DETERMINATION OF FAT SOLUBLE VITAMINS IN SOLVENT EXTRACTED MELON SEED OIL AND AMINO ACID PROFILE OF WHOLE MELON SEED FLOUR

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN, BENIN CITY.

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A PROJECT THESIS WRITTEN AND SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.SC.), DEGREE IN SCIENCE LABORATORY TECHNOLOGY (BIOTECHNOLOGY TECHNIQUES)

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CERTIFICATION

This is to certify that the project work was carried out by **Gift Uwaila OMORUYI (Miss)** with matriculation number **LSC1606019**, Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to Almighty God, for the gift of life, His mercies, protection, provision, for knowledge, wisdom and understanding through the period of compiling this project.

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I am indeed grateful to God Almighty for His infinite mercies, divine protection and love throughout the period of my study and preparation of this work. My profound gratitude goes to my Project Supervisor I, Dr. E. O. Oshomoh and Project Supervisor II, Mrs. P.O. Omozuwa for their guidance, encouragement, contributions and being very supportive in making my project work a success.

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ABSTRACT

This research study was carried out to evaluate the fat soluble vitamin contents (vitamin A, D, E and K) in solvent extracted melon seed oil and to determine the Amino acid profile of whole melon seed flour over a period of time. Results on fat soluble vitamins shows that, vitamin A had the highest value, followed by vitamin E, K and vitamin D had the lowest value of the fat soluble vitamin content and they were all relatively stable from month 0 to month 1. Result on whole melon seed flour shows that, it contains eleven essential amino acids and seven non-essential amino acids. Therefore solvent extracted melon seed oil contains fat soluble vitamins and whole melon seed flour contains the basic essential amino acids.

CHAPTER ONE

INTRODUCTION

A significant indigenous oil seed that is consumed by rural tribes in West Africa is melon seeds (*Citrullus colocynthis*) (Bankole and Adebanjo, 2003). Melon seeds have a high protein and oil content, with up to 35% protein and roughly 50% oil (Achu, 2005). The seeds have a small quantity of vitamins, protein, and oil (John, 2002). The majority of annual plant seeds yield large amounts of oil (Raziq *et al*., 2012). Due to their wide range of applications for both industrial and human requirements, oil and fat are in great demand worldwide. As a result, oil seeds play a significant role in the national economies of the nations that produce them (Corley *et al*., 2008).

Enhancing existing oil seed varieties and, more significantly, discovering new sources of oil are essential for increasing oil yields (Ziyada and Elhussien, 2018). Melon seeds include 4.5% moisture, 25.0% crude protein, 2.4% ash and 23.3% crude fibre. (Yantys *et al*., 2008). In addition to being used as a skin moisturiser and a common source of feedstock for the manufacturing of biodiesel, melon oils are also good for cooking (Clark, 2011).

A material is extracted from a matrix using one of two processes: mechanical (expression) extraction or chemical (solvent) extraction. Liquid-liquid and solid-liquid phase extraction are also included (Young *et al*., 1994). In this project, oil was extracted using a solvent.

1.1 AIM OF STUDY

To determine the presence of fat soluble vitamins (A, D, E and K) found in solvent extracted melon seed oil and also to determine the amino acids in whole melon seed flour.

1.2 OBJECTIVES OF STUDY

1. To prepare whole melon seeds
2. To chemically extract melon seed oil using a Soxhlet extractor in which Folch method is employed (using chloroform and methanol in the ratio of 2:1)
3. To determine the fat soluble vitamins present in the oil (vitamins A, D, E and K)
4. To determine the amino acids present in whole melon seed flour

CHAPTER TWO

LITERATURE REVIEW

Citrullus colocynthis, often known as melon seeds, is a member of the *Cucurbitaceae* family and is the traditional name for the native melon grown in Nigeria (Giwa *et al*., 2010). Small, flat, and oval in shape, melon seeds have a white cotyledon inside of a thin-walled shell that has a thick ring around the edges. The seed has a tapered end on one side and a rounded end on the other. As the flesh of gourds is neither delicious nor edible, they are mostly grown for their seeds. The gourds are left to ferment after being harvested, and the fermented flesh is removed from the seeds. Light brown husks are then manually or mechanically removed, and the seeds are dried (Oriaku *et al*., 2013).

Melon seed (*Citrulluscolocynthis*), a creeping but non-climbing annual herbaceous monoecious plant, belongs to the *Cucurbitaceae* family. Within three weeks of seeding, they totally cover the soil, and blossoming ensues. Insects carry out the pollination, and three to four months after planting, melon fruits, which are smooth, indehiscent berries that are frequently huge and seedy, are ready for harvest (Ogbonna *et al*., 2000). Proteins, oils, minerals, vitamins, fibre, and biologically active ingredients like phospholipids, sterols, and tocopherols are all abundant in melon seeds (Bande *et al*., 2013). In addition to having high oil content, melon seeds also contain roughly 30% pure protein. Protein gives the muscles and bones structure, preserving the skeletal system of the body (Oguntola 2010; Oluba *et al*., 2010).About 1-60% of plant seeds and occasionally the fleshy section of fruits like olive and oil palm contain a significant amount of oil (Higgings, 2003).

Melon contains glucose, fructose, vitamins A, D, C, K, and E, as well as certain group B vitamins. Melon contains 4.6 to 18% sugars and up to 4.5% pectin by weight. Melon also contains minerals like magnesium, sodium, phosphorus, potassium, selenium, calcium, and salt. Along with the components listed above, melon also contains a number of aromatic compounds (Ivanova, 2012).

2.1 SCIENTIFIC CLASSIFICATION

Scientific Name: *Citrullus colocynthis*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Citrullus
Species	colocynthis

Showing the Scientific classification of Melon *Citrullus colocynthis*

2.2 BOTANY AND MORPHOLOGY

A trailing, herbaceous annual crop is the melon (Agba *et al*., 2009). The leaves are inherently split (deeply cleft) into five irregular lobes that are roughly serrated and alternately arranged. They are 10 to 15 cm long, glabrous or somewhat scab ulous, and denticulate (Kehinde, 2008). They resemble watermelon leaves and have 3 to 7 split lobes (Schafferman *et al*., 1998). The stem is soft, hairy, and ridged, but at the base it turns woody (Rice *et al*., 1987).

While its fibrous root system grows out and climbs over neighbouring objects, its stem does not (Kehinde, 2008). Female flowers are somewhat larger than male blossoms in monoecious flowers (Lomova and Terekhina, 2009). The inferior ovary of female flowers is spherical and hairy. After germination, the first male flower opens 30 to 45 days later than the first female flower, which blooms 40 to 55 days later. After germination, flowering peaks 50 to 80 days later. Insects, primarily bees, typically pollinate the flowers in the morning. In the following 24 hours, the pedicel lengthens and the ovary swells (Vossen *et al*., 2004). The smooth, sphere-shaped fruit of the melon has a diameter of 5 to 10 cm. Depending on the kind, it has dull green or green skin with white markings, with a size range of 7 to 20 cm in diameter (Dupriez and Deleener, 1989).

