

**PROXIMATE COMPOSITION, BETA CAROTENE AND MINERAL CONTENT
OF DIFFERENT PUMPKIN (*Cucurbita spp.*) VARIETIES**

A THESIS

BY

SUNJIDA AFROSE

EXAMINATION ROLL NO.: 1705423

REGISTRATION NO.: 1705423

SESSION: 2017-2018

SEMESTER: JULY-DECEMBER, 2018

**MASTER OF SCIENCE
IN
FOOD SCIENCE AND NUTRITION**



**DEPARTMENT OF FOOD SCIENCE AND NUTRITION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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Dedicated

To

My Beloved

Parents

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The Author

ABSTRACT

Fruits and vegetables are important sources of nutrients for mankind. Proximate composition, Beta carotene and mineral content of three indigenous cultivar of Pumpkin. One factor variance (ANOVA) was conducted for all variable using SPSS software). The fruits of three indigenous cultivar S₁, S₂ and S₃ were collected. Physicochemical characteristics including moisture, ash, total fat, total fiber, protein, total carbohydrates, pH, acidity, total sugars and ascorbic acid was analyzed. Also sensory attributes (color, flavor, taste, texture and overall acceptance), Beta-carotene, Ca, Zn and energy was determined .No significant difference was found in moisture, fiber and protein. Ash content ($0.75667 \pm 0.1301\%$), fat content ($0.32 \pm 0.0300\%$), β -carotene content (0.455 ± 0.0271 mg/100g) were found higher in variety S₂. Higher vitamin - C content (87.833 ± 4.3466 mg/100g), total sugar content ($4.604 \pm 0.3130\%$), zinc content (87.177 ± 2.6866 %) and energy content (24.269 ± 2.5294 Kcal) were found in variety S₁. The variety S₃ contain highest amount of calcium (310.433 ± 16.3102 mg/100g). pH ranges from 6.347 to 6.527 and moisture ranges from 93 – 95% found to be significantly varied among the varieties. As for sensory attributes no significant change in the flavor and drastical changes found in overall acceptability. Least texture score (5.85 ± 0.671) was found in variety S₁ and highest (7.10 ± 0.641) in S₃. In taste and overall acceptance S₁ gives higher score rather than the two others. It can be concluded from the results of this study that all the pumpkin varieties are high in nutrition and beneficial compounds for the human health. This study shows that the variety S₁ gives more nutritional content and sensory scores than the respective varieties. Hence this study would help for the consumers, dietitian and industrial policymakers for further use.

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LIST OF ABBREVIATIONS

AOAC	:	Association of Analytical Chemists
ANOVA	:	Analysis of variance
<i>et al.</i>	:	And others
°C	:	Degree Celsius
min	:	Minute
spp.	:	Species
ml	:	milliliter
%	:	percentage
g	:	Gram
mg	:	Milligram
S1	:	<i>C. moschata</i>
S2	:	<i>C. maxima</i>
S3	:	<i>C. pepo</i>
RDA	:	Recommended Daily Allowance
kDa	:	Kilodalton
WSI	:	Water Solubility Index
WAI	:	Water Absorption Index
RVU	:	Rapid Visco Units
µm	:	Micrometer
ppm	:	Parts Per Million



CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Pumpkin belongs to the *Cucurbitaceae* family of the genus *Cucurbita* and is thought to be native of the South America. *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita pepo*, are three of the most cultivated species worldwide. Pumpkin fruit is one of the widely grown vegetables that is incredibly rich in vital antioxidants and vitamins. This humble backyard low calorie vegetable contains vitamin A, flavonoid poly-phenolic antioxidants such as leutin, xanthin and carotenes in abundance (Durante, 2014). *Cucurbitaceae* (Cucurbit) is an important family comprising one of the most genetically diverse groups of food plants. They are characterized by prostrate or climbing herbaceous vines with large fleshy fruits (Acquaah, 2004).

The pumpkin is a vegetable crop belonging to the *Cucurbitaceae* family. This family contains chemicals, including tetracyclic triterpenes, saponins, proteins, fibers, polysaccharides and minerals (iron, zinc, manganese, copper, etc) (Abuelgassim and Showayman, 2012). Some important Cucurbit family members include; gourd, melon, cucumber, squash and pumpkin (Robinson and Decker-Walters, 1999), the majority of the species in these families are used as food.

Botanically the pumpkin is a squash fruit, most commonly orange in colour when ripe and is appreciated when cooked, pureed, used in soups, breads, and many other dishes. In culinary terms, it is widely regarded as a vegetable. It has been found that 100 g of fresh pumpkin contain 80.0-96.0g moisture content, 4.6-6.5g sugars, 0.6-1.8g protein, 0.0-0.2g lipids and 0.5-1.3g fiber (Fennema *et al.*, 2004). This chemical composition associated with its antioxidants and vitamins allows the pumpkin to have an important health-protecting effect. In fact, the range of values of lipophilic substances as carotenoids presenting in pumpkin varieties can contribute significantly to the uptake of provitamin A and especially lutein, a carotenoid with special physiological functions (Murkovic *et al.*, 2005).

Food and Agriculture Organization of United Nation (FAO), the world production of pumpkins, squashes, and gourds in 2011 was estimated over 24.3 million tons harvested from 1.7 million hectares (FAOSTAT, 2013). *Cucurbita moschata* is the most heat-tolerant species and the most common in tropical Africa (Fedha *et al.*, 2010). It has great

economic potential as a food and as an industrial crop. It is utilized for its leaves, marrow, fruit pulp, seeds and flowers. The stem could be used as livestock feed. It has health enhancing properties (Chweya and Eyzaguirre, 1999; Mnzava and Mbewe, 1997).

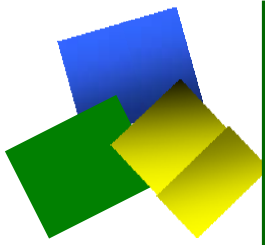
Pumpkin has an abundance of macro- and micro-nutrients, as well as antioxidants that boost the human body immunity against cancer and other deadly diseases (Oloyede *et al.*, 2013). It has such nutritional potential unequalled to any other single crop (Encyclopedia of Foods, 2004). Pumpkin is a traditional crop with high potential to overcome undernourishment and food poverty (Ondigi *et al.*, 2008), Reliable data on carotenoid analysis is urgently needed as this research area is developing its own potential to human health and also is important to the food and pharmaceutical industry. Carotenoid is recognized as a phytochemical compound which is responsible to reduce the risk of some degenerative diseases such as cancer and disease related to cardiovascular (Ames *et al.*, 1993).

Generally, carotenoid is a tetraterpenoid organic pigment which occurs naturally in the chloroplasts and chromoplasts of plants including some photosynthetic organisms like algae, some fungus, bacteria and at least one species of aphid (plant lice) (Nancy and Tyler, 2010).

Pumpkin is considered by Traditional Chinese Medicine as being immensely valuable for human health. In the Compendium of the Materia Medica, a classical work in the history of Chinese science and technology development, “Buzhong Yiqi, Runfei Huatan”, which means “it will do much good to our liver and lungs”, is recorded for pumpkin (Jian *et al.* 2005).

Hence the objective of the present study was:

1. To investigate physicochemical properties of pumpkin of three varieties
2. To evaluate the nutritional composition and sensory evaluation of these pumpkin varieties.
3. To evaluate the β -carotene and micronutrients of these varieties.



CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Pumpkin

2.1.1 Introduction of Pumpkin

Pumpkin (English), Kumbra (Bengali), Kohlu (Gujarati), Kaddu (Hindi), Kumbala (Kannada), Paarimal (Kashmiri), Mathan or Chakkara kumbalanga (Malayalam), Lal bhopla (Marathi), Kakharu (Oriya), Sitaphal (Punjabi), Purangikkai or Pooshanikai (Tamil), Gummadi kayi (Telugu), Dangaree (Sanskrit) (Gopalan *et al.*, 2007). Pumpkin is one of the most important crops of family *Cucurbitaceae*. *Cucurbitaceae* used as vegetable and medicine throughout the world. *Cucurbita* maxima (*C. maxima*) are an extremely diverse species. It has been suggested that it has more cultivated forms than any other crop. This species originated in South America from wild, free-living *Cucurbita* maxima species *andreaana*, over 4000 years ago, and apparently did not migrate from its continental origin during pre-Columbian era (Sanjur *et al.*, 2002). India, Bangladesh and Myanmar are considered to be secondary centers of diversity for *Cucurbita* maxima (Ferriol *et al.*, 2004).

The word pumpkin locally called “Elegede or Agbeje” in Yoruba land (Western part of Nigeria) originates from the *pepon* which is Greek for “large melon.” The French adapted this word *pompon*, which the British changed to *Pumpion* and later the American Colonists changed it to the word we use today “pumpkin”. The origin of pumpkin is not known, although pumpkins are said to have originated in North America. The oldest evidence, related to pumpkin seeds dated between 5500 and 7000 BC were found in Mexico. Pumpkins are squash-like fruit that range in size from less than 1 pound to over 1,000 pounds (Michael *et al.*, 2008). The genus *Cucurbita*, indigenous to the western hemisphere, is comprised of five domesticated species. Three of these, *Cucurbita pepo* L., *Cucurbita* maxima D. and *Cucurbita moschata* D. represent economically important species cultivated worldwide for human consumption (Whitaker and Davis, 1962; Robinson and Decker-Walters, 1997).

The family *Cucurbitaceae*, is *Cucurbita pepo*, (Common name: Pumpkin; Yoruba: Elegede), a medium sized plant grown for its Fruits and edible seeds. Hence, it is known

to be used as food and in herbal formulation in Nijeria. The ways of expanding the use of available local food sources are increasingly pursued, some of these local food sources contain seeds, many reports on some lesser known seeds and fruits indicated that they could be good sources of nutrients for both man and livestock (Elemo *et al.*, 2002).

2.1.2 Health Benefits of Pumpkin

Fokou *et al.*, (2004) assessed that pumpkin fruit has many nutritional components including pumpkin polysaccharides, active proteins, essential amino acids, carotenoids, and minerals. It has received considerable attention in the recent years because of the nutritional and health protective value of these components. Pumpkin origin in Beijing, containing 100g of edible part with protein 0.6g, fat 0.1g, carbohydrates 5.7g, crude fibre 1.1g, calcium 10 mg, phosphorus 32mg, iron 0.5mg, carotene, 0.53mg, vitamin B₁ 0.04mg, B₂ 0.05mg, niacin 0.7mg, vitamin C 5mg.

Attarde *et al.*, (2010) reported that pumpkin is very rich in carotenoid, which is known for keeping the immune system of an individual strong and healthy. Beta-carotene, found in pumpkin, is a powerful antioxidant as well as an anti-inflammatory agent. It prevents build up of cholesterol on the arterial walls, thus reducing chances of strokes. Being rich in alpha-carotene, pumpkin is believed to slow down the process of aging and also prevent cataract formation. Pumpkins have been known to reduce the risk of macular degeneration, a serious eye problem than usually results in blindness. The high amount of fibre, present in a pumpkin, is good for the bowel health of an individual. Being loaded with potassium, pumpkin is associated with lowering the risk of hypertension. The presence of zinc in pumpkins boosts up the immune system and also improves the bone density. Pulp of pumpkin applied to burns, scalds, inflammations, abscesses, boils and is remedy for migraine, neuralgia, haemoptysis and hemorrhages



Figure 2.1: Pumpkin sample

2.2 Varieties of Pumpkin

There are many varieties of pumpkin worldwide. Some are termed as Summer fruit, some are winter fruit and some are creamy fruits. Amongst them in Northern Bangladesh mainly three types are produced. Locally it is termed as “Misti kumra”. The varieties of pumpkin that are common are given in Table 2.1.

