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Enhancing functional characteristics and antioxidant activity of *Prosopis juliflora* pods' protein isolate through pH adjustment, while detecting the physicochemical properties and antibacterial inhibition activity

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ABSTRACT

This study evaluated the physicochemical properties of *Prosopis juliflora* pods and the preparation of its protein isolate. Changes in functional properties were observed when pH levels were adjusted to 5, 7, 8, and 10, with significant differences ($P \leq 0.05$) in functional characteristics and antioxidant and antibacterial activities. A high solubility value was achieved at pH 5.10 and lipid binding at pH 10, while the lowest value for both properties was found at pH 7. The highest foaming capacity was observed at pH 5 after 5 minutes of whipping, but decreased after 1 minute at pH 8. The emulsifying capacity was greatest at pH 10 and lowest at pH 7. The DPPH radical scavenging activity was high at pH 5, but decreased at pH 8 and also protein isolate showed inhibition activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. However, the highest inhibition was against Bacillus bacteria at 0.5% protein isolate concentration, while the protein digestibility was higher. The isolated protein had no noticeable cytotoxic effect on human blood cells. Besides, FTIR test was used to determine the structure of the protein.

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1-Introduction

The utilization of plants is experiencing growing and engaging accomplishments. Many plants remain largely unstudied yet hold significant economic, nutritional, and health value and potential. Several secondary source plants have been examined to create innovative eco-friendly products and alternative protein-rich feed stocks, as well as bioactive compounds (de Melo *et al*., 2021). The food industry can greatly benefit from food by-products, as stated by Cavalcante *et al*. (2022). Using fully processed foods can help enhance products and prevent waste while meeting basic nutritional needs and promoting good health. Minimizing the chances of illness (Iglesias and Alejandre, 2010). Numerous food compounds with high protein content, beneficial bioactive properties, and health-friendly characteristics from unconventional sources have been researched for the purpose of creating innovative products (de Melo *et al*., 2021). The Fabaceae or Legume family consists of the genus Prosopis, which comprises around 45 species of spiny trees and shrubs. This type of species is present in tropical and subtropical areas worldwide. Studies have looked into the feasibility of using whole mesquite grains as an ingredient because mesquite is mainly used in animal diets as an alternative food source. According to Gusmão *et al*. (2018) and Gonzalez Baron *et al*. (2020), mesquite flour is used in the making of cakes and bread for human consumption. It has antioxidant and antimicrobial properties to treat a variety of conditions such as asthma, labour/ postpartum pain, corns, conjunctivitis, diabetes, diarrhea, phlegm, fever, influenza, lactation, liver infection, malaria, and otitis. media, pain, lice, and rheumatism. Scabies infestation on the skin. Additional uses have been discovered (references 3-5). Cavalcante *et al*., 2019; Battista *et al*., 2018) demonstrate the technological possibilities of mesquite. In this instance, utilizing this product for human use is crucial (Cavalcante *et al*., 2020). Proteins play a crucial role in the development and overall well-being of the human body. Individuals require sufficient protein for regular body functions, development, maturation, pregnancy, breastfeeding, and healing from injuries and illnesses (Khan and Varshney, 2018). The need for alternative protein sources is driven by a growing global population and diminishing resources to meet protein demands. Plant proteins are significantly essential in the diet and more cost-effective to manufacture compared to animal proteins. Traditional legumes are important for food and feed in many countries but are not grown enough to keep up with increasing populations and demand from animal feed industries (Siddhuraju and Becker, 2003). The pH level can impact the structure and chemical characteristics of proteins, impacting how proteins interact in food systems throughout various stages like processing, storage, consumption, and preparation, ultimately affecting the overall quality and sensory aspects of food systems. Furthermore, protein can impact food characteristics like hydration, fat retention, emulsification, and foam formation (Feyzi *et al*., 2017). Nevertheless, limited scientific data exists on the process of preparing a protein isolate from musk pods. Therefore, this research focused on creating isolates from musk pods and analyzing the physical and chemical aspects of the pods, including protein amino acid quality, digestibility, cytotoxicity, and active groups. In addition, the effect of pH variations on functional characteristics and antioxidant activity was evaluated.

2- Materials and methods

2.1 Preparation of the sample

The pods of *P. juliflora* were cleaned and ground into powder and stored in a two-layer package of aluminum and

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polyethylene with obstacles against light and oxygen in the refrigerator until use.

2.2 Chemical composition

The total pH value measured by using a digital pH meter calibrated with buffers at pH 4.0 and 7.0. The protein content was evaluated using the Kjeldahl method, the total lipid content using hexane extraction in a Soxhlet, and the ash content using a muffle furnace at 550 C°. (Association of Official Agricultural Chemists, AOAC, 2000). The difference between the total amount of the other constituents (% moisture + % ash + % fat + % protein) and 100% was used to calculate the total amount of carbohydrates (Barbi *et al*., 2020). The measurements were performed with three replications.