White pulp that is supple and supportive covers the seeds in the mesocarp. An average plant produces 15 to 30 fruits (Schafferman *et al*.,, 1998). The type of seed is not based on the fruit's form. As a result, every fruit shape can contain any of the major seed kinds (Denton and Olufolaji, 2000). Fruit weight varies greatly, from very heavy (over 1500 g) to light (less than 300 g), and the white pulp, which contains 95% or more water, makes up the majority of this variation (Cobley, 1979). At maturity, the green fruit stem turns brown and contain fruit pulp that is bitter and inedible.

Figure 1: Melon seed fruit *Citrulluscolocynthis* (Picture courtesy: Zell, 2012)

Figure 2: Melon seed powder (Image courtesy: All Nigeria Recipe, 2012**)**

Figure 3: Melon seeds (Image courtesy: All Nigeria Recipe, 2012)

2.3 CULTIVATION

Melon seeds are offered all year long. This is due to the fact that it is dried after harvest, giving it a lengthy shelf life. Typically, planting takes place in April, while harvesting occurs in July. Before planting, fields are cleared and tilled. In farms, melon seeds are sown with 1 m × 1 m spacing, either on ridges, mounds, or flat surfaces.

Four to seven days after seeding, germination occurs (Adewusi *et al*., 2000).Three to four weeks following sowing, thinning is done. The plant controls weed due of its early ground coverage (Abbah *et al*., 2014). There is a 120-day growth period overall (Vossen *et al*., 2004).

2.4 SOIL AND CLIMATIC REQUIREMENTS

In sandy loam soil with a pH of 6 to 7, melon thrives best. In contrast to heavy rainfall or irrigation, which promotes disease on the field, well-aerated fertile soil is ideal for good and prolific growth of melons (Schippers, 2002). Low rainfall periods (at least 700 to 1000 mm) are tolerated and temperatures between 28 and 35 °C are needed (Adewusi *et al*., 2000).

2.5 HARVESTING, PROCESSING AND STORAGE OF MELON SEEDS

When the melon's fruit stem and auxiliary tendrils have dried out, the fruit is harvested. Sturdy wooden staffs are used to break fruits. They are then collected and covered for two weeks of fermentation. The seeds are taken out of the pulp, cleaned, and sun-dried. Harvested seeds are sold or kept in jars, lofts, baskets, and bags (Schippers, 2002). While seed without a seed coat had viability reduced to 12 months after storage, seed with a seed coat extended seed viability beyond 36 months (Adewusi *et al*., 2000).

2.6 NUTRITIONAL BENEFITS OF MELON SEEDS

Melon seed can be used as an oil seed or eaten whole because it is edible and high in fat and protein. It's interesting to note that melon seed oil primarily comprises unsaturated fatty acids, as has been widely reported. The oil in the seeds has a weight percentage of 17–19 % (Schafferman *et al*., 1998) and is made up of about 67–73 % linoleic acid, 10–16 % oleic acid, 5-8 % stearic acid, and 9–12 % palmitic acid.

It was also stated that its oil yield value is estimated at 250–400 L/hectare and that its oil constituent is comparable to safflower oil, with a total of 80–85% unsaturated fatty acids. According to (Oluba *et al*., 2008), the percentage composition of the oil by weight is: oleic, 14.50 %; linoleic, 56.94 %; palmitic, 0.78 %; myristic, 13.45 %; stearic, 13.71 %; and linolenic, 0.46 %. (Oguntola, 2010; Oluba *et al.,* 2010; Oluba *et al*., 2008; Bankole *et al*., 2005; Schafferman *et al*., 1998).

This composition contains 57.4% polyunsaturated fatty acids, making up approximately 72% of the weight (PUFA). The body cannot produce polyunsaturated fatty acids (PUFAs), hence it must be gotten from the food as they are necessary fatty acids. Unsaturated fats help the body absorb vitamins, specifically the fat-soluble vitamins A, D, E, and K. This is because the body accumulates fat soluble vitamins, it is possible to take excessive amounts of them and experience vitamin poisoning symptoms. The body absorbs fat-soluble vitamins when they are consumed and stores them in the fatty tissue (Zaykoski, 2011).

2.7 UTILIZATION OF MELON

In Nigeria, melon seed is used for a variety of dishes, including "egusi" soup and sauces (Denton and Olufolaji, 2000). Schippers (2002) stated that the crop has a significant impact on the ability of African peasant farmers to generate revenue. In Nigeria, seed production ranged from 131 to 1005 kg/ha (Ojo *et al*., 2002). It is frequently used in commercial and domestic cookery (for example, in the production of margarine, salad oil, and canned fish) (Adewusi *et al*., 2000). Other industrial applications include the production of candles, metal polish, lubricant, and pomade (Osunde and Kwaya, 2012).The seed is high in protein (69–78 % in the defatted meal), oil (53.1 %), and other nutrients (Ogbonna and Obi, 2007; Ogbona, 2013).

Some farmers in Republic of Benin claimed that *Citrullus lanatus sub sp*. mucosospermus has therapeutic characteristics and that the young, sliced fruit of Egusi is used to treat stomach issues (Achigan-Dako *et al*., 2008).

2.8 FAT SOLUBLE VITAMINS (A, D, E AND K) IN MELON SEED OIL

Vitamins are necessary nutrients that the body needs in small amounts for metabolism, protection, growth, and other processes as well as for regular development and operation of the body. Since the body cannot produce them, they must be taken from outside sources such as food, sunlight, and bacteria in the rumen. Additionally, it aids in the synthesis of genetic material, blood vessels, nervous system chemicals, and hormones. The majority of the time, they play the role of catalysts, joining with proteins to produce enzymes that are active in metabolism and vital for life reactions. Vitamins differ chemically from one another, some are alcohols, aldehydes, organic acids, and their derivatives, as well as derivatives of nucleotides.

Vitamins are categorised based on whether they can be absorbed better in fat or water. Both water-soluble and fat-soluble vitamins exist. For the body to operate properly, fat-soluble vitamins are crucial. These chemicals, which are hydrophobic and oily are retained in the liver and aren't expelled from the body. They require bile salts and lipids for absorption. Vitamins A, D, E, and K are examples of fat-soluble vitamins, whereas Vitamins B complex and C are examples of water-soluble vitamins (Kamangar and Emadi 2012).

