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Physico-chemical characterization, fatty acids composition and microbial analysis of pumpkin (*Cucurbita maxima*) seed oil harvested in Bangladesh

Md. Rashedul Azim, Mohammad Zubairul Alalm, Md. Moazzam Hossain, Kazal Baran Nath and Mohammad Helal Uddin

Abstract—Vegetable oils are an abundant source of fatty acids and other bioactive substances that have a variety of positive effects on human health. The seed oil of Pumpkin (*Cucurbita maxima*), was extracted using solvent extraction method and then characterized with regard to various physical and chemical parameters because vegetable oils have grown in importance due to their positive impacts on human health. It was observed that the pumpkin seed contained 40.87% oil. Fatty acid compositions of the oil sample were investigated by Gas-Liquid Chromatography (GLC). Pumpkin seed oil was found to contain Capric acid (18.28%), Undecanoic acid (17.15%), Stearic acid (29.81%) and Oleic acid (34.75%). The microbial activity of the oil sample was evaluated (seven bacteria were used for bacterial activity; seven fungi were used for fungal activity). The de-oiled cake of Pumpkin seed was studied quantitatively for the determination of percentage of ash, moisture, protein and minerals (N, P, Na, K, Ca and Fe) content. Consequently, it concludes with several significant findings in terms of industrial, pharmacological, and nutritional components.

Key words— Pumpkin, Solvent extraction, Fatty acid, GLC, Microbial activity, Protein, Mineral

1 Introduction

In addition to being used for many industrial and food uses, vegetable oils are crucial for supplying the world's nutritional needs [1]. Despite the wide variety of sources for vegetable oils, soybean, palm, rapeseed, and sunflower oils dominate global annual consumption with 31.6×10^6, 30.5×10^6, 15.5×10^6, and 8.6×10^6 tons respectively [2]. Such traditional vegetable oil sources can't keep up with the rising needs of the domestic and industrial sectors. As a result, finding additional sources to complement the supplies is necessary. From this vantage point, non-traditional oil seeds are very important to address this issue. In order to meet the growing demand for vegetable oil, the seeds of certain Cucurbitaceae species can beutilized as sources of edible oil [3]. Pumpkins are a member of the Cucurbitaceae family. In addition to being the most widely consumed vegetable in Bangladesh, pumpkin (*Cucurbita maxima*), also known as "Mistikumra," is also regarded as a useful food globally [4,5,6]. Despite being typically viewed as agricultural waste, pumpkin seeds are an amazingly rich source of bioactive compounds with remarkable nutraceutical properties [7]. Additionally, pumpkin seeds play a significant role in the human diet as a supplier of carbohydrates, proteins, lipids, and other nutrients required for maintaining good health [8].As stated by former studies on pumpkin seed, they may be beneficial for human health in a number of ways, including preventing prostate growth and shrinkage, delaying the development of hypertension, alleviating arthritis and hypercholesterolemia, lowering bladder and urethral pressure, relieving diabetes by boosting hypoglycemic effects, and decreasing the level of colorectal, gastric, breast, and lung cancer [9]. Many recent research studies have emphasized the health benefits of pumpkin seed oil against a variety of diseases, such as cancer, diabetes, and hypertension [10,11,12]. The purpose of the current study was to assess physico-chemical properties, nutritional composition, fatty acid composition and microbial activities of the oils extracted from pumpkin seed in light of the significance of vegetable oils in human diets and the lack of knowledge regarding less conventional sources of oil.

Md. Rashedul Azim[1], Mohammad Zubairul alam[1], Md. Moazzam Hossain[1], Kazal Baran Nath[2], Mohammad Helal Uddin[1,]*

1 Natural Products Research Laboratory (NPRL), Department of Applied Chemistry and Chemical Engineering (ACCE), Faculty of Science, University of Chittagong, Chattogram-4331, Bangladesh

2 Institute of Education and Research, University of Chittagong, Chattogram-4331, Bangladesh

*E-mail Address: mhuddin.acce@cu.ac.bd Mobile No: +8801554332302(Mohammad Helal Uddin) *Corresponding Author*

2 MATERIALS AND METHODS

2.1 Collection and processing

Pumpkin (*Cucurbita maxima*), locally known as "Mistikumra", was collected from Timebazar, Banshkhali (22° 01' 30.00" N, 91° 57' 15.12" E) in the district of Chattogram, Bangladesh. The seeds were separated and then dried in the sun for the following five days.

