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Research Article

Avocado (*persia americana*) seed processing into a third-generation functional food snack: Nutritional, antioxidative stress and safety potentials

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Abstract

Its food value and rich bioactives notwithstanding, processing avocado seeds (largely underutilized) into safe and acceptable 3G functional food snack has not been explored. The seeds(de-coated, blanched. dried, milled and sieved) were blended at different proportions with sweetened maize flour and subjected to extrusion (≈1000C, 100rpm) and hot air puffing (700C, 4hrs). Proximate, antioxidant properties and bioactive compounds of the resulting snack were evaluated. All were conducted in line with standard protocol. The snacks (mainly carbohydrate 47.3-61.8%) had low anti-nutrients with expansion ratios (2.8-3.1). Flavonoids (117.1-267.6mg/100g) and total antioxidant capacity (427.5-665.5 AAE) were significant ($p \leq 0.05$). A9, 12-Octadecadienoic acid was the predominant bioactive. Animal weight and malondialdehyde declined [110-102g; 10.18-3.58µm] in stressed avocado based snack-fed rats as against a rise in the control group. Overall organoleptically, 20% Avocado seed-based snack was most preferred. Safe and acceptable 3G avocado-based functional food snack with anti-stress and weight loss potentials is feasible, providing a novel outlet for the seed.

Keywords: Avocado seeds;3G Snack food; Antioxidative stress; Bioactive; Weight loss snacks

INTRODUCTION

Avocado fruit aptly called the 'green gold' is one of the most common tropical fruits grown around the world with production put at over 6 million metric tons and still rising (FAO,2018). A significant component of the fruit is the seed - conical, round or ovoid, 2-2.5cm in length, and enclosed in an outer layer (seed coat) that is papery and brown. The seeds represent some 13-18% of the fruit not often utilized but discarded. This seeming waste may pose a serious ecological problem (Ortiz et al., 2004). It may however be of interest to the food and pharmaceutical industries since it contains an array of substances some of which are useful and physiologically active.

Indeed, the compositional profile of Avocado seeds is multifarious and quite beneficial. According to Ramos et al. (2004), the seeds mostly comprise of triterpenes, fatty acids, and phytosterols. They have also been found to exhibit antidiabetic effect, antihypertensive properties, and a cholesterol-lowering propensity (Imafidon and Amaechina 2010). The seed contains starch with good physicochemical and rheological properties (Chel-Guerrero et al., 2016) along with fibrous residues that can retain water and oil several times its weight (Barbosa-Martins et al., 2016). This makes it a potential raw material for many food systems. Interestingly, some of the aforementioned positive characteristics of the seed have been translated into a successful application as food additive/flour substitute in biscuits (Mahawan et al., 2015), natural yellow food colorant (Dabas et al., 2011) as well as anti-oxidant and microbicide in pork burgers and meat chops(Weiss et al., 2010) Undoubtedly, the use of underexploited materials such as Avocado seeds has far-reaching consequences for the health and overall wellbeing of those who regularly consume them as food. A significant contributor in this respect is regular metabolic processes and extraneous factors generating reactive oxygen species (ROS) (free radicals) in cellular fluids. Indeed, these species have been linked to oxidation in biological systems culminating in diseased states like cancer, neurological degeneration as well as rancidity in foods. Anti-dotes to these phenomena has been associated with antioxidants that offer some measure of protection on biomolecules. When consumed regularly (as in fruits) they have been shown to slow down

cardiovascular degeneration and the aging process. This reduction is due to the presence of natural antioxidants such as phenolic acid, flavonoids, carotenoids abundant in fruits, vegetables, and their byproducts like avocado seeds (Rui, 2003).

In many African countries, Avocado seeds are traditionally consumed in soups and puddings because of their perceived antihypertensive benefits. Its bitter taste and brown color have however mitigated wide consumer acceptability (Anaka et al., 2009). There is therefore a need to explore possible processing methods of making the seeds more palatable and acceptable as with third-generation(3G) snack foods (eaten in-between meals), yet retaining most of its health-promoting properties. Notably, 3G snacks are semi-finished (half products) often in non-expanded pellets transformed to finished snacks only after expansion through exposure to either hot air, microwaving, or frying (Gandhi et al., 2016). Indeed, it remains one of the fastest-growing segments of the snack food subsector worldwide (Delgados- Nieblas, 2015; Watrous, 2019). It is the objective of this work therefore to evaluate the possibility of developing an acceptable 3G snack incorporating avocado seeds and ascertain their nutritional, antioxidative stress, and safety quality recent renewed interest in the use of several underexploited food materials such as avocado seeds beyond their nutritional value further underscores the need for this study.

MATERIALS AND METHODS

Raw material Inputs and sourcing

Avocado fruits and Maize grains are the principal raw material inputs. The fruit was sourced from a local market in Bodija, Ibadan Nigeria while the maize grains were obtained from the Institute of Agriculture Research and Training (IAR&T), Moor Plantation, Ibadan.

Processing of Maize- Avocado based 3G Snack

Conversion of Avocado Seed into Flour:

The seeds were obtained from the pulp by gently slicing through the fruit and the outer coat was carefully removed. Hot water (70-80^{0}C) blanching (1-2mins) of the seeds followed to reduce enzymatic browning, thereafter drained. It was next sliced into thin flakes, dried (cabinet drier, 60 °C for 48 h)(see supplementary files) milled and sieved (300 µm screen) to obtain a fine powder(packaged in polyethylene film and stored in a refrigerator).

Conversion of Maize grains into Flour:

The maize grains were first cleaned to remove the damaged kernel, stones, and other extraneous materials. They were next washed with potable water, dried (cabinet drier), milled (hammer mill) all in that order to obtain the flour. The resulting flour was sieved (300 µm screen) and stored in an airtight polythene bag.

Formulation and Processing of Maize- Avocado Seed based 3G Snack:

The formulation and extrusion of the Maize–avocado seed-based snack was based on the method of Balentic et al., (2018) with a slight adjustment. The snack was made by formulating composite blends of Maize and Avocado seed flour in selected ratios 90:10, 80:20, 70:30 respectively as well as 100% maize flour and 100% Avocado seed flour (Olatoye & Arueya,2018). Moisture content was adjusted to 14 g/100 g by adding distilled water and allowing the mixture to equilibrate for 4hrs at room temperature. The different proportions of the blends were mixed with other ingredients (see below). The mixture was subjected to a hot extrusion barrel at 110–115 °C with a Length to diameter (L/D) ratio of 12:1 and the screw speed of 100 rpm. The extruded snacks were then puffed at 70°C for 4 h, cooled, and properly stored in airtight polyethylene. The other ingredients added were vanilla (1%), salt (2%), sugar (5%) (Figure 1).

Proximate Analysis:

The moisture, ash, protein, fat, and crude fiber content in the sample were determined in triplicates according to the methods of the Association of Official Analytical Chemists (AOAC, 2005). The total carbohydrate content was then estimated by difference.