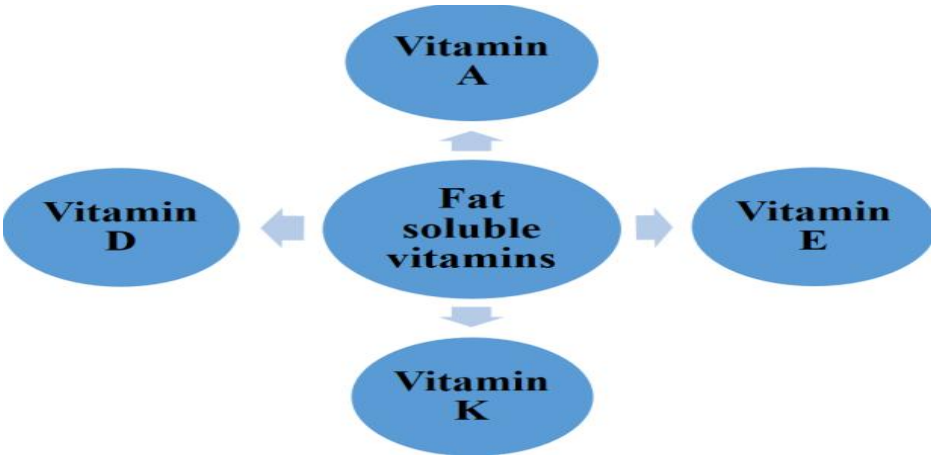

Figure 4: Examples of Fat Soluble Vitamins

2.8.1 Fat Soluble Vitamin A

A light-yellow primary alcohol made from carotene is vitamin A. Retinol (alcohol form), Retinal (aldehyde form), and Retinoic acid are all included (acidic form). You can get vitamin A from both plant and animal sources. Preformed vitamin A, such as retinoids, is known as vitamin A derived from animal sources (Rodriguez-Amaya, 2004), whereas provitamin A, such as carotenoids, is known as vitamin A derived from plant sources (Booth et al., 1992; Vuong 1997). Retinoids come in a variety of forms, including retinol, retinal, retinoic acid, and retinyl esters. The plant-derived provitamin A carotenoids beta-carotene, beta-carotene, and beta-cryptoxanthin are transformed by the human body's digestive process into preformed vitamin A retinoids. Vitamin A supports the immune system, the inflammatory system, cell growth and development, antioxidant activity, and correct cell communication in addition to assisting in the creation of red blood cells, skin, and bones. Red palm oil, sweet potatoes, carrots, spinach, amaranth, broccoli, pumpkin, squash, papaya, mango, bell pepper, parsley, sea vegetables, chilli peppers, tomatoes, shrimp, eggs, and grapefruit are all excellent sources of vitamin A. Sources of preformed

Vitamin A include, shrimp, eggs, cow's milk, cheese, yoghurt, salmon, sardines, chicken, turkey, cod, beef, and lamb.(World Health Foods, 2017).

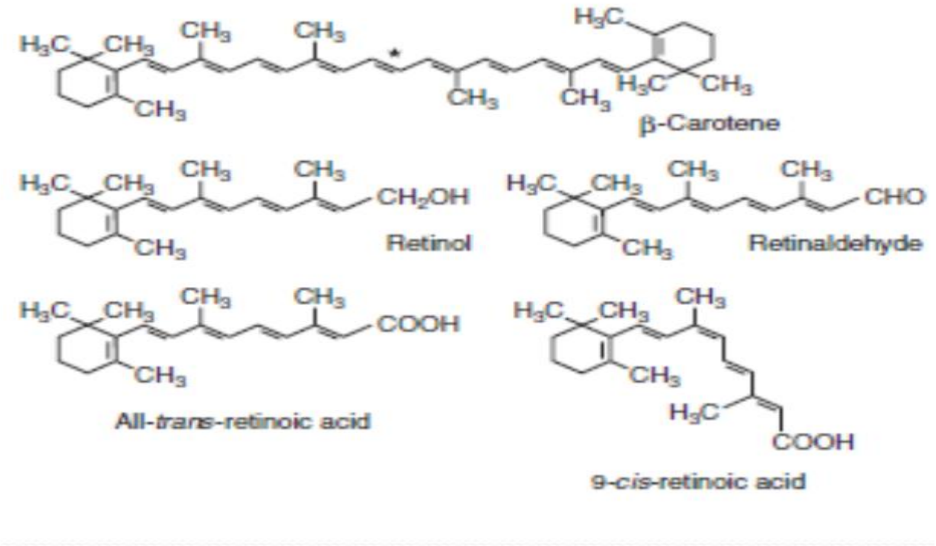

Figure 5: STRUCTURE OF VITAMIN A

2.8.2 Fat Soluble Vitamin D

This comprises a group of fat soluble sterol founds naturally in few foods. Ergocalciferol and D_3 are the two main forms of vitamin D that are physiologically significant (cholecalciferol). The body needs vitamin D, but the precise amount that must be consumed varies and follows no set pattern because sunlight exposure also causes the production of vitamin D in the human skin.

When sunlight's ultraviolet B (UVB) wavelength enters the body, it causes the human body to convert 7-dehydrocholesterol into cholecalciferol, a precursor of vitamin D. However, the amount of cholecalciferol produced by UV-B depends on the kind and makeup of skin pigments, the condition of the skin, and the characteristics of sunshine.

The conversion of cholecalciferol into the active form of vitamin D involves the kidney and liver, which further complicates prediction as cholecalciferol is not a fully active form of vitamin D. There are very few food sources that contain vitamin D, however sunlight exposure can enhance the body's level of this vitamin (World Health Foods, 2017).

Because it promotes healthy bones, the contraction of muscles and nerves, as well as general cellular function in all body cells, vitamin D is crucial for regular body function. Bone deformation and softening are brought on by its lack. Numerous diseases, including osteoporosis, rickets, osteomalacia, loss of balance, diabetes, rheumatoid arthritis, asthma, depression, epilepsy, and impaired immunological function are linked to vitamin D deficiency. In children, malformation of the bones is known as rickets, whereas in adults, it is known as osteomalacia (Wagner and Greer, 2008).

Vitamin D improves the digestion of calcium and decreases urinary calcium losses. It also keeps the blood calcium level stable by removing calcium from bones; however this method works best when there is enough vitamin D in the diet. Lack of vitamin D also increases the risk of diabetes and high blood sugar (Belenchia *et al*., 2013). The maturation of white blood cells, which are the first line of defence in immune responses, is influenced by vitamin D. Additionally; a consistent link between low vitamin D levels and an increased risk of respiratory infections was found (Jolliffe *et al*., 2013). Since the 1980s, there has reportedly been a rise in vitamin D deficiency because of greater sunscreen use and less exposure to sunlight (Faurschou *et al*., 2012).Egg yolks, cheese, cereals, salmon, sardines, mushrooms, cow's milk, soy milk, orange juice, and fortified foods are some examples of food sources (World Health Foods, 2017).