2.3 protein isolate

With minor modifications, extraction procedure was performed in accordance with Mariod *et al*. (2010). The material was mixed mechanically for one hour after being diluted in distilled water at a ratio of 1:20 (w/v), and pH 9.0 was achieved by adding a 1.0 M NaOH aqueous solution. The resulting filtrate was centrifuged at 10,000 rpm for 20 minutes at room temperature using white cheesecloth. In order to enhance protein precipitation, the supernatant was placed into a beaker and agitated once more for 20 minutes while the pH was adjusted to 4.5 with 0.1 M HCl. The sediment protein slurry was dialyzed overnight against water at 4°C after being centrifuged at 10,000 rpm for 10 min at room temperature. While changing pH, after bringing the protein precipitate's pH level to 7.0, it was collected and freeze-dried (Delta 2 24/LSC plus, Germany). For a subsequent experiment, the protein powder was vacuum-packed and kept at 18 °C.

2.4- Functional characteristics

2.4.1 Protein solubility

With slight adjustments, Kumar *et al*. (2014) approach was used to determine the proteins solubility. Accordingly, 100 mg of the material was dissolved in 5ml of distilled water. Using 0.1M HCl or 1M NaOH the pH of the mixture was changed to 5,7,8 and 10. The mixture was centrifuged at 4000g for 20 minutes after being agitated for an hour at room temperature. The Biuret was used to measure the protein levels in the supernatants. The protein content in isolated samples was measured with Kjeldahl method. The solubility of the protein was determined using the below equation:

$$\text{Solubility (\%)} = \frac{A}{B} \times 100$$

A represents the protein content found in the supernatant, while B corresponds to the total protein content present in the sample.

2.4.2 Foaming capacity (FC)

FC was measured according to the method described by Liu *et al*. (2021) with some modifications. The sample (500 mg) was added into 50 mL of distilled water, and the pH was adjusted to 5, 7, 8, and10 with either 0.1 M NaOH nor 0.1 M HCl. The solution was whipped using the maximum speed of a homogenizer (Sartorius Japan) for 2 min foaming ability It was expressed out as follows:

$$\text{FA (\%)} = \frac{V1-V0}{V0} \times 100$$

where V0 is the volume of liquid before homogenization (in ml), and V1 is the volume of foam after homogenization (in ml).

2.4.3 Emulsifying capacity (EC)

EC were calculated using a slightly modified version of the Lamsal *et al*. (2007) method. After being homogenized for 1

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minute in 20 mL of distilled water with the sample (300 mg), the pH was changed to 5, 7, 8, 10 and 15 mL of soybean oil were added to the protein solution, which was then homogenized for 1 minute. It was then centrifuged for three minutes at 5,000 rpm. EC was determined via the following formula.

$$EC\ (\%) = \frac{Hieght\ of\ emulsified\ layer}{Height\ of\ total\ \ content} \times 100$$

2.4.4 Oil holding capacity (OHC)

In a centrifuge tube, 10 ml of soybean oil and 0.5 g of the sample were combined, and the tubes were shaken vigorously (at 100 rpm) for 30 seconds each. At 3000 rpm for 30 minutes, the oil dispersion was centrifuged. Oil-holding capacity was determined as the amount of oil that was absorbed per gram of protein sample, which was assessed (Wasswa *et al.*, 2007).

2.5 Biochemical properties

2.5.1 DPPH assay:

The plant extracts' ability to scavenge DPPH radicals was assessed by mixing different concentrations of test extracts into 2.9 mL. of a DPPH solution with a 0.004% ethanol concentration. Following a 30-minute incubation period at ambient temperature, the absorption was evaluated at 517 nanometers. opposed to an empty [24]. IC50 values were determined based on the concentration of sample needed to eliminate 50% of free radicals. equation for regression. BHA was utilized as a positive control, with each test being conducted three times. DPPH's radical scavenging activity. The calculation of free radical inhibition (I%) was done using equation (1).

$$Inhibilation\ percentage = \frac{A\ blank - A\ sample}{A\ blank} \times 100$$

where A blank and A sample are respectively absorbance of control sample and absorbance of tested sample at the end of reaction.

