bioactive compounds, such as bioactive peptides, as well as for appearance, such as color, texture, and flavor, compensating for the high cost of equipment.

Currently, with the advancement of technology, several methods have been applied to protect food materials from deterioration, including decreasing water activity, applying preservatives, reducing pH, and increasing temperature [22]. One of the most modern methods for physically transforming food and preserving it stably is encapsulation. Encapsulation technology has been widely used to protect and create food barriers against external factors such as dust, light, and oxygen [23]. In the current market, there are several encapsulated products present in powder form, such as fruit and flavoring powder, which are beneficial to health. Encapsulation has also been widely used to obtain milk protein powder for instant usage and additional industrial purposes [24], coconut milk encapsulation [25], maintenance of the essential amino acid profile, and antioxidant components of whey powder [26], [27].

Dehydration of pomegranate juice enhances the stability of anti-inflammatory properties this fruit brings [28]. In other studies, the extraction of chokeberry pomace could be maintained to improve glucose metabolism and obesity treatment by making powder [29]. Hence, it should also be considered to make use of this advanced technology on almonds because the drying of powder might cause aggregation of particles, due to the presence of sugar inside almonds and nuts in general [30]. Therefore, the addition of carrier agents may reduce the stickiness. Moreover, the use of carrier agents can also increase the encapsulation of bioactive, heat-sensitive peptides in powder, avoid unfavorable conditions under ambient temperature, and reduce nutrient loss and instability of the powder [31]. The choice of carrier agents is diverse and MA, GA, and polymers such as starch, and pectin are frequently used as carrier agents [32]–[35]. However, using only one carrier agent is not as efficient as expected because there are advantages and disadvantages in cost and properties, with no carrier agent possessing all requirements [35]. Therefore, many combinations of carrier agents should be used to obtain the highest powder quality [36].

MA has always been considered an effective carrier agent for drying because of its efficient cost and satisfactory performance [37], [38]. MA is efficient in protecting and coating bioactive compounds from oxidation and degradation during storage [39]. Moreover, MA is used as wall material because of its low HY, and also by its high water solubility [34], [40]. For casein encapsulation, MA was effective as a carrier agent for

high solubility and reduction of HY, and efficiently stabilized and improved the functional properties [41]. MA also works quite effectively in combination with other carrier agents such as GA for chicken meat protein. Moisture content and water activity show a declining trend with the increasing enhancement of carrier agents to 30%, while the HY shows a strong reduction from 40% to 12% [42]. Therefore, MA can be applied to almond proteins to stabilize this powder.

IN can be found in wheat, barley, garlic, bananas, artichokes, etc. [43]–[45]. IN is a healthy food ingredient that promotes health benefits, as it is also seen as a prebiotic, and the structure of IN stays unchanged during the digestion process, which will further be fermented by gut bacteria [46]. Industrially, IN has potential for use in many drying processes [47]. According to Wang *et al.* [48], when comparing seven uses of carrier agents in the encapsulation of lutein, including trehalose, IN, sucrose, modified starch, MA, and three variations of dextrose equivalents, the results suggested that IN is the most suitable polysaccharide for encapsulating bioactive materials. Wang *et al.* [48] also reported IN as a possible encapsulation material, with a high retention rate of the powder. In another study, IN was also reported to be compatible with other carrier agents such as corn syrup solids, which resulted in positive data on the stability of the core material samples [49]. Similarly, Lacerda *et al.* [50] reported the great result of either combining or separating IN with MA to protect anthocyanins from Jussara pulp. Even though IN is still novel in the encapsulation of proteins, it is worth mentioning the successful effects of some other sensitive compounds; therefore, it could be transformed to encapsulate proteins in almonds.

GA is an edible polysaccharide that can be extracted from the Acacia tree, which is seen as an extrudate. The GA content is high in carbohydrates, accounting for 97%, and the remaining proportion is protein at 3% [51], [52]. From a nutritional perspective, GA is beneficial as a prebiotic for gastrointestinal conditions [42]. In addition, owing to its low viscosity, odorlessness, and tasteless nature, GA has been widely used in the food industry for the benefit of encapsulation [53], [54]. Based on a study by Subtil *et al.* [55], GA is powerful in providing low-water-content microcapsules and lessening the bitter taste by liberating protein chains. GA has been used to encapsulate several bioactive compounds in plants. In the extraction of citron, in the combination of MA and modified starch and GA, the microcapsules show high bulk density (BD), low porosity, and high retention of efficiency up to 87.2% [56]. In protein hydrolysate, GA is combined with MA for the encapsulation of chicken protein hydrolysate, and HY has been proposed to be reduced from 40% to 21% [57]. In recent years, GA has also been combined with MA to obtain coconut protein powder in spray drying [58] and whey protein isolate [59].

Similarly, protein casein hydrolysates with the same combination, also work effectively to encapsulate proteins and lower the bitter taste [42]. This indicates that the GA can cooperate well with the other agents to obtain the best results. GA can be used to encapsulate almond proteins.

Therefore, it is worth considering experimentation in this project to determine the impact of Flavourzyme concentration and incubation time on the protein content of the almond-defatted powder. Moreover, it is critical to understand how both conventional carrier agents, such as MA and GA, and their combination with novel encapsulants, such as IN, can impact the physical and functional properties of FD almond protein-based powders. Since protein from almond by-products has potential for application, it requires more scientific attention. This provides an opportunity to convert this nutrient-rich material into a useful product and reduce industrial food waste.

2 Materials and Methods

2.1 *Materials and chemicals*

Defatted ground almonds from the OFI company were stored and sealed aseptically with no contact with humidity or moisture until analysis commenced. Flavourzyme is an aminopeptidase from *Aspergillus Oryzae* purchased in liquid form from Novozymes, with declared activity (1000 LPU/mL), optimum temperature (30–65 °C), and pH (4–8). Folin reagent was purchased from Merck company. Albumin, Na_2CO_3, NaOH, $CuSO_4.5H_2O$, $Na3C_6H_5O_7$, and NaCl were purchased from Sigma – Aldrich Co. MA (DE 12), GA, and IN were purchased from Roquette and ACROS Organics, respectively. All the chemicals and reagents used were of analytical grade.