Table 2.1: Varieties of Pumpkin

Species	Plants	Native country	Type of pumpkin
<i>Cucurbita pepo</i>	Pumpkin, acorn squash, zucchini, spaghetti squash, delicate squash, small gourds	United States, Mexico	Bright orange skin. Hrad, woody, stem with ridges
<i>Cucurbita maxima</i>	Very large pumpkin, winter squash, buttercup squash, hubbard squash, turban squash, banana squash, large gourds	Chile, Argentina, Bolivia, Uruguay	Yellow skin, soft stem like a sponge or cork
<i>Cucurbita moschata</i>	Pumpkin, winter squash, butternut squash	Mexico, Peru	Long ,not round Tan skin stem has ridges

Pumpkin is classified according to grade into 3 classes:

2.2.1 “Extra” Class

Pumpkin shall be of superior quality. They shall be practically normal shape and free from skin defects, with the exception of very slight abnormality or superficial defects and not affect to the general appearance, the flesh quality, the keeping quality and presentation in the package.

2.2.2 Class I

Pumpkin shall be of good quality. The following slight abnormality or defects may be allowed:

- (1) Slight abnormality in shape;
- (2) Slight defects of shallow scars on skin; The abnormality or defects shall not affect the general appearance, the flesh quality, the keeping quality and presentation in the package.

2.2.3 Class II

Pumpkin which does not meet the requirement of higher classes shall satisfy the minimum requirements specified in Section 2.1, the following abnormality or defects may be allowed:

(1) Slight abnormality of shape;

(2) Skin defects of shallow scars, scratches, scabs, pressed marks and rubbing traces. The abnormality or defects shall not affect the general appearance, the flesh quality, the keeping quality and presentation in the package (Agricultural Standards, 2008).

2.3 Production of Pumpkin

Pumpkin is one of the important vegetable grown all around the world. It is a common vegetable widely grown in China, India, Russia, United States, Egypt and other countries. The total production of the pumpkin in the world is 24,724,859 tones, with China being the largest producer country with a production of 7,100,000 tones (Anon, 2015). The major pumpkin producing countries of the world are shown in Fig. 2.2. The production of pumpkin in India during 2015 was 4,900,000 tones (Saxena and Chander, 2015). In India, the vegetable is known with various names such as Sitaphal, Kashiphal, Mithakadhu etc. (Bhat and Bhat, 2013) and the major producing states are Orissa (85%), Assam (7%), West-Bengal (3%) and Karnataka (1.5%).

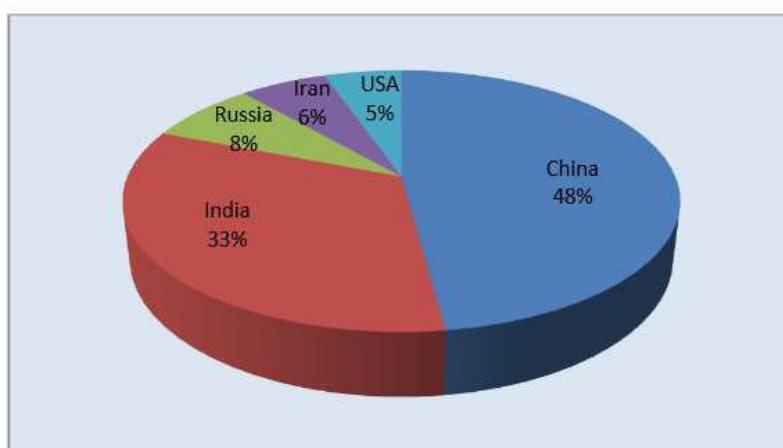


Figure 2.2: Major Pumpkin Producing Countries of the World and their Production

(Source: Anon, 2015)

Pumpkins are a vigorous, prostrate, annual vine with an extensive root system. They are able to put down peg roots to support the plant and their tendrils twine around other plants to prevent them from being blown around. Pumpkins have separate male and female flowers on the same plant. NSW has the second largest pumpkin crop in Australia with 40,718 t produced in 2007–08. The Murrumbidgee Irrigation Area (MIA) is the largest production area for pumpkins in NSW. In 2007–08 the MIA produced 22,197 t of pumpkins which is 54.5% of NSW production.

Table 2.2: Production of Pumpkin in different state of Australia

State	Area hectares	Production tones
Qld	2,751	43,783
NSW	2,057	40,718
WA	876	17,303
Vic	369	6,775
SA	188	2,586
Tas	102	1,478
NT	52	1,775
Aust	6,395	114,417

Source: ABS, 2007–08

2.4 Production of pumpkin in Bangladesh

Pumpkin is a very popular and one of the most important vegetable crops grown extensively throughout the tropical and subtropical countries. Due to its high nutritional content and lucrative market price, pumpkin may be considered as a high value crop. Both immature and mature vegetables are used as a vital ingredient for several culinary preparations in Bangladesh. Pumpkins are rich in carbohydrate and minerals and cheaper source of vitamins, especially carotenoid pigments, which have a major role in nutrition in the form of pro-vitamin A, antioxidants, when used at ripening stage. Thus, this vegetable can contribute to improve nutritional status of the people of Bangladesh, particularly the vulnerable group in respect of vitamin-A requirement. Among all the vegetables produced in the country, Pumpkin major area covered of total cropping area and production. It grows in all the districts of Bangladesh but plenty of Pumpkins are produced in the region of Jessore, Kustia, Chittagong and Dhaka.

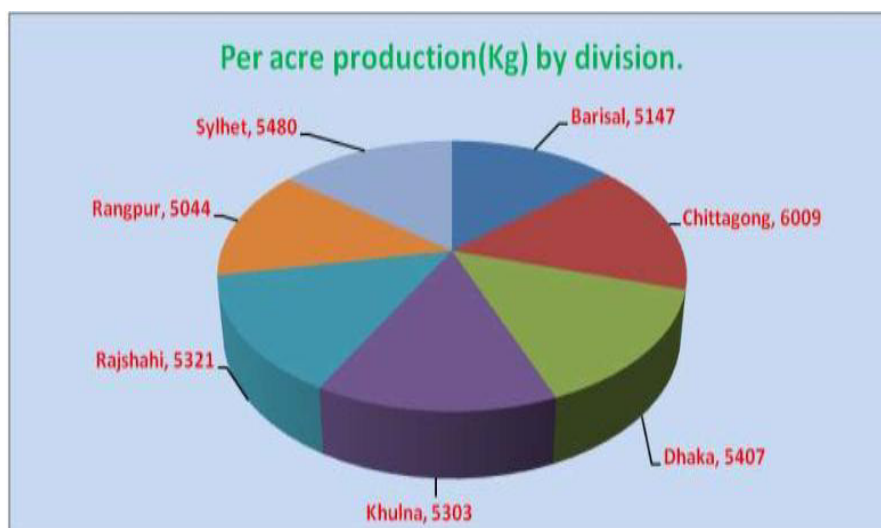


Figure 2.3: Production rate of pumpkin by divisions of Bangladesh

Table 2.3: Percentage distribution of areas under different varieties of Pumkin

Divisions	Varieties of pumpkin							
	Total		Local		Hybrid		Others	
	Area	%	Area	%	Area	%	Area	%
Bangladesh	120993	100	63598	52.56	56852	46.99	543	0.45
Barishal	10686	8.83	6145	5.08	4507	3.73	33	0.03
Chittagong	29885	24.70	18462	15.26	11343	9.37	81	0.07
Dhaka	24651	20.37	16671	13.78	7897	6.53	83	0.07
Khulna	27262	22.53	11575	9.57	15592	12.89	95	0.08
Rajshahi	12141	10.03	5664	4.68	6264	5.18	213	0.18
Rangpur	11493	9.50	3221	2.66	8254	6.82	18	0.01
Shylhet	4875	4.03	1860	1.54	2995	2.48	20	0.02

2.5 Composition of pumpkin

Pumpkin is one of the important vegetable with high nutritive value. It is a low-calorie vegetable with a rich source of carotene, vitamin, minerals, pectin and dietary fiber (Djutin, 1991). The vegetable contains vitamin A, B, C and E, flavonoid, poly-phenolic antioxidants such as lutein, xanthin etc. (Matus *et al.*, 1993).

Table 2.4: Chemical composition of pumpkin

Constituents	Quantity
Water(g/100g)	89
Protein (g/100g)	4.0
Fat(g/100g)	0.2
Carbohydra(g/100g)te	2.0
Fiber(g/100g)	2.4
Ca(mg/100gm)	475
P(mg/100gm)	175
Fe(g/100g)	0.8
β -carotene(mg/100gm)	1
Thiamine(mg/100gm)	0.08
Riboflavin(mg/100gm)	0.06
Niacin(mg/100gm)	0.03
Ascorbic acid(mg/100gm)	80
Al (mg/g dry weight)	9.21
Co (mg/g dry weight)	0.29
Cr (mg/g dry weight)	2.84
Cu(mg/g dry weight)	15.4
K (mg/g dry weight)	5.70
Mg (mg/g dry weight)	5.60
Na(mg/g dry weight)	6.90
Zn(mg/g dry weight)	113

Source: (<https://www.ars.usda> and de Escalada Pla *et al.*, 2007)

The major B complex group of vitamins present in pumpkin are folate, niacin, thiamine, pantothenic acid and vitamin B₆ (pyridoxine) (Foster and Flood, 1995). Further, the vegetable has been considered as beneficial to health because it contains various biologically active components such as polysaccharides, para-aminobenzoic acid, fixed oils, sterols, proteins and peptides (Buchbauer *et al.*, 1998; Murkovic *et al.*, 2002; Caili *et al.*, 2006). The vegetable is a good source of carotenoids and γ -amino butyric acid (Matus *et al.*, 1993; Murkovic *et al.*, 2002). Further, the seeds are also valued for their high protein content and essential fatty acids, like linoleic acid (Glew *et al.*, 2006). These seeds also contain large amounts of various essential micro-elements such as K, Crand Na along with Mg, Zn, Cu, Mo and Se etc. (de Escalada Pla *et al.*, 2007). Pumpkin also contains some bioactive compounds such as D-Chiro-inositol an antidiabetic which increase the insulin secretion and increase β -cell mass (Xia and Wang, 2007). The phenolic phytochemicals are also antidiabetic in function which helps in α -Glycosidase inhibition (Kwon *et al.*, 2007). Pumpkin polysaccharides are antioxidants that increase

serum SOD and GSH-PX and reduce malondialdehyde (Xu, 2000). Moschatin and cucurmosin are carcinogenic in function and helps in inhibition of cell tumor cell growth and works like ribosome inactivating protein (Xia *et al.*, 2003; Hou *et al.*, 2008).

2.6 Utilization of Pumpkin

2.6.1 General utilization of pumpkin

Pumpkin is widely cultivated in temperate and subtropical zones of the world for an edible and therapeutic purpose (Al-Rowais, 2002). It has been traditionally used as a vegetable as well as medicine in many countries, such as Mexico, India, China, Brazil, America, Argentina and Yugoslavia (Adolfo and Michael, 2005). In Mexico, *Cucurbita ficifolia* is consumed widely and several dishes and candies are prepared with the seeds or fruit (Andrade-Cetto and Heinrich, 2005). *Cucurbita moschata* is used for developing salty or sweet food products (de Escalada Pla *et al.*, 2007). Seeds of pumpkin are high in protein, oil and mineral content and are eaten raw or pressed to make oil (Hopkins *et al.*, 1996). Different products like jams, sweet, marmalade, beverage, baby food, ice-cream and chocolates are prepared from the pumpkin (Pongjanta *et al.*, 2006). It is processed in different ways for human consumption, such as fried, frozen, dried, candies or pickled. Pumpkin can be processed into flour, which has a larger shelf life. Further, pumpkin flour is used in preparation of different products because of its highly desirable flavour, sweetness and deep yellow orange colour. It has been reported to be used to supplement cereal flours in bakery products, preparing soups, sauces, instant noodle and spice as well as a natural colouring agent in pasta and flour mixes (Pongjanta *et al.*, 2006). Pumpkin powder can be used as the concentrated source of β -carotene in bakery and confectionery products (Sudipta and Soumitra, 2015).

