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Importance of introducing pumpkin seed powder from a microbial and nutritional standpoint in the biscuit industry

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ABSTRACT

The study results showed the possibility of introducing pumpkin seed powder into baked goods due to its effective antioxidant compounds and inhibitory capacity against pathogenic microbes. Using a 1.5% alcoholic extract significantly increased the inhibition diameters to 31 mm against Staphylococcus aureus compared to a 1% concentration, while the inhibitory effectiveness decreased to 10 mm for Pseudomonas aeruginosa. The antioxidant effectiveness results showed activity at a 1.5% concentration of the alcoholic extract due to the presence of phenolic compounds, tannins, flavonoids, and glycosides, which were identified using GC-MS with over 30 peaks in pumpkin seed powder. Given its high protein content of 16.4%, fat content of 46%, fiber content of 15.13%, and low moisture content of 4.5%, along with being gluten-free, it was introducing into biscuit production. Sensory evaluation results indicated that the best treatment was the second one with half the replacement ratio in terms of texture, taste, color, and spreadability, showing consumer acceptance of this product. Therefore, the current study aimed to find a healthy alternative to seeds for making beneficial and healthy foods. Baked products can be made from pumpkin seed flour due to its ability to enhance sensory properties and quality, in addition to the presence of active compounds in pumpkin seeds that increased the functional and nutritional properties of food products.

1- Introduction

Pumpkins belong to the genus Cucurbita moschata and the Cucurbitaceae family, which includes more than 130 genera and 800 species. They can be grown all over the world, especially in warmer regions, as pumpkins are one of the oldest cultivated species, dating back to 7000 BC. They are widely grown in Mexico, Central and North America, and are native to northern Mexico and the southwestern and eastern United States [1]. Pumpkin is a fruit that has many nutritional and medical benefits. From a nutritional point of view, unripe fruits are consumed as vegetables, while ripe fruits are eaten as sweets and can also be used in making sweets.These compounds are also converted inside the body into vitamin A, which plays an important role in the general health of the consumer in relation to the functions performed by the body. The two colors that give the pumpkin a bright yellow color. These fruits also contain a good amount of essential and non-essential amino acids. One of the most important essential amino acids available in pumpkin fruits is lysine [2]. The seeds may be white or brown in color, as pumpkin fruits are harvested after full ripeness to obtain high-quality seeds. Pumpkin fruits contain different percentages of seeds ranging from 3.52 to 4.27% of the pumpkin fruit volume. Despite their high nutritional value, they are not properly exploited and are sometimes disposed of as waste. The many nutritional and health components that pumpkin seeds possess, making them at the forefront of seeds that play an important role in the food industry as a healthy alternative to snacks. Pumpkin seeds can also be consumed roasted or raw and are used in cooking and making bread and cakes. They can also be added to salads [2 ; 3]. Pumpkin seeds are an excellent source of protein and a good source of minerals, dietary fiber and vitamins that are beneficial to the body. Pumpkin seeds are also used as an anthelmintic, treatment of urinary tract problems and high blood pressure, prevention of kidney stones, relief of prostate diseases and reduction of fever infection [4]. Pumpkin seeds contain many active compounds such as p-aminobenzoic acid, am-aminobutic acid, sugars, peptides, proteins and carotenoids. Due to the presence of the above-mentioned active compounds, pumpkin seeds have many health and therapeutic benefits and are used in many different food industries due to their importance [4]. In addition, pumpkin seeds are a rich source of minerals such as manganese, magnesium, copper, iron and zinc, and contain volatile oils, proteins and sugars. They also contain organic acids, fatty acids and flavonoids. Pumpkin seeds also contain vitamins [5]. Pumpkin seeds can be eaten raw or roasted and can be used in salads and in baking as an ingredient in bread and cakes. Pumpkin seeds have many benefits. They are dark green seeds with a chewy texture and a slightly sweet taste. They are available in many forms, including raw, shelled, roasted and unshelled seeds. In all cases, they have countless health benefits. This is why they are widely used in medicines and home remedies [6]. These seeds are not only a rich source of proteins, vitamins and minerals, but also contain healthy oils that are beneficial in case of nutritional deficiencies in your body. Pumpkin seeds contain minerals such as magnesium, phosphorus, iron, copper, zinc and manganese. They are also an important source of vitamins B and E. Because of their nutritional value, the seeds are added to salads. In addition, pumpkin seeds contain essential fatty acids and antioxidants. The main fatty acids are palmitic, oleic and linoleic acids. These fatty acids are important for metabolism and cell growth in the body [7].

Studies have shown that pumpkin seeds have antibacterial and antioxidant activity. Various

aqueous and alcoholic extracts are prepared from pumpkin plant parts and seeds [8]. The food industry is looking for new natural compounds to use as non-synthetic compounds that act as antimicrobial agents, with the aim of meeting the needs of consumers for healthy food products free from contaminants, [9]. The use of antimicrobial agents derived from plants and grains plays a major role in food safety and food security while providing additional functional properties to food products at the same time. For this reason, interest in studies has focused on different plant species and their grains, also considering obtaining new compounds from by-products of food processing industries and their products. [10] Bacteria, viruses, fungi and other parasites cause diseases in humans and as a result, these diseases have caused social, economic and health problems for millions of people as well as deaths for others. Although drugs are considered safe as a treatment for most diseases, several individuals still lack health safety due to the presence of allergic and other conditions and due to the development of drug-resistant pathogenic microorganisms, which is an alarm sign for scientists to develop new, more effective drugs for these infectious microorganisms. That is why natural sources are considered the best way to create new compositions for the isolation of new antimicrobial components. Pumpkin introduced several broad - spectrum antimicrobials against Gram-positive and Gram-negative pathological microbiota [11]. The study aimed to take advantage of pumpkin seeds, which are considered waste products that can reduce environmental pollution and it is economically feasible to introduce them as a powder in the bakery industry because they contain biologically active compounds, antioxidants, and bacteria inhibitors.