Fig 1: Pumpkin (*Cucurbita maxima*) seeds.

2.2 Extraction of seeds oil

The dried seed were cleaned, de-hulled and crushed in a glass mortar with a pestle. Using a continuous extraction process in a soxlet apparatus and petroleum ether as the solvent, oil from *Cucorbita maxima* seeds was extracted in accordance with the procedure established by the AOAC in 1990. Rotary evaporator was used to recover the extraction solvent at the end of operation.

Fig 2: Extracted oil of pumpkin seed

2.3 Physical characterization

The amount of total extracted oil was measured gravimetrically. The AOAC official method [13] was used to determine the seed cake's moisture, crude protein, crude fiber, crude fat and ash contents. The seed oil's moisture content, specific gravity, viscosity and refractive index were assessed using accepted techniques [14].

2.4 Chemical characterization

Numerous chemical properties of the oil sample were ascertained using the particular criteria of the traditional techniques. The oil's saponification value, saponification equivalent value, amount of unsaponifiable matter, acid value and free fatty acid (% as oleic acid), ester value, titre value, iodine value, acetyl value, peroxide value, Henher value, Polenske value, Reichert-Meissl value, Elaiden test and vegetable oil test were all determined using standard methods [15-19].

2.5 Mineral Analysis

Using recognized methods, the mineral contents (N, P, Na, K, Ca and Fe) of the pumpkin seed cake were determined [20].

2.6 Analysis of fatty acid composition

The chromatographic technique, especially gas liquid chromatography (GLC), has significantly improved the possibilities for obtaining compositional information from biological material. Because of ongoing advancements in accuracy and sophistication, chromatographic analyses have developed into a powerful evaluation method. Due to their simplicity and quickness, a great number of information on the fatty acid composition of oils is now available. Methanolic sulphuric acid (85:15, v/v) [21] was used to esterify the pumpkin seeds oil. Vials containing the reaction mixture were heated in an oven set to 80 °C for two hours. It was diluted with water after cooling, and then extracted with diethyl ether before being examined using gas liquid chromatography. The methylated esters of fatty acids were investigated by GLC and thus the fatty acid content of the oil sample was quantitatively analyzed [22].

2.7 Microbial analysis

The microbial activities of pumpkin seeds oil were studied against seven human pathogenic bacteria and seven phyto-pathogenic fungi.The antibacterial activities were assessed by disc diffusion method [23], using pumpkin seed oil to screen the experimental bacteria. In this case, Nutrient Agar (NA) was used as primary medium.The antifungal activities were evaluatedby food-poisoned technique, treating pumpkin seeds oil to screen the test fungi. In such case, Potato Dextrose Agar (PDA) was used as the fundamental medium. Using chloroform as a solvent, the lipid sample was made into the desired solution. To retain things in control, chloroform was used.

3 Results and discussion

The pumpkin seed oil (Cucurbita maxima) has been examined for a number of physical and chemical characteristics as well as microbial activities in order to determine its nature and assess its suitability for a specific application.

3.1 Physical characteristics

Knowledge of physical properties of fats and oils is essential for various reasons. Whether a fatty material can be used as an edible product or not, and many technical applications of them depend upon their physical properties. Table 1 presents the findings regarding the physical characteristics of pumpkin seed oil that was extracted.

The refractive index of the oil was observed to be 1.4675 at 30 °C, which is a comparatively high value and a symbol of modest unsaturated fatty acid concentrations in the fatty acid constituents. At 30 °C, the oil sample's specific gravity was found to be 0.897. The viscosity of the oil solution was determined to be 236.04 millipoise at 30 °C. From the data on viscosity, we deduced something about the intermolecular hydrogen bonding within pumpkin seed oil. According to the results of the current investigation we say that the oil sample may contain a few free acid molecules and hydroxyl groups. This observation is supported by the oil sample's minimal acid value and low acetyl value.The activation energy of viscous flow of the oil sample is found to be 24.64 $JK^{-1}mol^{-1}$. According to the Maxwell-Boltzmann distribution law the number of molecules possessing the necessary energy of flow is related to the factor $e^{-E/RT}$ where, E is the activation energy. Although other factors are also important, in general, the higher the energy of activation, the greater the viscosity of liquid.

