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Santhiya R
Department of Nutrition and Dietetics, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Sridevi J
Assistant Professor, Department of Nutrition and Dietetics, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Corresponding Author:
Santhiya R
Department of Nutrition and Dietetics, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India

Physico-Chemical characteristics of two varieties of pumpkin seeds

Santhiya R and Sridevi J

Abstract
Pumpkin seed is one among the plant foods that contain high levels of bioactive components such as beta-carotene, unsaturated fatty acids, phenolic compounds, phytosterols and tocopherols. Pumpkin seeds are generally considered as waste product but it is rich in bioactive compounds with neutraceutical properties and considered as a healthy source. The nutritional factors of pumpkin seed comprises of nutrients such as carbohydrates, fats and proteins, as well as minor components such as minerals, vitamins, fibres. The chemical composition of pumpkin varies from one cultivar or species to other. The study aimed to characterize the physico-chemical characterization of *Cucurbita maxima* Dushesne- CUCURBITACEAE i.e. orange pumpkin seed (OPS) and *Cucurbita pepo* L. – CUCURBITACEAE i.e. white pumpkin seed (WPS).

Keywords: Pumpkin seeds, macronutrients, micronutrients

1. Introduction
The pumpkin (*cucurbita)* belongs to the family *cucurbitaceae.* This family is one of the largest family in plant kingdom comprising of highest number of edible plant species. Pumpkins contain biologically active compounds like polysaccharides, para-aminobenzoic acid, fixed oils, sterols, proteins and peptides. They are also a good source of iron, vitamin A, proteins such as arginine, aspartate and glutamic acid but deficient in lysine and sulphur containing amino acids. It is a high yield vegetable which is easy to grow and is consequently inexpensive (Dar *et al*., 2017) [2]. They are cultivated for their flesh, seed oil and medicinal value. The chemical composition of pumpkin varies from one cultivar or species to other. The most valuable element of pumpkin are included in its part which is most commonly disregarded as waste, namely their seeds (Sharma G and Lakhawat S, 2017) [10]. These numerous off-white coloured seeds are placed in the central cavity of pumpkin in net like structure. The seed content varies from 3.52% to 4.27% (Devi *et al*., 2018) [19].
Pumpkin seeds are small, flat, edible seeds with varying colours and shape depending on the variety. Mostly these seeds are covered by a white husk, although some of the pumpkin varieties produce seeds without the husk. Pumpkin seeds can be found hulled or semi–hulled at most grocery stores (Okbi *et al*., 2016). It is consumed mostly raw or roasted according to the Mediterranean diet (Sakka and Karantonis, 2015) [6].
The nutritional factors of pumpkin seed comprises of nutrients such as carbohydrates, fats and proteins, as well as minor components such as minerals, vitamins, fibres (Sakka and Karantonis, 2015) [6]. Pumpkin seeds are an ample source of phytosterols, proteins, polyunsaturated fatty acids, antioxidants, vitamins, carotenoids, tocopherols and various other elements. It contains fatty acid components such as palmitic, palmitoleic, steric, oleic, linoleic, gadoleic, total saturated fatty acids and total unsaturated fatty acids (Ratnam *et al*., 2017). Pumpkin seeds are rich in medicinal and nutritive components, due to which they are used in most therapeutic purposes across the globe. They have pharmacological activities such as anti-diabetic, antibacterial, antifungal, anti-inflammatory and antioxidant activity (Dar *et al*., 2017) [2]. Due to their positive health benefit, research has been focused on the content and composition of fatty acids (FA) also (Montesano *et al*., 2018) [8].

2. Materials and Methods

2.1 Collection of sample and authentication

The orange pumpkin and white pumpkin was procured from the local market of Coimbatore during the months of December 2019, January 2020 and February 2020. The seeds were removed manually and the seeds were washed thoroughly, oven dried, powdered and stored in air tight plastic pouches. The seed samples were authenticated by Dr. C. Murugan, Scientist 'E'-in-charge, Botanical Survey of India, Southern Regional Centre, Government of India, Coimbatore. The orange pumpkin seed and white pumpkin seed was authenticated as *Cucurbita maxima* Dushesne-CUCURBITACEAE and *Cucurbita pepo* L. – CUCURBITACEAE respectively.

2.2 Processing of the sample

The seeds collected from orange and white pumpkins were washed thoroughly to remove the pulp particles and were oven dried at 110°C for about 4 hours. The dried seeds were grinded to coarse powder in a mixer grinder so that this grinding process makes the parts exposed to the solvents for easy penetration to extract the phytoconstituents. The powdered samples were stored in a sterile plastic pouches.