2-Materials and methods

The sample was purchased and prepared from local markets in Basrah governorate after cleaning, washing, and drying the seeds were kept in containers in the refrigerator until the completion of the study, this study was conducted in the laboratories of the Department of Food Sciences, grain laboratory for graduate studies and Microbiology Laboratory at the Faculty of Agriculture, University of Basra in the period between 2023 and 2024, four types of bacteria were selected to study the inhibitory effectiveness of: *Staphylococcus aureus* ، *Bacillus subtilis* ، *Escherichia coli* ، *Psudomonas aeruginosa*, Figure (1).

Figure (1): Steps for collecting and preparing pumpkin plants and their seeds

Chemical composition

Estimation of moisture content

The moisture content of pumpkin seed powder was estimated according to the method mentioned in [12] by weighing 5 g of samples and placing them in an electric drying oven for 3 hours until the weight stabilized at a temperature of 105°C.

Estimation of protein content

The nitrogen percentage in pumpkin seed powder was estimated using the microkjeldahl method according to what was mentioned in [13], then the result was multiplied by the protein coefficient of the grains [5.7] to extract the protein percentage.

Estimation of Ash Content

The ash content of the pumpkin seed powder under study was estimated based on the existing method[14] With a weight of 2-5 g. The sample was placed in the ceramic bowl in the incineration furnace at a temperature of 550 °C**.**

Estimation of the percentage of fat

The percentage of fat was estimated according to the method referred to in [14] using the Soxhlet continuous extraction device using petroleum ether solvent with a boiling point of 40-60 °C.

Fiber estimation

The percentage of fibre in pumpkin seed powder was estimated according to the method mentioned in [15] [The pellet was weighed again and the percentage of fibre was calculated based on the dry weight from the equation:-

w_1= weight of the sample before incineration and drying, w_2= weight of the sample after incineration and drying

Estimation of carbohydrate percentage

The percentage of carbohydrates was estimated by calculating the difference between the sum of the percentages of moisture, ash, fat and fibre.

Physical properties

pH

The pH of the samples was estimated using the pH-Meter device following the method mentioned in-.

[16]. This was done by taking 5 g of the sample and adding 20 ml to it. Then it was homogenized manually using a glass stick for 3 minutes. After the homogenization process was completed Estimate the pH.

Estimation of total acidity [titration method]

The total acidity was estimated according to the method mentioned in [14] By calculating the number of milliequivalents of acid

From the following equation

Extraction

Water extraction

The aqueous extract of dried pumpkin seed powder was prepared according to the method of [17], by weighing 100 g of pumpkin seed powder, mixing it with 500 ml of distilled water and leaving it for 2 hours, then incubating the powder at 40 °C in an incubator for 24 hours, placing it on a magnetic stirrer for 30 minutes, then passing the filter through filter paper, pouring it into a Petri dish and placing it in an electric oven at 40 °C, until the extract was dry, and storing the dry powder in the refrigerator at 4 °C until use.

Alcoholic extraction

The alcoholic extract of dried pumpkin seed powder was prepared according to the method mentioned in [17], by weighing 50 g of pumpkin seed powder and dissolving it in 500 ml of 70% methanol solution, then placing the mixture in a shaking incubator for 4 hours, and leaving the mixture at room temperature For 4 hours, then the mixture was filtered through filter paper (Whatman No. 1) The filtrate was collected in Petri dishes and dried in an electric oven at 40°C, the powder was collected and stored in containers in the refrigerator at 4°C until use. The concentrations of aqueous and alcoholic pumpkin seed extracts were prepared at concentrations of (100-1000) mg/ml and the yield percentage was calculated by weighing the sample before extraction and weighing the extracted product according to the equation below.

The yield percentage = (weight of dry extract/weight of sample) X 100

Qualitative detection of active compounds

Detection of phenols

The detection of phenols was carried out by adding two drops of 1% ferric chloride to 1 ml of the plant extract, the green color appeared and this is evidence of the presence of phenols [18].

Flavonoids detection

Flavonoid detection was done by soaking 10 g of dried pumpkin seed powder in 150 ml of 1% dilute hydrochloric acid for 24 hours, then filtration was done by taking 1 ml of the filtrate and adding 1 ml of alcoholic potassium hydroxide until an alcoholic layer was formed at the top of the test tube. If a light yellow colour appears, this is evidence of the presence of flavonoids in the plant extract [18]

Glycosides detection

Glycosides were detected using Benedict's reagent prepared by dissolving two solutions, the first consisting of 173 g of sodium citrate with 100 g of anhydrous sodium carbonate dissolved in 600 ml of distilled water and the second consisting of 17.3 g of copper sulphate dissolved in 150 ml of distilled water with gentle heating and stirring. The two solutions were mixed and the volume was completed to a litre then equal amounts of the prepared plant extracts were mixed with Benedict's reagent and placed in a boiling water bath for 5 minutes. The appearance of a brown or red precipitate was evidence that the test was positive. [19].

Tannin detection

Tannins were detected using 1 ml of lead acetate at a concentration of 1% mixed with 1 ml of plant extracts and the appearance of a white precipitate is evidence of the presence of tannins. [20].

Identification of biologically active compounds in pumpkin seed extracts

The active compounds in pumpkin seed powder were identified and their contents were characterized from the active compounds separated by the GS-MS device, by weighing 1 g of pumpkin seed powder samples and grinding them in the laboratory, then the samples were left at laboratory temperature and 10 ml of hexane solution was added to each sample, left for a period after which 4 ml of the extract were withdrawn and filtered using a 0.22 microliter filter, then 1 microliter of the samples were injected into the GS-MS device as in Table (1) which shows the operating conditions of the device. After obtaining the mass spectrum of the active compounds, the results were processed by the GS-MS solution program and matching the separated peaks with the spectra database in the NIST08.LIB program librar