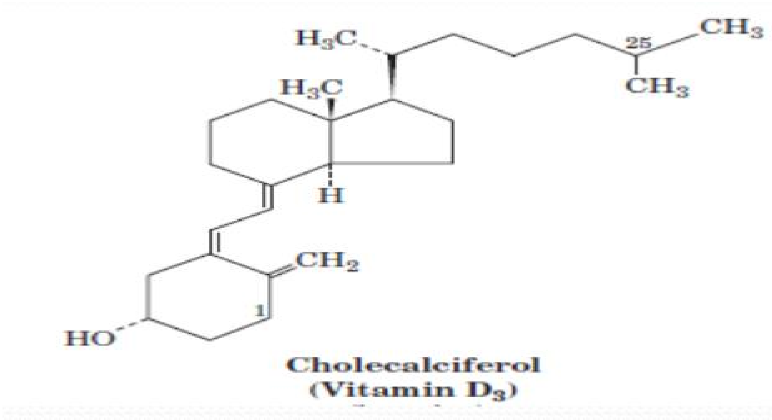

Figure 6: STRUCTURE OF VITAMIN D

2.8.3 Fat Soluble Vitamin E

Tocopherol and conception hormone are other names for vitamin E. Humans must consume vitamin E, however it is rarely deficient outside of pregnancy and the first few months of life, when it is linked to hemolytic anaemia. It is present in the diet as a blend of eight tocopherols, which are related molecules. Four separate tocopherols and four different tocotrienols make up the broad category of vitamin E.

These eight chemicals are collectively referred to as "tocochromanols," and they are fat-soluble antioxidants. The vitamin E component alpha-tocopherol is well recognised and extensively researched. Vitamin E prevents oxidative damage to membrane lipids and preserves cellular health thanks to its antioxidant properties. During storage and preparation, this vitamin shields the food against oxidative deterioration (World Health Foods, 2017). Vitamin E shields low-density lipoprotein (LDL) cholesterol against oxidative damage brought on by free radicals, blood vessel growth, and immune system stimulation. Low-density lipoprotein (LDL) cholesterols become more susceptible to oxidative damage and turn into oxidised low-density lipoprotein when vitamin E deficiency occurs.

Atherosclerosis, often known as hardening of the arteries, is caused by oxidised low-density lipoprotein building up in the blood vessels (World Health Foods, 2017).

Vegetable oils including sunflower, wheat germ, corn, safflower seeds, and soybean oils, nuts like almonds and peanuts, leafy greens like spinach and broccoli, tomatoes, avocado, shrimp, olives, olive oil, collard greens, raspberries, carrots, and green beans are all good sources of vitamin E. (World Health Foods, 2017).

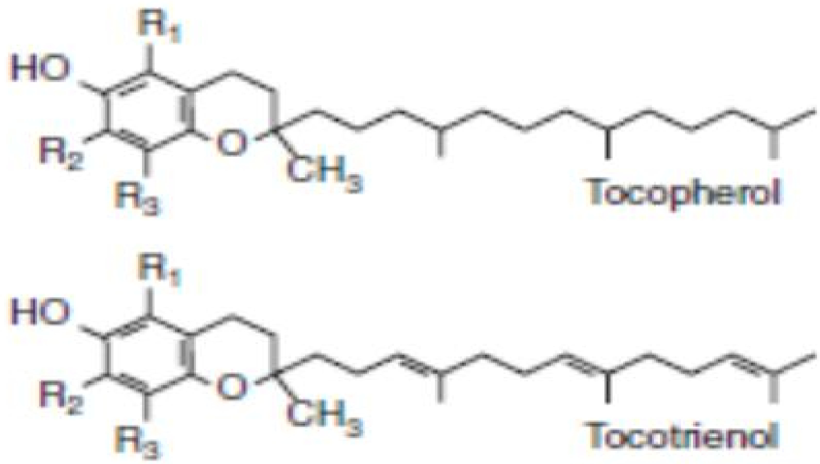

Figure 7: STRUCTURE OF VITAMIN E

2.8.4 Fat Soluble Vitamin K

Phylloquinone, anti-hemorragic vitamin, and coagulation vitamin are other names for vitamin K. The complex unsaturated hydrocarbon known as vitamin K is available in two forms: vitamin K_1 (phylloquinone) and vitamin K_2 (Menaquinone) Because vitamin K is derived from the German word "koagulation," which implies blood clotting, blood clotting factors are also known as vitamin K. (Shearer *et al*., 2012; Shearer, and Newman, 2014). Three fundamental varieties of vitamin K are K_1, K_2, and K_3. K_1 form is most prevalent and needed for photosynthesis of plants. K_2 form is synthesized from K_1 and K_3 form by bacteria and other microorganisms.

In human body, K_2 is also synthesized by biological conversion of K_1 and K_3. K_2 is not present in preformed form in plants but it is produced by fermenting bacteria through transformation of K_1 into K_2 (Hirota *et al.,* 2013). Key functions of vitamin K are photosynthesis, antioxidants and energy generation by electron movement (Kurosu and Begari, 2010).

Menaquinones, phylloquinones, and menadiones are the scientific names for K_1, K_2 and K_3, respectively. Depending on the circumstances, blood clotting can be advantageous or dangerous. Because twenty separate proteins are needed to complete the clotting process and four of those proteins need vitamin K to function, blood clotting is an extremely complicated process (Shearer *et al*., 2012; Shearer, and Newman, 2014).

A lack of vitamin K raises the chance of bone fracture, which is crucial for bone health. Vitamin K prevents the widespread formation of osteoclast cells, slows down demineralization, and controls osteoclasts by inducing programmed cell death (Atkins *et al*., 2009; Shearer, and Newman, 2014).

Bone strength is increased by vitamin K, particularly vitamins K_1 and K_2, which control the carboxylation of osteocalcin proteins. Dark green leafy vegetables are a good source of K_1, whereas eggs, meat, fish, dairy, fermented animal foods, and fermented plant foods are good sources of K_2. K_3 is not naturally present in dietary items (Shearer *et al*., 2012; Shearer, and Newman, 2014).

The following foods are typical dietary sources of vitamin K: spinach, mustard greens, collard greens, beet greens, swiss chard, turnip greens, broccoli, brussels sprouts, romaine lettuce, basil, cabbage, celery, kiwifruit, leeks, sage, green beans, cauliflower, cucumber, tomatoes, oregano, black pepper, green peas, blueberries, grapes, carrots, summer squash, clove (World Health Foods, 2017).