Anti-nutritionalFactors

Determination of saponin content:

The Spectrophotometric method of Brunner (1984) was used for saponin analysis. One gram of finely ground sample was weighed into a 250 ml beaker and 100 ml Isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Samples of between 0-10 ppm standard saponin solutions were prepared from the saponin stock solution. The absorbances of the sample as well as standard saponin solutions were read after color development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm. Percentage saponin was calculated using the formula:

Saponin (%) = Absorbance of sample x Average gradient x Dilution factor

Weight of sample x 10,000

Determination of tannin content:

The method of Talabi et al. (2016) was deployed for the determination of tannin contents with a slight adjustment. Aliquot amount (0.2 g) of finely ground sample was measured into a 50 ml beaker. About 20 ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80^{o}C for 1 h and stirred with a glass rod to prevent lumping. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as the 1 ml of the sample above. The absorbances of the Tannic acid standard solutions as

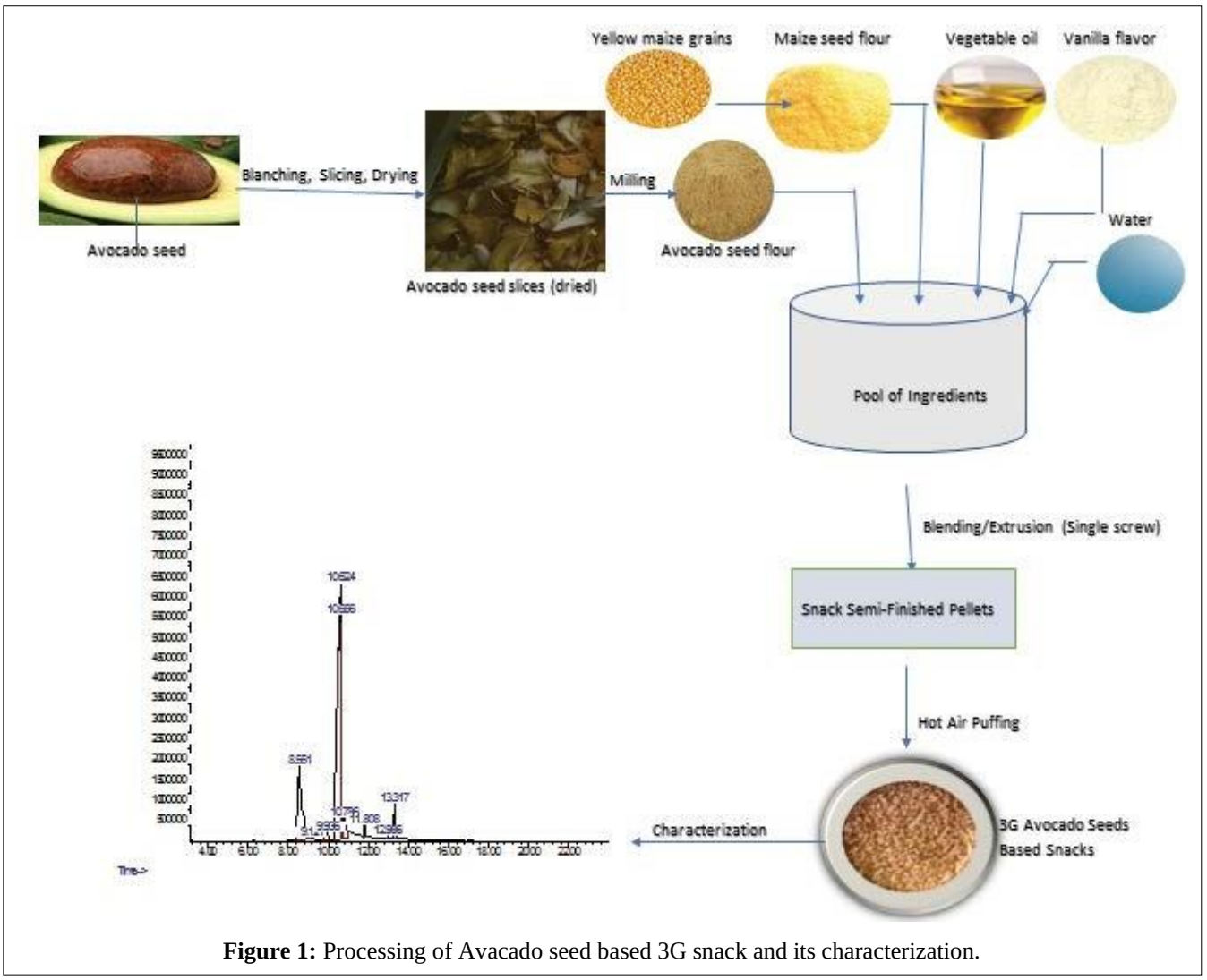


Figure 1: Processing of Avacado seed based 3G snack and its characterization.

well as samples were read after color development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated using the formula:

Tannin (%) = Absorbance of sample x Average gradient x Dilution factor

Weight of sample x 10,000

Determination of Phytate/Phytic acid:

Extraction and determination of phytate:

The extraction of phytate from the sample was carried out following a modified procedure of (Talabi et al., 2016). Some quantity (2.0 g) of the sample was extracted with 40 ml of 2.4% HCl (68.6 ml of 35% hydrochloric acid in a total volume of 1 liter of D_2O) under constant shaking at room temperature (25 ^{0}C) for 3 h. All extracts were then filtered using Whatman No. 1 filter paper. The content of phytate was determined using a spectrophotometric method, with an absorbance (A) wavelength at 640 nm (AOAC, 2005). The amount of phytic acid was calculated from the organic phosphorus by assuming that one molecule of phytic acid (containing six molecules of phosphorus (P)) was digested as shown in the equation below (AOAC, 2005):

Phytate mg/g sample

Mean = K" * A *20m

0:282 *1000

where A = absorbance;

‘‘K’’ = standard P (µg)/[A/volume (ml)];

Phytate = 28.2% P; 20 = extract volume (ml) of 1 g sample; 1000 = conversion from µg/g to mg/g. The results were reported in the percentage of phytate in 100 grams of sample.

Determination of Oxalate:

Oxalate was determined using the titration method described by AOAC (2005). Two grams of sample was suspended in a mixture of 190ml of distilled water in a 250ml volumetric flask. Some 6M HCl was added to 10ml of the suspension and heated for 1 hour at 100^0 C in a water bath. The mixture was cooled and made up to 250ml mark with distilled water before filtration. A duplicate portion of

125ml of the filtrate was measured into 250ml beakers. The filtrate was heated again to 90^{0}C on a hot water bath and 10ml of 5% calcium chloride solution was added while being stirred constantly. After heating, it was centrifuged at full speed (2500 rpm) for 5minutes. The supernatant was decanted and dissolved in 10ml of 20% (v/v) H_2SO_4 solution and the total filtrate resulting from 2g of the sample was made up to 300ml.

Permanganate titration: 125ml of the filtrate was heated until near boiling and then titrated against 0.05M $KMNO_4$ solution to a faint pink color.

Oxalic acid content was calculated using the formula, %Oxalic acid = T x (Vme) (Df) x 105 ME x Mf where, T = Titre of $KMNO_4$ (ml), Vme = volume - mass equivalent (1ml of 0.05M MNO4 solution is equivalent to 0.0022g anhydrous oxalic acid), Df = the dilution factor (i.e 300ml) 125ml, ME = the molar equivalent of $KMNO_4$ in oxalic acid ($KMNO_4$ redox reaction is 5), Mf = the mass of the sample use.

