

## OPTIMIZING ULTRASONIC ASSISTED EXTRACTION OF PROTEINS FROM PUMPKIN SEEDS FOR COOKIE FORTIFICATION

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### ABSTRACT

This research aimed to optimize ultrasonic-assisted extraction of proteins from pumpkin seeds for their effective use in cookie fortification. Ultrasonic treatment was applied to samples at varying time duration (10, 15, 20 and 25 minutes) while the other parameter like temperature and frequency remained constant at 32°C and 25kHz for all the 4 treatments. The maximum yield of (42.23%) was obtained at 25minutes. The protein isolates were then analyzed for their different functional properties including protein solubility, emulsifying properties, foaming properties and dispersibility. Ultrasonically extracted pumpkin seed protein isolates were then incorporated into wheat flour at substitution levels of 10%, 15%, 20%, and 25% to develop fortified cookies. Different analysis including proximate analysis, mineral composition, texture, color, physical properties, and sensory analyses was performed on cookies. Significant results were observed for proximate analysis. Iron, zinc, and potassium content increased with pumpkin seeds protein isolates addition, with iron peaking at (2.09%), zinc at (2.81%), and potassium at (127.70%). Texture hardness also increased from (43.15 to 51.22), while diameter and spread ratio decreased, indicating denser cookie structures. Color analysis confirmed a decrease in lightness (L\*) and an increase in redness (a\*) and yellowness (b\*) with higher pumpkin seeds protein isolates levels. On the basis of sensory evaluation, it was identified that T<sub>2</sub> (15%) pumpkin seeds protein isolates as the most acceptable formulation, with highest score in color (8.12), taste (8.08), texture (7.71), aroma (8.25), and overall acceptability (7.90). Higher substitution levels negatively affected sensory perception despite enhanced nutritional content. In conclusion, the optimal level of protein isolate from pumpkin seeds for cookie fortification was determined to be 15%, balancing improved nutritional value with consumer preferred sensory qualities. This research supports the potential of ultrasonic assisted extracted pumpkin seed protein as a sustainable and functional ingredient in baked goods.

**Keywords:** Ultrasonic assisted extraction, Plant based sources, Functional properties of protein, Fortification, Quality analysis of cookies

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### 1. INTRODUCTION

The growing demands for high protein foods have triggered the evolution of non-conventional sources of protein and the waste from food processing without causing any damage to the surroundings and the environment. Cereal waste and the waste from oilseeds are immensely used as a protein source nowadays, containing about 15–20% proteins after Defatting procedures (Singh *et al.*, 2022). Plant proteins which are recovered from the food processing by-products are an excellent substitute of animal proteins and also a sustainable approach in the food processing and production system. The extraction method to be followed depends on the designated use of the final product. For instance, enzymatic extraction of proteins is suitable for application in emulsion-based foods (Kleekayai *et al.*, 2023). Pumpkin seeds are considered highly nutritious and have good biological activity as they have high content of oil and protein. A by-product of pumpkin oil production, a seed cake contains up to 60% protein but is often sold cheaply as feed additives or abandoned, leading to waste of protein resources. Pumpkin seed proteins are rich in arginine and leucine, with an essential amino acid composition similar to the requirements

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of the human body. Various studies have demonstrated that pumpkin seed protein has several beneficial effects including their role as an antioxidant and angiotensin-converting enzyme inhibitor activity, making pumpkin seed protein isolates (PSPI) a promising and prominent ingredient in the food industry. However, research on pumpkin seed protein has mostly focused on solubility, emulsifying properties, and biological activity, with little work on heat-induced gelling properties (Habib *et al.*, 2025).

Ultrasonic-assisted extraction (UAE) has emerged as a sustainable technology for protein recovery, as it preserves structural and molecular properties during extraction, improves efficiency, requires less solvent, and provides high yield. UAE employs high-frequency waves which create cavitations, disrupting the cell walls and liberating the target compounds. Extraction proficiency is influenced by multiple factors such as frequency, wave intensity, solvent choice, extraction time, and temperature (Xiang *et al.*, 2024; Bhadange *et al.*, 2024). Current research suggest UAE as a promising method for extracting proteins from oilseeds like sunflower seeds, seeds of watermelon and pumpkin, soybean, groundnut, rice bran (Kumar *et al.*, 2021).

Public health guidelines highlight the role of fortification in food products in order to improve the nutritional intake. Application of plant by-products for fortification is rising as an alternative approach to combat nutritional deficient diseases like malnutrition and hidden hunger. Fortification is a cost-effective strategy to enhance public health and it also ensures sustainability in the food production systems (Darko *et al.*, 2024; Geny *et al.*, 2024). Plants are cheaper and more abundant sources of proteins than animal sources, provided they maintain acceptable functional properties. Studies have shown that pumpkin seed protein isolates hold better physicochemical properties, making them a valuable alternative for food industry applications (Habib *et al.*, 2025). Despite this, pumpkin seed proteins remain under-explored for fortification in bakery products, with most studies focusing on oil extraction. Thus, there is strong rationale for exploring UAE as a competent and efficient method for extracting pumpkin seed proteins and their application in cookie fortification (Sá *et al.*, 2023).

## 2. MATERIALS AND METHODS

### 2.1 Procurement of Raw Materials

Pumpkin seeds were procured from the local market of Faisalabad. All the required chemicals (hexane, sodium hydroxide and others) to perform analysis were acquired from the lab of University of Agriculture, Faisalabad and chemical store.

### 2.2 Preparation of Pumpkin Seeds Powder

Pumpkin seeds were firstly cleaned properly to remove any impurities like dust, stones etc. Then the seeds were sun dried and ground to make a powder by using a portable mixer grinder. Then the seed powder was placed in an air tight container and stored at room temperature for later use.

### 2.3 Proximate Analysis of Pumpkin Seeds

Moisture content, ash content, crude protein, crude fiber and the fat content were analyzed in pumpkin seeds powder by following methods as prescribed in AOAC (2016). All protocols regarding analysis are described below.

#### 2.3.1 Moisture Content

Moisture content of pumpkin seed powder sample was performed according to the method prescribed in AOAC (2016). Weight of china dish was recorded and labeled then took 5 grams sample of pumpkin seed powder in clean and dry china dish. Then the sample was taken in china dish and kept in an oven at 105°C for 24 hours. After 24 hours the china dish was taken away from oven and placed in desiccators for 5 to 10 minutes. Sample with china dish weight was noted and again kept into oven until constant weight. Triplicate readings were used to ensure the results. Moisture was estimated by using the following equation.

$$\text{Moisture Content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

#### 2.3.2 Crude Protein Content

Kjeldal Apparatus was used to find out the crude protein content in pumpkin seed powder as mentioned in AOAC (2016) method. 3 grams sample of pumpkin seed powder was taken into digestion flask with 30 mL concentrated sulfuric acid and digestion tablet. Digestion flask was placed into boiling roll at temperature of 370°C to 400°C. Mixture was boiled until fumes appeared and method was carried out for 1 hour until light green or transparent color of sample obtained. Then digestion flask was cooled by adding 250 mL of distilled water. The process of distillation was carried out through taking 10 mL diluted sample along with 40% NaOH solution at concentration of 10 mL in the flask. Then 4% boric acid was mixed with liberated ammonia and methyl red indicator was used to get final results. Mixture was converted to ammonium borate by distillation

process. In titration process finally the sample was titrated into the burette against 0.1N H<sub>2</sub>SO<sub>4</sub> for estimation of nitrogen content until the mixture color changed into pink. Crude protein was estimated by using the following equation.

