



## ALKALINE EXTRACTION AND QUALITY ASSESSMENT OF PROTEIN ISOLATE FROM ALMOND (*Prunus dulcis*) SEED

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### Abstract

The increasing demand for plant-based proteins with good functional properties has led to the exploration of alternative protein sources especially from plants like almonds (*Prunus dulcis*). Alkaline extraction method was used to extract protein from almond seed, and the extract obtained was freeze dried and the quality of the isolate was assessed. The functional properties, antioxidant activity and vitamin C content of the extracted protein were determined. Water absorption was  $0.77 \pm 0.08$  g/g, oil absorption  $1.81 \pm 0.38$  g/g, and foaming capacity  $0.78 \pm 0.07$  g/g. Antioxidant activity was  $161.34 \pm 1.67$  mg/g (saponin content),  $7.85 \pm 0.43$  mg GAE/g (phenolic content) and  $4.95 \pm 0.48$  mg QE/g (flavonoid content). Vitamin C content was  $3.29 \pm 0.05$  mg/g. The almond protein isolate has favorable functional properties and antioxidant activity.

**Keywords:** alkaline extraction, quality assessment, protein isolate, almond, functional property.

### Introduction

Protein is one of the macronutrients that people need to consume in their diet. It plays important role in various human body processes, including developing muscle mass, proper functioning of enzymes as well as immune system. Conventional protein sources have been focused primarily on animal foods since they are highly endowed with proteins and possess all the essential amino acids. However, due to environmental and ethical issues overarching the animal farming, there have been need to look for viable and sustainable sources in plant-based products (Bryant, 2022).

Over the last few years, proteins sourced from plant have become a subject of concern. According to Neji *et al.* (2022), consumers have shifted towards selecting plant protein-based products because of taste, and health. Furthermore, in the light of climate change and environment, plant protein is identified as having lower environmental effect than animal protein. The almonds (*Prunus dulcis*) are one good example of plant-based protein rich crop still waiting to breakthrough. Besides being healthy, almonds have protein that averages 16-22% (Yada *et al.*, 2011). In almonds, the proteins are mainly storage proteins in which a 14S globulin known as amandin dominates the almonds' protein content. The reserve proteins help in the provisions of

nutrients as soon as the seeds germinate as well as to the developing seedlings.

Several protein extraction strategies such as sonication (with high extraction rate but time, energy and solvent consuming, low extraction yield and inducement of thermolabile compounds), homogenization (with good emulsifying activity but negative nutrient degradation), detergent-based extraction (with good prospect to solubilize membrane proteins and lipids but negative protein denaturation) had been reported for protein isolation from plant seeds (Thakur *et al.*, 2024). Hexane solvent-extraction has been reported for extraction of protein from seeds (Jamdar *et al.*, 2010) with its negative environmental impact, safety concerns and impacts on protein functionality (Gravel *et al.*, 2023; Carre *et al.*, 2025). New methods like alkaline extraction, aqueous extraction as well as enzyme assisted aqueous extraction processes (AEP) are viable alternative to hexane extraction. These techniques involve the use of alkaline solutions, water and enzymes to extract proteins thereby reducing hazardous implications to the environment and human health. Alkaline extraction breaks the matrix in which the proteins are present; therefore, making protein more soluble (Momen *et al.*, 2021). Furthermore, AEP has a capability to extract protein, carbohydrate and oil independently from oil-bearing

materials; this increases the efficiency of the extraction and effectiveness as well as its sustainability (De Souza *et al.*, 2020). However, there are some constraints and, perhaps, research gaps the present investigation noted in the context of almond protein extraction. These are in aspects related to extraction parameters for higher yield and quality of the protein, the effect of extraction techniques on the functional properties of the extracted protein and the viability of largescale extraction (Cruz-Solis *et al.*, 2023). Hence, this research reports the functional properties, phytochemicals (flavonoid, tannin, saponin, alkaloids, phenol) and vitamin C content of protein obtained from almond seed using alkaline-extraction method.

