FORMULATION AND QUALITY EVALUATION OF BAKED TORTILLA CHIPS SUPPLEMENTED WITH PUMPKIN SEED FLOUR

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Roll No.: 0119/12

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Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

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December 2020

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This is to certify that we have examined the above Master's Thesis and have found that it is complete and satisfactory in all respects, and that all revisions required by the thesis examinations committee have been made.

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DEDICATED TO MY RESPECTED AND BELOVED FAMILY AND TEACHERS

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Abbreviations

CHO	:	Carbohydrate
N	:	Normality
B.P.	:	Boiling point
W	:	Watt
PUFA	:	Polyunsaturated Fatty Acids
DPPH	:	2,2 – diphenyl – 1 –picrylhydrazyl
UV	:	Ultraviolet
AOAC	:	Association of Official Analytical Chemists
℃	:	Degree celcius
T.E.	:	Trolox Equivalent
ANOVA	:	Analysis of Variance
SPSS	:	Statistical Package for Social Science
SD	:	Standard Deviation
RPM.	:	Revolutions Per Minute
RAE	:	Retinol Activity Equivalent
g	:	Gram
µg	:	Microgram

Abstract

A healthy person depends on a healthy food system. Malnutrition has become a great problem that imposes a high cost on society. Attention has been given to the right use of agricultural waste to overcome malnutrition. Pumpkin seeds are by-product which are rich in nutritional content. The study aimed to properly utilize pumpkin seed as a supplemental nutritious food i.e. baked tortilla chips. The proximate composition of pumpkin seed flour was determined and found 6.4% moisture, 5.3% ash, 38.5% protein, 37.9% fat, 0.4% fiber, 11.7% CHO and 542.8 kcal energy per 100 gm seed flour. Calcium, magnesium, sodium, phosphorus, iron, zinc, potassium, and chloride were 58, 380, 137, 260, 6.5, 7.98, 114, 34 mg/100g, respectively. Vitamin A level was found 397.55 µg RAE per 100 gm seed flour. Saponification (mg KOH/g oil) and iodine (g I_2/100g) values of the extracted oil from pumpkin seeds were 194.7 and 107.3, respectively. The saponification value indicates that the oil has high molecular weight fatty acids and the iodine value suggests a high degree of unsaturation. These make the oil an alternative good source of edible oil. Antioxidant capacity of seed flour was 2.7 mg TE/100gm. Due to the nutritive value of pumpkin seed flour, chips were prepared with three different substituted levels of pumpkin seed flour in all-purpose flour (Sample B=5%, Sample C=10% and Sample D=15%) were compared with control (Sample A=0% pumpkin seed flour). This substitution level was based on pumpkin seeds as excess seed flour will make chips less crunchy due to low gluten quantity of seed flour. Proximate composition and Vitamin A content of all formulations were evaluated and these showed that sample D has significantly higher protein (18.7%), fat (9.6%), fiber (3.1%), energy (390.73 kcal) and Vitamin A (432.6 µg RAE/100 gm). Sensory results also revealed sample C (10%) and D (15%) scored high in sensory parameters significantly and 15% incorporation was best accepted by panelists. Conclusively, healthy chips can be prepared successfully from pumpkin seed flour.

Keywords: Antioxidant capacity, Chips, Malnutrition, Nutritious, Sensory, Tortilla.

Chapter 1: Introduction

Snack food plays a vital role in providing supplemental nutrition and therefore, snack should contain complete nutrient content, including carbohydrates, protein, fat, vitamins and minerals (Hapsari, 2013). In the snack food industry pumpkin seeds are gaining popularity as a healthy alternative to other fried snacks. Addition of pumpkin seed flour to chips formulation will increase the nutritional value of this snack because of unsaturated fat content. Pumpkin seeds are a good source of polyunsaturated and monounsaturated fats, which contains important antioxidants and phytochemicals. Unsaturated fats are considered as beneficial fats because they can improve blood cholesterol levels and ease inflammation. Moreover, there are many healthy alternatives to unhealthy snacks. Baked chips are better to eat than deep-fried chips. Since baked chips are baked rather than fried, they are generally lower in cholesterol and fats (Munira and Tasnim, 2013). In addition, the baked tortilla chips formulation can be modified easily to meet the nutritional demand of the target consumers. Pumpkin belongs to the family of cucurbitaceae and is grown everywhere in the world. It has been used often as a functional food in several countries. Due to their nutritional value and health benefits, considerable attention has been given to pumpkin seeds recently. Pumpkin seeds, also referred to as pepitas are small, flat, green, edible seeds. Most pumpkin seeds are enclosed with white husk while some are without husk. Shelled pumpkin seeds are healthy because they are rich in fiber content. Pumpkin seed plays a crucial role in maintaining proper health as it is a source of lipids, proteins, carbohydrates and other nutrients (Alfawaz M, 2004). By evaluating proximate compositions of the seed, *Cucurbita maxima* reveal protein 33.48%, carbohydrate 28.68%, lipid 30.66%, fiber 3.07%, ash 3.98% and available energy 524.58 Kcal (Karaye *et al*., 2013). Pumpkin seeds are an excellent source of polyunsaturated fatty acids, vitamins, antioxidants (carotenoids and tocopherols) and micronutrients (Caili *et al*., 2006). Potassium, phosphorus and magnesium and moderately high amounts of other trace minerals (calcium, sodium, manganese, iron, zinc and copper) are found in seeds. These components make pumpkin seeds valuable for food supplements (Lazos, 1986). Zinc within pumpkin seed plays an essential role in raising memory and brain function (Shemi, 2014). Moreover, seed extract contains good quality and high levels of phytochemical sterols, which may benefit the immune system, reproductive health and other areas of health (El-Ghany *et al*., 2010). Pharmacological properties such as anti-diabetic, antifungal, antibacterial, anti-inflammation and antioxidant effects are inherited in pumpkin seeds (Nkosi *et al*., 2006). Nutrients like vitamin A, vitamin E, zinc, omega-3 fatty acids, omega-6 fatty acids are also found in seeds. One of the plant carotenoids is β-Carotene, which is converted to vitamin A. β-Carotene plays many functions in overall health. When combined with other carotenoids, it works efficiently and helps in reducing the risk of colon and lung cancer. The primary source of vitamin A is carotenoids and vitamin A deficiency is still common in many developing countries (Dhiman *et al*., 2009). Retinol performs an essential role in vision and it is the active form of vitamin A. Fats and oils belong to the cluster of naturally occurring compounds referred to as lipids. Lipids are the components of animal and plant cells that are soluble in an organic solvent such as ether, chloroform, benzene, hexane etc., however insoluble in water. Fats and oils are the necessary lipids found in nature and upon hydrolysis, these produce long chain fatty acid and glycerol. They are among the key 'food groups' required for the human body, the other two being proteins and carbohydrates. Fats and oils have great nutritional value and are widely distributed in food (Habib *et al*., 2015). Oil content in pumpkin seeds is 11 to 31%, of which 73.1 to 80.5% is a total unsaturated fatty acid (Patel *et al*., 2017). Pumpkin seed oil is a good quality edible oil. Due to its favorable fatty acid composition and variety of elements, the seed oils have high nutritive value and beneficial effects on the human organism (Kulaitiene *et al*., 2018). Seed oil is loaded with essential fatty acids that help maintain healthy blood vessels, nerves, and tissues (Levin and Rachel, 2008).

The demand of new nutritionally healthy and sustainable viable foods has been increased considerably. Special attention has been provided to the utilization of by-products. The proper use of by-products can contribute to new food products and minimize waste. Pumpkin seed is inexpensive and widely distributed. Pumpkin seed flour can be used to fortify chips, cookies, bread, biscuits, crackers, sauces etc. Many people are not familiar with pumpkin's products. Pumpkin seeds can be diversified into a wide range of processed products that can increase seeds' consumption. Snacks are popular food among children and adolescents, small amounts of food that are consumed between meals. Chips are dry snacks made from flour, fat and other ingredients and consumed extensively worldwide by all age groups. The popularity of chips comes from their attributes such as high palatable and nutritious attributes. Potato chips are prevalent snack food. But there are some health drawbacks to this popular food. A new study by Harvard researchers suggests that potato chips lead to more weight gain per serving than any other food (Mozaffarian *et al*., 2011). Potato chips, on average, a highly unbalanced nutritional composition being particularly biased towards an excess of fat. They were also low in sugar, high in saturated fat, low in protein, carbohydrate and fiber. Most of the chips are high in fats, particularly saturated fat. They are also high in calories, especially calories from fat. These calories are empty calories, meaning they do not provide any nutritional value (Munira and Tasnim, 2013). Saturated fats are responsible for increasing cholesterol levels in the body. Moreover, most chips are deep-fried, creating trans fats. Trans fats play a role in raising bad cholesterol, lowering the good cholesterol in the body, and increasing the risk of coronary heart disease. Saturated fats and trans fats promote inflammation and reduce the responsiveness of the cells lining blood vessels. Trans fats have also been implicated in promoting obesity and raising the risk of developing diabetes (Munira and Tasnim, 2013). That’s why this study aimed to formulate chips that are low in saturated fats but high in unsaturation and baked chips can be taken as a healthier option than other normal chips.

Roasted pumpkin seeds are consumed in Bangladesh by some people. In previous, there was no work done intensively and so this study is designed to utilize pumpkin seeds by developing baked tortilla chips supplemented with seed flour for nutritional improvement and evaluating the nutritional content and organoleptic attributes of supplemented baked chips.

Aims and Objectives

- To formulate baked tortilla chips incorporated with pumpkin seed flour and determine nutritional value of chips.
- To determine the proximate components, Vitamin A, mineral content and antioxidant activity of pumpkin seed flour.
- To evaluate the quality of oil extracted from the pumpkin seed.

Chapter 2: Review of Literature

Pumpkin seeds are tiny however, they are densely packed with helpful nutrients. In several countries, the consumption rate of pumpkin seed is rising. These will be eaten directly as snacks once seasoning or roasting. They will be additionally utilized in several foods to form other alimentary food.

