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Research Article

Okra seed protein isolate: functional properties

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ABSTRACT

In this paper, we dealt with the study of the okra plant in terms of chemical composition and the work of extracts, and the study of the effectiveness of these extracts against antioxidant activity and also against the growth of bacteria as was estimated total phenols and flavonoids total and the protein was separated from okra seeds and study the properties of the protein (Okra seeds contain a high percentage of protein and plant protein is in high demand due to the low costs of obtaining it compared to high animal protein, therefore, the okra protein is separated from the seeds to study the properties) and the combination of amino acids and its molecular weight and also the natural properties of protein isolate seed okra. The results obtained indicate that the percentage of protein (19.94%), total fiber (22.31%), total ash (8%), crude fat (2.9), total carbohydrates (40.43%), total phenols 130 mg/100 grams, total flavonoids and the antioxidant activity up to 0.711 mg/gram. The descriptive analysis of phytochemical compounds showed that the okra plant contains many second representative compounds such as steroids, glycosides, alkaloids, and terpenes, whether in the methanol or ethanol extract also showed an extract Ethanol against the activity of microbes can stabilize bacteria in concentrations up to 1000 micrograms. As for the properties of protein, Fatin pH = 2, the protein is at the lowest melting point and the highest degree of foam may reach its maximum after 25 minutes, and the protein contains a high percentage of essential amino acids such as amino acid lysine 16.13%. Thus, the experiments suggest that okra protein can be successfully used as a food ingredient.

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Introduction

Okra is a type of green plant (vegetable) known scientifically as "*Abelmoschus esculentus*" and also known as "Okra" in English. Okra belongs to the family of cotton (Malvaceae) and is a plant of tropical origin, usually grown in regions with warm climates. Okra is rich in nutrients and contains a range of vitamins and minerals, in addition to dietary fiber that benefits the digestive system and helps maintain heart health. Okra is consumed in many nations, where it can be used in a variety of cooked, grilled, fried, and pickled recipes. Okra is characterized by its content of soluble dietary fiber, as well as insoluble fiber and a high percentage of protein. (Eze and Akubor, 2012 and Akeem *et al.,* 2016).
Okra, also known as "ladies' fingers," is a vegetable that is widely used in various cuisines around the world. It is a green, elongated, and slightly curved vegetable that is typically 3-4 inches in length. Okra is a warm-season crop that is typically grown in tropical and subtropical regions. Okra is a good source of vitamins C and K, as well as folate and potassium. It is also high in fiber and low in calories, making it a popular choice for those looking to maintain a healthy diet. In cooking, okra is often used in soups, stews, and curries. It can also be fried, grilled, or pickled. Some people find okra to be slimy when cooked, but this can be minimized by cooking it quickly over high heat, such as in a stir-fry. Okra is also believed to have some health benefits. It has been traditionally used to treat digestive issues and has been found to have anti-inflammatory and antioxidant properties. However, more research is needed to confirm these potential health benefits (Gbadamosi *et al.,* 2012 and Petropoulos *et al.,* 2018).
Chemical analysis of okra can vary depending on several factors, such as the variety of okra, growing conditions, and maturity at harvest. Some of the differences in chemical analysis that can be observed in okra are:
The nutritional content of okra can vary depending on the growing conditions, fertilization, and harvest time. For example, a study showed that okra harvested 80 days after

planting had higher protein content, total ash, and fiber than those harvested at 60 or 70 days after planting (Akoja and Coker, 2018).

Okra plant is widely cultivated and consumed in many parts of the world, especially in Africa, Asia, and the Middle East. It is valued for its nutritional value, culinary uses, and potential health benefits. As I mentioned earlier, okra seeds, in particular, contain a variety of chemical compounds that may have health-promoting properties.

Moreover, the high antioxidant content of okra mucilage makes it a potential candidate for use in the development of functional foods and nutraceuticals. Antioxidants help to prevent cellular damage by neutralizing free radicals, which are highly reactive molecules that can cause oxidative stress and damage to cells (Abideen *et al.,* 2020).

Okra seeds are a potential source of oil and protein. The oil content of okra seeds ranges from 20 to 40%, depending on the variety, and the protein content ranges from 20 to 30%. The oil extracted from okra seeds is rich in unsaturated fatty acids, such as oleic and linoleic acids, which are beneficial for human health. Okra seed oil has been shown to have potential for use in food and industrial applications (Arapitsas 2008).

In addition to oil and protein, okra seeds also contain other beneficial compounds, such as dietary fiber and antioxidants, which have potential health benefits.

Okra is a popular food vegetable in many parts of the world, particularly in the Middle East, Africa, and South Asia. It is often used in soups, stews, and curries, and can be eaten raw or cooked. Okra is a low-calorie vegetable that is rich in nutrients and has many health benefits (Adelakun *et al.,* 2009).

Some of the health benefits of okra include: Lowering cholesterol levels: Okra is rich in soluble fiber, which can help reduce cholesterol levels in the blood. Helps regulate blood sugar levels: Okra is low in calories and high in fiber, which can help regulate blood sugar levels and prevent spikes in insulin. Promotes digestive health: Okra is high in dietary fiber, which can promote digestive health and prevent constipation. Boosts immune system: Okra is rich in antioxidants, such as vitamin C and beta-carotene, which can help boost the immune system and protect against diseases. Supports healthy pregnancy: Okra is rich in folate, which is essential for healthy fetal development during pregnancy. Overall, okra is a nutritious and healthy vegetable that can be a valuable addition to a balanced diet. (Khomsug and Thongjaroenbuangam, 2010)

Okra contains several phytochemical components that contribute to its potential antibacterial activity. These include Flavonoids: Okra is a rich source of flavonoids, which have been shown to have antibacterial properties. Some of the flavonoids found in okra include quercetin, kaempferol, and rutin. Tannins: Okra contains tannins, which are compounds that can bind to proteins and inhibit bacterial growth. Tannins have been found to have antibacterial activity against a range of bacteria. Saponins: Okra contains saponins, which are compounds that can form a lather when mixed with water. Saponins have been found to have antibacterial activity against a range of bacteria. Studies have shown that okra extracts have potential antibacterial activity against various bacterial strains, including Staphylococcus aureus, *Escherichia coli*, and *Pseudomonas aeruginosa*. The antibacterial activity of okra is believed to be due to the presence of these phytochemical components (Deuber *et al.,* 2012).