Table (1): Conditions for separating active compounds using GC-MS technique

Device Type	Shimadzu GS-MS2010,Gas Chromatography-Mass Spectrophotometry
Type of injection	Split at ratio 1:30
Separation column type	Rtx-5MS Ceapillary column [crossbond 5% dipheny1-95% dimethylpoly siloxane],30m[L] x 0.32[i.d.] with a 0.25µm film thickness
Injection temperature	250C
Column temperature	Starts at 40C and stabilizes for 5 min then increases by 5C every min until it reaches 250C
Gas used	Helium gas He with purity of 99.99%

Pressure	kpa57.4
Mass Spectrometer	Electron Impact Ionization[EI];recorded in intervals 30-170m/z
Sample volume at injection	1 microliter
mass spectrometer temperature	C200°

Antioxidant activity

The antioxidant activity was measured according to the method mentioned in [21[using DPPH free radicals prepared at a weight of 0.005 g dissolved in 100 ml of 98% methanol solution. 0.2 ml of the sample was taken and mixed with 3 ml of 0.5 mM DPPH solution. The mixture was mixed with a shaker and then the sample was kept in the dark for half an hour. After that, the absorbance was measured at a wavelength of 517 nm. The synthetic antioxidant ubiquitinated Hydroxy Toluene (BHT) was used at concentrations of 0-120 mg/ml for comparison. The control sample was prepared in the same way except for adding methanol instead of the sample. The percentage of effectiveness was calculated according to the following equation.

Inhibitory activity of microorganisms

McFarland solution

McFarland solution was prepared according to the method of [22] by dissolving 1% of aqueous barium chloride prepared from 1.17 g of barium chloride with the volume being completed with distilled water to the mark of 100] and 1% of sulfuric acid prepared from 0.55 ml of sulfuric acid with the volume being completed with distilled water to the mark of 100 and then the two solutions were mixed, Table (2).

Table [2]: Preparation of different concentrations of McFarland solution

Tube number	1 ml aqueous barium chloride 1%	1 ml sulfuric acid 1%	Bacteria count 810/ml
1	0.1	9.9	3
2	0.2	9.8	6
3	0.3	9.7	9
4	0.4	9.6	12
5	0.5	9.5	15

Liquid culture medium Nutrient brot

The nutrient medium was prepared by dissolving 28 g of the medium in 1 litre of distilled water and sterilized by an autoclave for 15 minutes at a temperature of 121°C and a pressure of 15 pounds/inch2 (Himedia Company).

Muller Hinton Agar

Prepared by dissolving 13 g of solid medium in 1 litre of distilled water and sterilized in an autoclave for 15 minutes at 121˚C and 15 pounds/inch^2 pressure (Oxoid Company).

Peptone solution

Used to make the required dilutions and prepared according to the instructions of the supplier company Himedia with a weight of 1 g dissolved in 1 litre of distilled water and sterilized in an autoclave at 121˚C for 15 minutes under a pressure of 15 pounds/inch ^{2}.

The absorbance was read by a spectrophotometer at a wavelength of 600 nm. The absorbance

results of the bacterial suspension were compared with the reading of McFarland's solution. Turbidity was noted. It was found that if the two readings were equal, this indicates that the number of bacteria had become 10^8/ml.

Pathogenic bacterial cultures used

Four bacterial isolates were selected in the microbiology laboratory of the Colleges of Agriculture and Sciences/University of Basra, including E. coli, Pseudomonas, Staph aureus, and Bacillus.

Kirby Bauer method

The previously selected pathogenic bacterial cultures were activated using liquid activation media by taking 1 ml of the bacterial culture and adding it to 9 ml of Nutrient broth and incubating for 18-24 hours at 37 °C, then pouring the solid culture medium for inhibition Muller Hinton Agar into Petri dishes and spreading a volume of 0.1 ml of the active bacterial culture on the surface of the solid medium using a glass diffuser and leaving the dishes for 5 minutes to dry, after which the previously selected antibiotic discs were transferred to each dish, taking into account leaving spaces between the discs. The plates were incubated at 37°C for 24 hours and the diameter of the inhibition halo [mm] was measured for each disc of plant extracts using a graduated ruler [23].

Estimation of gluten in pumpkin seed powder

Gluten was measured manually in the laboratory by weighing 20 g of pumpkin seed powder and mixing it with 12 ml of saline solution prepared at a concentration of 1%. After that, the dough was made manually into balls and then the manual washing process was carried out to get rid of starch and weak gluten residues. After that, the gluten coefficient was calculated as in the equation listed below. [24].

$$Clotin\ Factor = \frac{Total\ Gluten - Weak\ Gluten}{Total\ Clotin} * 100$$

Biscuit preparation, diffusion coefficient measurement and sensory evaluation form Samples of biscuits were prepared according to the method mentioned by [25], with some modifications in the proportions of substitution of wheat flour with pumpkin seed powder 50 and 100 g per 100 g of wheat flour with the added ingredients mentioned in the table [3].

Physical Properties of Biscuits

The physical properties of biscuits were studied, which include [diameter, thickness and diffusion coefficient] as mentioned in [26]

The diameter D was measured by taking 6 pieces of biscuits and measuring from the beginning of the edge to the end of the edge, while the thickness T was measured by placing 6 pieces of biscuits one on top of the other and then measuring in millimetres. After that, the diffusion coefficient was extracted by measuring the average value of the thickness and diameter D/. Diffusion coefficient = thickness diameter

Sensory Evaluation

The sensory evaluation of the biscuit samples was conducted according to colour, flavour, texture and general acceptance by experienced and specialized evaluators and undergraduate and graduate students according to the sensory evaluation .[27].

Table [3]: Components of laboratory-prepared biscuits

Raw material	Quantity % gm per 100
Pumpkin seed powder	In proportions of 50 and 100
Wheat flour	100
Ground sugar	30
Vegetable butter fat	20-25
Salt	1
Dry milk	5
Peak powder	4
Water	10-25 ml

Statistical analysis

The statistical analysis system [2^] was used to study the effects of different parameters. The least significant difference (LSD) between the mean values of treatments was determined.

