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Identification the Effective Compounds of Pumpkin Seeds and its Possibility of Application in Some Food Products

Hind Mohammed Abed[1], Luma khairy Hassan[2*] and Hanisah Kamilah Binti Abd Razak[3]
[1,2]Department of food science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
[3]Department of Crop Science, Faculty of Agricultural and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, Bintulu, Sarawak, Malaysia.
[3]Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
*Corresponding Author.

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Abstract

This study included the use of local pumpkin seeds of Iraqi origin, which were examined in the Seed Examination and Certification Department. It was found that they are from the plant family Cucurbitaceae Jass, and their exact scientific name is Cucuriba pepo L. This study aims to use the local Iraqi pumpkin seeds and study their effective compounds to apply these seeds to some foods. The pumpkin seeds were dried at room temperature after they were peeled, cleaned, and washed. The fiber and proximate analysis were conducted. After that, the aqueous and alcoholic extraction of the defatted and non-defatted pumpkin seeds was carried out to detect the active compounds such as tannins, glycosides, coumarins, flavonoids, resins, and saponins. For the phenols, only a positive test was given for the alcoholic extract of the defatted seeds as an indication of their presence in the oil. As for steroids and terpenes, the result is negative, and each of the extracts has a significance that is not found in the seeds. Then, the vitamins were detected in a few different proportions in pumpkin seeds, with vitamin D (26.21). As for phenols, their percentage is 14.90%, which is considered the highest result, and 13.07% for vanillin. Finally, the results of the sensory evaluation were done after cupcakes, cookies, and pudding products made from pumpkin seed flour were manufactured. The residents highly accepted those food products. Therefore, this current study aimed to find a healthy alternative to seeds to make valuable and nutritious foods. This study indicated that the manufacture of cupcakes, cookies, and pudding products from pumpkin seed flour could improve the sensory and quality properties.

Keywords: Effective compounds, pumpkin seeds, pumpkin fruit, phenols in pumpkin seed, Cucarbita pepo.

1. Introduction

The pumpkin plant belongs to the genus Cucurbita and the family Cucurbitaceae, which includes approximately 130 genera and 800 species. It can be grown all over the world, especially in warmer regions. It is widely cultivated in Mexico, Central, and North America,

being native to northern Mexico and the southwestern and eastern United States [1].

Pumpkin seeds contain various active compounds such as p-aminobenzoic acid, β-aminobutyric acid, polysaccharides, peptides, proteins, and carotenoids. Hence, they have many health and therapeutic benefits, and they are used in many different food industries due to their importance [2-3]. In addition, pumpkin seeds are a rich source of minerals such as manganese, magnesium, copper, iron, and zinc and contain volatile oils. They also contain organic acids, fatty acids, and flavonoids. Pumpkin seeds also contain vitamins B and C [4].

Pumpkin seeds have large amounts of essential vitamins, including vitamin C, A, and E. Pumpkin seeds are rich in nutrients and have various medical benefits such as lowering blood pressure, anti-inflammatory, anticoagulant, anti-allergic, anti-diabetic and anti-cancer [5, 6]. Polysaccharides, fixed oils, sterols, proteins, and peptides of the physiologically active components found in pumpkin seeds have been shown to have significant health benefits [7, 8]. Vitamin C is a powerful antioxidant that protects against cellular damage caused by reactive oxygen species by donating electrons. A fundamental difference is vitamin C found in the pulp, peel, and seeds of pumpkin species such as Cucarbita maxima (C. maxima) and Cucarbita pepo (C. pepo). On the other hand, vitamin A is an antioxidant that may be beneficial for those suffering from diabetes and vascular injuries [9]. A part from fat-soluble antioxidants such as tocopherols and β-carotenoids, there are other types of antioxidants that pumpkin seeds are rich in, such as manganese, flavonoids, flavones, catechins, polyphenols, and phytosterols [10]. Therefore, the objective of this study is to detect the active compounds and to evaluate the chemical composition of pumpkin seeds with the sensory properties of some food products made from pumpkin seeds.