2.6.2 Weaning Properties of Pumpkin

Pumpkin is second only to apple as the first fruit introduced into children's diets in Australia (Koh *et al.* 2014). Ezeji and Ojimekwe (1993) created a weaning mix using fluted pumpkin seed flour with Bambara ground nuts and fermented millet seed. Fernandez *et al.* (1998) developed a drink from whole dried milk and dried pumpkin flakes that would provide a 6-12 month old child drinking 400 ml/day with 100 percent vitamin A and protein RDA. Hashim and Pongjanta (2000) and Jirapa *et al.* (2001) have a more complex mix of cowpea flour, rice flour, banana-pumpkin, skim milk powder and

sugar in a 35:35:15:15:5 ratio. Freeze drying retained more nutrients than oven drying and 100g of freeze-dried weaning food provided an adequate amount of the recommended daily allowance (RDA) of vitamin A for infants. Usha *et al.* (2010) started with a mix of sorghum, green gram and rice in a 2:1:1 ratio. Pumpkin powder added at 20 percent significantly increased protein, fibre, carbohydrate, and antioxidant levels, while maintaining good sensory qualities. Ward and Ainsworth (1998) added pumpkin seed oil to a traditional Kenyan porridge to bring the energy and protein content to approved levels.

2.6.3 Medicinal Properties of Pumpkin

Most papers on the medicinal properties of pumpkin are based on the use of the oil extracted from the seeds.

Cancer

Aghaie *et al.* (2015) looked at mitigating the reproductive toxicity of cyclophosphamide used to treat cancer. Sharma *et al.* (2015) suggested a preventative effect of cucurbit on bowel cancer, but Tarrazo-Antelo *et al.* (2014), “observed a reduced risk for broccoli and pumpkin intake. Although fruit consumption does not seem to be associated with a lower lung cancer risk, only the frequent consumption of specific green leafy vegetables and other vegetables might be associated with a reduced risk of lung cancer.”

Diabetes

Beneficial action of *Cucurbita ficifolia* and probiotic yogurt, a traditional treatment in Iran, is described by Bayat *et al.* (2016). Boaduo *et al.* (2014) looked at the use of *Cucurbita pepo* (summer squash), a traditional treatment in South Africa and an unspecified *Cucurbita* on rats with diabetes in a study by Makni *et al.* (2011). Teugwa *et al.* (2013) concluded that “these findings showed that the selected *Cucurbitaceae* seeds *Cucurbita moschata* contained globulins with significant anti-hyper glycaemic activity. It is therefore highly encouraged to pursue investigations towards development of peptide-drugs and/or phyto medicines from these bioactive proteins which could be used as affordable alternative therapy against Diabetic Mellitus.”

Tapeworm

Li *et al.* (2012) showed pumpkin seed oil combined with areca nut expelled tape worms in nearly 90 percent of cases. However, nearly 50 percent of patients suffered mild reaction to the treatment such as dizziness and nausea.

Depression

Eagles (1990) and Axford *et al.* (1991) wrote on the mental health benefits of pumpkin seed; Kim *et al.* (2016) wrote on β -carotene benefits in Sweetme Sweet Pumpkin™ (baked *Cucurbita moschata*), which is used to treat patients with depression in Korea

Anti-medicinal Properties of Pumpkin

There are a number of papers on allergies with pumpkins, but these primarily concern chemicals in the skin of the fruit rather than consumption of the flesh. Arochena *et al.* (2012) wrote about supermarket workers affected by handling the fruit; La Shell *et al.* (2010) wrote about adolescents and Potter and Hashimoto (1994) wrote about dermatitis on adults.

2.7 Some Other Researches on Pumpkin

Karanja *et al.* (2013) conducted a study on the nutritional composition of the pumpkin (*Cucurbita* spp.) seed; The Objective of this work was to evaluate the nutritional quality of pumpkin seeds. Proximate composition was determined in accordance with AOAC methods, mineral composition by Atomic Absorption Spectrophotometry, tocopherol and fatty acid profile was analyzed using HPLC and GC, respectively. Data analysis was done by Genstat package. Significant differences ($p < 0.05$) were observed among the groups representing crude fibre (11.69-24.85%), crude fat (31.9 -41.37%), crude protein (14.05-33.29%) and carbohydrates (8.66-27.35%). Fatty acid profile showed a high content of unsaturated fatty acids and the dominant fatty acids were palmitic (1.16-20.81%), stearic (0.16-5.56%), oleic (15.56-30.79%), and linoleic acids (26.18-81.21%). The highest elemental minerals were potassium and sodium (124-335 and 70-148 mg/100 g) respectively. α -tocopherol content ranged between 8.33 and 122.65 $\mu\text{g/g}$ exhibiting significant differences ($p < 0.05$) among group 7 and the rest of the groups. The seeds were well endowed in crude oil, protein, carbohydrates and crude fibre. The oil contained unsaturated fatty acids and α -tocopherol. The pumpkin seed could be

incorporated in foods to increase the nutritional value especially in diets that are deficient in the said nutrients.

Elinge *et al.* (2012) performed a research on Proximate, Mineral and Anti-nutrient Composition of Pumpkin (*Cucurbita pepo* L) Seeds Extract. Pumpkin seeds were analysed for their nutritional and anti-nutritional composition, the results obtained were; moisture content (5.00%), ash (5.50%), crude lipid (38.00%), crude fibre (1.00%), crude protein (27.48%), Available carbohydrate (28.03%) and calorific value (564kcal/100g). Elemental analysis shows that potassium is the most abundant element in the sample (273mg/100g) and manganese is least (0.06mg/100g). The anti -nutritional parameters analysed are; phytate (35.06 mg/100 g), oxalate (0.02±0.10 mg/100 g), hydrocyanic acid content (0.22±0.04 mg/100 g) and nitrate (2.27±0.02 mg/100 g). The result shows that the pumpkin seeds if properly utilized can serve as good source of minerals.

Kwiri *et al.* (2014) conducted a comparative study on the proximate composition of pumpkin gourd (*Cucurbita pepo*) seeds from Zimbabwe; the proximate analysis including major nutrients and minerals were determined. The *C. pepo* seed had a moisture content of 5.662 ± 0.016 g/kg. Significantly, *Curcubita pepo* had high amounts of crude oil and proteins as compared to other edible oil rich seeds. The crude oil content and protein were 43.460 ± 0.098 g/kg and 32.860 ± 0.103 g/kg, respectively. Other components such as carbohydrates, crude fibre and energy were 12.160 ± 0.142 g/kg, 2.578 ± 0.007 g/kg and 562.82 ± 0.132 g/kg correspondingly. Ash content was 3.324 ± 0.010 g/kg which was further analyzed into various major minerals giving analyzed means as Na (67.956 ± 0.037 g/kg), Zn (1.244 ± 0.010 g/kg), P (1040.8 ± 0.663 g/kg), Fe (11.980 ± 0.086 g/kg), Ca (141 ± 0.316 g/kg) and Mg (344.6 ± 0.245 g/kg). The findings indicated that *C. pepo* seeds are a good alternative source of highly nutritious food for instance proteins and lipids as well as minerals (Mg, Ca, Zn, P and Fe) that could greatly contribute to human nutritional requirements.

Ovca *et al.* (2011) conducted an study on Speciation of zinc in pumpkin seeds (*Cucurbita pepo*) and degradation of its species in the human digestive tract; Pumpkin seeds are one of the foodstuffs recommended in diets which do not contain other Zinc-rich sources. The main objectives of this work were to get information on Zinc and its species in pumpkin seeds, and their possible degradation in the human gastrointestinal tract, indicative of Zinc bio accessibility. A sequential analytical approach was applied,

focusing on total Zinc, spatial Zinc distribution, extractability, speciation and bio accessibility of Zinc and its species. It was shown that water extracts of pumpkin seeds exhibit a specific Zinc species fingerprint with ca. 30% of a low-Molecular weight fraction 0.5–2 kDa (kilodalton) and ca. 60% of an intermediate/high- Molecular weight fraction 10–20 kDa (kilodalton). Digestion of Zinc species under simulated stomach conditions proved that Zn species identified in plant extracts were completely decomposed to Zn²⁺. The subsequent digestion under intestinal conditions showed that Zn becomes less accessible, indicating that antinutrients like naturally present phytate may be responsible for complex formation in the small intes-tines, thus reducing the potential for Zn bioavailability.

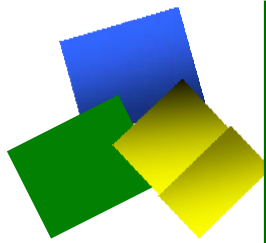
Kuchtová *et al.* (2016) on their Chemical composition and functional properties of pumpkin pomace-incorporated crackers; Pumpkin pomace obtained from cultivar (*Cucurbita moschata* Duch) was analyzed for their chemical composition and functional properties. Pumpkin pomace powder contained more than 50 mass% of total dietary fibre and showed high hydration properties such as water holding capacity (5.70 g/g) and swelling capacity (10.26 cm³/g). The effect of pumpkin pomace incorporation to wheat dough by replacement of wheat flour with pumpkin pomace (5 or 7.5%) on physical properties (volume, volume index, width, thickness, spread ratio) and sensory parameters (appearance, hardness, taste, odor, overall acceptability) of cracker were evaluated. The results indicated that the addition of higher amount (7.5% mass) of pumpkin pomace powder negatively affected the volume, volume index, spread ratio and reduced their overall acceptance. Pumpkin pomace is a good source of ash and dietary fibres and may be incorporated into baked goods as a functional ingredient.

Saeleaw and Schleining (2011) on their study upon Composition, Physicochemical and Morphological Characterization of Pumpkin Flour; Pumpkin flour exhibited high levels of carbohydrate (79.57 %), starch (48.30 %), dietary fiber (12.1%) protein (7.81%) and total ash (5.29 %); low contents of lipid (3.60%) and crude fiber (3.65 %). Vitamin A was 48.30 µg/100g. Pumpkin flour peak gelatinization temperature was 75.3°C, the water solubility index (WSI) was 27.58% and WAI (Water Absorption Index) was 491.75%. The pasting properties were: temperature: 45.5°C; peak viscosity: 87.55 RVU (Rapid Visco Units); breakdown: 3.22 RVU (Rapid Visco Units); setback: 56.58 RVU (Rapid Visco Units) and trough: 84.33 RVU (Rapid Visco Units). The morphology of the starch granules, observed by scanning electron microscopy, had less smooth granule

surfaces and appeared as a mixture of spherical, polyhedral and irregular shaped with sizes ranging from 5 to 15 μm .

Irwandi *et al.* (2012) investigated the Scheme of obtaining β -carotene standard from pumpkin (*Cucurbita moschata*) flesh; This study demonstrated that the purity of β -carotene standard; determined by HPLC was ranged from 92.21 to 97.95%. The standard curves with five different concentrations of β -carotene extract from pumpkins in triplicate were constructed by plotting the peak area against the concentration. The coefficient of correlation was 0.9936. Therefore, this study established that pumpkin can be a reliable source of beta-carotene standard as it is cheap and commonly available throughout the year.

