NUTRITIONAL QUALITY OF PUMPKIN (*Cucurbita pepo* L.) SEEDS BASED ON THE MINERAL AND FATTY ACID COMPOSITION

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DECLARATION

I, Pamela Akwap (15/U/GMFT/14516/PE) declare that this dissertation is my own original work and has never been submitted to any university or any other higher institution of learning for a similar or other academic award.

Signature…………………………………… Date…………………………………….

APPROVAL

This is to certify that the work presented herein is the student's own, done by her under my supervision and guidance, and is now ready for submission to the Board of Examiners of Kyambogo University.

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DEDICATION

Dedicated to my daughter, family and friends for their support which enabled me to accomplish the program.

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LIST OF ACRONYMS AND ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
EFA	Essential fatty acid(s)
FAME	Fatty Acid Methyl ester(s)
MUFA	Monounsaturated fatty acid(s)
PUFA	Polyunsaturated fatty acid(s)
SFA	Saturated fatty acid(s)
SCFA	Short chain Fatty acid(s)
USDA	United States Department of Agriculture

ABSTRACT

Pumpkin seeds contribute to the nutrition of human populations and are also used in medicine in many parts of the world. In Uganda, pumpkin seeds are nowadays promoted and consumed as a snack. The potential of a food to be recommended for human consumption is determined primarily by the composition of its nutrients. The probable contribution of pumpkin seed towards the mineral, and essential fatty acid and total dietary lipid intakes in Uganda is not well known. The objective of this study was to determine the mineral content and oil yield of pumpkin seeds, analyze the fatty acid composition, and assess the nutritional quality of the lipid fraction. Mineral content was determined using atomic absorption spectrophotometry (AAS). Fatty acid composition was determined using gas chromatography with flame ionization detection (GC-FID). Nutritional quality was determined using total polyunsaturated fatty acid (PUFA)/total saturated fatty acid (SFA), ω6/ω3 and hypocholesterolemic/hypercholesterolemic (h/H) ratios, and the atherogenicity (AI) and thrombogenicity indices.

Potassium (K) and magnesium (Mg) were the major macro-elements with mean concentrations of 416.45±27.66 and 407.56±58.93 mg/kg, respectively. Iron (Fe; 7.56±0.68 mg/kg) and zinc (Zn; 5.57±0.65 mg/kg) were major microelements. The oil yield ranged from 9.73% to 40.81%. The predominant fatty acids in decreasing order were linoleic (C18:2ω6), palmitic (C16:0), oleic (C18:1ω9) and stearic (C18:0) acids. Low linolenic acid levels (C18:3ω3; < 1%) were observed. The PUFA/SFA and h/H ratios, and the values of AI and TI as 0.23 and 0.90 repetitively for the pumpkin seeds were favorable. Pumpkin seeds are a health food for human consumption.

KEY WORDS: *Cucurbita pepo* L., mineral, oil yield, fatty acid, AI, TI, h/H ratio.

CHAPTER 1: INTRODUCTION

1.1 Background

Pumpkin (*Cucurbita* species) is an angiosperm belonging to the order *Cucurbitales*, family *Cucurbitaceae* and genus *Cucurbita* (Ondigi, Toili, Ijani, & Omuterema, 2008). Varieties of pumpkin fall under the species *Cucurbita pepo* (Linn), *Cucurbita maxima* (Duchesne), *Cucurbita moschata* (Duchesne) and *Cucurbita mixta* (Linn). *Cucurbita pepo* is the species with the greatest monetary value and among the ten leading vegetable crops grown around the world (Arslan, Akin, & Yilmaz, 2017). Pumpkin seeds are a high-energy source that contributes significantly to human nutrition in many parts of the world (Caili, Huan, & Quanhong, 2006; Srbinoska, Hrabovski, Rafajlovska, & Sinadinović-Fišer, 2012). They are rich in triterpenes, lignins, phytosterols, antioxidative phenolic compounds, carotenoids, tocopherol, dietary fibre and minerals (Abuelgassim & AL-showayman, 2012; Caili *et al*., 2006). They also have hepatoprotection, inhibition of benign prostatic hyperplasia, antioxidant, anticancer, antimicrobial, antiinflamatory, antidiabetic and antiulcer activities hence their application in ethnomedicine (Gutierrez, 2016). The medicinal properties of pumpkin seeds largely stem from their mineral composition, and the phytochemical composition of the pumpkin seed oil (Bardaa *et al*., 2016; Gutierrez, 2016; Caili *et al*., 2006).

The World Health Organization recommends consumption of between 300 and 400g fruits and vegetables per person per day because of their high concentrations of dietary fiber, vitamins, minerals such as zinc (Zn), magnesium (Mg), calcium (Ca), manganese (Mn), copper (Cu), iron (Fe), potassium (K) and sodium (Na) and more recently phytochemicals (Nishida, Uauy, Kumanyika, & Shetty, 2004). WHO also recommends favoring consumption of unsaturated fatty acids from vegetable oils to reduce the risk of developing non-communicable diseases such as diabetes and cardiovascular disease (WHO, 2015). The composition of dietary fat is important in determining health outcomes of persons with metabolic disorders (Tvrzicka *et al*., 2011). Dietary FA influence plasma lipoproteins, cell membranes, as well as adipose tissue fatty acid composition (Pedersen *et al*., 2011). Because of their effects on serum cholesterol and low density lipoprotein-cholesterol concentrations, FA can promote or prevent atherosclerosis and coronary thrombosis (Osmari, Cecato, Macedo, & Souza, 2011). The medium chain saturated fatty acids (SFA) lauric (C12:0) and myristic (C14:0) acids, and the long chain palmitic acid (C16:0) are atherogenic and are considered a health risk factor (Feddern *et al*., 2010). On the contrary, polyunsaturated fatty acids (PUFA) such as the linoleic acid (18:2ω6) have anti-atherogenic action (Garaffo *et al*., 2011). Linolenic (18:3ω3), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids, have anti-thrombogenic effects.

In Uganda, pumpkin seeds are nowadays promoted and consumed in substantial amounts as snacks. The seeds are rich in zinc which is essential for male fertility but cannot be stored in the body, thus requiring regular intake (Fallah, Mohammad-Hasani, & Colagar, 2018). The pumpkin seeds are also a rich source of phytosterols which prevent the enlargement of the prostate and secrete chemicals that prevent the transformation of testosterone into dihydrotestosterone (DHT), which can result into an enlarged prostate gland. Therefore, the roasted and seasoned seeds are promoted and eaten as a snack particularly among men in Uganda. Pumpkin seed oil is rich in unsaturated fatty acids (Murkovic, Hittebrand, Winkler, Leitner, & Pfannhauser, 1996). However, information on the nutritional quality of pumpkin seed varieties in Uganda is scanty. The PUFA/SFA, ω-6/ω-3 PUFA and hypocholesterolemic/hypercholesterolemic (h/H) ratios, and atherogenicity (AI) and thrombogenicity (TI) indices are commonly used to assess the nutritional quality of food lipids. Therefore, this study aimed to determine the mineral, oil content of the seeds from Nakawa, Kibuye, Kalerwe, and Nakasero markets in Kampala city, as well as analyze the FA composition, and assess the nutritional quality of the lipid fraction based on FA composition.

1.2 Statement of the problem

In Uganda, pumpkin seeds are largely considered an agro-waste with limited data on their composition. Although there is vast potential for pumpkin production and utilization, only a small proportion of households cultivate pumpkins as a source of livelihood. In addition, pumpkins and their seeds are generally underutilized. Because of the lack of scientific knowledge, pumpkin seeds are particularly not a popular source of oil in Uganda. There is significant association between consumption of saturated fatty acids (SFA) and risk of coronary heart disease (CHD). Therefore, nutritional bodies recommend replacing saturated fat in the diet with unsaturated fat. Pumpkin seed oil is rich in unsaturated fatty acids and could be used as such. While many studies such as Stevenson et al. (2007), Habib *et al*. (2015) and Bardaa et al. (2016) have reported on the composition of pumpkin seeds around the world, in Uganda, more attention has been focused on the pumpkin flesh. The luck of sufficient data on the chemical composition of pumpkin seeds in Uganda also means that the nutritional quality of especially the lipid fraction cannot be clearly known.

1.3 Justification

According to Muskesh, Shalini, Radha, Prasad and Hariom (2010), pumpkin seeds are the most important part of the pumpkin due to their nutrient and medicinal properties. In many parts of the world, the seeds are consumed at a domestic level when raw, roasted or cooked (Patel, 2013; Gutierrez, 2016). Extracts and metabolites of the pumpkin plant, particularly those from seeds and fruits possess useful pharmacological activities (Gutierrez, 2016). However, due to increased public awareness, pumpkin seeds have shown potential to capture a new and emerging market share in the snack food industry (Devi, Prasad, & Sagarika, 2018). Studies on pumpkins are now focused on the mineral composition of the seed, and the FA, tocopherol and sterol composition of the seed oil (Stevenson *et al*., 2007; Bardaa *et al*., 2016). There are no similar studies in literature reported for Uganda. Moreover pumpkin seeds are promoted as a health food (Habib *et al*., 2015). The results of this study were therefore expected to highlight the pumpkin seed potential as a functional food for nutritional improvement as well as health benefit for compromised individuals.

1.4 Objectives of the study

1.4.1 General objective

To assess the mineral, oil content, fatty acid profile and potential contribution of pumpkin seeds towards the total mineral and fatty acid intake.