2.2 *Sample preparation*

All juice extractions were performed directly using dry almond powder dissolved in distilled water at a ratio of 1:3 with different concentrations of enzymes (0, 0.5, 1, 1.5, 2, and 2.5%), incubated for different durations (30–180 min), and then deactivating enzymes at 90 °C for 5 min. After treatment with Flavourzyme, the almond juice extract was centrifuged at 7500 rpm for 15 min at 4 °C with some modifications [60]. The supernatant collected was mixed with different carrier agents in a total of 20% (w/w) [27], at different carrier levels, as shown in Table 1. The solution containing GA and IN was heated to 55 °C because IN and GA are difficult to dissolve at room temperature. The whole mixture was homogenized until it completely dissolved before storing at –40 °C, and dried at –40 °C using DAIHAN Scientific freeze-dryer, Korea.

2.3 *Effects of Flavourzyme enzyme concentrations and incubation time on the protein content of defatted almond powder*

2.3.1 Effects of Flavourzyme enzyme concentrations

Different enzyme concentrations of Flavorzyme were used to extract protein from defatted almond powder. Briefly, 0.5%, 1%, 1.5%, 2%, and 2.5% Flavourzyme was added to a mixture of almond powder and distilled water at a ratio of 1:3 (w/v). Control samples were treated without enzyme addition. Enzyme-assisted extraction was performed in a shaking incubator at 50 °C for 120 min and then heated to 90 °C for 10 min for enzyme deactivation [16].

2.3.2 Effects of Flavourzyme enzyme incubation time

Different enzyme incubation spans of Flavourzyme were used to assist in protein extraction from almond powder. Specifically, almond powder was mixed with distilled water at a powder-to-water ratio of 1:3 [16]. The whole mixture was incubated with the chosen concentrations for different durations of 30 min, 60 min, 90 min, 120 min, and 180 min. The enzymatic extraction was performed in a water bath at 50 °C. Subsequently, enzymes were inactivated at 90 °C for 5 min. The optimal extraction duration was determined in the next experiment.

Table 1: Ratio of MA, GA and IN as carrier agents

Carrier Agents	%MA	%GA	%IN	Overall Concentration (%, w/w)
Control	0.0	0.0	0.0	0.0
MA 20%	20.0	0.0	0.0	20.0
GA 20%	0.0	20.0	0.0	20.0
IN 20%	0.0	0.0	20.0	20.0
MA:GA (2:1)	13.3	6.7	0.0	20.0
MA:IN (2:1)	13.3	0.0	6.7	20.0
MA:GA:IN (2:0.5:0.5)	10.0	5.0	5.0	20.0

2.4 *Effects of different carrier agents on chemical, physical and functional properties of FDAP*

The final quality of the almond juice powder was determined using carrier agents. The formulation of carried agents added to the almond powder extract was fortified as follows with a total concentration of 20% compared to the control sample without the addition of carrier agents [27]. The mixtures of the almond protein hydrolysate were freeze-dried using a freeze-dryer (DAIHAN Scientific, Korea), and further examined for their physical properties.

2.5 *Analytical analysis*

2.5.1 Quantification of protein

Protein quantification was performed using the Lowry method [61] with some modifications. Reagent A was prepared by adding 2 g of Na_2CO_3 (Merck, Darmstadt, Germany) in 0.1M NaOH (Merck, Darmstadt, Germany) to make a 100 ml solution. Reagent B was prepared by dissolving 0.5 g $CuSO_4.5H_2O$ (Sigma-Aldrich Co., St. Louis, MO, USA) in 1% $Na3C_6H_5O_7$ (Sigma-Aldrich Co., St. Louis, MO, USA). Briefly, 0.4 mL of dilute protein samples was added to 2 mL of reagent C (a mixture of reagents A and B). After 10 minutes, the whole solution was added with 0.2 mL of the Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO, USA). After a 10 min interval, 2.4 mL of distilled water was added before measurement. A solution of Albumin 0.1% (Sigma-Aldrich Co., St. Louis, MO, USA) was used as the standard. The samples were analyzed in triplicate and measured at A517 nm using a UV-Vis spectrophotometer (Genesys 10S, Thermo, USA).

2.5.2 Determination of the protein recovery yield

The protein recovery yield was determined by Adetoro *et al.* [28] with some modifications. The concentration of protein obtained from different carrier agents was divided by the initial protein concentration of the defatted raw almond powder on a dry basis, as follows Equation (1):

$$Protein\,recovery\,yield\left(\frac{g}{100g}\right)=\left(\frac{Protein\,concentration\,of\,FD\,(d.b)\,(g)}{Protein\,concentration\,of\,initial\,almond\,\,powder(d.b)\,(g)}\right)\times 100 \quad (1)$$

2.5.3 Determination of moisture content

The moisture content (MC) of the freeze-dried almond powder was determined using the AOAC method [62]. Briefly, 2 g of FD almond protein-based powder was weighed and placed in a vacuum oven at 100 °C for drying. The drying process was repeated every 15 min until a constant weight was obtained. The amount of MC was equal to the amount of water that evaporated during drying. The MC was calculated as follows Equation (2):

$$MC=\frac{Weight\,of\,\,absorbed\,water\,\,(g)}{Weight\,of\,\,freeze-dried\,\,powder\,\,(g)}\times 100 \quad (2)$$

2.5.4 Determination of bulk density

Bulk density (BD) (g/mL) was determined from the mass per volume the powder occupied. It was reported by Şahin-Nadeem *et al.* [63], with some modifications. Briefly, 2 g of freeze-dried almond powder was poured into 10 mL graduated cylinders. The experiment was performed in triplicate at room temperature, as follows Equation (3):

$$BD\left(\frac{g}{ml}\right)=\frac{Weight\,of\,\,freeze-dried\,\,powder\,\,(g)}{Volume\,of\,occupied\,\,(ml)} \quad (3)$$

2.5.5 Determination of bulk tapped density

The bulk tapped density (BTD) (g/mL) was determined by the mass per volume occupied by the powder. It was reported by Santana *et al.* [25] with some modifications. Briefly, 2 g of freeze-dried almond protein-based almond powder was poured into a 10 mL graduated cylinder. Samples were tapped continuously at a distance of 10 mm. The experiment was performed in triplicate at room temperature, as follows Equation (4):

$$BTD\left(\frac{g}{ml}\right)=\frac{Weight\,of\,\,freeze-dried\,\,powder\,\,(g)}{Volume\,of\,\,occupied\,after\,tapping\,\,(ml)} \quad (4)$$

