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Phytosterol, phenolic acid, amino acid and sugar profiles of the unripe fruits of *Solanum melongena* L. (Round variety)

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ABSTRACT: Numerous health benefits of consuming eggplant fruits (*Solanum melongena* L.) have been reported. This research was carried out to determine some of the constituents responsible for its nutritional and medicinal properties. The unripe fruits of *Solanum melongena* L. (round variety) were evaluated for its phytosterol, phenolic acid, amino acid and sugar profiles by gas chromatography. The Phytosterol analysis showed the presence of seven phytosterols and sitosterol (233 ± 0.852 mg/100g) was found to be significantly higher ($p<0.01$) than the other phytosterols. The phenolic acid profile of the fruit also showed the presence of resorcinol, chlorogenic acid and other phenolic acid, with phenol having the highest concentration (764 ± 0.872 mg/100g). The Amino acid profile showed the presence of seventeen amino acids. Glutamate (11.76 ± 0.08 g/100g) and aspartate (10.49 ± 0.06 g/100g), were found to be significantly higher ($p<0.01$) than the other amino acids. Amino acids play central role in the synthesis of proteins and as intermediates in metabolism. The sugar profile showed the presence of ribose, xylose and other sugars, with fructose having the highest concentration (3.34±0 .0267 mg/100g).
Keywords: Phytosterol, phenolic acid, amino acid, sugar, eggplant, *Solanum melongena*

Introduction

Fruits and vegetables contain variety of nutrients and phytochemicals, which are key components of human daily diet (Craig and Beck, 2009; Wargovich, 2000). Quantitative estimation of these nutrients and phytochemicals that are present in these fruits and vegetables could be of great importance in managing certain diseases. *Solanum* is one of the largest genera of the family *Solanaceae*, comprising of over 1400 species of which the eggplant is one (Hideki *et al*., 2014). The family *Solanaceae* is ranked as the third in economic importance among plants and it is regarded as a source of many morphologically different domesticated crop species that are beneficial to human health, diet, beauty and ornamental use (Mueller *et al*., 2005; Sekara *et al*., 2007).

Solanum melongena L is a popular variety of eggplant known for its beautiful round or oval shape fruits that are white in colour as well as unique in taste and texture. It is consumed on daily basis by urban families and also represents the main source of income for households in West Africa (Danquah-Jones, 2000). Fruits of *Solanum melongena* are used in the preparation of several delicacies and even eaten raw as a snack. It is an inexpensive but major food component of diet (Chinedu *et al*., 2011). Eggplants ranks highly amongst the many fruits that are nutritionally rich in bioactive compounds and in antioxidant properties (Ossamulu *et a*l., 2014a), it is ranked among the top 10 vegetables in terms of oxygen radical absorbance capacity (Salerno *et al*., 2014). Besides being used as an important fruit or vegetable, eggplant has been extensively exploited in traditional medicine for treatment of many diseases (Kashyap *et a*l., 2003). Its medicinal properties are strongly linked to its nutrients and phytochemical compounds (Ossamulu *et al*., 2014a). Some of the reported medicinal properties of eggplant include: analgesic, anti-flammatory, anti-asthmatic properties, hypoglycemic, antioxidative and hypolipidemic properties (Igwe *et al*., 2003; Bello *et al*., 2005). The

medicinal properties possessed by most plants are linked to the quality and quantity of nutrients and phytochemicals they contain (Evans, 2005; Doughari, 2012).