2. 5.1 The *in vitro* protein digestibility:

Method was established based on Algadi and Yousif's (2017) explanation. 200 mg of the protein from the aliquot was suspended in 15 mL of 0.1 N HCl that also contained 1.5 mg of pepsin. For up to three hours, the mixture was submerged in water heated to 37 ^{o} C. In addition, the hydrolysate from the pepsin digestion was neutralized with 7.5 mL of 0.2 N NaOH, and then 4 mg of pancreatin was added to 7.5 mL of phosphate buffer (pH 8.0) before the samples were incubated (MIR154, Sanyo, Japan) at 37 °C for an additional 24 hours. After incubation, the sample was treated with 10 mL of a 10% trichloroacetic acid solution, centrifuged for 20 min. at 5,000g at room temperature, and the collected supernatant was used to calculate the total nitrogen content using the Kjeldahl method (Nielsen, 2010). Casein from isolated bovine milk was employed as a standard reference for comparison purposes. The following equation was used to determine the IVPD values:

$$\text{Protein digestibility (\%)} = \frac{Nitrogen\ supernatat}{Nitrogen\ in\ sample} \times 100$$

2.5.2 Toxicity of the isolated protein

The toxicity of the isolated protein was determined by placing 1 ml of fresh human blood in 20 ml of normal saline. To detect the toxicity of the isolated protein, a range of concentrations were prepared, ranging from 100 to 1000 micrograms/ml, and 100 microliters of each concentration were mixed with 2 ml of human blood. A control sample was prepared by adding 100 µL of distilled water. The mixture was heated to a temperature of 37 °C, and the turbidity was

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checked after 10, 30, and 60 minutes. (Nair *et al*., 1989)

2.6 Antibacterial assay:

Different bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*) were collected from the Department of Food Science at the College of Agriculture, University of Basrah. Experiment with the excitatory impact of the protein isolate made at various concentrations (0.2, 0.3, and 0.5%) through the co-etching technique as described in the study by De Oliveira *et al.* (2011). The traditional McFarland solutions with a turbidity level of 0.5 and a cell integrity of 1.5 x 108 cells were utilized. The level of antibacterial activity was assessed with creating a 6 mm-diameter hole on the Mueller-Hinton medium's surface including a 50-microliter protein bolus for each concentration. Treatment involved the use of distilled water. The plates were incubated at 37 °C for 24 hours. The measurement of the diaphoresis diameter was taken in mm.

2.7 Characterization of proteins

2.7.1. Amino acid analysis

The amino acids of protein isolation were identified using the Levin and Grushka (1985) technique through High-Performance Liquid Chromatographic Analysis.

2.7.2. Fourier-transform infrared spectroscopy (FTIR) test

After mixing KBr with freeze-dried samples, tablets were made. The band 400–4000 cm^{-1} was scanned using an FTIR (Thermo Scientific Nicolet iS5, USA) (Lin *et al*., 2021).

2.8 Statistical Analysis:

Statistical methods were utilized to examine the variation in the data from Gen Stat Release 12.1. All comparisons were performed using the least significant difference (LSD) to evaluate specific differences, with a significance threshold of 0.05.

3. Result and discussion

3.1 Physicochemical tests

The physico-chemical characteristics of the *P. juliflora* pods were determined (Table 1). The moisture 8.37%, protein 11.93%, fat 3.65%, ash 5.41%, and carbohydrate 70.64% content, while pH value was 6.43. It was observed that the protein and fat were higher, found at 9.7% protein and 1.9% fat, according to Choge *et al*. (2007). Silva *et al*. (2007) examined *P. juliflora* pods as well and discovered average values of 2.1 and 7.2% for crude protein and fat. The protein content indicated in other studies can be seen to be lower than the levels obtained in this study.

Table 1: Physico-chemical properties of the *P. juliflora* pods.

Component	Percent (%)
Moisture	**8.37**
Protein	**11.93**
fat	**3.65**
Ash	**5.41**
Carbohydrate	**70.64**
pH	**6.43**

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The presence of proteins indicates its potential as a food source. According to Summo *et al.* (2019), mesquite seed values are comparable to those of black chickpea (18,2%), a commercial source of vegetative protein. A food product can be regarded as a source of proteins if it contains at least 6 g of this component in a 100-gram serving, according to the Technical Regulation on Nutritional Information of Foods (ANVISA, 2012).

3.2 Functional characteristics:

3.2.1 Solubility:

From the (1) figure, it can be observed that the pH value change influences the solubility of the protein isolate. Solubility increases as we move further from neutrality towards either acidity or basicity. The solubility was observed to rise at pH 5 and 10, reaching 40.21% and 40.22% each, while it decreased at pH 7, reaching 35.45%. It is nearing the neutralization point of the protein isolate. The solubility varies depending on the pH level. Mune *et al*. (2016) reported a decrease in protein solubility near the protein's isoelectric point, with higher protein solubility observed outside this point. There was a statistically significant difference ($p \leq 0.05$) observed between the pH groups.