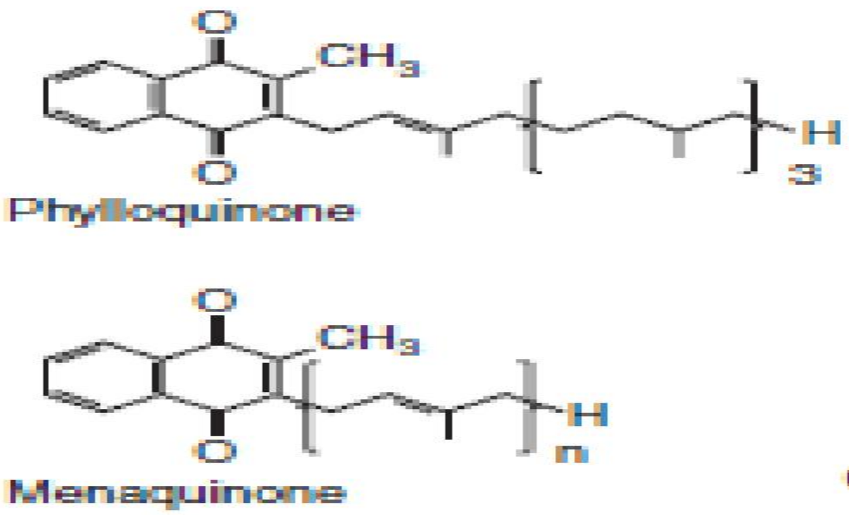

Figure 8: Structure of Vitamin K

2.9 AMINO ACIDS IN WHOLE MELON SEED FLOUR

However, the phrase "amino acid" is always used to refer to an alpha-amino carboxylic acid. The term "amino acid" can refer to any molecule that contains both an amino group and any sort of acid group.

Glycine, an amino acetic acid, is the most basic alpha-amino. There are side chains (symbolised by R) replaced on the alpha-carbon atom in other popular amino acids. For instance, the amino acid alanine has a methyl side chain. The alpha-amino acids are all chiral, excluding glycine.

About half of the amino acids required to form proteins can be produced by humans. The diet must contain additional amino acids known as the essential amino acids. The following chart lists the essential amino acids:

Table 2: Amino acid composition (g/100g protein) of melon seed flour

Amino Acid	Concentration (g/100g Protein)
Histidine*	2.0
Alanine *	5.6
Arginine*	9.0
Lysine*	0.4
Glycine	2.2
Serine	2.4
Threonine*	3.1
Methionine*	0.3
Aspartic acid	16.3
Isoleucine*	4.8
Leucine*	4.2
Glutamic acid	16.9
Proline	3.2
Phenylalanine*	3.2
Tyrosine	2.2
Valine*	1.3
Cysteine*	1.1

*Essential Amino Acids

Source: (Ojieh *et al.,* 2008)

Complete proteins are defined as having all the essential amino acids in roughly the correct amounts for human nutrition. Meat, fish, milk, and eggs are some sources of complete proteins. Adult people need about 50g of complete protein per day. Incomplete proteins are those that are severely lacking in one or more of the necessary amino acids. The amount of human protein that can be produced is constrained by the levels of the insufficient amino acids if the majority of the protein in a person's diet comes from one imperfect source. In general, plant proteins are unfinished. Lysine is lacking in all three grains: wheat, corn, and rice.

Corn and rice both lack the amino acid tryptophan. The most complete proteins among common plants are found in beans, peas, and other legumes; however they are lacking in methionine.

If vegetarians eat a variety of plant foods, they can obtain an appropriate intake of the essential amino acids. When choosing plant proteins, keep in mind that some meals may provide amino acids that other foods do not. An alternative is to include a rich supply of complete protein, like milk or eggs, in the vegetarian diet as a supplement.

Citrullus colocynthis seed protein content was determined to be 8.25% and to be rich in lysine, leucine, and sulfo-amino acids, specifically methionine (Shaheemet *et al*., 2000). The oil (52 %), protein (28.4 %), fibre (2.7 %), ash (3.6 %), and carbohydrates (8.2 %) are all present in the melon (*Citrullus colocynthis*) kernels. These are good providers of minerals (Ca, Mg, Mn, K, P, Fe and Zn), vitamins B_1, B_2, and Niacin, as well as vital amino acids like arginine, tryptophan, and methionine (Simmons *et al*., 1982). Especially sunflower and safflower meals, which compare favourably with those of other oil seeds, for their important amino acids. (Sosulski and Sarwar, 1973). In contrast to most oil seeds, melon seeds have higher concentrations of the amino acids arginine, tryptophan, and amino acids containing sulphur.

The flour is a rich source of vegetable protein since the proteins contain important amino acids. When melon seed flour was added to traditional West African diets, either alone or with other plant proteins, there was a noticeable improvement in growth (Sanchez *et al*., 1972). Lysine and threonine are the first and second limiting amino acids in the flour, respectively. This partially corroborates the findings of Oyenuga and Fetuga (1975), which noticed that the first and second limiting amino acids of watermelon seeds - a close relative of melon, were respectively, lysine and methionine.

Several micronutrients (vitamins and minerals) included in melon seed flour could greatly improve diet (Kopple and Swendseid, 1974).

2.10 OIL EXTRACTION METHOD

2.10.1 Old Traditional Methods

The ancient traditional or natural wet extraction techniques employed in remote populations in the world are reffered to as ineffective in terms of oil recovery and oil output, frequently earning below the range of plant oil content documented in literature (Alonge and Olaniyan, 2006; Olaniyan, 2010). Olaniyan (2010) identified wet extraction (hot water or steam extraction), solvent extraction, and mechanical expression as the three main methods for extracting oil from oleaginous materials derived from plants. Regarding the wet extraction method, (Oluwole *et al*., 2012) suggested nine major operations that are involved in the extraction of castor oil by the old ancient method, including gathering seed pods, shelling the pods/winnowing, boiling the seeds to reduce moisture, grinding the seeds to form paste, mixing the paste with water/boiling to extract oil, scooping the oil, and drying the oil by heating.

According to (Olaniyan and Yusuf, 2012), the old-fashioned method of extracting seed oils involved roasting the seed kernels using a mortar and pestle or between two stones, mixing the crushed mass with water, cooking the mixed paste to extract the oil by floating and skimming, and then drying the oil through additional heating. They went on to say that this strategy was time-consuming, energy-draining, prone to drudgery, ineffective, and low in income and quality. In other words, the out-dated, conventional procedures are imprecise, mostly non-scientific, ineffective, and produce extracted oil of subpar quality.

2.10.2 Conventional Methods

The formal methods are the well-known and commonly utilised procedure of oil extraction namely, solvent extraction and mechanical expression. Either of the two techniques, or a combination of the two, is used to extract a lot of seed oils. Although possibly not as commonly utilised, rendering may also fall under this group (Ali *et al*., 2005).No matter the extraction technique, the physical-chemical characteristics of the extracted and refined oil must be assessed to determine its application or consumption.

2.10.3 Solvent Extraction Method

A traditional extraction technique used frequently on oilseeds with low oil content (20 %), like soybean, is solvent extraction. This technique is thought to be one of the most effective ways to extract vegetable oil because it leaves little leftover oil in the cake or meal (Buenrostro and Lopez-Munguia, 1986; Tayde *et al*., 2011). The preferred solute substrate's maximal leaching properties are the primary factor in the solvent selection (Dutta *et al*., 2015). Hexane, diethyl ether, petroleum ether, and ethanol are examples of regularly used solvents. High solvent-solute ratios, the volatility of the solvent in relation to the oil, the viscosity and polarity of the oil, cost, and market accessibility are other factors (Muzenda *et al*., 2012; Takadas and Doker, 2017).