Phytochemicals are a wide variety of bioactive compounds such as flavonoids, alkaloids, tannins, saponins, phenolics, anthraquinones, and phytosterols that are produced by plants that have protective or disease preventing properties (Doughari, 2012; Ghoson, 2015). Phytochemicals are associated with the prevention of the leading causes of death in many regions of the world, which are cancer, diabetes, cardiovascular disease and hypertension (Liu, 2004). Phytochemicals have been reported to act synergistically with nutrients (Liu, 2003). Phenolic acids belong to a class of phytochemicals known as Phenolics, phenols or polyphenols. They occur ubiquitously in plants. Phenolics in plants are mostly synthesized from phenylalanine or tyrosine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Puupponen-Pimiä *et al*., 2008). Foods containing phenolics are widely reported to prevent chronic diseases, such as atherosclerosis, oxidative damage, cardiovascular disease, hormone dependent cancers (Liu, 2004), arthritis, neuro-degeneration, and osteoporosis (Middleton *et al*., 2000). Phenolics are some of the most potent antioxidants (Salerno *et al*., 2014). Phytosterols are steroidal compounds similar to cholesterol, which occur in plants and vary only in carbon side chains and the presence or absence of a double bond. They are found in plant cell membranes, due their structural similarity to cholesterol. Phytosterol esters in food reduce LDL-cholesterol by 10% at a maximum effective dose of 2 g/day (Ostlund, 2004). Phytosterols include β-sitosterol, campesterol, stigmasterol, stigmastanol and 5-avenasterol.

Sugars and Amino acids are important classes of macro nutrients. They are obtained from diet either as free sugars or amino acids or as breakdown products of carbohydrates and proteins respectively, they are important and are required in relatively larger quantities than most other nutrients. Sugars, many of which are found freely in most fruits, are also found in the tissues of most plants. Amino acids are either used to synthesize proteins and other biomolecules or are oxidized to urea and carbon dioxide as a source of energy (Brosnan, 2000). Out of the twenty amino acids that are commonly found in nature, isoleucine, leucine, valine, tryptophan, phenylalanine, threonine, lysine, methionine are essential and must be provided in diet because they cannot be synthesized by the body. These essential amino acids therefore make the consumption of dietary amino acids very importance. Also, the chemical properties of the amino acids determine the biological activity of proteins (Baldwin and Lapointe, 2003).

Nutritional and phytochemical studies that involve the identification and quantification of the specific types of nutrient and phytochemicals in food are important in determining the nutritional quality of food. They can be used for information, education and as a tool to orient food consumption; they could also help to point scientists in the right direction in searching for ample sources of therapeutic agents (WHO, 2011). Since nutrient and phytochemical profiles show the specific types of nutrients and phytochemicals and their levels in food the aims of this study was to determine the phytosterol, phenolic acid, amino acid and sugar profiles of the unripe fruits of *Solanum melongena* L. (round variety) .

Materials and Methods

Plant materials

Mature, fresh unripe fruits of *Solanum melongena* (round variety) were purchased from Oba market in Oredo Local Government Area of Edo state. The eggplants were washed thoroughly with distilled water and their crowns were plucked off and each fruit was diced into thin slices and then transferred into the oven for drying at 60 °C. After drying, the eggplant samples were ground to a smooth powder using a blender and then stored in an airtight glass container to prevent it from reabsorbing moisture before analysis.

Nutritional and phytochemical profiles of *Solanum melongena*

Phytosterol, phenolic acid, amino acid and sugar profiles of the unripe fruits of *Solanum melongena* L, where estimated with a gas chromatograph (GC) equipped with flame ionisation detector (FID). The GC HP 6890 Powered with HP Chem Station Rev. A 09.01 [1206] Software was used for the analyses. GC conditions varied depending on the compound being analyzed. The retention time and peak area compared with that of selected standards were used to estimate the type and concentration of compounds present in the eggplant. Only compounds with available standards were identified and quantified.

Phytosterol Profile

Extraction of the phytosterols was carried out by the method of AOAC (1996). A measured quantity of 20 g of the pulverized eggplant was weighed and transferred into a Stoppard flask and treated with petroleum ether until the powder was fully immersed. The flask was shaken every hour for the first six hours and was then kept aside and shaken after 24 hours. The process was repeated for three days and the extract was filtered. The extract was collected and evaporated to dryness using nitrogen stream. 0.5 g of the concentrated extract was added to a screw-capped test-tube. The extract was saponified at 95°C for 30 min using 3ml of 10% KOH in ethanol to which 0.2 m1 of benzene was also added to ensure miscibility. 3ml of de-ionised water was also added and 2 ml of hexane was used to extracting the non-

saponifiable materials, which are phytosterols. Three extractions with 2 ml of hexane each were carried out for 1 hour, 30 min and 30 min, respectively to achieve complete extraction of the phytosterols. The hexane fraction was concentrated to 2 ml in Agilent vial for gas chromatographic analysis. 1 microlitre of the hexane fraction was injected into the injection port for the analysis.