$$\text{Nitrogen \%} = \frac{\text{Vol. of 0.1N H}_2\text{SO}_4 \text{ used} \times 0.0014 \times \text{Vol. of dilution (250 mL)}}{\text{Vol. of distillate sample taken} \times \text{Weight of sample}} \times 100$$

$$\text{Crude Protein \%} = \text{Nitrogen \%} \times 6.25$$

### 2.3.3 Crude Fat

Pumpkin seed powder was analyzed for fat content according to the method prescribed by AOAC (2016). The method employed for fat determination included the following steps. First of all, cleaned all glass wares with a petroleum spirit, drained them out and then dried them in hot air oven at 102°C for about 30 minutes and then cooled them in desiccators so the sample does not absorb the atmospheric moisture. After cooling, piece of cotton wool was placed at bottom of the flask. Then extraction thimble was stood in flask by placing wedge of cotton wool in bottom of thimble. After that, 5 grams of properly blended sample was taken in an extraction thimble, coated with filter paper. Then dried sample was put into hot air oven at the temperature of 102°C for 5 hours and cooled the sample in desiccators used in laboratory. After that the washing of the soxhlet was started for this purpose, the thimble was embedded in a soxhlet extractor. Perfectly weighed 150 mL round bottom flask was then thoroughly cleaned and dried. Put approximately 90 mL of hexane in the round bottom flask and assembled the extraction unit over an electric mantle for heating. Then the solvent was heated until hexane evaporated and condensed in a tube having the sample. The hexane washed out and came back to round bottom flask. Total 6 washings were done for fat extraction from the sample. The process continued for 2 hours till all the fat was extracted from sample. After that removed sample and put sample into oven for drying at 105°C for 1 hour and weighed. Given equation was then used to determine the crude fat content.

$$\text{Crude Fat \%} = \frac{\text{Sample weight (before extraction)} - \text{Sample weight (after extraction)}}{\text{Weight of sample}} \times 100$$

### 2.3.4 Crude Fiber

Crude fiber content of seed powder was checked for crude fat content through method described in AOAC, (2016). Fat free sample along with 2.5 mL sulfuric acid was taken into 500 mL of beaker and made volume up to 200 mL by addition of distilled water. Mixture was boiled for half hour. Boiled sample was made acid free by 2 to 3 washings of distilled water. 1.25% NaOH solution was then added and again sample was boiled for half hour and washed by distilled water to make it alkali free. Then the Sample was taken into crucible and placed into oven at temperature of 105°C for 2 hours. After moisture removal sample was weighed and put into muffle furnace for 5 to 6 hours at 550-650°C. Then sample was taken out from muffle furnace, cooled and weighed. Crude fiber was estimated by following equation.

$$\text{Crude Fiber \%} = \frac{\text{Sample weight before ashing} - \text{Sample weight after ashing}}{\text{Weight of sample}} \times 100$$

### 2.3.5 Ash Content

Ash content in pumpkin seed powder was quantified according to procedure mentioned in method of AOAC, (2016). 3 grams of oven dried moisture free sample was taken in pre-weighed crucible dish. Sample was burnt to make them smoke free this process is called "charring". Then sample was put at 550-600°C for 5 to 6 hours in muffle furnace till grayish ash was obtained. Equation mentioned below was used to determine amount of ash.

$$\text{Ash \%} = \frac{\text{Weight of Ash}}{\text{Sample weight}} \times 100$$

### 2.3.6 Nitrogen Free Extract (NFE)

Determination of Nitrogen free extract was done in accordance to the given equation.

$$\text{NFE} = 100 - (\text{moisture \%age} + \text{ash \%age} + \text{crude fiber \%age} + \text{crude fat \%age} + \text{crude protein \%age})$$

## 2.4 UAE of Pumpkin Seeds

### 2.4.1 Preparation of Pumpkin Seeds for Analysis

The procedure proposed by (Güldane, 2024) was used with slight modifications for the preparation of pumpkin seed samples for protein extraction. First of all, oil from the pumpkin seeds was removed by following the Soxhlet extraction process. The pumpkin seeds were crushed by using blender apparatus. The resulting sample was then mixed with hexane in a ratio of 10:1 (v/w) and then subjected to Soxhlet apparatus for the purpose of extraction. After the defatting procedure the seeds were dried at 40°C for 24 hours in an oven. After the drying process, the seeds were properly pulverized. The defatted seed powder was then packed in an airtight or polyethylene bags and then stored at +4°C up till further use.

#### 2.4.2 Protein Extraction Process

UAE of pumpkin seeds was performed at varying time duration to obtain the maximum yield. UAE of protein from the pumpkin seeds was carried out for 10, 15, and 20 and 25 minutes for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively while the frequency remains the same at 25 kHz.

#### 2.4.3 Ultrasound Treatment

UAE of protein was executed by using ultrasonic homogenizer which has 20-25 kHz frequency and a probe of 6 mm diameter. In a 100 mL beaker, 5 grams sample of defatted pumpkin seeds and 50 mL of sodium hydroxide was taken. The ultrasound probe was inserted in the beaker for a maximum of 1 cm deep into the solution. The solution was treated by using the ultrasonic homogenizer at varying treatment time of 10, 15, 20 and 25 minutes keeping the temperature fixed at 32°C and the ultrasound power 200W. The solution's temperature was monitored continuously and was managed by employing the water bath. The solution was stirred continuously for the selected period of 20 by using a magnetic stirrer. The sample was then centrifuged at 5000 × g for 15 minutes at 4°C. Then the supernatant was assembled, and the precipitate was again dissolved in the 0.1N NaOH and stirred for about 1 hour. It was then again centrifuged for about 10 minutes at 5000 × g. Then the collected supernatant and the previously collected were combined, for the further isolation of proteins. After the centrifugation the combined supernatant was acidified to pH 5 by using 0.1 HCl. It was then centrifuged for 25 minutes at 7000 × g at 4°C. The precipitates were the pumpkin seeds protein isolates (PSPI).

#### 2.4.4 Estimation of PSPI

Kjeldhal method was followed for the estimation of crude protein content. The crude protein yield was estimated by using the following equation.

$$\text{Protein yield (\%)} = \frac{\text{Weight (g) of PSPI}}{\text{Weight (g) of meal}} \times 100$$

#### 2.5 Analysis of Proteins

The PSPI were analyzed for different functional properties as discussed below

##### 2.5.1 Protein Solubility

The solubility of PSPI was determined by pursuing the prescribed method in (Lv *et al.*, 2019). The protein solution (w/v, 2% in distilled water) was centrifuged at around 10,000 × g for 30 minutes at 4°C. After determining the protein content, the solubility was estimated by the following equation.

$$\text{Solubility (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein in sample}} \times 100$$

##### 2.5.2 Emulsifying Properties

Emulsification properties of the PSPI were figured out by following the prescribed method in (Fatima *et al.*, 2023). The emulsion capacity (EC) was determined by taking 1 gram of sample and dissolving it with 50 mL of 0.1 N NaOH in a 250 mL beaker. Fifty mL corn oil was incorporated in the mixture. It was then homogenized at 10000 rpm for 2 minutes to make a stable emulsion. Then take the 100-measuring cylinder and transfer the emulsion into it. The emulsion capacity was estimated by using the equation given below.

$$\text{EC} = \frac{(\text{Initial volume of oil} - \text{Released volume of oil})}{\text{Weight of the sample}}$$

##### 2.5.3 Foaming Properties

Foaming properties of proteins from pumpkin seeds was determined by using the prescribed method in (Lv *et al.*, 2019). 50 mL of protein solution (w/v, 2% in distilled water) was taken and homogenized it with the homogenizer at 20000 rpm/min for 2 min. It was then transferred into the measuring cylinder of 100 mL. The foam volume was recorded at 0 minute and at 30minutes. Foaming capacity was calculated as.