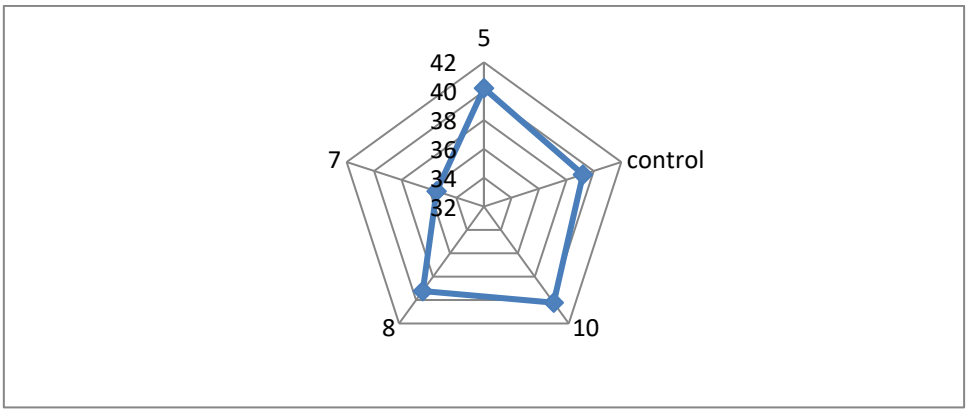

Figure 1: Effect of deferent pH on protein isolate solubility

3.2.2 Foaming capacity:

The foaming capacity of the isolated protein was measured as shown in the figure 2 at a time of 1 min as a starting point. It was highly dependent on different levels of pH. The lowest foaming capacity was recorded at pH 5 at 1 minute of whipping 66.67% followed by pH 4 (71.43) while the highest foaming capacity was obtained at pH 5 at 5 minutes of whipping 140% followed by pH 7 (130.77%) with a significant difference ($p \leq 0.05$). The higher foaming capacity at pH 5 may be due to increases in the net charge of the protein molecules, which weakens hydrophobic interactions and also increases protein flexibility. In this case, it is allowed to diffuse to the air-water interface quickly, thus encapsulating air molecules and increasing foam formation (Lawal, 2004).

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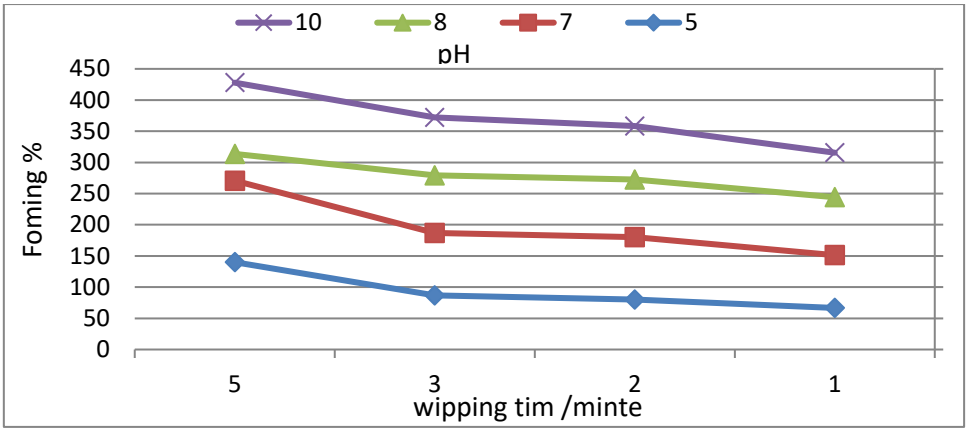

Figure (2) Effect of deferent pH on protein isolate foaming capacity.

3.2.3 Oil holding capability:

It can be observed in figure (3) that the oil holding capacity depended on the pH. When the pH nears the pH 10, there is an increase in oil holding of 2.3 g/ml, and the lowest value occurred at pH 7 (1.7g/ml). Also observed was that the oil holding capacity of the protein isolated was significantly ($P<0.05$) . The presence of several non-polar side chains many bind the hydrocarbon chain of fat, thereby resulting in higher absorption of oil (Sathe *et al*., 1982).