2.1 History of snack food

The snack food industry has been around for hundreds of years. Popcorn has been around since just about 3000 B.C. In the late 1890s, potato chips were discovered and became a well-liked American snack. It started as a tiny business venture to urge chips to locals and caught on and spanned the globe. Thousands of varieties of chips, fruit snacks, cookies and anything our hearts desire are available (McCarthy, 2001). Now the industry looks after healthier foods for snacking. Low sodium, low oil and low calories containing foods are most desired now. Companies are focusing on baking chips to provide lower oil chips. Naturally, families with youngsters and teenagers are the biggest customers of chips.

2.2 History of tortilla chips

Either the Aztecs or the Zapotecs created tortilla first. The Zapotecs were an ancient civilization that lived close to Oaxaca in the Monte Alban ruins. They made Totopochtli by roasting tortillas on a flat grill. This tortilla had lower shelf life such as one or two days. The fried tortilla was considered a tostado that improved the flavor and prolonged storage time. If the tostados were cut into pieces they were referred to as Totopos. Baking and frying of tortilla chips give the chips a firmer texture (Quintero-Fuentes, 1997).

2.3 Pumpkin – general aspects

Classification of pumpkin embody,

Family	:	Cucurbitaceae
Botanical Name	:	*Cucurbita Maxima*
Kingdom	:	Plantae
Order	:	Cucurbitales
Genus	:	Cucurbita
Color	:	Light yellow orange to bright orange

Pumpkin belongs to the family Cucurbitaceae, an angiosperm, genus Cucurbita with totally different varieties. Usually, people consider pumpkin seeds a waste, but these are good sources of nutrients and oil and might be used as a food (Younis *et al.,* 2000).

Figure 2.1 Pumpkin seeds

After eliminating pulp, pumpkin seeds and rinds that stay in massive quantities as the waste matter may be consumed for edible purposes (Abd El-Aziz and El-Kalek, 2011). Seeds are large, flat and oval in shape and end in a tip. Pumpkin seeds are enriched with dietary fiber and monounsaturated fatty acids suitable for good heart health. Additionally, the seeds are high in protein, minerals and vitamins which are suitable for health (Shemi, 2014). In several countries, fried or seasoned pumpkin seeds are sold and consumed, very similar to peanuts or sunflower seeds, and hold the best food and nutritional worth. As a flavor enhancer, kernels of pumpkin seeds are used in gravies and soups, yogurts, creams, sauces, dressings and utilized in cooking, baking and ground meat formulations as a wholesome supplement and a portion of functional food (Montesano *et al*., 2018). Seed flours were used as protein supplements in a variety of native foods. In vitro protein digestibility of bread improved when pumpkin seed proteins were added (Ceclu *et al*., 2020).

2.4 Constituents of pumpkin seeds

Seeds that are nutrient dense foods, contain large quantities of protein and fat, mainly unsaturated fatty acids. These are also rich in different form of other nutrients and supply dietary fiber, vitamins (e.g. vitamin E, folic acid, vitamin B6, niacin), minerals (e.g. copper, magnesium, potassium, zinc) and plenty of bio-active compounds like antioxidants, phytosterols and different phytochemicals (Dreher *et al*. 1996).

The moisture content of pumpkin seeds will vary by type and region. *Cucurbita maxima* seeds are chiefly characterized by a high concentration of fatty acids and proteins (Stevenson *et al*., 2007). Table 2.1 shows the nutritional composition reported in numerous articles proved that seeds have a high nutritional value, serve good quality oil and tocopherols and have a good vegetable cover (Agatemor, 2006).

Table 2.1: Proximal composition of Cucurbita maxima seeds

Component	**100 g**			
	Amoo *et al*. 2004	**Alfawaz, 2004**	**Lazos, 1986**	**Rezig *et al*. 2012**
Moisture (%)	3.08	5.97	5.40	8.46
Energy (kcal)	628	453	676	455
Crude protein (g)	14.31	39.25	32.3	33.92
Crude fat (g)	52.13	27.83	45.4	31.57
Carbohydrate (g)	24.45	11.48	5.55	0.11
Crude fiber (g)	2.55	16.84	12.1	21.97
Total ash (mg)	3.60	4.59	4.65	3.97

Amino acids are noteworthy in *Cucurbita maxima* seeds, with the most abundant glutamic acid and arginine. Cucurbitaceae family have an uncommon organic compound known as cucurbitin (amino acid), chemically defined as (-)-3-amino-3-carboxipirrolidine; and has anti-inflammatory and anti-parasitic functional properties (Alfawaz, 2004).

Every organism requires minerals in a minimal amount for their growth and maintenance of functional activity. Food and vegetables provide the necessary supply of minerals for individuals and exist in food as inorganic and organic combination. Pumpkin seeds are packed with minerals that are fight diseases like arthritis, inflammatory disease, and glandular carcinoma (Maheshwari *et al.*, 2015). Table 2.2 shows the mineral content of *Cucurbita maxima* seeds. The variations ascertained may well be explained by soil conditions, seed conditions and mineral determination methodology, among others (Salama, 2006).

Table 2.2*:* Mineral content of Cucurbita maxima seeds

Mineral	Seed Amount (mg/100g)		
	Amoo *et al*. 2004	**Alfawaz, 2004**	**Rezig *et al*. 2012**
Sodium	29.69	68.58	356.75
Potassium	35.87	753.11	886.56
Magnesium	34.87	364.43	146.13
Calcium	29.47	139.70	271.89
Phosphorus	224.14	1036.82	824.53
Manganese	1.79	-	3.42
Zinc	3.98	1.09	25.19
Iron	4.27	13.66	15.37

2.5 Vitamin A content in pumpkin seeds

Vitamin A, known as beta carotene, is present in food and is a fat soluble vitamin. It helps in retarding the ageing process, reducing tumor progression and strengthening the immune system. For the proper functioning of the heart, kidneys and other organs, vitamin A plays an important role. Food rich in beta carotene lowers individuals' risk of certain kinds of cancer, including prostate and lung. Vitamin A content in processed seed was 36.83 µg/100 gm and in unprocessed seed 33.72 µg/100 gm (Ambi *et al*., 2021). Preschool children are the most affected by Vitamin A deficiency and hence they have poor immune system being prone to infection, physical growth and impaired cognitive (Berti *et al*., 2014). Vitamin A deficiency is prevalent in rural areas due to food insecurity, poverty, unavailability, and quality of food. Pumpkin seed and flesh are both good sources of proteins, minerals, vitamins, and antioxidants (β-Carotene and tocopherols) (Kim *et al*., 2012). Fortification of foods with products which are rich in carotene will be helpful for reducing health problems like night blindness and low body immunity that are related with vitamin A deficiency.

2.6 Chemical properties of pumpkin seed oil

Fatty acids content is up to 50% of the total weight of *Cucurbita maxima* seed, with a high percentage of unsaturated short chain fatty acids (Amoo *et al*., 2004). Therefore, seeds have a high stability to oxidative rancidity process. Oil can be extracted by solvent extraction process and oil content within the seeds is 40-60%. There are several factors that affect the chemical composition of extracted oil. The chemical composition of oil indicates the purity of the oil yield and might be affected by different extraction methods and parameters. The pumpkin oil is beneficial in treating diarrhea. Other advantages of oil consumption were reported in Korea to treat depression. Japanese said an increased consumption of pumpkin seed oil provides vitamin E (tocopherol) in their daily diet (Dar *et al*., 2017).

2.6.1 Saponification value of pumpkin seed oil

The saponification value of oil refers to the weight of potassium hydroxide expressed in milligram, needed to saponify one gram of oil or fat. It is a parameter for checking adulteration of the oil sample (Amoo *et al*., 2004). The saponification index, varied from 126 to 201, shows that fatty acids within the examined oils have more carbon atoms.

2.6.2 Iodine value of pumpkin seed oil

The iodine value equals the quantity of grams of iodine needed to saturate the fatty acid present in one hundred gram of oil or fat. It is the value of iodine in grams, absorbed by 100 grams of a given oil sample. Oils having high iodine values means a high degree of unsaturation. This unsaturation is in double bonds that react with iodine compounds. The more C=C bonds are present in the fat if there are higher iodine numbers (Mbatchou and Kosoono, 2012). The iodine value is used in industry, as it indicates the oil's stability and health properties and the higher iodine value means the greater amount of unsaturation (Akububugwo and Ugbogu, 2007). Table 2.3 shows the iodine and saponification value of pumpkin seed oil. These values differ according to the place of origin and the references analyzed.

Table 2.3*: Physicochemical characterization of pumpkin seed oil*

Parameter	Value	
	Amoo *et al*. 2004	Regiz *et.al.* 2012
Saponification value	126.09	199.3
Iodine value	18.66	153.66

2.7 Antioxidant activity of pumpkin seed

Antioxidants play an essential role human diet. Numerous extracts of pumpkin could play a crucial role in pre-diabetics, people with diabetes and vascular injury due to potential antioxidant activity. To evaluate the antioxidant activity, DPPH free radical assay is used (Sanchez *et al*., 1998).

Antioxidants are the compounds that sabotage the free radicals and act as a shield by protecting our body from numerous free radical associated diseases. The mechanism is related to free radical mediated oxidative process through initiation, propagation and termination. Production of antioxidants might occur within the body and naturally in several foods (Alam *et al*., 2020). The pumpkin contains antioxidants such as carotenoids, tocopherols, phenolic acids and flavonols. It is unremarkably full- grown in Europe, Asia, South America, North America and Africa (Tanaka *et al*., 2013). The rinds of *Cucurbita maxima* had the higher antioxidant activity (33.8%), measured by DPPH and the pulp of *Cucurbita maxima* and seed of *Cucurbita moschata* had 29.6% and 25.4% respectively (Indrianingsih *et al*., 2019).