Physical Properties of Maize- Avocado Seed based 3G snack food

Bulk Density

Loosed and packed bulk densities were determined according to the procedures of Arueya & Ugwu (2017). Some 10grams of the sample(W) were weighed into a 50ml measuring cylinder and the sample inside the cylinder was tapped several times for 10 minutes to eliminate spaces within the flour till a constant volume (V) was obtained.

$$\text{Bulk Density} = \frac{\text{Weight of sample (g)}}{\text{Volume (ml)}}$$

Swelling capacity and solubility power:

Following the method described by AOAC (2000), one gram of each sample was weighed into a conical flask, and 15ml of distilled water was added. The mixtures were shaken for 5 minutes and heated on a water bath for 40 minutes at 80 – 85^{0}C with consistent stirring. The resulting solutions were then transferred into pre-weighed centrifuge tubes. Some 7.5ml of distilled water was added into each centrifuge tube and centrifuged at 2,200 rpm for 20 minutes. The resulting supernatant was decanted into pre-weighed cans and dried at 100^{0}C to a constant weight. These were then cooled in a desiccator and weighed.

$$\text{Swelling Capacity} = \frac{\text{weight of sediment}}{\text{sample weight} - \text{weight of soluble}}$$

$$\%\text{Solubility} = \frac{\text{Weight of soluble}}{\text{Weight of sample}} \text{X } 100$$

Expansion Ratio

The diameter of the snack food products was measured using calipers. The expansion ratio (ER) of the snack food sample was calculated by dividing their diameter (D) by the extruder die diameter (d^2). Each value was an average of ten measurements.

$$\text{Expansion ratio} = \frac{D}{d}$$

Antioxidant Potential

Total Phenol Content:

The Folin- Ciocalteu method was used for the analysis of the total phenol content of the samples with few adjustments (Arueya et al., 2017). One milliliter of appropriate dilutions (10–1000 µg) of the sample extract and 1ml Folin-Ciocalteu reagent were mixed. The resulting mixture after some three minutes was neutralized through the addition of 1ml of a saturated solution of Sodium Carbonate (15%). The overall mix was made up of 5ml using 2ml distilled water and then incubated for 20mins at 40 ^{0}C. The absorbance of the resulting solution was read at 760nm in a UV visible spectrophotometer (Spectrum lab 752s). The total phenolic value was expressed in terms of Gallic acid equivalent (mg of Gallic acid/g of the extracted compound) (Chel-Guerrero et al., 2016).

Total flavonoid content:

Total flavonoid was determined using the method of Ordonez et al. (2006) for the formation of a complex flavonoid. A volume of 0.5 mL of 2% $AlCl_3$-ethanol solution was mixed with 0.5 ml of the extract (1 mg/mL). The absorbance was measured at 420 nm using a UV-VIS spectrophotometer. Total flavonoid content was calculated as catechin equivalent (mg/100g) using the equation obtained from the curve Y=0.255x, R_2=0.981 2, where x is the absorbance and Y is the catechin equivalent

DPPH scavenging assay

The method of Shen et al. (2010) was used for the determination of the scavenging activity of DPPH radicals in the extract solution. A portion (0.1ml) of 0.135 mM DPPH was prepared in methanol containing 0.5 mL of the extracts. The reaction mixture was vortexed thoroughly and thereafter left in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the food sample on DPPH was calculated using the equation:

DPPH scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control)] ×100,

Where Abs control is the absorbance of DPPH + methanol;

Abs sample is the absorbance of DPPH radical + sample extract or standard.

The results were expressed in % inhibition = DPPH scavenging activity

Total antioxidant capacity:

The total antioxidant capacity of the samples was analyzed using the method described by Prieto et al., (1999) with minor changes. An aliquot (0.3mL) of the sample extract was mixed with 3mL of the reagent (containing 0.6M sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) and was incubated at 95^{0}C for 90

mins. The mixture was cooled to room temperature and the absorbance was taken at 695nm spectrophotometrically using Optima SP-3000 (UV/VIS-SP-3000). The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

Ferric Reducing Antioxidant Potential (FRAP):

The reducing antioxidant potential of the sample was determined by assessing the ability of the sample to reduce iron chloride (Pulido et al., 2000). One milliliter of the sample (0.5g of the sample in 20ml ethanol) was added to 2.5ml of 200mM sodium phosphate buffer and 2.5ml of 1g/100ml potassium ferrocyanide. The absorbance of the mixture thereof was taken at 700nm indicating the reducing power of the sample. Decreased absorbance of the reaction mixture indicated higher reducing power of the sample extract.

Animal Study

Experimental design:

A total number of 35 Wistar albino rats were obtained from the Animal House, Physiology department of the University of Ibadan weighing between (95-110g). They were acclimatized for 10 days under controlled conditions: temperature of 30 ± 5^0 C, 55- 60% relative humidity with 12 hours light/ dark cycles. The rats were randomly selected into 7 groups, housed in standard cages, fed with standard rat feed (Ladokun feed) and water ad libitum after which the test diet was administered. The experiments were carried out following ethical clearance and directions obtained for this study from the Animal Care Unit and Research Ethical Committee, University of Ibadan, (ACUREC-UI).

Animal grouping and 3G snack food administration

The animals were selected into groups as follows.

Group (1): Negative Control: Rats unstressed and fed on standard Rat diet

Group (2): Positive Control: Rats stressed and fed on standard Rat diet

Group (3): Treatment 1: Rats stressed and fed on 10% Avocado seed-based 3G snack food

Group (4): Treatment 2: Rats stressed and fed on 20% Avocado seed-based 3G snack food

Group (5): Treatment 3: Rats stressed and fed on 30% Avocado seed-based 3G snack food

Group (6): Treatment 4: Rats stressed and fed on 100% Maize based 3G snack food

Group (7): Treatment 5: Rats stressed and fed on 100 % Avocado based 3G snack food

Induction of oxidative stress:

The method described by Al-Rejaie et al. (2012) was used with slight changes. Immobilization was used to induce oxidative stress in the animals by restraining each rat in a well-ventilated / plastic cage of the same size (2.5 in. X 4 in.) for 2 hours per day for 5 days in a week between the periods of 9 am and 12 noon. The movements of the control rats were however not restrained throughout the experimental period. When stress was being induced, the animals were deprived of food and water (Liu et al., 1996). The experiment lasted for 4 weeks.

Sample collection:

Four milliliters of blood samples were collected from each experimental rat by ocular puncture using heparinized capillary tubes. The blood was then dispensed into lithium heparin-coated tubes and centrifuged at 2000rpm for 15 mins. The plasma was separated and stored at -18^0 C and used for all the assays.

Biochemical assays

Estimation of malondialdehyde (MDA):

The method described by Al-Rejaie et al. (2012) was used for the estimation of malondialdehyde. One milliliter of 10 % trichloroacetic acid was mixed with two milliliters of plasma and boiled on a water bath for 20 mins. The resulting mixture was cooled and centrifuged at 3000 rpm for 15 mins. The optical density of the supernatant was next measured at 532nm using a spectrophotometer (Optima-3000). The malondialdehyde (MDA) level was calculated using the molar extinction coefficient of (1.56×10^5 M^{-1} cm^{-1}) (Arueya & Ugwu, 2017).

Measurement of antioxidant marker enzymes activity:

Determination of superoxide dismutase activity:

The method described by Oyedemi et al. (2010) was used to carry out this essay. The reaction mixture contained 0.5 ml of hepatic PMS (phenazinemethosulphate), 1 ml of 50 mM sodium carbonate, 0.2 ml of freshly prepared 0.1mM hydroxylamine hydrochloride, and 0.4 ml of 25 µm nitro blue tetrazolium. The reaction mixture was quickly mixed by inversion, following which a clear supernatant of 0.1ml of plasma (10% w/v) was added and centrifuged at 4000 rpm for 10 min. The change in absorbance was recorded at 560 nm.

Determination of catalase activity:

The method described by Onyeka et al. (2012) was used to estimate the catalase activity by measuring with a UV spectrophotometer the decrease in absorbance following decomposition of H_2O_2 at 240nm. The reaction mixture (3 ml) contained 2.9 ml of 30 mM H_2O_2 in phosphate buffer (pH 7.0) and 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0). To calculate a reduction in absorbance, 240nm of 40 M^{-1} cm^{-1} or extinction coefficient for H_2O_2 was used. A mole of H_2O_2 reduced per minute per mg. protein was applied to express catalase activity.