$$\text{FC (\%)} = \frac{\text{Vol. after homogenization} - \text{Initial volume}}{\text{Initial volume}} \times 100$$

##### 2.5.4 Dispersibility

The dispersibility of PSPI was evaluated at pH 9-12. Defatted pumpkin seeds sample and the 3 grams of PSPI were taken and were mixed with distilled water in measuring cylinder. pH was adjusted to the desired level by adding a few drops of dil. HCl and sodium hydroxide solution. Then distilled water was added to make the final volume of 30 mL. Then the solution was stirred incessantly and then stand for 2 hours to settle down. The dispersibility was then measured by the formula given below.

$$\text{Dispersibility (\%)} = \frac{\text{Total volume} - \text{Settle volume}}{\text{Total volume}} \times 100$$

**2.6 Preparation of Pumpkin Seeds Protein Isolates (PSPI) Fortified Cookies**

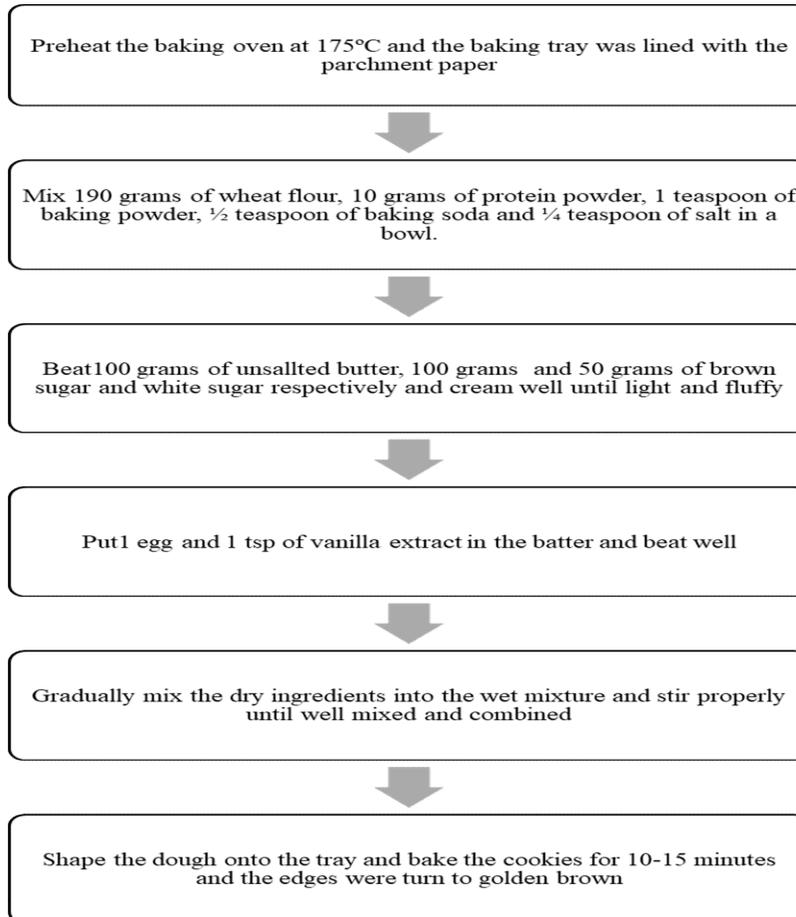
Cookies were prepared by varying percentage of protein isolates extracted from pumpkin seeds according to the treatment plan given below in the (Table 1).

**Table 1:** Treatment Plan for PSPI Fortified Cookies

Treatment	Wheat Flour (%)	PSPI (%)
T <sub>0</sub>	100	0
T <sub>1</sub>	90	10
T <sub>2</sub>	85	15
T <sub>3</sub>	80	20
T <sub>4</sub>	75	25

**2.6.1 Preparation of Dough**

In a bowl, all the dry ingredients including wheat flour, pumpkin seed protein powder, baking powder, baking soda, pinch of salt was sifted and set aside. Then in a separate bowl the unsalted butter was creamed and then granulated sugar was added, then cream for about 2-3 minutes. Then the egg and the vanilla extract were added. Dry ingredients were gradually added to the wet mixture and stirred until well combined. The dough was then scooped onto the tray, spacing them about 2 inches apart. Baked the cookies for 10-15 minutes until the edges was golden brown. A step-by-step procedure for making cookies is described below in the (Fig 1).



**Fig 1:** Preparation of PSPI Fortified Cookies.

**2.7 Analysis of PSPI Fortified Cookies**

**2.7.1 Proximate Analysis**

Cookies were subjected to proximate analysis as mentioned earlier by following the standard AOAC method.

### 2.7.2 Mineral Analysis

Wet digestion was followed by a mineral analysis of the samples using the AOAC (2023). For zinc, potassium, and iron, a flame photometer and an atomic absorption spectrophotometer were employed. The dried samples of 0.5 grams were digested in a volumetric flask at low temperature 60-70°C for 20 minutes with HNO<sub>3</sub> (10 mL/100mL), then heated at high temperature 190°C with HClO<sub>4</sub>, until the contents were fully evident. The samples were moved to a volumetric flask of 100 mL after the digestion, diluted with double-distilled water, and then filtered. Standards of known intensity were initially tested to get a standard curve for each mineral. All the samples were analyzed for potassium using flame photometer and the iron and the zinc content were assessed by operating absorption spectrophotometer.

### 2.7.3 Textural Analysis

The developed product was subjected to texture analysis using a texture analyzer (Khan *et al.*, 2024). The cookies were cut into equal shapes and thickness. The compression test was carried out. To record the data analyzer was coupled with computer. Each treatment was repeated for three measurements and mean value was computed. Based on the curves, various attributes were determined.

### 2.7.4 Color Analysis

The color of cookies sample was determined by utilizing a colorimeter (Ranasinghe *et al.*, 2024). Color analysis was performed by colorimeter by directing them to the path of light. In Petri dishes, grounded product samples were placed. Samples were leveled to remove the extra sample so the optimum reflection of light could achieve. The L\*, a\*, and b\* value was noted for color measurement of the product.

### 2.7.5 Analysis for Physical Characters

#### 2.7.5.1 Diameter

The diameter of PSPI fortified cookies was measured in accordance to the procedure described by (Ikuomola *et al.*, 2017). Put the four cookies edge to edge and their combined diameter was measured with the ruler. Then the cookies were turned at 90-degree angles for duplicate reading. The average diameter was measured in millimeter after repeating the experiment.

#### 2.7.5.2 Thickness

The thickness of the fortified cookies was determined based on the procedure (Ikuomola *et al.*, 2017). It was determined using a digital vernier caliper with 0.01 mm accuracy.

#### 2.7.5.3 Spread Ratio

Spread ratio of the cookie samples fortified with PSPI was measured as described by Ikuomola *et al.*, (2017). Two rows of four cookies (that were well formed) were made and then their height was taken. They were arranged horizontally, edge to edge and their sum (total) of diameters were taken. The spread ratio was obtained as by dividing the diameter by height.

#### 2.7.5.4 Breaking Strength

Breaking strength was estimated by following the method outlined by Ikuomola *et al.*, (2017). Cookies of which the thickness was known were positioned centrally between two parallel metal strips about 3 cm apart and weights were loaded until the cookies broke. The minimum weight resulted in smashing of the cookies was considered the breaking strength of those cookies.