GC conditions for the analysis of phytosterols

Column HP INNOWax with dimensions 30m×0.25mm×0.25 µm was used. Nitrogen was used as the carrier gas at a constant flow of 1.0ml/min and split ratio of 20:1. The injector temperature was set at 250 ^{o}C and the detector temperature was 320 ^{o}C. The oven temperature was programmed from 60^{o}C for 5 min with a decrease to 18^{o}C/min for 15 min and to 12 ^{o}C/min for 4 min. Hydrogen pressure was 22 psi and compressed air was 35 psi.

Phenolic acids profile

Two stage extraction procedures were used for the effective extraction of the phenolic compounds as described as follows; Stage l: 50 mg of the pulverized eggplant was extracted with 5 ml of 1M NaOH for 16 hr on a shaker at ambient temperature as described by Kellev *et a1*. (1994) and Provan *et a1*. (1994). After extraction, the extract was centrifuged at 5000 x g, rinsed with water and centrifuged again. The supernatants were combined and placed in a disposable glass test tube and heated at 90°C for 2 hr to release the conjugated phenolic compounds. The heated extract was cooled and titrated with 4 M HCl to give a pH<2.0, it was diluted to 10 ml, with deionised water and centrifuged to remove the precipitate or sediment or residue. The supernatant was saved for subsequent purification while the residue was extracted further in stage 2. Stage 2: The residue from stage 1 was extracted with 5 ml 4 M NaOH, heated to 160°C in Teflon as described by Provan *et a1*. (1994). After cooling, the mixture was filtered and the filtrate was collected, the residue washed with deionised water and filtered again. The supernatants and the filtrates were combined and adjusted to pH <2.0 with 4 M HCl for further purification. Purification of extracted phenolics: An aliquot (5 ml.) of the supernatants was passed through solid state extraction tube. The tubes were placed under a vaccum (60 kPa) until the resin was thoroughly dried .The Phenolic acids were eluted with l.0ml of ethyl acetate into gas chromatography auto sampler vial. The concentrated extract in the gas chromatography vial was derivatized by adding 20 µL of derivatising agent bis(trimethylsilyl)trifluoroacetamide (BSTFA). Silicone septum corked vial was lowered into the water bath with hanger to stand upright in the water bath with a magnetic stirrer at 45 °C for the derivatization period of 10 min. 1.0 µL was injected into the gas chromatograph through the injection port.

GC Conditions for Phenolic acid analysis:

Column Type was HP-1 Capillary and the column dimensions were 30 m × 0.25 mm × 0.25µm. The split ratio was 20:1 and the carrier gas was nitrogen with a flow rate of 1.0 ml/min. The inlet temperature was 250 °C, The Oven Program was an initial temperature of 60 °C for 5 min, first ramping at 15°C/min for 15min, second ramping at 10 °C/min for 4 min. Detector was FID and detector temperature was 325 °C. Hydrogen Pressure was 28 psi, compressed air was 32 psi.

Amino Acid Profile

The modified method of AOAC (2005) and Obreshkova *et al.* (2012) were used in the extraction and estimation of the amino acids. 10 g of the pulverized eggplant was weighed into a 250 ml conical flask capacity. The sample was defatted by extracting the fat content of the sample with 30 ml of petroleum spirit three times with soxhlet extractor that was equipped with thimble. The defatted sample was hydrolyzed thrice for complete hydrolysis, by soaking with 30 ml of 1M potasssium hydroxide solution and incubating for 48hours at 110 ^{0}C in a borosilicate glass container. After hydrolysis, the hydrolysate was neautralized to get a pH in the range of 2.5-5.0. The solution was purified by cation-exchange solid phase extraction. The amino acids in the purified solution were deriviatized for volatility by reacting with sufficient quantity of the derivatizing agent ethylchloroformate. The derivatizing reagent was removed by scavenging with Nitrogen gas to mop up the excess reagent. The derivatized amino acids that were free of derivatizing reagent were made up to 1 ml in a vial for gas chromatography analysis. 1.0 µL of the derivatised extract was injected into the gas chromatograph equipped with Flame Ionization Detector (FID).