2.5.6 Determination of flowability and cohesiveness

The flowability and cohesiveness of the powder were determined in terms of Carr index (CI) and Hausner ratio (HR) [64] using the bulk and tapped density, using the following Equations (5) and (6):
Carr Index:

$$CI=\left(\frac{\rho t-\rho b}{\rho t}\right)\times 100 \quad (5)$$

Hausner's ratio:

$$HR=\frac{\rho t}{\rho b} \quad (6)$$

Table 2: Flowability and Cohesiveness indicators according to Jinapong *et al.* [65]

CI	Flowability	HR	Cohesiveness
<15	Very good	<1.2	Low
15–20	Good	1.2–1.4	Immediate
20–35	Fair	>1.4	High
35–45	Bad		
>45	Very bad		

2.5.7 Determination of hygroscopicity

The hygroscopicity (HY) is the ability to absorb water at room temperature. This was determined according to the method described by Bhat *et al.* [64]. Approximately 2 g of FD almond protein-based almond powder was spread evenly on a petri dish and placed in a 25 °C jar with NaCl saturated solution (75.29% relative humidity). The weight difference was measured at 30 min intervals until the weight on the petri dish reached a constant value. The samples were done in triplicates, placed in the desiccator, and were examined every 7 days. HY (%) of the powder is calculated following the Equation (7):

$$HY\,(\%)=\frac{Weight\,of\,\,absorbed\,moisture\,\,(g)}{weight\,of\,\,dry\,solid\,\,(g)}\times 100 \quad (7)$$

2.5.8 Determination of water solubility index

The water solubility index (WSI) of almond protein powder is determined by the method of Cano-Chauca *et al.* [66]. 2 g of freeze-dried almond powder was dispersed in 50 mL distilled water and blended at high speed until it became homogeneous in 5 min. The solution was centrifuged at 8000 rpm for 10 min at 20 °C. The supernatant was placed in aluminum foil and dried in the oven at 105 °C until it reached a constant weight. The solubility is calculated based on weight difference and is expressed as a percentage, as follows Equation (8):

$$WSI\,(\%) = \frac{Weight\ of\ dried\ supernatant\ (g)}{Weight\ of\ sample\ (g)} \times 100 \quad (8)$$

2.5.9 Determination of water-holding capacity and oil-holding capacity

The method of analysis was from Diniz and Martin [67] with some modifications. Briefly, 0.5 g of freeze-dried almond-based protein sample was mixed with 50 mL of distilled water or oil at room temperature, the whole solution was left for 30 min under the same condition. After centrifuging the mixture at 8000 rpm for 15 min at 20 °C, the volume of the resulting supernatant was measured. The quantity that resulted from subtracting the beginning volume from the final volume. The ability of WHC/OHC was calculated as the difference in volume divided by the mass of FD powder, as follows Equation (9):

$$WHC/OHC\left(\frac{ml}{g}\right) = \frac{Initial\ volume - Final\ volume\ \ (ml)}{Weight\ of\ sample\ (g)} \quad (9)$$

2.6 *Statistical analysis*

Statistical analysis was carried out using SPSS Statistics software (version 2018) with a level of 95% confidence to evaluate the differences between the quality of almond juice concerning enzymatic and carrier agents. One-way Analysis of variance [1] using Fisher LSD was used to compare treatments. Means and significant differences were accepted at p-value ≤ 0.05. All determinations were conducted in triplicate for each treatment and data were reported as ± standard deviations (SD).

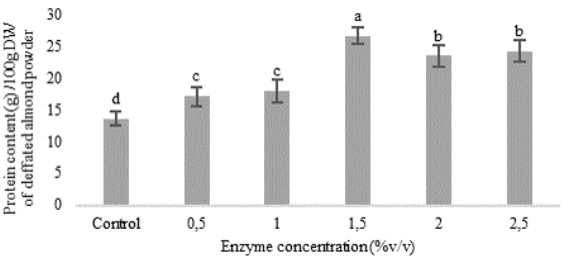

Figure 1: Enzyme concentration on protein content extracted from the defatted almond powder. The values were means ± SD. The same letters indicate insignificant differences with p-value > 0.05.

3 Results and Discussions

3.1 *Effects of enzyme concentrations and incubation durations on protein content of defatted almond powder*

3.1.1 Effects of enzyme concentrations

From Figure 1, it is clear that there are significant differences in protein contents between treated and non-treated samples. The control sample without enzyme contained the lowest amount of protein, (13.65 ± 1.0 g/100 g dried weight (DW). Meanwhile, with the first treatment at 0.5%, it was shown to increase by 3.55%, be statistically different from non-treated samples, 17.20 ± 0.015 g/100g DW. However, there was an insignificant distinction between the 1% and 0.5% samples in protein concentrations. Positive results were indicated when enhancing samples at 1.5, 2, and 2.5% Flavourzyme, in which, 1.5% was seen to be the most ideal concentration of Flavourzyme for defatted almond samples. It showed the greatest extracted protein at 26.73 ± 0.012 g/100g DW, higher than 2 and 2.5% Flavourzyme, which were 23.55 and 24.30 g/100g DW, accordingly. The decrease in protein concentration at 2% and 2.5% enzyme treatments can be explained through the enzyme-substrate binding mechanism. The study of Guérard *et al.* [68] using Alcalase on tuna waste hydrolysis also showed that the addition of enzyme at 1.5% resulted in the highest degree of hydrolysis. Flavourzyme contains both endopeptidases and exopeptidases for hydrolysis [69], for which exopeptidases account for the majority [13]. Hence, the liberation of proteins to amino acids at increased concentrations can create enzyme

inhibitors, such as dipeptides, peptides, or L-amino acids of L-Leucine, that act as potential inhibitors of Flavourzyme [70]. Giesler *et al.* [71] also found that the activities of Flavourzyme can be inhibited by the presence of amino acids L-leucine, L-isoleucine, L-phenylalanine, L-valine, L-histidine, and L-glutamic acid in wheat protein hydrolysis. Since wheat protein and almonds have quite similar amino acid profiles, the explanation could be inferred from those of almond protein that also contains L-leucine and L-isoleucine [69]. Moreover, it was stated by Marquez Moreno & Fernandez Cuadrado [72] that the diminishing of the rate would occur when the amount of substrate is excessive, causing substrate inhibition. The reaction between enzymes and the formation of inhibitory peptides was said to happen, hence, the reaction rate will eventually decline compared to that of hydrolysis without inhibitors. This is also in agreement with Chavan & Hejgaard [73] in finding subtilisin as an inhibitor during hydrolysis, which could be traced back to the possibility of Flavourzyme inhibitor formation, apart from those of inhibitory peptides and amino acids mentioned above.