Determination of glutathione:

The method was premised on the reduction of Ellman's Reagent (5,5'-dithio-bis- [2-nitrobenzoic acid]) (DTNB) with reduced glutathione (GSH) to generate a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance measured at 405 nm using a commercial kit (Biodiagnostic, Egypt) (Noemann et al., 2011).

Liver function tests:

The standard procedure described by Burtis et al. (2007) was employed to determine the activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alanine phosphates (ALP). This was complemented using commercial Randox diagnostic kits (Randox Laboratories, USA).

Elucidation of the structure of possible bioactive compounds in the Snack

Following a slightly modified method of Ciudad-Mulero et al (2018), five grams of the sample were extracted in 30ml of hexane for 2 hrs, and the extracts filtered through cheesecloth. The filtrate was collected and re-filtered through Whatman paper No. 1. The resulting filtrate was evaporated under reduced pressure using a rotary evaporator at a low temperature of 40 ^{o}C until a dry mass was obtained. The mass was collected and stored at 4 $^{\circ}$C for the elucidation of possible bioactive compounds. Before the elucidation, the mass extract was re-extracted with methanol before injection into gas chromatography-mass spectrometer (GCMS) (Model GC 7890A, MSD 5975). The column (Agilent 190915- 433) specifications were length 30 m, internal diameter 0.320 mm (i.d.), and thickness of 0.25 mm.

The elucidation of the identity of possible bioactive compounds was carried out using the method of Kim et al. (2007) with slight modification. An aliquot of 1.0 ml of the extract solution was injected. The identification of the components was done by comparing their Kovats GC retention indices and mass spectra with those obtained from the corresponding standards and data from the NIST MS library 14.

Sensory Evaluation

The sensory properties of the 3G snack food were evaluated using thirty (30) panelists (Iwe, 2002; Sharif et al., 2017). Each panelist was presented with different coded samples (max. four per session) of maize – avocado seed based 3G snacks. They were provided with a glass of water to rinse mouth in between sample evaluation. Sensory evaluation was carried out on a 9-point hedonic scale for Appearance, texture, taste, aroma, and overall acceptability (where 9=extremely liked and 1= extremely disliked).

Statistical analysis

One–way analysis of variance (ANOVA) SPSS version 20.0 software (Coakes, 2017) was used to analyze the data obtained in this study. The means were separated using Duncan's multiple range tests ($p < 0.05$) and presented as Mean ± standard deviation.

RESULTS AND DISCUSSION

Proximate composition

The 3G snack food was mainly carbohydrate (60.4–75.8%) (Table 1). These values compared favorably with those obtained by Reddy (2014) for maize–black gram snacks (68.22–77.62%). An overall evaluation of the nutrient profile reflects low-calorie snack food (347-433kcal/100g), similar to functional snack bars with weight loss potentials (Ramirez-jimenez et al., 2018). The low moisture content (7.19–7.69%) played a vital role in maintaining the crispness and freshness of the snacks as well as their keeping quality.

Anti-nutritional factors in Maize- Avocado seed based 3G snack food.

Tannins:

The tannin content of the raw flour (119.00- 684.33mg/100g) and 3G snacks (34.99–49.10 mg/100g) are clearly evident (Table 2). The snack food had tannin content that rose with an increasing level of ASF substitution (34.99, 40.46, 49.10 mg/100g for 10%, 20%, and 30% ASF substitution). The control samples were not significantly different ($P<0.05$) These values are lower than those obtained by Kumar et al. (2018) for extruded sorghum- soya blends, thus making the proteins more bioavailable.

The tannin content of the feed material/raw flour was highest in the 100% avocado seed flour. The low level of the tannin in the 3G snacks could be associated with several factors including feed composition and the extrusion condition itself. The loss of tannin may be traceable to thermal degradation of molecules, the formation of insoluble complexes during extrusion, or changes in their chemical reactivity. Tannins are phytochemical compounds known for their propensity for causing bitterness and binding proteins, rendering them indigestible.

Phytate:

Both maize and avocado raw seed flours contain a considerable amount of phytic acid (296.83- 345.50 mg/100g). The process conditions were however found to have significantly reduced the phytic acid to between 109.5 and 121mg/100g. This compares with the 66.67 % reduction of phytate observed in the extrusion of a maize-soybean-African breadfruit snack (James & Nwabueze, 2013). Phytate reduction following extrusion could be linked to fragmentation and formation of insoluble complexes. Studies have shown that several processing techniques (soaking, boiling) as obtained in this study can significantly reduce the phytic acid content of plant products (Talabi et al., 2016). Phytic acid is an antinutrient that forms complexes with multivalent cations (iron, zinc, calcium) and endogenous enzymes making them unavailable for biological use.

Table 1. Proximate Composition of Maize- Avocado Seed based 3G Snack food.

Sample	Moisture Content %	Ash Content %	Crude Fibre %	Crude Fat %	Protein %	Total CHO% (by difference)
100% ASF	7.69± 0.01^{c}	0.04± 0.014^{a}	1.32±0.165^{bc}	19.59 ± 0.5^{c}	11.05 ± 0.02^{d}	47.31
100% MF	7.19 ± 0.015^{a}	0.07± 0.028^{a}	0.51± 0.015^{a}	13.5 ± 0.5^{b}	8.75 ± 0.05^{a}	57.08
70%MF:30% ASF	7.64 ± 0.02^{cb}	0.03±0.003^{a}	1.74 ± 0.24^{bc}	8.70± 0.2^{a}	10.03± 0.05^{d}	59.66
80%MF:20% ASF	7.48± 0.03^{b}	0.02±0.025^{a}	1.33± 0.14^{ba}	7.44±0.02^{a}	9.71± 0.03^{c}	60.24
90%MF:10%ASF	7.38±0.02^{b}	0.01±0.034^{a}	0.97± 0.136^{a}	6.20±0.70^{a}	9.25 ± 0.03^{b}	61.786

± Standard deviation of 3 replicates

Means in the same column with different superscripts are significantly different at p<0.05

MF: MAIZE FLOUR

ASF: AVOCADO SEED FLOUR

Table 2. Antinutritional factors in Raw and Extruded Maize- Avocado Seed based Snacks.

SAMPLES	TANNIN (mg/100g)	PHYTATE (mg/100g)	SAPONIN (mg/100g)	OXALATE (mg/100g)
100% MF RF	139.55±1.48^{d}	296.59±3.34^{b}	268.40±3.11^{b}	212.30±3.82^{d}
100% MF	43.30±1.039^{fg}	110.50±4,95^{c}	22.20±0.57^{e}	23.65±2.47^{f}
100% ASF RF	684.33±5.61^{a}	341.75±46.32^{a}	484.70±17.82^{a}	409.55±3.61^{a}
100% ASF	36.73±1.93^{g}	120.50±3.54^{c}	20.80±0.14^{e}	27.25±0.35^{f}
70%MF:30% ASF RF	342.15±4.89^{b}	310.00±1.41^{ab}	280.55±6.30^{b}	254.60±3.11^{b}
70%MF:30% ASF	49.10±0.66^{f}	109.50±7.77^{c}	22.05±0.91^{e}	16.15±1.06^{g}
80%MF:20% ASF RF	222.20±5.09^{c}	318.50±4.95^{ab}	193.70±1.56^{c}	240.25±2.33^{c}
80%MF:20% ASF	40.46±0.14^{fg}	121.00±8.49^{c}	22.00±0.28^{e}	26.60±0.42^{f}
90%MF:10%ASF RF	119.00±8.62^{e}	345.50±3.54^{a}	116.30±4.80^{d}	195.70±0.71^{e}
90%MF:10%ASF	35.00±0.33^{g}	115.00±4.24^{c}	20.65±0.35^{e}	7.15±0.21^{h}