Kiharason *et al.*, (2017) on effect of drying method on nutrient integrity of selected components of pumpkin (*Cucurbita moschata* Duch.) fruit flour; Oven drying took shortest time of 7.25 hours to attain 15.15% final moisture content (MC), while OSD took 9.5 hours to attain 14.91% MC, but these MC were above safe levels. Enhanced solar drying achieved safest 12.82% MC, but in a longer time of 13.2 hours. A significant ($P < 0.05$) difference resulted in β -carotene, protein and zinc contents of the four flours. There was consistent increase of β -carotene and protein contents in dried flour compared to fresh fruit, while minerals and energy slightly reduced ($P > 0.05$). Oven dried flour had 74.84 μg , while fresh fruit had 16.6 μg β -carotene. Protein ranged from 13.8% to 16.5% in dry flours compared to 2.6% in fresh fruit. Zinc, iron, calcium and energy decreased in dry flours compared to fresh fruit, and ranged from: 9 to 44 ppm zinc, 49.5 to 94.5 ppm iron, 525 to 1,116.82 ppm calcium, and 3.6 to 4.2 kcal/g energy.



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This chapter deals with the materials and methods used to carry out the present research work. The materials and methods used to determine the Proximate Composition, Beta carotene and mineral content of different varieties of pumpkins grown in northern region of Bangladesh.

3.1 Experimental Site

The experiment was conducted in the Food and Process Engineering Lab-1 and Laboratory of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.2 Sample Collection

Fresh and mature pumpkin of different varieties *i.e.* *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita pepo* were collected from Thakurgaon district during the harvesting season (August-September 2018). These pumpkins were stored at room temperature before analysis.



a) *Cucurbitace Maxima*

b) *Cucurbitace Pepo*

c) *Cucurbitace Moschata*

Figure 3.1: Freshly collected Pumpkins

3.3 Proximate analysis

The proximate chemical composition represents the gross content of important chemical constituents such as moisture, fat, crude fiber, ash, protein and total carbohydrate. The study of proximate composition serves as an important base to study the nutritive quality of pumpkin.



a) *Cucurbitace Moschata* b) *Cucurbitace Pepo* c) *Cucurbitace Maxima*

Figure 3.2: Sample after cutting

3.3.1 Determination of Moisture Content

The moisture content of the samples were determined in accordance to moisture measurement by AOAC (1984) method.

Procedure:

About 5g sample of each variety was taken in a previously dried crucible. Then the crucible was dried in a oven at 100-105⁰C for 24 hrs or more till constant weight. After drying the crucible was removed from the oven and cooled in desiccator. The crucibles were removed from the desiccator and weighed soon after reaching room temperature.

$$\% \text{ Moisture} = \frac{\text{Loss of weight}}{\text{Weight of sample}} \times 100$$

3.3.2 Determination of Ash Content:

Ash content of food stuff represents inorganic residues remaining after destruction of organic matter. The ash was determined by the method of AOAC (1975) with some modification. Muffle furnace was used to determination of the ash content.

Procedure:

At first 5g moisture free sample was weighed and transferred into a clean, dry and pre-weighed crucible. Then the crucible with sample placed in a muffle furnace and dried the sample at 550 °C for 5-6 hours. When the time was accomplished, the sample with crucible cooled in desiccator for 15 minutes and again weighed. By using the weight of sample ash content was calculated.

The ash content was calculated by the following formula:

$$\% \text{ Ash} = \frac{W_1 - W_2}{W} \times 100$$

Here,

W= Weight of sample

W₁ = Weight of ash with crucible

W₂ = Weight of empty crucible

3.3.3 Determination of Crude Fiber

The method is based on the procedure developed by *Hennaberg, Stohman and Rautenberg in the agricultural experiment station at Weendebei Gottinger in Germany in 1864*. Only slight modifications have been made in the Weende method since its development.

Reagents:

- a) 1.25% sulfuric acid solution: 12.5ml conc. sulfuric was added in about 200ml distilled water and the volume was made up to the 1000ml.
- b) 1.25% sodium hydroxide solution: 12.5 g sodium hydroxide was dissolved in about 200ml distilled water and the volume was made up to the 1000ml.

Procedure:

About 20g moisture and fat free sample was taken in a 500ml beaker, about 200ml 1.25% sulfuric acid was added in the beaker and boiled for 30 min. Keeping the volume constant by addition of distilled water at the frequent intervals. Then the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. Then the materials was transferred in to the same beaker and about 200ml 1.25% NaOH solution was added in the beaker and boiled for 30 min. (Keeping the volume constant as before). Then the mixture was filtered through same cloth. The residue was washed with hot water till free from alkali. Then the residue was transferred in a dried preweighed crucible and dried about 24 hrs at 100-105⁰C in an oven. Then the crucible was cooled in a desiccator and weighed. Finally then the crucible was heated in a muffle

furnace at 600⁰C for 3-5 hrs, cooled and weighed again until two consecutive weight was same.

Calculation:

$$\% \text{ Crude fiber} = \frac{\text{Dry Residue Wt. (g)} \times \text{Ignited Residue Wt. (g)} \times \text{Blank Wt Loss(g)} \times 100}{\text{Weight of sample (g)} \times \text{Sample Moisture (\%)}} \times 100$$

3.3.4 Estimation of Protein from Pumpkin Sample by Modified Kjeldahl Method

Principle:

The modified kjeldahl method is used to determine the total nitrogen consisting of organic and ammonium forms. It is a wet oxidation procedure, where complex form of nitrogen in plant protein is converted to nitrogen. Three steps are involved in this method.

1. Digestion.
2. Distillation.
3. Titration.

Apparatus required.

1. Kjeldahl flask'
2. Kjeldahl digestion stand.
3. Conical flask.
4. Volumetric flask
5. Measuring cylinder
6. Burette
7. Distillation apparatus
8. Electrical Balance.

Chemical required:

1. Sulphuric acid
2. Potassium sulphate
3. Copper sulphate
4. Selenium powder.
5. Sodium hydroxide
6. Boric acid.

Preparation of different reagents:

- 1) 4% Boric acid solution: 20g Boric acid (H_3BO_3) was taken in a 250ml beaker about 150ml distilled water was added to it and heated until the solution became clear and cooled. Then the solution was transferred in a 500ml volumetric flask and volume made up to the mark with distilled water.
- 2) Mixed indicator: 0.5g bromocresol green and 0.1g methyl red was taken in a 150ml beaker 30-40ml ethanol was added to it and was dissolved. Then the solution was transferred in a 100ml volumetric flask volume was made up to the mark with ethanol.
- 3) 40% sodium hydroxide (NaOH) solution: 400g NaOH plate was taken in a 500ml beaker, about 300ml distilled water was added to it and stirred with a glass rod until dissolved the NaOH and cooled the solution. Then the solution was transferred in one litre volumetric flask and volume was made up to the mark with distilled water.
- 4) Standard (0.2N) sulphuric acid solution: About 200ml distilled water was taken in a 1000ml volumetric flask, 5.6ml conc. sulphuric acid was added to it and volume made up to the mark with distilled water.

Procedure:

- A) Digestion** 1g previously oven dried sample was taken in a digestion flask. 10g potassium sulphate (K_2SO_4), 0.1g copper sulphate (CuSO_4), 1g selenium powder and 25ml conc. H_2SO_4 was added to it and heated until the solution became clear. Then the flask was cooled.
- B) Distillation** After digestion about 300ml distilled water and 125ml 40% NaOH solution was added to it. Then the flask was attached quickly to the distillation set and heated the flask continuously. In the meantime a 250ml conical flask (containing 25ml of 4% boric acid and 4-5 drops of mixed indicator) was placed at the jet of the condenser. About 150ml distillate was collected and was titrated with 0.2 N H_2SO_4 solution.

Calculation:

$$\% \text{ Nitrogen} = \frac{\text{Titrate value} \times N \times 0.014 \times 100}{\text{Weight of sample}} \%$$

$$\text{Protein} = \% \text{ Nitrogen} \times 6.25$$

Here,

$N = \text{Normality of the } H_2SO_4$

3.3.5 Determination of Crude Fat content

The most popular extraction procedure is that of (Folch *et al.*, 1957).

Folch method

1. The tissue is homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (1 g in 20 ml of solvent mixture). After dispersion, the whole mixture is agitated during 15-20 min in an orbital shaker at room temperature.
2. The homogenate is either filtrated (funnel with a folded filter paper) or centrifuged to recover the liquid phase.
3. The solvent is washed with 0.2 volume (4ml for 20ml) of water or better 0.9% NaCl solution. After vortexing some seconds, the mixture is centrifuged at low speed (2000rpm) to separate the two phases. Remove the upper phase by siphoning and kept it to analyze gangliosides or small organic polar molecules. If necessary (need of removing labelled molecules), rinse the interface one or two times with methanol/water (1/1) without mixing the whole preparation.
4. After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids is evaporated under vacuum in a rotary evaporator or under a nitrogen stream if the volume is under 2-3ml.

3.3.6 Carbohydrate content:

Carbohydrate was calculated based on difference.

$\text{Carbohydrate} = 100 - [\text{crude protein } (\%) + \text{crude fat } (\%) + \text{crude fiber } (\%) + \text{ash } (\%)]$

But in case of available carbohydrate the calculation was quite different and given as follows:

$\text{Available carbohydrate} = 100 - (\text{moisture} + \text{protein} + \text{ash} + \text{fat} + \text{dietary fiber})$

3.4 Energy calculation:

Standard procedures were used for the calculation of protein energy, lipid (fat) energy and carbohydrate energy. The Atwater factors 4, 4 and 9 for protein, carbohydrate and fat, respectively expressed in kilocalories were used. The values for carbohydrate, protein and fat obtained in proximate analysis were multiplied by their respectively Atwater factor to give their corresponding energy values in kcal.

The energy was calculated by using this calculation:

$$\text{Energy} = \text{Total carbohydrate} \times 4 + \text{protein} \times 4 + \text{fat} \times 9$$

But in case of high fiber food energy is calculated as follows:

$$\text{Energy} = (\text{Total carbohydrate} - \text{dietary fiber}) \times 4 + \text{protein} \times 4 + \text{fat} \times 9$$

3.5 Determination of Sugar by Lane and Eynon Method

Chemicals Required:-

1. Fehling's Solution 1:

Fehling's Solution 1=Copper Sulphate 34.64g dissolved in 500 ml distilled water.

Fehling's Solution 2= Rochelle salt (Sodium potassium tartrate) 173g and NaOH 125g dissolve in 500ml distilled water.

2. Fehling's Solution 2: A mixture of equal volume of Fehling's solution 1 and Fehling's solution 2.

2. Methyl blue indicator.

3. 4.5% Neutral lead acetate solution.

4. 2.2% potassium oxalate solution.

Preparation of Standard Glucose Solution: 10 g previous dried glucose was taken in a liter volumetric flask about 200ml distilled water was added to it and shaken thoroughly. Then volume made up to the mark with distilled water. Concentration of this solution is 10mg/ml.

Standardization of Fehling's Solution with Standard Reducing (Glucose) Sugar:

10ml of Fehling's solution and 50ml of distilled water was taken in a 250ml conical flask, heated and titrated with standard glucose solution. At the end point brick like red ppt. was appeared.

$$\text{Fehling's Factor} = \frac{\text{Titrate Value} \times 10}{1000}$$

Preparation of Standard Invert Sugar Solution:

10g sucrose was taken in a 250ml beaker 5ml conc. HCl was added to it. Then it was transferred in one litre volumetric flask and was volume up to the mark with distilled water.

Then 250ml of this solution was taken in a 250ml beaker and heated for about 5 min. Then cooled and neutralized with 1N NaOH solution. Then 5ml lead acetate was added to it, waited for 10min. Then 5ml potassium oxalate solution was added to it, filtered and volume was made 1000 ml with distilled water. Concentration of this solution was 2.5mg invert sugar/ml.