3.1.2 Effects of incubation durations

From Figure 2, the extraction duration was screened in the range of 30–180 min. There was no significant difference in protein content when treating Flavourzyme 1.5% from 30 min to 90 min. Whereas, using 1.5% enzyme at 120 min yields the highest protein, at 22.9 g/100g DW.

However, the yield was highest (p-value ≤ 0.05) at 120 min, indicating this duration was the most suitable for extracting protein from the defatted almond powder using Flavourzyme. Anh *et al.* [16] also found 120 min to be the most favored time for soybean extraction using Flavourzyme for non-heat samples. However, when exposing the defatted almond powder for a prolonged duration (180 min), protein concentration decreased substantially. The mechanism for this situation was explained by [71], in which 5.5 h long proteolysis of Alcalase on tuna not only did not yield higher hydrolysis but also lowered the rate. The possible explanation came down to the ability of enzymes to hydrolyze themselves when being over-exposed since enzymes are also a protein nature [68], and up to 70% of the dry matter is protein [74].

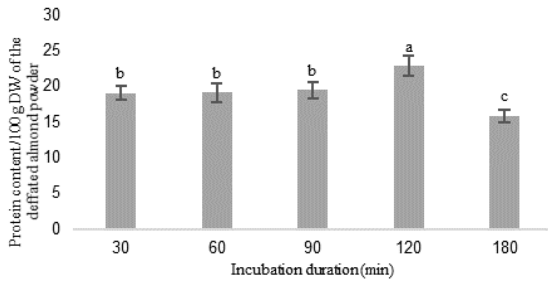

Figure 2: Extraction duration on protein content extracted from the defatted almond powder. The values were means ± SD. The same letters indicate insignificant differences with p-value > 0.05.

Meanwhile, the liberation of amino acids at prolonged durations could cause enzyme inhibitors, because Flavourzyme is a mixture of both endo and exopeptidases, that could release more inhibitory peptides and amino acids at 180 min compared to that at 120 min (15.9 and 22.9 g). Regarding the finding of Guérard *et al.* [68], the author also explained the capability of the lack of hydrolyzable peptide bonds where protease could attack, in combination with enzyme denaturation when enhancing the duration of proteolysis to cause such degradation. This was also supported by Diniz and Martin [67] on goldfish protein. Over-exposure of protein with time under heat could also degrade enzyme quality, which is inactivated easily by the presence of other chemical compounds formed during hydrolysis processes. Moreover, the protein structure (including enzymes) is linked and stabilized by weak forces, this inadequate stability implies that proteins are energetically close to multiple alternatives. Consequently, it could have less physiologically active structures [75].

3.2 *Effects of carrier agents on the chemical, physical, and functional properties of FDAP*

3.2.1 Protein recovery yield

From Figure 3, it can be observed that there is no significant difference between the protein recovery yield of the encapsulated powder samples from different carrier agents, which ranged from 77.57–83.92% respectively (p-value ≤ 0.05). Hence, it can be concluded using carrier agents has no significant effect on retaining protein rather than other physical effects.

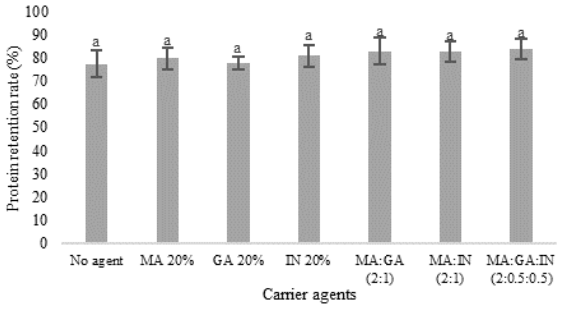

Figure 3: Protein recovery yield using carrier agents The values were means ± SD. The same letters indicate insignificant differences with p-value > 0.05.

To compare, the study showed it acceptable protein retention rate since it was higher than the previous study using the same enzyme on protein, which had 62% yield, under the same extracting conditions [76].

This, in turn, indicates the ability to use the FD method to recover protein. Whether there is limited research in drying protein, almond is a plant-based product the mechanism of retaining can be inferred from other plant materials since it also contains polysaccharide cell wall, that is said to be rich in lignin and cellulose [77]. Cellulose is very abundant in the vegetable cell wall, which has strong mechanical characteristics related to the cell structure, which directly contributes to the overall drying rate if being frozen at the freezing state properly. This polysaccharide layer acts as a prevention of component loss, gas exchange, and protection for the internal parts from degradation. Particularly, mechanical attributes and structural maintenance play an integral part in retaining product molecules to avoid the collapse of the structure during the first and second stages of drying [21]. In the situation of almonds, protein is captured by a cytoplasmic polysaccharide network, including a membrane shaped like a hexagon. [78]. In this case, the structural strength of this polysaccharide network served the role of minimizing the damage during the first and the second drying state, it prevents the collapse in the properties of food when drying or storing FD plant products [21].

Even though there has been only a handful of research using FD on protein until recently, having to understand the mechanism, this drying method is superior and has been executed on many types of plant-based food. For instance, according to Araya-Farias *et al.* [79], FD has significantly retained up to 93% carotenoid content, 34% Vitamin C, and 11% more phenolics than using hot air. Those compounds are said to be volatile, and heat-sensitive, where their nature is similar to proteins [80], [81]. In vegetables, many authors have elucidated the potential of FD in preserving nutritional content. Gümüşay *et al.* [82] have compared the effects of 4 different drying methods: sun drying, oven-drying, vacuum oven, and FD on phenolic contents, antioxidant capacity, and ascorbic acid amount. Consequently, FD was the most significant method where the retentive phenolic acid doubled other drying mechanisms [82]. On the one hand, specialty foods such as coffee or spice were also sufficiently made use of by FD. Kraujalytė *et al.* [83] have reported the remarkable retention of phenolics in coffee regarding other drying methods. Generally, FD is a superior method to retain sensitive bio compounds such as protein, by having a polysaccharide network, protein is bound inside the membrane and retained efficiently if freezing initially is done properly. When compared to air drying, it is found that freeze-drying improved the retention of anthocyanins, phenolics, and antioxidant activity during the processing of conventional as opposed to organic blueberries and raspberries. In some instances, freeze drying even increased the concentration of phytochemicals [21]. As a result, the coating of carrier agents such as MA, GA, IN, or their combination imposed no significant effects on the retention of protein content in almond protein extracts in this study. This is due to the natural barrier of almonds, combined with the efficiency of the FD method, which made it sufficient for the extract to keep its protein content.