± Standard deviation of 3 replicates

Means in the same column with different superscripts are significantly different at p<0.05

MF: MAIZE FLOUR

ASF: AVOCADO SEED FLOUR

RF: RAW FLOUR

Oxalate

The raw flour had high oxalate content (195.70-409.55mg/100g) with the 100% ASF flour having the highest (Table 2). The oxalate levels of the MF: ASF 3G snacks ranged from 7.15 to 27.25 mg/100g while the control samples were 23.65 mg/100g (100% MF) and 27.25mg/100g (100% ASF). The oxalate content profile: 7.15, 26.60,16.15 mg/100g for 10%, 20%, and 30 % ASF substitution levels showed no definite pattern for varying levels of ASF substitution. The extrusion - led -decrease in the oxalate levels of the 3G snacks may be a function of mechanical shearing of the raw materials. According to Hui (1992) at certain concentrations (above 5g/100g), oxalates begin to chelate multivalent ions making them precipitated or unabsorbable.

Saponin

The 100% avocado seed flour-based 3G snack had the highest saponin content while MF: ASF snack variants increased in saponin content with increasing ASF substitution (20.65, 22.00,22.05 mg/100g for 10%,20%, and 30% ASF substitution) (Table 2). The raw MF: ASF flour had saponin content ranging between 116.3 and 484.70mg/100g. The saponin content of the 3G snacks is considerably lower compared to that of the raw flour. This can be attributed to extrusion which traditionally employs a combination of heat, pressure, and mechanical shearing. Saponins, like most antinutrients, have been found to exhibit some deleterious effects such as interference or reduction in the uptake of certain nutrients particularly cholesterol and glucose in the gut through intraluminal physicochemical interaction (Price et al., 1987). According to Lalitha et al. (1990), high levels (300mg/kg body weight in rats) caused diarrhea, restlessness, and histopathological changes in the kidney and liver.

Physical properties of Maize-Avocado seed extrudates.

3Bulk Density:

The bulk density of the 3G snacks ranged from 0.51 ±0.03 to 0.59±0.02 g/cm^{3}. The overall results indicate that the bulk density of the maize- avocado seed-based snacks rose with increasing % substitution of ASF in the feed composition. This is understandably so, being a function of the Fat content of the ASF. The lower the bulk density the better the crispness of the snacks (Dokic et al. (2009). High bulk density is important for certain products like pasta and noodles while low bulk density is preferable for others like breakfast cereals associated with high expansion ratios.

Expansion ratio of the 3G snacks:

The snacks exhibited a porous structure and cylindrical shape. The expansion ratio of the snacks ranged from 2.75 ± 0.06 to 3.08±0.03. These values are lower than those obtained for maize – amaranth grit extrudates (1.83± 0.18) (Dokic et al. (2009) The study reveals that increasing the addition of ASF to the feed formulation resulted in decreased expansion ratio of the 3G snacks.

Ostensibly, this development may have come about by the inherent fat playing the role of a lubricant/plasticizer - reducing shearing and friction of biopolymers(starch and proteins), culminating in reduced product expansion at the die (Ilo et al., 2000). Notably, the expansion ratio dropped with increasing high fat-laden ASF levels.

Water solubility index:

The water solubility index is an excellent pointer of the breakdown of ingredients at the molecular level and the extent of the gelatinization of starch. The water solubility index of the maize- avocado seed based 3G snacks ranged from 9.0 to 11.88 %. The highest water solubility indices were those of the 100% MF and 100% ASF control samples (11% and 11.88% respectively). The water solubility of the snacks did not follow the pattern of varying feed composition.

Swelling capacity:

Swelling is a property of amylose and amylopectin as it's directly related to polysaccharide leached from the starch granules. The swelling capacity of the 3G snacks ranged from 4.07 ±0.06 – 6.19 ± 0.03 g/g. The feed composition affected the swelling capacity of the MF: ASF 3G based snacks as reflected in the increasing swelling capacity with higher ASF content. Snack samples with the highest ASF correlated with higher solubility.

Antioxidant potentials of Maize- Avocado 3G snack

Total Phenol content (TPC):

The maize-avocado 3G snacks increased significantly ($P<0.05$) with the rise in the ASF fraction in the snack (313.36, 392.505, 346.41mg/100g) at 10%, 20%, and 30% ASF levels (Table 3). Several researchers have found that extrusion had no significant effect on the phenolic content of extrudates (Ozer et al., 2016).

Total flavonoids content:

This study shows that the 3G snacks are rich in flavonoids ranging between 117.06 and 267.61mg/100g (Table 3). The flavonoids content of the 3G snacks increased to approximately 268mg/100g with a rising ASF inclusion level(10%) and thereafter started to decline to attain 117mg/100g sample(100% ASF). The peak value is about two-fold of the flavonoid content of the 100%MF or 100% ASF based 3G snacks. This may be attributable to the interplay of factors namely extrusion conditions, optimal release of bound flavonoids from the food matrix at this inclusion level as well as covalent interactions (Nayak et al., 2011). Food products rich in flavonoids such as the 3G snacks under study are often associated with the prevention of free radical damage. The significant amounts of flavonoids present after extrusion of maize-avocado seed demonstrates the thermostability of these flavonoids at temperatures between 110-115^{0}C.

DPPH (2,2- Di Phenyl -1- Picrylhydrazyl) radical scavenging activity:

As shown (Table 3) the DPPH values increased with increasing substitution of ASF (54.09%, 50.69%, 45.49% at 70:30, 80:20 and 90:10 MF: ASF). Phenolic compounds are known to show very strong activities against DPPH radicals since they provide hydrogen atoms or donate electrons that will quench the free radicals that have been associated with oxidative damage of cells in the human body.

Total Antioxidant Capacity (TAC):

The 3G snack had a total antioxidant capacity of 427.5, 537.5, 554.0 for the samples with 90:10, 80:20, 70:30 MF: ASF an indication that the total antioxidant capacity increased with increasing ASF inclusion levels. In some cases, TAC provides more information or maybe more useful than the determination of specific antioxidant species giving a more holistic view of antioxidant capacity.

Ferric Reducing Antioxidant Power (FRAP)

The MF: ASF based 3G snacks had reducing power in the following order 80:20 > 70:30 > 90:10 implying that it did not follow the pattern of increasing level of inclusion (Table 3). It has been established that antioxidant species do differ in their activity in biological systems and so while extrusion feed composition may not have a direct impact on the reducing power, the antagonistic or synergistic effect of the

Table 3. Antioxidant Properties of the 3G Snacks.

Samples	DDPH (%)	Total Antioxidant (Vit-C Equivalent)	Reducing Power (Abs values nm)	Total Phenol (mg/100g)	Total Flavonoid (mg/100g)
100% MF	39.04±0.042^e	421.00±4.24^d	0.24±0.01^{ab}	252.74±1.63^d	138.17±2.76^b
100% ASF	43.16±0.22^d	665.50±6.36^a	0.34±0.03^b	409.98±2.20^a	117.06±8.25^c
70%MF:30% ASF	54.09±0.41^a	554.00±5.66^b	0.300±0.14^{ab}	392.51±15.93^a	146.23±2.35^b
80%MF:20% ASF	50.69±0.05^b	537.500±4.95^c	0.128±0.01^b	346.41±3.09^b	265.39±10.83^a
90%MF:10%ASF	45.49±0.28^c	427.50±3.54^d	0.27±0.09^b	313.37±10.81^c	267.61±1.17^a

± Standard deviation of 3 replicates
Means in the same column with different superscripts are significantly different at $p<0.05$
MF: MAIZE FLOUR
ASF: AVOCADO SEED FLOUR

inherent bioactive compounds contributing to antioxidation do (Sanjust et al., 2008). The bioactive compounds with ferric reducing prowess are indeed advantageous as they prevent redox imbalance capable of causing cell and tissue damage.