3-Results and discussion

Chemical characterization of pumpkin seed powder

The results of the statistical analysis showed significant differences at the probability level $p \leq 0.05$ for the pumpkin seed powder as in figure (2) Determination of physico-chemical properties of as the moisture content in the powder reached 4.576%, which is less than 18%, which is evidence of the dryness of the seeds and the decrease in their water content, which reduces the percentage of powder spoilage and extends its storage life, while the ash percentage was 11.14%, while the fat percentage in the pumpkin seed powder reached 45.11%, which is considered high in seeds, so the grains are considered a source of Omega-3, which affects the health of the body, while the fibre percentage was 15.13%, which plays a role in facilitating the digestion process and thus can be included in the healthy diet system. The results showed a significant increase in the protein percentage, which reached 16.03%, which is evidence that it contains a group of essential and non-essential amino acids. The type and variety of seeds, the method of planting, the place and environmental conditions had a clear effect on showing the above results. It is close to what was reached by [29].The differences in the nutritional parameters might be due to the differences in species, varieties, ripening stages, fertilization system, and growing conditions. The results of the present study agreed with [29] who reported that pumpkin contained 5% moisture, 38% fat, protein 27.48%, however higher than [29]. pumpkin contained 5.2% moisture, 41% fat, 25% protein, and ash 5.3% Also, [29]. reported that , the obtained values lower than that showed by [29] where is the pumpkin contained 26.3% protein, 43.1% fat, 3.8% ash.However, higher than that reported by [29]. the pumpkin contained 26.3%protein, 43.1% fat, 3.8% ash, who reported that ,The obtained results in the current study emphasized the high nutritional quality of the investigated seed as a good source of protein, fat and fiber which is encourage their utilization in food applications. The results of the physical properties of pumpkin seed powder showed that the pH is within the required range for the studied samples [5-6], while the total acidity was estimated at 78.34%. [30] and [31]. Active compounds in pumpkin seed powder extracts The results in Table (4) showed the presence of tannin and glycoside compounds after qualitative detection of some active compounds of defatted pumpkin seed powder extracts, as phenols were

observed in the oil and seeds of the alcoholic extract of whole pumpkin seed powder, and the appearance of a light green colour in the aqueous extract was positive evidence of the presence of flavonoids compared to the alcoholic extract, and these results agreed with [32] who stated that the difference in the results is due to genetic and environmental factors such as soil components, acidity, temperature, light intensity, and duration of exposure to light that affect the type and quantity of these compounds and also affected the metabolic pathways within the body [33].

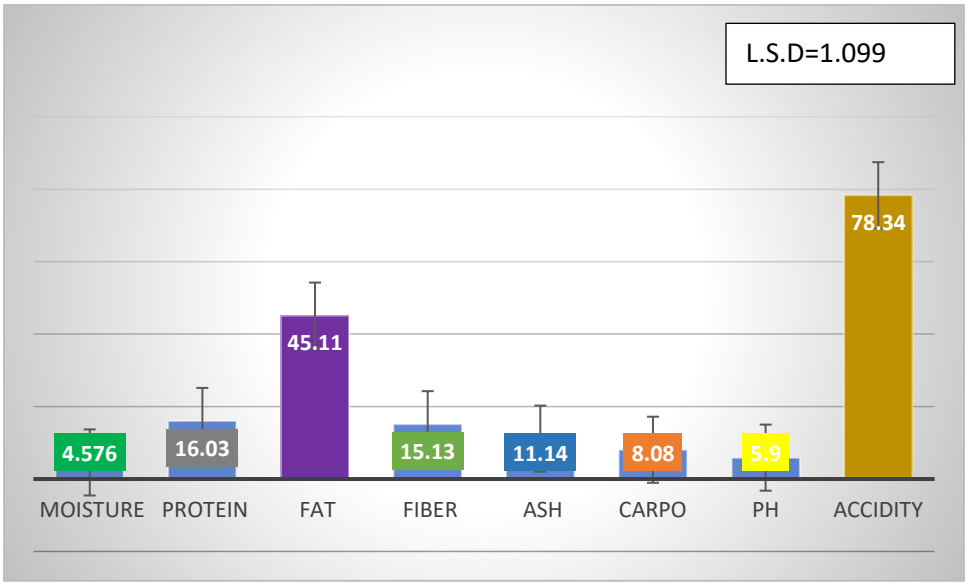


Figure (2): Chemical and physical content of pumpkin seed powder

Table (4): Qualitative detection of active compounds in pumpkin seed powder

Active compounds	Aqueous extract of defatted pumpkin seed powder	Alcoholic extract of defatted pumpkin seed powder	Aqueous extract of non-defatted pumpkin seed powder	Alcoholic extract of non-defatted pumpkin seed powder
Glycosides	+	+	+	+
Tannin	+	+	+	+
Phenols	-	-	-	+
Flavonoids	+	+	-	+

Estimation of the value of the gluten coefficient

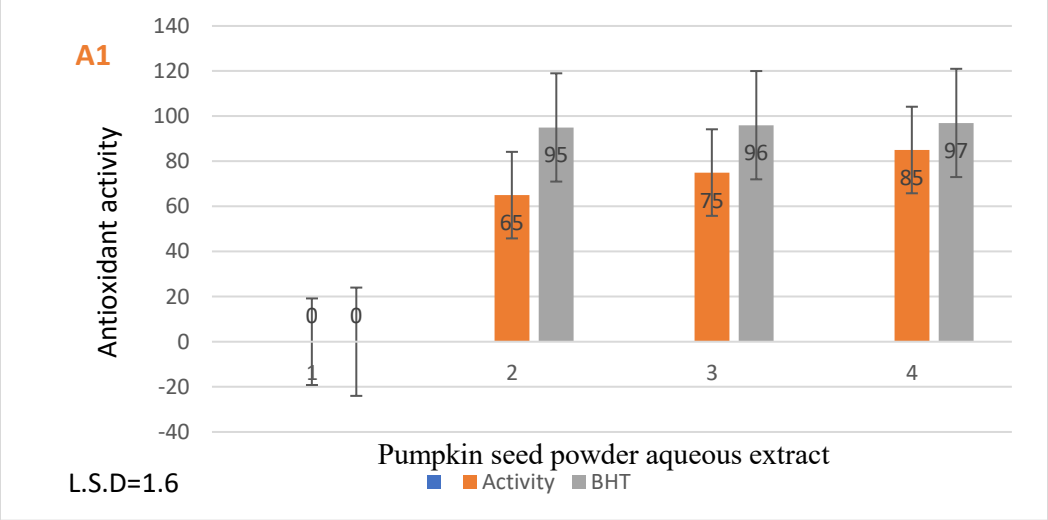
Tests were conducted on pumpkin seed powder, and it was noted that it is free of gluten, so this product is considered a health safety for patients with wheat gluten allergy, it also has an important role in the diet system, so gluten-free baked goods can be produced from pumpkin seed powder [24].