## Materials and Methods

### Materials

Almond (*Prunus dulcis*, sweet) seed was obtained from Oja Oba market, Akure, Ondo State. Sodium hydroxide; NaOH (0.1M, purity >99%) and hydrochloric acid; HCl (1.0M, purity >99%) were obtained from (Merck, Germany). Distilled water was used throughout the experiment.

### Methodology

Preparation of almond seed flour. Figure 1 shows the process route for production of almond seed flour. The almond seeds were screened, washed to remove foreign or unwanted items, blanched, deskinning and grinded using attrition mill. The grinded seeds were sieved through a 12 mesh, 1.70 mm sieve size, fine granulometry (Cruz-Solis *et al.*, 2023).

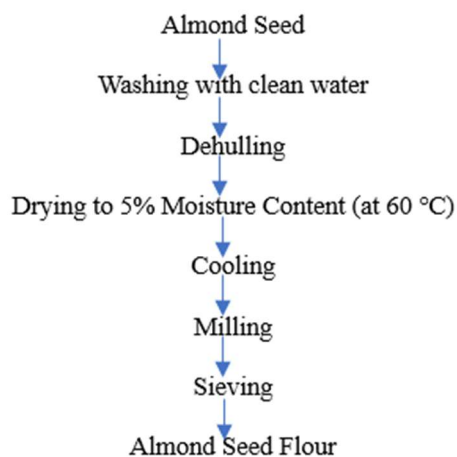


Figure.1: Flow process for the production of almond seed flour (Cruz-Solis *et al.*, 2023)

### Alkaline extraction of protein isolate from almond seed flour

Almond protein extract was produced using the alkaline extraction process from non-deffated almond flour (Juul *et al.*, 2023). To achieve this, 700

g of almond flour was dispersed into 7000 cm<sup>3</sup> of water to create a 1:10 solids-to-liquid ratio (w/v) in a 10-L jacketed glass reactor. The extraction was carried out with 0.1M NaOH at pH 10, using a pH meter (S-610L Series, Peak Scientific Instruments, UK) and a temperature of 50 °C for 60 mins (Juul *et al.*, 2023). The glass reactor was continuously stirred at 120 rpm. Following the extraction, the slurry was centrifuged with a centrifuge (GYTD-4, Xiamen Guoyi Scientific Instrument, China) at 10000 × g for 30 mins (Juul *et al.*, 2023). The supernatant was kept, while the residue was re-dissolved, pH readjusted as before and centrifuged again. The supernatants from the two steps were then combined. The combined supernatant's pH was then adjusted to 4.5 using 1M of hydrochloric acid. Then, the solution was mixed for 2 hours and centrifuged to allow the protein to precipitate (Juul *et al.*, 2023). The precipitated protein was re-dissolved in water and the solution then neutralized to pH 7.0 using 0.1M of NaOH at room temperature. The solution was dialyzed, centrifuged and freeze-dried to obtain the protein isolate (Juul *et al.*, 2023).

### Determination of foaming property of extracted protein isolate

Foaming properties was measured following the modified method of Falade *et al.*, 2003). Twenty-five mL of a 1% (w/w) dispersed protein isolate was placed in a 250 mL plastic beaker. The pH was adjusted to different pH values ranging from 2.0 to 10.0 by adding 1N HCl or 1N NaOH. The adjusted dispersed protein isolates were continuously whipped at 10,000 rpm for 2 minutes using a Polytron PT 2500 Homogenizer (Kinematica AG, Lucerne, Switzerland), and the mixture was immediately transferred to a 100 mL graduated cylinder. The foam volume was recorded at time zero for foaming capacity (FC) and at 60 minutes for foaming stability (FS). Samples were evaluated in triplicate (Falade *et al.*, 2003). The following equations was used to determine foaming capacity and stability:

$$FC (\%) = \frac{V_a - V_b}{V_b} \times 100 \quad (1)$$

$$FS (\%) = \frac{V_{60min} - V_b}{V_b} \times 100 \quad (2)$$

where  $V_a$  is the foaming volume immediately after whipping,  $V_b$  is the volume before whipping, and  $V_{60min}$  is the volume 60 minutes after whipping (Falade *et al.*, 2003).