1.4.2 Specific objectives of the study

1. To determine the levels of macro and micro elements (Na, K, Mg, Ca, Fe, Mn, Cu, Zn and P) in pumpkin seeds obtained from Nakasero, Kalerwe, Nakawa and Kibuye markets.

2. To determine the oil yield of the pumpkin seeds from the four markets in 1.
3. To determine the fatty acid composition and nutritional quality of the pumpkin seed oil based on fatty acid composition.

1.5 Hypotheses

1. Pumpkin seeds are a good source of minerals.
2. Pumpkin seeds are a rich source of vegetable oil.
3. Pumpkin seed oil contains high amounts of polyunsaturated fatty acids.

CHAPTER 2: LITERATURE REVIEW

2.1 General aspects of the pumpkin

Pumpkin is the fruit of the species *Cucurbita pepo* or *Cucurbita mixta* (Caili, Huan, & Quanhong, 2006). It can also refer to a specific variety of the species *Cucurbita maxima* or *Cucurbita moschata*, which are all of the genus *Cucurbita* and the family *Cucurbitaceae*. Among these species, *Cucurbita pepo* L. is an economically important member of the family and among the ten leading vegetable crops worldwide (Saavedra *et al*., 2015). It is also the most frequently consumed *Cucurbita* species in Uganda.

Pumpkins are warm-season crop that must not be seeded until the soil temperature reaches 15.6°C three inches beneath the soil surface (Deyo & O‘Malley, 2008). Therefore, in America, they are generally seeded in the field during the first couple weeks of July. Pumpkin crops may suffer if there is lack of water and at temperatures below 18.3°C (Deyo & O‘Malley, 2008). They also do not do well in sandy soil with poor water retention or poorly drained soils that become waterlogged after heavy rain. However, pumpkins are rather hardy, and even if many leaves and portions of the vine are damaged, the plant quickly re-grows secondary vines to replace what was lost (Williams & Roger, 2013). Pumpkins are grown all around the world for a number of reasons ranging from agricultural purposes (such as animal feed) to commercial, and also for ornamental reasons (Košťálová, Hromádková, & Ebringerová, 2009).

Cucurbita pepo pumpkins generally weigh between 2.7 and 8.2 kg, although the largest cultivars (of the species *C. maxima*) regularly reach weights of over 34 kg. Mature pumpkin fruits contain many flat, dark green seeds. The seed content of pumpkin fruit varies from 3.5% to 4.27% of fruit weight (Devi, Prasad, & Sagarika, 2018). This is because the number of seeds produced by a fruit depends on pollination efficiency and growing conditions (Kiramana, Isutsa, & Nyende, 2017). Seeds are encased in yellow-white husks although some varieties of pumpkins produce seeds without shells.

2.2 Pumpkin production

There is a growing trend in the world production of pumpkins. In 2016, world production of pumpkins was 26,486,616 tons. China, India, Russia, Ukraine and United States of America are the five highest producers. Africa's pumpkin production was estimated at 2,346,145 tons, reflecting 8.9% of the world production. Egypt is the leading producer in Africa, followed by South Africa, Rwanda, Algeria, Morocco, Cameroon, Niger, Tunisia, South Sudan and Sudan in that order (Table 1). However, East Africa (Kenya, Tanzania, Rwanda, Burundi, South Sudan and Uganda) contributes 381,112 tons towards the global production (FAOSTAT, 2017). According to FAOSTAT (2017), global pumpkin production has been increasing at an annual rate of 2.6%. Data on pumpkin production in Uganda is scarce but in line with global trends, utilization of pumpkins and their products, as well as production are on the increase.

Table 1: Pumpkin production in the world and the top ten African producers (tons)

Country	2012	2013	2014	2015	2016
World	23,917,255	24,522,500	25,107,419	25,256,633	26,486,616
Egypt	559,606	494,664	444,653	458,218	463,451
South Africa	244,000	247000	423,074	446,900	419,791
Rwanda	236,000	230,694	269,951	273,250	280,502
Algeria	227,789	260,913	285,293	302,449	271,054
Morocco	256,520	224,315	215,503	201,227	189,256
Cameroon	166,845	171,609	176,373	181,137	185,901
Niger	30,085	69,636	53,345	133,005	123,366
Tunisia	78,300	80,300	86,400	89,700	90,080
South Sudan	50,000	58,000	65,000	72,273	80,430

Source: FAOSTAT (2017)

2.3 Uses of pumpkin plant and pumpkin fruit

The use of *Cucurbita* species and their active constituents in various clinical and pharmacological studies revealed the presence of multiple, effective and useful compounds, which provide the opportunity for further production of antidiabetic, analgesic, anti-inflammatory and cardio protective drugs and foods (Salehi *et al*., 2019). *C. pepo*, as the whole plant, is applied in the folk medicine of Mesoamerica and Caribbean for the therapy of fitness due to its pancreatic lipase inhibition activity. Topical use of *C. pepo* fruit as an external antiseptic was reported in Spain, whereas in the same location the flowers of this plant are used for antigenic, antidermatitic, antiecchymotic, antiophidian, antipyretic and anti-toxic purposes

(Salehi *et al*., 2019). Pumpkins are used in a varied number of ways in different countries due to their nutritional and medicinal value (Ardabili, Khodaparast, & Farhoosh, 2011). Immature fruits are consumed as a vegetable (Gutierrez, 2016). The mature fruit which is usually sweet is ground into a powder and used to make confectioneries, beverages baked goods and baby foods (Gutierrez, 2016; Mukesh, Shalini, Radha, Prasad, & Hariom, 2010). On industrial scale, pumpkin flesh is used in soups, purees, jams and pies (Young, Jin, Kim, Choi, & Lee, 2012; Alfawaz, 2004). In East Africa, pumpkins are mainly used for food or pudding/dessert and as a supplementary food (Ondigi, Toili, Ijani, & Omuterema, 2008). In Uganda, pumpkin leaves are used to make a local sauce when cut into small pieces. The fruit flesh is served either as a side dish or a main course normally with beans. Pumpkin pulp has also locally been used as a weaning/complementary baby food. Pumpkin based products such as porridges, baby foods and baked products in which pumpkin pulp or seed flour is used, are on the increase in Uganda.

2.4 Overview of pumpkin seeds

Pumpkin seeds are a valuable part of the pumpkin not just because of their nutritional benefits but also for their medicinal properties (Stevenson *et al.*, 2007). According to Bisognin (2002) pumpkin seeds were the first pumpkin part to be used as food. The seeds are green in colour with an oblong shape and in most varieties, the seeds are covered with a plump and tan or dull white testa that serves as a protectant around the seeds (Elinge *et al*., 2012; Alfawaz, 2004). However some seeds have no testa, they are hull-less (Ardabili, Khodaparast, & Farhoosh, 2011). *Cucurbita pepo* seeds are mainly regarded as agro-industrial waste, while in some parts of the world they are used raw, roasted or cooked, at a domestic scale (Salehi *et al*., 2019).

Pumpkin seeds have a malleable, chewy texture and a subtly sweet, nutty flavor. They are used in culinary practices mainly in Europe (Austria, Slovenia, and Hungary and in many African countries, Roasted pumpkin seeds are a popular snack in many African countries (Rezig *et al*., 2012; Apostol, Moșoiu, Iorga, & Martínez, 2017). The seed powder is used in China and the United States as an ingredient of salad dressings and in baked products (Raihana, Marikkar, Amin, & Shuhaimi, 2015). In Nigeria, seeds form a major part of the diet and are consumed as a meal as well as ingredients in local soups (Oloyede, Obisesan, Agbaje, & Obuotor, 2012). Industrially, pumpkin seeds have been used in a number of countries largely for oil and protein production. Oil from pumpkin seeds has long been considered for use in the prevention of various ailments, especially for prostate diseases because of its nutritional and medicinal values (El-Mosallamy, Sleem, Omar, Shaffie, & Kenawy, 2012; Shaban & Sahu, 2017). Pumpkin seed oil has a special place among edible oils produced in many countries including Yugoslavia, Austria, Hungary, Nigeria and southern parts of the former USSR (Tsaknis, Lalas, & Lazos, 1997). The production of oil from pumpkin seed provides a means of utilization of a renewable resource while adding value to the agricultural byproducts (Raihana, Marikkar, Amin, & Shuhaimi, 2015). The oil has been used for cooking in some countries in Africa and the Middle East, and as a salad oil in the south of Austria and the adjacent regions in Slovenia and Hungary (Wenzl, Prettner, Schweiger, & Wagner, 2002). In Uganda, no oil has been produced from pumpkin seeds, but the pooled seeds are available for sale in many open air markets and are consumed in significant amounts as a snack in the form of salted roasted seeds like in many other countries, constituting a food rich in oil (Gutierrez, 2016).

2.4.1 Proximate composition of pumpkin seeds

The main nutritionally relevant components of pumpkin seed are proteins and oil (Montesano, Blasi, Simonetti, Santini, & Cossignani, 2018; Srbinoska, Hrabovski, Rafajlovska, & Sinadinović-Fišer, 2012). Their moisture, crude fibre and ash levels are similar to those of soybean, peanuts, sesame and sunflower seeds, but their carbohydrate levels are lower (up to 10 %; Achu, Fokou, Tchiégang, Fotso, & Tchouanguep, 2006; Srbinoska, Hrabovski, Rafajlovska, & Sinadinović-Fišer, 2012).