Protein in okra seeds is considered a complete protein, meaning that it contains all of the essential amino acids that the human body needs to function properly. Okra seed protein has been found to have a good balance of amino acids, with high levels of glutamic acid, aspartic acid, arginine, and leucine.

Protein in okra seeds has also been found to have functional properties, such as emulsifying and foaming capacity, which make it useful in food applications such as in the preparation of beverages, sauces, and bakery products. Additionally, okra seed protein has been studied for its potential use as a source of bioactive peptides, which may have health benefits such as antioxidant and antihypertensive activities.

Protein in okra seeds is a nutritious and versatile ingredient that can be used in a variety of food applications (Gbadegesin *et al.,* 2018).

Proteins can be isolated from defatted okra seeds. The protein composition of okra seeds can vary depending on factors such as the cultivar, growing conditions, and processing methods. Some of the major proteins found in okra seeds include globulin, albumin, and glutelin. These proteins have been shown to have various functional properties, such as emulsifying, foaming, and gelling abilities, which make them useful in food applications.

The paper aims to identify the chemical composition of the okra plant in Egypt, as well as the plant's content of total phenolic compounds and total flavonoids, as well as antioxidant activity, as well as antibacterial activity, and finally to separate the protein from okra seeds and identify the natural properties of it and what it contains amino acids.

Materials and Methods

Samples preparation of the okra seeds

The okra (*Abelmoschus esculentus* L.) of a unique Egyptian variety (*Alruwmiu*) was obtained from a farm, in Menoufia Governorate - Menoufia City, Egypt (during the summer, of 2020). Cleaning, the harvested pods are cleaned to remove any debris or foreign matter. The pods are usually washed with water and then dried. The dried pods are then opened to extract the seeds. The seeds are usually separated from the capsule by wrapping or cutting the pod open. Seeds can be collected in a container or on a clean surface. Extracted seeds are cleaned to remove any debris or residual plant material. Seeds can be washed with water and then dried. Dried and clean okra seeds can be ground into a fine powder using a mortar and pestle, mixer, or grinder. The grinding process can be improved depending on the intended use of the powder. For example, fine powder may be required for cosmetic or pharmaceutical applications, while coarse powder may be sufficient for food applications. Okra seed powder can be stored in an airtight container in a cool, dry place. The powder must be protected from moisture, heat, and light to prevent deterioration

Chemicals and reagents

The chemicals used in the paper were obtained through Al-Gomhoria Company - Zagazig Branch - Sharkia – Egypt. The solvent was distilled before use – all chemicals were of high purity. The bacteria (*E. coli* and *S. aureus*) were obtained from the Microbiology Laboratory - Department of Microbiology - Faculty of Science - Zagazig University.

Proximate analysis

Chemical analysis refers to the process of examining a substance to determine its components and properties, and it is performed sequentially the following processes:

First, the determination of total protein moisture, total ash, crude fat, and total fiber, and finally the estimation of total carbohydrates (on a dry weight basis) was calculated by difference, and all these estimates are estimated in AOAC official methods (Adams *et al.,* 1985).

Phytochemical Analysis

The extracts of the okra were tested for carbohydrates, proteins, steroids, glycosides, flavonoids, alkaloids, saponins, terpenoids, and cardiac glycosides. This phytochemical screening of the extracts is carried out by standard methods (Mensah *et al.,* 2009; Riaz *et al.,* 2015).

Preparation of extracts

To 5 g of a well-ground okra sample and then placed in an Erlenmeyer sanded capacity of 250 ml and then add 100 ml suitable solvent (methanol - ethanol - hexane) and the Erlenmeyer is placed on the shaker at a speed of 100 rpm for 24 hs after that the dissolved part is separated from the in-dissolved part by centrifugation at a speed of 5000 rpm for 15 min and the previous steps are repeated with the insoluble part and combines the dissolved part with some and then A solvent evaporation process rotary evaporator standard Buchi r-200 at 40°C. Store the extract in a closed bottle and temperature at 0°C (Riaz *et al.,* 2015).

Quantification of polyphenols

Estimation phenols compound

Sample preparation: The first step is to prepare the sample. was usually dried, ground, and then extracted with a suitable solvent (methanol and ether). The extract is mixed with Folin-Ciocalteu reagent and a solution of sodium carbonate was described by (Jalande and Gachande, 2012). The Folin-Ciocalteu reagent reacts with the phenolic compounds in the sample, resulting in the formation of a blue-colored complex. The absorbance of the blue-colored complex is measured at a specific wavelength (usually 765 nm) using a spectrophotometer. Absorbance is proportional to the concentration of phenolic compounds in the sample. The concentration of phenolic compounds in the sample is calculated using a standard curve generated with known concentrations of a standard phenolic compound such as gallic acid. The total phenols content is expressed as mg of gallic acid equivalents (GAE) per gram of sample. Total phenols assay provides a quick and simple way to measure the concentration of phenolic compounds in a sample. Gallic acid was used as a standard for the calibration curve Figure (1).

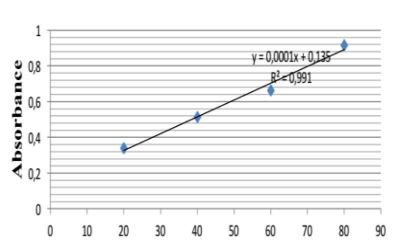

Figure 1. Calibration curve of total phenol.

Determination of Flavonoids

Zheng *et al.,* (2014) method is used to estimate total flavonoids, which depend on the interaction of aluminum chloride compound with flavonoids in an alkaline medium and a complex formation between aluminum and flavonoids with a yellow color measured at a visible of 510 nm. Procedure; The first step is to prepare the sample. The sample can be a plant extract or a food product. The sample is usually dried, ground, and then extracted with a suitable solvent such as (methanol, and ether). The extract is mixed with a solution of aluminum chloride and a sodium acetate buffer. The aluminum chloride reacts with the flavonoid compounds in the sample, resulting in the formation of a yellow-colored complex. The absorbance of the yellow-colored complex is measured at a specific wavelength (usually 510 nm) using a spectrophotometer. The absorbance is proportional to the concentration of flavonoid compounds in the sample.