Table 1: Physical parameters of the pumpkin seed oil

Physical properties	**Experimental results**
Physical state	Liquid
Color	Light yellow
Odor	Odorless
Moisture content	2.2%
Refractive index (at 30 °C)	1.4675
Specific gravity (at 30 °C)	0.897
Viscosity (at 30 °C)	236.04 millipoise
Energy of activation	24.64 $JK^{-1}mol^{-1}$

3.2 Chemical Characteristics

Study of chemical properties is a very important part of oil analysis, as oils and fats are identified and distinguished by tests shown in Table 2.The findings regarding the chemical properties of the pumpkin seeds oil are presented in Table

2.The relatively high values of 191.46 and 293.01 for saponification and saponification equivalent, respectively, show that the seed oil contains a higher proportion of fatty acids with high molecular weight. The sample oil only contains a small amount of tocopherols, unsaponifiable sterols, hydrocarbons, vitamins A and D and other substances, as evidenced by the minimal rate of unsaponifiable matter (1.29%). The acid value and the amount of free fatty acid (as oleic) were found to be 1.60 and 0.801%, respectively. The free fatty acid is produced by the hydrolytic breakdown of the oil or fat. As a result, the oil's low acid value and percentage of free fatty acids (as oleic) indicate that it is safe for consumption. The ester value for the sample oil was found to be 189.86. The amount of ester in the lipid sample is indicated by this number. The titre value of oils is the solidifying point of the mixed fatty acids. It is a value for characterizing oils and assumes the hardness. As shown table 3, the titre value of Pumpkin seeds oil was found to be 29.5 °C. Both the iodine value of 92.80 and the Elaiden Test demonstrate that the oil is of the semidrying type and contains a moderate amount of unsaturated fatty acid constituents. The acetyl value of the experimental oil is found to be 6.35, which indicates that the oil does not contain any fatty acid with hydroxyl group and the number of free hydroxyl group in the oil are low. The peroxides value is used as an indicator of rancidity. It is also an indication of unsaturation of an oil or fat. As shown in Table 3, the peroxide value the sample oil was determined to be 20.23 which indicates that the oil sample contain large amount of unsaturated fatty acids and a little amount of saturated fatty acids.Henher value is the measure of water insoluble fatty acids present in oil or fat. The Henher value of the experimental pumpkin seeds oil was 84.00%, which indicates the presence of higher proportion of water insoluble fatty acids. The Polenske value of the pumpkin seeds oil was found to be 1.002, which indicates the presence of low amount of steam-distillable water insoluble fatty acid. The Reichert-Meissl value is a measure of the volatile, water-soluble acid constituents of the oil or fat. The Reichert-Meissl value of the sample oil was found to be 0.40, which indicates that the oil contains very low amount of steam volatile fatty acids. The low Reichert-Meissl value and low polenske value of the pumpkin seed oil indicate the presence of comparatively higher fatty acids in the oil. As per studies examining the effects of storage time, peroxide and acid values increase while iodine and R-M values decrease. According to this, oils' quality deteriorates over time as they are stored.

Table 2: Chemical parameters of Pumpkin seed oil

Chemical properties	Values for the experimental oil
Saponification value (S.V.)	191.46
Saponification equivalent	293.01
Unsaponifiable matter (%)	1.29
Acid value	1.6
Free fatty acid (% as oleic acid)	0.801
Ester value	189.86
Titre value	29.50 °C
Iodine value	92.80
Acetyl value	6.35
Peroxide value	20.23
Henher Value (%)	84.00 %
Polenske Value	1.002
Reichert-Meissl Vaule	0.40
Elaiden test	Semi-drying oil
Vegetable oil test	Vegetable oil

3.3 Quantitative analysis of seed cake

3.3.1 Pumpkin's de-oiled seed cake analysis

The de-oiled seed cake of Pumpkin has been studied by quantitative analysis for the determination of percentage of ash content, protein, and moisture content. The results were given in Table 3. From the result as shown in Table 3, we get an idea about the percentage ash content, protein and moisture content of de-oiled seed cake of Pumpkin.