2.4.1.1 Protein

Cucurbitaceae seeds and their defatted cakes are rich in proteins 28 to 40.49 to 73.59% respectively; (Kwiri *et al.*, 2014). However, these authors also reported that cucurbit seeds from Sudan had a low protein content ranging from 14 - 17.5% on dry weight basis, implying that the nutritional content of these seeds depends on their origin. Nevertheless, food plants that provide more than 12% of their calorific value from protein are a good source of protein (Aberoumand, 2011). In that context, pumpkin seeds are a good source of protein (Table 2). The supply of animal protein is inadequate to meet the protein needs of the rapidly growing population in many developing countries (Devi, Prasad, & Sagarika, 2018). This has necessitated contemporary research efforts geared towards the study of the food properties and potential utilization of protein from locally available food crops, especially from underutilized or relatively neglected high protein oilseeds and legumes.

Table 2: Chemical composition of pumpkin seeds from data of different countries in Africa (%)

Country	Species	Lipids	Carb.	Crude fibre	Ash	Protein
Nigeria[1]	*C.pepo* L	46.05	16.65	2.20	3.55	23.35
Egypt[2]	*C.maxima*	35.2-41.95	4.8-10.96	4.12-4.69	5.3	34.19-39.75
Burkina Faso[3]	*C.pepo* L	41.07	-	-	-	18.87
Zimbabwe[4]	*C.pepo* L	43.46	12.16	2.58	3.324	32.86
Ghana[5]	*C.pepo* L	46.0	4.28	2.40	4.90	36.0
Kenya[6]	-	31.9-41.37	8.66-27.35	11.69-24.85	-	14.05-33.29
Kenya[7]	*C. maxima*	2.19	35.58	20.64	5.29	31.60
Tunisia[8]	*C.maxima*	31.57	0.11	21.97	3.97	33.92
Nigeria[9]	*C.pepo* L	38	28.03	1.0	5.6	27.48
Egypt[10]	*C.maxima*	35.7	10.56	14.9	3.8	35.0
Cameroon[11]	*C.maxima*	49.05	8.62	3.44	3.95	34.93
Rep. of Niger[12]	*C.pepo*.L	29.8	-	-	-	58.8
Egypt[13]	-	51.01	4.88	4.43	3.21	36.47

Source: [1]Ogunbanjo, Awotoye, Jayeoba, & Jeminiwa (2016); [2]Al-Anoos, et al. (2015); [3]Ouattara et al. (2015); [4]Kwiri, et al. (2014); [5]Steiner-Asiedu et al. (2014); [6]Khamis, Muchugi, & Mugendi (2013); [7]Kunyanga, Imungi, & Vellingiri, 2013; [8]Rezig *et al*. (2012); [9]Elinge *et al*. (2012); [10]Hegazy & Kinawy (2011); [11]Achu, Fokou, Tchiégang, Fotso, & Tchouanguep (2005); [12]Glew *et al*. (2006); [13]El-Adawy & Taha (2001)

Among the Cucurbits, C. pepo was reported to have a Net Protein Utilization (NPU) comparable to that of soybean seeds (61.0 vs 67.7; Steiner-Asiedu, Nuro-Ameyaw, Agbemafle, Hammond, & Tano-Debrah, 2014). According to the authors, NPU is used as a means of predicting the quality of a protein. The NPU ranges from 0 to 1 (or 100), with a value of 1 (or 100) indicating 100% utilization of dietary nitrogen as protein and a value of 0 indicating that none of the nitrogen supplied was converted to protein (Steiner-Asiedu, Nuro-Ameyaw, Agbemafle, Hammond, & Tano-Debrah, 2014). Cucurbita pepo is thus regarded as the best protein source for providing much essential amino acids for the growth and maintenance of consumers especially vulnerable people like children, pregnant women, the elderly and the sick and thus should be promoted (Steiner-Asiedu *et al*., 2014). However, lysine and isoleucine were first and second limiting amino acids in pumpkin seeds (El-Adawy & Taha, 2001; Glew *et al*., 2006). It is also noteworthy that a peptide from pumpkin seeds was proved to anti-microbial properties that inhibit Botrytis cinerea, Fusarium oxysporum and Mycosphaerella arachidicola (Ratnam, Vandana, Najibullah, & Ibrahim, 2017). The extract showed moderate to high activity against all the investigated microbial strains.

2.4.1.2Characteristics of Pumpkin seed oil

2.4.1.2.1 Oil content and general properties of the oil

The oil content of different Cucurbita species ranges from 9.8 to 52.1% while different varieties of *C.pepo* contain oil in the range of 31.2 to 51.0% (Ardabili, Farhoosh, & Khodaparast, 2011). Studies in Africa also reported the oil content of *C.pepo*, *C.maxima* and *C.moschata* pumpkin seeds in the range of 15 to 55% (El-Adawy & Taha, 2001; Achu *et al*., 2006; Glew *et al*., 2006; Hegazy & Kinawy, 2011; Rezig *et al*., 2012; Karanja *et al*., 2014; Ouattara *et al*., 2015; Benalia *et al*., 2015; Kukeera *et al*., 2015).

Pumpkin (*Cucurbita pepo* L.) seed oil belongs to the group of oils of high nutritive value due to its favourable fatty acid composition and different minor components which have certain beneficial effects on the human health (Vujasinovic, Djilas, Dimic, Romanic, & Takaci, 2010). Besides the positive nutritive-pharmacological properties, pumpkin seed oil is characterized by specific sensory properties, such as colour, odour, taste, and aroma. Pumpkin seed oil varies from dark green to brown in color (Stevenson *et al*., 2007). The oil has a relative density *ca.* 0.920 g/cm³ at 20°C, and has a typical pumpkin seed aroma (Ardabili, Farhoosh, & Khodaparast, 2011). The refractive index (40°C) of the oil ranges from 1.465-1.474 (Aktaş, Gerçekaslan, & Uzlaşır, 2018). Pumpkin seed oil has high iodine number (> 100g of KOH/100 g), an indication of high unsaturated fatty acid content (Ibrahim, Faeq, Ibraheem, & Al-Noor, 2017; Devi, Prasad, & Sagarika, 2018).

2.4.1.2.2 Fatty acid composition of pumpkin seed oil

Lipids consist of fatty acids classified mostly according to the presence or absence of double bonds as saturated (SFA; without double bonds), monounsaturated (MUFA; with one double bond) and polyunsaturated fatty acids (PUFA; more than one bonds). They can further be classified as *cis* or *trans* based on the configuration of the double bonds and as ω-3 or ω-6 PUFA based on the position of the first double bond from the fatty acid methyl-end (Orsavova, Misurcova, Ambrozova, Vicha, & Mlcek, 2015). Data on pumpkin seed oils shows that the content of PUFA is higher than the content of MUFA or SFA (Tables 3 and 4).

Table 3: Saturated fatty acid composition of pumpkin seed oils from other countries

Country	Pumpkin	C10:0	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	C22:0	Ref.
Tunisia	*C.pepo*	-	-	0.23	14.83	-	6.68	0.43	0.06	2
Burkina-Faso	*C.pepo*	-	-	-	10.5	-	8.89	-	-	3
Egypt	*C. pepo*	ND-3.06	ND-2.80	ND-1.9	9.6-16.56	ND-1.3	3.8-6.93	-	-	4
Kenya	*C.*species	-	-	-	1.16-20.81	-	15.56-30.79	-	-	5
Nigeria	*C.pepo*	-	-	0.18	16.41	-	11.14	-	-	6
Tunisia	*C.pepo*	-	-	-	15.96	-	4.68	0.41	-	7
Egypt	*C.maxima*	-	-	-	15.52	-	9.27	-	-	8
Cameroon	*C.maxima*	-	-	0.1	12.6	-	8.5	0.5	-	9
	C.moshata	-	-	0.3	19.2	-	9.2	1.7	-	
Rep. of Niger	*C.species*	-	0.02	0.16	13	-	7.8	0.58	0.16	10
Cameroon	*C.maxima*	-	-	0.1	12.6	-	8.53	0.47	-	11
	C.moshata	-	-	0.35	19.05	-	9.1	1.7	-	
Egypt	*C. pepo*	-	-	0.17	13.4	-	-	-	-	12

Source: 1. This study; 2. Bardaa et al. (2016); 3. Ouattara, Somda, Moyen, & Traore (2015); 4. Al-Anoos et al. (2015); 5. Karanja *et al.* (2013); 6. Elinge *et al*. (2012); 7. Rezig *et al*. (2012); 8. Hegazy & Kinawy (2011); 9. Fokou *et al*. (2009); 10. Glew *et al*. (2006); 11. Achu, Fokou, Tchiégang, Fotso, & Tchouanguep (2006); 12. El-Adawy & Taha (2001).