Calculation of total flavonoid content: The concentration of flavonoid compounds in the sample is calculated using a standard curve generated with known concentrations of a standard curve (Figure 2) of flavonoid compounds such as quercetin. The total flavonoid content is expressed as mg of quercetin equivalents (QE) per gram of sample.

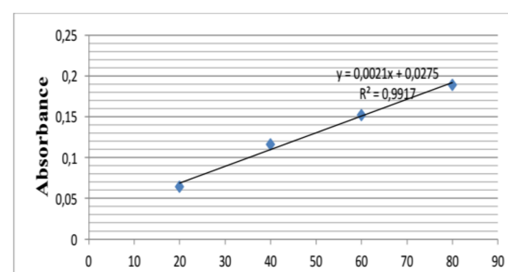

Figure 2. Calibration curve of Total Flavonoids.

Estimation of antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method DPPH radical scavenging activities were determined by the method described by (Pownall *et al.,* 2010).

Prepared different concentrations of the sample by dissolving it in the selected solvent (ethanol) to obtain a range of concentrations. Prepared a control solution without the sample. This will be used to measure the maximum absorbance of DPPH at a specific wavelength. One milliliter of the sample solution was mixed with 1 mL of 0.3 mM DPPH in methanol. The mixture was vortexed for 60 s and incubated in the dark for 30 min. After the incubation period, measure the absorbance of each cuvette at a specific wavelength (usually around 517 nm) using a spectrophotometer. The absorbance is proportional to the remaining DPPH concentration.

Calculation: Calculate the percentage of DPPH scavenged by the sample at each concentration using the following formula: % Scavenged DPPH = [(Abs_control - Abs_sample) / Abs_control] × 100

Methanol was used as a blank. The free radical scavenging ability was calculated using the equation below (Figure 3).

IC_{50} determination: IC_{50} is the concentration of the sample required to scavenge 50% of the DPPH radicals. This value indicates the antioxidant activity, whereas lower IC50 values indicate higher antioxidant potential.

$$\text{DPPH} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

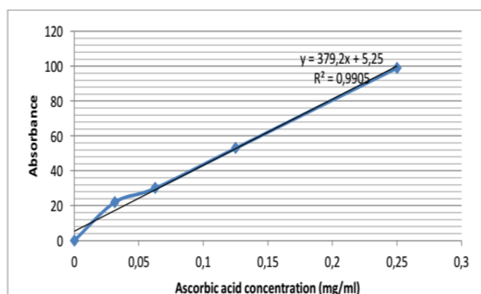

Figure 3. Calibration curve of antioxidants.

Screening the extracts for antibacterial activity

Antibacterial activity is determined by the Agar diffusion method (Khomsug and Thongjaroenbuangam, 2010). Two bacterial cultures were used namely Staphylococcus *aureus* and *Escherichia coli.* The methanolic and ethanolic extracts of okra seeds were tested against the two strains of bacteria.

Procedure

10 mg/ml of sample is prepared. The concentrations in which the bacteria screened are 1000, 500, 250, 100, 50, 25 µg. Ciprofloxacin is used as the standard antibiotic. Media Used are Peptone-10 g, NaCl-10g, Yeast extract 5g, and Agar 20g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37ºC for 18 hrs. After incubation, observe the Petri dishes for clear zones of inhibition around the paper discs. These zones indicate that the bacterial growth has been inhibited by the compounds present in the okra extract. Finally, measure the diameter of the inhibition zones around each paper disc using a ruler or caliper. Larger zones indicate stronger antibacterial activity.

Protein isolation okra seeds

The first step is to remove fat from okra seeds. This can be done using a solvent, such as petroleum ether, which selectively dissolves fats. Then, the defatted okra seeds are ground into a fine powder and mixed with an insulating solution, such as Tris-HCl or phosphate solution, to extract the proteins. The mixture is then filtered through a fine mesh or cotton cloth to remove any solid particles. Extracted proteins are then precipitated using a suitable agent, such as ammonium sulfate or acetone, causing the proteins to exit the solution. Dialysis: The precipitated proteins are then washed and analyzed against a buffer solution to remove any remaining salt or other contaminants. The protein solution is then concentrated using techniques such as ultrafiltration or freeze-drying (Hagerman and Butler, 1978; Rajalingam *et al.,* 2009).

Determination of functional groups by Fourier transform infrared

The FTIR spectra of protein isolate okra seeds were recorded in an FT-IR instrument (Model/ Make: IFS 25, Bruker, Germany), with PC-based software-controlled instrument operation and data processing. Firstly analyze the obtained FTIR spectra using appropriate software or tools. Then after identifying characteristic peaks in the spectrum that correspond to specific functional groups present in the okra seed sample. The data on infrared transmittance was collected over a wave number ranging from 4000 cm^{-1} to 500 cm^{-1} (Saguer *et al.,* 2012).

Determination of protein solubility

Malomo *et al.,* (2015) method was used to determine the degree of solubility of the isolate okra protein and the method depends on dissolving its weight (0.1 g) in a test tube with buffer solutions at different pH values (2 – 4 – 6 – 8 – 10) and dissolving the protein using shaking on a pipe shaker at a speed of 50 rpm and then centrifugal work at a speed of 5000 rpm for 15 min and then measure the protein concentration in the clear part (suspension) using the Lowry method (1951)

Determination of protein functional properties

Determining the functional properties of proteins is crucial in various industries, especially in the food and beverage sector. Several methods are used to assess protein functional properties, such as solubility, emulsifying ability, foaming capacity, and water-holding capacity.

Water absorption capacity (WAC) The WAC of the samples at room temperature and elevated temperatures (50,60,70, 80, 90, and 100°C) was determined following the AOAC (2020) method. One gram (1 g) (w1) of each sample was weighed into a centrifuge tube and the sample was weighed (W2). Ten milliliters (10 mL) of distilled water was added to the sample in a tube. Then after centrifuge (BOSCH centrifuge, TLD-500, England at 3500×g for 20 min). the sample to separate the absorbed water from the protein, and measure the weight of the water retained by the protein. Finally, water-holding capacity is calculated as the amount of water retained per unit weight of the protein. The WAC was expressed as a percentage of the volume of water absorbed by the sample (Gbadamosi *et al.,* 2012).

$$\text{Water absorption capacity (\%)} = \frac{W3 - W2}{W1} \times 100$$

W_3 = weight of tube + sample after centrifuging and decanting W_2 = weight of tube + sample before water was added, W1 = weight of sample.