There are several benefits to the solvent extraction technique. The solvent extraction process is a very efficient method, with high yield and consistent performance, though cost of production was relatively higher than mechanical press methods due to the high cost of solvent, according to Bhuiya *et al*. (2015) who conducted research on the optimization of oil extraction process from Australian Native Beauty Leaf Seed (*Calophylluminno phyllum*).

The ability of the solvent to extract oil during solvent extraction is said to be enhanced with an increase in extraction time, with the solvent-solute ratio preferably being in the solvent's favour by a factor of 6:1, according to Muzenda *et al*. (2012) in their work on the optimization of process parameters for the production of castor oil. The impact of extraction techniques on the quantity and quality attributes of oils from shear nuts were investigated by (Ikya *et al*., 2013). Results of oil extracted using solvent extraction and oil extracted using more conventional methods were compared in terms of physical, chemical, and sensory aspects.

They reported an improved oil holding quality for the solvent-extracted oil and a greater oil production of 47.5 % compared to the older traditional method's 34.1% (lower moisture content and lower flash and fire point values). Akpan *et al*., (2006) used the solvent extraction method to separate castor seed oil from castor bean paste using a Soxhlet extractor as part of their research on the extraction, characterisation, and modification of castor seed oil. Their 33.2 % oil output was within the range predicted by the literature for castor beans. They came to the conclusion that the manner of extraction and seed variety are crucial factors influencing oil yield. Its reliability and reproducibility are further benefits.

However, this process has a few drawbacks for industry, including extended extraction times, relatively high solvent consumption, high investment, high energy needs, issues with plant security, emissions of volatile organic compounds into the atmosphere, high operating costs, poor product quality brought on by high processing temperatures, and a disproportionately high number of processing steps (Buenrostro and Lopez-Munguia, 1986; Del Valle and Aguilera, 1999; Dawidowicz *et al*., 2008; Takadas and Doker, 2017). The procedure also employs organic solvents, whose clean-up add to the cost and labour (Gibbins *et al*., 2012).

The main method for extracting vegetable oil from oleaginous materials is the soxhlet-based solvent extraction procedure. The Soxhlet procedure is frequently employed for small-scale oil extraction in laboratories (Abdelazize *et al*., 2014), but for large-scale operation, a solvent extractor would be needed (Ogunniyi, 2006). The Soxhlet process' primary benefit is solvent recycling (repeatedly) during extraction. However, the Soxhlet approach has drawbacks such as a high solvent need, time and energy consumption, and sample dilution in large volumes of solvent (Takadas and Doker, 2017; Rassem *et al*., 2016).

2.10.4 Oil Seed Pre-treatment

No matter the extraction technique chosen, oil seed pre-treatment is essential. Basic phases in this process are dehulling, pod or seed coat removal, winnowing, sorting, cleaning, grinding or milling and preheating (Ogunniyi, 2006; Yusuf *et al*., 2015). Prior to extraction, oilseeds are ground or crushed to ensure that tiny cells that contain oil and are embedded in fibrous structures are ruptured or broken, releasing the oil (Akpan *et al*., 2006; Tayde *et al*., 2011). Heat treatment reduces moisture content and hardens the inside of the oilseed, which helps the oil release process even more (Patel *et al*., 2016). In recent years, microwave-assisted heat treatment has replaced conventional hot air oven preheating of oilseeds since it offers some advantages (Mgudu *et al*., 2012). Furthermore, size reduction or grinding before solvent extraction enhances the surface area for solvent penetration to leach the oil out. Oil output from oleaginous seed material is often influenced by the oilseeds' quality. To increase oil yield, certain pre-treatment variables can be changed, including temperature, particle size, and moisture content of the raw material.

Oil output and quality are typically impacted by oil seed pre-treatment before oil extraction, according to Olaniyan (2010). Similar to this, Faugno *et al*. (2016) showed that the combination of seed preheating and high extraction temperature, among other things, had a substantial impact on oil output when they analysed the key extraction parameters on yield of mechanically pressed tobacco (*Nicotianatabacum L*) seed oil. Therefore, oilseed processing or pre-treatment offers a way to control important variables and circumstances for improved oil yield and quality.

2.11 SOXHLET EXTRACTOR

Franz von Soxhlet created a laboratory tool called a Soxhlet Extractor in 1879. (Soxhlet, 1879). Its original purpose was to remove lipids and other compounds from solid samples. A Soxhlet extractor is based on the idea of a Pythagorean cup, sometimes known as a "greedy cup" or "joke cup." When the container is overfilled, the liquid begins to flow out of a hole in the bottom (Harwood, 1987).

An exhaustive extraction method frequently used with analytes that are sufficiently thermally stable is soxhlet extraction. The sample is collected in the hot solvent as the extraction solvent is cycled continuously through the matrix through boiling and condensation. The presence of both non-polar and polar lipids in the oils recovered from plant material at room temperature employing a combination of solvents, such as chloroform and methanol, may increase the extraction yield (Soxhlet, 1879).

2.11.1 Description of a Soxhlet Extractor

A Soxhlet extractor has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a syphon mechanism, which periodically empties the thimble.

2.11.2 Assembly of a Soxhlet Extractor

- The source material containing the compound to be extracted is placed inside the thimble
- The thimble is loaded into the main chamber of the Soxhlet extractor
- The extraction solvent to be used is placed in a distillation flask
- The flask is placed on the heating element
- The Soxhlet extractor is placed on top the extractor

2.11.3 Working Principle of a Soxhlet Extractor

As soon as the solvent reaches reflux, the solvent vapour climbs a distillation arm and pours into the chamber holding the solid's thimble. Any solvent vapour is made to cool and drip back down into the chamber containing the solid substance by the condenser. Warm solvent progressively fills the chamber containing the contaminated material. In the heated solvent, some of the desired chemical dissolves. The Soxhlet chamber is emptied by the syphon when it is almost full. The distillation flask receives the solvent back. The thimble makes sure that no solids are transported to the still pot by the solvent's rapid velocity. This cycle may be permitted to repeat numerous times, over hours or days.

A fraction of the non-volatile component dissolves in the solvent throughout each cycle. The target component is concentrated in the distillation flask after several cycles. The benefit of this method is that only one batch of the warm solvent is recycled, as opposed to numerous portions being passed through the sample. The extracted component is obtained after the solvent has been eliminated, often using a rotary evaporator. The extracted soil's insoluble component is often left in the thimble and thrown away (Jensen, 2007).

2.11.4 A Soxhlet Extractor

Plate 1: A Soxhlet Extractor (Picture Courtesy: Chemistry Annex Lab, 2022)

2.12 HIGH PERFORMANANCE LIQUID CHROMATOGRAPHY (HPLC)

High Performance Liquid Chromatography is known as HPLC. Chromatography is a separation process, a chromatogram is the end product, and a chromatograph is the equipment used to carry out chromatography. In order to separate, recognise, and measure the active chemicals, High Performance Liquid Chromatography (HPLC), a particular type of column chromatography, is frequently employed in biochemistry and analysis. A mobile phase/solvent reservoir, a pump, an injection valve, an HPLC column, a detector, a solvent delivery system, and a PC with a liquid chromatography data system installed are some of the parts that make up high pressure liquid chromatography (HPLC) (Martin, 2005).