Table 4: Unsaturated fatty acid composition (%) of pumpkin seed oils from other countries

Country	Pumpkin	C16:1	C17:1	C18:1	C20:1	C22:1	C18:2	C18:3	C20:4	Ref.
Tunisia	*C.pepo*	0.15	0.08	25.82	-	0.06	50.88	-	-	2
Burkina-Faso	*C.*pepo	ND	-	17.22	-	-	62.97	-	-	3
Egypt	*C.pepo*	ND-0.8	ND-1.3	8.18-50.0	-	-	22.79	ND-0.7	0.3-42.94	4
Kenya	*C.*species	-	-	15.56-30.79	-	-	26.18-81.21	-	-	5
Nigeria	*C.*pepo	0.16	-	18.41	-	0.76	52.69	1.27	-	6
Tunisia	*C.pepo*	Trace	-	44.11	-	-	34.77	Trace	-	7
Egypt	*C.pepo*	0.44	-	20.4	-	-	55.6	-	-	8
Cameroon	*C.maxima*	0.1	-	25.3	-	-	52.4	0.2	0.3	9
	C.moshata	0.1	-	25.3	-	-	49.5	0.2	0.2	
Rep. of Niger	*C.species*	0.17	-	45.4	0.13	0.08	31	0.19	-	10
Cameroon	*C.maxima*	0.07	-	25.23	-	-	52.17	0.2	0.27	11
	C.moshata	0.1	-	19.3	-	-	48.95	0.2	0.23	
Egypt	*C.maxima*	-	-	33.16	-	-	42.05	-	-	12

Source: 1. This study; 2. Bardaa et al. (2016); 3. Ouattara, Somda, Moyen, & Traore (2015); 4. Al-Anoos et al. (2015); 5. Karanja *et al*. (2013); 6. Elinge *et al*. (2012); 7. Rezig *et al*. (2012); 8. Hegazy & Kinawy (2011); 9. Fokou *et al*. (2009); 10. Glew *et al*. (2006); 11. Achu, Fokou, Tchiégang, Fotso, & Tchouanguep (2006); 12. El-Adawy & Taha (2001).

The levels of oleic and linoleic acids range from 60% to 90%. Linoleic acid, oleic acid, palmitic acid, and stearic acid are the predominant fatty acids in pumpkin seed oil (Fruhwirth & Hermetter, 2008). Linoleic acid, a precursor of arachidonic acid, is important in the inflammatory cascade as it is the starting material for the synthesis of prostaglandins, thromboxanes, and leukotrienes (Bardaa *et al*., 2016). These substances act as inflammatory mediators and accelerate the inflammatory process. However, there is some evidence that in some species and under some conditions linoleic acid may act as a promoter of tumor growth (Jandacek, 2017). According to Matus, Moln, & Szab (2001), the oleic acid content of pumpkin seeds is negatively correlated with the amount of linoleic acid due to the substrate-product relationship of the two fatty acids. The ratio of linoleic acid to oleic acid significantly influences the oxidative stability of pumpkin seed oil (Murkovic & Pfannhauser, 2000). The composition of fatty acids is dependent on several factors including the variety, soil fertility, climate, and state of ripeness (Stevenson *et al*., 2007).

2.4.1.2.3 Health effects of pumpkin seed oil

Pumpkin seed oil has been implicated in providing many health benefits. The oil obtained from the seed could be used as a preservative and as a functional ingredient in cosmetics, food and nutraceuticals (Rezig *et al*., 2018). The most remarkable health benefits of pumpkin seed oil are prostate disease prevention, retardation of hypertension progression, reduction of bladder and urethral pressure, urinary disorders prevention, and the alleviation of diabetes by promoting hypoglycaemic activity (Nishimura *et al.*, 2014). Oil extracted from *Cucurbita pepo* is particularly useful for the treatment of urinary disorders (Nishimura, Ohkawara, Sato, Takeda, & Nishihira, 2014). Pumpkin seed oil also retards the progression of hypertension and mitigates hypercholesterolemia and arthritis (Dar, Sofi, & rafiq, 2017). In addition, diets high in pumpkin seeds have been associated with lower levels of gastric, breast, lung, and colorectal cancer (Kim, Kim, Kim, Choi, & Lee, 2012).

2.4.1.2.4 Antimicrobial effect of pumpkin seed oil

Pumpkin seed oil exhibits a broad spectrum of antimicrobial activity. The oil is effective against *Actinetobacter baumanii, Aeromonas veronii biogroup sobria, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica serotype typhimurium, Serratia marcescens* and *Staphylococcus aureus* at the concentration of 2·0% (v/v) (Zoué, Bédikou1, Gonnety, Faulet, & Niamké, 2012; Ratnam, Vandana, Najibullah, & Ibrahim, 2017). Methanolic seeds extracts of un-irradiated samples have been reported to possess antibacterial effects against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* local isolates at concentration levels of 1.0, 2.0, 2.0 and 3.0 mg/ml, respectively. In addition, the oils are reported to show antimicrobial activity against *Aspergillus fumigatus* and *Trichophyton mentagrophytes* (Zoué, Bédikou1, Gonnety, Faulet, & Niamké, 2012). Pumpkin seed oil also has antifungal activity against *Rhodotorula rubra* and *Candida albicans* at 0.5 and 1.0 mg/ml concentrations, respectively (Dar, Sofi, & Rafiq, 2017). EI-Aziz & EI-Kalek (2011) also reported effectiveness of pumpkin oil seeds extracts of 1.0 mg/ml against *Penicillium chrysogenum* and *Aspergillus parasiticus*, and 2.0 mg/ml against *Aspergillus flavus (A)*. Chemotherapy has revolutionized modern medicine and has significantly reduced death and ailments from infectious diseases. However, microorganisms have progressively diminished the effectiveness of previously successful antibiotics by developing resistance (EI-Aziz & EI-Kalek, 2011). The infection caused by multidrug-resistant and pan drug-resistant strains are often hard to treat. Currently, there is a great interest in natural antimicrobial products in hope that they may provide useful leads into anti-infective drug candidates (Gutierrez, 2016).

2.4.3 Carbohydrates

According to Achu, Tchiégang, Fotso, & Tchouanguep (2005), the carbohydrate content of Cucurbitaceae seeds ranges from 7.11 to 10.01% dry weight, irrespective of the area of cultivation. However, values as low as 0.11% were reported for *C.maxima* seeds (Rezig *et al*., 2012), while values greater than 25% have also been reported (Elinge *et al*., 2012; Khamis *et al*., 2013). Carbohydrate plays an important role on the physiological activities of plants, and glucose and glycogen serve as important source of energy for vital activities (Habib *et al*., 2015). In humans, carbohydrates provide the necessary calories in diets and promote the utilization of dietary fats in addition to sparing proteins. They provide easily available energy for oxidative metabolism moreover carbohydrate-containing foods such as pumpkin seeds are vehicles for important micronutrients and phytochemicals (Habib *et al.*, 2015). Dietary carbohydrate is also important to maintain glycemic homeostasis and for gastrointestinal integrity and function. Further, the higher the carbohydrate content the higher the degree of sweetness of a food (Kim, Kim, Kim, Choi, & Lee, 2012).

2.4.4 Crude fiber

Pumpkin seeds contain high amounts of fibre (Nyam, Lau, & Tan, 2013). Dietary fibre encompasses a range of divergent compounds that differentially affect numerous important gastrointestinal (GI) and systemic body processes (Brownlee, 2011). Dietary fibres that inhibit intestinal digestive processes result in decreased upper GI transit times, which may affect satiety and satiation. The large intestine houses a varied microflora. Dietary fibre is a major energy source for these bacteria and markedly affects the GIT microfloral diversity (Brownlee, 2011). Dietary fibres can also affect innate immune responses of the gut mucosa both directly and indirectly, which as a result may impact on cardiovascular/systemic health. Fibre present in pumpkin seeds and rinds can prevent constipation, reduce blood glucose and cholesterol level, prolong intestinal transit time and provide satiety (Nyam, Lau, & Tan, 2013).

Dietary fibre also has many functional properties such as water holding, oil holding, emulsifying and gel formation, and technological functions such as fat binding, gel binding, chelating and texturizing agent (Nyam, Lau, & Tan, 2013). Incorporation of dietary fibre into food products would therefore help to modify the textural properties of the food, avoid syneresis and stabilize high fat content food and emulsion. Pumpkin seed flour can be added into food products, such as dairy, bakery jam and meat products to enhance the texture and flavour of the products (Elleuch *et al*., 2010).

2.4.5 Minerals

Mineral elements are very important in the body since they are involved in various physiological and biochemical processes in the body (Bailey, West Jr., & Black, 2015). Pumpkin seeds are a good source of minerals as illustrated by the high ash content (2.47 to 4.21%; Elinge et al., 2012). They are rich in potassium, phosphorus and magnesium (Bailey, West Jr., & Black, 2015). They also contain modest amounts of iron and zinc. Iron and zinc deficiencies are the most common micronutrient deficiencies in the world, affecting more than 30% of the world's population (Bailey, West Jr., & Black, 2015). Zinc plays a critical role in protein and carbohydrate metabolism and also vitamin A mobilization from its storage sites in the liver, moreover it facilitates the synthesis of DNA and RNA (Elinge *et al*., 2012). It is also required for normal growth and development from in utero until puberty. Iron is an essential component of hemoglobin, myoglobin, enzymes, and cytochromes and is necessary for oxygen transport and cellular respiration. It is critical for optimal growth and cognitive function (Bailey, West Jr., & Black, 2015). Iron deficiency is the most important cause of nutritional anemia in infancy (Bamuwamye, Ogwok, & Tumuhairwe, 2015). It is associated with impaired health, immunocompetence and performance, and mental and motor development delays (Dijkhuizen, Wieringa, West, & Muhilal, 2001). Pumpkin seeds can supply the required Recommended Dietary Allowance (RDA) of 8 mg Fe/day for men and for women over 50 years, 18 mg/day for the girls and women of about 11 to 50 years old (Karanja, Mugendi, Khamis, & Muchugi, 2014). Pumpkin seeds can also supply the RDA of 2-5 mg per day Mn for infants, children, pregnant and breastfeeding mothers. Table 5 shows the mineral composition of pumpkin seeds from different countries in Africa.