Emulsifying Ability: a. Prepared an oil-in-water emulsion by mixing the protein sample with an oil phase (e.g., vegetable oil) and a water phase (e.g., water or buffer). b. Homogenize the mixture to form a stable emulsion. c. Measure the stability of the emulsion over time by monitoring changes in the droplet size or visual observation. d. The emulsifying ability is determined by the ability of the protein to stabilize the emulsion and prevent droplet coalescence.

Foaming properties Foaming properties were estimated using the method described by Miller and Groninger (1976) with modifications. Protein solutions (15 mL; 1% w/w, pH 7.0) were homogenized at 8000 rpm for 5 min. Then, the contents were immediately poured into a 50 mL measuring cylinder. The foaming capability (FC) and foaming stability (FS) were calculated using Eqs.

FC (%) = (Foam volume / 15) *100

FS (%) = (Foam volume after 30 or 60 minutes / Foam initial volume) *100

Amino acid determination

Amino acid composition was determined following the method described by Gbadamosi *et al.,* (2012) using S433 Amino Acid Analyzer (SYKAM, Eresing, Germany). Samples were freeze-dried and then hydrolysis of the Protein Isolate**:** a. Weigh a specific amount of the okra seed protein isolate (e.g., 10 mg) and transfer it to a centrifuge tube. b. If using HCl hydrolysis, add 6 N HCl to the tube to cover the

protein isolate completely. c. If using an amino acid analysis kit, follow the manufacturer's instructions for hydrolysis reagents and conditions. d. Seal the tube tightly and heat it at a specific temperature (e.g., 110°C) for a defined time (e.g., 24 hours) to hydrolyze the proteins into individual amino acids, If HCl hydrolysis was used, neutralize the hydrolysate by adding sodium hydroxide (NaOH) to reach a neutral pH. b. If derivatization is required for amino acid analysis, follow the kit instructions for derivatization reagents and conditions. When ready for analysis, 50 µL of the hydrolysates was directly injected into the analyzer. Tryptophan was determined separately by hydrolysis of the sample with sodium hydroxide (Girgih, *et al.,* 2011).

Results and Discussion

Chemical composition analysis

The chemical composition of the sample (okra) is determined to identify the possibility of directing the sample to a specific use somewhat with the rest of the different analyses, and therefore through our results, described in Table (1). It was found that the percentage of crude protein in okra reaches 19.94% and also the percentage of moisture in the sample under study is 11.52% The presence of moisture in this ratio can be a dangerous factor in helping the growth of bacteria and then fungi, and this is what harms the food sample The results of the chemical analysis of the sample, of course, depend on many factors, including those related to the analyzer, including those related to the plant in terms of variety, place, harvest conditions and storage conditions, and this is what (Arapitsas 2008). Ash total content of (5.64%) indicates that the leaves are rich in mineral elements and the high percentage of total fiber in okra (39.21%) indicates the importance of its use in food systems because of its great role in fiber (Acikgoz *et al.,* 2016).

Table 1. Chemical composition of contents in okra.

Content	Moisture	Protein	Lipid	Fiber	Ash	Carbohydrates
Units	11.52	19.94	0.36	39.21	5.64	23.33

Phytochemical analysis

Okra seeds revealed the presence of carbohydrates, glycosides, flavonoids, alkaloids, saponins, terpenoids, and cardiac glycosides. Carbohydrate is present in ethanolic extract and methanolic extract of okra. Phytochemical constituents that are absent in both the extracts of okra are proteins, starch, amino acids, steroids, tannins, and gums. The ethanolic extract exhibited the absence of flavonoid whereas the same is present in methanolic extract. Glycosides, alkaloids, saponins, terpenoids, phlorotannins, and cardiac glycosides are present in both the solvent extractions of okra (Table 2).

Table 2. Analysis of Phytochemical Screening Done In Ethanol and Methanol Extract of *Okra.*

	Phytochemicals	Ethanolic extract	Methanolic extract
1	Carbohydrates	+	+
2	Proteins	-	-
3	Steroids	-	-
4	Glycosides	++	+++
5	Flavonoids	-	-
6	Alkaloids	++	+++
7	Saponins	+	++
8	Terpenoid	+++	+++
9	Cardiac glycosides	+++	+++

+++ Appreciable amount, ++ Moderate amount, + Trace amount, -Absen

Looking at the results presented in Table 2, it is found that the concentration of cardiac glycosides as well as terpenes are present in the extracts at a high concentration. While the presence of imports and flavonoids was not found in this type of extract, and finally the presence of Cardiac glycosides, total increases, and alkaloids in medium concentrations was found in the extracts. These descriptive results of phytochemical compounds are consistent with previous studies of both (Hagerman and Butler, 1978 and Akeem *et al.,* 2016), where they made extracts of okra seeds and identified phytochemical compounds, and the results were close to what was reached in this research. It is known that the presence of secondary metabolic compounds in plant extracts in high or moderate concentrations indicates the possibility of using these extracts in various biological activities such as anticancer-antibacterial activity - antioxidant activity and others, as well as the possibility of their entry into the treatment of many diseases such as heart disease and others (Abideen *et al.,* 2020).

Carbohydrates are present along with alkaloids and steroids whereas flavonoids are absent in ethanol extract of okra seeds (Abideen *et al.,* 2020). In the present study, flavonoids were found in moderate amounts. *okra* contains many phytochemical constituents which are responsible for several health effects. In both plants, methanolic extract answers for more positive results. The phytochemicals that are equally present in okra are carbohydrates, alkaloids, flavonoids, saponins, terpenoids, and cardiac glycosides (Elsohaimy *et al.,* 2015).