### 2.7.6 Sensory Evaluation

The developed product was subjected to sensory evaluation by the testing panel for various quality characters (such as appearance, aroma, taste, texture, and overall acceptability) using the 9-Point Hedonic Score System (Sik *et al.*, 2024). Faculty members and postgraduate students of NIFSAT were the part of evaluation to choose the best treatment combination. The scale ranged from 1 (extremely disliked) to 9 (extremely liked). The product was put in random boxes and presented in front of evaluation panel for unbiased opinion. The water was provided to the panelists to enhance the result's accuracy.

### 2.8 Statistical Analysis

The results collected for each parameter was statistically analyzed subjecting to one-way ANOVA under CRD to check the level of significance as described by Montgomery (2017). Each treatment was repeated for three measurements, and the mean was computed.

### 3. RESULTS AND DISCUSSION

#### 3.1 Proximate Analysis of the Raw Material

##### 3.1.1 Moisture Content

According to proximate analysis the results revealed that moisture content of pumpkin seed powder was (4.89±0.03). Almost same result (5.2±0.05) was determined for moisture content of pumpkin seed powder by Dhiman *et al.* (2018). The result is also very close to the result (5.00%) of the research conducted by Elinge *et al.* (2012).

##### 3.1.2 Crude Protein Content

Results obtained from analysis observed that the protein content of pumpkin seed powder was (28.70±0.54). According to previous research the protein content present in pumpkin seed protein of two species were (27.27±0.14) and (27.78±0.14) respectively as same to present data Elkhedir and Mustafa, (2015). Research done by Abd-Elnoor, (2019) also found similar result (28.50±0.33) for crude protein content.

##### 3.1.3 Crude Fat Content

Results of the proximate composition analysis revealed that the fat content of pumpkin seed powder was (44.31±0.12). The data of current study is in accordance to the result (48.13±0.57) found by Dhiman *et al.* (2018) with slight difference. Similar result (47.03%) was found by Badr *et al.* (2011). The variation might be because of the drying and grinding effects.

##### 3.1.4 Crude Fiber Content

Analysis determined that the result for crude fiber content of pumpkin seed powder was (2.63±0.15). The same result (2.70%) was determined in research conducted by Besaar *et al.* (2008). The research executed by Hamed *et al.* (2008) also found the same result (2.98±0.06) to this study.

##### 3.1.5 Ash Content

Analysis regarding ash content of seed powder revealed that the ash content of the powder was (4.76±0.77). The value for ash content of pumpkin seed powder was almost equal to the value of ash content (5.53±0.0) described by Elkhedir and Mustafa, (2015). The same result (5.16±0.40) was determined in a past study observed by Uduwerella *et al.* (2021).

##### 3.1.6 Nitrogen Free Extract (NFE)

Proximate analysis results showed that the NFE value for pumpkin seed powder was (13.51±0.04). The obtained data was in compliance with result (9.91±0.03) determined by Vaishali and Parameshwari (2019) with little variation. Another study conducted by Hamed *et al.* (2008) found value (15.63±0.03) having a variation as compared to present data. Variation in the NFE content might be because of the drying, processing and grinding effects of seeds.

#### 3.2 Analysis of Pumpkin Seed Protein Isolates

##### 3.2.1 Protein Yield

Protein yield is an important parameter for describing the efficiency of the UAE process. UAE greatly accelerates cell wall disruption and allows intracellular protein content release, thus enhancing the extraction yield. The result found that the highly significant difference ( $p < 0.01$ ) exists between different treatments. Protein yield in this research varied between (33%-42%) as shown in the (Fig 2), keeping the ultrasound parameters like frequency 25 kHz and temperature 32°C at constant and the time were variable. T<sub>4</sub> gave the maximum yield (42.23%) which was treated for 25 minutes, followed by T<sub>3</sub> (40.46%), T<sub>2</sub> (36.42%) and T<sub>1</sub> (33.48%). Results (54.19%) observed by Lv *et al.* (2019) was close to this research with some difference. The research study conducted by Das *et al.* (2022) also generates the same result (48.10%) with little difference. The variation in protein yield may be attributed to differences in experimental parameters or equipment scale.

##### 3.2.2 Protein Solubility

Protein solubility is a basic functional property that, in turn, affects others, like emulsification and foaming. Solubility was greatly enhanced by UAE because of exposure of hydrophilic groups and less molecular aggregation. The results of the experiment were highly significant ( $p < 0.01$ ) between different treatments. Protein solubility in this research varied between (18.92%-24.78%) shown in (Fig 2), keeping the ultrasound parameters like frequency 25 kHz and temperature 32°C at constant and the time were variable. T<sub>4</sub> gave the maximum protein solubility (24.78%) which was treated for 25 minutes followed by T<sub>3</sub> (23.46%), T<sub>2</sub> (21.79%) and T<sub>1</sub> (18.92%). These findings

suggest that optimal UAE conditions can provide more functional and bioavailable protein fractions ideal for food fortification. The UAE of *Moringa oleifera* seeds was conducted. A significant rise was found in the solubility of *M. oleifera* seeds protein isolated by the ultrasonic process, which was (29.82%). The result of the present research was comparable to the study conducted by Fatima *et al.* (2023) on *M. oleifera* seeds. The results were also close to the study conducted by Lv *et al.* (2019) which was (28.76%) with small difference. The variation might be due to time variation and equipment setup.

Functional properties of protein isolates across treatments (T<sub>1</sub>-T<sub>4</sub>)

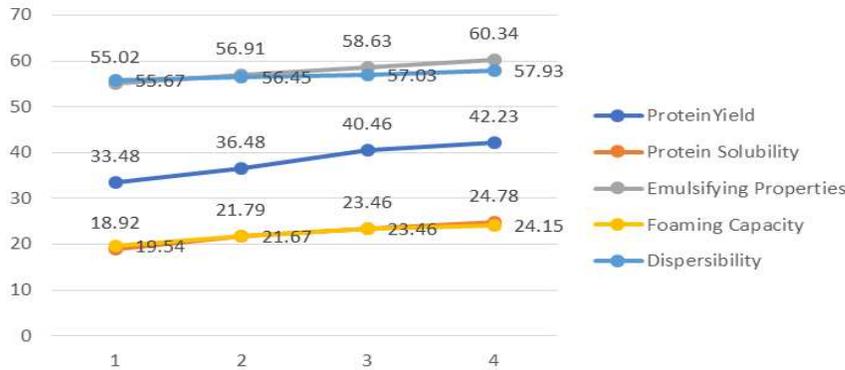


Fig 2: Results of Functional Properties of PSPI.

### 3.2.3 Emulsifying properties

The emulsifying activity is the capacity to create an emulsion and to maintain the stability of the resultant emulsion. It is a significant factor in the manufacturing of numerous fabricated foods. The emulsion capacity of ultrasound-extracted pumpkin seed protein was found to be (57.16-65.47 mg/mL) as illustrated in (Fig 2), keeping the ultrasound parameters like frequency 25 kHz and temperature 32°C at constant and the time were variable, which reflects its capacity to stabilize the emulsions. UAE enhanced the surface activity of the protein by partially denaturing protein structures, which would expose hydrophobic groups improving interaction at the oil/water interface. These findings indicate the promise of pumpkin seed protein in food applications requiring emulsification, for example, cookie dough with fats. The results of the experiment were highly significant ( $p < 0.01$ ) between different treatments. T<sub>4</sub> gave the maximum emulsion capacity (60.34 mg/mL) which was treated for 25 minutes followed by T<sub>3</sub> (58.63 mg/mL), T<sub>2</sub> (56.91 mg/mL) and T<sub>1</sub> (55.02 mg/mL). The study performed by the Fatima *et al.* (2023) also observed the similar results (75.93 mg/mL) with some variation. The study concluded by Naik *et al.* (2022) also observed the same trend for the emulsification activity of bitter melon seeds meal protein. The variation might be due to the extended time, equipment setup or the denaturation of proteins.