Table 5: Mineral composition (mg/100g) of pumpkin seeds as reported by different studies in Africa

Country	Species	K	Ca	Mg	P	Fe	Zn	Mn	Na	Cu
Nigeria[1]	*C.Pepo*	237.24	9.78	67.1	47.68	3.75	14.14	0.06	170.35	-
Kenya[2]	*C. spp*	124.8-335.7	5.24-38.50	33.65-86.11	-	13.71-35.65	0.96-0.42	3.39-8.91	70.5-148.7	0.24-2.67
Egypt[3]	*C.maxima*	260	-	96.85	-	17	2.2	0.85	-	0.94
Egypt[4]	*C.maxima*	267	-	88	-	10.5	2.18	0.80	-	0.40
Egypt[5]	*C.pepo*	982	130	483	1090	10.9	8.2	8.9	38	1.7
Ghana[6]	*C.pepo*	610.0	31.0	530.0	-	6.50	7.10	3.20	8.50	7.90
Zimbabwe[7]	*C.pepo*	-	141	344.60	1040	11.98	1.24	-	67.96	-
Kenya[8]	*C.maxima*	-	25.0	405.0	845.0	7.4	8.4	-	0.85	-

Source: [1]Elinge *et al*. (2012); [2]Karanja *et al*. (2013); [3]Al-Anoos, El-dengawy, & Fahmy (2015); [4]Al-Anoos, El-dengawy, & Fahmy, 2015; [5]El-Adawy and Taha (2001); [6]Steiner-Asiedu *et al*. (2014); [7]Kwiri et al. (2014); [8]Kunyanga, Imungi, & Vellingiri (2013).

2.4.6 Vitamins

Pumpkin seeds and their oil are very good sources of vitamins especially carotenoids and tocopherols and it is partly for this reason that they are used as natural supplements (Shaban and Sahu, 2017). Stevenson *et al*. (2007) reported the vitamin E content of pumpkin seed oil to be in the range of 58.94 to 123.4 mg/100g. Tocopherols are of significant nutritional value because they impart oxidative stability to pumpkin seed oil and also prevent oxidative damage to body tissues due to their powerful antioxidant activity (Ardabili, Khodaparast, & Farhoosh, 2011; Kim, Kim, Kim, Choi, & Lee, 2012). Pumpkin seeds from the Lake Victoria basin have a vitamin C content in the range of 11.62 to 26.23 mg/100 g, a β-carotene content in the range of 0.02 to 0.42 mg/100 g, and α-tocopherol content of 2.39 mg/100 g (Echessa *et al*., 2013). These authors also reported substantial amounts of the B-complex vitamins; thiamine, riboflavin, niacin and pyridoxine in the pumpkin seeds (Table 6).

Table 6: Vitamin content (μg/100 g) of pumpkin seeds compared with the recommended dietary allowances (RDA)

Vitamin	1	2	3	4	RDA
β-carotene	9.6	40	9.6	20-420.0	600
Thiamine (B1)	270	370	273	190-540	1400
Riboflavin (B2)	153	-	153	370-700	1600
Niacin (B3)	4987	3120	4987	430-1030	18000
Pantothenic acid (B5)	750	-	750	-	6000
Pyridoxine (B6)	143	-	143	130-260	2000
Folic acid (B9)	58	n.d.	58	-	4000
Ascorbic acid (C)	1.9	1000	1.9	11620-26230	75000
α-tocopherol (E)	-	-	35100	2390-6040	15000

Source: 1. Dar, Sofi, & Rafiq (2017); 2. Kunyanga, Imungi, & Vellingiri (2013); 3. USDA (2018). 4. Echessa *et al*. (2013).

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

Pooled seeds of different varieties of *Cucurbita pepo* were obtained from Nakawa, Kibuye, Kalerwe, and Nakasero markets in Kampala city. All chemicals used were of analytical grade. Pumpkin seed oil was extracted with n-hexane (boiling range 62-68°C, 96%) manufactured by Sharlab S. L. Spain. Nitric acid (Sigma-Aldrich, analytical grade) and Hydrochloric acid (Sigma-Aldrich, analytical grade) were used to digest pumpkin seeds for mineral determination and were obtained from the Natural Chemotherapeutics Research Institute (NCRI) Chemistry laboratory. Distilled water was used for preparation solutions and cleaning of glassware. Pure standards of fatty acid methyl esters (FAME); FAME standard mixtures (Supelco 37 component FAME MIX) obtained from Supelco/Sigma-Aldrich (USA) were used to identify the fatty acids.

3.2 Methods

3.2.1 Sampling and sample preparation

Twelve (12) samples of pooled pumpkin seeds weighing 4800 g were purchased from Nakawa, Kibuye, Kalerwe and Nakasero markets. Three fractions of 400 g each, were bought from either of the markets. The pumpkin seeds were sealed in airtight polythene bags and transported to the Natural Chemotherapeutics Research Institute chemistry laboratory. Pumpkin seeds were dried in a hot air oven to a constant weight at 70°C for 6 hours (Peninah *et al*., 2018). The dried pumpkin seeds were stored in labelled dry airtight bags under cool and dry conditions until analysis.

3.2.2 Determination of minerals content

The dried pumpkin seeds were ground using a pre-cleaned pestle and mortar. The powder (2 g), was weighed out into a Pyrex boiling tube. About 5 ml of distilled water was added, followed by 25 ml of concentrated nitric acid (Sigma-Aldrich, analytical grade) added in a fume hood. The sample was then digested on a water bath at boiling point for 2 hrs. Approximately 2 ml of hydrochloric acid (Sigma-Aldrich, analytical grade) was then added followed by 50 ml of distilled water. The digest was allowed to cool, filtered into a 100 ml standard flask on a Whatman filter paper No.1 and the filtrate diluted to the mark with distilled water. A blank was also prepared using the procedure above without adding the sample.

Standard solutions for each of the mineral elements (Ca, Cu, Fe, Zn, Mn, Mg, K, and Na) analyzed, were prepared at five different concentrations; 0.1, 0.5, 1.0, 2.0, and 4.0 mg/L. The absorbance was determined and in each case a calibration curve was generated. The calibration curve was used to relate the absorbance for the analyte samples to mineral concentration.

Analysis was done at wave length (λ) 422.5, 324.7, 248.2, 213.7, 279.2, 285.1, 766.1 and 588.6 nm for Ca, Cu, Fe, Zn, Mn, Mg, K, and Na, respectively. Each of the diluted samples was aspirated into the AAS and the absorbance measured was used to determine the concentration of the mineral element from the standard curves. Mineral concentrations for the pumpkin seeds expressed in mg/L were converted to mg/100g using equation (1).

$$Mineral\ \ conc.\ in\ \left(\frac{mg}{100g}\right) = \frac{conc. of\ mineral\ \left(\frac{mg}{l}\right) \times vol.(ml) \times 100g}{(sample\ weight\ (g) \times 1000\ ml)} \qquad (1)$$

3.2.3 Estimated daily intake of essential dietary minerals via pumpkin seed consumption

The daily intake of mineral elements from a food depends on the mineral concentration in the food and the ingestion rate of the food (Bamuwamye, Ogwok, & Tumuhairwe, 2015). The actual daily intake of pumpkin seeds per individual in Uganda is not known. However, the recommended calorie intake for all adults is between 2,000 and 2,800 calories per day (Van Horn *et al*., 2016). The American heart association recommends 141.7g per week for nuts, seeds such as pumpkin seeds and legumes at a calorie intake level of 2,000 to 2,800 calories (Van Horn *et al.*, 2016). The ingestion rate was therefore calculated as 20.2 g per day. The daily intake of essential dietary minerals via pumpkin seed consumption was then estimated using this ingestion rate and calculated as follows using equation (2).

$$\mathrm{EDI} = \frac{(\mathrm{C} \times \mathrm{IR})}{\mathrm{BWa}} \qquad (2)$$

Where: EDI is the estimated daily intake of the mineral element (mg/kg BW day^{-1}), C is the mineral element concentration (mg/kg) in pumpkin seeds and IR is the mass (kg) of pumpkin seeds consumed daily and BWa, the average body weight (70 kg by convention).

3.2.4 Oil extraction

Pumpkin seeds were dried for 4 h at 105°C in a Neo-Tech SA hot air oven manufactured in Germany. The dry seeds were pounded to fine powder using a pestle and motor, and the powder (10 g), was extracted over 24 hrs by cold maceration using n-hexane. This procedure was repeated three times and the extracts combined into one fraction. The solvent was evaporated on a rotary evaporator and the residual oil dried in a hot air oven at 80°C for 30 min. The oil yield was expressed as a percentage on pumpkin seed weight used for extraction.