GC conditions for amino acid analysis

The column type was EZ and the dimensions were 10 m x 0.25 mm x 0.2µm . The temperature program of analytical column was 110 °C, ramping from 110 °C to 320°Cf or 5 min. The temperature of injector was 250°C and that of flame – ionization detector was 320°C. Carrier gas was Hydrogen. Hydrogen pressure was 20 psi, Compressed air was 35 psi.

Sugar Profile

The modified method of Raessler *et al.* (2010) was employed. 5 mL of ultra pure water was added to 20 mg of the pulverized eggplant. The suspension was incubated in a water bath at 80 °C for 30 min. After cooling to room temperature, the suspension was centrifuged at 4500 rpm for 10 min. The liquid phase was transferred into a 50 ml flask and filled to mark with ultra pure water. An aliquot of this solution was extracted with 80% ethanol. The extract obtained was derivatized for volatility by reacting with 0.50 mL mixture of pyridine: hexamethyldisilane:trimethylchlorosilane in the ratio 10:2:1. The derivatized extract was concentrated to l mL in the

vial for chromatography analysis. 1.0 µL of the derivatized extract was injected into the injection port of the gas chromatograph.

GC Conditions for Sugar analysis

The Column Type was BPX70 and column dimensions was 12 m × 0.32 mm × 0.25 µm .The Oven Program was Isothermal at 210 °C, the detector was FID and detector temperature was 325 °C . The split Ratio was 50:1 and the carrier gas was Hydrogen at a flow rate of 1.0 ml/min. The inlet temperature was 250 °C. Hydrogen was 25 psi and compressed air was 35 psi.

Statistical analysis

Data obtained from this study were subjected to statistical evaluation. Analysis of variance of data was done by the statistical analysis system (INSTAT Software). Tukey-Kramer multiple comparison test was employed (INSTAT Software) to determine the statistical differences among the means. Results were expressed as Mean Values of triplicates ± Standard Error of Mean (SEM).