### Determination of functional properties of extracted protein isolate

**Determination of water absorption capacity:** The water absorption capacity (WAC) of protein isolates

defines their functionality in foods and especially in bakery and meat replacement products. The WAC was obtained through the modified method of Sze-Tao and Sathe, (2000). In this method, 1 g of the sample was dissolved in 10 ml distilled water in a previously weighed centrifuge tube. The two solutions were mixed by vortexing for 30 seconds and allowed to stand for another 30 minutes at room temperature. Finally, after the samples were stood, these samples were centrifuged at 3000 rpm for 30

minutes, and which the supernatant was siphoned off and the residue was weighed (Sze-Tao and Sathe, 2000). The water absorption capacity was calculated using the following formula (Sze-Tao and Sathe, 2000):

$$\text{WAC (g/g)} = \frac{\text{weight of water absorbed by product}}{\text{weight of dry protein sample}} \quad (3)$$

Table 1: Foaming capacity and functional properties of extracted protein isolate

Sample	Foaming capacity	Water absorption capacity (g/g)	Oil absorption capacity (g/g)
TOL	0.78 ± 0	0.77 ± 0.08	1.81 ± 0.38

Values are presented as mean ± SD (n=3); TOL: Sample of protein isolate from almond.

**Determination of oil absorption capacity:** The absorption capacity of oil (OAC) in the protein isolates was analyzed based on the technique outlined by Sze-Tao and Sathe, (2000) with slight modifications. This property is crucial in establishing the functional characteristics of protein in food matrices especially in systems containing emulsified products. For determination of OAC, 1 g of the protein isolate was dissolved in 10 mL vegetable oil on a previously weighed centrifuge tube. After that, the mixture was stirred for half a minute and then left standing for 30 minutes at room temperature. It was followed by spinning of the sample for 30 minutes at 3000 rpm. The upper layer of oil was very carefully aspirated and the weight of the tube with the adsorbed oil was noted (Sze-Tao and Sathe, 2000). The oil absorption capacity was calculated using the formula (Sze-Tao and Sathe, 2000):

$$\text{OAC (g/g)} = \frac{\text{weight of oil absorbed by product}}{\text{weight of dry protein sample}} \quad (4)$$

#### Antioxidant analyses of protein extract

**Determination of saponin content:** The spectrophotometric method of Borokinni et al. (2022) was used for saponin determination. 2 g of finely grinded sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol or (But-2-ol) was added. The mixture was shaken for 5 hrs to ensure homogeneity, and then filtered, with No 1 Whatman filter paper, into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate (MgCO<sub>3</sub>). The mixture obtained was filtered again, through No 1 Whatman filter paper, to obtain a clean colourless solution. 1 ml of the colourless solution was taken into 50 ml volumetric flask using pipette and 2 ml of 5% iron(III)chloride

(FeCl<sub>3</sub>) solution was added, then made up to the mark with distill water. It was allowed to stand for 30 min for the colour to develop. The absorbance is read against the blank at 380nm (Borokinni et al., 2022).

$$\text{Saponin Content} = \frac{A \times DF \times V_{\text{total}}}{\epsilon \times l \times W} \quad (5)$$

where A is the absorbance at 380 nm, DF is the dilution factor, V<sub>total</sub> is the total volume of extract (mL),  $\epsilon$  is the molar extinction coefficient of saponin (L/mol/cm), l is the path length of the cuvette (cm) and W is the weight of the sample (g) (Borokinni et al., 2022).