2.8 Use and application of pumpkin seeds by-products: Flour

One of the most common ways to utilize pumpkin waste is flour, obtained from seeds. Pumpkin shells are high in protein and fiber content, in addition to ascorbic acid and calcium, that are given relevant concentrations compared to the pulp, a commonly consumed part (Monteiro, 2009). Seeds are vital source of flour and by processing seeds like fermenting, germinating or roasting flour can be obtained (Akintade *et al*., 2019). The essential amino acid content of the processed pumpkin seed flour was quite good for all the processes. All the processes aimed to make longer the shelf life of pumpkin seeds and extend the bioavailability of the bioactive compounds concerned in anti-diabetic, antifungal, antibacterial, anti-inflammation and antioxidant mechanisms (Ceclu *et al*., 2020).

A study showed a positive result through partial replacement of wheat flour for pumpkin seed flour in muffins for children because the higher substitution of wheat flour for pumpkin seed improved the nutritional value of muffins. Within the muffins, increased pumpkin seed content caused a decrease in saturated short chain fatty acids. The partial replacement of wheat flour with pumpkin seed flour reduced long chain fatty acid content from extracted muffin fat. Furthermore, after evaluating sensory parameters, over 71% of the children liked muffins with 33% of pumpkin seed flour and reported as tasty. And also muffins supplemented with seed flour can be stored for two weeks without special condition packaging (Bialek *et al*., 2015). So the utilization of pumpkin seed in flour production can lead to higher nutrient rich food products.

2.9 Pumpkin seed in tortilla chips

The pumpkin seeds are distinctive in seasoned and nutty taste and consumed salted and roasted as a snack in regions of Mexico, Canada, United States, China and Europe. Pumpkin seeds are rich in iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorus, PUFA, c-tocopherol, carotenoids, and so currently, these are selling as fermented, baked and concentrated forms of protein (Sayed *et al*., 2019). Various food products mainly based on pumpkin seeds, like vegetables salad, granola chunks, bread, quinoa salad, tortilla chips and cookies are promoted by many food shops of the United States such as Walmart, Costco and Trader Joe (Patel, 2013).

2.10 Evaluating quality of tortilla chips

The acceptability of tortilla chips depends on flavor, texture and appearance. A variety of tests developed to give an excellent correlation to sensory evaluation of texture in limited numbers of foods. Tortilla chips are strongly affected by texture and objective measures of texture properties (Quintero-Fuentes, 1997). Sensory panels are needed to examine for taste, texture and appearance of products. In these panels, people taste samples and describe their reactions to the feel like crunchiness. They are also asked however they feel regarding the flavor and the appearance of the food product.

Conclusion:

Although pumpkin seeds are underused, these are beneficial due to their chemical composition, therapeutic and industrial properties. They are used to produce oil, in the bakery and can be consumed as a snack. Pumpkin seed flour is regarded as a functional, healthy and sustainable food ingredient due to its fiber, protein, fat and energy content. Selecting a healthy snack in a good portion can increase energy level and balance hunger by providing regular fuel to the body.

Chapter 3: Materials and Methods

3.1 Study period and study area

This research work was conducted for five months from August 2021 to November 2021. Experimental procedures were carried out in the laboratory of the Department of Applied Food Science and Nutrition, Department of Food Processing and Engineering, Department of Applied Chemistry and Chemical Technology, Department of Physiology, Pharmacology and Biochemistry, Department of Fishing and Post-Harvest Technology at Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram.

3.2 Collection of samples and preparation of pumpkin seed flour

Pumpkins were collected from the local market of Chattogram. The seeds were dissected from the vegetable and washed thoroughly to remove dirt and any foreign material.

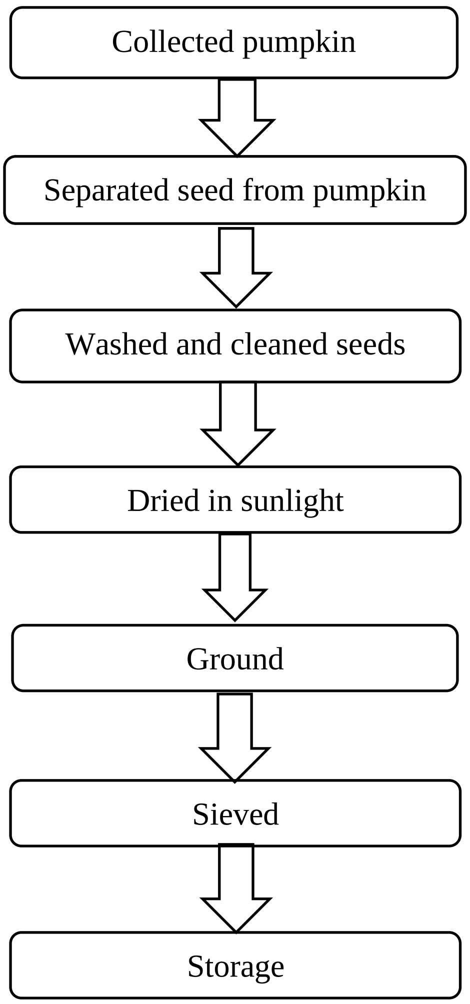

Figure 3.1: Flow sheet for pumpkin seed powder production

After then, the seeds were placed on a tray and dried in the sunlight for seven consecutive days. Dried seeds were taken and ground into powder form with the help of a grinder and then sieved them. After that the seed flour was kept into zip-lock plastic bag and kept at 4°C in the refrigerator for further examinations.

3.3 Preparation of baked tortilla chips supplemented with pumpkin seed flour

The baked tortilla chips were prepared by mixing pumpkin seed flour, all-purpose flour and other ingredients including salt, sugar, baking powder, spices (garlic powder, onion powder, paprika), soyabean oil, water according to the method described by (Adedapo *et al.* 2014) with slight modification. Four formulations of chips were made to compare if the different substitution level of seed flour had any effect on chips. Sample A contained 100 gm all-purpose flour but no pumpkin seed flour and remarked as control. Sample B contained 5 gm seed flour and 95 gm all-purpose flour. Sample C had 10 gm seed flour and 90 gm all-purpose flour. Sample D contained 15 gm and 85 gm pumpkin seed flour and all-purpose flour respectively. Other ingredients were remain same in each formulation. Chips were developed substituting all-purpose flour at 5, 10 and 15% with pumpkin seed flour, represented in Table 3.1.

Table 3.1: Formulation of tortilla chips

Ingredients (g)	Sample A (Control)	Sample B	Sample C	Sample D
Pumpkin seed flour	0	5	10	15
All-purpose flour	100	95	90	85
Salt	3	3	3	3
Sugar	2	2	2	2
Oil	4.2	4.2	4.2	4.2
Spices mixture	0.3	0.3	0.3	0.3
Baking powder	1	1	1	1

All ingredients were weighed first and then mixed until getting a smooth dough. After 10 minutes of resting dough, a piece of baking paper was placed on the dough and then the dough was sheeted continuously to form a thin tortilla. After that, the tortilla was formed into triangular pieces with a knife and baked in the electric oven at 180°C for 12 minutes, then cooled, packaged and labeled. For better taste, some chat-masala was sprinkled on the chips. Fig 3.2 shows the development of pumpkin seed tortilla chips.

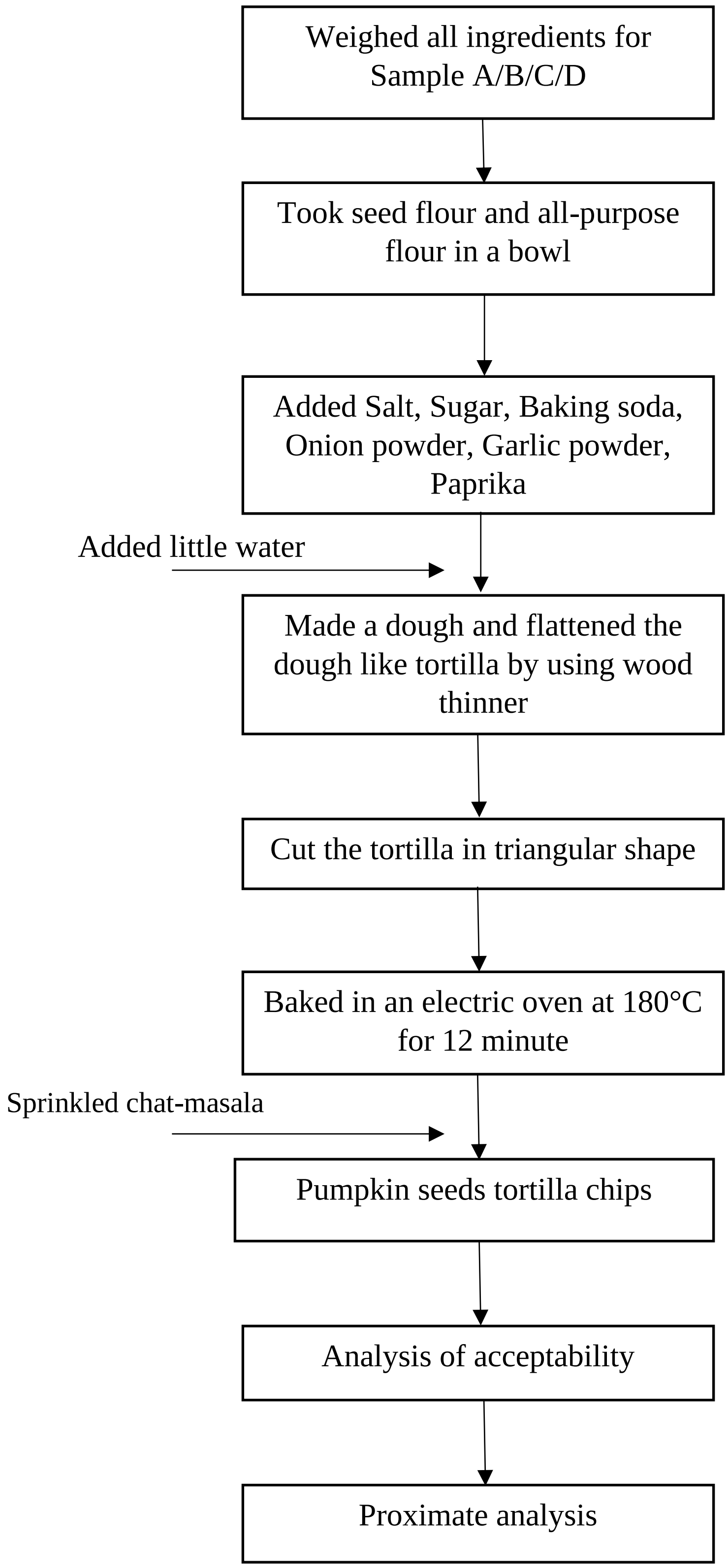

Figure 3.2: The development of pumpkin seed tortilla chips

3.4 Proximate composition analysis

The moisture content of the sample was determined using the standard method of AOAC (Association of Official Analytical Chemists). The protein, fat, fiber and ash content of seed were analyzed on dry weight basis according to the Association of Official Analytical Chemists (AOAC, 2016). The moisture was measured by oven drying at 105ºC to constant weight. The crude protein content was measured by Kjeldahl procedure (percentage of Nitrogen is multiplied by 5.85). Total lipid was extracted by the AOAC method using Soxhlet apparatus. Ash was measured gravimetrically in a muffle furnace by heating at 550ºC to constant weight. All determinations were done in triplicate and the result was expressed as the average value.