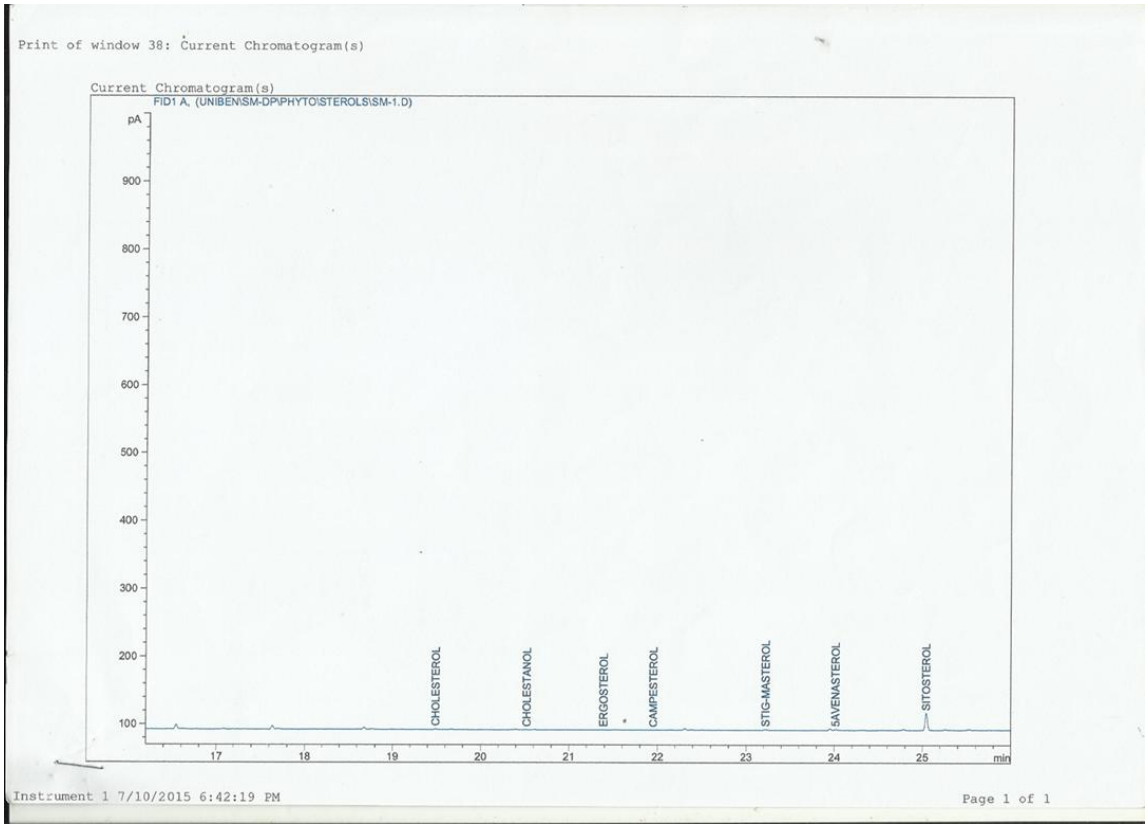
Results

The whole and sliced fruits of *Solanum melongena* L. (round variety) that were used in this study are shown in Figs 1 and 2.

Fig. 1. Unripe fruits of *Solanum melongena* (round variety)

Fig. 2. Sliced unripe fruits of *Solanum melongena* (round variety) that were oven dried and pulverized to produce dried fruit powder

The chromatogram of the phytosterol profile of *Solanum melongena* L. (round variety) is shown in Fig. 3. The phytosterols that were detected incude campesterol, stigmasterol, 5-avenasterol and sitosterol. The level of sitosterol was the highest (Table 1).



Legend: Y axia = Area, X axis = Retention time

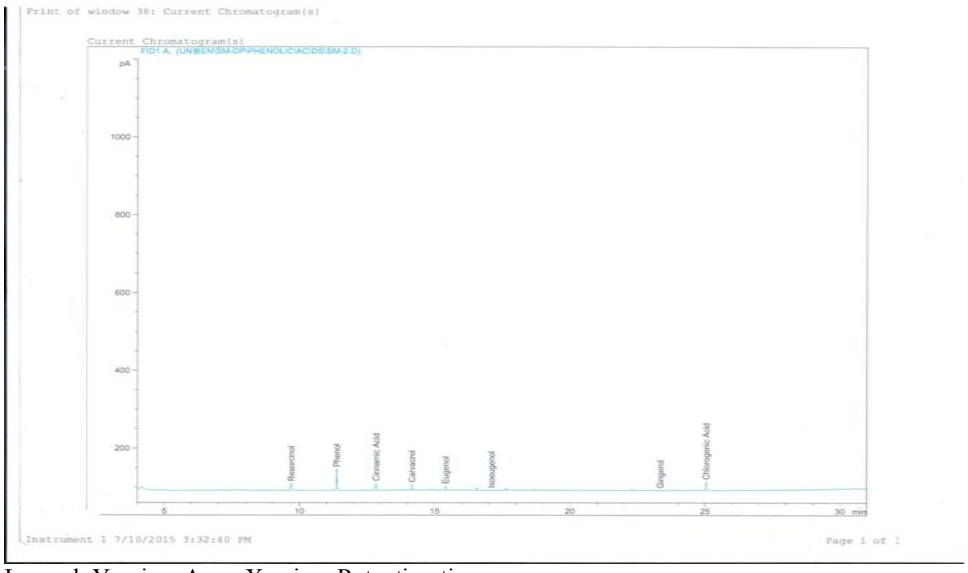
Fig. 3. Chromatogram of phytosterol profile of the unripe fruits of *Solanum melongena* L (round variety)

Table 1. Phytosterol profile of the unripe fruit of *Solanum melongena* (round variety)

Types of Phytosterols	Amount (mg/100g Dry weight)
Cholesterol	0.000000406 ± 0.000000153
Cholestanol	0.00137 ± 0.000000910
Ergosterol	0.00138 ± 0.00000194
Campesterol	55.3 ± 0.185
Stigmasterol	43.7 ± 0.505
5-Avenasterol	23.4 ± 0.160
Sitosterol	233 ± 0.852

Means ± SEM for three determinations

Chromatogram of the phenolic acid profile of *Solanum melongena* L. (round variety) is shown in Fig. 4. Chlorogenic acid, phenol and cinnamic acid were presemt in high amounts, while other phenolic acids were either present in low amounts (Table 2).