Table 3: Percentage of ash, protein and moisture content in de-oiled seed cake

Contents	Values of seed cake (%)
Ash content	8.895
Protein	9.00

Moisture content	11.20

3.3.2 Minerals (N, P, Na, K, Ca, and Fe) analysis of de-oiled seed cake

Standard techniques were used to calculate the percentages of N, P, Na, K, Ca and Fe in the de-oiled seed cake of the pumpkin. The de-oiled seed cake of Pumpkin has been studied by quantitative analysis for the determination of percentage of minerals. The results are given in Table 4. Serious problems with protein deficiency affect the majority of people in Bangladesh. Table 4 makes it abundantly clear that pumpkin seeds are properly balanced in contexts of essential amino acids and have a sufficient amount (12.65%) of protein (proteineous nitrogen). The de-oiled seed cake of pumpkin seeds was determined to contain 2.4551 percent phosphorus. The results of the experiment suggest that the de-oiled seed cake may contain phospholipid. The de-oiled pumpkin seed cake was found to have 1.33 percent potassium content. People with low blood pressure may be able to raise their readings by eating these pumpkin seeds. As a result, treating low blood pressure might involve using that one. The de-oiled seed cake of pumpkin seeds contained 1.895 percent calcium, according to the analysis. Children who eat pumpkin seeds during their growing years may have stronger bones. Iron content in the de-oiled seed cake of pumpkin seeds was found to be 0.017 percent.

Table 4: Percentage of minerals of de-oiled seed cake

Minerals	**Values of seed cake (%)**
Nitrogen	12.650
Phosphorous	2.455
Sodium	2.207
Potassium	1.330
Calcium	1.895
Iron	0.017

3.4 Analysis of fatty acid composition

Gas Liquid Chromatography (GLC) was used to identify and measure the fatty acids in the pumpkin seed oil, and the results are shown in Table 5.It was found that the sample contain the highest amount of Oleic acid (34.37%); other fatty acids i.e. Undecanoic acid (17.15%), Stearic acid (29.81%) and Capric acid (18.28%) were also found. From this it is evident that the oil sample contains high proportion of higher fatty acids. It has been found that the oil sample contains mostly saturated fatty acids (65.24%) and unsaturated fatty acids 34.75%. Diets enriched in saturated fatty acids inhibit cytochrome P450_2E1 and lipid peroxidation and ameliorate established alcoholic liver disease. Vitamin E may also play a role in modulating lipid peroxidation and liver injury. Additionally, plasma levels of endotoxin liver mRNAs for pro-inflamatory cytokines are also down regulated after treatment with saturated fatty acids. Thus saturated fatty acids are a potential therapeutic intervention in inflammatory liver injury [24].

Table 5: Fatty acid composition of the fatty acid methyl ester mixture derived from the Pumpkin seed oil obtained by GLC analysis

Name of the oil	**Peak Number**	**Types of fatty acid**	**Name of the fatty acid**	**Retention time (RT)**	**Area**	**Relative area (%)**	**Total composition (%)**
The oil sample of pumpkin seed	1	Saturated fatty acids	Capric	6.08	1147	18.28	65.24
	2		Undecanoic	8.43	1076	17.15	
	3		Stearic	25.17	1870	29.81	
	4	Unsaturated fatty acids	Oleic	34.75	2180	34.75	34.75

3.5 Microbial activities of the Pumpkin seed oil

Antibacterial activity of the seed oil sample was studied against seven pathogenic bacteria. For antifungal activity, the oil sample was also studied against seven plant pathogenic fungus.