2.12.1 Working Principle of HPLC

The distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase is the foundation of the HPLC separation principle (packing material of the column). The molecules travel through the stationary phase more slowly depending on the chemical makeup of the analyte.

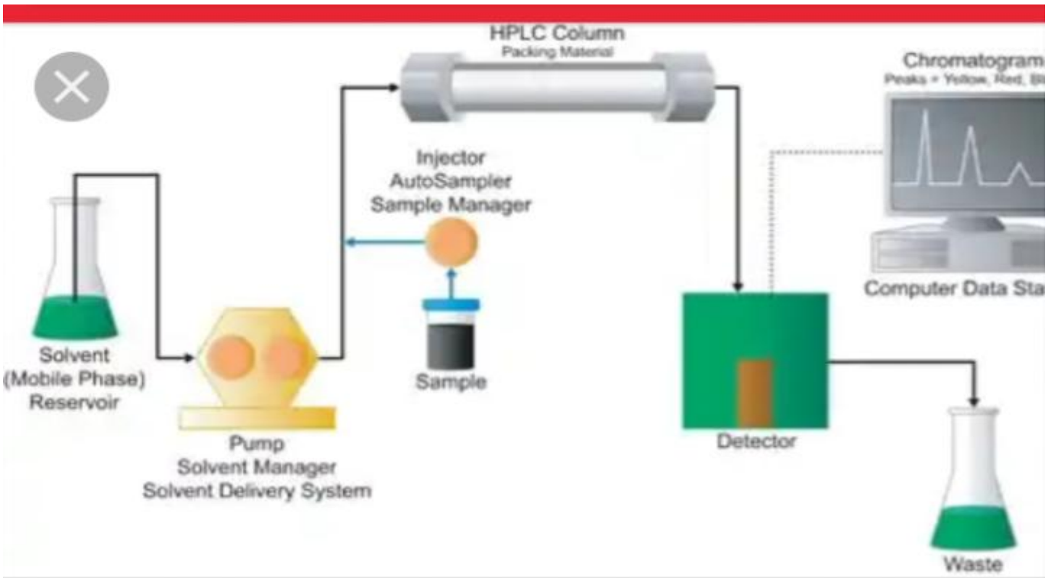

Figure 9: Working principle of HPLC

Figure 10: High Pressure Liquid Chromatography (HPLC)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Dried grounded melon seeds

3.1.1 Chemicals and reagents

Chloroform

Methanol

All chemicals were of analytical grade

3.1.2 Apparatus

Conical Flask

Round bottom flask

Volumetric Flask

Kjeldahl flask

Transparent plastic containers

Plastic Sample/Specimen bottles

3.1.3 Equipment

Weigh balance

Electric blender

Water bath

Heating mantle

Soxhlet Extractor

Rotary Evaporator

High Performance Liquid Chromatography (HPLC)

3.2 METHODOLOGY

3.2.1 Seed Collection and Preparation

The Melon seeds (*Citrullus colocynthis*) were obtained from New Benin Market in Oredo Local Government Area, Benin City, Edo State, Nigeria. The melon seed (C*itrullus colocynthis*) were screened to remove the bad ones and sun dried for about 30minutes at ambient temperature (37°c) to reduce moisture content, and then it was weighed using automated weigh balance and grounded using an electric blender to increase the surface area for oil extraction.

3.2.2 Seed processing and oil extraction

The grounded melon was extracted by continuous extraction using a Soxhlet apparatus for 8 hours using Folch method (a mixture of methanol and chloroform) in a ratio of 2:1 as solvent. The oil was obtained using solvent extraction method. At the end of the extraction, the solvent was evaporated using a water bath leaving the oil and lipid sample as residue.

3.2.3 Extraction of Fat soluble vitamins (A, D, E and K)

The samples were out of the less than 4°c compartment in the laboratory and then placed on the laboratory bench to acclimatize to the laboratory conditions. 0.125g of the sample was accurately weighed and was added to a 10ml volumetric flask, then 8ml of methanol/methylene chloride (CH_3OH-CH_2CL_2) (1:1, v/v) was also added to the flask. After about 15minutes of ultrasonic extraction, 5ml of methanol/methylene chloride (CH_3OH-CH_2CL_2) (1:1, v/v) was added to the volumetric flask mark. Then the prepared sample solution was stored in the dark. Prior to injection, the solutions were then filtered through a 0.2µm filter (Millex-GN).

3.2.4 Determination of Fat soluble vitamins (A, D, E and K)

High Performance Liquid Chromatography was used to determine the fat soluble vitamins (A, D, E and K).

A) (CH_3OH-CH_3CN) methanol/methyl cyanide (8:2, v/v), mobile phase

B) Methyl tert-butyl ether (MTBE)

Injection volume: 10µl

3.2.5 Determination of Amino Acid Profile described by micro Kjeldahl method according to AOAC (2005) slightly modified

A known weight of sample (250mg) was placed in Kjeldahl flask and about (200mg) of catalyst mixture (potassium sulphate, copper sulphate and selenium powder) was added. Then 10.0cm^2concentrated sulphuric acid was added to the content of the flask. It was heated gently for few minutes until frothing ceases then heat was increased to the digest for 1hour 30 minutes.

Then it was allowed to cool and made to a known volume with distilled water (100cm^3). (10.0$cm^{3)}$ aliquot was distilled to the diluted solution of the digest by pipetting the volume into a distillation chamber of micro Kjeldahl distillation apparatus. Then (10.0$cm^{3)}$ of 40% sodium hydroxide solution was added and steam distill into (10.0cm^3) of 4% boric acid containing mixed indicator (a colour change from red to green was noted). It was titrated with standard 0.01N or 0.02N hydrochloric acid to grey endpoint.

$$\%N = \frac{(a-b) \times 0.01 \times 14.0057 \times c \times 100}{dxe}$$

a- Titre value of the sample

b- Titre value of the blank

c- Volume to which digest is made up with distilled water d- Aliquot taken for distillation

e- Weight of dried sample (mg)

Hydrolysis of the Sample

7ml of 6N HCL was added and oxygen was expelled by passing nitrogen into the ampoule (to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°c ±5°c for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. (Note: Tryphtophan was destroyed by 6N HCL during hydrolysis). The filtrate was then evaporated to dryness using a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading Hydrolysate into Amino Acid Analyser

The amount loaded was 60microlitre which was dispensed into the cartridge of the analyser. The analyser is designed to separate and analyse free acidic, neutral and basic amino acids of the hydrolysate. An integrator attached to the Analyser, calculates the peak area proportional to the concentration of each of the amino acids.