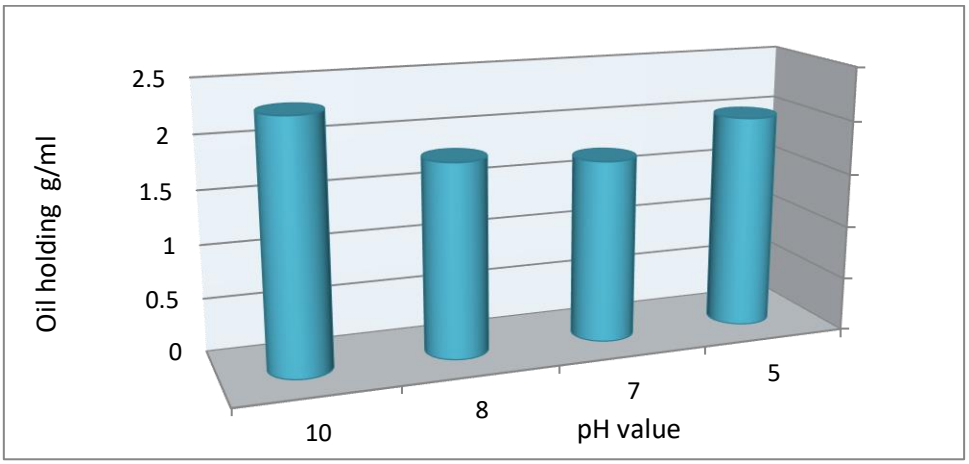

Figure 3: Effect of deferent pH on protein isolate oil holding capacity

3.2.4 Emulation capacity:

Emulation capacity evaluates how well protein-based emulsifiers work by assessing the absorption of protein at the interface. Emulation capacity is a characteristic of protein molecules that is dependent on pH and affects the balance between hydrophobic and hydrophilic properties (Figure 4). In this outcome, significant levels ($p\leq 0.05$) of emulation capacity were detected at both low and high pH levels, with the highest emulation capacity value recorded at pH 5 and 10. Therefore, the hydrophobic force decreased due to the increased net charge and flexibility. Therefore, proteins can quickly spread to the air-water interface, leading to low emulation capacity, according to Yuliana *et al.* (2014). The discovery is comparable to the outcome documented by Feyzi *et al*. (2015) concerning the protein isolate of Fenugreek (*Trigonella foencem graecum*) seed and Lupinus luteus (Burgos

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Diaz *et al*., 2016), wherein the greatest EC was detected in extremely acidic and alkaline environments.

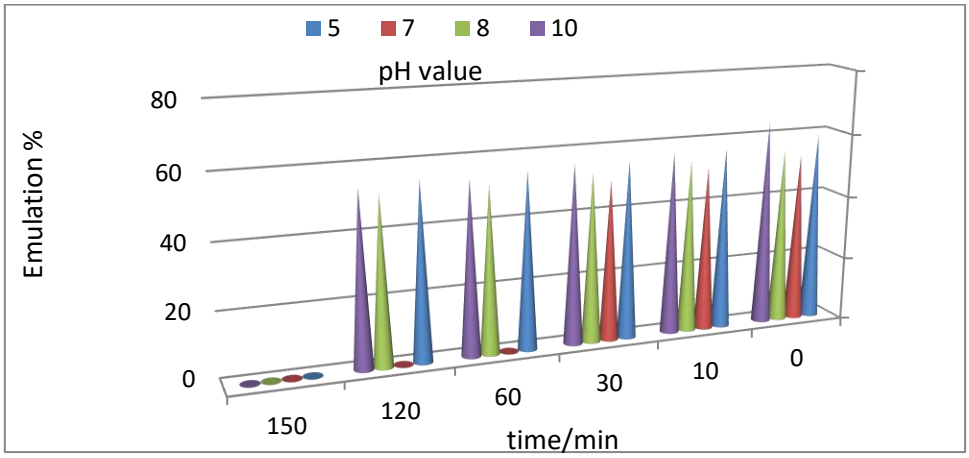

Figure 4: Effect of deferent pH on protein isolate emulation capacity.

3.3 Biochemical properties:

3.3.1 Antioxidant activity:

Antioxidant activity of protein isolation was determined through the analysis of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals for free radical scavenging activity. The percentage of DPPH radical inhibition represented the radical scavenging activity (Figure 5). The results showed that the free radical scavenging activity was most effective at pH 5 (31.44%), followed by pH 7, with the lowest inhibition of DPPH radical at pH 8 (8.03%) with a significant difference ($p \leq 0.05$). This demonstrates the pH variation effect of antioxidant activity. showing that these protein isolates have a higher number of amino acid residues exposed, leading to an increased amount of active sites for scavenging DPPH radicals. The analysis of amino acid composition in Figure also reinforces these findings, with the protein isolate having the highest levels of antioxidant amino acids (Trp, Met, His, Tyr, and Lys). These amino acids stabilize free radicals by providing protons or electrons (You *et al*., 2009). Variables that may impact antioxidant effectiveness could be the type of protein, the arrangement of amino acids, and the protein's level of hydrophobic amino acids. Proteins rich in hydrophobic amino acids like phenylalanine, tryptophan, and methionine exhibit antioxidant properties. The act of attaching amino acids to fats increases, leading to a decrease in the presence of free radicals and an increase in antioxidant activity at a pH of 4.5-7.5-10. The protein has significant amounts of hydrophobic amino acids like leucine, iso-leucine, cysteine, methionine, and basic amino acids including histidine, arginine, and lysine. that antioxidant activity depends upon the oxidation rate of antioxidant compounds, and this oxidation rate was influenced by the surrounding pH (Jovanovic *et al*. 1994).