Measuring the antioxidant activity of pumpkin seed powder extract

The results in Figure (B2-3) showed a significant increase in the alcoholic extract of pumpkin seed powder by capturing the radicals DPPH1,1-diphenyl-2-picrylhydrazy, as the results of the statistical analysis showed that pumpkin seed powder had a significant effect $p \leq 0.05$ on the

antioxidant activity, as the alcoholic extract of pumpkin seed powder had a higher activity on capturing the free radical compared to DPPH in the alcoholic extract of the powder, meaning that the activity increases as the ability to capture increases For free radicals at a concentration of 0.1-0.7 mg/ml, the percentages increased significantly with increasing concentration, reaching 76%, 83%, 87% for concentrations 0.1, 0.3 and 0.7 for the above extract, respectively. This is close to the standard antioxidant Butylated Hydroxy Toluene BHT. As for the ability to capture the free radical DPPH for the aqueous extract of pumpkin seed powder, as shown in Figure (A1-3), the results showed significant differences at the probability level $p \leq 0.05$ in capturing the free radical DPPH, as the aqueous extract of pumpkin seed powder gave a significant increase in antioxidant activity with increasing concentration, as antioxidant activity reached 56-68% for the two concentrations 0.1-0.7 mg/ml.

In comparison to the standard antioxidant Butylated Hydroxytoluene BHT. This is due to the ability of pumpkin seed powder to capture free radicals by oxidizing hydroxyl groups and phenols present in foods that have an effective role as antioxidants, which have a clear effect in protecting the body from various chronic diseases, inflammation, tumours, etc. Phenols also play a role in regulating several vital processes in living cells and enzymes inside the body. The presence of these compounds increases the nutritional value of the manufactured product, in addition to the powder containing hydroxybenzoic acid, which is an antioxidant and a preservative. Vanillic acid is a biologically active compound and reduces the incidence of cancer, diabetes, obesity, neurological diseases, heart diseases, autoimmune diseases, bacterial and fungal infections, etc. [34]



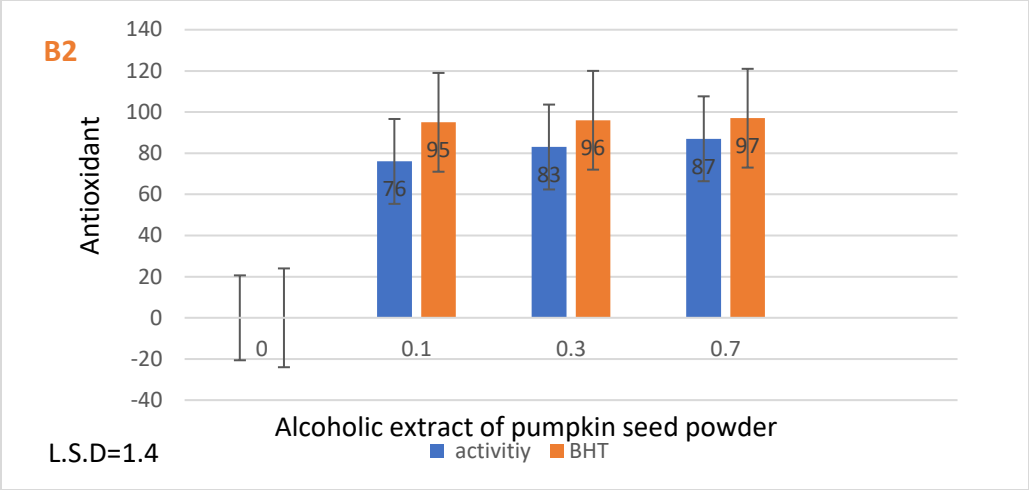


Figure (3): Antioxidant activity of pumpkin seed powder alcoholic extract A1 and aqueous extract B2

Active compounds identified by GC-MS technique for pumpkin seed powder

The results in Figure (4) and Table (5) showed that the active compounds identified by gas chromatography technique connected to the mass spectrometry of pumpkin seed powder had 30 peaks belonging to biologically active compounds, and the highest peak 9 was for the compound 1 ,2Propanediol, 3-benzyloxy-1,2-diacetyl at a concentration of 17.57%, which is an effective phenolic hydrocarbon compound containing a benzyl ring with a similarity percentage of 82% and its common names are N-Benzylidenebenzylamine and Benzonitrile, m-phenethyl, , that peak 22 of the compound reached 16.47% 2,3-Diphenylcyclopropyl methyl phenyl sulfoxide, trans- It is a five-membered cyclic phenolic compound that contains a sulfur sulfate group with a similarity rate of 84% and its common names are 1-benzylindole and 1-Propene, 3-[2-cyclopentenyl]-2-methyl-1,1-diphenyl-, while peak number 1 reached 14.52% for the compound Styrene, which is a cyclic compound that contains an amine group in its structure and is considered one of the effective chemical phenolic compounds found in foods, nuts and vegetables and its names are Benzeneethanamine,N-[4-hydroxy] hydrocinnamo. We note from Table (5) that most of the identified compounds are due to the effective phenolic compounds as antioxidants with high and low concentrations, the percentage reached 0.26% at peak 2 for the compound Tribenzyl-N-acetyl-N, 1-dibenzoyl galactosamine, which has a low similarity rate of 54%, While peak 3 It gave a concentration of 0.49% for the organic cyclic hydrocarbon compound beta.-d-Glucopyranose pentabenzoate with a low identity percentage of 35%, while small percentages were observed, reaching 0.80-0.76% for peaks 29 and 30, respectively , for the compound with a similarity percentage of up to 78%, for the compound 1,2-Propanediol,3-benzyloxy-1,2-diacetyl,which is a compound that contains a chlorine group in its composition and is a hydrocarbon compound, some of its common names are Dichloroacetic acid, 3-phenylpropyl ester. We notice from Figure (4)and table(5) that the majority of the active compounds are antioxidant phenolic compounds, including the compound Phenol, 2,2'-methylenebis[6-[1,1-dimethylethyl]-4-methyl, while there are at peak 15 a cyclic compound of phenolic compounds that has 23 carbon atoms and its names include The common [p-Cresol,2,2'-methylenebis 6-tert-butyl], and the compound 1,3,14,16-