3.5 Mineral analysis

This method involves the extraction of minerals from the organic food matrix by digestion. Powdered sample of pumpkin seed was digested in an acid solution consisting of HNO_3 and $HClO_4$ into 2:1 ratio. One gram of pumpkin seed sample was weighted in a conical flask. 7 ml HNO_3 and 3 ml $HClO_4$ were added and then the flask was placed in a hot plate at 200W for 3 minutes until complete digestion. The solution was cooled down and filtered through filter paper into a 100 ml standard flask and diluted to the volume with distilled water. This solution was used for mineral content determination. Mineral contents (sodium, potassium, magnesium, calcium, phosphorus, iron and zinc) were determined by using a biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox) was used for biochemical assay. All the analyses were expressed in mg/100g.

3.5.1 Determination of Sodium (Na^+)

Sodium is precipitated as a triple salt with magnesium and uranyl acetate. The excess of uranyl ions is reacted with ferrocyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to the sodium concentration in the sample. In the precipitation step, 1 ml precipitating reagent and 0.02 ml sodium standard were added into the cuvette by using a pipette for standard. For sample, 1 ml precipitating reagent and 0.02 ml sample were added into the cuvette. These were mixed well and let them stand at retention time for 5 minutes with shaking well intermittently. Later these were centrifuged at 2500 to 3000 RPM to obtain a clear supernatant. In the color development step, for blank 1 ml acid reagent, 0.02 ml precipitating reagent and 0.1 ml color reagent were added into the cuvette with the help of a pipette. Similarly, 1 ml acid reagent, 0.02 ml supernatant, 0.1 ml color reagent were taken into cuvette for standard and sample preparation. After mixing them, incubated at R.T. for 5 minutes. The absorbance of blank, standard and sample were measured against distilled water within 15 minutes. The ratio of sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and the concentration of sodium was obtained in mmol/L.

3.5.2 Determination of Calcium (Ca^{++})

Calcium ions form a violet complex with O-Cresolphthalein in an alkaline medium. For the preparation of reagent blank solution, 25 µL distilled water and 1 ml working reagent were added into cuvette. For standard, 25 µL (Ca^{++}) standard and 1 ml working reagent were added. 25 µL sample extract and 1 ml working reagent were added for the preparation of sample solution. The absorbance of sample and standard were measured. The ratio of sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and concentration of calcium was obtained in mg/dl.

3.5.3 Determination of Potassium (K^+)

Sodium tetraphenylboron reacts with potassium to produce a fine turbidity of potassium tetraphenylboron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample. For the preparation of blank solution, 1 ml potassium reagent and 0.02 ml deionized water added into cuvette by pipette. For standard solution, 1 ml potassium reagent and 0.02 ml potassium standard and for sample solution, 1 ml potassium reagent and 0.02 ml sample extract were added into cuvette. After mixing these were incubated at retention time for 5 minutes. The absorbance of standard and sample were measured against blank within 15 minutes. The ratio of sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and potassium concentration was obtained in mg/dl.

3.5.4 Determination of Magnesium (Mg)

The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the cromophore formed is proportional to the concentration of magnesium in the sample. For the preparation of reagent blank solution 1 ml reagent was taken in cuvette. 1 ml reagent and 10 µL sample extract were added into cuvette for the preparation sample solution. For the standard solution preparation, 1 ml reagent and 10 µL magnesium standard were taken in cuvette. After mixing let these cuvettes stand for 2 minutes at room temperature. The absorbance of sample and standard at 520 nm were measured against the reagent blank. The ratio of sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and concentration of magnesium was obtained in mg/dl.

3.5.5 Determination of Phosphorus (P)

For blank solution preparation only 1 ml phosphorus reagent, for standard 1 ml phosphorus reagent, 10 µL phosphorus standard and for sample solution 1 ml phosphorus reagent, 10 µL sample extract were added into cuvette by pipette. Later these were mixed and incubated for 5 minutes. The absorbance of sample and standard were measured against the blank. The ratio of sample absorbance to standard absorbance is multiplied by standard concentration (mg/dl) and concentration of phosphorus was obtained in mg/dl.

3.5.6 Determination of Iron (Fe)

The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent from by means of ascorbic acid. Ferrous ions give with Ferrozine a colored complex. The intensity of the color formed is proportional to the iron concentration in the sample. For the preparation of blank solution, 1 ml reagent was added into cuvette with the help of pipette. For standard preparation, 200 µL standard and 1 ml reagent were added. 200 µL sample extract and 1 ml reagent were added for the preparation of sample solution. After mixing, incubated these for 10 minutes at room temperature. The absorbance of standard and sample were measured against blank. Concentration of iron was obtained in µg/dl.

3.5.7 Determination of Chloride (Cl^-)

Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red brown ferric thiocyanate complex. Intensity of the color formed is directly proportional to the amount of chloride present in the sample. For the preparation of blank solution, 1 ml chloride reagent, 0.01 ml deionized water were added into cuvette. 1 ml chloride reagent and 0.01 ml chloride standard were taken for standard solution preparation. 1 ml chloride reagent and 0.01 ml sample extract for sample solution preparation. After mixing these incubated at retention time for 2 minutes. The absorbance of standard and sample were measure against blank within 60 minutes. Chloride concentration in mmol/L was found by multiplying standard concentration (mg/dl) with the ratio of sample absorbance to standard absorbance.

3.5.8 Determination of Zinc

Zinc is an alkaline medium reacts with nitro – PAPS to form a purple colored complex. Intensity of the complex formed is directly proportional to the amount of zinc present in sample. For the preparation of blank solution, 1 ml working reagent, 0.05 ml distilled water were added into cuvette by pipette. For standard solution preparation, 1 ml working reagent and 0.05 ml zinc standard were taken into cuvette. For sample solution preparation, 1 ml working reagent and 0.05 ml sample extract were taken into cuvette. After mixing well, incubated at retention time for 5 minutes. The absorbance of the standard and sample were measured against the blank within 20 minutes. Concentration of zinc (µg/dl) was obtained by multiplying 200 with the ratio of sample absorbance to standard absorbance.

3.6 Vitamin A determination

Vitamin A was measured using colorimeter. The contribution of both retinol and beta-carotene is used to determine the total Vitamin A content of a specific food. Proteins are precipitated with alcohol and retinol and carotenes extracted into light petroleum. After reading the intensity of the yellow color due to carotenes, the light petroleum is evaporated and the residue dissolved in chloroform before carrying out the color reaction. Allowance is made for the carotene contribution to the reaction (Bradley and Hornback, 1973). Retinol present in sample reacts with trifluoroacetic acid (TFA). During the reaction of sample and TFA, a blue color is observed indicating the presence of retinol in sample. The blue color is transient, so if the color develops, it must be observed within 2 seconds after adding the reagent (Guamuch *et al*., 2007). For each sample preparation 100 mg sample, 1 ml distilled water and 2 ml ethanol was taken in a tube and mixed with a vortex mixer. The tube was centrifuged for 15 minutes at 3000 rpm and then 1 ml supernatant was taken. First carotene was determined. For blank solution preparation 6 ml S2 reagent and for standard preparation 6 ml standard reagent were taken into cuvette by pipette. For sample solution preparation 1 ml sample extract, 2 ml S1 reagent and 3 ml S2 reagent were taken into a cuvette by pipette. All mixed well with a vortex mixer and a mechanical shaker for 10 minutes. The tubes were centrifuged for 10 minutes at 3000 RPM. Then 2 ml blank, standard and supernatant from sample were collected and the absorbance was read at 420 nm against the blank. This was done without delay to prevent solvent evaporation and destruction of carotenoids by light. Then the retinol was determined. For the preparation of sample solution 2 ml sample extract (S3) that was prepared in carotene determination, was taken and evaporated the contents of the sample cuvette to dryness in a 50°C water bath. After evaporation, 100 µl S4 reagent and 1 ml S5 reagent were added in the sample cuvette. For the blank solution preparation 100 µl S4 reagent and 1 ml S5 reagent were taken into cuvette by pipette. For standard solution preparation 100 µl standard reagent and 1.0 ml S5 reagent were taken. These were mixed well with a vortex mixer. The absorbance was recorded at 620 nm at exactly 2s after adding the reagent. Because S5 reagent is a strong acid with an irritant vapor. The carotene, retinol and total vitamin content were measured as follows,

Retinol (mg/l) = (0.0759 × Absorbance) + 0.1023

Carotene (mg/l) = (- 0.0167 × Absorbance) + 0.0091

Where, 0.0759 and 0.0167 are slope; 0.1023 and 0.0091 are intercept

Total vitamin A (RAE) = µg of retinol + (µg of beta-carotene / 6)

3.7 Antioxidant capacity determination

The antioxidant activity of pumpkin seed was determined on the basis of radical scavenging capacity on the DPPH (2,2-Diphenyl-1-picrylhydrazyl) stable free radical. Antioxidant capacity of the extracts was determined by the method described by (Azlim *et al*., 2010) with slight modification. 1 gm sample was taken in falcon tube. After that 10 ml absolute methanol was added and left for 72 hours. Continuous straining was done after 4 hours intervals. The filtrate was collected after 72 hours, and the methanolic extract was found. Stock solution (1 mg/ml) of extract was diluted to concentrations of (0.50, 1.00, 1.50, 2.00, 2.50) mg/ml in methanol. DPPH solution was prepared by dissolving 6.0 mg of DPPH in 100 ml absolute methanol. The methanolic DPPH solution (2 ml) was added to 1 ml of each extract solution of different concentrations and the mixture was left for 30 minutes. The absorbance was read at wavelength 517 nm. Control was prepared by adding 1 ml of methanol to 2 ml of DPPH solution. Trolox was used as standard. Antioxidant capacity based on the DPPH free radical scavenging activity of extracts was calculated and expressed as milligrams of Trolox equivalents (TE) per gram of extracts (mg TE/g).