Standardization of Fehling's Solution with Standard Invert Sugar:

10 ml of Fehling's solution and 50ml of distilled water was taken in a 250ml conical flask, heated and titrated with standard glucose solution. At the end point brick like red precipitated was appeared.

$$\text{Fehling's Factor} = \frac{\text{Titrate Value} \times 2.5}{1000}$$

Preparation of Sample: 5-10g sample was taken in a 250ml beaker about 100ml distilled water was added to it and boiled for 10 minutes, cooled and filtered and transferred in a 250ml volumetric flask. Then 5ml lead acetate solution and 5ml potassium oxalate solution was added to it and made volume up to the mark with distilled water.

Titration of Reducing Sugar: 10ml of Fehling's solution was taken in a 250ml conical flask .50ml distilled water was added to it and heated to boiling point and titrated with plant extract sugar solution. At the end point brick like red precipitated will be appear.

$$\% \text{Reducing Sugar} = \frac{\text{Fehling's factor} \times \text{dilution made up} \times 100}{\text{Titrate value} \times \text{wt. of sample} \times 1000}$$

Preparation of Sample for Titration of Total Invert Sugar: 50ml of previously prepared sample was taken in a 250ml Beaker 5g of citric acid and 50ml of distilled water was added to it and boiled for 10 minutes then cooled and neutralized by 1N NaOH solution then the solution transferred in a 250ml volumetric flask and volume made up to the mark with distilled water.

Titration: 10ml of Fehling's solution was taken in a 250ml conical flask 50ml distilled water was added to it and heated at the boiling point and titrated with unknown sugar solution.

$$\% \text{ Total Invert Sugar} = \frac{\text{Fehling's factor} \times \text{dilution made up} \times 100}{\text{Titrate value} \times \text{wt. of sample} \times 1000}$$

$$\% \text{Non - reducing Sugar} = \% \text{Total Invert sugar} - \% \text{Total reducing sugar.}$$

$$\% \text{Total Sugar} = \% \text{Reducing sugar} + \% \text{Non - Reducing sugar}$$

3.6 Estimation of Vitamin 'C'

L-Ascorbic acid was determined following the method of Ranganna (2007).

Chemical Required:

1. Metaphosphoric acid solution: 30g Metaphosphoric acid dissolved in 80ml glacial acetic then this solution transferred in a 1000ml volumetric flask volume up to the mark with distilled water.
2. Dye solution: 260mg 2, 6 di-chloro phenol Indophenol and 210mg NaHCO₃ dissolved in 1000ml distilled water.
3. Standard Vitamin C solution: 100mg Vitamin C (L-ascorbic acid) dissolved in 1000ml meta phosphoric acid solution.

Standardization of dye solution:

5ml of standard vitamin C solution was taken in a 100 conical flask and titrated with dye solution from Burette.

$$\text{Dye factor} : \frac{0.5}{\text{Titrate value}}$$

Preparation of Sample: 10g of fruit sample was blended with about 50ml metaphosphoric solution then it was filtered with white thin cloth and transferred in a 100ml volumetric flask volume up to the mark with metaphosphoric acid solution .

Titration with Unknown Solution: 10ml of fruit sample was taken in a conical flask and titrated with dye solution from Burette.



Figure 3.4: Homogenizer

3.7 Determination of Beta Carotene

Procedure 1

Chemicals:

1. Acetone.
2. n-Hexane

Preparation of solvent:

Acetone : n-Hexane (4:6).

Procedure: One gram of each sample in triplicate were taken in a test tube with stopper about 15ml solvent was added to it. Then it was heated for 15 minutes, filtered and absorbance was measured at 663 nm, 505 nm and 453 nm respectively. Beta carotene content was estimated in mg/100g by using the following equation (Barros *et al.*, 2007).

Calculation:

$$\beta - \text{Carotene} = \frac{0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453} \text{ (mg/g)}}{(\text{mg/100ml if the sample is liquid}) \times (\text{mg/100gm})}$$

3.8 Estimation of minerals

The presence of minerals (such as Zn, Ca) indicates nutritive quality of pumpkin fruit. The study of minerals serves as an important base to study the nutritive quality of pumpkin.

3.8.1 Estimation of Calcium (Ca) from Pumpkin Fruit Complexometric Method

Estimation of Calcium (Ca) from Pumpkin Fruit Complexometric Method of Titration Using Na₂-EDTA as a Complexing Agent (Page *et al.*, 1982)

Apparatus required

1. Burette with stand,
2. Pipette,
3. Conical flask or Erlenmeyer flask,
4. Volumetric flask,
5. Measuring cylinder,
6. Beaker with watch glass,
7. Electrical balance and
8. Glass rod etc.

Chemicals required

1. Disodium EDTA
2. Sodium Hydroxide (NaOH 10%)
3. Calcon Indicator
4. Methanol
5. Masking agent:
 - a) Hydroxylamine hydrogen chloride
 - b) Potassium ferrocyanide
 - c) Triethanolamine (TEA)

Preparation of Different Reagents or Chemicals

1. Preparation of Na₂-EDTA solution (0.02 M): 7.44g of disodium salt of EDTA (Na₂-EDTA) was exactly dissolved in a liter volumetric flask containing about 400ml distilled water. Shaken the flask thoroughly until Na₂ EDTA completely dissolved. The volume made up to the mark with distilled water. This solution gave 0.02M Na₂-EDTA solution.

2. Preparation of calcon indicator solution: 400mg calcon indicator powder of AR grade was taken in a 100ml volumetric flask containing about 30ml methanol or ethanol. Shaken the flask thoroughly and made the volume up to the mark with methanol or ethanol.

3. Preparation of NaOH Solution (10%): Dissolve 50g NaOH AR grade in a 500ml volumetric flask containing approximately 200ml distilled water. Shaken the flask thoroughly to mix NaOH with water. Make the volume made up to the mark with distilled water.

Procedure:

1. 5ml of plant extract solution was taken in a 250ml conical flask.
2. 20ml distilled water was added into the conical flask and shaken thoroughly.
3. 10 drops of each masking agent of a) Hydroxylamine hydrogen chloride b) Potassium ferrocyanide c) Triethanolamine(TEA) was added
4. 5ml NaOH buffer solution and shake thoroughly was added
5. 5-6 drops calcon indicator was added into the conical flask (depending on the concentration of the indicator solution) and shaken the flask thoroughly.
6. Then titrated against 0.01M Na₂-EDTA solution from burette.
7. Continue the titration until pink color of the solution completely turned to pure blue color.
8. Repeated the experiment at least 3 times.
9. Conducted a blank experiment also by taking all the reagents as above except calcium stock solution. Taken 5ml more distilled water into conical flask instead of calcium solution.
10. Tabulated the data and calculate the amount of calcium present in the prepared solution.

Formula of Calculation:

$$1\text{ml } 1\text{M EDTA} \equiv 1\text{ml } 1\text{M Ca} = 40.08\text{mg Ca}$$

3.8.2 Complexometric Titration of Zinc

Chemicals and Setup: Zn powder, EDTA solution, Erio T indicator, $\text{NH}_3/\text{NH}_4\text{Cl}$ buffer (pH 10), unknown Zn^{2+} solution. 250 mL volumetric flask, 50 mL buret, 25 mL volumetric pipet, three clean 125 mL (or 250 mL) Erlenmeyer flasks, 50 mL beaker, polyethylene dropping bottle, buret stand.

Procedure

This should be done One Lab Period in Advance:

1. Accurately weighed between 0.25 and 0.40 g of pure Zinc and dissolve it in about 5mL of 6M HCl in a 50ml beaker covered with a watch glass. Label and store in your locker.
2. Transferred the Zn solution (prepared before) quantitatively to a 250 ml volumetric flask. Dilute to the mark and mix well.
3. Prepared a comparison solution containing 25ml distilled water, 25ml $\text{NH}_4\text{Cl}/\text{NH}_3$ buffer solution, 1ml of EDTA solution, and one drop of Erio T indicator in a 250ml Erlenmeyer flask. All end-point determinations will be made in comparison to this solution.
 - a) Pipetted 10.00 mL of the standard Zn solution into a 250 mL Erlenmeyer flask, added 15 mL of water, 25 ml of buffer and 1 drop of Erio T indicator. Titrate with EDTA until the red color was completely gone and the solution was clear blue, identical (in hue but not necessarily in intensity) to the comparison solution color.
 - b) Repeated this titration two additional times.
 - c) Obtained a sample of unknown Zn solution from the stock room. Pipetted 10.00ml of that unknown solution, into a 250ml Erlenmeyer flask, then add 15ml of water and 25ml buffer, and one drop of Erio T indicator. Titrated with EDTA to the clear blue end-point. Three such titrations was done

3.9 Determination of pH

Digital pH (HANNA, pH-211) meter was used to determine the pH value of the sample by performing two point calibrations (with buffer 7.0 and buffer 4.0) before measuring the samples pH value.



Figure 3.5: pH meter

3.10 Sensory Evaluation of cooked pumpkin

A test panel evaluated the consumer's acceptability of three varieties of cooked pumpkin. The test panel (20) were selected from the students of faculty of food technology, Thakurgaon Polytechnic Institute, Thakurgaon. The panelist were requested to assign score for characteristics color, flavor, texture, taste and overall acceptability of the cooked pumpkin.

The scale was arranged such

- 9 = Like extremely
- 8 = Like very much
- 7 = Like moderately
- 6 = Like slightly
- 5 = Neither Like nor Dislike
- 4 = Dislike slightly
- 3 = Dislike moderately
- 2 = Dislike very much
- 1 = Dislike extremely

Table 3.1: Sesory Evaluation Score Card

Attributes	F1	F2	F3
Color			
Flavor			
Texture			
Taste			
Overall acceptance			

F1= *C. pepo*

F2=*C. maxima*

F3=*C. moschata*



Figure 3.6: Cooked Pumpkin



Figure 3.7: Dried Pumpkins

3.11 Statistical Analysis

Each experiment included three varieties and one way ANOVA was used. Data were analyzed using statistical software (SPSS-Version 22). A descriptive analysis was carried out. DMRT method was used for calculation. Differences were considered to be significant at $P < 0.05$.



CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Proximate Composition of Different Pumpkin Variety

4.1.1 Moisture Content

The moisture content of different pumpkin varieties differed significantly ($P \leq 0.05$) as shown in Table 4.1. Moisture content varies above 90% in all varieties. However, the highest and lowest moisture content was found in *C. pepo* (95.41%) and *C. maxima* (93.07%), respectively. The results are almost in agreement with the findings of Yadav *et al.* (2010) and Young *et al.*, (2004) 89% to 96%.

4.1.2 Ash Content

Table 4.1 represents the ash content of different pumpkin varieties. The results showed that ash content varied significantly ($p \leq 0.05$) were highest (0.757%) in *C. maxima* and lowest (0.366%) in *C. moschata*. Young *et al.*, (2004) found ash content in pumpkin 0.34 to 1.43%, respectively.

4.1.3 Protein Content

Protein in three varieties of pumpkin shown in Table 4.1 significant ($p \leq 0.05$) differences in protein among pumpkin varieties were found in this study. The highest value of protein was found in *C. maxima* (2.1%) and lowest value in *C. pepo* (1.53%). Hussain *et al.* (2017) has gotten protein content in pumpkin 3.043%.

4.1.4 Fat Content

The total fat ranged from 0.180 to 0.320% Table 4.1 which was found to be varied significantly ($p \leq 0.05$). According to Yadav *et al.* (2010) fat content in pumpkin 0.20%; usually fat content of different fruit is not greater than 1% (Norman, 1976) which supports this findings.

4.1.5 Carbohydrate Content

Generally, carbohydrate of vegetables is less concentrated than cereal because of their carbohydrate content. Vegetables rich in carbohydrate provides a high amount of energy.