**Determination of total flavonoid:** The total flavonoid content of the extract was determined using a colorimeter assay developed by Meda et al. (2004). 0.2 ml of the extract was added to 0.3 ml of 5% NaNO<sub>3</sub> at zero time. After 5 min, 0.6 ml of 10% AlCl<sub>3</sub> was added and after 6 min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank and flavonoid content was expressed as mg rutin equivalent. The total flavonoid was determined according to the following equation (Meda et al., 2004).

$$\text{Flavonoid content (mg/g)} = \frac{\text{Absorbance} \times \text{Dilution Factor} \times \text{Volume of Extract (L)}}{\text{Slope of standard curve} \times \text{Weight of sample (g)}} \quad (6)$$

**Determination of total phenol:** The total phenol content of the extract was determined by the method of Oboh and Omoregie, (2008). 0.2 ml of the extract was mix with 0.5 ml of 10% Folin Ciocalteu's reagent and 2 ml of 7.5% sodium carbonate. The

reaction mixture was subsequently incubated at 45 °C for 40 mins, and the absorbance was measured at 700 nm in a spectrophotometer. Gallic acid was used as standard phenol and total titratable acid, expressed as milligrams of gallic acid, was determined according to Padhi et al. (2017) using Folin Ciocalteu phenol reagent. The total phenol was calculated using the following equation (Obloh and Omoregie, 2008).

$$\text{Total phenol (mg/g)} = \frac{\text{Absorbance} \times \text{Dilution Factor} \times \text{Volume of Extract (L)}}{\text{Slope of standard curve} \times \text{Weight of sample (g)}} \quad (7)$$

**Determination of tannin content:** Tannin content was also estimated from the method described by Obloh and Omoregie (2008). The extract was filtered using Whatman No.1 filter paper. 0.5 ml Folin-Denis reagent was added to 0.1 ml of the filtrate and 1 ml of saturated sodium carbonate solution was also added. The above mixture was made up to 10 ml with distilled water and allowed to develop color for 30 minutes at room temperature. The absorbance was recorded at 760 nm using UV-Visible spectrophotometer (Obloh and Omoregie, 2008). Tannin content was determined using a calibration curve based on tannic acid up to a concentration of 100 mg/L and was reported as milligrams of tannic acid equivalent per gram of sample (mg TAE/g) (Obloh and Omoregie, 2008).

$$\text{Tannin content (mg/g)} = \frac{\text{Absorbance} \times \text{Dilution Factor} \times \text{Volume of Extract (L)}}{\text{Slope of standard curve} \times \text{Weight of sample (g)}} \quad (8)$$

**Determination of Phytic Acid:** The phytic acid content was determined using the method described by Lai et al. (2013). 0.5 g protein isolate sample was precipitated in 100 mL of 0.2 M hydrochloric acid (HCl) and shaken for 3 hrs at room temperature. The obtained mixture was filtered using Whatman No. 1 filter paper. 25 mL of the filtrate was titrated against 0.1 M ferric chloride solution (FeCl<sub>3</sub>) using 5 mL of 0.3 % ammonium thiocyanate as indicator. The end point of the titration was inferred by persistent formation of yellow colour which is characteristic of ferric phytate (Lai et al., 2013). The phytic acid content was calculated using the following formula (Lai et al., 2013)

$$\text{Phytic acid content} = \frac{V \times M \times 660}{W} \quad (9)$$

where V is the volume of ferric chloride solution used (mL), M is the molarity of ferric chloride solution and W is the weight of the sample (g).

**Determination of alkaloid content:** Alkaloid content was determined according to the description of Li et al. (2017). 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 hrs. The mixture was filtered, and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the concentrated extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Li et al., 2017).

$$\text{Alkaloid content} = \frac{W_3 - W_2}{W_1} \times 100 \quad (10)$$

where W<sub>1</sub>, W<sub>2</sub> and W<sub>3</sub> are weight of initial sample, weight of empty filter paper used to collect alkaloid precipitate (g), and weight of dried filter paper and dried alkaloid precipitate (g), respectively (Li et al., 2017).