2. Materials and Methods

2.1 Preparing the sample

The study was conducted at the University of Baghdad, College of Agricultural Engineering Sciences, and a local sample was obtained for 8 months. The sample was peeled, purified, washed, and dried completely at room temperature, then crushed with an electric grinder and preserved in bags.

2.2 Proximate analysis of pumpkin seeds

2.2.1 Estimation of fiber content

The percentage of fibers was estimated, 1 g of the sample in a glass beaker was, then 200 mL of diluted H_2SO_4 acid at 1.25% was added and left for 30 minutes. Then, filtered and NaOH at 1.25% was added. The volume was completed to 200 mL and left for 30 minutes. Then, it was filtered into glass lids and placed in a drying oven at a temperature of 105 °C for 16 hours. After that, the weight of 1 mg of the sample was taken after drying. It was placed in an incineration oven at a temperature of 550 °C for 30 minutes [12]. The weight was recorded after incineration, and the percentage of fibers was estimated using the equation:

$$\% \text{ Fiber} = \frac{Weight\ of\ the\ eyelid\ with\ the\ sediment\ after\ drying - Weight\ of\ the\ eyelid\ with\ the\ ashes\ after\ burning}{sample\ weight} \times 100$$

2.3 Dehydrated and unrefined seed extracts

2.3.1 Aqueous extract

According to what was stated in [13], the aqueous extract was prepared with a modification in drying the filtrate instead of lyophilizing it, as 100 g of each of the pumpkin seeds powder, removed and not de-oiled, was taken separately, mixed with 500 mL of distilled water, and left for two hours, after which the mixture was incubated at a temperature of 40°C in an incubator for 24 hours, then placed on a magnetic stirrer for 30 minutes, after which the filtrate is passed through filter paper (Whatman No. 1). The filtrate is collected, then poured into a Petri dish and placed in an electric oven at a temperature of 40°C until the extract is dry and the powder is scraped off. Dried, then collected in dry bottles and placed in the refrigerator until use.

2.3.2 Alcoholic extract

Alcohol extraction was carried out according to the method of [13], whereby 50 gm of pumpkin seed powder, both de-oiled and not de-oiled, were placed separately with 500 mL of 70% ethanol. The mixture was then placed in a shaking incubator for 4 hours, and the mixture was left at room temperature for 4 hours. 24 hours; after that, the mixture was filtered through filter paper (Whatman No. 1), then the filtrate was collected in Petri dishes and dried in an electric oven at 40 °C, then scraped and collected in dry bottles and kept in the refrigerator until use with the injector temperature 250 °C, scan range m/z 50-500 and injection type splitless. The oven was programmed as follows: 55 °C for 2 min, 55°C -180°C for 7°C/min, 180°C -280°C for 8°C/min and 280°C for 2 min.

2.4 Test for gluten in pumpkin seed flour

The examination was carried out at the Ministry of Commerce/General Company for Seed Manufacturing, and the method is as follows: A sample of pumpkin seed flour was mixed well and weighed 10 g. It was placed in a plastic washing container using a fine sieve (88 microns), and a saline solution was added to it (the saline solution (2%) was prepared by dissolving 20 g of table salt in 1 liter of distilled water) at 4.8 mm using an automatic pipette (DISPENSER).

The first stage of washing is done in a machine automatically, and after the machine stops, the sample is transferred to the washing bowl of a coarse sieve (840 microns). After that, the sample was transferred to the centrifuge to separate the weak gluten from the vital gluten. The part of the sample passing through the sieve (weak gluten) was weighed, and the remaining sample was weighed without zeroing the scale (total gluten), and the results were recorded and applied in the equation:

$$\text{Calculation of the coefficient of gluten} = \frac{total\ gluten - Weak\ gluten}{total\ gluten} \times 100$$

In the calculation of dry gluten, the sample was placed in a gluten dryer and weighed after losing water.

2.5 Preparation of the products made from pumpkin seeds

2.5.1 Preparation of cupcake sample

A cupcake was manufactured in a laboratory from local pumpkin seeds, according to the ingredients and amounts shown in **Table 1**.