Impact of Maize- Avocado seed 3G based snack on animal weight

By the end of the study duration, there was a significant difference in the weight of the rats (Table 4). The administration of the MF: ASF 3G based snacks to animals stressed by immobilization reduced their body weights by 3.5%, 7.17%, 7.94% when fed 90:10, 80:20, 70:30 MF: ASF diets respectively. The positive (stressed and fed with a standard diet (Gp2)) and negative (unstressed and fed with a standard diet (Gp 1)) control animals experienced an increase in their body weights by 12.40% and 13.92% respectively. There was a significant difference (Table 4) between the stressed groups 6 and 7 fed with the control diets (100%MF and 100%ASF). The reason for this has to do more with the nature of the diet rather than the quantum of feed consume (Uzukwu et al., 2017). Daily treatment of rats with avocado seed for 14 days was reported to have decreased their food consumption and body weight(Taha et al.,2008) These results also support previous works that repeated immobilization inducing stress in rats' do cause a reduction in their intake of water and food after their release (Bhattahagar et al., 2006). The reduced intake of food and water may have also played a part in suppressing their growth hormones (Al-Rejaie et al., 2012.

Effect of Maize-Avocado seed based 3G snack on oxidative stress markers (MDA)

Malondialdehyde (MDA) (an oxidative stress marker) increased in the positive control animals (Gp.1) (stressed and fed on a standard diet) 10.32 µM compared to the 4.22µM observed in the negative control animals (Gp.2) (unstressed and fed on a standard diet) (Table 5). There were marginal decreases in the lipid peroxidation of the groups subjected to restraint- induced stress and fed with the varying compositions of MF: ASF based 3G snacks as epitomized by the declining MDA levels in the groups (3,4,5) (10.18, 9.80, 8.25 µM for 10%,20% and 30% ASF inclusion respectively). The groups (6&7) stressed and fed with the snacks (100% MF and 100% ASF based snacks) had MDA levels of 12.17 and 3.58 µM respectively. When compared with other groups, 100% ASF had the best effect on reducing lipid peroxidation while 100% MF had the least impact. These could be linked to the high density of antioxidants in the ASF based 3G snack variant. Similar studies have shown a reduction in MDA levels of experimental animals when there was an increase in the levels of bioactive compounds (Arueya and Ugwu 2017).

Effect of Maize-Avocado seed based 3G snack on total glutathione (GSH)

This study showed that GSH was lower in the positive control group (Gp.1) (1.13mM) (unstressed) compared to the value in the negative control group (Gp.2) (1.41mM) (stressed) although both groups were fed with the same standard diet (Table 5). Understandably, the rise in Glutathione levels in Gp. 2 is a natural response to stress as it is known to protect tissues against lipid peroxidation and oxidative stress. The Glutathione levels in Rats stressed and fed the ASF based snack food(10%,20%,30%,100%) rose to between 5%(1.48mM) and 18%(1.67mM) higher and above the 1.41mM value for Gp. 2 (stressed and fed the standard Rat Diet). It may well be said therefore that the ASF based snack food enhances the metabolic production of Glutathione, in sharp contrast to the effect of the 100% MF based 3G snack. Glutathione is a naturally occurring tripeptide, a non-enzymatic biological antioxidant that presents largely in the

Table 4. Impact of Avocado seed based 3G Snacks on Weight of Rats.

Groups	Daily Feed Consumption(g)	Initial Weight (g)	Final Weight (g)	Weight Difference (%)
1	100±5.0^{a}	129.3 ± 10.3^{c}	147..3± 17.5^{d}	13.9
2	100±4.8^{a}	113.0± 16.24^{a}	127.0± 20.16^{c}	12.4
3	100±5.2^{a}	118.4± 8.4^{a}	115.8±8.52^{b}	-3.58
4	90±3.6^{b}	106.0± 21.45^{a}	98.4±13.24^{a}	-7.17
5	86±2.9^{b}	110.8±4.85^{a}	102.0±18.92^{a}	-7.94
6	100±5.1^{a}	112.8±13.37^{a}	139.2±14.32^{c}	23.40
7	95±2.2^{b}	89.6±15.73^{b}	83.8±18.48^{a}	-6.25

± Standard deviation of 4 replicates

Means in the same column with different superscripts are significantly different (p≥0.05)

Group (1): Rats unstressed and fed on standard Rat diet

Group (2): Rats stressed and fed on standard Rat diet

Group (3): Rats stressed and fed on 90:10 MF:ASF

Group (4): Rats stressed and fed on 80:20 MF:ASF

Group (5): Rats stressed and fed on 70:30 MF:ASF

Group (6): Rats stressed and fed on 100% MF

Group (7): Rats stressed and fed on 100% ASF

Table 5. Effect of Avocado seed 3G based snack on Oxidative stress marker (MDA) and Antioxidant Enzymes levels in Rats.

SAMPLES	Superoxide Dismutase (SOD)	Catalase (CAT)	Malondialdehyde (MDA)	Glutathione (GSH)
Group 1	12.90±0.04^{a}	23.64±0.85^{c}	4.22±3.6^{a}	1.13±0.36^{a}
Group 2	8.79±0.09^{e}	33.19±0.46^{c}	10.32±3.13^{b}	1.41±0.20^{a}
Group 3	10.35±0.19^{c}	34.10±0.26^{a}	10.18±0.65^{b}	1.48±0.29^{a}
Group 4	10.58±0.16^{c}	34.93±0.20^{a}	9.80±5.87^{b}	1.53±0.34^{a}
Group 5	10.93±0.11^{c}	34.49±0.24^{a}	8.25±3.56^{c}	1.57±0.37^{a}
Group 6	10.17±0.02^{d}	130.37±4.78^{b}	12.17±4.52^{d}	1.35±0.23^{a}
Group 7	11.76±0.24^{b}	33.89±0.58^{a}	3.583 ±5.67^{a}	1.67±1.01^{a}

Means in the same column with different superscripts are significantly different at $p<0.05$

Group (1): Rats unstressed and fed on standard Rat diet

Group (2): Rats stressed and fed on standard Rat diet

Group (3): Rats stressed and fed on 90:10 MF:ASF

Group (4): Rats stressed and fed on 80:20 MF:ASF

Group (5): Rats stressed and fed on 70:30 MF:ASF

Group (6): Rats stressed and fed on 100% MF

Group (7): Rats stressed and fed on 100% ASF

liver. It functions removing free radicals, biotransformation of drugs, maintenance of membrane protein, and detoxification of foreign chemicals (Ramani et al., 2011).

Effect of Maize- Avocado Seed based 3G Snack on Antioxidant Enzymes

Superoxide Dismutase Activity (SOD):

The groups fed with the MF: ASF 3G based snacks had their SOD levels increased (18-34%) with rising fractions of ASF in the snacks (10.35, 10.58, 10.90 µ/ml for rats fed with 90:10, 80:20, 70:30 MF: ASF diet). This is in contrast to Rats in Gp. 2(stressed and fed standard rat diet). This trend is similar to that observed in immobilized rats fed green and black tea (Al-Rejaie et al., 2012).