### 3.2.4 Foaming Properties

Foaming capacity is the ability to trap air and create stable foam upon whipping or agitation. Pumpkin seed protein isolated by UAE in this research had a foaming capacity of (19.54%-24.15%) as seen in (Fig 2), which suggests its capacity to create a protein film at the air-water interface. The increased foaming seen in UAE-treated samples can be associated to the partial unfolding of protein, which strengthens surface hydrophobicity and flexibility, enhancing air entrapment. This functional attribute is particularly valuable in baked foods, where foam enhances volume and structure. UAE improved this characteristic by decreasing protein aggregation and enhancing surface flexibility such that proteins could quickly adsorb at the air-water interface and create stable foams. However, extremely high-power ultrasound intensity decreased foam volume slightly, perhaps owing to the denaturation of the protein. The results of the experiment were significant ( $p < 0.05$ ) between different treatments. T<sub>4</sub> gave the maximum result (24.15%) which was treated for 25 minutes and T<sub>1</sub> gave the minimum foaming capacity of (19.54%). The study directed by the Lv *et al.* (2019) also observed the similar results (20.03%) with minor difference. The variation might be due to the extended time of UAE. Similar increasing trend also found in the research conducted by Fatima *et al.*, (2023) who apply ultrasonication on moringa seeds for protein extraction.

### 3.2.5 Dispersibility

Dispersibility may refer to the ease with which the protein powder rehydrates and disperses homogeneously in aqueous solutions. It is desirable to have good dispersibility to add proteins to cookie dough without clumping.

UAE treatment enhanced the dispersibility index, presumably because of smaller particle size and more homogeneous powder morphology. The enhanced dispersibility of pumpkin seed protein facilitates more uniform mixing and incorporation in cookie recipes, improving texture and nutritional quality. The results of the experiment were significant ( $p < 0.05$ ) between different treatments. Dispersibility of protein in this research varied between (55.67%-57.93%), see (Fig 2), keeping the ultrasound parameters like frequency 25 kHz and temperature 32°C at constant and the time were variable. T<sub>4</sub> gave the maximum dispersibility results (57.93%) which was treated for 25 minutes followed by T<sub>3</sub> (57.03%), T<sub>2</sub> (56.45%) and T<sub>1</sub> (55.67%). The UAE of proteins from pumpkin seeds were conducted by Das *et al.* (2022). The maximum dispersibility observed was (68%).

### 3.3 Proximate Analysis of PSPI Fortified Cookies

#### 3.3.1 Moisture Content

The results of the moisture content of cookies found that significant difference ( $p < 0.05$ ) exists between different treatments. Moisture content of cookies was ranged from (3.16% to 5.19%) between different treatments as depicted in (Table 2). The treatment sample T<sub>4</sub> containing maximum moisture content of (5.19%) which was holding 25% pumpkin seed protein powder followed by T<sub>3</sub> (4.61%), T<sub>2</sub> (4.05%), and the T<sub>1</sub> (3.95%) while the minimum level of moisture content was observed for control sample T<sub>0</sub> (3.16%), having 0% pumpkin seed protein powder. The results of various treatments regarding moisture content are related to the findings noted by Dhiman *et al.*, (2018) who prepared weaning mix and cookies supplemented with pumpkin powder and pumpkin seed kernel powder and showed that moisture content of weaning mix and cookies as (4.05% to 4.17%) and (2.14% to 2.17%) respectively for weaning mix and cookies. It illustrates that moisture contents increased when increasing the pumpkin seed powder in the flour blends. In another study, Kumari *et al.* (2020) developed and evaluated nutritional enriched biscuits by incorporating seed flour of germinated pumpkin seeds and showed that moisture contents of biscuits are in the range of (2.20% to 2.94%) and reported that moisture content of biscuits increase with increased concentration of pumpkin seed flour supplementation. In another study, Hayat *et al.* (2022) prepared cookies by incorporating plant-based protein isolates from red kidney beans at different concentration. It also revealed that moisture content in cookies varied from (3.14% to 5.59%) showing that the moisture level risen as the concentration of protein isolates increased. The variation might be due to baking conditions, instrument calibration and temperature fluctuations which influenced the moisture level of cookies.

**Table 2:** Mean Values for the Proximate Analysis of PSPI Fortified Cookies

Treatment	Moisture	Protein	Fat	Fiber	Ash	Carbohydrates
T <sub>0</sub> (Control)	3.16±0.27 <sup>c</sup>	9.78±0.66 <sup>d</sup>	26.92±1.14 <sup>a</sup>	1.61±0.93 <sup>a</sup>	0.23±0.09 <sup>d</sup>	56.83±1.89 <sup>a</sup>
T <sub>1</sub> (90% Wheat Flour, 10% PSPI)	3.95±0.46 <sup>bc</sup>	11.85±1.12 <sup>c</sup>	25.39±0.74 <sup>ab</sup>	1.42±0.42 <sup>ab</sup>	0.44±0.12 <sup>c</sup>	55.62±1.96 <sup>ab</sup>
T <sub>2</sub> (85% Wheat Flour, 15% PSPI)	4.05±0.58 <sup>b</sup>	13.83±0.90 <sup>b</sup>	24.65±0.11 <sup>bc</sup>	1.23±0.57 <sup>ab</sup>	0.59±0.16 <sup>bc</sup>	53.68±1.56 <sup>bc</sup>
T <sub>3</sub> (80% Wheat Flour, 20% PSPI)	4.61±0.32 <sup>ab</sup>	14.86±0.32 <sup>b</sup>	24.02±0.64 <sup>b</sup>	0.82±0.39 <sup>ab</sup>	0.71±0.04 <sup>ab</sup>	51.70±1.29 <sup>b</sup>
T <sub>4</sub> (75% Wheat Flour, 25% PSPI)	5.19±0.57 <sup>a</sup>	16.16±0.59 <sup>a</sup>	22.96±1.59 <sup>c</sup>	0.46±0.29 <sup>b</sup>	0.83±0.07 <sup>a</sup>	50.90±1.12 <sup>c</sup>

#### 3.3.2 Crude Protein Content

The data observed significant difference ( $p < 0.05$ ) among the five treatments of cookies. Mean values seen after the analysis were ranged from (9.78% to 16.16%) for the crude protein as illustrated in (Table 2). The data revealed that protein content of the PSPI fortified cookies was increased when increasing the supplementation of pumpkin seed protein powder. The protein level was highest for T<sub>4</sub> (16.16%), with 25% pumpkin seed protein powder followed by T<sub>3</sub> (14.86%), T<sub>2</sub> (13.83%), T<sub>1</sub> (11.85%) having 20%, 15% and 10% pumpkin seed protein powder respectively and lowest value was in treatment T<sub>0</sub> (9.78%) which was control treatment without pumpkin seed protein powder supplementation. The findings of present research were closed to the results of Dhiman *et al.* (2018) who showed that crude protein contents were increased ranging from (8.47% to 14.21%), when he prepared cookies with different treatments of pumpkin seed kernel powder. In another study, Kanwal *et al.* (2015) supplemented pumpkin seeds in biscuits to combat childhood malnutrition and showed related data as obtained in current study and also reported that the crude protein content of biscuits was slightly increased with increasing supplementation of pumpkin seeds. In another study, Park *et al.* (2021) prepared cookies by incorporating soy and whey protein isolates at varying ratios. The highest crude protein content among all the samples was (26.66%), concluding that amount of protein increased as the ratio of soy protein isolates was increased. The variation might be due to baking conditions, instrument calibration and temperature fluctuations which influenced the protein level of cookies.