3.2.2 Moisture content

When MC is altered, it may result in changes to a powder's BD, flowability, and appearance [84]. Overall, all coated samples performed well and were in an acceptable range of commercial MC for FD powder that is lower than 10% for microbial safety [85]. From Figure 4, non-coated control powder had higher moisture content, compared to coated samples. The phenomenon can be explained due to the stickiness of powder with no absorption of coating agents and has more protein-like characteristics. As mentioned above, without encapsulating agents, protein hydrolysate is

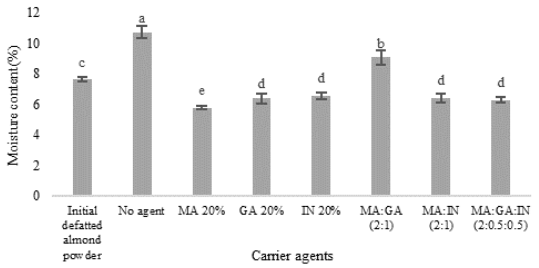

Figure 4: Moisture content of FDAP. The values were means ± SD. The same letters indicate insignificant differences with a *p*-value > 0.05.

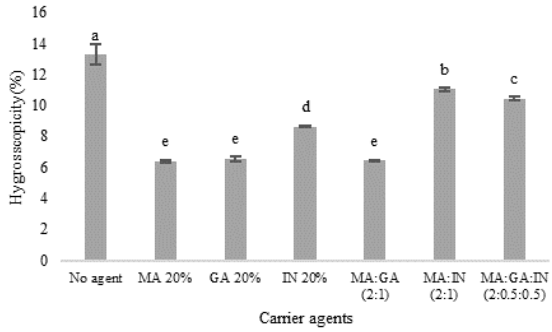

Figure 5: Hygroscopicity of FDAP. The values were means ± SD. The same letters indicate insignificant differences with *p*-value > 0.05.

highly hygroscopic and unstable. As a result, the high MC of non-encapsulated powder can also come from water from the surrounding environment re-binding again onto the protein-based powder surface [18]. The result was agreed that drying mussel protein powder using carrier agents significantly reduced the MC compared to that of non-treatments [84]. Meanwhile, it also comes down to the FD system that produces more porous due to low-temperature exposure, alongside higher MC than other drying methods [86]. A similar result was obtained when using FD for apple and watermelon juice powder, where SP powder would obtain 87% lower MC [87]. Compared to the initial MC of defatted almond powder, all coated samples resulted in lower MC of powder, the only exception was the combination of MA: GA at a ratio 2:1, with 9.10 ± 0.47%, which has a significantly higher amount of moisture, to be unfavorable for any dried powder. Quoc [88] also obtained the same result of MC for the addition of both MA and GA together at 7.12 ± 0.32%, compared to the value of 5.98 ± 0.42% using only MA. This is the indication that GA and MA have hydrophilic groups generally, which are easy to absorb moisture from the environment [88]. The result was supported by Ferrari *et al.* [32] who stated GA is a complex heteropolysaccharide matrix with ramifications in structure, containing multiple hydrophilic groups. This interaction acts as an initiative that modifies hydrogen bonding and hydroxyl groups of the crystalline and amorphous matrix of macromolecules [89]. Moreover, GA was assumed to have a higher capacity to absorb water from the surrounding environment than MA [90]. Additionally, there is no significant difference between using IN 20% only or MA: IN 2:1 simultaneously. The MC of the MA: IN sample was similar to the experimental data of [29], which also resulted in around 7.86 ± 0.53% MC for the addition of 15% and 6.87 ± 1.29% for 25% of the MA: IN mixture of agents. Respectively, The FD powder from IN also contained higher MC than that of the MA containing one. When mixing all three components altogether, MC would not be statistically different, the data only stood at 6.30 ± 0.17%. Therefore, it could be drawn from the data that GA would be the main reason causing the high MC in MA:GA powder.

3.2.3 Hygroscopicity (HY)

One of the most significant drawbacks of enzymatic hydrolysates is that they are hygroscopic, which restricts their physicochemical qualities, as well as their flowability, storage time, and durability, as well as their industrial applications.

From Figure 5, it can be seen the uncoated powder has considerably higher HY than other samples, which peaked at 13.30 ± 0.65%. Whereas the coated samples presented lower HY, which ranged from 6.41 to 11.06%, respectively. The study showed that the encapsulated almond-protein-based products were lower in HY. For instance, after a 7-day interval, the absorption of humidity stayed at 6.41 ± 0.067%, and 6.59 ± 0.14% for MA and GA, respectively. The data is also supported by Yang *et al.* [27] in drying whey protein hydrolysate, using encapsulating agents also improved the performance. The optimization of shark protein hydrolysate by Rodríguez-Díaz *et al.* [91] also indicated the inversely proportional relationship between MA addition and HY. When adding more

MA, the HY value degraded. This is because MA is stated to be non-hygroscopic by its nature [92]. Another explanation for the lower HY value when adding carrier agents is that encapsulated proteolyzed products would contain short chains of peptides, and the molecular weight of the additional agents that would have higher molecular weight such as MA with 1800 g/mol or GA (47000–3000000 g/mol) [93]. Glass transition temperature (T_g) is elevated proportionally with molecular weights, the addition would increase the stability of the product, reducing the stickiness. T_g is linked with the amorphous matrix including water, soluble solids, and food polymeric networks, above this temperature, food would change from a glassy to a rubbery state, that has a high viscosity and is directly related to stickiness and agglomeration [94]. the fortification of more carrier agents would increase T_g, hence increasing powder stability.

According to Cai and Corke [95], when using different molecular weights MA (10, 15, 20, and 25 DE), when MW decreased, T_g followed. Lower T_g would also result in greater HY of powder because lower MW MA has shorter chains and is more hydrophilic. Generally, food that is low in MC with T_g higher than room temperature is stable. The addition of MA or GA countered the unfavorable ramifications ion, increased T_g. The effective aid of MA in vacuum drying mango pulp has been clarified by Jaya and Das in 2004 [96]. For sugar-rich products, stickiness is a phenomenon that is challenging to tackle, due to the low T_g in its composition. The addition of carrier agents reduced the phenomenon, hence, ameliorating the stickiness issue. Another study by Yang *et al.* [27] also reported a reduction of HY in encapsulated powder from 64.3% to 43.1% and 36.9%.