3.5.1 Bacterial activity test

As test organisms, three gram positive and four gram negative pathogenic bacteria were used to assess the antibacterial effects of the oil sample. The results are presented in Table 5. We employed paper discs that had been immersed in 5% and 10% sample oil solutions. It was discovered that the oil sample solution had antimicrobial activity against *Escherichia coli, Staphylococcus aureus,* *Micrococcus luteus, Bacillus subtilis, Klebsiella pneumonia, Kluyveromyces fragilis*. The sample lipid showed a higher (20 mm) inhibitory zone against *Klebsiella pneumonia* when compared to other test bacteria. Pumpkin seed oil has no inhibition on *Pseudomonas aeruginosa*.

Table 5: Antibacterial activity of Pumpkin seed oil

Name of bacteria	Type of sample	After 48 hours, Inhibitory zone (diameter in mm)		
		Treatment	Control	Differences
Escherichia coli	10%	14	0	14
	5%	7	0	7
Pseudomonas aeruginosa	10%	0	0	0
	5%	0	0	0
Staphylococcus aureus	10%	15	0	15
	5%	7	0	7
Micrococcus luteus	10%	16	0	16
	5%	8	0	8
Bacillus subtilis	10%	14	0	14
	5%	6	0	6
Klebsiella pneumonia	10%	20	0	20
	5%	10	0	10
Kluyveromyces fragilis	10%	18	0	18
	5%	9	0	9

3.5.2 Fungal activity test

We examined the antifungal effects of the oil sample against seven phyto-pathogenic fungi. The results have been shown in the following Table 6.It is evident from the Table 6 that, when concentration of oil is low the percentage of inhibition is low and when concentration is high, the percentage of inhibition or stimulation is also high (i.e. inhibition increases with increasing concentration of the seed oil).The results indicated that Pumpkin seed oil sample showed inhibition of mycelial growth of *Colletotrichum corchori,Alternaria alternata, Aspergillus niger*. The growth of *Curvularia lunata, Aspergillus funiculosus, Fusarium equiseti* and *Macrophomina phaseolina* was stimulated by the oil sample. From the table highest inhibition (32mm) was recorded in the case of *Alternaria alternate* where the oil was used at 10% concentration and highest stimulation (-154.04 mm) was recorded against *Macrophomina phaseolina* at 10% sample concentration.

Table 6: Percent growth of seven phyto-pathogenic fungi by the Pumpkin seed oil

Name of Fungi	Concentration of oil sample (%)	% inhibition of Pumpkin seed oil (after 5 days)
Colletotrichum corchori	1%	23.5
	10%	16.8
Curvularia lunata	1%	-12
	10%	-18.4
Macrophomina phaseolina	1%	-129.78
	10%	-154.04
Aspergillus niger	1%	4.4
	10%	0
Alternaria alternate	1%	28.7
	10%	32
Aspergillus funiculosus	1%	-41.5
	10%	-22.12
Fusarium equiseti	1%	-51.4
	10%	-47

Due to lack of laboratory resources and facilities, it was not possible to continue the further research of the antibacterial and antifungal activities of the lipid sample. From the result we can conclude that this research work will provide valuable information about the prospect of derivation of pesticides and pharmaceuticals from the oil of Pumpkin seed.

4 Conclusions

Physico-chemical Characterization and microbial studies of pumpkin seed (*Cucurbita maxima*) oil have been done in this investigation. The presence of significant amount of unsaturated fatty acids in the pumpkin seed oil sample was confirmed by Refractive index and Iodine value. Sterols, vitamins A, D, and E are taken into consideration based on the value of Unsaponifiable matter (%); and the sample's low hydroxyl group content was confirmed by its acetyl value. Gas liquid chromatographic analysis supported the presence of a significant amount of higher fatty acids in pumpkin seed oil. The results of the current study showed that bacterial and fungal growth was inhibited by pumpkin seed oil. As a result, the extracted pumpkin seed oil can be used to produce pesticides and pharmaceutical components (such as antibiotics, germicides, antifungal ointments, antibacterial creams, etc.) with further research. Protein and other important minerals (N, P, Na, K, Ca and Fe) also found with significant values in the pumpkin seed oil. Considering their values as nutraceuticals and food supplements, pumpkin seeds should be encouraged to be consumed.

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