From this study, the total carbohydrate content ranged from 2.3 to 4.60% (Table 4.1), which was found to be verified significantly ($p \leq 0.05$) among the varieties. Since carbohydrate is calculated by subtracting the percent moisture, ash, protein and fat from 100%, so its totally dependent on the other components content.

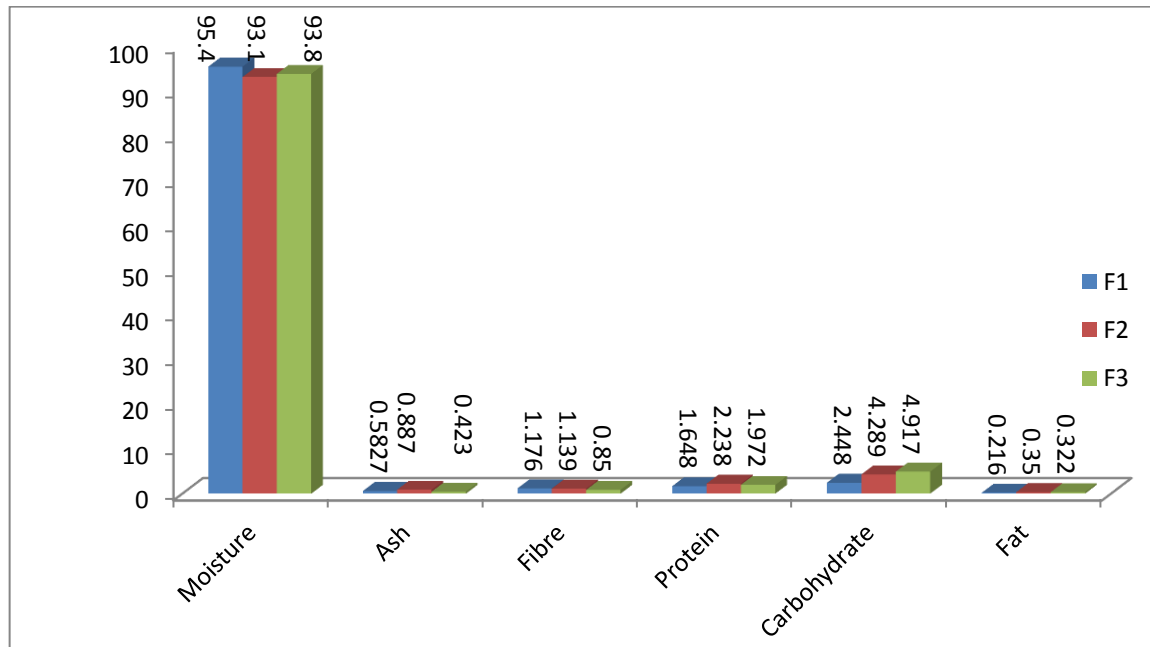


Figure 4.1: Graphical Representation of moisture, ash, protein, fat, fiber and carbohydrate after preparation

Here,

F1= *C. pepo*

F2=*C. maxima*

F3=*C. moschata*

4.1.6 Total Fiber Content

Total fiber content results obtained from different varieties are shown in Table 4.1. The total fiber content according to analysis ranged from 1 -1.160% which varied significantly ($p \leq 0.05$) among the varieties. Higher content found in *C. pepo*.

Table 4.1: Values of the components of three varieties

Components	<i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>
%Moisture	94.510±0.9086	92.240±0.8314	92.243±1.5822
%Ash	0.512±0.0707 ^a	0.757±0.1301 ^b	0.366±0.0751 ^a
%Fibre	1.063±0.1131	0.970±0.1697	0.659±0.1919
%Protein	1.453±0.1955	1.913±0.3252	1.680±0.2921
Vit-C mg/100g	50.700±6.4969 ^a	62.500±4.8125 ^a	87.833±4.3466 ^b
%Fat	0.180±0.0361 ^a	0.320±0.0300 ^b	0.291±0.0317 ^b
%Carbohydrate	2.332±0.1161 ^a	3.974±0.3056 ^b	4.604±0.3130 ^c
β-Carotene mg/100g	0.324±0.0259 ^a	0.455±0.0271 ^b	0.410±0.0153 ^b
Ca mg/100g	310.433±16.3102 ^c	161.233±12.0272 ^a	230.667±1.7502 ^b
Zn mg/100g	64.550±1.7755 ^a	78.380±0.8450 ^b	87.177±2.6866 ^c
Energy	18.827±4.4448 ^a	26.109±0.7102 ^{ab}	24.269±2.5294 ^b
pH	6.527±0.0058 ^b	6.343±0.0058 ^a	6.347±0.0058 ^a

4.1.7 Energy Content

According to the analysis we found that the highest value found in *C. maxima* (26.819Kcal) and lowest value in *C. pepo* (23.26Kcal) which varies significantly ($p \leq 0.05$) among the varieties. Hussain *et al.* (2017) found energy 28 Kcal, respectively.

4.2 Bio chemical properties of pumpkin

4.2.1 pH Content

Value of pH varied significantly among some variety of pumpkin which ranged from 6.34 - 6.52 in *C. maxima* and *C. pepo*, respectively.

4.3 Bioactive compounds in different pumpkin varieties

4.3.1 Vitamin C

Vitamin C or Ascorbic acid is an important anti-oxidant in food and it is the major vitamin present in citrus fruits. Ascorbic acid plays a major role as an antioxidant in the detoxification of hydrogen peroxide, super oxide (O_2^-) and hydroxile radicals (OH^-) that are generated from different reactive oxygen species in the plant tissue (Moldau, 1993). This study found that vitamin C in different pumpkins cultivar were varied significantly ($p \leq 0.05$) ranged from 57mg/100g in *C. pepo* and 93mg/100g in *C. moschata*. According to previous report Yadav *et al.* (2010) found value of Ascorbic acid is 80 mg/100g in *C. moschata*.

4.3.2 β - carotene Content

β -carotene is ubiquitously present in green leafy and yellow orange fruit and vegetables. β -carotene content of different variety was found in the range of 0.349-0.482 mg/100g (figure 4.2). The β -carotene content of fruits may be influenced by growing condition, maturity index, post-harvest handling conditions, as well as variety of cultivar (Mangels *et al.*, 1993).

4.4 Mineral Content

4.4.1 Calcium Content

Calcium is very important nutrient for the building of bone and teeth. From this study it was found that *C. maxima* contain significantly less Ca (173mg/100g) and *C. pepo* contain highest amount of Ca (326.74 mg/100g). Yadav *et al.* (2010) reported that value of Ca was 475mg/100g.

4.4.2 Zinc Content

Zinc is another important mineral for the human body. From the study we found the value ranged (66-89 mg/100g) for *C. pepo* and *C. moschata* respectively which varied significantly among different varieties. Research conducted by Yadav *et al.* (2010) they found value of Zinc was 113 mg/100g.

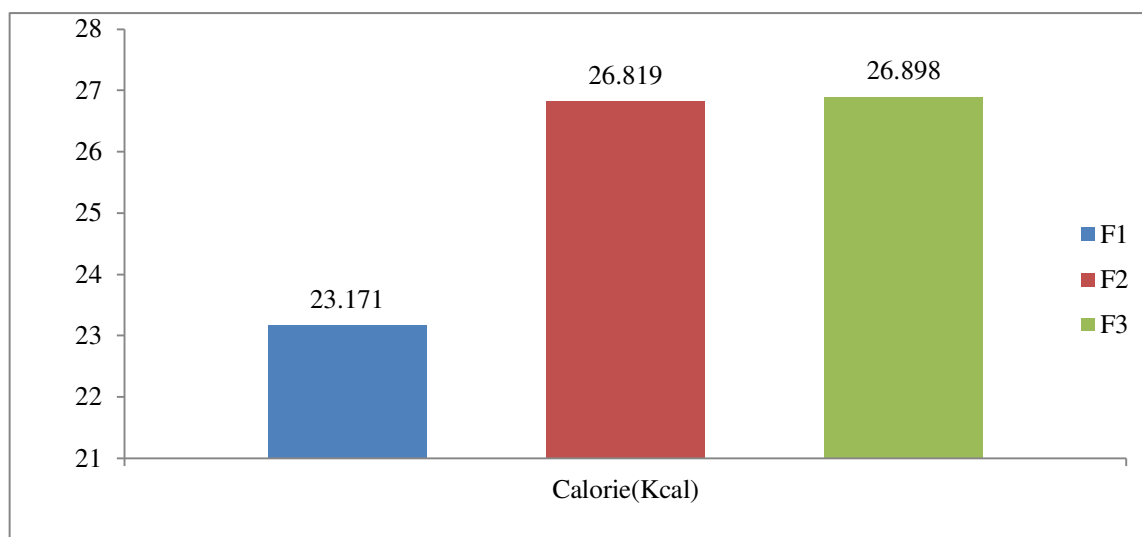


Figure 4.2: Graphical representation of energy content

Here,

F1= *C. pepo*

F2=*C. maxima*

F3=*C. moschata*

4.5 Sensory Acceptance

The level of consumers acceptance is often assed by asking consumer the rate how much they like a product overall, using a nine point hedonic scale (Popper and Gibes, 2004). It is known that affective test help to answer whether the product has commercial potential.

4.5.1 Color

Sensory evaluation of the present study reported that Color of different varieties differed significantly and *C. moschata* has highest score (8.6) whereas lowest rating obtained by *C. pepo* (7.6) from table 4.2.

Table 4.2: Sensory Attributes for Different Pumpkin Verities

Components	<i>C. pepo</i>	<i>C. maxima</i>	<i>C. moschata</i>
Color	6.90±0.718 ^a	7.05±0.759 ^a	7.90±0.788 ^b
Flavor	7.60±0.681	7.50±0.688	7.90±0.641
Texture	7.10±0.641 ^b	7.05±0.686 ^b	5.85±0.671 ^a
Taste	6.40±0.681 ^a	7.45±0.510 ^b	7.70±0.470 ^b
Overall	6.40±0.503 ^a	7.00±0.459 ^b	7.65±0.489 ^c

4.5.2 Flavour

Sensory evaluation of the present study calculated that flavour of different varieties differed significantly and *C. moschata* has highest score (8.5) and lowest score has *C. maxima* (8.1) from Table 4.2.

4.5.3 Texture

Sensory evaluation of the present study reported that Texture of different varieties differed significantly and the highest score has obtained by *C. pepo* (7.7) and lowest score by *C. moschata* (6.52) from Table 4.2.

4.5.4 Taste

Sensory evaluation of the present study calculated that Taste of different varieties differed significantly and *C. moschata* has highest score (8.1) and lowest rating scored by *C. pepo* (7.1) from Table 4.2.

4.5.5 Overall Acceptance

Sensory evaluation of the present study reported that Overall acceptance of different varieties differed significantly and the variety *C. moschata* has obtained highest score (8.1) and lowest by *C. pepo* (6.9) from Table 4.2.

