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Effect of processing methods on the antioxidant potentials of netlespurge (*Jatropha tanjorensis*) and black nightshade (*Solanum nigrum*) vegetables in peanut burger

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ABSTRACT

Two lesser known indigenous vegetables: netlespurge (*Jatropha tanjorensis*) and black nightshade (*Solanum nigrum*) were each processed into flour samples and were incorporated into wheat flour in coating peanut. These veggie peanut burgers were analysed for their antioxidant properties such as DPPH, FRAP and Metal Chelating ability assay and their linoleic acid inhibition. Veggie peanut burger with netlespurge vegetable had values between 44.62 to 68.32%, 0.0106 to 0.0253 mg AAE/g sample and 49.03 to 63.30% for DPPH, FRAP and Metal chelating assay, respectively, while veggie peanut burger with black nightshade had values between 54.97 to 72.70%, 0.0105 to 0.0173 mg AAE/g sample and 47.13 to 64.01% for DPPH, FRAP and Metal chelating assay respectively. The result of the inhibition of linoleic acid of the peanut burger incorporated with vegetables revealed the abilities of the vegetables to inhibit propagation of peroxides up till the fourth day. Netlespurge vegetable had absorbance values of 2.3220 μm initially rising to 2.6845 μm on the fourth day and a fall absorbance value of 1.1845 μm on the sixth day while Black Nightshade vegetable had absorbance values of 2.3670 μm initially rising to 2.7490 μm on the fourth day and a fall absorbance value of 1.2355 μm on the sixth day. This study concluded that processing methods does not eliminate the antioxidative properties of vegetables and the inclusion of vegetables into peanut burgers increased their antioxidative properties.

Introduction

Peanuts (*Arachis hypogaea* L.) also called groundnuts or monkey-nuts belong to the *Leguminosae* family and is a tropical annual legume (Ntare et al., 2008). They are loaded in protein, oil and fibres (Suchoszek et al., 2011) the utilization everywhere throughout the world in on the increase (Arya et al., 2016). They have been developed into different items like peanut/groundnut burger, roasted peanuts, nut oil, peanut paste, peanut sauce, peanut milk, peanut drink, peanut snacks (salted and sweet bars), peanut flour, etc. (Arya et al., 2016).

Lipid oxidation of peanut is a major source of spoilage in peanuts owing to the high degree of fatty acid unsaturation (Talcott et al., 2005). Polyunsaturated

fatty acids, uniquely linoleic and linolenic, are exceptionally prone to oxidation even under slight ambient conditions and are effectively consolidated into the chain component of lipid peroxidation, to yield free and peroxy radicals (Talcott et al., 2005). It has been reported that oxidation of lipid parts of food materials is the main cause of reduced shelf-stability, adverse tastes, nutrient loss and creation of objectionable aromas while prolonging peanut meals (Reed et al., 2002). Peanuts are also prone to contamination by aflatoxin due to the growth of fungi in the peanut structure (Reed et al., 2002).

Hence, it is imperative to develop means of preserving peanut meals. According to Han et al. (2009), peanut has successfully been coated with protein based isolate with a view to reducing oil migration. However, its

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effect on shelf-stability was found questionable. Arya et al. (2016) thus suggested further coating processes to be evaluated on peanut in order to extend its shelf-stability. This study proposes coating peanut with vegetable flour in addition to regular flour. Two lesser-known vegetables are employed: Netlespurge (*Jatropha tanjorensis*) and Black nightshade (*Solanum nigrium*).

Jatropha tanjorensis belongs to the family of *Euphorbiaceae*. It has been reported to delay the onset of degenerative diseases thereby functioning as an antioxidant (Ezengige, 2017). Because of its hypoglycemic action, it is used in treating diabetes mellitus (Olayiwola et al., 2004). It has been long used as malaria cure (Oyewole et al., 2012); there have been cases of people using it as preventive measure to manage their hypertensive status (Omoregie and Osagie, 2012) and it also support spermatogenesis (formation of sperm cells in the testes) (Ezengige, 2017). Arun and Brindha (2012) studied the antioxidant properties of this vegetable and discovered that it provides an opportunity of been used as a medicinal plant and as a promising basis of natural antioxidant and anti-inflammatory agents.