Nonadecatetraene with a concentration of 0.47% and a similarity of 91%, while the results showed that peak 17 gave a concentration of 0.48% 3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester, which is an organic compound It contains a double bond and has a similarity of up to 86% and its common names are 1,E-8,Z-10Tridecatriene, And another compound was found, which is -Hydroxypentadecanoic acid, at peak 18 with a percentage of 0.47% and a similarity of 77%, which is a long-chain hydroxy fatty acid from omega acids, one of its most important common names is Pentadecanoic acid, while the compound d-Ribose, 2-deoxy-bis [thioheptyl]-dithioacetal, which reached 0.96% with a similarity percentage of 68%. It was noted that it contains in its composition a pentose sugar, which is important for the formation of nitrogenous bases and a sulfur group. Among its common names is 1-Hexadecylindane. The results below showed that peaks 25, 26 and 27 gave percentages of 0.46, 2.41 and 1.04 with a similarity percentage of 94%, 61% and 61% respectively. These are due to phenolic cyclic compounds containing a benzene ring and an amine group, among their names are [35]. The common 2-Phenylcyclobutyl]benzene and 2-[4-benzyloxybenzylidenamino Benzo- nitrile, while peaks 4, 5 and 6 which belong to the compounds Benzene, 1,1'-[1,2-cyclobutanediyl] bis-cis- and Benzene, 3-butenyl and Benzene, 1,1'-[1,2-cyclobutanediyl] bis-trans with percentages of 0.36 and 3.28 and similarity percentages of 95%, 80% and 5.27% respectively are hydrocarbon compounds containing a cyclic benzene group and are characterized by containing carbon and hydrogen atoms [aldehyde hydrocarbon compound]. The results also indicated that the compound 2-Cyclohexen-1-one, 4-[3-[beta. -d-appeared at peak 8 at percentage and peak 8 at percentage 0.13% and a low similarity percentage 50glucopyranosyloxy]1butenyl]-4-hydroxy-3,5,5-tri and through Figure [4] shows some of the identified active compounds and the extent of similarity of the active compound. [35].