3.3 Determination of fatty acid profile of the oil

The FA profile was determined using gas chromatography with flame ionization detection (GLC-FID)/capillary column based on the ISO 5509:2000 transesterification method. Fatty acids were determined as FAME on a Varian 3800 GC chromatograph with flame ionization detection (GC-FID) manufactured by Varian Chromatography System, USA; using a Chrompack CP-Sil 88 TM fused silica capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness; Varian Inc., Walnut Creek, CA, USA). Analysis was done at the Uganda National Bureau of Standards (UNBS) chemistry laboratory. The FA profile was analyzed with a Chrompack CP 9001 chromatograph equipped with a split-splitless injector, an FID and a Chrompack CP-9050 autosampler manufactured by Varian Inc. (USA). The temperature of the injector and detector was 250°C. Separation was achieved on a 50 m (l) × 0.25 mm (i.d.)-fused silica capillary column coated with a 0.91 mm film of CP-Sil 88. Nitrogen was used as a carrier gas at an internal pressure of 120 kPa. The column temperature was 140°C for a 5 min hold. It was then programmed to increase to 220°C at a rate of 4°C/min and then held for 10 min. The split ratio was 1:20, and the injected volume was 1.2 mL. The results were expressed as relative percentage of each FA, calculated by internal normalization of the chromatographic peak area. Fatty acid identification was made by comparing the relative retention times of fatty acid methyl ester (FAME) peaks from the samples with those of standards. A Supelco mixture of 37 FAME (standard 47885-U) was used.

3.4 Evaluation of fatty acid-based nutrition indices

Nutritional quality of the pumpkin seed oil was evaluated based on fatty acid composition of the oil. The PUFA/SFA and ω6/ω3 ratios, and the atherogenicity index (AI), thrombogenicity index (TI) and the ratio between the hypocholesterolemic FA and hypercholesterolemic FA; (h/H) were used to evaluate the nutritional value of the fat portion (Popova, Ignatova, Petkov, & Staniši´, 2016). Calculation of these factors was carried out based on the levels of particular fatty acids. The AI and TI were calculated using Equations 1 and 2, respectively (Ulbricht and Southgate, 1991), and the h/H ratio was calculated according to Equation 3 (Santos-Silva, Bessa, & Santos-Silva. 2002).

$$AI = (l2:0 + (4 \times l4:0) + 16:0) / (\omega\text{-}6\ PUFA + \omega\text{-}3\ PUFA + MUFA) \qquad (3)$$

$$TI = (14:0 + 16:0 + 18:0)/[(0.5*MUFA) + (0.5*\omega\text{-}6\ PUFA) + (3*\omega\text{-}3\ PUFA) + (\omega\text{-}3PUFA/\omega\text{-}6\ PUFA)] \qquad (4)$$

$$h/H = [(\text{sum of } C18:1\omega9c,\ C18:1\omega7c,\ C18:2\omega6c,\ C18:3\omega6,\ C18:3\omega3,\ C20:3\omega6,\ C20:4\omega6,\ C20:5\omega3,\ C22:4\omega6,\ C22:5\omega3 \text{ and } C22:6\omega3)/ (\text{sum of } C14:0 \text{ and } C16:0)] \qquad (5)$$

3.5 Data analysis

All experiments were performed in triplicate and results expressed as mean ± standard deviation. Data was analyzed using SPSS software (IBM SPSS Statistics version 22, IBM corporations). Analysis of variance (ANOVA) was used to determine differences in oil yield, mineral content among the pumpkin seed varieties. Mean differences were considered significant at $p < 0.05$.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Mineral elements in pumpkin seeds

4.1.1 Macro element composition of pumpkin seeds

Phosphorus was the most abundant element in the pumpkin seeds with concentration ranging between 1398.00 and 2092.67 mg per 100 g (Table 7). Phosphorus content of the pumpkin seeds of this study was higher than reported in previous studies (El-Adawy & Taha, 2001; Elinge *et al*., 2012; Kwiri *et al*., 2014). Phosphorus is the second most abundant essential mineral in the human body after calcium. According to Koricanac *et al*. (2015), 80% to 90% of phosphorus is present in the bones and teeth in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Many enzymes and hormones also contain phosphorus as a structural component (Kwiri *et al*., 2014). Phosphates (compounds containing the phosphate ion, PO_4^{3-}) are a component of DNA, RNA, ATP, and phospholipids such as phosphatidylcholine that is a major structural components of cell membranes (Raina *et al*., 2012). Hemoglobin, the important oxygen-carrying protein in the bloodstream, also depends upon phosphorus contained in its structure for proper function. In addition, phosphorus acts as a buffer, neutralizing acids to maintain normal pH in the blood (Raina *et al*., 2012).

Potassium was the second most abundant element in the pumpkin seeds with concentration ranging from 381.10 to 429.83 mg/100 g (table 7) and a mean concentration of 416.45 mg/100 g. Pumpkin seeds from Kibuye market had the highest potassium content while those from Nakawa market had significantly ($p < 0.05$) lower concentration of potassium. The mean concentration of potassium of this study was lower than that reported by El-Adawy and Taha (2001) and Aliu *et* *al*. (2016), but lower than reported by Elinge et al. (2012), Karanja, Mugendi, Khamis, & Muchugi (2013), Kwiri *et al*. (2014) and Al-Anoos, El-dengawy, & Fahmy (2015). Potassium is essential for the proper functioning of cells, tissues, and organs in the human body (Karanja, Mugendi, Khamis, & Muchugi, 2014). It helps us synthesize protein from amino acids in cells, and also to convert glucose to glycogen for storage in the liver as a future supply of energy. It is also crucial for heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function. Therefore from the data obtained in this study regular consumption of pumpkin seeds would increase the potassium uptake in the end leading to reduced high blood pressure.

Table 7. Macro element concentrations (mg/100 g) of pumpkin seeds from Nakasero, Kalerwe, Kibuye and Nakawa markets

Element	Nakasero	Kalerwe	Kibuye	Nakawa	Mean± SD
P	2092.67±220.21[a]	1549.00±71.27[b]	1702.00±55.02[b]	1398.00±12.53[c]	1685.42±152.00
K	429.83±13.49[a]	421.50±3.55[a]	433.38±25.03[a]	381.10±26.76[b]	416.45±27.66
Mg	352.27±9.63[a]	354.35±7.27[a]	473.70±19.00[b]	449.95±22.48[b]	407.56±58.93
Na	8.89±0.03[a]	23.25±1.41[b]	12.16±0.14[a]	12.56±0.15[a]	14.21±5.68
Ca	6.27±0.07[a]	8.76±0.05[b]	6.51±0.10[a]	6.33±0.06[a]	6.97±1.09

Values are recorded as mean ± standard deviation of three values. Values within a row with different superscripts are significantly different ($p<0.05$)

Magnesium concentration in the studied pumpkin seeds ranged from 352.27 to 473.70 mg/100 g and a mean concentration of 407.56 mg/100 g. Pumpkin seeds were evenly divided into two segments on the basis of their Mg content. Seeds from Nakasero and Kalerwe markets had a magnesium content that was significantly lower than those from Kibuye and Nakawa markets. Magnesium concentrations were in agreement with those reported by El-Adawy & Taha (2001) but higher than reported elsewhere in Africa (Elinge *et al*., 2012; Al-Anoos, Steiner-Asiedu, Nuro-Ameyaw, Agbemafle, Hammond, & Tano-Debrah, 2014; El-dengawy, & Hasanin, 2015). Magnesium plays an important role in electrolyte homeostasis being necessary for the activation of ATP/ATPase pumps such as Na+/K+ pump, Na+/Ca++, Na+/Mg++ and Mg++/Ca++ pumps (Ismail & Ismail, 2016). It is also necessary for bone mineral density and strength, macronutrient metabolism, energy transfer, storage and use. In addition, Mg encourages bone formation, directly and indirectly through its effect on bone hormones such as parathyroid hormone (Ismail & Ismail, 2016).

Sodium content varied between 8.89 and 23.25 mg/100 g of the pumpkin seeds with a mean concentration of 14.21 mg/100 g. It was highest in seeds from Kalerwe market and lowest in seeds from Nakasero market. The Na concentration showed variability among pumpkin seeds from different markets although there was no significant difference in the sodium content of pumpkin seeds from Kibuye and Nakawa markets. Sodium concentration determined was lower than that reported by previous studies (Ahsan *et al*., 2015; Al-Anoos, El-dengawy and Fahmy 2015; Kwiri *et al*., 2014; Karanja *et al*., 2013; Elinge *et al*., 2012). Like potassium, Na in moderate amounts is an essential element, important for nerve transmission and muscle function (Karanja, Mugendi, Khamis, & Muchugi, 2014).

Calcium was in the concentration range of 6.27 to 8.76 mg/100 g. Pumpkin seeds from Kalerwe market had the highest concentration while those obtained from Nakasero market had the lowest.

The pumpkin seeds from Kalerwe had a significantly ($P < 0.05$) higher Ca content when compared with those from the other markets. Calcium levels observed in the pumpkin seeds of this study were in line with those reported by Karanja *et al.* (2013) but lower than reported in other studies in Africa (El-Adawy & Taha, 2001; Kwiri *et al*., 2014; Al-Anoos, El-dengawy, & Fahmy, 2015). Calcium is the most important structural element, occurring not only in combination with phosphate in bone and teeth but also with phospholipids and proteins in cell membranes (Pua, Chenb, & Xuea, 2016). It is widely involved in many physiological and biochemical processes throughout the body including the coagulation of blood, the coupling of muscle excitation and contraction, the regulation of nerve excitability, the motility of spermatozoa, the fertilization of ova, cell reproduction, the control of many enzyme reactions and the transmission of many hormone actions at the appropriate receptor site on the cell membrane.