The groups fed snack samples (100% MF, 100%ASF) had SOD levels of 10.12 and 11.75 µ/ml respectively. The ASF based 3G snack had a better salutary effect on SOD than the 100% MF based 3G snack. SOD enzymes protect the cells against the negative effects of Reactive oxygen species (ROS) by catalyzing the rapid removal of free radicals (Moore & Roberts 1998).

Catalase activity:

The catalase enzyme activity showed a similar pattern to that of SOD activity by raising the catalase enzyme levels in the groups fed with MF: ASF 3G based snack food (Table 5). The marginal increase was not in the same proportion with the quantum of substitution (34.49, 34.98,34.10µmol/min/ml for the groups fed with 70:30, 80:20, 90:10 MF: ASF). The positive control group had a lesser SOD activity (23.19 µmol/min/ml) compared to the negative control group–33.89 µmol/min/ml. The animals fed with control diets (100% ASF & 100% MF) had catalase levels of 33.88 and 30.37 µmol/min/ml respectively. The reduced catalase activity in Gp.1 (unstressed and fed standard rat diet) may be attributed to the low antioxidant content of the standard diet compared to those of the MF: ASF based 3G snacks. Catalase enzymes operate by hastening the breakdown of hydrogen peroxide to water and oxygen devoid of any harm.

Activities of Liver enzymes in rats fed with Maize-Avocado seed based 3G snacks

The effect of oxidative stress on the Alanine transaminase (ALT), Aspartate transaminase (ASP), Alkaline phosphatase (ALP) markers in the plasma of the groups fed with MF: ASF based 3G snacks decreased significantly with an increase in ASF inclusion levels (Figure 2). This decrease could be due to bioactive compounds in the samples, which had a positive effect on the liver markers due to their antioxidative properties. Liver damage results in increased serum levels particularly of Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline Phosphatase (ALP) found in serum bilirubin, cytosol, and during necrosis of hepatic cells. This study revealed that Wistar rats fed with the MF: ASF snacks showed no signs of toxicity during the period of administration. They rather appeared to be physically healthy. The comparison of the two groups fed with control diets (100% MF and 100% ASF) showed that the group fed with 100% ASF had lower ALT, AST, and ALP levels. These results indicate that rats fed with the MF: ASF based 3G snack variants had better protection against oxidative stress. The liver is one of the organs in biological systems that are highly susceptible to oxidative stress-induced damage.

Elucidation of the possible bioactive compounds in the snack

The maize- avocado seed based 3G snack is a rich source of bioactive compounds such as fatty acid esters, phenolic compounds, and furans that have potential health benefits. Some of the identified bioactive compounds of the MF: ASF snacks (20% ASF as representative) were 9,12 octadecadienoic acids (Z,Z)(440mg/kg, n- hexadecanoic acid (25mg/kg), epicatechin (9.5mg/kg), cis–vaccenic acid(4.1mg/kg) and N-(5- oxo- tetrahydro-furan – 2yl–

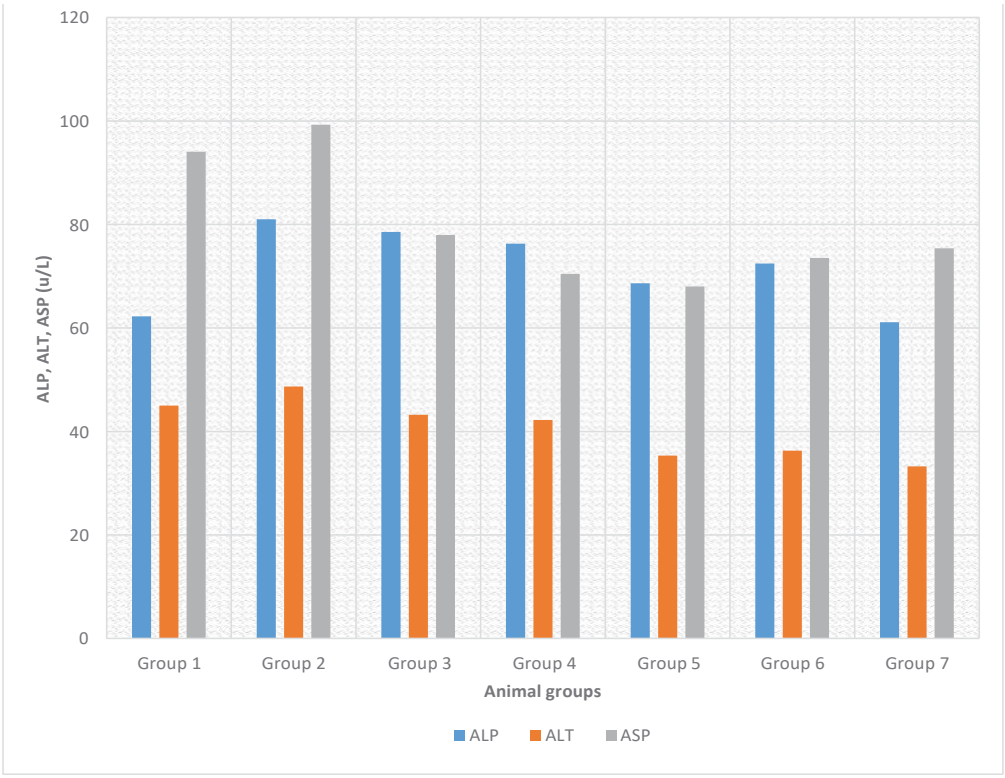


Figure 2: Effect of Avocado based 3G Snack on Liver Enzymes.

Group (1): Rats unstressed and fed on standard Rat diet
Group (2): Rats stressed and fed on standard Rat diet
Group (3): Rats stressed and fed on 90:10 MF:ASF
Group (4): Rats stressed and fed on 80:20 MF:ASF
Group (5): Rats stressed and fed on 70:30 MF:ASF
Group (6): Rats stressed and fed on 100% MF
Group (7): Rats stressed and fed on 100% ASF

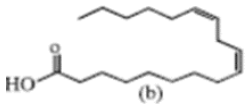
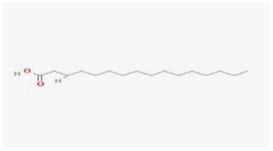
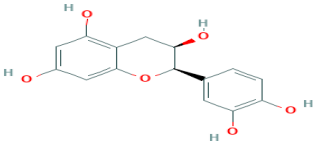
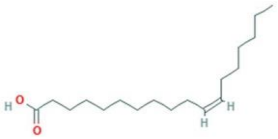
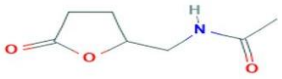
methyl) – acetamide(1.4mg/kg) (Table 6). These compounds contributed about 61.6%, 20.8 %, 7.9%, 3.4%, and 1.2% respectively of the peak areas of the chromatogram (Figure 3).

The activity of these bioactive compounds is a function of subunits of their structural moieties. A dominant bioactive constituent of the snack-9,12 octadecadienoic acids (Z, Z) is a doubly unsaturated n- 6 fatty acids (Table 6). A key structural phenomenon here is the one H-bond donor (H atoms connected to the donor atoms) and two acceptors (atoms with lone pair(s) of electrons capable of establishing H-bonds) counts. Its inherent double bonds (rich in electrons) facilitate attraction for singlet oxygen. This upon oxidation produces compounds that form binary mixtures with other phenols, leading to antioxidant effects (synergistic or antagonistic) towards lipid radicals (Berber et al., 2014). Interestingly, this same compound has been linked to body fat reduction in animals by boosting specific enzymes and proteins involved in the fat breakdown (Whighan et al., 2007).