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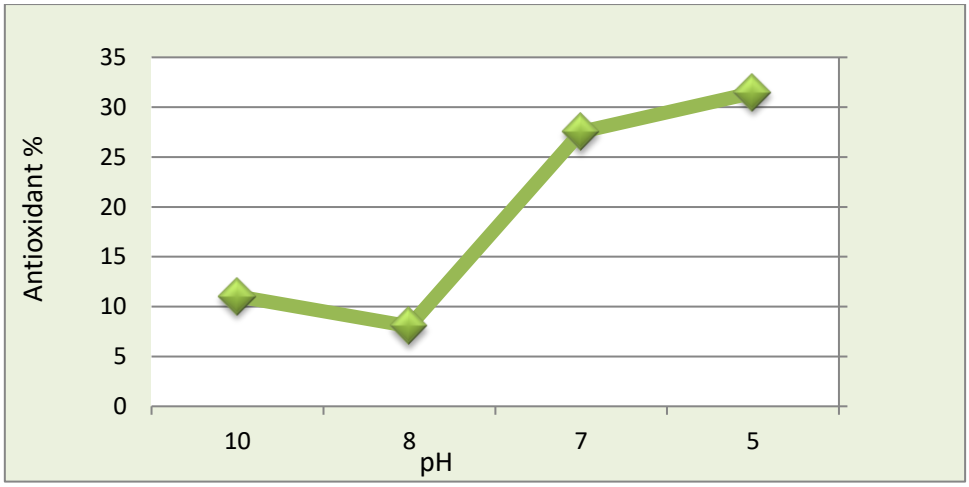

Figure (5) Effect of deferent pH on protein isolate antioxidant activity

3.3.2 Protein digestibility:

Bioavailability is the fraction of consumed food components that can be used for various physiological functions at their intended site of action (Guerra *et al.*,2012). Bioavailability involves the readiness of a substance to be absorbed and used by the body. The entire journey of a nutrient after it is consumed, which includes its breakdown and absorption in the digestive system. In this study, the digestibility of the isolated protein of *P. juliflora* sprouts was determined, and the digested protein of *P. juliflora* sprouts was 80.18%, and this value was much higher to that of soy protein isolate (71.04%) (Wang *et al.*, 2010).

3.3.3 Cytotoxic protein detection

Figure 6 displays the findings of cytotoxicity detection in *P. juliflora* protein isolated. The findings indicated that the protein isolated at concentrations ranging from 100 to 1000 micrograms/ml did not induce any noticeable alterations in human blood cells, such as sedimentation or degradation, when exposed to 37°C for 10, 30, and 60 minutes, in comparison to the control group.

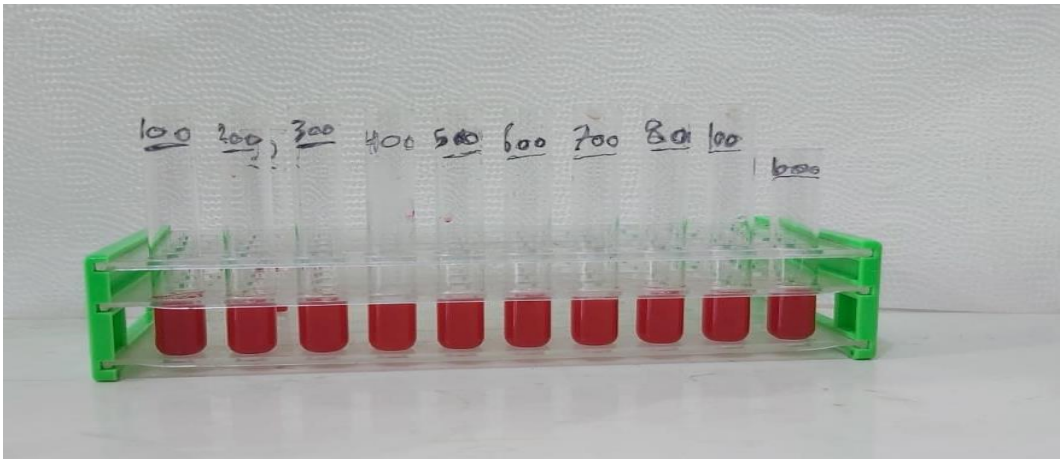

Figure 6: Cytotoxic activity of protein isolate at different concentrations

3.4 Inhibitory activity of the protein isolates against different bacteria

The table 7 displays the protein isolate's ability to stimulate activity against three different bacteria strains. The protein

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isolate had varying stimulating effects depending on the bacteria type, with a significant difference ($p \leq 0.05$) observed and the largest halo diameters of 2.5, 2, and 2 mm observed with concentrations of 0.5% for *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*, respectively, in pigeons. A concentration of 0.2% resulted in a minimum areola diameter of 0.5 mm. The protein's ability to inhibit bacteria could be attributed to its high levels of amino acids, particularly lysine, arginine, and histidine, which are positively charged. The positive charge network boosts the interaction between the protein and the bacteria's surface negative charge, resulting in an initial bond via electrical interaction with the positive protein. Lipopolysaccharides lead to membrane disruption, creating an unstable area that results in membrane destruction and cell death.