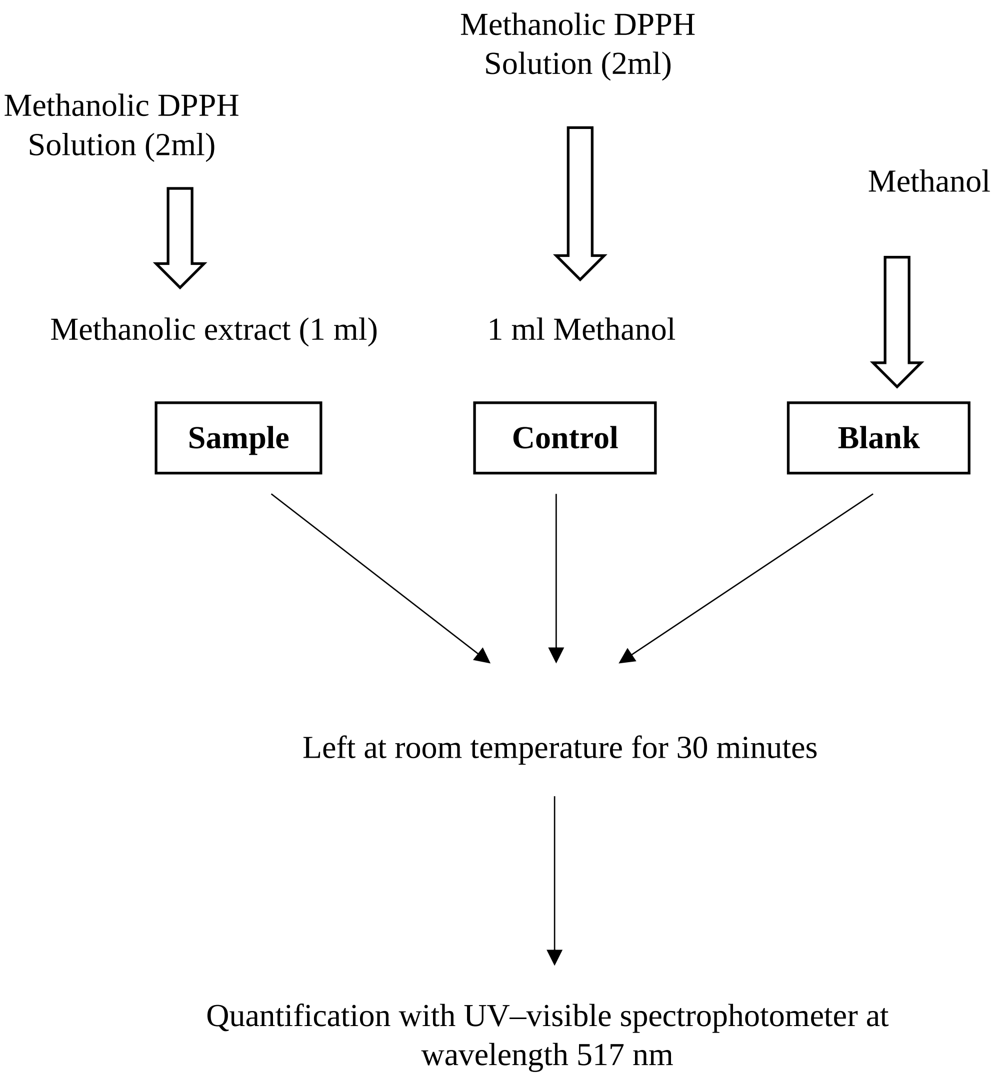

Figure 3.3: Determination of antioxidant capacity

3.8 Determination of chemical properties of oil

Oil extraction from pumpkin seed was done by Soxhlet extractor. Petroleum ether (b.p. 40°C – 60°C) was used as an extraction solvent. 1.6 gm oil was found from 4 gm pumpkin seed powder. The petroleum ether extract obtained was evaporated under reduced pressure to obtain oil.

3.8.1 Saponification value determination

A weighted quantity of the oil was saponified with a known amount of potassium hydroxide, excess of which was determined by titration. Saponification value of oil was determined by the method described by (Ogungbenle, 2003) with some modification. 7.125 g KOH pallets were dissolved in 4 ml of water and the solution was mixed with

250 ml of 95% ethanol. The solution was allowed to stand for overnight. The clear supernatant liquid was filtered off and the filtered solution was kept in a dark place for further use. Accurately weighed 0.314 g of oil sample was taken into a round bottomed flask. 25 ml of alcoholic KOH solution was added into a flask through a pipette, taking a definite draining time. Then it was boiled continuously for 1 h under a reflux condenser and swirling the contents of the flask at frequent intervals. The excess alkali was determined while the solution was stilled hot by titration with the 0.5 N HCl solution using 0.5 ml of the chosen indicator. Blank determination was carried out with the same KOH solution at the same time under the same condition. Saponification value can be measured as follows,

$$\text{Saponification value} = \frac{56.1 \times \text{N} \times (\text{B} - \text{A})}{\text{W}}$$

Where,

N = Normality of HCL solution

B = Volume of HCL (in ml) used for blank titration

A = Volume of HCL (in ml) used for the sample

W = Weight of sample taken (in g)

3.8.2 Iodine value determination

Several variations of iodine value have been developed, among them Hanus method is still widely used. The iodine number is determinate with a carrier for the iodine in which case the solution may be iodine monobromide in glacial acetic acid (Yildiz *et al*., 2019). 13.2 g of pure resublimed iodine was added to glacial acetic acid taken in a 500 ml volumetric flask by warning over water bath. When the iodine was completely dissolved, the solution was cooled and then about 1.5 ml of pure Br_2 was added to the solution. The solution was diluted to 500 ml with glacial acetic acid. The whole operation was conducted in a fume hood. 0.86 gm sample oil was taken in a well-stoppered bottle. The oil was dissolved 10 ml of chloroform. 25 ml Hanus solution was added to the stoppered bottle from a burette and the solution was allowed to stand for half an hour in dark place with occasional shaking. At the end of this period, 10 ml KI (15%) solution was added and shaken thoroughly and 100 ml of distilled water was also added by washing and free iodine on stopper. The solution was titrated with standard 0.1N $Na_2S_2O_3$ solutions, was added gradually with constant shaking until yellow color of the solution was almost disappeared. A few drops of starch solution were added and the titration was continued until the color was initially disappeared. The blank determination was also carried out by observing the same condition omitting the oil. The difference between the volumes, in ml, of 0.1 N sodium thiosulfate vs. consumed by the blank test and the actual test, multiplied by 12.69 (atomic weight of iodine) and divided by the weight in g of the substance taken for test is the iodine value. Iodine value of oil can be measured as follows,

$$\text{Iodine value} = \frac{(B - S) \times N \times 12.69}{W}$$

Where,

B = Volume of $Na_2S_2O_3$ required for the blank titration

S = Volume of $Na_2S_2O_3$ required for sample

N = Strength of the $Na_2S_2O_3$ solution

W = weight of oil taken in g

3.9 Sensory evaluation of Baked Tortilla Chips

Sensory evaluation was done by hedonic scale. The hedonic 7-point scale is a useful tool for any examiner of food preference or overall liking of food. The scale is easy for the panelist to understand and use. The scale is self-explanatory with little instructions from the moderator of the test (Lawless and Heymann, 1998). Sensory evaluation was assessed using 20 trained panelists in the CVASU premises, where the panelists were both the teacher and students of CVASU. They evaluated a total of 4 formulations of tortilla chips for overall acceptability, appearance, odor, taste, saltiness, crispness and hardness. Four formulations were encoded with sample A, sample B, sample C, sample D. The panelists tested the four samples without informing them of the formulations. Panelists were given the definitions of crunchiness and hardness to minimize judgement variability. Crunchiness was defined as when the product fractures after applying more force to break the sample than normal (Pineda, 2007). The panelists were asked to score the above sensory attributes. Water was given to each panelist in between samples. The products were rated using a seven point hedonic scale and the scale was arranged such that: Like extremely = 7, Like moderately = 6, Like slightly = 5, Neither like nor dislike = 4, Dislike slightly = 3, Dislike moderately = 2, Dislike extremely = 1. While scoring, highest score (7) was assigned to most preferred characteristic and least score (1) to the least desired characteristic. This method does not reflect actual consumer perception, but it strongly indicates attributes that a good quality product should possess.