Solanum nigrium is nurtured as a food crop belonging to *Solanaceae* (night shadow plants) family. It is an extensively employed plant as it had been envisaged to be antipyretic, diuretic, antitumorogenic, antioxidant, hepatoprotective and anti-inflammatory (Jain et al., 2011). Olayinka et al. (2012) examined the antioxidant properties of *Solanum nigrium*. It was reported to have high antioxidant content.

The objective of this work is to determine the effect of antioxidant abilities of these vegetables on coated peanut burger.

Materials and methods

Netlespurge vegetable was harvested from Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Nigeria. Black nightshade vegetable was bought in situ from a farmer in Ile-Ife, Nigeria. Flour, vegetable oil, salt, eggs, peanuts/groundnuts and sugar were obtained from retail outlets in Ile-Ife, Nigeria. All chemicals used were of analytical grade.

Sample Preparation

Netlespurge and black nightshade vegetable samples were washed, destalked, sliced and blanched (at 100 °C for 1 minute). They were then oven-dried (using Gallenham oven, Model OV-420, England) and sun-dried (from 9:00 am to 5:00 pm when the sun's intensity was high). The samples were prepared according to the modified method of Okpala and Ekechi (2014) shown in

Figure 1. Abbreviation used in text: Unblanched sun-dried "Iyana Ipaja" (*Jatropha tanjorensis*); BSI: Blanched sun-dried "Iyana Ipaja" (*Jatropha tanjorensis*); ULI: Unblanched "Iyana Ipaja" (*Jatropha tanjorensis*) oven-dried at 50 °C; BLI: Blanched "Iyana Ipaja" (*Jatropha tanjorensis*) oven-dried at 50 °C; UHI: Unblanched "Iyana Ipaja" (*Jatropha tanjorensis*) oven-dried at 60 °C; BHI: Blanched "Iyana Ipaja" (*Jatropha tanjorensis*) oven-dried at 60 °C; USO: Unblanched sun-dried "Odu" (*Solanum nigrium*); BSO: Blanched sun-dried "Odu" (*Solanum nigrium*); ULO: Unblanched "Odu" (*Solanum nigrium*) oven-dried at 50 °C; BLO: Blanched "Odu" (*Solanum nigrium*) oven-dried at 50 °C; UHO: Unblanched "Odu" (*Solanum nigrium*) oven-dried at 60 °C; BHO: Blanched "Odu" (*Solanum nigrium*) oven-dried at 60 °C.

Production of veggie peanut burger

Peanut burger was prepared according to the modified method described by Christina (2016) using the recipe itemized in Table 1. Dried vegetable leaves prepared according to Figure 1 were milled and sieved using a mesh aperture of 500 µm diameter. Fresh peanuts were sorted, rinsed, blanched at 100 °C for 15 minutes, strained and air-dried at room temperature (27 °C ± 2 °C). Sugar was stirred with whisked egg and the solution was simultaneously sprinkled alongside with flour (wheat and vegetable) and salt on the peanuts. The process was repeated till all the peanuts were evenly coated after which it was deep fried, strained and packaged. Veggie peanut burger was ground into powder form using mortar and pestle.

Table 1. Veggie peanut production recipe

Ingredients	Quantity (g)
Peanut	100.0
Wheat flour	50.0
Sugar	20.0
Eggs	30.0
Salt	2.0
Vegetable flour	1.0

Antioxidant properties determination

Total Phenolic Content (TPC) was carried out using Folin-Ciocalteu's phenol reagent reaction as described by Singleton et al. (1999). Extraction of sample was done using 0.01 g of vegetable flour in 10 mL of distilled water.