N-hexadecanoic acid, another bioactive contained in the snack is a straight-chain, sixteen carbon fatty acid. It is characterized by one H-bond donor and two acceptor counts, similar to the earlier mentioned one (Table 6). This bioactive has been known to exhibit significant anti-carcinogenic and cytotoxic activity (Ravi & Krishnan, 2017). This puts the maize- avocado-based 3G snack on stead to not only be potentially cytotoxic but also anti-inflammatory.

(-) Epicatechin is yet another bioactive constituent of the 3G snack (Table 6). It is a flavonoid having 4 Hydroxyl groups at positions that are critical in determining antioxidant activity, all within two aromatic rings linked by an oxygenated heterocycle. The H-bond donor and H-bond acceptor sites are 5 and 6 respectively. The propensity to scavenge ROS has been widely acknowledged as one of its key properties. This compound has also been associated with eliciting satiety, weight loss, and depressing appetite in a randomized placebo-controlled trial (Greenberg 2016). The precise mechanism for these phenomena is largely unknown.

Table 6. Major Bioactives identified in Maize – Avocado Seed Snacks.

RT (mins)	Name of compound	Peak area (%)	Molecular Structure	Molecular Weight (g/mol)
10.624	9,12 octadecadienoic acid (Z, Z)	61.599		280.452
8.551	N – hexadecanoic acid	20.767		256.4
10.656	Epicatechin	7.925		290.271
10.786	Cis –Vaccenic acid	3.375		282.468
11.808	N-(5- oxo- tetrahydro-furan – 2 yl – methyl) – acetamide	1.155		157.169

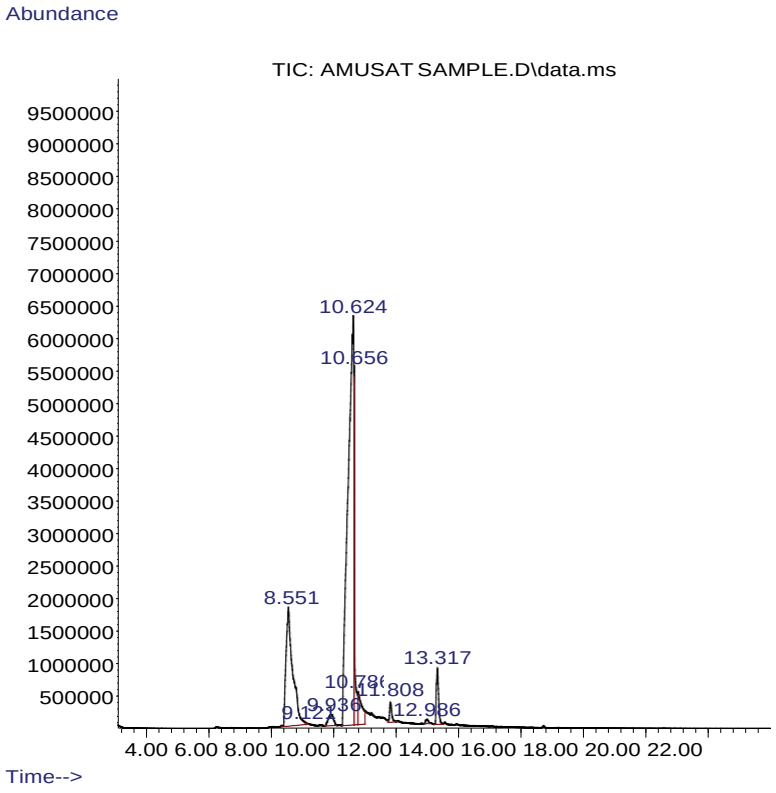


Figure 3: GCMS – chromatogram of Major Bioactives in Avocado seed based 3G snack.

Table 7. Sensory Scores for Maize-Avocado Seed based 3G Snack.

	Texture	Appearance	Aroma	Taste	Acceptability
70 MF: 30 ASF	5.40 ± 1.67^{c}	5.53 ± 2.16^{b}	6.00 ± 1.37^{b}	6.37 ± 1.73^{bc}	5.90 ± 1.49^{c}
90MF :10 ASF	5.53 ±1.50^{bc}	5.73 ± 1.01^{b}	6.63 ± 1.19^{a}	7.00 ± 1.15^{ab}	6.83 ± 0.91^{b}
80 MF: 20 ASF	6.47 ± 1.55^{a}	6.17 ± 0.95^{b}	7.20 ± 0.93^{a}	7.53± 1.01 ^{a}	7.07 ± 1.08^{ab}
100% ASF	4.90 ± 1.63^{c}	5.83 ± 2.15^{b}	5.80 ± 1.16^{b}	5.77 ± 1.17^{c}	5.83 ± 1.29 ^{c}
100%MF	6.27 ± 1.34^{ab}	7.07± 1.14^{a}	6.93 ± 1.17^{a}	7.40 ± 1.16 ^{a}	7.50 ± 1.11^{a}

Values are expressed as Mean ± SD

MF: MAIZE FLOUR

ASF: AVOCADO SEED FLOUR

Cis- vaccenic acid also known as Z-Octa-dec-11-enoic acid is an omega -7-fatty acid and the stereoisomer of vaccenic acid. The H-bond donor count is one while the acceptor count stands at 2. This bioactive has been reported to exhibit anti-carcinogenic properties and some salutary effects on several endogenous antioxidant enzymes (Mirmiranpour et al. 2018).

Aside from fatty acids, the 3G snacks also contained furfural derived compound N-(5-oxo- tetrahydro-furan–2yl–methyl–acetamide. Characterized with one H-bond donor and 3 acceptor counts, this compound has a furan ring oxygen with an affinity for electrons as well as double bonds with attraction for non-free radical singlet oxygen (Table 6). This bioactive compound (a Maillard reaction product) is not natural but has been linked to prolonged storage and thermal processes such as extrusion (a high-temperature operation, enhanced by low water activity and the presence of saccharide) (Matic et al., 2009). This compound is beneficial to human health, although some concerns have been raised for and against their safety. No case reports or studies have linked exposure to these products with cancer development (Arueya & Ugwu, 2017).

Sensory evaluation

With a mean sensory score of 7.07± 1.14, the MF based 3G snack was the most preferred in terms of appearance closely followed by 20%,100%,10%,30% ASF inclusion level in that order(Table 7). Most were not significantly different ($p \leq 0.05$) from each other. The 20% ASF based 3G snack was rated the best for texture with a mean score of 6.47± 1.55 while the 100% ASF snack sample had the least score 4.90 ±1.63. Of all the samples 20% ASF based snack was the most flavorful [aroma (7.53±0.93), taste (sweet) 7.53± 1.01)], followed by the 100% ASF whose sensory scores were not significantly different ($p \leq 0.05$). The inclusion of ASF is a contributory factor to the flavor of these snack samples when compared to the control (100% MF).In terms of overall acceptability, panelists opined that the 100% MF snack is the most preferred (7.5±1.11) immediately after the 20% ASF sample variant with a mean sensory score of 7.07±1.08 comparing favorably with each other. The organoleptic properties of newly developed food products have an important bearing on whether it will be accepted and used by the target consumer.

CONCLUSION

The seed of the avocado fruit can be utilized beneficially in the production of a functional food while also reducing its negative effect on the environment. This study revealed that the inclusion of avocado seed (up to 20%) in 3G snacks (see supplementary files) was most acceptable, safe, and exhibited a considerable impact on its weight loss and antioxidative potentials.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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