### 3.3.3 Crude Fat Content

The results showed that relationship regarding fat content among different treatments was significant ( $p < 0.05$ ). Mean values observed for crude fat contents of cookies were ranged from (26.92%- 22.96%) as shown in (Table 2). The fat content of cookies was decreased as the supplementation pumpkin seed protein powder increased. T<sub>4</sub> contain the minimum fat content (22.96%) which has 25% pumpkin seeds protein powder while T<sub>0</sub> contain the maximum (26.92%) containing 0% protein powder. Similar readings have been recorded by previous study in which Hayat *et al.* (2022) prepared cookies from protein isolates of red kidney beans. It demonstrated that as the concentration of protein isolates increased fat content decreased to (25.11%). In another study by Park *et al.* (2021), cookies were prepared from soy to whey protein ratio. It also observed the same decreasing trend as concentration of protein isolates increased. The results were in compliance with reported decreasing trend in fat contents.

### 3.3.4 Crude Fiber Content

Non-Significant results ( $p > 0.05$ ) were found between various trials. The mean values calculated for crude fiber contents of cookies were ranged between (1.61% - 0.46%) as seen in the (Table 2). Fiber content decreased as the pumpkin seed protein powder increased. The highest value of crude fiber was observed for the control sample T<sub>0</sub> (1.61%) followed by T<sub>1</sub> (1.42), T<sub>2</sub> (1.23%), T<sub>3</sub> (0.82%) while minimum was noted in T<sub>4</sub> (0.46%). The results were quite similar to the results of Hayat *et al.* (2022) who showed, the crude fiber contents of cookies samples incorporated with red kidney beans protein isolates were decreased from (0.50% to 0.43%) comparable to control sample of cookies. In another study, Park *et al.* (2021) also revealed that the fiber contents of the cookies were decreases soy protein isolates concentration increased.

### 3.3.5 Ash Content

Ash content present in product shows presence of mineral and many other trace elements present in food items that play important role in body. Minerals are essential for skeleton, nerve functioning, enzyme as co-factor and oxygen transport (iron). It also effects on properties of food like texture, outlook, stability and flavor. The ash contents of cookies were ranged between (0.23% - 0.83%) as illustrated in (Table 2). The data values obtained were highly significant ( $p < 0.01$ ) between each treatment. Mean values for ash showed increasing trend with increasing supplementation of pumpkin seed protein powder. The highest value was recorded for T<sub>4</sub> (0.83%) followed by T<sub>3</sub> (0.71%), T<sub>2</sub> (0.59%), T<sub>1</sub> (0.44%) and T<sub>0</sub> (0.23%) which was without pumpkin seed protein powder. In the study by Park *et al.* (2021), revealed that the ash contents of the cookies were increased to (1.89%) as soy protein isolates concentration increased. In another study Kanwal *et al.* (2015) also reported increasing trend in ash content in fortified cookies.

### 3.3.6 Carbohydrates Content

The carbohydrate contents of cookies were ranged between (56.83% - 50.90%) as depicted in (Table 2). The data values obtained were significant ( $p < 0.05$ ) between each treatment. Mean values for carbohydrates showed decreasing trend with increasing supplementation of pumpkin seed protein powder. Carbohydrate content decreased as the pumpkin seed protein powder increased. The maximum value of carbohydrate was seen for the control treatment T<sub>0</sub> (56.83%) while minimum was noted in T<sub>4</sub> (50.90%). In the study by Park *et al.* (2021), it was recorded that carbohydrate contents of the cookies were decreased to (40.93%) as soy protein isolates concentration increased. In another study, Hayat *et al.* (2022) reported that carbohydrate content was decreased from (61.61% to 51.59%) when protein isolates of red kidney bean increased. The variation might be due to baking conditions, instrument calibration and temperature fluctuations.

## 3.4 Mineral Analysis

Minerals play essential part in human nutrition and are particularly important in the context of functional and nutritional food development aimed at addressing micronutrient deficiencies. Minerals are crucial for various physiological functions and have a significant part in enhancing the nutritional value of functional foods.

### 3.4.1 Iron Content

Iron is a crucial mineral helps in transporting oxygen in the blood and also supports energy production and brain function. Iron obtained from the plant sources has better absorption ability when it is paired with vitamin C, so including ingredients containing iron can help. Enhancing cookies with iron-rich ingredients can make them a slightly healthier treat. The iron contents of cookies were ranged between (0.77% - 2.09%). The data values obtained were non-significant ( $p > 0.05$ ) between each treatment. Mean values for iron showed increasing trend with increasing supplementation of pumpkin seed protein powder. The level of iron content raised as the pumpkin seed protein powder increased. The highest value of iron was observed for the treatment T<sub>4</sub> (2.09%) while minimum was

noted in T<sub>0</sub> (0.77%). In the study by Alshehry (2020), it was recorded that iron contents of the cookies were increased to (2.69%) as pumpkin seed powder increased.

### 3.4.2 Zinc Content

Zinc, though not a primary ingredient in cookies, plays a significant role in nutrition. It supports immune function, wound healing, and cell growth, making it an essential mineral for overall health. While cookies generally do not provide much zinc, adding ingredients like whole grains, oats, nuts, or seeds can help increase zinc content. Including these can slightly enhance the nutritional value of cookies without compromising taste. The zinc content of cookies showed the increasing trend. The zinc content was ranged between (1.30%- 2.81%). The results are significant ( $p < 0.05$ ) between the treatments. The maximum level of zinc was observed for the treatment T<sub>4</sub> (2.81%) while minimum was noted in T<sub>0</sub> (1.30%). In the study by Alshehry (2020), it was recorded that zinc contents of the cookies were increased to (4.31%) as pumpkin seed powder increased.

### 3.4.3 Potassium Content

Potassium has a valuable role in both the baking process and in the nutritional profile of cookies. In baking, it can act as a leavening agent (such as potassium bicarbonate) and a salt substitute to reduce sodium content. Nutritionally, potassium is essential for maintaining fluid balance, healthy blood pressure, and proper muscle function. While cookies are not typically high in potassium, adding ingredients like bananas, nuts, or dried fruits can boost their potassium content. This makes the treat slightly more beneficial while still being enjoyable. Potassium content also boosted as the concentration of pumpkin seed protein powder raised. The results were highly significant ( $p < 0.01$ ). Potassium content in cookies was ranged between (120.92% - 127.70%). The maximum value of potassium was observed for T<sub>4</sub> (127.70%). In the study by Alshehry (2020), it was recorded that potassium contents of the cookies were increased to (185.97%) as pumpkin seed powder increased. The variation might be due to baking conditions, instrument calibration and temperature fluctuations.

### 3.5 Texture Analysis

Results were highly significant ( $p < 0.01$ ) among various treatments samples. Mean values for texture (hardness) of cookies were ranged between (43.15- 51.22). Increasing flow was resulted as the concentration of pumpkin seed protein powder gets increased. The maximum value of texture (hardness) was found for T<sub>4</sub> (51.22) which was supplemented with 25% pumpkin seed protein powder followed by T<sub>3</sub> (49.68), T<sub>2</sub> (48.17) and T<sub>1</sub> (45.19), while minimum value was noted for T<sub>0</sub> (43.15) which was without supplementation. The results are in accordance to the results of Alshehry (2020) who found that texture (hardness) of cookies was increased from (45.27 to 58.39) by increasing the pumpkin seed powder concentration. In another study Khan *et al.* (2024) also found increasing trend in texture (hardness) from (42.18 to 53.31) in flaxseeds fortified buns. The variation could be due to the pumpkin seed protein powder concentration, mixing of ingredients and quality of dough or due to baking conditions of the product.