Legend: Y axis = Area, X axis = Retention time

Fig. 4 Chromatogram of the phenolic acid profile of the unripe fruit of *Solanum melongena* L. (round variety).

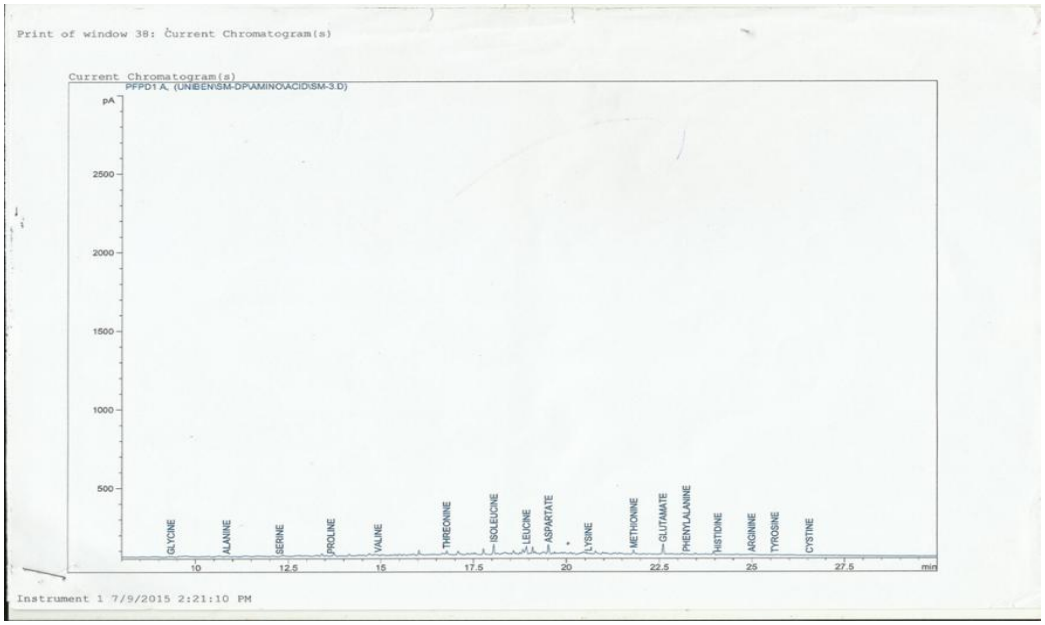
Phenolic acid Profile

Table 2. Phenolic acid profile of the unripe fruits of *Solanum melongena* L. (round variety).

Types of Phenolic acids	Amount mg/100g
Resorcinol	0.000343 ± 0.00000364
Phenol	764 ± 0.872
Cinnamic acid	145 ± 0.155
Carvacrol	0.207 ± 0.00336
Eugenol	0.000183 ± 0.00000109
Isoeugenol	0.0000313 ± 0.000000433
Gingerol	0.000433 ± 0.00000618
Chlorogenic acid	532 ± 1.26

Means± SEM for three determinations

The chromatogram for amino acid profile of *Solanum melongena* L. (round variety) is shown in Fig. 5. The amino acids, aspartate, glutamate and leucine were present in high amounts (Table 3).