The radical scavenging ability of the vegetable samples was determined using the stable radical DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) as described by

Pownall et al. (2010). Vegetable flour samples were extracted using 0.025 g in 10 mL of methanol.

The ferric reducing antioxidant power (FRAP) assay was carried out according to the method of Benzie and Strain (1999). The principle of FRAP method was based on the reduction of a colourless ferric tripyridyltriazine complex to its blue ferrous coloured form due to the donation of electron by antioxidant compounds. 10 mg of sample was extracted in 10 mL distilled water.

The metal-chelating assay of the samples was carried out according to the method of Singh and Rajini (2004). The sample was prepared by extracting 1.0 g in 10 mL of distilled water.

Inhibition of linoleic acid oxidation

Linoleic acid oxidation was measured using the method described by Li et al. (2008). Sample (1.0 g) each was dissolved in 1.5 mL of 0.1 M sodium phosphate buffer (pH 7.0) and the mixture added to 1.0 mL of 50 mM linoleic acid dissolved in 99.5% ethanol. For the control assay, 1.5 mL of buffer was added to the ethanolic linoleic acid solution.

The mixtures were kept at 60 °C in the dark for 7 days. At 24-h intervals, 100 µL of the assay solution was mixed with 4.7 mL of 75% aqueous ethanol, 0.1 mL of ammonia thiocyanate (30 % w/v) and 0.1 mL of 0.02 M ferrous chloride were dissolved in 1 M HCl.

The degree of colour development of the sample was measured using the spectrophotometer (INESA UV-VIS) at 500 nm after 3 min incubation at room temperature. An increased absorbance implied an increase in the level of linoleic acid oxidation.



Fig 1. Flowchart for (a) blanched and (b) unblanched vegetable flour processing

Results and discussion

Antioxidant properties

The results of the antioxidant properties are presented in Table 2. The TPC of veggie peanut burger from netlespurge (*Jatropha tanjorensis*) vegetable samples had value ranged from 0.0509 to 0.1267 mg GAE/g extract of sample. Those from black nightshade (*Solanum nigrum*) vegetable ranged from 0.0343 to 0.0525 mg GAE/g extract of sample. Control sample ranged from 0.017 to 0.069 mg GAE/g extract of sample. It was observed that the inclusion of vegetables to the peanut burger increased the TPC of the peanut burger as compared with the control sample (peanut burger without vegetable). Similar results were reported by Alertor et al. (2013) on some value-added Nigerian traditional snacks (*kulikuli* and *kokoro*) enriched with nutraceuticals.

Blanching reduced the TPC value of samples as unblanched samples are higher in TPC (values) than the blanched samples (values). This agrees with reports of other authors: Fletcher and Hunter (2002) reported a loss of approximately 50%, also, Amin et al. (2006) reported raw samples been higher than the blanched samples. Olayinka et al. (2012) suggested that the reduction of TPC might be due to leaching during blanching. Francisco et al. (2010) however stated that the leaching is due to the disruption of the cell wall of the plant. Exposure to intense heat affected the TPC of the samples. Samples oven-dried at 50 °C had the highest TPC followed by samples oven-dried at 60 °C, samples sun-dried had the least TPC values. Olayinka et al. (2012) reported that temperature is one

of the critical factors that affect the antioxidant properties.

Veggie peanut burger of netlespurge vegetable had FRAP values ranged from 0.01 to 0.03 mg AAE/g sample. The FRAP of veggie peanut burger of black nightshade vegetable ranged from 0.01 to 0.02 mg AAE/g sample. Peanut burger sample without vegetable had an average FRAP value of 0.01 mg AAE/g sample. Veggie peanut from netlespurge vegetable ranged from 4.73 to 10.59% while veggie peanut from black nightshade vegetable ranged from 54.72 to 74.39%. Control sample (peanut burger without vegetable) had values from -17.32 to -22.20% and this showed their inability to scavenge free radicals.