3.10 Statistical analysis

Data were recorded and entered into the MS Excel -2013 and exported to Statistical Package for Social Sciences (SPSS version 20.0). Descriptive statistics (mean and standard deviation) were performed for proximate composition, antioxidant capacity and sensory evaluation of seed and pumpkin seed chips. Proximate composition and sensory evaluation data of chips were analyzed by using One-way ANOVA procedures to assess a significant level of variation at 95% confidence level. Level of significance was shown at $P<0.05$.

Chapter 4: Results

4.1 Proximate composition of pumpkin seed flour

The proximate composition showed that moisture content of pumpkin seed flour was quite low and was found to be (6.4 ± 0.64%), which may be advantageous in view of the product's shelf life. The result seed flour was rich in protein (38.5± 0.69%), which shows that seed can serve as a source of protein considering the level of protein deficiency in the society. The ash content of seed was (5.3± 1.11%), giving an idea of the inorganic content of the seed flour from where the mineral content could be obtained. Highest amount of fat content was exhibited in seed flour (37.9± 0.01%). Fats are essential as they increase the palatability of foods. The fiber and CHO content were found to be (0.4± 0.09%) and (11.7± 1.12%) respectively. The energy content of seed flour was found to be (542.8± 0.81) Kcal per 100 gm seed.

4.2 Mineral and Vitamin A composition of pumpkin seed flour

The result of nutritionally valuable minerals is presented in Table 4.1. Seed flour contained moderate concentrations of minerals. Although only fair amounts of zinc and iron were present, seed flour was a good source of some other minerals, especially magnesium (380 mg/100g), phosphorus (260 mg/100g), potassium (114 mg/100g) and calcium (58 mg/100g). The sodium content was quite high. The Vitamin A content of pumpkin seed was found to be 397.55 µg RAE per 100 gm. These variations may be occurred due to soil condition, seed condition and methodology.

Table 4.1: Mineral composition of pumpkin seed flour

Minerals	Amount (mg/100g)
Calcium	58
Magnesium	380
Sodium	137
Phosphorus	260
Iron	6.5
Zinc	7.98
Potassium	114
Chloride	34

4.3 Chemical properties of pumpkin seed oil

To identify the edibility and stability of oil, chemical properties play an essential role. The high iodine value indicates that the oil has a high content of unsaturated fatty acid, which enhances the nutritional value of food products in which it is used. High saponification value indicates oil suitability for industrial use. The mean value for chemical properties, saponification value and iodine value are showed in Table 4.2.

Table 4.2: Saponification and iodine value of pumpkin seed oil

Chemical parameters	Value
Saponification value (mg KOH/g)	194.7 ± 2.21
Iodine value (g I_2/100g oil)	107.3 ± 2.11

Legends: All values showed ME ± SD of data. ME = Mean. SD = Standard Deviation

4.4 Antioxidant activity of pumpkin seed flour

Antioxidant capacity of pumpkin seed flour at different wavelengths is shown in Table 4.3. From the table, the mean value of antioxidant capacity of pumpkin seed flour was found to be (2.7 ± 0.07) mg TE/100gm. (This value showed ME ± SD of data. ME = Mean. SD = Standard Deviation).

Table 4.3: Antioxidant capacity of pumpkin seed flour

Sl. no	Conc (mg/100g)	WL 517.0
1.	2.722	0.071
2.	2.729	0.070
3.	2.735	0.070

4.5 Nutritional composition of Baked Tortilla Chips supplemented with pumpkin seed flour

Nutritional value of Baked Tortilla Chips is shown in Table 4.4, almost all samples are significantly different. Sample A had the highest moisture content (1.5 ± 0.19) %, whereas Sample B had the lowest (0.5 ± 0.38) %. The highest value of ash content was found in Sample B (14.7 ± 1.00) % and the lowest value (9.9 ± 2.16) % was for sample D. Fiber content was higher in Sample D (3.1 ± 0.02) % and lower in Sample A (0.2 ± 3.14) %. Protein content was higher in Sample D (18.7 ± 0.09) % comparatively than Sample A, B and C. Fat content (9.6 ± 1.05) % was also higher in Sample D than other formulations. Carbohydrate content was higher in Sample A (64.7 ± 7.03) % and lower in Sample D (58.2 ± 0.71) %.

Table 4.4: Proximate analysis of Baked Tortilla Chips

Parameters	Sample A	Sample B	Sample C	Sample D
Moisture (%)	1.5 ± 0.19^{a}	0.5 ± 0.38^{d}	0.8 ± 0.50^{c}	1.3 ± 0.01^{b}
Ash (%)	13.4 ± 3.14^{b}	14.7 ± 1.00^{a}	10.5 ± 0.92^{c}	9.9 ± 2.16^{d}
Fiber (%)	0.2 ± 0.06^{d}	1.4 ± 0.01^{c}	2.3 ± 0.06^{b}	3.1 ± 0.02^{a}
Protein (%)	14.8 ± 0.85^{d}	16.5 ± 0.07^{c}	17.7 ± 0.15^{b}	18.7 ± 0.09^{a}
Fat (%)	5.1 ± 1.77^{d}	5.4 ± 1.37^{c}	6.1 ± 2.45^{b}	9.6 ± 1.05^{a}
CHO (%)	64.7 ± 7.03^{a}	61.8 ± 2.52^{b}	61.8 ± 1.31^{b}	58.2 ± 0.71^{c}

Legends: All values showed ME ± SD of data. ME = Mean. SD = Standard Deviation. The presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at $P<0.05$.

A, B, C and D represented Formulation 1 (0% pumpkin seed flour), Formulation 2 (5% pumpkin seed flour), Formula 3 (10% pumpkin seed flour) and Formula 4 (15% pumpkin seed flour) respectively.

4.6 Energy content of Baked Tortilla Chips supplemented with seed flour

Figure 4.1 shows the energy content of four formulations of chips. Energy content in Sample D was calculated in the highest amount (390.73 Kcal/100 g) and lowest (364.07 Kcal/100 g) in Sample B.

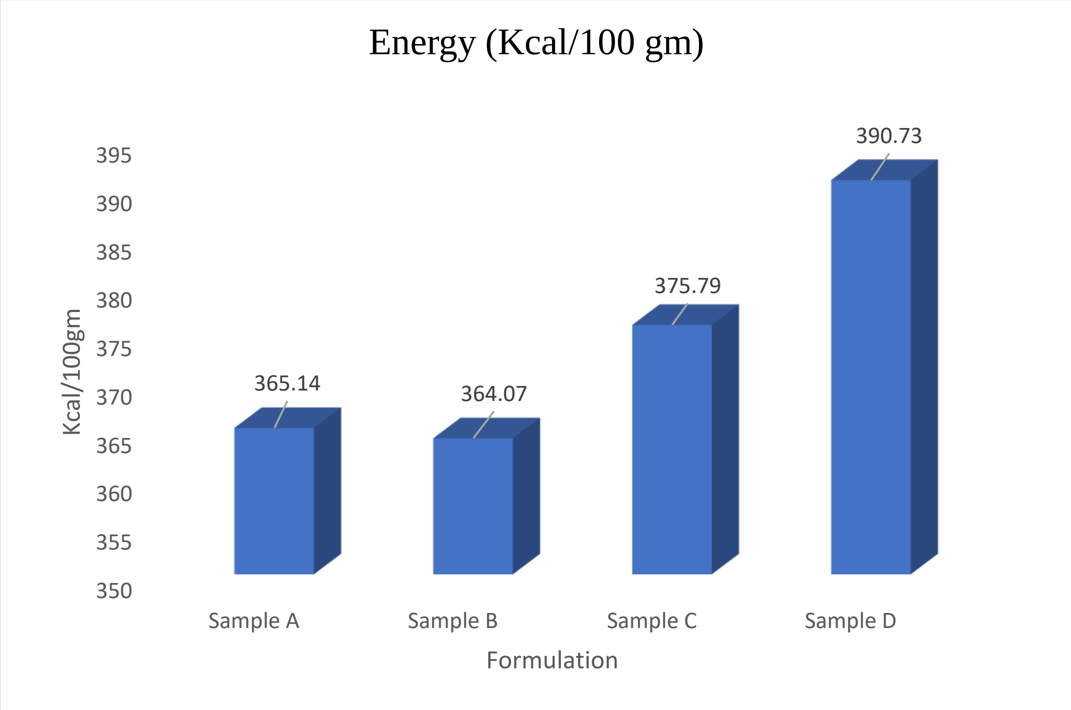

Figure 4.1: Comparison of energy content in Baked Tortilla Chips

4.7 Vitamin A content in Baked Tortilla Chips supplemented with seed flour

The findings for Vitamin A content in baked chips were presented in Table 4.5. From the table, a significant increase was observed. Sample A (Control) had the lowest value where Sample D had the highest value which was supplemented with 15% seed flour.

Table 4.5 Vitamin A content in Baked Tortilla Chips

Formulations	Vitamin A (µg RAE/100g)
Sample A	409.6 ± 0.01^{d}
Sample B	413.6 ± 0.02^{c}
Sample C	416.6 ± 0.01^{b}
Sample D	432.6 ± 0.01^{a}

Legends: All values showed ME ± SD of data. ME = Mean. SD = Standard Deviation. The presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at $P<0.05$.

4.8 Sensory evaluation of Baked Tortilla Chips

In Table 4.6 highest (ME ± SD) score for appearance, saltiness, crispness, hardness was recorded 6.1 ± 0.83, 5.4 ± 1.36, 5.9 ± 0.76, 5.8 ± 0.89 respectively in the case of Sample D. Sample C scored highest in odor (5.5 ± 0.95) and taste (5.6 ± 0.99). On the other hand, lowest score for all attributes was recorded in Sample A. Sample D had the highest acceptance rate (5.8 ± 0.86). However, Sample A scored the least acceptance (4.6 ± 1.05) compared to other samples.