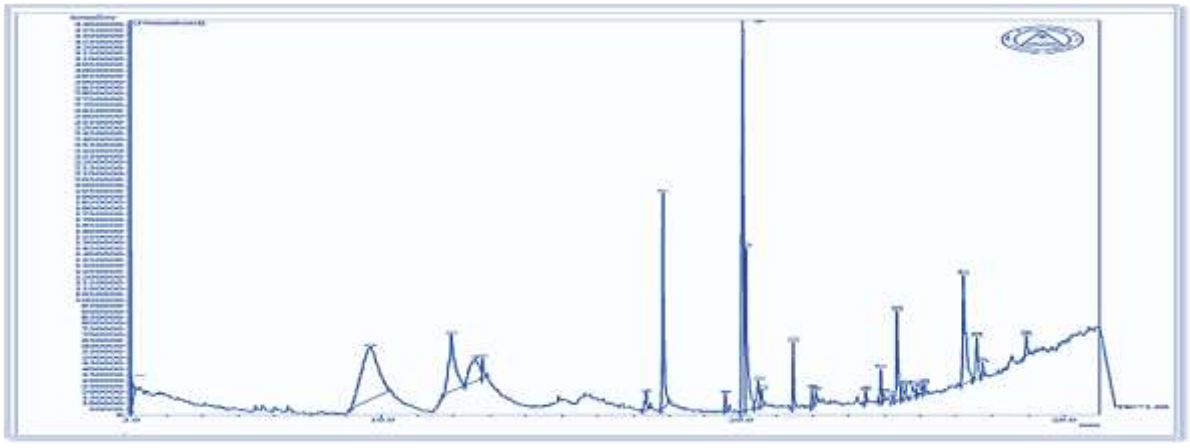


Figure (4): Profile of active compounds identified by GC-MS technique

Table (5) Active compounds in pumpkin seed powder

Name	Area%	R-Time	Peak

3-Allyloxy-1,2 propanediol	14.52	3.169	1
1,3-Propanediol, 2-[hydroxymethyl]-2-nitro-	0.26	3.914	2
alpha.-D-Galactopyranoside, methyl	0.49	15.102	3
3,4-Altrosan	0.36	15.571	4
Acetamide, 2-[2-hydroxyethoxy	3.28	15.930	5
Benzene, 1,1'-[1,2-cyclobutanediyl]bis-, trans-	5.27	16.271	6
n-Hexadecanoic acid	8.91	17.829	7
9,12-Octadecadienoic acid, methyl ester, [E,E]-	0.13	20.108	8
Linoelaidic acid	15.94	20.193	9
cis-13-Octadecenoic acid	8.60	20.148	10
- Octadecanoic acid	5.54	20.664	11
Tributyl acetylcitrate	0.29	22.126	13
Carbamic acid, 2-[dimethylamino]ethyl ester	0.20	22.292	14
13-Tetradece-11-yn-1-ol	0.47	23.158	15
Oxiraneoctanoic acid, 3-octyl-, methyl ester	17.57	23.422	16
3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.48	23.692	17
Carbamic acid, 2-[dimethylamino]ethyl ester	0.47	23.744	18
4-Cyanobenzoic acid, undec-10-enyl ester	0.96	23.900	19
Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl]ethyl ester	4.58	24.349	20
2-[2-Hydroxyethylamino]-5-nitrobenzonitrile-	10.27	24.188	21
Carbonic acid, decyl 2-ethylhexyl ester	16.47	24.329	22
Bis[2-ethylhexyl] phthalate	4.18	24.375	23
10-Undecenoic acid, 2-hydroxy-, methyl ester	5.67	24.488	24
E-8-Methyl-9-tetradecen-1-ol acetate	0.46	25.175	25
Octadecanoic acid, 9,10-dihydroxy-, methyl ester, [R*,R*]-	2.41	25.295	26
9,12-Octadecadienoic acid [Z,Z]-, 2,3-dihydroxypropy	8.14	26.201	27
Octadecanoic acid, 2,3-dihydroxypropyl ester	0.40	25.592	28
2-Ethylhexyl stearate	0.80	28.858	29
Squalene	0.76	28.992	30

Characterization of fatty acids in pumpkin seed powder

We note from Table (6) the presence of saturated and unsaturated fatty acids identi- fied by gas chromatography technique connected to mass spectrometry GC-MS with the presence of a group of peaks, some of which contained fatty acids. At peak 7, there was a branched fatty acid with a percentage of 17.8% n-Hexadecanoic acid, the compound at peak 8 was a saturated fatty acid with a retention time of 20.1% 9,12-Octadecadienoic acid, methyl ester, E, E-

Peak 9 showed an unsaturated fatty acid, which is n-Hexadecanoic acid, with a percentage of 15.94%, while another branched fatty acid Octadecanoic acid, appeared at peak 11 with a percentage of 20.9%, while the unsaturated fatty acid cis-13-Octadecenoic acid, was present at

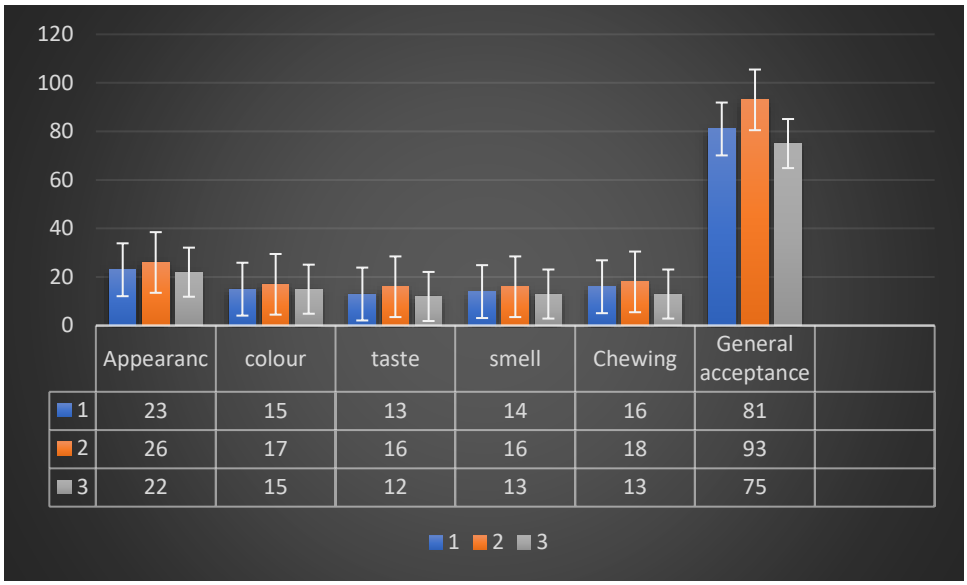
peak 10. The results below showed the presence of a saturated fatty acid at peak 20 4.3% Hexadecanoic acid, 2hydroxy 1[hydroxym- eth-yl]ethyl ester, at the top 26 Octadecanoic acid, 9,10-dihydroxy-, methyl ester, R*, R*-, saturated fatty acid 2.41% Saturated and unsaturated fatty acids play an effective role as antioxidants and antimicrobials. [35].

Table (6): Saturated and unsaturated fatty acids

Saturated and unsaturated fatty acids	Hexadecanoic acid, methyl ester	9,12-Octadecadienoic acid, methyl ester, [E,E]-	cis-13-Octadecenoic acid	11,14-Eicosadienoic acid, methyl ester	-Octadecanoic acid	Hexadecanoic acid, 2-hydroxy-1-[hydroxymethyl]ethyl ester	Octadecanoic acid, 9,10-dihydroxy-, methyl ester, [R*,R*]-
R-Time	17.829	20.108	20.148	20.598	20.664	24.349	25.295
Area%	8.91	0.13	8.60	15.94	5.54	4.58	2.41
Peak	7	8	10	9	11	20	26

Sensory evaluation of biscuits

The sensory evaluation results in Figure (5) for biscuits made by replacing part of the flour with pumpkin seed powder at a replacement rate of 50 and 100%, as the results of the statistical analysis showed the presence of significant differences at the probability level $p \leq 0.05$, as the second treatment showed a significant increase in the sensory qualities listed below: appearance, colour, taste, smell, chewiness and general acceptance at a replacement rate of 100% compared to the control sample. The results showed that the biscuit gave an attractive and crunchy appearance, while the colour was a good indicator of consumer acceptance of the product, the yellow-green colour due to the presence of chlorophyll and carotene pigments in pumpkin seeds, which are affected by thermal treatments and may be exposed to oxidation processes during manufacturing, while the smell and chewing qualities were accepted by the specialists in food science at the replacement rates of 50 and 100% of pumpkin seed powder. The results showed that treatment No. 2 is the best in terms of sensory evaluation, followed by treatment No. 1 compared to the control treatment, and this is consistent with what [36] reached**.** Similar results were obtained for color and other qualities when [40] prepared biscuits with 5, 10, 15, 20, and 25% substitution levels of wheat flour with decorative pumpkin seed powder. Acceptable biscuits with 5 and 10% substitution levels scored relatively high for color, very close to the control sample. Substitution of 10% wheat flour with pumpkin seed powder gave the best color scores, and the biscuits prepared were also very similar in taste, flavor, and texture to control biscuits made with 100% wheat flour. [42] reported that substitution of 10% pumpkin flour with wheat flour for biscuits scored good overall acceptability. Similar overall acceptability results were obtained when [40] added pumpkin seed powder to wheat flour to check the quality of biscuits. Similar results to our study were found in [40] when they studied the effect of mixing pumpkin powder with wheat flour, noting that the 10% substitution level achieved good results in sensory criteria