**Determination of vitamin C of extracted protein isolate:** The determination of vitamin C content was carried out as described by Obloh and Omoregie, (2008). The protein isolate was precipitated according to the method of Maehre et al., (2018) with slight modification. 1 g of the protein isolate was diluted with 6% trichloroacetic acid to make up 10 ml and then centrifuged at 3,000 rpm for 10 minutes. The supernatant was collected for the purpose of identification, and 9 mL of 2,6-dichlorophenolindophenol (DCPIP) dye solution was added to 1 mL of the supernatant. The mixture was thoroughly mixed, and the absorbance was measured at 520 nm using a UV-Vis spectrophotometer.

Concentration of vitamin C was determined using a standard calibration curve prepared with ascorbic acid and expressed as milligrams of ascorbic acid equivalent per gram of sample (mg AAE/g) (Obloh and Omoregie, 2008).

## Results and Discussion

### Foaming properties of extracted protein isolate from almond seed

Foaming capacity (FC) is a desirable attribute for products such as whipped toppings, mousses, cakes etc. where it is desirable to incorporate air into a system. The foaming capacity of the almond protein isolates from the present study, as presented in Table 1, was 0.78 ± 0.07 g/g, which is relatively lower than that reported for almond and soybean isolate (1.20 to 1.60 g/g) by Sze-Tao and Sathe (2000) while

evaluating the functional properties of almond and protein isolates, using alkaline extraction at pH of 8.1.

It was observed that foaming capacity depends mostly on the ability of the protein to be adsorbed at air-water interface in order to reduce the surface tension and stabilize the dispersion of air bubbles. The lower foaming capacity attained in this study may mean that the protein surface characteristics changed due to extraction, probably through denaturation or aggregation of the protein (Mæhre *et al.*, 2018). This could have lowered the stability of the protein foams because the denatured proteins are not capable of forming a strong layer around the foam bubbles (Mæhre *et al.*, 2018). Furthermore, the interval of pH in the foaming test, which is expected to reduce protein solubility and interaction at interfaces, could not have been well regulated and, therefore, low foaming characteristics recorded (Hadinoto *et al.*, 2024).

### **Functional properties of extracted protein isolate from almond seed**

#### *Water absorption capacity*

The water absorption capacity (WAC) of protein isolates is important since it determines the convenience of the protein in functional foods such as baked goods and meat analogs. The amount of water absorbed in this study, as shown in Table 1, was  $0.77 \pm 0.08$  g/g and is slightly lower than the values (1.20 to 1.42 g/g) reported by Sze-Tao and Sathe, (2000) in the enzyme assisted aqueous extraction of almond protein isolates.

There are several factors that dictate the WAC of protein isolates. Loss of structural configuration by protein through heat during extraction might also have hampered its ability to bind water due to variation in the extent of hydration of the extracted protein. Another potential factor is the low surface area of the protein isolate hence it cannot adsorb water molecules effectively. Other proposed factor is the pH of alkaline employed for solubilization of flour. The observation in this study is in congruent with the findings of Devnani *et al.*, (2021), who reported that, when the pH level is regulated at its proper level (8.1), protein's ability to absorb water increases. This implies that extraction procedure could alter the protein structure and hence reducing its overall hydrophilic and surface-active characteristics, since water absorption depends on the polarity of the protein surface and the availability of hydrophilic groups. The lower water absorption capacity in this research might be due to the formation of protein aggregates which decreased the percent protein solubility, as a result of the increased pH of alkaline solution used during extraction although there was no influence on

protein secondary structure (Gao *et al.*, 2020). Therefore, current processing conditions in this study might not be favorable in enhancing water binding as evidenced in earlier studies (Wagner and Anon, 1990), but favourable to obtain protein isolates with less swelling ability and viscosity. This low viscosity and WAC, even at high protein isolate concentration, are reportedly useful in development of protein-dense drinks (Zayas, 1997).