2.3 Physical characterization of pumpkin seeds

The physical characteristics was measured based on the method of Devi *et al*., 2018 [19].

2.3.1 Geometric mean diameter

Three dimensions viz. length, thickness, width of orange and white pumpkin seeds were measured with Vernier caliper and thickness with screw gauge. Measurement was done with 20 randomly drawn orange and white pumpkin seeds. From these values the geometric mean were calculated using the formula:

$$\text{Geometric mean diameter (Dg)} = (LWT)^{1/3}$$

Where L is the length, W is the width and T is the thickness.

2.3.2 True density

The true density of both orange and white pumpkin seeds were determined separately using water displacement method. 50 ml of water was placed in a 100ml graduated measuring cylinder and 5g of pumpkin seeds were immersed in that water. The amount of displaced water was recorded from the graduated scale of cylinder. The ratio of weight of seeds to the volume of displaced water gave the true density.

$$\text{True density} = \frac{\text{Weight of the sample in air (g)}}{\text{Volume of displaced fluid (ml)}}$$

2.3.3 Bulk density

The bulk density for both orange and white pumpkin seeds were determined separately. Each of the seed (5g) was weighed into a 10 ml measuring cylinder. The bottom of the cylinder was gently tapped for 5 minutes from a height of 5 cm. The bulk density was taken as mass per unit of the sample.

$$\text{True density} = \frac{\text{Weight of the sample in air (g)}}{\text{Volume of displaced fluid (ml)}}$$

2.3.4 Porosity

The porosity (ε) of the orange and white pumpkin seeds was computed separately from the values of true and bulk density with the relationship

$$\text{Porosity } (\varepsilon) = \frac{1 - \text{Bulk density}}{\text{True density}} \times 100$$

2.4 Chemical characterization of pumpkin seeds

2.4.1 Quantitative Analysis of Macronutrients

i) Determination of moisture content:

The test was done based on AACC (2010) [1]. The moisture content was analysed using the moisture analyser. Each samples were kept in plate present in the analyser and the time was set to 10 minutes and the results were noted.

ii) Determination of carbohydrates

Total carbohydrate content of foods has been calculated by difference method. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula:

Total carbohydrates = 100 - (Weight in grams [protein + fat + water + ash] in 100 g of food. (http://www.fao.org/3/y5022e/y5022e03.htm).

iii) Determination of proteins

Protein was estimated by the procedure as described by Lowry's method. Hydrolysing the protein and estimating the amino acids alone will give the exact quantification. The method is sensitive enough to give a moderately constant value and hence largely followed. Protein content of enzyme extracts is usually determined by this method. The method is based on the blue color developed by the reduction of the phosphomolybdicphosphotungstic components in the Folin - Cioealteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate.

iv) Determination of fat

This method was based on Das and Biswas. (2019) [7]. Dry and finely grounded feed when mixed with diethyl ether dissolves fats and fat soluble substances at a temperature of 2000°C as the boiling point of Diethyl ether is 3000°C. Subsequently evaporation of ether from the fat solution leaves the resulting residue referred to as crude fat. Approximately 2 grams of feed was weighed and grinded to fine powder. In a conical flask finely grounded feed was mixed with 10ml of Diethyl Ether and boiled at temperature of 200° C on a heating mantle for 20 minutes. Prior to extraction a petri dish was weighed and after heating the supernatant was poured on the pre weighted petri dish and weight was taken. The difference in weight of the petri dish indicates the quantity of fats. For confirmation of fats in the sample Sudan III test 9–11 was done which gives a positive results.

$$\text{Fat content percent} = \frac{\text{B- C} \times 100}{\text{A}}$$

Where A=Sample weight B=Weight of the glass plate after extraction C=Weight of the glass plate prior to extraction.

v) Determination of ash content

The test was done based on Carpenter and Ward. (2010) [5].The crucibles were weighed separately for each samples. 2 grams of the each of the samples were weighed. The crucibles were placed in electric Bunsen burner and heated till the samples were completely charred. Then it was heated for five hours at 600°C in a muffle furnace in order to obtain the ash. Later it was cooled and weighed. The ash obtained was greyish in color.

100 - (Weight in grams [protein + fat + water + ash] in 100 g of food.