Table 4.6: Hedonic rating test for sensory evaluation of Baked Tortilla Chips

Parameters	Sample A	Sample B	Sample C	Sample D
Appearance	5.1 ± 1.82^{c}	5.4 ± 1.04^{b}	5.6 ± 0.74^{b}	6.1 ± 0.83^{a}
Odor	5.3 ± 1.13^{a}	5.4 ± 1.23^{a}	5.5 ± 0.95^{a}	5.4 ± 1.14^{a}
Taste	4.3 ± 1.27^{c}	5.2 ± 1.11^{b}	5.6 ± 0.99^{a}	5.4 ± 1.05^{a}
Saltiness	5.0 ± 1.21^{b}	5.2 ± 1.41^{a}	5.4 ± 1.31^{a}	5.4 ± 1.36^{a}
Crispness	5.2 ± 1.48^{c}	5.6 ± 1.23^{b}	5.8 ± 0.95^{a}	5.9 ± 0.76^{a}
Hardness	4.3 ± 1.78^{d}	5.2 ± 1.33^{c}	5.5 ± 1.00^{b}	5.8 ± 0.89^{a}
Overall Acceptability	4.6 ± 1.05^{c}	5.1 ± 1.12^{b}	5.6 ± 0.93^{a}	5.8 ± 0.86^{a}

Legends: All values showed in ME ± SD of data. ME = Mean, SD = Standard Deviation. The presence of different superscript along a row indicates a significant difference and the same superscripts are not significantly different at $P<0.05$.

Chapter 5: Discussions

5.1 Proximate composition of pumpkin seed flour

The dried pumpkin seed contained (6.4 ± 0.64%) moisture. They were safe for long period of storage without spoilage. Generally, dried pumpkin seeds with this low moisture content are not highly susceptible to microorganism's attack (Ajayi *et al*., 2006). Moisture content may vary as it depends on environmental factors.

The lipid content was found to be (37.9 ± 0.01%) fell in the range of (9.8 – 52.1%) reported for various species of cucurbita (Stevenson *et al*., 2007). Due to genetic diversity and climate conditions such differences in the oil content can be observed (Stevenson *et al*., 2007). Also, the lipid content of the pumpkin seed in the recent study was found to exceed, or be comparable to, that of some common edible oils such as cottonseed (22 -24%), safflower (30 – 35%), soybean (18 – 20%) (Nichols and Sanderson, 2003). That's why the pumpkin seed can be regarded as a potential source of vegetable oil.

The total protein content of *Cucurbita maxima* seed was quantified to be (38.5 ± 0.69%) which shows that pumpkin seed is an excellent source of protein. Pumpkin seed is high in crude protein, about 35% reflects a significant and different amount of amino acids (Jafari *et al*., 2012). The study showed that the protein content of the pumpkin seed was higher than those of other oil seeds such as cashew nuts (22.8%), cottonseed (21.9%) and sesame (18.7%) and that of animal protein (16.0 – 18.0%) such as lamb, fish and beef (Ajayi *et al*., 2006). Seed can contribute to the daily protein need of 23.6 g/100 g for adults as recommended by some authorities (Ajayi *et al*., 2006). Due to the high protein content, seed flour can be an essential ingredient in formulating nutritious food recipes and can solve the protein malnutrition problem.

Total carbohydrate content was found to be (11.7 ± 1.12%) which was higher than 5.6% reported by (Lazos, 1986). In addition, it was lower than the CHO content of cashew nut (26.2%) and sesame (26.0%) (Achu *et al*., 2005). Most of the inorganic constituents or minerals remain in ash. Total ash content was obtained (5.3 ± 1.11%), which was closed to that obtained by some researchers (Alfawaz, 2004; Lazos, 1986). The presence of key minerals mainly characterizes ash content. Fiber is a crucial component of many complex carbohydrates. It is always found only in plants, particularly vegetables, fruits, nuts and legumes. Fiber content of pumpkin seed was found to be

(0.4 ± 0.09%) which was low compared to (Lazos, 1986). As energy was found to be (542.8 ± 0.81%), pumpkin seed can meet the daily energy need of an adult.

5.2 Mineral and Vitamin A content of pumpkin seed flour

A significant amount of valuable minerals are found in pumpkin seed. Table 4.1 gave detailed information of pumpkin seeds. The seeds are rich in potassium, magnesium, high in calcium, phosphorus. Pumpkin seeds are also good source of trace elements such as zinc, iron. Minerals such as zinc, iron, manganese possess antioxidant potential hence act as cofactors of crucial antioxidation - dependant biocatalyst (Seyman *et al*., 2016). Magnesium plays an essential role in the human body as a cofactor of many enzymes, proteins, RNA and DNA synthesis. Magnesium is a component of bone and teeth and is related with phosphorus and calcium (Elinge *et al*., 2012). The magnesium content of pumpkin seed was 380 mg/100 gm. High potassium content in the seed flour is beneficial for improving cardiovascular health. High amount of potassium is beneficial to people taking diuretics to control hypertension (Elinge *et al*., 2012). Calcium is an important mineral for the growth, maintenance of teeth, bone and muscle. Potassium and calcium were found 114 and 58 mg per 100g respectively. 7.98 mg/100gm of zinc was quantified from this study. The concentration of phosphorus in the seed flour was estimated as 260 mg/100 gm, which acts as a buffer that prevents change in the acidity of the body fluids (Elinge *et al*., 2012). Zinc is essential in male reproduction, structural proteins and cellular protection (Aghaei *et al*., 2014). Zinc was found 7.98 mg/100gm. The concentration of sodium in seed flour was 137 mg/100 gm. Sodium is needed by the body to regulate blood pressure and blood volume. Iron content was found 6.5 mg/100 gm. Iron helps to formulate blood and transfer oxygen and carbon dioxide from one tissue to another. Retinol Equivalent Activity (RAE) represents Vitamin A activity as retinol. The level of Vitamin A in seed flour was found to be 397.55 µg RAE per 100 gm. This may vary due to geographical and analysis differences (Elinge *et al*., 2012). Pumpkin seed can be useful for food fortification or bakery products because of mineral concentration.

5.3 Chemical properties of pumpkin seed oil

The pumpkin seed oil is greenish brown in color with nut like taste, liquid at room temperature and also in refrigerator. From this study, the pumpkin seed oil had an iodine value of 107.3±2.11 g I_2 / 100 gm oil, indicating a high degree of unsaturation. The higher degree of unsaturation means the higher iodine value. So the greater is the liability of the oil or fat to become rancid by oxidation. The pumpkin seed oil has a higher chance of becoming rancid by oxidation.

Oil's saponification value was 194.7±2.21 mg KOH/g oil and fell in the 174 – 197 range reported for the pumpkin seed oils (Nichols and Sanderson, 2003). This value showed that the pumpkin seed oil had fatty acids with higher number of carbon atoms in comparison with coconut (248 -265) and palm kernel (230 – 254) oils (Nichols and Sanderson, 2003). High saponification value shows the presence of low proportion of lower chain fatty acids. The present result also indicates that pumpkin seed oil contains high proportion of higher chain fatty acids.

5.4 Antioxidant capacity of pumpkin seed

DPPH was an extensively used substrate to evaluate antioxidant activity especially for investigating the free radical scavenging activities of biological as well as chemical substances. From the result of antioxidant capacity (2.7 ± 0.07) mg TE/100gm, pumpkin seed is a good source of antioxidant compounds. Tocopherols and tocotrienols in pumpkin seeds are powerful antioxidant with the ability to deactivate highly active radicals by releasing H^+ ions from its ring (Bharti *et al*., 2013). If pumpkin seed is added to a food product especially to lipids or lipid containing foods, can increase the shelf life by retarding the lipid peroxidation and prevent deterioration of food products due to natural antioxidant properties.

5.5 Nutritional composition of Baked Tortilla Chips supplemented with pumpkin seed flour

Four formulations of supplemented baked tortilla chips made from all-purpose flour and pumpkin seeds flour were evaluated. Chemical analysis of nutritious chips revealed that the addition of pumpkin seeds in chips affected the moisture content of chips. Sample B (0.5±0.38 %), C (0.8±0.50 %) and D (1.3±0.01 %) had low moisture content than Sample A (1.5±0.19 %) as sample A had no pumpkin seed flour. Low level of moisture in pumpkin seeds causes a decrease in moisture in chips which enables them to preserve for long time (Kanwal *et al*., 2015). The final moisture content in the chips must be less than 2% to ensure a crisp texture (Pineda, 2010). Higher moisture content result in tough, chewy texture. Ash content was found quite good in Sample B (14.7±1.00 %), C (10.5±0.92 %), D (9.9±2.16 %), which were incorporated with pumpkin seed flour. It suggests that baked tortilla chip made with pumpkin seed flour is a better source of minerals. Fiber content of the chips increased with increasing fortification level while the control (Sample A) had the least value of fiber (0.2±0.06 %) due to low fiber content in all-purpose flour. Highest fiber content was recorded (3.1±0.02 %).in Sample D. Increasing fiber content is desirable because fiber is a good source to control cholesterol level, many digestive problems, and decrease risk of cancer (Kanwal *et al.*, 2015).

Protein content became high with the increasing substitution of pumpkin seed flour. High protein content was observed in Sample D (15% pumpkin seed flour + 85% all-purpose flour). Sample D contained (18.7±0.09 %) protein while in control (14.8±0.85 %). It is desirable as it will increase the protein content in the diet. Proteins obtained from the chips have excellent digestibility and high biological value (Mansour *et al.*, 1993). With an increase of supplementation level in the chips the mean values for fat contents of nutritious chips increase and highest fat content were found in sample D (9.6±1.05 %) than sample A (5.1±1.77 %), sample B (5.4±1.37 %) and sample C (6.1±2.45 %). Fat is a concentrated source of energy which is stored in the body as reserves to be used when the body requires energy supply. Generally fat helps in the protection of internal organs such as heart, kidney, lungs and subcutaneous tissues of the skin (Mohammed *et al.*, 2017). The carbohydrate content ranged from 58.2% in the sample D to 64.7% in sample A. There were no significant differences in sample B and C. The Recommended Dietary Allowances for carbohydrate is 130g/day for adults and children aged one year or older (Slavin, 2018). Energy content in chips made with pumpkin seed flour (Sample B to Sample D) ranged from 364.07% to 390.73%, which states that these chips are a good source of energy to meet the daily need.