Quantification of total phenolic and total flavonoid extract of okra seed

The content of the sample of total phenols as well as total flavonoids gives a good indication that this sample (okra) can have many applications against many diseases and also has many diverse biological activities, so we have known the content of these compounds in okra seeds and it was found by presenting the results in Table 3 that the total phenols that were expressed as gallic acid equivalent has a concentration of 2.142 mg / g extract, while total flavonoids were expressed as the equivalent of the assistant compound. The total concentration of flavonoids reached 3.546 mg / g extract was expressed as catechin equivalent. This indicates that the methanol extract of okra seeds has biological activity and can be applied to a variety of biological activities and these results that have been reached are consistent with the findings of the results of the content of total phenols, flavonoids and condensed tannins confirmed those obtained by (Kumar *et al.,* 2010 and Graham, *et al.,* 2017). On okra respectively. Where he studied the content of okra extracts of phenolic compounds total and also flavonoids and the results were close to what was reached in this paper.

Table 3. Total phenolic, and flavonoid contents of different extracts of okra seeds.

Sample	Total phenolic (mg GAE/g Ext)	Total flavonoids (mg CE/g Ext)
Methanol extract	**2.142**	**3.546**
Ethanol extract	**3.456**	**4.672**

(mg GAE/gExt=mg acid gallic equivalent/ g of extract, mg CE/gExt = mg catechin equivalent/g of extract)

Antioxidant Activity extract okra seed

The free radical scavenging activity of seed okra extract refers to its ability to neutralize and eliminate free radicals.

Free radicals are highly reactive molecules that can cause damage to cells and contribute to oxidative stress, which is associated with various health issues, including aging, cancer, and cardiovascular diseases. Seed okra extract is known to contain various bioactive compounds, such as polyphenols, flavonoids, carotenoids, vitamins, and minerals, which have antioxidant properties. These antioxidants can help counteract the harmful effects of free radicals by donating electrons or hydrogen atoms, stabilizing the free radicals, and preventing them from causing damage to cellular structures. To evaluate the free radical scavenging activity of seed okra extract, researchers commonly use assays such as the DPPH method. In these assays, the extract is tested for its ability to quench or reduce the free radical DPPH. The antioxidant activity is measured by the decrease in the absorbance or color change, indicating the scavenging of free radicals by the methanol extract (Figure 4) (Graham, *et al.*, 2017).

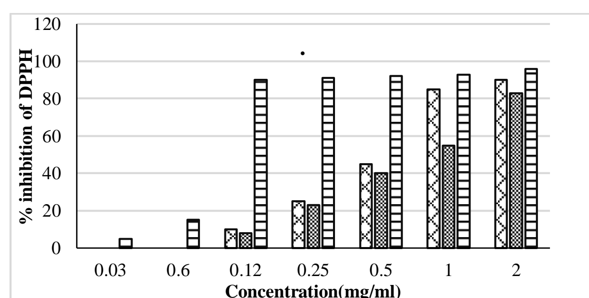

Figure 4. Antioxidant activity of okra by (DPPH method). ethanol extract; methanol extract and ascorbic acid.

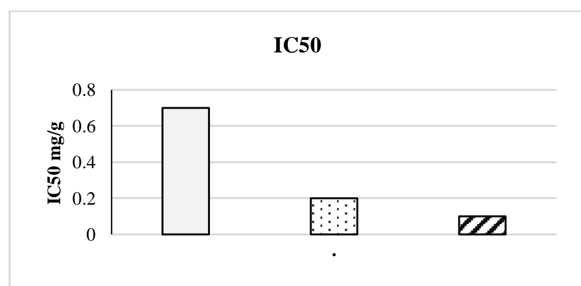

Figure 5. IC_{50} values of DPPH method antioxidant activity of okra. ethanol extract; ME, methanol extract, and ascorbic acid.

Value IC_{50} is an abbreviation for Inhibitory Concentration 50, a term used to estimate the antioxidant activity of chemical compounds. The term is used in a wide range of biotests to evaluate the power of oxidative inhibitors, which aim to protect cells and tissues from damage caused by harmful oxidation.

The relationship between the value of IC_{50} and the antioxidant activity is inverse, meaning the lower the value of IC_{50}, this is indication that the sample under study has antioxidant activity.

It is shown in Figure (5) that the results of IC_{50} for okra extracts, whether methanol and ethanol, as well as ascorbic acid as a material for comparison, that methanol extract has higher IC_{50} values than values of ethanol extract, and of course the lowest value is ascorbic acid at all concentrations used as shown in Figure 4. These results are similar to what was obtained by Graham *et al.*, (2017), where he studied the ability of okra seed extract on antioxidant activity and calculated the value of IC_{50} for methanol extract.

Antibacterial activity

Antibacterial activity is tested for okra extracts by the Agar diffusion method. Methanolic and ethanolic extracts of okra exhibited antibacterial activity against *Escherichia coli* and *staphylococcus aureus.* A maximum inhibition zone is observed in ethanol extract of okra in the concentration of 1000 µg against *Escherichia coli* bacteria. Inhibition zones observed in other concentrations (500, 250, 100, 50, 25µg) were slightly lesser when compared to a concentration of 1000µg. Ethanolic extract showed inhibition zones in all the concentrations except at 25µg, where the inhibition zone is very low. Aqueous and ethanolic extract of okra showed no inhibition activity for *Escherichia coli* and *Staphylococcus aureus* (Chaudhari, *et al.*, 2011) whereas a mixture of spices exhibited inhibition zone for the same bacteria. The diameter of the inhibition zone in 1000µg concentration obtained was 9mm (Khomsug *et al.*, 2010). In the present study, 12 mm is obtained in a concentration of 1000 µg which is an increased inhibition zone compared to previous works carried out in the same species. An inhibition zone of 6 mm was observed against *S. aureus* (Khomsug *et al.*, 2010) which is considered less active (Table 4).