2.4.2 Quantitative analysis of micronutrients

Determination of minerals

The ash solutions were prepared by the method of Carpenter and Ward. (2010) [5] by washing the crucibles containing the ash residues after removing from the muffle furnace using small amount of distilled water and 5ml of concentrated Hcl and was made up to 50 ml with distilled water.

a. Determination of β-carotene

The test was performed by AOAC (1980) [4] method. Weigh 2 grams of each sample. The extraction is done with 25 ml of acetone separately for each samples using mortar and pestle until the residues become colorless. Centrifuge for 20 minutes. Transfer the supernatant to a separating funnel containing 20 ml of petroleum ether and it was mix gently. 20ml of 5% sodium sulphate solution was added and it was shaken gently. The obtained extracts for each samples were pooled and it was made up to 50ml with petroleum ether. 10 grams of anhydrous sodium sulphate was added and it was kept for 30 minutes. The absorbance was measured at 453 nm using petroleum ether as blank.

b. Estimation of iron

The estimation was based on Wong. (1928) [22]. The stock iron solution was made and from which the working standard solution was prepared. The reaction mixture (10ml) contains different aliquots of working standard solutions such as 1, 2, 3, 4, 5 corresponding to 5, 10, 15, 20and 25γ of iron concentrations respectively. 1ml of saturated potassium persulphate was added and 1ml of 30% H_2SO_4 was added. Finally 1.5ml of 3N potassium thiocyanate was added for development of red colour. The sample ash solutions of each sample was taken 5 ml and done by same process as above. The blank comprises of the same procedure without the addition of working standard or sample ash solutions. The reaction mixtures was kept for 10 minutes. The absorbance was measured at 530nm.

c. Estimation of phosphorus

The test was based on the Fiske *et al*. (1925) [9]. The stock phosphorus solution was made and from which the working standard solution was prepared. The reaction mixture (10ml) consists of 1, 2, 3, 4 and 5ml of working standard solutions corresponding to 10, 20, 30, 40 and 50γ of phosphorus concentrations.1ml of ammonium molybdate I (12.5 grams of ammonium molybdate in 100 distilled water with 250ml of 10N H_2SO_4 and made up in 500ml standard flask) was added. 0.4 ml of ANSA was added. The sample solution (10ml) contains 1ml of ash solution with 1ml of ammonium molybdate II (12.5 grams of ammonium molybdate in 100 distilled water with 150ml of $10NH_2SO_4$ and made up in 500ml standard flask) and 0.4ml of ANSA. The blank consists of 1ml of ammonium molybdate I, 8.6 ml of distilled water and 0.4ml of ANSA. The solutions were allowed to stand for 20 minutes. The absorbance was measured at 660nm.

d. Estimation of zinc

The ash solutions of both orange and white pumpkin seed powders were estimated through atomic absorption spectroscopy (model name: AA-6300). This uses principally the air– acetylene flame with a temperature of about 2300 °C and the nitrous dioxide system (N_2O) – acetylene flame with a temperature of about 2700 °C. The sample solutions were aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which were introduced into a spray chamber, where they were mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (<10 µm) enter the flame. This conditioning process is responsible that only about 5% of the aspirated sample solution reaches the flame, but it also guarantees a relatively high freedom from interference. On top of the spray chamber is a burner head that produces a flame that is laterally long (usually 5 – 10 cm) and only a few mm deep. The radiation beam passes through this flame at its longest axis, and the flame gas flow – rates may be adjusted to produce the highest concentration of free atoms. The burner height may also be adjusted, so that the radiation beam passes through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity. Thus, the zinc results for both the samples were estimated.

e. Estimation of magnesium

The ash solutions of both orange and white pumpkin seed powders were estimated through atomic absorption spectroscopy (model name: AA-6300). The magnesium results were obtained by following the same procedure how zinc was obtained.

f. Estimation of potassium

The ash solutions of both orange and white pumpkin seed powders were estimated through atomic absorption spectroscopy (model name: AA-6300) and the potassium results for both the samples were estimated by following the same procedure of that of zinc.

3. Results and Discussion

The physical properties of the seed are the pre-requisites for the design of equipment for handling, dehulling and other processes (Devi *et al*., 2018) [19].

The physical characteristics includes parameters such as length, width, thickness, geometric mean diameter, true density, bulk density and porosity were estimated and tabulated in Table 1.