#### *Oil absorption capacity*

Oil absorption capacity (OAC) is another functional property that partly governs protein ability to emulsify oil in food systems and then impact product texture, flavor, and mouthfeel. The oil absorption capacity of the almond protein isolate in the present study, as shown in Table 1, was determined to be  $1.81 \pm 0.38$  g/g and it can be said that the value obtained falls within the range (1.50 – 2.30 g/g) reported by Sze-Tao and Sathe (2000). This is consistent with the comparison of the oil absorption capacity and indicates that the protein isolate maintained its lipophilic character, which is so important in their applications as plant-based meats and dairy products.

The capacity of proteins to soak up oil is based on the hydrophobic side chains of amino acids that bear fat (Shire *et al.*, 2004). The almost identical absorption capacities in the studies suggest that the alkaline extraction process employed in this study retains the facet of the protein that binds oil (Shire *et al.*, 2004). Nonetheless, it can be noted that the present study has slightly higher standard deviation of  $\pm 0.38$ , which indicates that the ability of protein isolates to bind oil was not uniform or constant. This may be attributed to the small differences in the composition or particle size of the protein isolates at the extract facility (Vishwanathan *et al.*, 2011).

### **Antinutrient properties of extracted protein isolate from almond seed flour**

#### *Saponin content*

The saponin content and other antinutrient properties of the extracted protein isolate are reported in Table 2. Saponins are natural glycosides with health promoting activities such as anti-hyper cholesterol and authoritative effects. In this study, the saponin content of the almond protein isolate was  $161.34 \pm 1.67$  mg/g which was significantly higher than the values reported in the literature for almond protein (Amirshaghghi *et al.*, 2017). It is possible that the almond used in this study belongs to a specific type with higher saponin content or whether the processing conditions of almonds induce higher retention of saponins. Although, saponins play a role in the antioxidant activity of proteins (Amirshaghghi *et al.*, 2017; Del Hierroi *et*

al., 2018), through their bioactive qualities that act as free radicals' harbingers, and its inclusion at these high levels could further improve the nutritional value of the almond protein isolate for use in functional foods aimed at correcting cardiovascular diseases or strengthening the immune system.

However, the current research also revealed that high concentration of saponins could negatively impact the nutritional value of protein isolate by decreasing the protein digestibility (Timilsena et al., 2023).

Table 2: Antioxidant properties of extracted protein isolate from almond

Sample	Saponin (mg/g)	Tannin (mg/g)	Flavonoid (mg (QE)/g)	Phytate (mg/g)	Alkaloid (%)	Phenol (mg GAE/g)
TOL	161.34 ± 1.67	0.05 ± 0.01	4.95 ± 0.48	12.88 ± 0.89	2.32 ± 0.46	7.85 ± 0.43

Values are presented as mean ± SD (n=3); TOL: Sample of

#### Flavonoid content

Flavonoids are considered polyphenolic compounds that have preeminent free-radical neutralization capability. The flavonoid content of the almond protein isolate in this study was determined to be  $4.95 \pm 0.48$  mg (QE)/g. As observed from another study by Arangia et al. (2023), almond skin extracts higher flavonoids content, this study estimated a relatively low flavonoid level. This might be due to the fact that the study was conducted on the protein isolate and flavonoids are known to be more concentrated in almond or almond skin, rather than the almond protein isolate. However, the flavonoid content which is relatively low still makes a contribution to the antioxidant capacity of the protein isolate, which is ideal for foods to be consumed in order to minimize oxidative pressure on the body (Polisak et al., 2021).

#### protein isolate produced from almond seed flour

##### Phenolic content

Phenols are another category of compounds often used in foods due to their great antioxidant abilities. The phenol content of the almond protein isolate used in this study was  $7.85 \pm 0.43$  mg (GAE) per g, which falls within the normal range of values of other studies such as Arangia et al. (2023). The phenolic compounds which are found in almond proteins are likely to confer several potential health promoting effects; foremost of these is the ability to remove reactive oxygen species which cause chronic illnesses such as cardiovascular ailments and cancers (Matsumura et al., 2023). The moderate phenolic content established in this study supports the recommendation that almond protein isolate could serve as a functional component in food products that boost overall health especially in products that promote antioxidant rich diets (Matsumura et al., 2023).