CHAPTER FOUR

RESULTS

Table 4.1: Results on Vitamin Composition of Fat Soluble Vitamins in Solvent Extracted Melon Seed Oil (Month 0)

Sample	Amount
Vitamin A(IU/ML)	$8.64227e^{-3}$
Vitamin D(IU/ML)	$1.59287e^{-5}$
Vitamin E(IU/ML)	$6.03572e^{-1}$
Vitamin K(IU/ML)	$5.20404e^{-3}$

Table 4.2: Results on Vitamin Composition of Fat Soluble Vitamins in Solvent Extracted Melon Seed Oil (Month 1)

Sample	Amount
Vitamin A(IU/ML)	$8.41274e^{-3}$
Vitamin D(IU/ML)	$1.59031e^{-5}$
Vitamin E(IU/ML)	$5.90320e^{-1}$
Vitamin K(IU/ML)	$4.90619e^{-3}$

Table 4.3: Results on Vitamin Composition of Fat Soluble Vitamins in Solvent Extracted Melon Seed Oil over a Period of Time

Sample	Amount (Month 0)	Amount (Month 1)

Vitamin A(IU/ML)	$8.64227e^{-3}$	$8.41274e^{-3}$
Vitamin D(IU/ML)	$1.59287e^{-5}$	$1.59031e^{-5}$
Vitamin E(IU/ML)	$6.03572e^{-1}$	$5.90320e^{-1}$
Vitamin K(IU/ML)	$5.20404e^{-3}$	$4.90619e^{-3}$

Table 4.4: Amino Acid Profile of Whole Melon Seed Flour (Month 0)

Amino Acid (concg/100gprotein)	Month 0

Leucine*	6.63
Lysine*	3.43
Isoleucine*	5.19
Phenylalanine*	4.43
Tryptophan	1.59
Valine*	4.24
Methionine*	2.67
Proline	2.96
Arginine*	13.84
Tyrosine	2.42
Histidine*	2.21
Cystine*	1.33
Alanine*	4.03
Glutamic Acid	13.26
Glycine	5.08
Threonine*	3.17
Serine	3.25
Aspartic Acid	8.66

* = **Essential Amino Acids**

Table 4.5: Amino Acid Profile of Whole Melon Seed Flour (Month 1)

Amino Acid (conc g/100g protein)	Month 1
Leucine*	6.35
Lysine*	2.93
Isoleucine*	4.63
Phenylalanine*	4.19
Tryptophan	1.55
Valine*	4.08
Methionine*	2.52
Proline	2.72
Arginine*	13.31
Tyrosine	2.42
Histidine*	2.14
Cystine*	1.60
Alanine*	4.39
Glutamic Acid	13.61
Glycine	4.77
Threonine*	3.23
Serine	3.43
Aspartic Acid	8.34

* = **Essential Amino Acids**

Table 4.6: Amino Acid Profile of Whole Melon Seed Flour over a Period of Time

Amino Acid(conc g/100g protein)	Month 0	Month 1
Leucine*	6.63	6.35
Lysine*	3.43	2.93
Isoleucine*	5.19	4.63
Phenylalanine*	4.43	4.19
Tryptophan	1.59	1.55
Valine*	4.24	4.08
Methionine*	2.67	2.52
Proline	2.96	2.72
Arginine*	13.84	13.31
Tyrosine	2.42	2.42
Histidine*	2.21	2.14
Cystine*	1.33	1.60
Alanine*	4.03	4.39
Glutamic Acid	13.26	13.61
Glycine	5.08	4.77
Threonine*	3.17	3.23
Serine	3.25	3.43
Aspartic Acid	8.66	8.34

* = **Essential Amino Acids**

CHAPTER FIVE

DISCUSSION

To paraphrase it in a nutshell, the following is true: The fat-soluble vitamins A, D, E, and K are crucial for the body's efficient operation. They are oily and hydrophobic compounds that are stored in the liver and not excreted out of the body (Kamanger and Emadi, 2012).

High Performance Liquid Chromatography was used to analyse the determination of Fat Soluble Vitamins. In month 0, the amount of vitamin A obtained from the analysis was approximately 8.64227e-^{3}, while that of vitamin D was approximately 1.59287e-^{5}, vitamin E was 6.03572e-^{1}, and vitamin K was 5.20404e-^{3}. In month 1, the amounts of vitamin A were respectively 8.41274e-^{3}, 1.59031e-^{5}, 5.90320e-^{1}, and 4.90619e-^{3}.

It was found that the melon seed oil has the highest concentration of vitamin A, followed by vitamin E, and the lowest concentrations of vitamins K and D. As indicated in Table 3, it was also noted that the level of fat-soluble vitamins in the melon seed remained largely steady from month 0 to month 1. As a result, the fat-soluble vitamin content for A, D, E, and K remained largely consistent over the course of storage.

The necessary and non-essential amino acids are present in whole melon seed flour. Since the body cannot produce essential amino acids, we must get them from our diet. After being analysed with an amino acid analyser for this investigation, the Whole Melon Seed Flour contained roughly eleven necessary amino acids. In various amounts in months 0 and 1 are Leucine, Lysine, Isoleucine, Phenylalanine, Valine, Methionine, Arginine, Histidine, Cysteine, Alanine, and Threonine, which are all included in Table 6.

Melon seeds contain a lot of oil (53.1%) (Ogbonna and Obi, 2007). 8.25% of the seeds of melon seed oil were discovered to contain protein, with high concentrations of the amino acids methionine, leucine, and lysine (Shaheem *et al*.,2000).In this study, it was observed that the melon seed oil obtained after extraction was also rich in Lysine 3.43g/100gprotein,Leucine6.63g/100gprotein and Methionine 2.67g/100gprotein shown in Table 4.

Sosulki and Sarwar (1973) reported that melon seed contains higher amount of Arginine, Tryptophan and Sulphur containing amino acids. In this study, it was observed that, Arginine, Isoleucine and Leucine contained higher amount of the essential amino acid as shown in Table 4 and Table 5. The content of essential amino acids in the proteins of the melon seed flour makes it a good vegetable protein ingredient (Sanchez *et al.,* 1972). In this study, results show that Whole Melon Seed flour is vital for human consumption.

According to the findings of (Oyenuga and Fetuga, 1975), the first and second most scarce amino acids in water melon seed - a close relative of melon - were respectively lysine and methionine. Methionine, Histidine, and Cystine were the Whole Melon Seed flour's limiting amino acids in this investigation because they appear in lesser quantities than other essential amino acids, as indicated in Table 6.

5.1 CONCLUSION

There was no deterioration during the storage period since the fat-soluble vitamins (A, D, E, and K) for months 0 and 1 were comparatively stable. The necessary and non-essential amino acids required in a human diet are found in the whole melon seed flour.

5.2 RECOMMENDATION

Further studies can be done on the Melon Seed Oil and Whole Melon Seed flour to determine if there will be deterioration if kept for a long period of time and also determine water soluble vitamins and other nutritional properties.

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