Table 1: Physical characteristics

Parameters	Physical characteristics	
	Orange pumpkin seed	White pumpkin seed
Length (cm)	1.67 ± 0.04	1.184 ± 0.01
Width (cm)	0.81 ± 0.04	0.742 ± 0.02
Thickness (cm)	0.317 ± 0.008	0.284 ± 0.018
Geometric mean diameter(cm)$^{1/3}$	0.7540 ± 0.001	0.6295 ± 0.0004
True density (g/cm^3)	0.53 ± 0.025	0.8 ± 0.1
Bulk density (g/ml)	0.5185 ± 0.0005	0.5711 ± 0.0005
Porosity (%)	58.0120 ± 0.64	85.78 ± 0.70

From the results obtained the length, width, thickness and geometric mean diameter of orange seeds are 1.67cm, 0.81cm, 0.317cm and 0.7540 cm$^{1/3}$ respectively. Likewise, for white pumpkin seeds it is 1.184cm, 0.742cm, 0.284cm and 0.6295 cm $^{1/3}$ respectively. The true density of the orange and white pumpkin seeds are 0.83 g/cm^3 and 0.5 g/cm$^{3.}$ The bulk density of orange and white pumpkin seeds are 0.5185 g/ml and 0.5711 g/ml. The porosity of orange and white pumpkin seeds are 58.0120% and 85.78% respectively orange pumpkin seeds shows greater length, width, thickness, true and bulk density whereas the porosity is higher in case of white pumpkin seeds.

Devi *et al*., 2018 [19] reported the length, width, thickness and Dg as 1.681cm, 0.88 cm 0.275cm and 0.742(cm) $^{1/3}$. Similarly, the true density, the bulk density and porosity as 1.157g/cm^3, 0.398 g/ml and 65.60%. The results obtained in table 1 were slightly in accordance with Devi *et al*., 2018 [19].

3.2 Determination of Nutritional Composition

3.2.1 Macronutrient analysis

The macronutrient analysis includes analysis of carbohydrates, proteins, fats, ash, and moisture content of the samples. Carbohydrates are the important components of storage and structural materials in the plants. Total carbohydrates include all the different types of carb in a food or meal. These include starches, sugars etc. (http://www.fao.org/3/y5022e/y5022e03.htm)

Proteins perform a variety of functions, including enzymatic catalysis, transporting ions and molecules from one organ to another, nutrients, contractile system of muscles, tendons, cartilage, antibodies, and regulating cellular and physiological activities (https://www.sciencedirect.com/topics/neuroscience/protein.

Fat is an important foodstuff for many forms of life, and fats serve both structural and metabolic functions. They are a necessary part of the diet of most heterotrophs (including humans) and are the most energy dense, thus the most efficient form of energy storage (https://en.wikipedia.org/wiki/Fat).

Moisture is the mass of water molecules present in the food. It is commonly measured for number of reasons like sensory quality and stability of foods (AACC, 2010 and Bradley, 2010) [1]. Moisture plays an important part in the growth of trees. Water is indispensable for the absorption and transport of food, to carry out photosynthesis, to metabolize materials and to regulate moisture in plants, as in all other living system. It contributes as much as to the essential properties of life as do the other constituents like protein, carbohydrate. Moisture is also essential for most of the physiological reactions in plant tissue and in its absence life does not exist (Habib *et al*., 2015) [3].

Ash is the measure of the total amount of minerals within a food. It is the inorganic residue remaining after removing the water and organic matter by heating in the presence of oxidizing agents (Carpenter and Ward, 2010) [5].

The values obtained in the macronutrient analysis of various extracts of orange and white pumpkin seeds are tabulated in the table 2.

Table 2: Macronutrient analysis

Sample	Macronutrients				
	Total Carbohydrates (g/100g)	Proteins (g/100g)	Fat (g/100g)	Moisture content (g%/100g)	Ash content (g/100g)
Orange pumpkin seed	56.0824 ±0.04	0.0576 ±0.001	1.4 ± 0.1	43.61 ± 0.01	3.85 ± 0.01
White pumpkin seed	41.2226 ±0.1	0.0474 ±0.001	1.0 ± 0.25	54.38 ± 0.01	3.35 0.01

According to the table 2, the macronutrient contents such as carbohydrates 56.0824 g/100g, proteins 0.0576 g/100g, fat 1.4g/100g, ash content 3.85 g/100g of orange pumpkin seed were found to be higher than those values of white pumpkin seed except for the moisture content. This is because the white pumpkin has more moisture texture in comparison.

Syed *et al*. (2019) [20] reported carbohydrate 10.71g/100g, total fats 49.05g/100g, protein 30.23g/100g. Malkanthi *et al*. (2018) [18] elucidated ash content as 3.65g%/100g which was in accordance with the result in table 4.2. Devi *et al*. (2018) [19] showed the moisture % to be 5.53% which was less than the value obtained in table 2.