Legend: Y axis = Area, X axis = Retention time

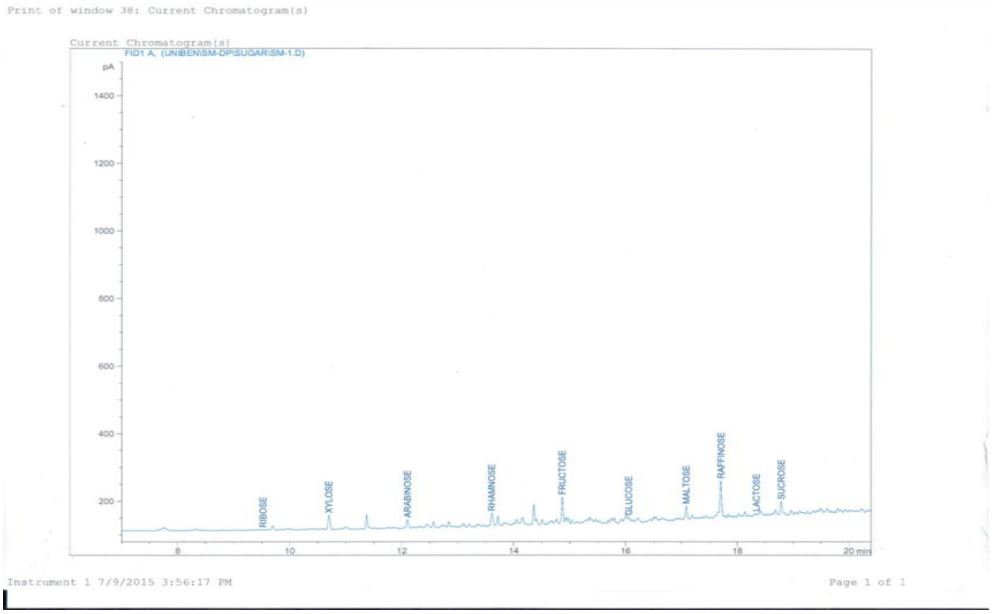
Fig. 5. Chromatogram of the amino acid profile of the unripe fruits of *Solanum melongena* L (round variety)

Table. 3. Amino acid profile of the unripe fruit of *Solanum melongena* l. (round variety)

Types of Amino acid	Amount (mg/100g Dry weight)
Glycine	3.40 ± 0.06
Alanine	4.93 ± 0.03
Serine	2.66 ± 0.01
Proline	4.01 ± 0.06
Valine	3.73 ± 0.06
Threonine	3.95 ± 0.03
Isoleucine	3.56 ± 0.06
Leucine	8.77 ± 0.09
Aspartate	10.49 ± 0.06
Lysine	4.07 ± 0.07
Methionine	0.59 ± 0.25
Glutamate	11.76 ± 0.08
Phenylalanine	4.00 ± 0.04
Histidine	3.02 ± 0.00
Arginine	6.62 ± 0.02
Tyrosine	4.29 ± 0.02
Cystine	0.36 ± 0.00

Means ± SEM for three determinations

The chromatogram for the sugar profile of *Solanum melongena* L. (round variety) is shown in Fig. 6. Fructose and raffinose were present in high amounts (Table 4).



Legend: Y axis = Area, X axis = Retention time

Fig. 6. Chromatogram of the sugar profile of the unripe fruit of *Solanum melongena* L (round variety)

Table 4. Sugar profile of the unripe fruit of *Solanum melongena* L. (round variety).

Types of sugars	Amount (mg/100g Dry weight)
Ribose	0.000208 ± 0.00
Xylose	1.36 ± 0.006
Arabinose	1.25 ± 0.00954
Rhamnose	0.0000374 ± 0.00000124
Fructose	3.34 ± 0.0267
Glucose	0.46 ± 0.00837
Maltose	0.0000623 ± 0.0000001
Raffinose	3.24 ± 0.0176
Lactose	0.0000291 ± 0.0000000882
Sucrose	2.48 ± 0.0234

Means ± SEM for three determinations

Discussion

Gas Chromatographic analysis of the phytosterols in the unripe fruits of *Solanum melongena* L showed the presence of cholesterol but the level was very minute, other phytosterol that were found include cholestanol, ergosterol, campesterol, stigmasterol, 5-avenasterol and sitosterol (Fig, 3 and Table 1). Sitosterol was significantly higher ($p<0.01$) than the other phytosterols. The levels of campesterol and stigmasterol were also high and this result agrees with previous studies by Muhammet *et al*., (2006) who reported that the predominating phytosterols in plants are campesterol, sitosterol and stigmasterol. Phytosterols have been reported to be helpful in lowering cholesterol level and decreasing serum total and LDL cholesterol levels due to their ability to lower the systemic absorption of cholesterol (Miettinen, 2001; Nissinen *et a*l., 2002; Trautwein *et al*., 2003). Phytosterols compete with cholesterol for the micellar phase of the small intestine, thereby reducing its absorption. Micelles are the essentially small aggregates that carry a mixture of lipids and bile salts in the intestinal lumen (Bartnikowska, 2009). Some studies have demonstrated that the intake of phytosterols was inversely associated with the development of esophageal, stomach and breast cancers. (De Stefani *et al*., 2000a; De Stefani *et al*., 2000b)) Sitosterol has also been implicated in anticancer activity (Awad and Fink , 2000). Cell culture and animal studies have shown that phytosterols may attennuate the inflammatory activity of immune cells (Navarro *et al*., 2001; Awad *et al*., 2004). The potential role of phytosterols both in the etiology as well as