Meanwhile, HY is highly dependent upon the additional agents in their encapsulating behaviors. In this situation, IN did not possess an ideal characteristic. Using IN at 20% resulted in 8.66 ± 0.05%, and the combination of MA:IN (2:1) also significantly increased HY content by 11.06 ± 0.098%. This is because inulin is highly hygroscopic due to the branching structure, that initiates hydrogen bonding and absorbs MC from the air. Per high HY, IN would bind water to create a gelled network [97]. The explanation would be transparent, however, if only considering the gelled network formation as the only explanation for the unwanted HY of MA:IN, that would be insufficient since GA is also a hydrocolloid that attracts water. It also comes down to the MW difference between IN and GA, where GA has a higher MW than IN (3600–5200 g/mol) [98]. This would signify the disparity of T_g between samples as mentioned above, and the T (Tout-Tg), whereas Tout for FD is 25 °C. When T_g gets higher, the difference gets smaller, hence declining stickiness [42]. The combination of all coating agents indicates the intricate complex between polysaccharides, hydrocolloids, and protein matrices. At the ratio of 2:1 when MA is still the determining coating saccharide, the result would be more positive, this trend backfired when the ratio of MA: Hydrocolloid equals 1:1 (with 0.5 for IN and 0.5 for GA), this would cause the synergy of absorbing water in hydrocolloids, indicating the synergetic effect of both IN and GA [99].

3.2.4 Bulk density and bulk tapped density and flowing performance

When it comes to powder goods, BD is an especially significant metric because of its association with mixing, packing, transportation, and storage [34]. BD is the mass of solid particles and moisture divided by the volume occupied by those particles, including pores and surface moisture, to the surrounding environment. To illustrate, powder with high BD will have higher packing volume, whereas lower BD powder will be prone to product oxidation, because of the air that is trapped within [100]. BD values of almond protein-based powder varied widely from 0.16 to 0.42 g/cm^3.

Regarding Table 3, non-added powder shows the lowest BD, higher BD values were seen when adding different types of carrier agents. The highest BD value was obtained by the formation of MA:IN at a ratio of 2:1 into the powder, followed by 0.43 ± 0.03 g/cm^3. The observation is supported by Michalska-Ciechanowska *et al.* [29] in FD Chokeberry pomace extracts, the experimental results differentiated BD between MA and IN, which was 0.12 ± 0.03 g/mL and 0.17 ± 0.01 g/mL, respectively. Furthermore, in the extraction of cranberry, the lower value would be in MA-coated powder, meanwhile, the contribution of both agents MA and IN caused higher BD. These authors also stated the profound effect of different types of carrier agents on the BD compared to the added concentration [87].

If using only one type of carrier agent, the result was statistically different at p-value < 0.05, where MA resulted in 0.32 ± 0.02 g/mL, GA was 0.22 ± 0.01 g/mL, and IN 0.42 ± 0.03g/mL BD. To clarify, IN would possess the highest value of BD, followed up by GA and MA consecutively. Likewise, pure IN had a higher BD than MA, due to MC difference, hence limiting space between particles [79]. To further explain the distinction between these agents, particle size is one of the parameters that should be taken into consideration. All powders containing GA resulted in lower BD values, either used alone or in combination. There was no significant difference in BD between the MA:GA or MA:GA:IN mixtures, which hovered around 0.25 ± 0.02 and 0.24 ± 0.009 g/mL. This was solidified by Tonon *et al.* [101] in drying acai juice, in which GA also possessed lower BD compared to MA with different Dextrose Equivalent. They also indicated smaller particles would, in turn, have higher BD, because the shaking action during the density retightens the spaces among molecules, forcing powder particles to fit in smaller volumes [102]. Microcapsules of Moringa protein produced with GA also had the lowest BD, which might be related to the viscous characteristic of GA, meaning that large storage is needed for GA-coated powder [103]. The higher the solid content, the lower the BD [29]. The coating's molecular weight and internal structural bond may also have a role, as may the coating's underlying chemical nature [104]. There were notable distinctions between samples with various coating ingredients, and this may have been due to the microcapsules' irregular and amorphous form because of the freeze-drying method. The results indicated that the BD of all generated microcapsules is affected by the coating material [103].

In FD, the material was frozen before coming to the drying stages, with no heat exposure, the products come directly to the sublimation stage [105]. No liquid transformation is observed because the vapor changes from solid to vapor which removes the moisture of the food structure and automatically lets it be below the collapse temperature [106]. As a result, the collapse and shrinking effects of the product are avoided, creating a porous structure of the material [107]. This non-linear relationship is also supported by Michalska-Ciechanowska *et al.* [29] and Araya-Farias *et al.* [79]. High BD is said to be beneficial economically, since this would require smaller packing materials, hence, transportation and storage costs are cheaper. In the meantime, lower BD is correlated to the trapped air particles, initiating oxidation and instability.

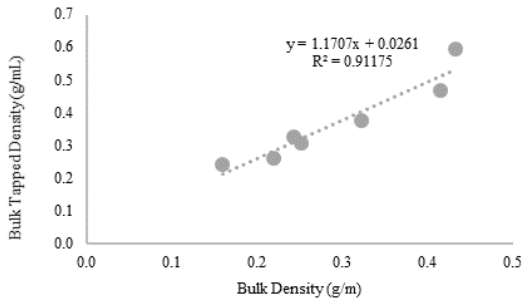

Figure 6: Correlation between Bulk Density and Tapped Bulk Density of FDAP.

Tapped bulk density (TBD) is quite different from BD since BD also considers the possibility of interspaces between particles. Meanwhile, TBD measures the tapped value of powder, excluding more external factors of spacing between molecules [32].