3.2.2 Micronutrient analysis

The estimation of minerals such as iron, potassium, zinc, magnesium and phosphorus were done in the micronutrient analysis. Minerals are inorganic substance required by the organism in very small amount for their growth and maintenance of functional activity. Food and vegetables are the important source of mineral for human beings and exist in food as organic and inorganic combination. In foods mineral elements are present as salt (Habib *et al*., 2015) [3]

Iron is a chemical element. It is a metal that belongs to the first transition series. The iron is present in the ferric state (Wong, 1928) [22]. Phosphorus is a non-metallic element of the nitrogen family with atomic number 15 that occurs widely in combination especially as phosphates, that is essential for life in all known organisms, and that is used especially in fertilizers and organophosphorus compounds (https: //www. merriam-webster. Com /dictionary/

phosphorus). Zinc is an essential mineral involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division. It also supports normal growth and development. (https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/). Magnesium is a silver-white malleable ductile light metallic element that occurs abundantly in nature and is used in metallurgical and chemical processes (https://www.merriam-webster.com/dictionary/ magnesium). Potassium is the third most abundant mineral in the body. It helps the body regulate fluid, send nerve signals and regulate muscle contractions. Roughly 98% of the potassium in the body is found in the cells. Of this, 80% is found in the muscle cells, while the other 20% can be found in the bones, liver and red blood cells (https://www.healthline.com/nutrition/what-does-potassium-do#section1). B-carotene is an organic, strongly coloured red-orange pigment. It is a member of the carotenes, which are Terpenoids (isoprenoids), synthesized biochemically from eight isoprene units and thus having 40 carbons. Among the carotenes, β-carotene is distinguished by having the beta-rings at both ends of the molecule (AOAC, 1980) [4]. The values were tabulated in table 3.

Table 3: Micronutrient analysis

Sample	Micronutrients(mg/100g)					
	β-carotene	**Iron**	**Phosphorus**	**Zinc**	**Magnesium**	**Potassium**
Orange pumpkin seed	2.25 ± 0.16	2 ± 0.25	65 ± 1	78 ± 1	845 ± 1	505 ± 1
White pumpkin seed	0.25 ± 0.01	2 ± 0.25	255 ± 0.15	116 ± 0.2	790 ± 0.20	206 ± 0.25

From the table 3, β-carotene 2.25mg/100g, magnesium 845 mg/100g, potassium 505 mg/100g values were greater in orange pumpkin seed whereas phosphorus 255 mg/100g and zinc 116 mg/100g values were found to be greater in white pumpkin seed. Iron content 2 mg/100g was found to be equal in both the samples.

According to Devi *et al*. (2018) [19] the mineral content of iron, phosphorus, zinc, magnesium, potassium are 16.1 mg/100g, 848.6 mg/100g, 907 mg/100g, 335.6 mg/100g, 404.9 mg/100g. Syed *et al*., 2019 [20] concluded β-carotene value as 0.009mg/100g. The results obtained in table 2 have lesser values in iron, phosphorus, zinc and higher value than magnesium and it is slightly in accordance with potassium value. The β-carotene value was lesser in comparison with the table 2 values.

4. Summary and Conclusion

Pumpkin is a cultivar of squash which is plump with smooth, slightly ribbed skin, and ranges in different colors mostly between deep yellow to orange. The seeds obtained from the pumpkins being small in size, they are packed full of valuable nutrients such as healthy fats, magnesium, zinc etc. There are more than 100 varieties of pumpkin and its seeds. In this study, the seed varieties of OPS {orange pumpkin (*Cucurbita maxima* Dushesne-CUCURBITACEAE)} and WPS {white pumpkin (*Cucurbita pepo* L. – CUCURBITACEAE)} were selected. This study aimed to estimate the characters such as physical characteristics, macronutrient profile and micronutrient profile.

The physical characters of OPS and WPS such as the length, width, thickness and geometric mean diameter, true density, bulk density and porosity of OPS and WPS were determined. The OPS seems to be greater in physical dimensions except bulk density and porosity.

Macronutrient analysis results of OPS and WPS were analysed. In comparison, the OPS showed good composition except moisture content. This shows the seeds has good amount of macronutrient composition. Micronutrient determination of OPS and WPS were estimated. This shows the seeds has good amount of selected micronutrients in them.

Pumpkin seeds which can be separated manually from economically cheap pumpkins are generally considered as a waste material and is commonly discarded during cooking or processing. These seeds may have medicinal properties and may be a booster for health benefits which can be consumed as a therapeutic agent with minimal side effects. Further research on these lines must be illustrated to know more about the possible mechanism of action of these seeds for the purpose of higher incorporation levels in food products with minimizing losses and to bring about the invention of therapeutic drugs from this underutilized pumpkin seeds.

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