Table 4. Antibacterial activity of okra against *Escherichia coli* and *Staphylococcus aureus.*

Sample	*E. coli*					*S. aureus*				
	25 µg	50 µg	100 µg	250 µg	500 µg	25 µg	50 µg	100 µg	250 µg	500 µg
Ethanol extract of okra	0.5	4	6	8	13	3	3	5	2	8
Methanol extract of okra	0.5	3	5	6	11	4	5	7	2	7
Standard antibiotic	17	19	22	25	29	33	25	22	19	17

Fourier transformed infrared spectra of okra protein isolate extracted

Infrared spectroscopy (FTIR) technology is a powerful analytical tool used to study the molecular structure of proteins and the functional groups active in their part. This technique is based on measuring the absorption of infrared radiation by the chemical bonds contained in the protein. The infrared spectrum provides information on the secondary and three-dimensional structure of the protein, including effective functional aggregates. When the infrared spectrum is applied to a particular protein, the functional groups that are effective in the protein can be determined using spectral peaks produced by different chemical bond reactions. Here are some common functional aggregates that can be determined using the infrared spectrum: Peptide bonds: Characteristic peaks appear in the region of 1600-1700 cm^{-1} and represent the peptide bonds by which amino acids are joined together to form protein chains. Hydrogen bonds: Peaks from hydrogen bonds range from 3000 to 4000 cm^{-1} and usually appear when a protein reacts with water. Carbonyl bonds: The characteristic peaks of carbonyl bonds appear about 1700 cm^{-1}, representing carbon-oxygen bonds in carbonyl groups such as ketones and esters. Sulfur bonds: Sulfur bonds can be detected in sulfur chemical aggregates,

such as thiol (-SH) groups, which usually appear at a frequency of about 2500 cm^{-1} (Abideen *et al.*, 2020).
The appearance of signals in the infrared spectrum at 1648 and 1539 cm^{-1} shows that the okra protein contains amide-type bonds and also carbonyl-type bonds, and this confirms the possibility that the okra protein contains a small percentage of triglycerides.
Okra protein contains the remnants of the amino acid as well as lysine and histidine, and also the percentage of their presence is high compared to other amino acids that are present in the protein, so we find that the signal of the amino group is strong as shown through the infrared spectrum Figure 6. Therefore, an explanation can be given for the ability of okra protein to stop antioxidant activity, due to the availability of the amino group in the side chain of the okra protein peptide chain (Barron *et al.*, 2005; Armstrong *et al.*, 2006).

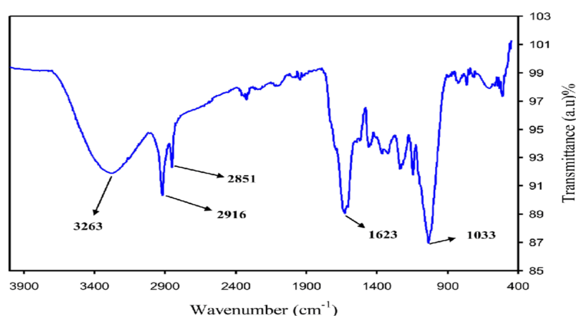

Figure 6. FTIR-spectrum-of okra protein extract.

Electrophoresis patterns of okra protein

In a Native PAGE of okra protein, you would observe proteins in their native forms. The migration of proteins depends on both size and charge. Therefore, the bands would not strictly be ordered by molecular weight but rather by the overall charge and size of the proteins. Native PAGE can provide valuable information about the protein composition and molecular weight distribution in the okra extract, allowing researchers to compare different samples or detect any changes in protein content under different conditions.
The electrophoresis patterns of okra-extracted protein in an aqueous extract with different pHs are shown in (Figure 7). It is well known that extraction methods change the chemical characteristics of proteins. To characterize the protein profile in non-reducing and reducing conditions, SDS-PAGE electrophoresis was performed for okra protein (Figure 6). In the gel, bands with different molecular weights are depicted and agree with electrophoretic profiles previously published by different authors for other okra varieties (Deuber *et al.*, 2012). According to (Oyelade *et al.*, 2003), one of the protein fractions present in okra is 11S globulin, with a molecular weight of 50 kDa. On the other hand, (Hagerman *et al.*, 1978) reported the presence of a 31–33 kDa hemoprotein and a 55 kDa protein, which correspond to globulins. Elsohaimy *et al.* (2015) reported that the protein bands smaller than 20 kDa belong to albumin components. The protein with MW 85 KDa was not found in all pHs while the protein with 55 KDa corresponding to globulin according to (Ijarotimi *et al.*, 2023) was found in all pHs. The protein with MW 33 KDa was found in all pHs with high expression. Proteins with 31–33 KDa correspond to hemoprotein according to (Girgih *et al.*, 2011). While protein with MW 22 KDa was found in all pH values but in high expression in pHs 7,9 and 10. All protein bands less than 20 KDa corresponding to albumin components according to (Ijarotimi *et al.*, 2023) were found in all pH values with very high expression.

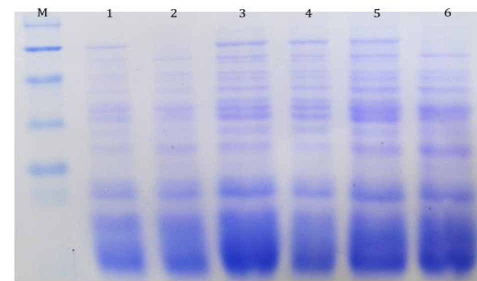

Figure 7. SDS–Page of protein extractability on different pHs: Lane 1 – pH 5, Lane 2 – pH 6, Lane 3 – pH 7, Lane 4 – pH 8, Lane 5 – pH 9, and Lane 6 – pH 10.

Emulsifying property

The result illustrated in Figures (8, 9, and 10) clarified that protein okra seeds have low emulsification activity (3.2%) but it has higher emulsification stability (98.9%). The decrease in the emulsifying activity of the protein may be due to the protein reaching the electrolyte point, which causes it to precipitate and reduce its emulsifying activity. The emulsifying property of protein is important to form a gel structure with water by imbibing it and binding to it, Protein prevents slow melting.

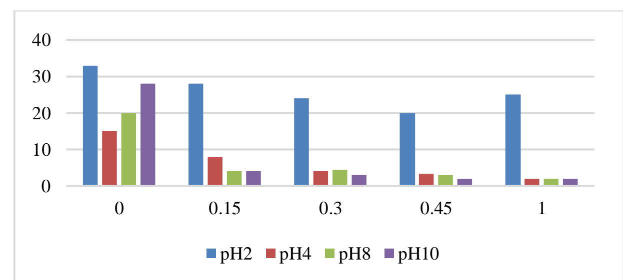

Figure 8. Foam stability of protein okra seed isolate.