### 3.6 Color Analysis

Color has major impact on the acceptability and quality of product. Color can be determined by human senses or by using equipments. In current study the color of cookies was measured by colorimeter. It has three parameters as L, a\* and b\*, determines lightness to darkness, redness to greenness and yellowness to blueness respectively.

#### 3.6.1 L-value

The analysis revealed that results have highly significant ( $p < 0.01$ ) relationship. Results showed that T<sub>0</sub> has highest value of L (49.30), while T<sub>4</sub> has the lowest (38.80). L value decreased from T<sub>0</sub> to T<sub>4</sub> in an order that T<sub>0</sub> (45.97) > T<sub>1</sub> (43.92) > T<sub>2</sub> (41.34) > T<sub>3</sub> (40.15) > T<sub>4</sub> (38.14). Variation in L value of color observed was due to the varying concentration of pumpkin seed protein powder. So, it is evident that increasing the amount of pumpkin seed protein powder tends to increase darkness of biscuits and lower the lightness of biscuits. The findings of Das *et al.* (2021) support the results of this research that showed similar shift in color values (as increase in darkness and decrease in lightness of biscuits) with slight variation. In other study Khan *et al.* (2024) also found decreasing trend in L-value in flaxseeds fortified buns. Another study concluded by Čakarević *et al.* (2021) also noted the same decreasing trend for the L-value of cookies formed by substituting wheat flour to pumpkin seed oil cake proteins.

#### 3.6.2 a-value

According to the results, highly significant ( $p < 0.01$ ) relationship was indicated between a-values of various treatments samples. a-value of fortified cookies was at the range of (6.96 to 10.79). Results from statistical analysis indicated that T<sub>4</sub> contains maximum a-value of color (10.79) which was supplemented with 25% of pumpkin seed protein powder

while T<sub>0</sub> has lowest a-value (6.96) having 0% pumpkin seed protein powder. Increased in redness and decrease in greenness in the color of cookies was monitored while increasing the pumpkin seed protein powder concentration in various treatments samples. Alshehry (2020) observed the same trend for the a-value of cookies prepared by replacing wheat flour to pumpkin seeds powder. The experiment conducted by Čakarević *et al.* (2021) also observed the increasing trend for the a-value of color of cookies made by replacing wheat flour to pumpkin seed oil cake proteins.

### 3.6.3 b-value

It was observed that there was significant ( $p < 0.05$ ) relationship between b-values of different treatments samples. Mean values noted for b-value of cookies were ranged from (24.96 to 33.12). Outcomes of statistical analysis indicated that T<sub>4</sub> contains highest b-value of color (33.12) followed by T<sub>3</sub> (31.48), T<sub>2</sub> (29.33) and T<sub>1</sub> (26.95) while T<sub>0</sub> has minimum b-value (24.96) was without pumpkin seed protein powder. It was seen that increasing the concentration of pumpkin seed protein powder also increased the b-value of color in the supplemented treatments samples. The conclusion made by the recent study were also indicating close relationship to the results of Alshehry (2020), who produced nutritionally enriched cookies by replacing wheat flour to pumpkin seeds and observed increasing trend in b-value of color in cookies with little differences. The difference might be due to various factors such as difference in the concentration of pumpkin seed flour, temperature fluctuation and change in baking conditions of the product. The study conducted by Čakarević *et al.* (2021) also noted the similar results made by replacing wheat flour to pumpkin seed oil cake proteins.

## 3.7 Analysis for Physical Characters

### 3.7.1 Diameter of Cookies

Results were significant ( $p < 0.05$ ) between different treatments. Mean values for diameter of cookies were ranged from (6.73-4.54). Decreasing flow resulted by increasing the concentration of pumpkin seed protein isolates as seen in (Fig 3). The maximum value of diameter was found for T<sub>0</sub> (6.73) which was without pumpkin seed protein powder while minimum value was noted for T<sub>4</sub> (4.54) which contain 25% pumpkin seed protein powder. The results are in accordance to the results of Alshehry (2020) who found that diameter of cookies was decreased from (11.35-6.65) by increasing the pumpkin seed powder concentration. In another study Hayat *et al.* (2022) also found decreasing trend in diameter of cookies fortified with protein isolates of red kidney bean.

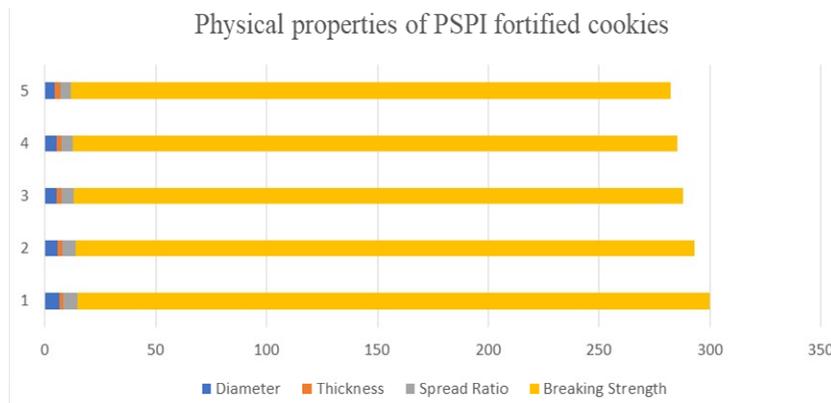


Fig 3: Physical Properties of PSPI Fortified Cookies.

### 3.7.2 Thickness of Cookies

Results were highly significant ( $p < 0.01$ ) between various treatments samples. Mean values observed for thickness of cookies were ranging from (1.39-2.53). Increasing flow was resulted when the concentration of pumpkin seed protein powder increased as seen in (Fig 3). The maximum value of thickness was found for T<sub>4</sub> (2.53) which contain 25% pumpkin seed protein powder while minimum value was noted for T<sub>0</sub> (1.39) which was without pumpkin seed protein powder. The results are according to the results of Hayat *et al.* (2022), also found increasing shift in thickness of cookies fortified with protein isolates of red kidney bean. A study proposed by Alshehry (2020) also found the decreasing trend in cookies prepared by replacing wheat flour to pumpkin seeds powder.

### 3.7.3 Spread Ratio of Cookies

Results were highly significant ( $p < 0.01$ ) between different treatments samples. Mean values for spread ratio of fortified cookies were ranging from (6.66 to 4.77). Decreasing flow was resulted by increasing the concentration of

pumpkin seed protein powder as shown in (Fig 3). The maximum value of spread ratio was found for T<sub>0</sub> (6.66) which was without pumpkin seed protein powder while minimum value was noted for T<sub>4</sub> (4.77) which contain 25% pumpkin seed protein powder. The findings are similar to the results of the study by Hayat *et al.* (2022), it also found decreasing trend in spread ratio of cookies fortified with protein isolates of red kidney bean. In another study Alshehry (2020) also observed the same decreasing trend from (6.30-5.54) for cookies prepared by replacing wheat flour to the powder of pumpkin seeds.