4.1.2 Microelement composition of pumpkin seeds

The micronutrients, which are needed only in trace amounts and were quantified in pumpkin seeds, are Fe, Zn, Mn and Cu. Iron was the most abundant in the pumpkin seeds (Table 8). Iron content ranged between 6.93 to 8.57 mg/100g, with seeds from Nakasero market having a significantly higher Fe content (8.57 mg/100 g) than seeds from other markets; while those obtained from Nakawa had the lowest iron content (6.93 mg/100 g). The mean iron (7.56 mg/100 g) was lower than that reported by Karanja *et al*. (2013), Ahsan *et al*. (2015), Chung *et al*. (2013) and Al-Anoos *et al*. (2015) but higher than reported by Elinge *et al*. (2012) and Aliu *et al*. (2016). These differences can be attributed to by differences in environmental source. Iron is an essential micronutrient for almost all living organisms because it plays a critical role in metabolic processes such as DNA synthesis and respiration (Bamuwamye *et al.*, 2017). However, Fe deficiency is one of the most common nutritional deficiencies leading to severe anemia in children, women of child bearing age, and pregnant women (Bamuwamye, Ogwok, & Tumuhairwe, 2015).

Table 8. Micro elements concentration (mg/100 g) of pumpkin seeds from Nakasero, Kalerwe, Kibuye and Nakawa markets

Element	Nakasero	Kalerwe	Kibuye	Nakawa	Mean± SD
Fe	8.57 ± 0.13^{c}	7.09 ± 0.09^{a}	7.65 ± 0.04^{b}	6.93 ± 0.05^{a}	7.56±0.68
Zn	6.41 ± 0.10^{a}	4.68 ± 0.04^{a}	5.76 ± 0.09^{a}	5.44 ± 0.03^{a}	5.57±0.65
Mn	3.11 ± 0.16^{a}	2.64 ± 0.11^{a}	4.31 ± 0.28^{b}	3.53 ± 0.14^{a}	3.40±0.66
Cu	0.60 ± 0.03^{a}	0.54 ± 0.01^{a}	$0.77 \pm .05^{b}$	0.56 ± 0.02^{a}	0.62±0.10

Values are recorded as mean ± standard deviation of three values. Values within a row with different superscripts are significantly different ($p<0.05$)

There was variability in the zinc content of the pumpkin seeds from the different markets. The highest zinc content was 6.421 mg/100 g recorded in seeds from Nakasero market while the lowest of 4.68 mg/100g was recorded in seeds from Kalerwe market. The observed mean zinc concentration (5.57 mg/100 g) was lower than that reported for pumpkin seeds in Nigeria (Elinge *et al.*, 2012) and in Egypt (Al-Anoos, El-dengawy, & Fahmy, 2015). However, it was higher than that reported in other countries in Africa (Ahsan *et al.*, 2015; Kwiri *et al.*, 2014; Karanja, Mugendi, Khamis, & Muchugi, 2013). Zinc is the second most abundant trace element after Fe, and is essential for all living organisms (Kambe, Tsuji, Hashimoto, & Itsumura, 2015). It is necessary for growth, appetite, testicular maturation, skin integrity, mental activity, wound healing and immunocompetence. It has also been proposed that zinc is required for multiple steps in insulin synthesis and release hence recommended in management of diabetic patients (George & Friendgood, 2018).

There was variability in the Mn content of pumpkin seeds from the different markets. Manganese levels ranged from 2.64 to 4.31 mg/100 g with a mean concentration of 3.40 mg/100 g. Pumpkin seeds from Kibuye market had the highest Mn level while those form Kalerwe market had the lowest Mn level. The content of Mn observed in the pumpkin seeds in this study was lower than the reported by El-Adawy & Taha (2001) and Karanja, Mugendi, Khamis, & Muchugi (2013) and higher than that reported elsewhere in Africa. Manganese is a major mineral related to carbohydrate and lipid metabolism, and is essential for normal functioning of nerve, heartbeat and the central nervous system in humans (Bamuwamye *et al*., 2017). It is a good anti-oxidant, and is required for bone formation, red blood cell regeneration and aids enzymatic actions.

Copper was the mineral with the least concentration in the studied pumpkin seeds with concentrations ranging from 0.54 to 0.77 mg/100 g. Seeds from Kibuye market had the highest Cu content and those from Kalerwe had the lowest content. Seeds from Kibuye market had a higher Cu content than seeds from other markets. The Cu content of the studied seeds was within the same range of 0.24 to 2.67 mg/100 g reported previously by Al-Anoos, El-dengawy, & Fahmy (2015), Kwiri *et al*. (2014), Karanja, Mugendi, Khamis, & Muchugi, (2013) and Alfawaz (2004). Copper is required for adequate growth, cardiovascular integrity, lung elasticity, neovascularization, neuroendocrine function, and iron metabolism (Bost *et al*., 2016).

4.1.3 Dietary contribution of pumpkin seeds towards mineral intake

The Estimated Daily Intake (EDI) of essential mineral elements from the studied pumpkin seeds followed the order P > K > Mg > Na > Fe > Ca > Zn > Mn > Cu (Table 9). The EDI was compared with the Dietary Reference Intakes (DRI) suggested for the essential elements by the Institute of Medicine (IOM, 2006). The observed EDI values for pumpkin seeds indicate that daily consumption of 20.2 g of pumpkin seeds would contribute substantially towards P, Mn, Mg, Zn, Cu, and to a limited extent, Fe, but would not be an important source for dietary calcium. Habib *et al*. (2015) earlier reported similar findings although according to these aurthers , pumpkin seeds are also a good dietary source of Ca.

Table 9. Estimated daily intake (EDI) of essential elements by an adult person weighing 70 kg via consumption of 20.2 g of pumpkin seeds per day compared with the DRI (mg/day).

Element	RDA/AI (mg/day)			% of RDA/AI	
	Men[1]	Women[1]	EDI[2]	Men	Women
P	700	700	337.08	48.15	48.15
Ca	1000	1000	1.41	0.14	0.14
Mg	410	315	82.33	20.08	26.14
K	4700	4700	84.12	1.79	1.79
Na	1500	1500	2.87	0.19	0.19
Zn	11	8	1.13	10.23	14.06
Cu	0.9	0.9	0.13	13.92	13.92
Mn	2.3	1.8	0.69	29.86	38.16
Fe	8	18	1.53	19.09	8.48

Source: 1. Institute of medicine: Dietary reference intakes for calcium, magnesium, potassium, sodium, zinc, copper, manganese and iron, Washington DC, 2006, National Academy Press; 2. Calculated from data of this study. EDI Estimated daily intake (mg/day).

4.2 Oil yield

The oil yield of the pumpkin seeds ranged between 31.14 and 36.78%. Pumpkin seeds obtained from Nakawa market registered the highest average oil content of 36.78±2.77%, while those from Nakasero market had the lowest content, 31.14±1.16% on average. The seeds obtained from Kalerwe and Kibuye markets had an average oil yield of 35.53±2.89 and 33.90±0.59%, respectively. The oil yield in the present study was higher than previously reported for pumpkins in Uganda (Kukeera, Banadda, Tumutegyereize, Kiggundu, & Asuman, 2015). It was also higher than reported in Algeria (Benalia *et al*., 2015), Tunisia (Rezig, Chouaibi, Msaada, & Hamdi, 2012), and the Republic of Niger (Glew et al., 2006). However, it was lower than observed in pumpkin seeds from Nigeria, Burkina Faso, Zimbabwe, Cameroon and Egypt (Adnan *et al*., 2017; Ouattara, Somda, Moyen, & Traore, 2015; Kwiri et al., 2014; Achu, Fokou, Tchiégang, Fotso, & Tchouanguep, 2006; El-Adawy & Taha, 2001). Differences in foil content of pumpkin seeds could be due to varying growth conditions and differences in genetic makeup of the pumpkins (Al-Anoos, El-dengawy, & Hasanin, 2015). Crop yields and chemical composition are highly sensitive to changes in temperature and water availability with significant reduction after drought periods (Bavec, Mlakar, Rozman, & Bavec, 2007). Differences in the level of maturity at the time of harvest could also affect chemical compositions of plant materials (Petkova & Antova, 2015).

4.3 Fatty acid composition of pumpkin seed oil

Eight fatty acids were determined in the pumpkin seed oil (Table 10). The amount of unsaturated fatty acids (70.29%) was higher than the SaFA proportion (27.94%). Linoleic acid (18:2ω6; *cis*-9-*cis*-12-octadecadienoic acid) followed by oleic acid (C18:1ω9; *cis*-9-octadecenoic acid), were the major unsaturated FA with mean concentrations of 43.09±4.62% and 27.20±3.30%, respectively. Alpha linolenic acid (C18:3ω3; *all-cis*-9,12,15-octadecatrienoic acid) was present in minute quantities (0.51±0.45%). Palmitic (C16:0) and stearic (C18:0) acids were the major SFA with corresponding mean values of 16.50±1.87% and 10.32±1.26%. Palmitic, stearic, oleic and linoleic acids together accounted for 97.11% of the total fatty acids. Arachidic acid (C20:0), behenic acid (C22:0) and tricosanoic acid (C23:0) were present but, in minute quantities (< 1.0%).