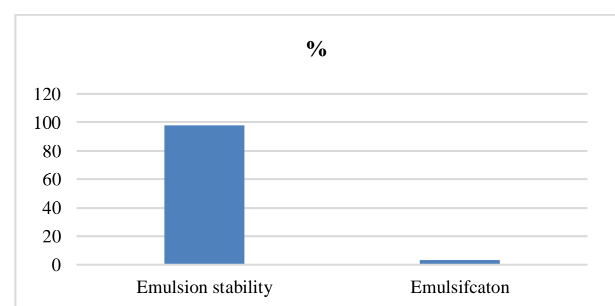

Figure 9. Emulsion stability (A) and Emulsification (B) of isolating okra seed protein.

Thus, reducing the free water in the mixture. As for the stability of the emulsification, it was clear from the results that protein okra seeds have a high percentage of stability, and the percentage of stability of the emulsifier was high in the medium at pH=7. It was explained by (Mensah *et al.*, 2009) that the stability of the emulsion depends on the formation of a charged layer through the dissolved proteins around the oil droplets, leading to the repulsion of the droplets with each other. Which has an effective surface with the ability to emulsify and its stability through the stable

electrostatic force on the surface of the fat drop (Oyelade *et al.,* 2003).

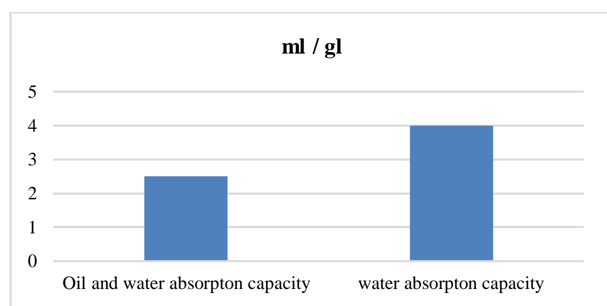

Figure 10. Oil and water absorption capacity.

Content protein okra from Amino acid

The amino acid composition of okra protein isolate is presented in (Table 4). Proteins in okra contain different ratios of amino acids, which vary between different varieties of okra and also depending on the different growing conditions. However, okra is considered a good source of essential amino acids such as lysine, threonine, valine, and isoleucine. Some studies suggest that okra protein contains higher levels of lysine compared to other plant proteins. Lysine deficiency is a common problem in plant proteins, so the presence of high levels of lysine in okra protein is an important advantage for vegetarians and vegans. The following are the ratios of the major amino acids in known okra protein.

Okra protein had a high level of lysine (16.13%), which registered the highest amino acid score (AAS), glutamic acid (6.70%), and aspartic acid (5.54%), while had a very low level of proline (0.10%) and arginine (0.03%). On the other hand, okra protein had a moderate level of glycine (9.60%), alanine (5.34%) and phenylalanine (5.67%). From the obtained results appeared that the okra protein had reasonable concentrations of essential amino acids (Table 5). The results obtained are consistent with what (Ijarotimi *et al.,* 2023) when they studied the amino acid components of the okra seed protein isolate, and the results were close to what was obtained, while the results were far from what was done by (Yao *et al.,* 2021)

Table 5. Amino acid composition of okra protein isolate (g/100 g protein).

Necessary amino acids	g/100 g	Unnecessary amino acids	g/100 g
Histidine	**2.76**	Serine	**2.57**
Lysine	**16.13**	Glycine	**9.60**
Leucine	**4.60**	Alanine	**5.34**
Isoleucine	**1.30**	Glutamic	**6.70**
Methionine	**0.89**	Proline	**0.10**
cystine	**0.67**	Aspartic	**5.54**
Phenylalanine	**5.67**	Tyrosine	**2.88**
tyrosine	**3.25**	Arginine	**0.03**
Threonine	**1.47**		
Valine	**2.03**		
Tryptophan	**0.5**		

Conclusion

The current study studies the nutritional importance of the okra plant, as okra is an available source that can be obtained, as it contains high percentages of protein, carbohydrates, minerals, vitamins, fibers, and fatty acids, and the focus here was on protein and studying the structural and functional properties and molecular weight of protein as well as knowing the functional groups through the infrared spectrum, as well as knowing the content of total phenols, as well as total flavonoids and antioxidant activity. It was also found that the phytochemicals in okra seeds show antibacterial activity and antioxidant activity and these extracts (aqueous and alcoholic) may be useful in the development of a new antibacterial agent from natural sources as the components of amino acids involved in the composition of okra seed protein were also identified, where it was found that the basic amino acid is not present at a higher rate than the rest of the acids and reached (17.13%) followed by the amino acid glycine by (9.6%) and was the least acid Amino ratio is arginine (0.05%).

References

Abideen AA, Oyekanmi HA, Uthman TA, Muraina TA, Opeyemi SA and Sheriffdeen AA (2020). Characterization and Phytochemical Property of Okra Fruits. International J of Academic Engineering Res. 4(5), 34-39

Elsohaimy SA, Refaay TM and Zaytoun MA (2015). Physicochemical and functional properties of quinoa protein isolate. Annals of Agricultural Sciences, 60(2), 297–305.

Acikgoz C, Borazan AA, Andoglu EM and Gokdai D (2016). Chemical composition of turkish okra seeds (*hibiscus esculenta)* and the total phenolic content of okra seeds flour. Anadolu University Journal of Science and Technology A-Applied Sciences and Engineering, 17(5), 766-774.

Adams CA, Norby SW and Rinne RW (1985). Production of multiple vacuoles as an early event in the ontogeny of protein bodies in developing soybean seeds. Crop Sci 25: 255-262

Adelakun OE, Oyelade OJ, Ade-Omowaye BO, Adeyemi IA and Van de Venter M (2009). Chemical composition and the antioxidative properties of Nigerian Okra seed (*Abelmoschusesculentus Moench*) flour. Food Chem Toxicol 47: 1123-1126.

Akoja SS and Coker OJ (2018). Physicochemical, functional, pasting and sensory properties of wheat flour biscuit incorporated with Okra powder. International J of Food Science and Nutrition. 3(5), 64-70.