Vitamins are organic compounds necessary for growth, metabolism and nutrition. A small amount of vitamins are required in the diet (Bardaa *et al.*, 2016). Vitamin A level was highest in Sample D (15% seed flour). Sample D had (432.6±0.01) µg RAE Vitamin A per 100 gm sample. Sample B and Sample C contain (413.6±0.02) µg RAE and (416.6±0.01) µg RAE Vitamin A respectively. On the other hand, Sample A with no seed flour had the lowest Vitamin A content (409.6±0.01) µg RAE per 100 gm than other samples. Vitamin A content found in control due to using soyabean oil that was already Vitamin A fortified. So it was observed that addition of pumpkin seed flour significantly increased the level of Vitamin A in the baked chips. The recommended daily amount of Vitamin A is in the range of (600 – 900 µg RAE) for adults (male and female) and (300 – 400 µg RAE) for children (Russell *et al*., 2001). Therefore, these chips can provide a good amount of Vitamin A in the diet.

5.6 Sensory evaluation of Baked Tortilla Chips

The utilization of pumpkin seed flour with different proportions to produce chips was sensory evaluated and then compared to control chips which contained 0% pumpkin seed flour (Sample A). Supplementation of chips with different ratios of pumpkin seed flour significantly affected the sensory attributes of produced chips.

In case of appearance, the control chips, Sample A, had the lowest score (5.1±1.82), while the chips sample supplemented with 15% of pumpkin seed flour (Sample D) had the highest score (6.1±0.83). Sensory evaluation findings indicated that the increase of supplementation ratio up to 15% of pumpkin seed flour prominently increased the acceptance of chips appearance as color became gradually better.

Odor of supplemented chips scored quite similar to control and no significant difference was found as there was no extra flavor added and the seeds were not roasted. The development of flavor could be attributed to the roasting of pumpkin seeds during processing, which is necessary for the development of aroma characteristics in seeds (Siegmund and Murkovic, 2004). These aromatic seeds lead to good results aroma in chips production.

Regarding the taste, significant differences were observed among all supplemented chips. The chips sample supplemented with 10% of pumpkin seed flour (Sample C) recorded highest score (5.6±0.99) while the control (Sample A) recorded the lowest score (4.3±1.27). Sample D (5.4±1.14) also scored close to sample C and no significant difference was observed. In case of saltiness, crispness and hardness sample D supplemented with 15% pumpkin seed flour recorded the highest score than sample B and sample C, whereas the control recorded the lowest score.

The highest score for overall acceptability was recorded by sample D (5.8±0.86), which contained 15% of pumpkin seed flour and the samples supplemented with 5% and 10% of pumpkin seed flour were sample B (5.1±1.12) and sample C (5.6±0.93) respectively. In contrast, control sample A was the lowest score (4.6±1.05). Findings indicated that an increase of pumpkin seed flour in the formulation improved the overall acceptability of chips for panelists. Overall acceptability was different quality parameters and was not affected by the individual trend of color, flavor and texture (Kanwal *et al*., 2015).

Chapter 6: Conclusion

This study concludes that pumpkin seed based – baked tortilla chips can be used to increase supplemental nutritional intake. The results of the study mirrored the potentiality of supplementation baked tortilla chips using different ratios of pumpkin seed flour. The nutritional analysis of supplemented chips revealed a significant increase in protein, fat, fiber, energy and Vitamin A as the level of pumpkin seed flour increased. These chips can be a good option for protein fortification. In terms of acceptability, the panelists gave preference to the sample supplemented with pumpkin seed flour which is superior to the control. Pumpkin seed itself is rich in protein, fat, CHO, energy, calcium, potassium, magnesium, phosphorus, iron, zinc which can be eaten as raw or roasted and make the baked tortilla chips suitable for improving nutrient intake without compromising flavor. This baked tortilla chips can provide a new era for introducing pumpkin seed to consumers who normally resist eating them as raw. So to improve the nutritional status especially protein and energy, these baked tortilla chips supplemented with pumpkin seed flour could be considered one of the best types of healthy chips ready-to-eat for children and adults alike.

Chapter 7: Recommendations and Future Perspectives

Pumpkin seeds have received considerable attention in recent years due to their nutritional value. Half of the people are suffering from malnutrition in our country and in this case, tortilla chips made with pumpkin seed could be a great source of nutrients and energy. The present study is conducted to investigate the formulation and quality (proximate analysis of seed and chips, mineral analysis of seed, chemical constituents such as iodine and saponification value of seed, sensory) evaluation of baked tortilla chips made from pumpkin seed flour. In our country, pumpkins are widely available and we get pumpkin seed as a by-product. This pumpkin seed can be developed into a snack food as this can be a healthy snack choice for malnourished children and people by considering the nutritional factors. Based on the present investigation, the following suggestions and prospects are made for further research work:

- Mineral parameters of chips should be analyzed including selenium, manganese.
- Vitamin C and fat soluble vitamins like D, E, K should be analyzed.
- Bioactive compounds of chips should be observed.
- The physical characteristics test and the shelf life of chips should be analyzed.
- The findings will be helpful from a therapeutic point of view as they have medicinal value.
- The composition may be modified with a different flavor for better taste.
- Modern packaging and storage conditions would be developed to better tortilla chips.
- Awareness should be created about the health benefits of pumpkin seed and its possibility of being scope for the food industry.

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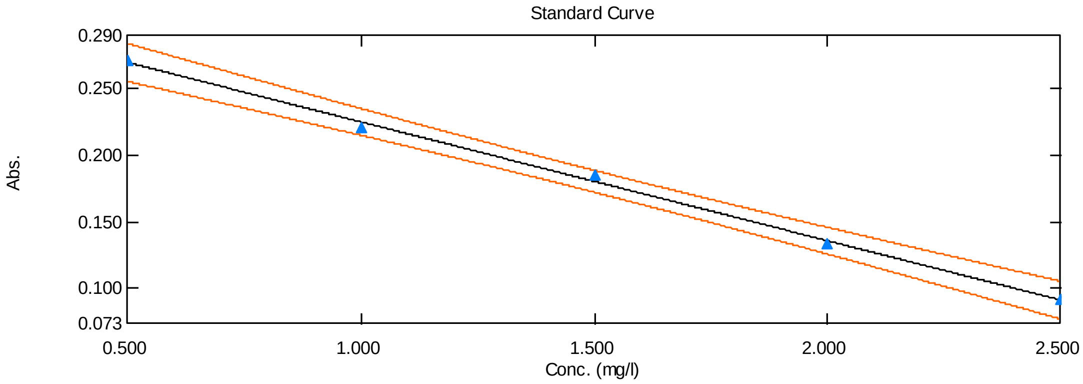
Appendices

Appendix A: Antioxidant capacity of Baked Tortilla Chips

Standard table of Trolox

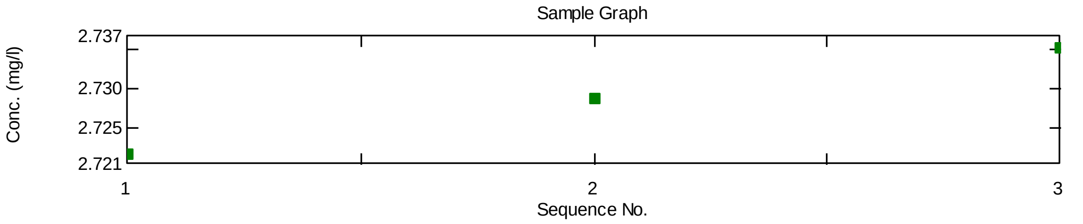
Sample ID	Type	Conc.	WL517.0
STD 1	Standard	0.500	0.272
STD 2	Standard	1.000	0.221
STD 3	Standard	1.500	0.185
STD 4	Standard	2.000	0.133
STD 5	Standard	2.500	0.092

Standard curve of Trolox



y = - 0.0894539 x + 0.314536
r2 = 0.99735

Sample graph



Appendix B: Hedonic test for Baked Tortilla Chips

Date: **Sample code:**

Gender: **Age:**

Instruction: You are given four samples. Please start your evaluation from left to right. Evaluate each attribute by circling the appropriate scale which indicates your degree of liking. Rinse your mouth with plain water before testing each sample.

(1 – Dislike extremely, 2 – Dislike moderately, 3 – Dislike slightly, 4 – neither like nor dislike, 5 – Like slightly, 6 – Like moderately, 7 – Like extremely).

Appearance

Dislike extremely Neither like nor dislike Like extremely

Odor

Dislike extremely Neither like nor dislike Like extremely

Taste

Dislike extremely Neither like nor dislike Like extremely

Saltiness

Dislike extremely Neither like nor dislike Like extremely

Crispness

Dislike extremely Neither like nor dislike Like extremely

Hardness

Dislike extremely Neither like nor dislike Like extremely

Overall acceptability

Dislike extremely Neither like nor dislike Like extremely

Comment (if any):

Appendix C: Photo Gallery

Washing

Sun drying

Grinding

Sieving

Dough making

Baking in oven

Sample A (no seed flour)

Sample B (5% seed flour)

Sample C (10% seed flour)

Sample D (15% seed flour)

Baked Tortilla Chips

Sensory evaluation

Digestion
Mineral determination

Fat determination

Protein determination

Digestion
Protein determination

Distillation
Protein determination

Titration
Protein determination

Fiber determination

Preparing samples for spectrophotometric

Working in UV - spectrophotometer

Soxhlet extractor

Reflux condenser (Saponification determination)

Iodine value determination

Vitamin A determination by colorimeter

Brief Biography

Tahira Tasnim Fariha passed the Secondary School Certificate Examination in 2011 and then Higher Secondary Certificate Examination in 2013. She obtained her B.Sc (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest in improving the health status of poor people through proper guidance and suggestions and creating awareness about food science and nutrition.