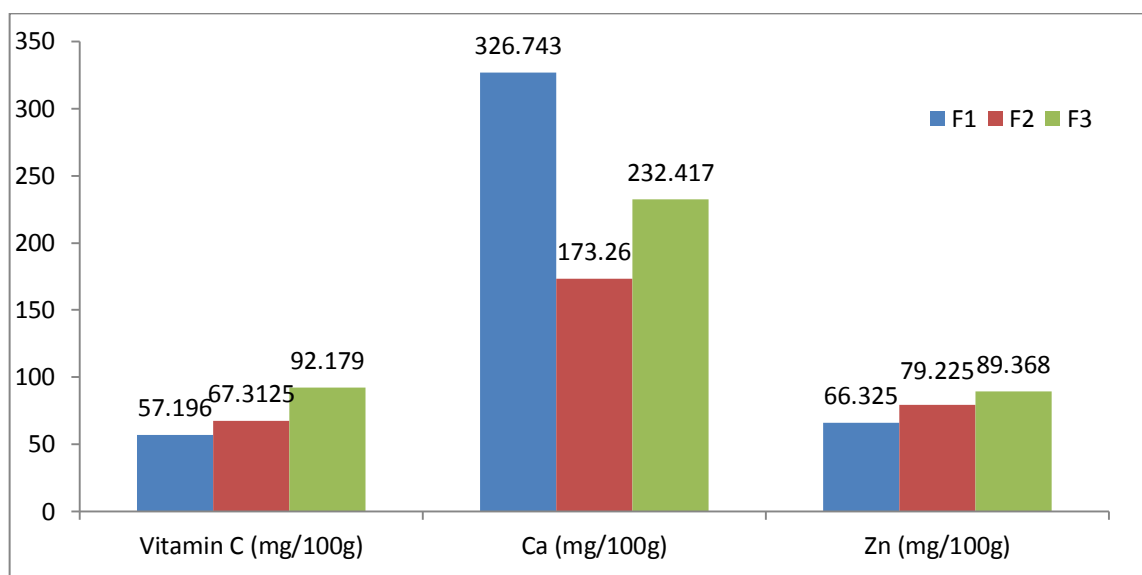


Figure 4.3: Graphical representation of vitamin C, Ca and Zn

Here,

F1= *C. pepo*

F2=*C. maxima*

F3=*C. moschata*

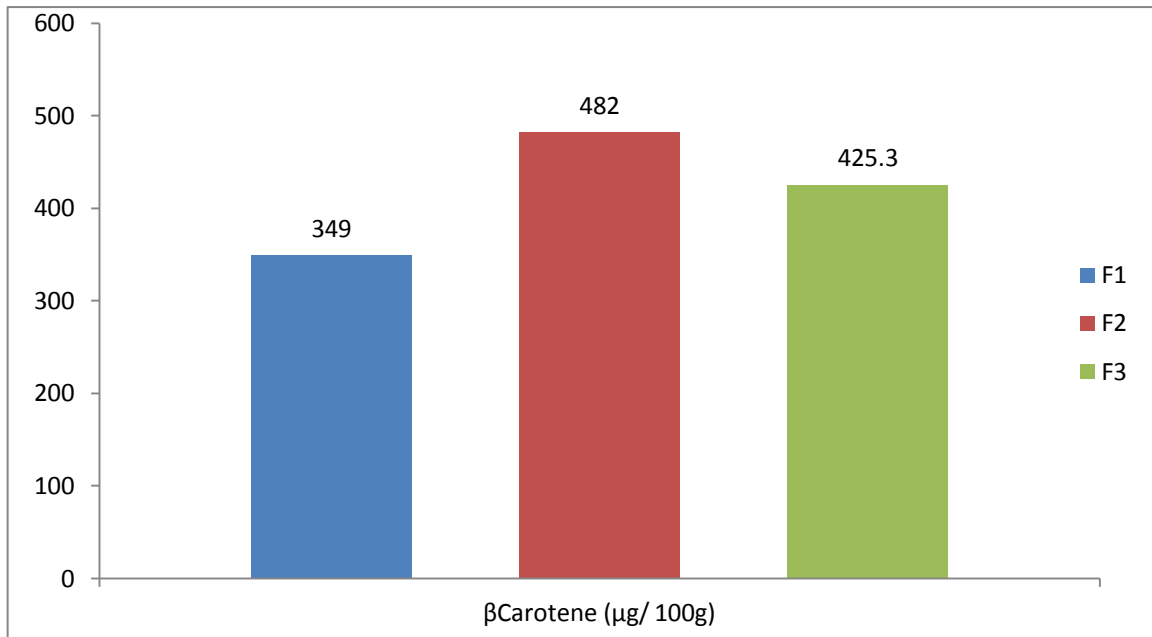


Figure 4.4: Graphical representation of β- Carotene

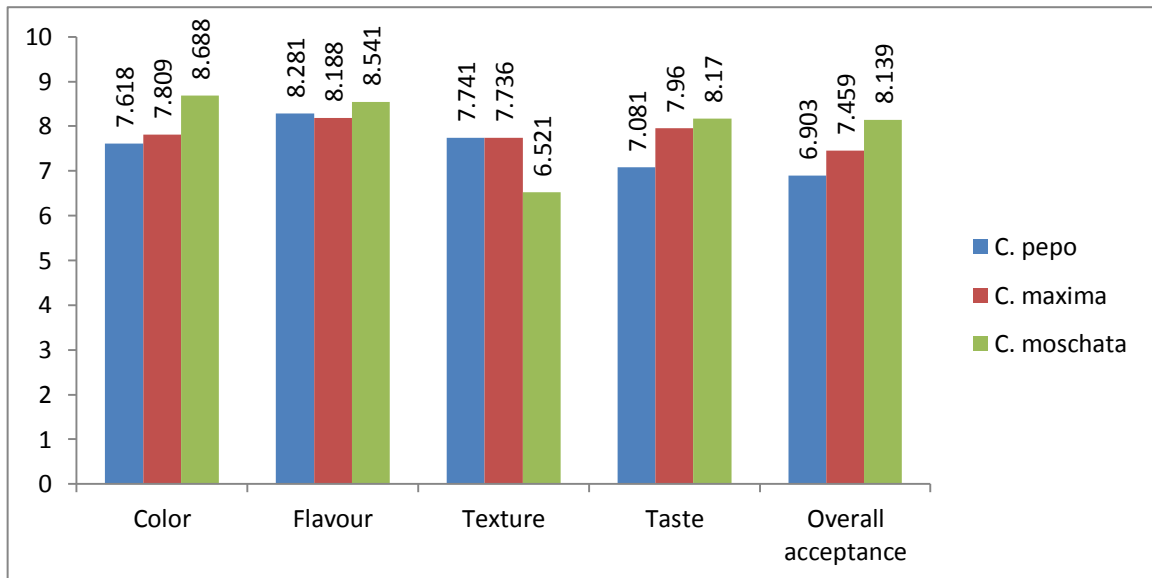
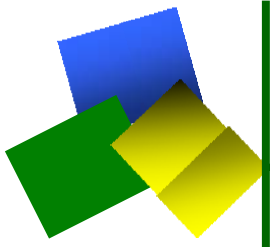


Figure 4.5: Graphical representation of sensory attributes (color, flavor, texture, taste and overall acceptance)



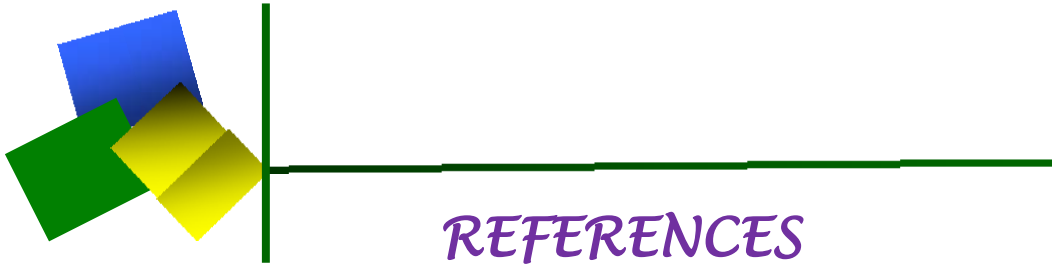
CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

This research shows that different varieties gave different value of proximate composition, β carotene and minerals (calcium and zinc). *C. moschata* gives higher proximate values such as highest Vit-C (92.179mg/100gm of sample), total sugar (4.917%) as well as highest energy and Zinc (26.798kcal and 89.763mg/100g) respectively. Also according to the score obtained from sensory evaluation *C. moschata* has got highest scoring. Considering all values obtained from this research *C. moschata* is most optimized variety rich in nutrition and as well as consumers acceptance.



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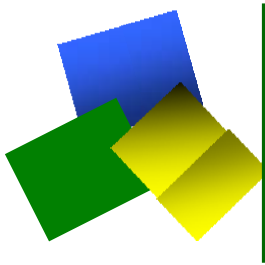
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APPENDICES

APPENDICES

Appendix I: Descriptive Analysis of different components (mean, standard deviation, standard error minimum and maximum values) of three indigenous cultivar of pumpkin.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Moisture	<i>C. pepo</i>	3	94.51000	0.908625	0.524595	92.25285	96.76715	93.470	95.150
	<i>C. maxima</i>	3	92.24000	0.831445	0.480035	90.17458	94.30542	91.280	92.730
	<i>C. moschata</i>	3	92.24333	1.582224	0.913498	88.31287	96.17380	90.560	93.700
	Total	9	92.99778	1.513744	0.504581	91.83421	94.16134	90.560	95.150
Ash	<i>C. pepo</i>	3	0.51200	0.070704	0.040821	0.33636	0.68764	0.435	0.574
	<i>C. maxima</i>	3	0.75667	0.130128	0.075130	0.43341	1.07992	0.630	0.890
	<i>C. moschata</i>	3	0.36567	0.075036	0.043322	0.17927	0.55207	0.286	0.435
	Total	9	0.54478	0.190158	0.063386	0.39861	0.69095	0.286	0.890
Fibre	<i>C. pepo</i>	3	1.06300	0.113119	0.065310	0.78200	1.34400	0.947	1.173
	<i>C. maxima</i>	3	0.97000	0.169679	0.097964	0.54849	1.39151	0.796	1.135
	<i>C. moschata</i>	3	0.65900	0.191948	0.110821	0.18217	1.13583	0.457	0.839
	Total	9	0.89733	0.230610	0.076870	0.72007	1.07460	0.457	1.173
Protein	<i>C. pepo</i>	3	1.45333	0.195533	0.112891	0.96760	1.93907	1.290	1.670
	<i>C. maxima</i>	3	1.91333	0.325167	0.187735	1.10557	2.72109	1.560	2.200
	<i>C. moschata</i>	3	1.68000	0.292062	0.168622	0.95448	2.40552	1.370	1.950
	Total	9	1.68222	0.311439	0.103813	1.44283	1.92162	1.290	2.200
VitC	<i>C. pepo</i>	3	50.70000	6.496922	3.751000	34.56075	66.83925	44.700	57.600
	<i>C. maxima</i>	3	62.50000	4.812484	2.778489	50.54513	74.45487	57.900	67.500
	<i>C. moschata</i>	3	87.83333	4.346646	2.509537	77.03567	98.63100	83.900	92.500
	Total	9	67.01111	17.060293	5.686764	53.89741	80.12481	44.700	92.500

Appendix I: Descriptive Analysis of different components (mean, standard deviation, standard error minimum and maximum values) of three indigenous cultivar of pumpkin (Contd.)