Table 2: Antibacterial activity of protein isolate at different concentrations

Inhibitory zone (mm)			**Concentration**
Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	**%**
0.5	1	2	0.2
1	1.5	2	0.3
2	2	2.5	0.5
-	-	-	0

(-) Indicates no inhibition

3.5 Protein characterization

3.5.1 Amino acids profile:

The amino acid composition (µg/g protein) of *P. juliflora* pods protein isolate is shown in the table 3 and figure 8. Proline was predominant among the nonessential amino acid (97.47µ/g protein),while the essential amino acid are isoleucine and histidine (97.44 and 96. 66 µ/g protein) and the presence of hydrophobic amino acid ,such as leaucine, methionine ,proline and alanine ,also showed significant performance in the functional properties of food proteins ,amino acid composition .Hashim *et al*.(2024) observed that the amino acids found in the trypsin –hydrolyzed pod of *P. juliflora* include aspartic acid 11.4%,glutamic acid 2.8% serine 5% histidine 2.6% tyrosine 4.9% , 27.1% cysteine ,39.9%valine,5.7%methionine ,0.1% isoleucine and 0.1%leucine. The hydrolyzed papain pod of *P. juliflora* contains 30% aspartic acid ,2.1% glutamic acid, 5.8% serine ,5% histidine ,7.7% cysteine ,9.2% phenylalanine ,18.1% isoleucine ,12.8% leucine and 3.1% lysine.

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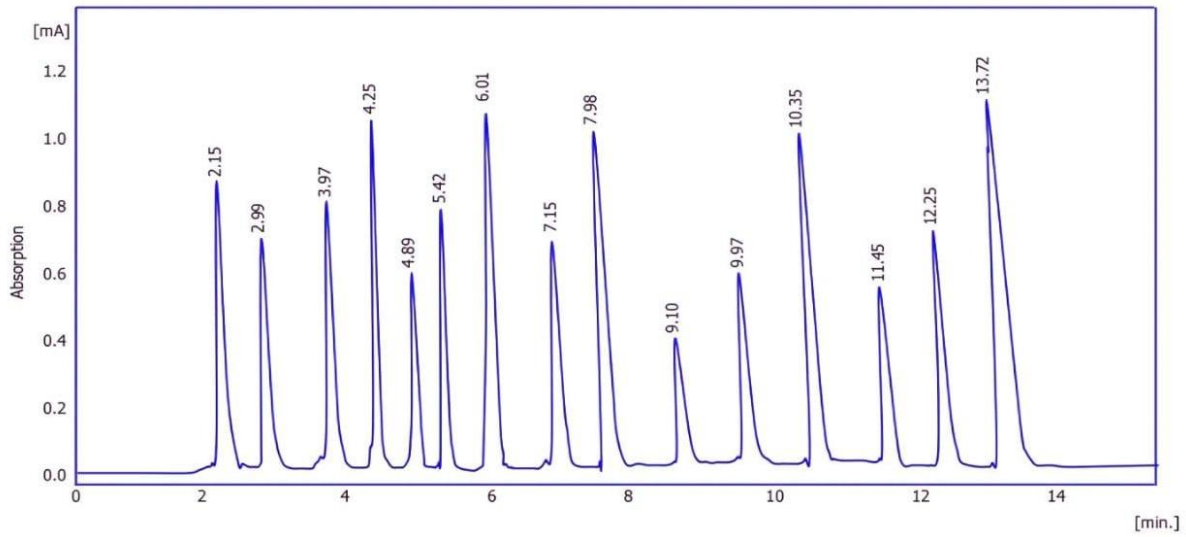

Figure 8: Content of protein isolate of amino acid

Table 3. Content of *P. juliflora* pods protein isolate of amino acid

No.	Reten. Time (min)	Area(mAU.s)	Compound name	Amount (µg/gm)
1	2.15	1256.89	Lysine	90.25
2	2.99	2541.00	Methionine	85.46
3	3.97	1985.48	Trptophan	75.99
4	4.25	3526.44	Arginine	94.28
5	4.89	4256.98	Threonine	82.13
6	5.42	5214.89	Valine	94.56
7	6.01	1623.25	Isoleucine	97.44
8	7.12	4265.28	Lucin	90.25
9	7.98	24513.65	Pheynlalanine	48.97
10	9.10	2013.65	Glutamic acid	62.58
11	9.97	4215.98	Aspartic acid	84.15
12	10.35	3201.46	Histidine	96.66
13	11.45	2155.98	Serine	42.58
14	12.25	1954.78	Proline	97.47
15	13.72	2013.65	Cysteine	88.05

3.5. 2 FTIR test

The arrangement of atoms in a biological compound can be identified. Use FTIR spectroscopy to analyze the infrared radiation absorbed by the sample based on wavelength and intensity. This method is commonly used to analyze the secondary structure of proteins or polypeptides and their hydrolases. The FT-IR spectra of the isolated protein are shown in Figure 8. Characteristic IR signals of amides A, B, and I–VI were identified. The two most important vibrational bands in the protein backbone are the amide I and II bands. The Amide I region, located in the 1700-1600 cm spectral range, is the most sensitive region for analysis of secondary structural elements of polypeptides due to its association with C=O stretching vibrations. Protein functional groups (N-H and C=O)