TBD varied from 0.24–0.36 g/mL, with a significant difference between encapsulating agents. Modification and tapping methods have already disintegrated the values, despite the sequential combination of different agents. There is a strong correlation between BD and TBD in the production of this almond protein-based powder with $R^2 = 0.91$ (Figure 6). Hence, there might be an implication that the rooted reasons causing the differences in BD can also be applied to explain the disintegration in TBD. From a general viewpoint, using IN or the combination of IN and MA also released the highest TBD, where the value lies at 0.59 ± 0.04 g/mL, 0.46 ± 0.04 g/mL, compatibly. To illustrate, the highest TBD was extracted by using the combination of MA and IN resulting in smaller particles of powder, creating greater TBD values. On the contrary, Coated-GA samples showed the lowest BD value compared to other components. Higher values of TBD were observed in the usage of IN and MA at 20%, this was strengthened by Sarabandi *et al.* [41] and George *et al.* [103] that MA could cause higher TBD than GA, or this combination would decrease the value of this parameter. The presence of GA lowered any TBD value to 0.33 ± 0.01 g/mL, including the final mixture of all three coating ingredients. BD and TBD are highly correlated to one another and determine the flowability of a powder product.

Flowability is a vital measuring factor, which is used to predict and decide the most suitable condition for processing, formulating recipes, packaging, and product logistic management [38]. All powders can flow, which helps them resist the differential movement of their particles under external forces. In this circumstance, flowability is determined through Carr's index and Hausner's ratio, since one single method would be inefficient in deciding whether that type of carrier agent is appropriate. Overall, CI ranged from 12.60–33.19%, and HR was from 1.13–1.51.

To illustrate, the flowability of powder with no agent was a worse performance than those added with different types of carrier agents. According to Table 2, in the mono-use of powder, it can be observed that Inulin gave out the best flowability of powder, followed by MA and GA, the value agrees with the trend in BD, that higher BD gives out better flowability in CI. Gum Arabic would create heavier particles hence degrading flowing performance in all 3 agents since particle size and distribution might impact BD [100]. If the Carr's Index is lower than <15, it indicates a better-flowing nature. In this experiment, only using MA and IN separately had a positive effect on flowability, which had 12.60 ± 0.55% and 13.13 ± 0.86%, on the one hand, GA and its mixture with MA indicated good flowability, which was 19.62 ± 0.95% and 15.74 ± 1.49%, respectively. Using various wall materials increased Hausner's ratio and Carr's indexes, because the flow rate of a material is dependent upon a wide variety of elements, including the particle structure and morphology, and the conditions of the drying process, in this situation, are freeze-drying. The compressibility of a freeze-dried powder may alter its flow characteristics on the micro-scale via adhesion forces between the particles, which are generated when the powder is compressed. It is possible for the flowability of mixtures containing two or more coating materials to vary as a result of interactions between the encapsulants. Because freeze-drying removes so little moisture compared to other methods, the resulting powders may have poor flowability because of the porosity this drying technique brings [108]. The value of cohesiveness lies between 1.13 to 1.17 with no significant difference at p-value > 0.05. Lower HR values for the addition of using one agent MA, GA, and IN are indicative of desired cohesiveness features that are associated with greater flowability characteristics and are therefore indicative of superior handling properties. Meanwhile, the addition of more than 2 materials caused ramifications in cohesiveness, the data ranged from 1.22–1.34, respectively. On a good note, without the aid of agents, the cohesiveness would not give a great performance.

Table 3: Bulk density, Tapped Bulk Density, Carr index, and Hausner's Ratio of FDAP

Carrier Agents (Total Concentration 20%)	Bulk Density (g/mL)	Tapped Bulk Density (g/mL)	Carr Index (%)	Hausner's Ratio
No agents	0.16 ± 0.004^{d}	0.24 ± 0.01^{f}	33.19 ± 2.082^{a}	1.51 ± 0.07^{a}
MA	0.32 ± 0.02^{b}	0.38 ± 0.03^{c}	13.13 ± 0.86^{d}	1.17 ± 0.03^{d}
GA	$0.22 \pm 0,01^{c}$	0.26 ± 0.03^{f}	19.62 ± 0.95^{c}	1.19 ± 0.07^{d}
IN	0.42 ± 0.03^{a}	0.47 ± 0.03^{b}	12.60 ± 0.55^{f}	1.13 ± 0.05^{d}
MA:GA (2:1)	0.25 ± 0.02^{c}	0.31 ± 0.02^{e}	15.74 ± 1.49^{e}	1.22 ± 0.1^{c}
MA:IN (2:1)	0.43 ± 0.03^{a}	0.59 ± 0.04^{a}	26.14 ± 1.25^{b}	1.34 ± 0.09^{b}
MA:GA:IN (2:0.5:0.5)	0.24 ± 0.009^{c}	0.33 ± 0.01^{d}	26.46 ± 2.64^{b}	1.33 ± 0.05^{b}

Note: The values were means ± SD. The same letters indicate insignificant differences with p-value > 0.05.

3.2.5 Water solubility index

Enzymatic proteolysis can increase soluble capability, since enzymes have the potential to improve the digestibility, absorption, and functionality of proteins, allowing these hydrolysates to be widely applicable in food formulation [109] in the production of casein and whey protein, WSI is enhanced due to the breakage of protein into small amino acids, facilitating the accessibility of water-loving amino acids in water [42].

Regarding Figure 7, the addition of a carrier agent genuinely increased the solubilization of powder. Non-added powder presents lower solubility compared to other encapsulated products. This is because when formulating with coating agents, due to their high water-soluble nature, the solubility of powder is also enhanced [110], [111]. Respectively, the WSI ranged from 42.8–58.89%, with the highest value belonging to the usage of MA and GA at a ratio of 2:1 (58.89%). According to Do and Nguyen [112], the

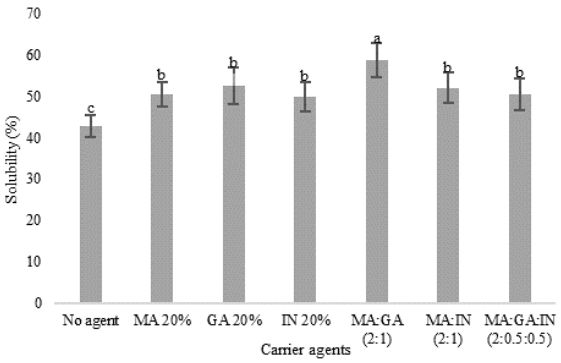

Figure 7: Solubility of FDAP The values were means ± SD. The same letters indicate insignificant differences with *p*-value > 0.05.