Table 1. The ingredients were used in cupcakes**.**

The ingredients	Weight (g)
Pumpkin seed flour	100
Sugar	30
Salt	1
Dry milk	5
Cake Mohsen	10
B.P	4
Vanilla	4
Eggs	200
Foam	30

2.5.2 Preparation of cookies sample

The local pumpkin seed cookies were manufactured in a laboratory; the experimenter suggested the materials and their proportions, as shown in **Table 2**.

Table 2. The ingredients were used in cookies**.**

The ingredients	Weights (g)
Pumpkin seed flour	100
Brown sugar	20
White sugar	10
Salt	1
Vanilla	4
Backing powder	4
Eggs (2)	80
Foam	10

The white and brown sugar, eggs, and baking powder were mixed for several minutes in the electric kneading machine. The small balls were formed and put in the electric oven at 180 °C for 30 min.

2.5.3 Preparation of pudding sample

The pudding was made from pumpkin seeds, as mentioned in [20], with some modifications in type and ingredient proportions, as shown in **Table 3**.

Table 3. The ingredients were used in the pudding.

The ingredients	Weight
Ground seeds	10 g
Skimmed milk	10 mL
Sweetener (honey)	4 g

About 10 g of pumpkin seeds were weighed and ground in an electric grinder for a minute. After that, the ground seeds were placed in an oven at a temperature of 140 °C for 10 minutes in order to get rid of unwanted tastes (metallic taste). A glass containing 10 mL of skim milk with manual stirring, adding a continuous flow of honey or molasses during the stirring process, After which the product is in tightly closed glass containers.

2.6 Sensory evaluation

The sensory evaluation for the pumpkin seeds sample was in line with [21]. The panelists in the Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad evaluated all products. The panelists were asked to consider the products using a taste panel for appearance, upper surface, texture, pulp color, taste and flavor, and freshness (appearance = 20; upper surface = 15; texture = 10; color pulp = 10; taste and flavor = 20; and freshness = 10).

2.7 Statistical analysis

The statistical analysis system [22] 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

3.1 Proximate analysis of pumpkin seeds

Table 4 shows the chemical composition of pumpkin seeds. The results showed that the percentage of moisture was 4.97%, which was higher than the range mentioned, as it was 2.50% in pumpkin seeds. The fat was 49.11%, and this result was higher than what was stated in [23], as it was 41.00% in pumpkin seeds. Meanwhile, the fiber percentage was 18.15%, and this result is much higher than the range mentioned previously [26]. It was 5.34%, which is a protein percentage of 25.00%, which is higher than the observed value of 12.03%. Finally, the rate of carbohydrates was 10.92%, and this percentage is less than what was reached by [23], as it was 25.00%. The differences in the obtained results compared to what others have reached can be attributed to differences in seed variety, place of cultivation, and environmental conditions [24].

Table 4. Chemical composition of pumpkin seed.

Components	%
Moisture	4.97
Protein	12,03
Fat	49.11
Fibers	18,15
Ash	4,82
Carbohydrates	10.92

3.2 Active compounds of pumpkin seed extracts

The results confirmed the qualitative detection of some active compounds of defatted pumpkin seed extracts that contained tannins, glycosides, coumarins, and saponins because the results of the detections have been for these compounds, while the compounds terpenes and steroids gave a terrible result for all samples and phenols gave only an excellent impact at the alcoholic extract of complete pumpkin seeds, that's evidence of their presence in the seed oil, as in **Table 5**. As for the resins, they gave a positive test for the alcoholic extract because they only dissolve in it; as for alkaloids, a positive test was given. At the same time, the flavonoids were given a positive test, except for the aqueous extract [10]. The reason for the difference in the results compared to the research results may be due to genetic and environmental factors such as soil components, pH, temperature, light intensity, and photoperiod that affect the type and quantity of those compounds as they affect the metabolic pathways of the active compounds [25]. These effective compounds are considered antioxidants and inhibit the free radicals that enter or form inside the

body. These compounds raise the nutritional value of pumpkin seeds and, thus, the product made from them [26].

Table 5. Active compounds in pumpkin seeds.