AOAC (2016). Association of Official Analytical Chemists. Official Methods of Analysis 20th edition, Washington DC. USA.

Arapitsas P (2008). Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chem., 110: 1041–1045.

Armstrong PR, Maghirang E, Xie F and Dowell FC (2006). Comparison of dispersive and Fourier-transform NIR instruments for measuring grain and flour attributes. Applied Engineering and Agriculture, 22(3), 453–459.

Barron C, Parker ML, Mills ENC, Rouau X and Wilson RH (2005). FT-IR imaging of wheat endosperm cell walls in situ reveals compositional and architectural heterogeneity related to grain hardness. Planta, 220(5), 667–677.

Chaudhari Y, Kumar EP, Badhe M, Mody RH and Vamshikrishna BA (2011). An Evaluation of Antibacterial Activity of Abelmoschus esculentus on clinically isolated infection disease causing Bacteria pathogen from Hospital. Inte J of Pharmaceutical and Phytopharmacological Res. 1 (3), 107-111.

Deuber H, Guignard C, Hoffmann L and Evers D (2012). Polyphenol and glycoalkaloid contents in potato cultivars grown in Luxembourg. *Food Chemistry, 135*(*4*), 2814–2824.

Eze JI and Akubor PI (2012). Effect of drying methods and storage on the physicochemical properties of okra. J Food Process Techno 3:177.

Gbadamosi SO, Abiose SH and Aluko RE (2012). Amino acid profle, protein digestibility thermal and functional properties of Conophor nut (*Tetracarpidium conophorum*) defatted flour, protein concentrate and isolates. International J of Food Science and Technology, 47(4), 731–739.

Gbadegesin AR, Odunlade TV and Otunola ET (2018). Nutritive and sensory properties of okra fortified instant fufu. International Food Research J 25(3): 1031-1035.

Gemede HF, Ratta N, Haki GD, Beyene F, Woldegiorgis AZ and Rakshit SK (2016). Proximate, mineral, and anti-nutrient compositions of indigenous okra (*Abelmoschus esculentus*) pod accessions: implications for mineral bioavailability. Food Science and Nutrition.; 4(2): 223–233.

Girgih A, Udenigwe C, Li H, Adebiyi A and Aluko R (2011). Kinetics of enzyme inhibition and antihypertensive effects of hemp seed (*Cannabis sativa* L.) protein hydrolysates. Journal of the American Oil Chemists’ Society, 88(11), 1767–1774.

Hagerman AE and Butler LG (1978). Protein precipitation method for the quantitative determination of tannins. J of Agricultural and Food Chemistry, 26: 809 – 812.

Riaz H, Almas B, Syed AR and Zia MK (2015). Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan International Current Pharmaceutical J, 2015, 405-409.

Jalande V and Gachande BD (2012). Effect of Aqueous leaf extracts of Datura Sp. Against two plant pathogenic fungi. International J of Food, Agriculture and Veterinary Sciences. 2 (3), 131- 134.

Khomsug P and Thongjaroenbuangam W (2010). Antioxidative activities and Phenolic content of extracts from Okra (*Abelmoschus esculentus* L.). Res J of Biological Sciences. 5 (4), 310-313.

Kumar S, Dagnoko S, Haougui S, Ratnadass A, Pasternak D and Kouame C (2010). Okra (*Abelmoschus spp.*) in West and Central Africa: potential and progress on its improvement. Afr J Agric Res, 5:3590-3598

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J of Biological Chemistry, 193*(*1*), 265–275.

Malomo SA, He R and Aluko RE (2015). Structural and functional properties of hemp seed protein products. J of Food Science, 79, 1512–1521.

Mensah JK, Okoli RI, Turay AA, Ogie-Odia EA (2009). Phytochemical analysis of medicinal plants used for the management of hypertension by Esan people of Edo State, Nigeria, 48: 648 - 661

Oyelade OJ, Ade-Omowaye BI and Adeomi VF (2003). Influence of variety on protein, fat contents and some physical characteristics of okra seeds. Food Chem., 57: 111-114.

Petropoulos S, Fernandes A, Barros L and Ferreira I (2018). Chemical composition, nutritional value and antioxidant properties of Mediterranean okra genotypes in relation to harvest stage. Food Chemistry 242 , 466–474.

Pownall TL, Udenigwe CC and Aluko RE (2010). Amino acid composition and antioxidant properties of pea seed (*Pisum sativum* L.) enzymatic protein hydrolysate fractions. J of Agricultural Food Chemistry, 58(8), 4712–4718.

Rajalingam D, Loftis C, Xu JJ and Kumar TKS (2009). Trichloroacetic acid-induced protein precipitation involves the reversible association of a stable partially structured intermediate. *Protein Science: A Publication of the Protein Society, 18*(*5*), 980–993.

Saguer E, Alvarez P and Ismail AA (2012). Heat-induced denaturation / aggregation of porcine plasma and its fractions studied by FTIR spectroscopy. Food Hydrocolloids, 27(1), 208–219.

Akeem S, John J, Rowland K and Fausat K (2016) Comparative phytochemical analysis and use of some Nigerian spices Croatian. Journal of Food Technology, Biotechnology and Nutrition.; 11 (3-4), 145-151.

Zheng W, Zhao T, Feng W, Wang W, Zou Y, Zheng D and Wu X (2014). Purification, characterization and immunomodulating activity of a polysaccharide from flowers of *Abelmoschus esculentus*. *Carbohydrate Polymers, 106*(*1*), 335–342.

Jochebed OG, Jacob KA and Fidelis MK (2017). Total Phenol Content and Antioxidant Activity of Okra Seeds from Different Genotypes American J of Food and Nutrition, 5(3), 90-94

Ijarotimi OS, Abimbola OA and Timilehin DO (2023). Okra seeds proteins: Amino acid profile, free radical scavenging activities and inhibition of diabetes and hypertensive converting enzymes indices Measurement: Food 11,100-111.

Yao HL, Jia NY, Zhan J, Qu L, Su M and Jiang YJ (2021). Preparation, amino acid composition, and in Vitro antioxidant activity of okra seed meal protein hydrolysates Food Sci Nutr. 9, 3059–3070.