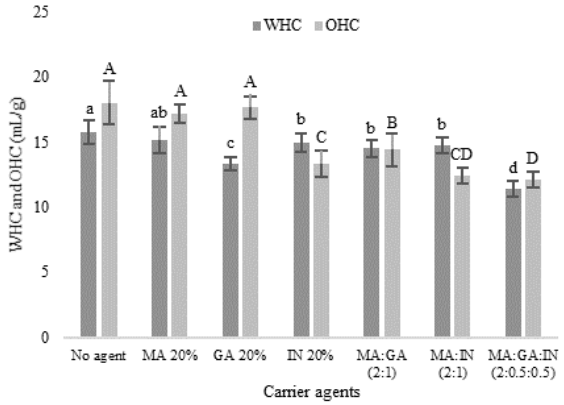

Figure 8: WHC and OHC of FDAP. The values were means ± SD. Lowercase indicates data about WHC, and uppercase indicates data about OHC. The same letters indicate insignificant differences with *p*-value > 0.05.

optimal value when using both MA and GA can be seen from their amorphous structure, hence better binding surfaces, require lower thermodynamic energy to dissolve, which creates exceptional solubility. Further support from Daza *et al.* [110] also clarified the good use of IN and GA in enhancing the solubility of encapsulated Cagaita. However, even though the percentage of WSI got inclined, compared to other dried fruit powders, the solubility was not competitive. A possible explanation for the mechanism is the polysaccharide-protein network that differentiates the compounds from other dried products. To clarify, electrostatic attraction is the fundamental force between proteins and polysaccharides. However, it also comes down to the hydrogen bonding and hydrophobic interaction that stabilize the aggregation of polysaccharide-protein [113]. Hence, there could be a possible explanation that the creation of proteins using Flavourzyme creates more cationic amino acid chains than anionic ones. On the one hand, using more than one polymer can separate the system, resulting in 2 insoluble precipitating phases that degrade the solubility of a product [114].

On the one hand, the other explanation for the low solubility of almond-protein-based products could also come down to the bound structure of polysaccharides that remained stable during the FD process. According to Saura-Calixto *et al.* [115], almond meat also contains a considerable amount of cellulose, lignin, and hemicellulose. Generally, the insolubility of cellulose is because it can form hydrogen bonds within and outside the molecules, which bind together and restrain it from absorbing water. Furthermore, it is supported as the crystalline structure of cellulose which makes it difficult to dissolute in water [116]. Hydrophobic surfaces were also indicated to have hydrophobic lipids and aroma molecules in cellulose crystalline structure [117]. The contributions of both hydrogen bonding and hydrophobic interaction were determined to be the crucial factors in its insolubility [118]. Meanwhile, lignin is also found in almond seeds as a coating polysaccharide. Similar to cellulose, lignin is also insoluble in water and cannot be broken down into smaller chains [119]. Combining all these factors, it could be observed that it is not only about the complex matrix of protein and polysaccharide, but it could also be the nature of the polysaccharide itself that makes the whole solution insoluble in water. The addition of polysaccharides as carrier agents only enhances the complexity of this matrix.

3.2.6 Water holding capacity and oil holding capacity

Water Holding Capacity (WHC) and Oil Holding Capacity (OHC) are necessary parameters to understand the characteristics of the powder and its application in the food industry. According to Figure 8, it can be observed the WHC of almond protein-based powder ranged from 11.44–15.83 mL/1 g with the highest value coming from control powder with no agents. The WHC does not show any significant difference between MA 20%, IN 20%, or the combination

of MA and IN at a ratio of 2:1. This indicates there is no difference in water absorption using IN, MA, or its combination. This could be related to the initial MC of these products that initial MC is quite similar with no significant distinction. Hence the ability of water binding could be similar. On the one hand, as mentioned above, using IN 20%, or IN and MA being combined are highly hygroscopic, hence absorbing water significantly [97]. In the meantime, adding IN into the powder enhanced the result of WHC, despite the value was still lower than those control samples. Another study also indicated strong water encapsulation in IN due to its branching structure, which facilitates hydrogen bonding and absorption of water from the surroundings [50]. Meanwhile, the lower value for WHC is seen in the GA-encapsulated sample and gets deducted notably when combining all three agents. This can be explained by the high complexity of the structure by combining all three components, as free hydrolysates would have more free-binding sites, which can absorb either water or oil better [55].

By contrast, when WHC is enhanced, OHC is declined. In other words, powder coated by GA has better OHC than MA and IN. This is in agreement with Adetoro *et al.* [28] that GA also resulted in higher OHC than MA and other studied carrier agents. Similar results were also seen in the encapsulation of raspberry juice powder, where GA-coated samples also gave out higher OHC than other drying aids, which was 3.5-fold higher than using MA [120]. Furthermore, there has been a disparity between WHC and OHC in almond-protein-based FD products. Using no agents for drying consequently produced more hydrophobic powder, since the value of OHC is higher than WHC with no agents ($18.056 \pm 1.66 > 15.833 \pm 0.90$). As a result, it could be inferred from the initial proteolysis process, that the production using Flavourzyme resulted in more hydrophobic chains than hydrophilic ones. The reducing result of WHC compared to OHC can be seen as the breakdown of peptide bonds in making polar groups will be not as significant as non-polar amino acid and peptide chains, which degraded WHC value [121]. On a note, using agents enhanced WHC property, due to the hydrophilicity and water-binding characteristics of polysaccharides such as MA or IN even though the results were not as significant, and the samples produced from MA still gave out their hydrophobicity.

4 Conclusions

The use of Flavourzyme imposed a positive effect on the extraction of almond hydrolysate, which has a specific ideal concentration at 1.5% and 120 min on almond proteolysis. The recovery yield of powder was the highest in using MA and IN whereas the protein concentration signified no differences statistically (p-value > 0.05). The physical properties of almonds were enhanced by encapsulating them with agents even though different carrier agents determined the properties of the product. MC and HY were unfavorable if the sample were uncoated, due to the hygroscopic and unstable nature of protein hydrolysate. BD and flowing properties were statistically different between the encapsulated and control samples, using no agent would decrease the BD of the product, resulting in poor flowing performance. Solubility was ameliorated by encapsulants because of the nature of these binding powders. On the contrary, WT and functional properties such as WHC or OHC were decreased by encapsulation. This might be because coating multiple agents would intricate the matrix and make it harder to absorb or immerse in water quickly.

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Author contribution

T.Y.G.N.: investigation, writing-original draft preparation; H.V.H.N.: conceptualization, writing-review, and editing.

Conflicts of Interest

The authors declare no conflict of interest.

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