in the prevention of immunological diseases is due to the anti-inflammatory activity of phytosterols that resuls in the inhibition of secretion of inflammatory mediators, such as IL-6 and TNFα (Bouic, 2001). Phytosterols also possess antioxidative properties. *In vitro* phytosterols decreased lipid peroxidation of platelet membranes in the presence of iron (van Rensburg *et a*l., 2000; Wang *et al*., 2002).).

In the phenolic acid profile, phenol and chlorogenic acid had the highest concentration (Fig 4 and Table 2) .This is similar to the result of the previous studies by Stommel and Whitaker (2003) which showed that phenol and chlorogenic acid were higher than other phenolic acids in *Solanum melongena* after chromatography. Phenols are ranked among the top ten phytochemicals in vegetables in terms of having protective effects against oxidative damage and are effective in disease prevention. High phenol content has been associated with higher antioxidant capacity (Santas *et a*l., 2008). Chlorogenic acid, which was significantly high in this study is one of the most potent free radical scavenger found in plants. Eggplant has been reported as having a very high content of chlorogenic acid, which constitutes the major phenolic compound in the fruit (Plazas *et al*., 2013; Ahn *et al*., 2011; Stommel and Whitaker, 2003). Chlorogenic acid is a plant compound that is known to show multiple beneficial properties such as analgesic, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-microbial, anti-obesity, cardioprotective, hypotensive and neuroprotective effects (Suzuki *et al*., 2006; Cho *et al*., 2010; Sato *et al*., 2011; Ahn *et al*., 2011; Burgos-Moron *et al*., 2012; Coman *et al*., 2012; Zhao *et al*., 2012; Plazas *et al*., 2013;). Furthermore, chlorogenic acid is highly bioavailable for humans when ingested (Dos Santos *et al*., 2006). Cinnamic acid is used in flavours. Phenols and sugars are important determinants of the flavour of eggplant fruits (Esteban *et al*., 2000).

Nine of the amino acids present in the amino acid profile of *Solanum melongena* are essential amino acids, which are needed by the body for cellular functions (Fig. 5 and Table 3). Glutamate and aspartate were significantly higher ($p<0.01$) than other amino acids and both are acidic amino acids. This is consistent with previous research that found Aspartic acid and Glutamic acid as having the larger percentage 16.9% in ripe *Solanum* species. Leucine was found to be the highest essential amino acid present in the fruit. This result also correlated with the result of the research carried out by Adeyeye and Adanlawo (2011). The Leucine content in eggplant meets the Dietary Reference Intake (DRI, 2002) standards which is 23-43mg/Kg body weight. Leucine is gaining prominence in the sporting world as vital for muscle building. Arginine is an essential amino acid for children and reasonable level was observed in the fruit. Eggplants are therefore good sources of both essential and non- essential amino acids.

The fructose level of of unripe *Solanum melongena*.L was higher than that of other sugars (Fig. 6 and Table 4). Other sugars that were also significanlty present in the fruit include raffinose, sucrose, xylose and arabinose. The glucose level of *S. melongena* was significantly low, as was rhamnose, maltose, lactose and ribose levels. Agoreyo and Fregene (2014) also reported lower glucose levels in the round variety of the unripe fruit of *S. melongena*. The low glucose level in *S. Melongena* makes it useful to diabetic patients and individuals on weight loss diets.

Conclusion

The levels of phytosterols, phenolic acids, amino acids and sugars in the unripe fruit of *Solanum melongena.* L (round variety) in this study showed that these nutrients and phytochemicals contribute significantly to the nutritional and medicinal values of these fruits.

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