### 3.7.4 Breaking Strength

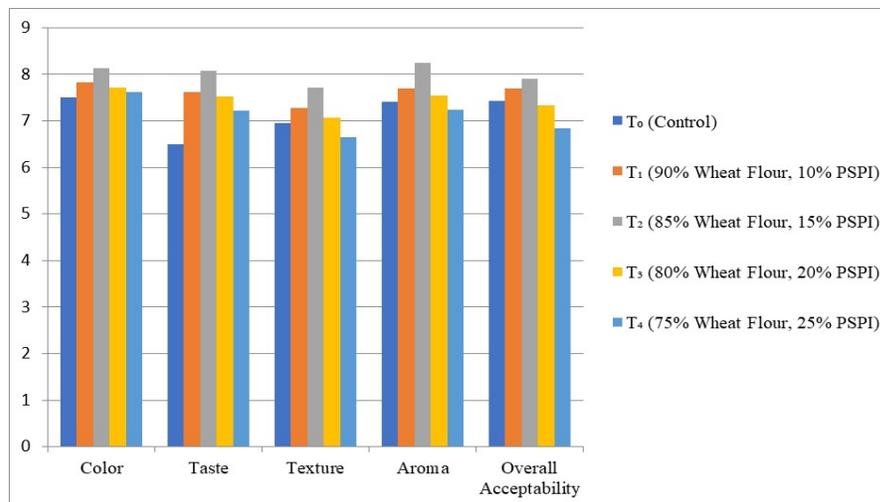
Results were highly significant ( $p < 0.01$ ) among the treatments. Breaking strength of cookies was varied from (285.06-270.64). Decreasing flow resulted by raising the concentration of pumpkin seed protein powder as described in (Fig 3). The maximum value of breaking strength was found for T<sub>0</sub> (285.06) which was without pumpkin seed protein powder while minimum value was noted for T<sub>4</sub> (270.64) which contain 25% pumpkin seed protein powder. The outcomes are similar to the results of the study by Ikuomola *et al.* (2017), it also found decreasing trend in breaking strength of cookies fortified with protein isolates of red kidney bean. In another study Čakarević *et al.* (2021) also observed the same decreasing trend for cookies prepared by replacing wheat flour to pumpkin seed oil cake proteins.

### 3.8 Sensory Evaluation

Sensory evaluation is considered as an important tool in the food product development. These types of analyses are conducted to know about consumers preferences. Consumer's perception of texture, color, flavor, taste and overall acceptability plays an important role in accepting or rejecting the food product as these factors shaped the overall impression of the quality. In the study, Sensory parameters of cookies were evaluated by the trained panel using 9-hedonic scale.

#### 3.8.1 Color

Color is the first thing to be noted and is significantly influences the acceptance of the product in the market. Color of the product influences the initial impression of the costumer indicating the products attractiveness. Therefore, in sensory evaluation color of the product is an important factor to be evaluated. Attractive and good color of cookies significantly influences the acceptance of cookies. The results indicated that the impact of color is substantial ( $p < 0.05$ ) across different samples. Mean values recorded for the color of cookies were ranging from (6.17-8.12). As shown in (Fig 4) the data recorded highest score for T<sub>2</sub> (8.12±0.32), supplemented with 15% pumpkin seed protein powder followed by T<sub>3</sub> (7.82±0.39), T<sub>1</sub> (7.82±0.43) and T<sub>4</sub> (7.61±0.21) while lowest score was for T<sub>0</sub> (7.51±0.66). The observed values of this study were same to the results of Park *et al.* (2021). The study performed by Hayat *et al.* (2022) also noted the same scores comparable to the present study.



**Fig 4:** Results of Sensory Analysis of PSPI Fortified Cookies

#### 3.8.2 Taste

The sensory features of food, especially taste, have a big impact on the consumer's choices. Acceptability and the value of product greatly influence by the taste of the product. There are different factors affecting the taste of the end product such as the quantity of the ingredients and the concentration of specific components. Good taste of the

product is the reliability of its acceptance in the market. This research indicated that significant difference ( $p < 0.05$ ) was occurred between the different treatment's samples of fortified cookies as far as taste was concerned. Mean values recorded for the taste of cookies were ranged from (6.50 to 8.08) as shown in (Fig 4). The maximum score was observed for T<sub>2</sub> (8.08) which contain 15% pumpkin seed protein powder. The minimum score was observed for T<sub>0</sub> (6.50). The readings of the present work were similar to the findings of Alshehry (2020) who also observed the same trend. The work conducted by Hayat *et al.* (2022) also stated the same results as compared to present study.

### 3.8.3 Texture

Determining the texture of the product is an integral element for its acceptability. In sensory evaluation, texture is the only characteristic that can detect by human. To differentiate the texture of any product tongue and fingers are used. The uniform and consistent texture also adds to the width and spread factor of cookies, which is crucial for high quality cookies. The data showed significant difference ( $p < 0.05$ ) between texture values of different treatment. Mean values for the scores of texture analysis of cookies were ranging from (6.96 to 6.64), illustrated in (Fig 4). The maximum value was seen for T<sub>2</sub> (7.71) and the minimum value was observed for T<sub>4</sub> (6.64). The results of the current research were much similar to the results of work conducted by Hayat *et al.* (2022). In a previous research the same findings were achieved by Malkanthi *et al.* (2018).

### 3.8.4 Aroma

Aroma attracts the customers toward product. It also has as important role in the marketing of the product. The results revealed that the aroma of cookies have significant ( $p < 0.05$ ) relationship between different treatments. Aroma of cookies was ranged from (7.24 to 8.25), shown in (Fig 4). Results indicated that T<sub>2</sub> has the highest value of (8.25), which contains 15% pumpkin seeds protein powder. The minimum score was observed for T<sub>4</sub> (7.24) which contain 25% pumpkin seeds protein powder. The results of the current study were much similar to the results of study conducted by Hayat *et al.* (2022). The results of the current study were also in accordance to the findings of the study conducted by Park *et al.* (2021).

### 3.8.5 Overall acceptability

Overall acceptability generally refers to the overall evaluation of all the marked scores and the feedback given by the panelists on the basis of various parameters including color, aroma, taste and the texture of the product. In overall acceptability the effects of the attributes may be overlapped by one another. Analysis showed that there exists a significant effect ( $p < 0.05$ ) for overall acceptability across various treatments samples. Mean values recorded for overall acceptability of cookies were ranged between (6.83- 7.90) as depicted in (Fig 4). The highest value was seen for T<sub>2</sub> (7.90) and the lowest value was seen for T<sub>4</sub> (6.83) while scores shown by T<sub>0</sub> (7.43), T<sub>1</sub> (7.69) and T<sub>3</sub> (7.34). The outcomes achieved from current study are same to the results of Alshehry (2020) who prepared cookies by replacing wheat flour to pumpkin seeds powder and got alike findings as compared to current study. The results quantified by Hayat *et al.* (2022) were also the same who developed enriched biscuits.

## 4. CONCLUSION

In conclusion, this study successfully optimized the UAE parameters to improve protein yield from pumpkin seeds, demonstrating the technique's efficiency and sustainability as a green extraction method. The extracted pumpkin seed protein exhibited promising functional and nutritional properties, promising a valuable ingredient for food fortification. Its incorporation into cookie formulations not only enhanced the protein content but also improved the nutritional profile without compromising sensory acceptability. The observed findings highlight the capacity and the potential of pumpkin seed protein as an economic and health-promoting ingredient in bakery products, contributing to the development of nutritional and functional foods that cater to the growing requirements for plant-based protein sources. Future research may explore the scale-up of UAE processes and the application of pumpkin seed protein in a wider range of food systems.

### Declarations

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**Data Availability:** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Authors' Contributions:** Sara was responsible for the study conceptualization, Reviewing, editing, Muhammad Waseem, and Murtaza Ali; methodology design, data collection, formal data analysis, and Mehwish Arshad; preparation of the original draft, and manuscript review and editing.

**Generative AI Statement:** The authors declare that no generative artificial intelligence tools, including DeepSeek, were used in the preparation of this manuscript.

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