These vegetables have high scavenging abilities and their inclusion into peanut burger snacks could have been responsible for the scavenging abilities of the peanut burger snacks. It therefore might be a health promoting food/snack as it reduced the risk of lifestyle diseases that might have been initiated by the free radical scavenged by DPPH. The DPPH is a stable free radical mostly utilized in examining the antioxidant activities of materials (Olagunju et al., 2018).

For chelating abilities of the veggie peanut burgers; peanut burgers from netlespurge ranged from 47.52 to 69.94% while those from black nightshade ranged from 14.07 to 27.62%. Control sample (peanut burger without vegetable) had values ranged from 12.65 to 13.68%. A decrease of 0.49 to 11.66 was recorded in chelating abilities of the blanched samples. Chelating ability measures the effectiveness of a compound to compete with ferrozine for ferrous ion and thus chelating the ferrous ion to prevent reactive oxygen species (ROS) formation (Singh and Rajini, 2004).

Table 2. Antioxidant properties of veggie peanut burgers

Sample	TPC(mg GAE/g extract)	FRAP (mg AAE/g sample)	DPPH (%)	MCA (%)
USI	0.1925±0.00 ^{de}	0.0143±0.00 ^e	7.8322±0.09 ^{fg}	60.0460±0.09 ^d
BSI	0.0957±0.01 ^g	0.0106±0.00 ^h	4.7572±0.02 ^h	48.3859±0.14 ^e
ULI	0.2985±0.04 ^c	0.0255±0.00 ^a	10.5341±0.07 ^e	69.3490±0.06 ^a
BLI	0.1794±0.03 ^e	0.0219±0.00 ^b	9.4952±0.03 ^{ef}	65.7476±0.28 ^b
UHI	0.2348±0.00 ^d	0.0214±0.00 ^b	8.6459±0.15 ^{efg}	63.7922±0.18 ^c
BHI	0.1056±0.03 ^g	0.0184±0.00 ^c	7.13267±0.01 ^g	59.8895±0.05 ^d
USO	0.1260±0.03 ^g	0.0133±0.00 ^f	64.5970±0.014 ^c	14.75427±0.22 ^j
BSO	0.1164±0.03 ^g	0.0105±0.00 ^h	55.4205±0.08 ^d	14.2676±0.17 ^j
ULO	0.6638±0.03 ^a	0.0172±0.00 ^d	73.2637±0.15 ^a	27.5101±0.014 ^f
BLO	0.6220±0.02 ^a	0.0165±0.00 ^d	55.4205±0.08 ^d	26.2075±0.05 ^g
UHO	0.3642±0.02 ^b	0.0127±0.00 ^f	73.2637±0.015 ^a	23.2918±0.15 ^h
BHO	0.2638±0.03 ^{cd}	0.0116±0.00 ^g	67.6938±0.23 ^b	18.7363±0.17 ⁱ
Control	0.0507±0.03 ^{fg}	0.0097±0.00 ^h	-19.3634±0.25 ⁱ	13.03707±0.06 ^k

Values reported are means ± standard deviation of triplicate determinations. Mean values with different superscript within same column are significantly (p < 0.05) different.

Table 3. Inhibition (nm) of linoleic acid oxidation of veggie peanut burger from netlespurge and black nightshade vegetables