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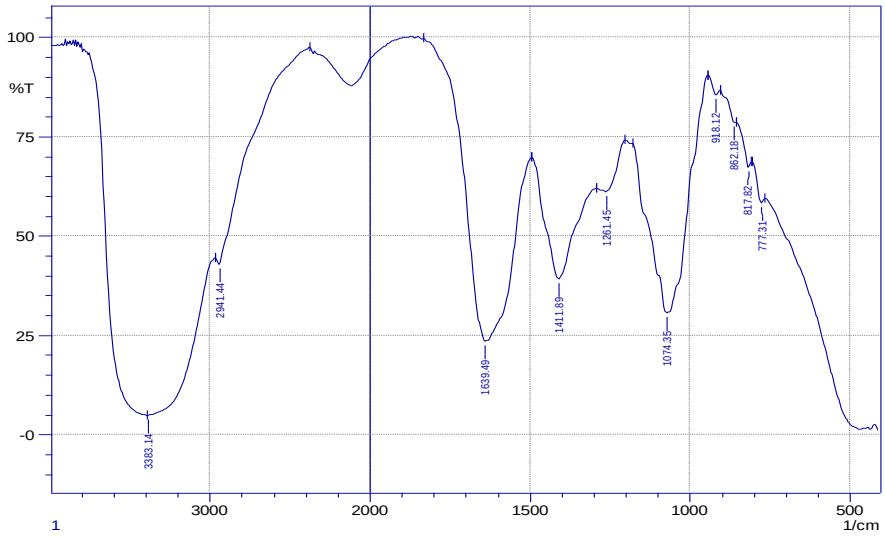
were analyzed in isolated *P. juliflora* pods protein through FT-MIR spectroscopy. The associated IR bands were identified at 1411.89 cm -1 (amide II bands), 1639.49 cm -1 (amide I band), between 3333.14 and 2941.44 cm -1 (amide A and B bands), and 1261.45-777.31 cm -1 -1 (Tiwari and Singh, 2012). Food components such as carbohydrates and fats have distinct intramolecular bonds. Carbohydrates generate a peak around 1200-900 cm-1, indicating stretching of CO-C and CO-OH (Nugrahani *et al*., 2020).



4. Conclusion

P. juliflora pods can serve as a source of protein due to their high protein content. The solubility, foaming, oil binding and emulsifying properties and also antioxidant activity of proteins are greatly affected by the pH level. Besides, the protein isolates showed the ability to inactivate three types of bacteria, excellent digestibility, and a high level of essential amino acids necessary for human body growth, without any cytotoxic effects.

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مقاله علمی_پژوهشی

افزایش خواص عملکردی و فعالیت آنتی اکسیدانی ایزوله پروتئین غلاف *Prosopis juliflora* از طریق تنظیم pH، همزمان با ارزیابی ویژگی‌های فیزیکوشیمیایی و فعالیت باکتریواستاتیک.

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چکیده

این مطالعه به بررسی خواص فیزیکوشیمیایی غلاف *Prosopis juliflora* و تهیه ایزوله پروتئینی آن پرداخته است. هنگامی که سطوح pH در ۵، ۷، ۸ و ۱۰ تنظیم شد، تغییرات در خواص عملکردی با تفاوت معنی دار ($P \leq ۰.۰۵$) در ویژگی‌های عملکردی و فعالیت های آنتی اکسیدانی و ضد باکتریایی مشاهده گردید. مقدار حلالیت بالا در pH 5 و10 و اتصال لیپید در pH 10 به دست آمد، در حالی که کمترین مقدار برای هر دو ویژگی در pH 7 یافت شد. بالا ترین ظرفیت کف کردن در pH 5 پس از ۵ دقیقه مشاهده اما پس از ۱ دقیقه در pH 8 مقدار آن کاهش یافت. ظرفیت امولسیون کنندگی در pH 10 و فعالیت مهار رادیکال DPPH در pH 5 بالا بدست آمد، اما در pH 8 مقدار آن کاهش یافت و همچنین ایزوله پروتئین فعالیت مهارکنندگی را در برابر اشریشیا کلی، باسیلوس سوبتیلیس و استافیلوکوکوس اورئوس از خود نشان داد. با این حال، بیشترین بازدارندگی در برابر باکتری باسیلوس در غلظت پروتئین ایزوله ۰.۵ در صد مشاهده شد، در حالی که قابلیت هضم پروتئین بالاتر بود. پروتئین جدا شده هیچ اثر سیتوتوکسیک قابل توجهی بر سلول های خونی انسان نداشت. همچنین برای تعیین ساختار پروتئین از آزمون FTIR استفاده گردید.

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