##### Tannin content

The tannin content in this study ( $0.05 \pm 0.01$  mg/g) is significantly lower than values reported in other plant-based proteins. For example, previous studies

have found that soybean protein isolates contain tannins ranging between 0.2 and 1.5 mg/g, depending on processing methods (De Souza et al., 2020). Tannins are known to reduce protein digestibility by forming insoluble complexes with proteins and minerals, but the low levels in almond protein isolate indicate minimal interference with nutrient bioavailability (Zayed et al., 2025; Cosme et al., 2025).

##### Phytic acid content

The phytic acid content ( $12.88 \pm 0.89$  mg/g) observed in this study is comparable to the values in legumes such as chickpeas, which range from 10 to 15 mg/g (Lai et al., 2013). While phytic acid is an antinutrient that chelates essential minerals, it also possesses antioxidant properties that can contribute positively to the health benefits of almond protein isolate (Chen and Xu., 2023).

##### Alkaloid content

The alkaloid content ( $2.32 \pm 0.46\%$ ) is higher than values typically reported for legumes, which are often below 1% (Borokinni et al., 2022). Alkaloids enhance the antioxidant capacity of plant-based proteins, providing additional health benefits such as anti-inflammatory and antimicrobial properties. This makes almond protein isolate a valuable ingredient for functional foods (Singh and Sharma., 2013; Sychrová et al., 2020).

##### Vitamin C

Vitamin C is also regarded as a potent antioxidant that also supports immune function. In this study, the vitamin C content, as shown in Table 3, was found to be  $3.29 \pm 0.05$  mg/g. While almond proteins are not typically recognized as major sources of vitamin C, the presence of this antioxidant adds to the overall antioxidant profile of the protein isolate (Didier et al., 2023). This makes the almond protein isolate a more comprehensive functional ingredient, contributing not only to protein enrichment but also to antioxidant fortification in food products (Każmierczak-Barańska et al., 2020).

When compared to other plant protein isolates, vitamin C levels in almond protein isolate are competitive. For example, studies on legume protein isolates such as soy and chickpeas typically show negligible vitamin C content (Begum et al., 2023). In contrast, extracts from seeds like moringa or

baobab, known for their high antioxidant capacities, may contain higher levels of vitamin C, albeit as part of a broader phytochemical profile (Neji et al., 2022; Cruz-Solis et al., 2023).

Table 3: Vitamin C composition of extracted protein isolate from almond

Sample	Vit. C (mg/g)
TOL	3.29 ± 0.05

Values are presented as mean ± SD (n=3); TOL: *Sample of protein isolate from almond seed flour*

## Conclusion

The effect of alkaline extraction method for protein extraction from almonds was successfully conducted in this study and the biological functions, antioxidant activity, and vitamin C content were also determined. The almond protein isolate demonstrated excellent oil absorption ( $1.81 \pm 0.38$  g/g) for its use in the food items that require emulsifying properties such as plant-based meats and dairy products. This radical scavenging activity, in conjunction with moderate antioxidant compositions, saponins which averages  $161.34 \pm 1.67$  mg/g and phenolic compounds that averages  $7.85 \pm 0.43$  mg GAE/g, make it a suitable functional ingredient for foods with potential to minimize oxidative stress and improve the cardiovascular system.

Nonetheless, the water absorption capacity was just  $0.77 \pm 0.08$  g/g and the foaming capacity  $0.78 \pm 0.07$  g/g is lower than the values reported in the literature. These differences underline the necessity for improving extraction conditions, such as extractant concentration and extraction temperature, and its attendant effects on the structural nature (denaturing or otherwise), WAC, OAC and phytochemicals of the extracted isolates in order to positively influence functional characteristic of almond protein.

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