Table 10: Fatty acid composition (%) of pumpkin (Cucurbita pepo) seed oil

Fatty acid	1	2	3	4	Min	Max	Mean± SD
C16:0	17.95	14.82	18.27	14.94	14.82	18.27	16.50±1.87
C18:0	9.78	8.81	11.14	11.56	8.81	11.56	10.32±1.26
C20:0	0.59	0.58	0.95	0.69	0.58	0.95	0.70±0.17
C22:0	0.17	0.22	0.45	0.21	0.17	0.45	0.26±0.13
C23:0	0.61	< 0.05	< 0.05	< 0.05	< 0.05	0.61	-
C18:1ω9*cis*	32.1	25.7	24.98	26.02	24.98	32.1	27.20±3.30
C18:2ω6*cis*	38.45	48.48	40.14	45.28	38.45	48.48	43.09±4.62
C18:3ω3 all *cis*	0.21	0.35	1.17	0.3	0.21	1.17	0.51±0.45
SaFA	29.1	24.43	30.81	27.4	24.43	30.81	27.94±2.72
MUFA	32.1	25.7	24.98	26.02	24.98	32.1	27.20±3.30
PUFA	38.45	48.48	40.14	45.28	38.45	48.48	43.09±4.62

1. Kalerwe market; 2. Nakasero market; 3. Kibuye market; 4. Nakawa market; SaFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LOD: Limit of detection = 0.05%

Studies on pumpkin seeds in Africa documented linoleic, oleic, palmitic and stearic acids as the major fatty acids in pumpkin seed oil (Glew et al., 2006; Ouattara, Somda, Moyen, & Traore, 2015; Bardaa *et al*., 2016). These fatty acids have been reported to account for > 97% of the total fatty acids in pumpkin seeds (Al-Khalifa, 1996; Lelley, Loy, & Murkovic, 2009; Elinge *et al.*, 2012; Rezig et al., 2012; Bardaa *et al*., 2016). The majority of literature reports indicated C18:2ω6 as most predominant fatty acid but Glew *et al*. (2006), Sabudak (2007) and Rezig, Chouaibi, Msaada, & Hamdi (2012) reported C18:1ω9 as the predominant fatty acid. Murkovic *et al*. (1996) and Fruhwirth & Hermetter (2008) observed that the relative amounts of C18:1ω9 and C18:2ω6 are always negatively correlated because of the precursor-product relationship of the two FA. Environmental factors affect the quality of seeds during seed development (Welbaum and Bradford, 1991). Low temperatures during the last weeks of seed filling cause a shift from C18:1ω9 to C18:2ω6. According to Younis *et al*. (2000), the content of C18:1ω9 and C16:0 decreases as the average growing temperature decreases. Despite the high UFA content, pumpkin seed oils were low (< 1%) in C18:3ω3, which is in agreement with other studies on pumpkin seed oil (Glew *et al.*, 2006; Fokou *et al*., 2009; Rezig *et al*., 2018). A low content of the oxidatively unstable linolenic acid, makes the pumpkin seed oil suitable for food and other industrial applications (Tsaknis, Lalas, & Lazos, 1997; Stevenson *et al*., 2007). However, ALA is an important essential fatty acid that is converted to the long chain ω-3 fatty acids; eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3), both of which possess cardio-protective properties (Ivanova, Marinova, & Batchvarov, 2016).

4.4 Nutritional quality of pumpkin seed oil

The potential of a particular food to be recommended for human consumption is determined primarily by the composition of its nutrients. The ratios of PUFA/SFA (P/S), ω6/ω3 PUFA, h/H, and AI and TI (Table 11), are widely used to evaluate the nutritional value of fat for human consumption (Pestana et al., 2012; Popova, Ignatova, Petkov, & Staniši´, 2016).

Table 11**:** Ratios of P/S, ω6/ω3 PUFA, h/H, and atherogenic and thrombogenic indices of the pumpkins compared with those from data of other countries

Country	∑SFA	∑PUFA	ω6	ω3	P/S	ω6/ω3	AI	TI	h/H
Uganda	27.78	43.60	43.09	0.51	1.57	84.90	0.23	0.90	4.29
Kenya	34.17	53.69	53.69	0.35	1.57	153.4	0.14	0.89	7.00
Egypt	22.48	44.76	44.41	-	1.99	-	0.25	0.51	5.26
Egypt	24.79	55.6	55.6	-	2.24	-	0.20	0.65	4.90
Egypt	13.57	42.05	42.05	4.01	3.10	10.49	0.18	0.36	5.54
Tunisia	22.23	50.88	50.88	-	2.29	-	-	-	5.09
Tunisia	21.05	34.77	34.77	0.11	1.65	316.09	-	-	-
Burkina-Faso	19.39	62.97	62.97	0.19	3.25	331.42	0.13	0.48	7.64
Rep. of Niger	21.72	31.19	31	0.2	1.44	155	0.18	0.54	5.83
Cameroon	21.7	52.64	52.44	0.2	2.43	262.2	0.17	0.54	6.13
	30.2	49.38	49.18	0.2	1.64	245.9	0.30	0.82	3.54
Cameroon	21.7	52.9	52.7	0.2	2.44	263.5	0.17	0.53	6.16
	30.4	49.9	49.7	1.27	1.64	39.13	0.27	0.75	3.86
Nigeria	27.73	53.96	52.69	-	1.95	-	0.24	0.70	4.36

∑SFA = sum of the saturated fatty; ∑PUFA = sum of polyunsaturated fatty acids; ω6 = sum of omega-6 fatty acids (linoleic acid); ω3 = sum of omega-3 fatty acids (ALA); P/S = ∑PUFA/∑SFA; AI = atherogenic index; TI = thrombogenic index; h/H = hypocholesterolemic/ hypercholesterolemic ratio.

The minimum ratio of PUFA to SFA recommended by the British Department of Health and WHO/FAO is 0.45 (Okrouhlá et al., 2013). Diets with higher PUFA/SaFA ratios have stronger hypocholesterolemic effect than those with lower ratios (Chang & Huang, 1998). However, the beneficial effect of C18:2ω6 on health is produced only when the PUFA/SFA ratio is < 1.50 (Romero, Romero, Doval, & Judis, 2013; Naydenova, Kaishev, Iliev, & Mihaylova, 2014). Very high-PUFA diets (PUFA/SFA ≥ 2.0) decrease both Low-density lipoprotein (LDL) and High-density lipoprotein (HDL) while the LDL/HDL ratio does not improve moreover there is enhanced oxidative stress because PUFA are highly susceptible to peroxidation (Kang, Shin, Park, & Lee, 2005). The observed PUFA/SFA ratio of the pumpkin seeds in the study (1.57) would favor the reduction of the risk of cardiovascular disease (CVD; Kang, 2005). However, the high ω-6 proportion largely made up by LA (ω6/ω3 > 10), was far from optimal (Simopoulos, 2016). Linoleic acid competes with ALA for endogenous conversion to EPA and DHA, and LA also inhibits incorporation of DHA and EPA into tissues (Lopes *et al*., 2014).

According to Ganzaroli, Sanchez, & Silva (2017), the utilization of indices based on the functional effects of the fatty acids is a better approach for the nutritional evaluation of fat. According to Ulbricht and Southgate (1991), C12:0, C14:0, and C16:0 FA are atherogenic, while C14:0, C16:0, and C18:0 are thrombogenic. PUFA ω-3, PUFA ω-6, and MUFA are antiatherogenic and antithrombogenic (Santos-Silva, Bessa, & Santos-Silva, 2002). Different authors reported varying recommendations for the AI and TI. Sinanoglou, Batrinou, Mantis, Bizelis, & Miniadis-Meimaroglou (2013) reported the appropriate values for a healthy diet to be less than 1.0. However, because atherogenic indices are strong determinants of the risk of coronary heart disease, Ulbricht & Southgate (1991) and Topuz, Yerlikaya, Yatmaz, Kaya, & Alp (2017) suggested the respective levels of AI and TI considered beneficial for humans to be less than 0.5. Low values were observed for these indices implying that the pumpkin seed oil had high quantities of fatty acids with anti-atherogenic properties (Kabašinskienė, Liutkevičius, Sekmokienė, Zaborskienė, & Šlapkauskaitė, 2015). On the other hand, the seeds had a h/H ratio of 4.29. Hypocholesterolemic/hypercholesterolemic ratio is specifically related to cholesterol metabolism, thus from the nutritional point of view, the higher the value the better the oil (Feddern *et al*., 2010; Ganzaroli *et al*., 2017; Hashempour-Baltork, Torbati, Azadmard-Damirchi, & Savage, 2018). When compared with data from other countries, the results from this study showed a favorable nutritional balance, implying that the inclusion of pumpkin seeds in the human diet could prevent the development of CVD among consumers of pumpkin seeds in Uganda.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Pumpkin seeds would contribute significantly towards mineral especially phosphorus, manganese, magnesium, zinc, and iron dietary. The oil yield of the pumpkin seeds was high, making them a potentially valuable source of edible oil. The oil was rich in linoleic acid, an important nutritional aspect since linoleic acid is an essential ω-6 fatty acid. Favorable nutritional quality indices based on the lipid and fatty acid composition confirmed the good nutritional quality of pumpkin seeds. Therefore, pumpkin seeds are a health food for human consumption.

5.2 Recommendations

Pumpkin seeds could be included as a functional ingredient in foods for the enhancement of food security. However, because pumpkin seed oil is generally poor in ω-3 PUFA, blending with other oils rich in these fatty acids would create a more positive nutritional effect. Further studies are recommended in respect of the anti-nutrient composition of pumpkin seeds and their impact on micronutrient bioavailability, and the potential allergenic reactions that may be associated with pumpkin seeds especially among children.

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