Active compounds	Alcoholic extract of non-defatted pumpkin seed	Aqueous extract of non-defatted pumpkin seed	Alcoholic extract of defatted pumpkin seeds	Aqueous extract of defatted pumpkin seeds
Tanins	+	+	+	+
Glycoside	+	+	+	+
Phenols	+	-	-	-
Resins	+	-	+	-
Saponin	+	+	+	+
Alkaloid	+	+	-	+
Comarins	+	+	+	+
Terpenes	-	-	-	-
Steroids	-	-	-	-
Flavonoid	+	-	+	+

* (+) denotes the presence of the compound, and (-) denotes its non-existence

3.3 Vitamins in pumpkin seeds extract

Table 6 shows the percentages of some vitamins in pumpkin seed extracts. It was found that the values of vitamin C, B_1, B_2, and D_3 were 0.385 mg/100 g, 0.018 mg/100 g, 0.021 mg/100 g, and 26.205 IU, respectively. The amount of vitamin C was 0.27 mg, vitamin B_1 was 0.27 mg, vitamin B_2 was 0.15 mg, and some other vitamins [27]. They found the value of vitamin C was 1.9, B_1 was 0.27 mg, B_2 was 0.15 mg, and some other vitamins [28]. These differences in results may be attributed to genetic and environmental factors such as soil components, pH, temperature, light intensity, photoperiod, and also the conditions of the experiment. The absence of vitamins in the extracts can be explained by the quality and source of the seeds used [27]. These vitamins are essential, especially vitamin D; without them, the body cannot absorb calcium. It is also an antioxidant, anti-inflammatory, and good for the health of the immune system and brain activity [29].

Table 6. The percentages of some vitamins in pumpkin seed.

Vitamins	Percentages
C	0.385 mg/100g
Thiamin (B_1)	0.018 mg/100g
B_2	0.021 mg/100g
D3	26.205 IU

3.4 Phenols in pumpkin seed extracts

Table 7 shows the percentage of phenols present in pumpkin seed extracts. The highest value was recorded for vanillic acid, which was 14.90 mg/100 g; vanillin, 13.07 mg/100 g; and ferulic acid, which was 10.43 mg/100 g. As for kaempferol and lignin, the lowest percentage was recorded at 0.21 and 0.23%, respectively. The phenolic substances contents were 6.86, 35.20, 55.34, and 0.27 mg/kg for different cultivars due to several factors; the most important were seed quality and various factors such as seeds were exposed during examination [30, 31]. Phenols are foodstuffs with antioxidant properties that play many roles in protecting the body from various chronic diseases, infections, tumors, etc. Phenols also regulate a number of vital processes at the

level of cells and enzymes, so they raise the nutritional value of pumpkin seeds and the products made from them. Hydroxybenzoic acid is an antioxidant and a preservative for seeds and the product made from it, vanillic acid exerts a diverse biological activity against cancer, diabetes, obesity, neurodegenerative diseases, heart diseases, and diseases. Vascular and liver, through inhibiting the molecular pathways related to it, its derivatives also have the therapeutic capacity to treat autoimmune diseases as well as fungal and bacterial infections [32]. Chlorogenic acid may provide a wide range of potential health benefits, including its anti-diabetic, anti-cancer, anti-inflammatory, and anti-obesity effects, providing a non-drug, non-invasive approach to the treatment or prevention of certain chronic diseases [33].

Table 7. The percentage of phenols present in pumpkin seed.

Phenols	Percentage (mg/100 g)
Pyrogallol	0.84
Gallic acid	1.39
Luteolin	2.76
Kaempferol	0.21
Vanillic acid	14.90
Ferulic acid	10.43
Vanillin	13.07
Caffeic acid	0.61
Cinnamic acid	0.40
Catechol	0.49
4-hydroxy benzoic acid	2.24
Qurctin	0.58
Cinnamaldehyde	0.28
Tyrosol	8.22
Eugno	0.61
Lignin	0.23
Chlorognic	0.79
LSD-value	1.893 *

* ($p \leq 0.05$)

3.5 Test for gluten of pumpkin seed flour

It was concluded that pumpkin seeds are free of gluten and are safe for patients with gluten sensitivity [34,35]. The cause of the disease is not known until now, except that one of the doctors found a relationship between the consumption of bread and grains and diarrhea. It is also suitable for followers of the keto diet in a certain amount, and this thing is helpful in manufacturing gluten-free products for many patients whose disease is associated with this protein, such as patients with brain disorders and autoimmune diseases [35,36]. It was concluded that pumpkin seeds are free of gluten and pumpkin seeds are safe for patients with gluten sensitivity [34-37].