Figure(5): Sensory evaluation form

Study of functional properties of biscuits

The results of the statistical analysis in Table (7) showed significant differences at the probability level p≤0.05 in the average thickness and diameter rates of biscuits when increasing the percentage of pumpkin seed powder replacement compared to the control sample No. 3, as it was noted that the best average was 42.437 mm for diameter and 6.5 for thickness, while the diffusion coefficient increased with increasing the percentage of replacement from [5.5 to 8.7%], and this is due to the formation of the gluten network present in wheat flour protein, which led to an increase in the viscosity of the dough, and The results were consistent with those of [37], as biscuit width decreased significantly with increasing levels of pumpkin seed powder, while thickness increased. This decrease in width and the increase in thickness also resulted in a decrease in thickness. Similar results were obtained [40] when using pumpkin seed powder at levels of 5, 10, 15, 20, and 25% in wheat flour for biscuit preparation. They observed a decrease in the width, thickness, and thickness of biscuits. Supporting results were also obtained when [41] studied the physical and chemical composition of biscuits mixed with pumpkin seed powder. They studied the effect of mixing pumpkin powder with wheat flour, and observed a significant decrease in the width and increase in the thickness of the biscuits produced. [42] reported that during mixing the ingredients, greater water absorption occurs, which increases the viscosity of the dough, leading to a decrease in the biscuit spreading coefficient. In the case of pumpkin peel, pulp, and seed powder, the fiber content is higher than in white flour, and this fiber may be the reason for the lower biscuit spreading coefficient. When wheat flour is replaced with some non-wheat flour in biscuit preparation, its breadth decreases due to its greater water absorption during mixing when its powder and seeds are added to white flour.[42] Others reported that increasing the fiber content in biscuit preparation reduced its spreading factor

Table (7): Physical properties of laboratory-made biscuits

T	Samples	thickness ml	Diameter ml	Diffusion coefficient
1	A	6.9±0.005	41.632±0.035	5.889±0.010

2	B	5.5±0.005	42.437±0.035	7.752±0.100
3	C	6.1±0.015	39.412±0.095	6.412±0.035

Microbial effect inhibitory activity of pumpkin seed powder

We note from the tables (8 , 9) that the alcoholic extract of pumpkin seed powder gave higher effectiveness compared to the aqueous extract and for all the microorganisms selected in the study. The results showed a significant increase in the diameters of inhibition for the aqueous extract of pumpkin seed powder, as the highest diameters of inhibition for the concentration of 1.5% reached [18 and 21] mm for *E. coli* and Staph aureus bacteria, respectively. As for the diameters of inhibition for the pumpkin seed extract for the alcoholic extract, they increased significantly with increasing concentration, reaching 31 mm against Staph aureus bacteria. At the same time, they decreased to the lowest diameter of inhibition against Pseudomonas bacteria, reaching 10 mm at a concentration of 1.5% compared to a concentration of 1%. The reason for the anti-pathogenic effect on microorganisms is that pumpkin seed powder contains a group of effective and biologically active compounds such as flavonoids, phenols, saponins, alkaloids, and others. [38]. Plant extracts also damage the cell wall of pathogenic microorganisms as they enter the microbial cell, affecting the cytoplasm and thus destroying the cell membrane. This depends on the type of bacteria, whether Gram-negative or Gram-positive, due to their dependence on the composition of the bacterial cell wall. [39].

Table (8): Inhibition diameters in millimeters of the aqueous extract of pumpkin seeds against pathogenic microorganisms

Concentration\Type of bacteria	*Staph. aurous*	*E.coli*	*Pseudomonas*	*Bacillus*
1%	7	16	7	11
1.5%	21	18	10	16

L.S.D =1.851

Table (9): Inhibition diameters in millimeters of the alcoholic extract of pumpkin seeds against pathogenic microorganisms

Concentration\Type of bacteria	*Staph. aurous*	*E.coli*	*Pseudomonas*	*Bacillus*
1%	10	15	7	24
1.5%	31	27	10	27

L.S.D =1.309

4-Conclusions

Research results have shown that pumpkin seed powder has high nutritional value and contains significant amounts of functional and dietary components responsible for multifunctional roles in the human body. The proteins, polysaccharides, oils, vitamins, carotenoids, minerals, and phenolic compounds found in pumpkin fruits exhibit immunomodulatory, anti-inflammatory, antioxidant, antimicrobial, and

antiviral activities. Since pumpkin is gluten-free, pumpkin powders, extracts, isolates, and derived food products are suitable ways to benefit from this nutritious fruit. The use of innovative and modern techniques, such as the manufacture of edible coatings, vacuum packaging, nanoparticles, and bio preservation, can be useful processes to extend the shelf life of this perishable food while increasing the bioavailability of nutrients. The current urgent need is to incorporate these pumpkin-derived ingredients into the daily human diet to maintain body balance, supported by an enhanced immune system. Therefore, it has been introduced into the banking industry as it is considered safe for patients with gluten allergies.

Finding
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Authors Contribution

SHFA and S.S.G: Suggested a title of the research, sample preparation, Laboratory methodology, Statistical analysis and writing the manuscript,
ALY: Assistant in translation of research and publication.

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