Fat	<i>C. pepo</i>	3	0.18000	0.036056	0.020817	0.09043	0.26957	0.140	0.210
	<i>C. maxima</i>	3	0.32000	0.030000	0.017321	0.24548	0.39452	0.290	0.350
	<i>C. moschata</i>	3	0.29133	0.031660	0.018279	0.21269	0.36998	0.258	0.321
	Total	9	0.26378	0.070019	0.023340	0.20996	0.31760	0.140	0.350
Carbohydrate	<i>C. pepo</i>	3	2.33233	0.116092	0.067026	2.04395	2.62072	2.231	2.459
	<i>C. maxima</i>	3	3.97433	0.305638	0.176460	3.21509	4.73358	3.650	4.257
	<i>C. moschata</i>	3	4.60433	0.313009	0.180716	3.82678	5.38189	4.271	4.892
	Total	9	3.63700	1.040720	0.346907	2.83703	4.43697	2.231	4.892
BCarotene	<i>C. pepo</i>	3	0.32400	0.025942	0.014978	0.25956	0.38844	0.295	0.345
	<i>C. maxima</i>	3	0.45467	0.027099	0.015645	0.38735	0.52198	0.429	0.483
	<i>C. moschata</i>	3	0.41033	0.015275	0.008819	0.37239	0.44828	0.397	0.427
	Total	9	0.39633	0.061006	0.020335	0.34944	0.44323	0.295	0.483
Calcium	<i>C. pepo</i>	3	310.43333	16.310222	9.416711	269.91650	350.95017	291.900	322.600
	<i>C. maxima</i>	3	161.23333	12.027191	6.943902	131.35613	191.11053	149.700	173.700
	<i>C. moschata</i>	3	230.66667	1.750238	1.010500	226.31883	235.01450	228.900	232.400
	Total	9	234.11111	65.452109	21.817370	183.80017	284.42206	149.700	322.600
Zinc	<i>C. pepo</i>	3	64.55000	1.775528	1.025102	60.13934	68.96066	62.750	66.300
	<i>C. maxima</i>	3	78.38000	0.845044	0.487887	76.28079	80.47921	77.540	79.230
	<i>C. moschata</i>	3	87.17667	2.686584	1.551100	80.50282	93.85051	84.230	89.490
	Total	9	76.70222	10.017391	3.339130	69.00217	84.40227	62.750	89.490
Energy	<i>C. pepo</i>	3	18.82667	4.444843	2.566232	7.78506	29.86827	16.000	23.950
	<i>C. maxima</i>	3	26.10933	0.710229	0.410051	24.34503	27.87364	25.454	26.864
	<i>C. moschata</i>	3	27.87033	2.529407	1.460354	21.58694	34.15373	25.219	30.257
	Total	9	24.26878	4.889327	1.629776	20.51051	28.02705	16.000	30.257
pH	<i>C. pepo</i>	3	6.52667	0.005774	0.003333	6.51232	6.54101	6.520	6.530
	<i>C. maxima</i>	3	6.34333	0.005774	0.003333	6.32899	6.35768	6.340	6.350
	<i>C. moschata</i>	3	6.34667	0.005774	0.003333	6.33232	6.36101	6.340	6.350
	Total	9	6.40556	0.090982	0.030327	6.33562	6.47549	6.340	6.530

Appendix II: ANOVA (Analysis of variance) for Moisture, Ash, Fibre and protein

		Sum of Squares	df	Mean Square	F	Sig.
Moisture	Between Groups	10.291	2	5.145	3.839	0.084
	Within Groups	8.041	6	1.340		
	Total	18.331	8			
Ash	Between Groups	0.234	2	0.117	12.743	0.007
	Within Groups	0.055	6	0.009		
	Total	0.289	8			
Fibre	Between Groups	0.269	2	0.134	5.137	0.050
	Within Groups	0.157	6	0.026		
	Total	0.425	8			
Protein	Between Groups	0.317	2	0.159	2.077	0.206
	Within Groups	0.459	6	0.076		
	Total	0.776	8			

Appendix III: ANOVA (Analysis of variance) for Vit C, Fat, Carbohydrate and β -carotene

VitC	Between Groups	2159.902	2	1079.951	38.449	.000
	Within Groups	168.527	6	28.088		
	Total	2328.429	8			
Fat	Between Groups	.033	2	.016	15.372	.004
	Within Groups	.006	6	.001		
	Total	.039	8			
Carbohydrate	Between Groups	8.255	2	4.128	60.442	.000
	Within Groups	.410	6	.068		
	Total	8.665	8			
β Carotene	Between Groups	.026	2	.013	24.221	.001
	Within Groups	.003	6	.001		
	Total	.030	8			

Appendix IV: ANOVA (Analysis of variance) for Calcium, Zinc, Energy, pH

Calcium	Between Groups	33444.349	2	16722.174	121.251	.000
	Within Groups	827.480	6	137.913		
	Total	34271.829	8			
Zinc	Between Groups	780.616	2	390.308	105.638	.000
	Within Groups	22.169	6	3.695		
	Total	802.785	8			
Energy	Between Groups	137.926	2	68.963	7.761	.022
	Within Groups	53.318	6	8.886		
	Total	191.244	8			
pH	Between Groups	.066	2	.033	990.333	.000
	Within Groups	.000	6	.000		
	Total	.066	8			

Appendix V: Least Significant difference for Moisture, Ash, Fibre and protein

Moisture		
Tukey B ^a		
variety	N	Subset for alpha = 0.05
		1
<i>C. maxima</i>	3	92.24000
<i>C. moschata</i>	3	92.24333
<i>C. pepo</i>	3	94.51000
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

Ash			
Tukey B ^a			
variety	N	Subset for alpha = 0.05	
		1	2
<i>C. moschata</i>	3	.36567	
<i>C. pepo</i>	3	.51200	
<i>C. maxima</i>	3		.75667
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

Fibre		
Tukey B ^a		
variety	N	Subset for alpha = 0.05
		1
<i>C. moschata</i>	3	.65900
<i>C. maxima</i>	3	.97000
<i>C. pepo</i>	3	1.06300
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

Protein		
Tukey B ^a		
variety	N	Subset for alpha = 0.05
		1
<i>C. pepo</i>	3	1.45333
<i>C. moschata</i>	3	1.68000
<i>C. maxima</i>	3	1.91333
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 3.000.		

Appendix VI: Least Significant difference Of Vit C, Fat, Carbohydrate and β -carotene

VitC			
Tukey B ^a			
variety	N	Subset for alpha = 0.05	
		1	2
<i>C. pepo</i>	3	50.70000	
<i>C. maxima</i>	3	62.50000	
<i>C. moschata</i>	3		87.83333
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

Fat			
Tukey B ^a			
variety	N	Subset for alpha = 0.05	
		1	2
<i>C. pepo</i>	3	.18000	
<i>C. moschata</i>	3		.29133
<i>C. maxima</i>	3		.32000
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

Carbohydrate				
Tukey B ^a				
Variety	N	Subset for alpha = 0.05		
		1	2	3
<i>C. pepo</i>	3	2.33233		
<i>C. maxima</i>	3		3.97433	
<i>C. moschata</i>	3			4.60433
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

βCarotene			
Tukey B ^a			
variety	N	Subset for alpha = 0.05	
		1	2
<i>C. pepo</i>	3	.32400	
<i>C. moschata</i>	3		.41033
<i>C. maxima</i>	3		.45467
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

Appendix VII: Least Significant difference for Calcium, Zinc, Energy, pH

Calcium				
Tukey B ^a				
Variety	N	Subset for alpha = 0.05		
		1	2	3
<i>C. maxima</i>	3	161.23333		
<i>C. moschata</i>	3		230.66667	
<i>C. pepo</i>	3			310.43333
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

Zinc				
Tukey B ^a				
variety	N	Subset for alpha = 0.05		
		1	2	3
<i>C. pepo</i>	3	64.55000		
<i>C. maxima</i>	3		78.38000	
<i>C. moschata</i>	3			87.17667
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

Energy				
Tukey HSD ^a				
variety	N	Subset for alpha = 0.05		
		1	2	
<i>C. pepo</i>	3	18.82667		
<i>C. maxima</i>	3	26.10933	26.10933	
<i>C. moschata</i>	3		27.87033	
Sig.		.055	.759	
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

pH				
Tukey HSD ^a				
variety	N	Subset for alpha = 0.05		
		1	2	
<i>C. maxima</i>	3	6.34333		
<i>C. moschata</i>	3	6.34667		
<i>C. pepo</i>	3		6.52667	
Sig.		.768	1.000	
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 3.000.				

Appendix VIII: ANOVA (Analysis of variance) for Color, Flavour, Texture, Taste and Overall acceptance

		Sum of Squares	df	Mean Square	F	Sig.
Color	Between Groups	11.633	2	5.817	10.186	.000
	Within Groups	32.550	57	.571		
	Total	44.183	59			
Flavor	Between Groups	1.733	2	.867	1.930	.155
	Within Groups	25.600	57	.449		
	Total	27.333	59			
Texture	Between Groups	20.033	2	10.017	22.567	.000
	Within Groups	25.300	57	.444		
	Total	45.333	59			
Taste	Between Groups	19.033	2	9.517	30.220	.000
	Within Groups	17.950	57	.315		
	Total	36.983	59			
Overall	Between Groups	15.633	2	7.817	33.375	.000
	Within Groups	13.350	57	.234		
	Total	28.983	59			

Appendix IX: Descriptive Analysis of Color, Flavour, Texture, Taste and Overall acceptance

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Color	<i>C. pepo</i>	20	6.90	.718	.161	6.56	7.24	6	8
	<i>C. moschata</i>	20	7.90	.788	.176	7.53	8.27	7	9
	<i>C. maxima</i>	20	7.05	.759	.170	6.69	7.41	6	8
	Total	60	7.28	.865	.112	7.06	7.51	6	9
Flavor	<i>C. pepo</i>	20	7.60	.681	.152	7.28	7.92	7	9
	<i>C. moschata</i>	20	7.90	.641	.143	7.60	8.20	7	9
	<i>C. maxima</i>	20	7.50	.688	.154	7.18	7.82	6	9
	Total	60	7.67	.681	.088	7.49	7.84	6	9
Texture	<i>C. pepo</i>	20	7.10	.641	.143	6.80	7.40	6	8
	<i>C. moschata</i>	20	5.85	.671	.150	5.54	6.16	5	7
	<i>C. maxima</i>	20	7.05	.686	.153	6.73	7.37	6	8
	Total	60	6.67	.877	.113	6.44	6.89	5	8
Taste	<i>C. pepo</i>	20	6.40	.681	.152	6.08	6.72	5	7
	<i>C. moschata</i>	20	7.70	.470	.105	7.48	7.92	7	8
	<i>C. maxima</i>	20	7.45	.510	.114	7.21	7.69	7	8
	Total	60	7.18	.792	.102	6.98	7.39	5	8
Overall	<i>C. pepo</i>	20	6.40	.503	.112	6.16	6.64	6	7
	<i>C. moschata</i>	20	7.65	.489	.109	7.42	7.88	7	8
	<i>C. maxima</i>	20	7.00	.459	.103	6.79	7.21	6	8
	Total	60	7.02	.701	.090	6.84	7.20	6	8

Appendix X: Least Significant difference for Color, Flavour, Texture, Taste and Overall acceptance

Color			
Tukey HSD ^a			
sensory	N	Subset for alpha = 0.05	
		1	2
<i>C. pepo</i>	20	6.90	
<i>C. maxima</i>	20	7.05	
<i>C. moschata</i>	20		7.90
Sig.		.806	1.000
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 20.000.			

Flavor		
Tukey HSD ^a		
sensory	N	Subset for alpha = 0.05
		1
<i>C. maxima</i>	20	7.50
<i>C. pepo</i>	20	7.60
<i>C. moschata</i>	20	7.90
Sig.		.152
Means for groups in homogeneous subsets are displayed.		
a. Uses Harmonic Mean Sample Size = 20.000.		

Texture			
Tukey HSD ^a			
sensory	N	Subset for alpha = 0.05	
		1	2
<i>C. moschata</i>	20	5.85	
<i>C. maxima</i>	20		7.05
<i>C. pepo</i>	20		7.10
Sig.		1.000	.969
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 20.000.			

Taste			
Tukey HSD ^a			
sensory	N	Subset for alpha = 0.05	
		1	2
<i>C. pepo</i>	20	6.40	
<i>C. maxima</i>	20		7.45
<i>C. moschata</i>	20		7.70
Sig.		1.000	.343
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 20.000.			

Overall				
Tukey HSD ^a				
sensory	N	Subset for alpha = 0.05		
		1	2	3
<i>C. pepo</i>	20	6.40		
<i>C. maxima</i>	20		7.00	
<i>C. moschata</i>	20			7.65
Sig.		1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 20.000.				