The cause of the disease is not known until now, except that one of the doctors found a relationship between the consumption of bread and grains and diarrhea. It is also suitable for followers of the keto diet in a certain amount, and this thing is helpful in manufacturing gluten-free products for many patients whose disease is associated with this protein, such as patients with brain disorders and autoimmune diseases [35-38].

3.6 Sensory evaluation for products made from pumpkin seeds

3.6.1 Cupcake product

An evaluation form for the studied sensory characteristics of a cupcake product that was made from local pumpkin seeds. The results of the sensory evaluation received excellent acceptance by the assessors, as shown in **Table 8**, which included all the characteristics shown in the table.

Table 8. Sensory properties of cupcake made from pumpkin seed**.**

Adjectives	Degree	Degree of the sensory
Regularity of external shape	20	20
Qasra quality	10	10
Soft pulp tissue	10	9
Freshness	10	10
Regular pulp tissue (granulation)	20	10
Pulp color	10	9
Taste and Flavor	20	20
Total	100	98

3.6.2 Cookies product

Table 9 shows the evaluation of the cookie product that was made from local pumpkin seeds for the characteristics studied. The consequences of the sensory assessment acquired excellent recognition by using the evaluators.

Table 9. Sensory properties of cookies made from pumpkin seed**.**

Adjectives	Degree	Degree of sensory
External appearance and oppearance	20	19
The nature of the upper surface crack	20	18
Freshness	20	18
Taste and Flavor	20	20
Pulp color	20	20
Total	100	95

3.6.3 Pudding product

Table 10 shows the results of the sensory evaluation of the studied sensory characteristics of the pudding product made from pumpkin seeds, as in **Figure 1**, where the evaluation process was carried out for the traits (general appearance, color, texture, and flavor).

Table 10. Sensory properties of pudding made from pumpkin seed**.**

Adjective	Degree	Degree of sensory
General appearance	25	25
Color	25	25
Strength	25	25
Flavor	25	25
Total	100	100

Figure 1. pudding made from pumpkin seed.

4. Conclusion

In this work, the effective compounds, phenols, and vitamins for the Iraqi pumpkin seed were characterized after the aqueous and alcoholic extraction of the defatted and non-defatted pumpkin seeds were carried out. Then, practice the seeds in cupcakes, cookies, and pudding products. The outcomes confirmed that pumpkin seed extracts active compounds; the most crucial are glycosides, alkaloids, and cumarine. After that, the detection of a few phenols contained in pumpkin seeds via gas chromatography (GC), the most crucial of which can be vanillic acid (14.90 mg/100 g), vanillin (13.07 mg/100 g), and ferulic (10.43 mg/100 g). Hence, pumpkin seeds are free of gluten, which causes allergies in many consumers of baked goods and pastries. In addition, the detection of vitamins contained in pumpkin seeds through the use of excessive-performance liquid chromatography (HPLC), such as vitamin C (0.385 mg/100 g), B_1 (0.018 mg/100 g), B_2 (0.021 mg/100 g), and D_3 (26.205 IU).

In accordance with these results, healthy food products can be made from pumpkin seeds due to their richness in nutrients, as they can be considered healthy and functional foods, as well as improve sensory characteristics such as flavor, taste, and texture when applied to different products. Therefore, pumpkin seeds are important nutritionally, healthily, and functionally.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

This study was carried out after approval obtaining from the Scientific Committee in the Department of Food Sciences/College of Agricultural Engineering Sciences/University of Baghdad.

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