Sample	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
USI	2.4870 ± 0.000 ^d	2.5510 ± 0.001 ^b	2.6250 ± 0.000 ^b	2.6525 ± 0.001 ^c	2.6815 ± 0.002 ^b	1.2810 ± 0.001 ^g	1.2395 ± 0.001 ^f
BSI	2.3220 ± 0.003 ^f	2.5265 ± 0.002 ^c	2.5985 ± 0.001 ^c	2.6225 ± 0.000 ^d	2.6510 ± 0.003 ^c	1.2690 ± 0.001 ^h	1.1845 ± 0.001 ^e
ULI	2.5755 ± 0.002 ^a	2.5995 ± 0.001 ^a	2.6540 ± 0.001 ^a	2.6835 ± 0.001 ^b	2.6845 ± 0.002 ^b	1.4305 ± 0.001 ^a	1.2725 ± 0.001 ^b
BLI	2.5505 ± 0.001 ^b	2.5985 ± 0.001 ^a	2.6250 ± 0.000 ^b	2.6255 ± 0.002 ^d	2.6815 ± 0.001 ^b	1.3635 ± 0.002 ^d	1.2550 ± 0.000 ^d
UHI	2.5730 ± 0.001 ^a	2.5990 ± 0.001 ^a	2.6275 ± 0.004 ^b	2.6540 ± 0.001 ^c	2.6820 ± 0.002 ^b	1.3815 ± 0.001 ^c	1.2610 ± 0.001 ^c
BHI	2.5035 ± 0.005 ^e	2.5980 ± 0.000 ^a	2.6020 ± 0.004 ^c	2.6250 ± 0.001 ^d	2.6540 ± 0.001 ^c	1.3190 ± 0.001 ^e	1.2495 ± 0.002 ^e
USO	2.4870 ± 0.000 ^e	2.5080 ± 0.002 ^d	2.5985 ± 0.001 ^c	2.6235 ± 0.001 ^c	2.6815 ± 0.002 ^c	1.3015 ± 0.002 ^f	1.2620 ± 0.000 ^e
BSO	2.3670 ± 0.000 ^f	2.4130 ± 0.001 ^e	2.4880 ± 0.001 ^e	2.5995 ± 0.001 ^d	2.6815 ± 0.002 ^c	1.2975 ± 0.001 ^g	1.2355 ± 0.001 ^g
ULO	2.5290 ± 0.001 ^b	2.5980 ± 0.001 ^a	2.6540 ± 0.001 ^a	2.6815 ± 0.002 ^b	2.7490 ± 0.001 ^a	1.7615 ± 0.002 ^a	1.4470 ± 0.003 ^a
BLO	2.5280 ± 0.000 ^b	2.5490 ± 0.001 ^c	2.5745 ± 0.001 ^d	2.6240 ± 0.002 ^c	2.7140 ± 0.001 ^b	1.3865 ± 0.002 ^d	1.3125 ± 0.002 ^c
UHO	2.5745 ± 0.001 ^a	2.5755 ± 0.002 ^b	2.5985 ± 0.001 ^c	2.6815 ± 0.002 ^b	2.7145 ± 0.001 ^b	1.4850 ± 0.000 ^b	1.3120 ± 0.001 ^c
BHO	2.4305 ± 0.001 ^e	2.5085 ± 0.001 ^d	2.5740 ± 0.000 ^d	2.6240 ± 0.001 ^c	2.7135 ± 0.002 ^b	1.3165 ± 0.002 ^e	1.3005 ± 0.001 ^d
Control	1.9920 ± 0.000 ^g	2.3230 ± 0.001 ^d	2.4665 ± 0.001 ^d	2.5755 ± 0.002 ^e	2.6540 ± 0.001 ^c	1.2980 ± 0.000 ^f	1.2485 ± 0.002 ^e
Ascorbic	1.6210 ± 0.001 ^h	1.6390 ± 0.001 ^e	1.6545 ± 0.001 ^e	1.6720 ± 0.003 ^f	1.7030 ± 0.004 ^d	0.0045 ± 0.000 ⁱ	-0.0100 ± 0.001 ^g
Blank	2.4655 ± 0.002 ^e	2.5985 ± 0.001 ^a	2.6270 ± 0.003 ^b	2.7510 ± 0.001 ^a	2.7140 ± 0.001 ^a	1.4055 ± 0.001 ^b	1.3310 ± 0.001 ^a

Values reported are means ± standard deviation of triplicate determinations. Mean values with different superscript within same column are significantly ($p < 0.05$) different.

Inhibition of linoleic acid oxidation

The effect of the antioxidative properties of the vegetables were determined via the ability of the veggie peanuts to inhibit the oxidation of linoleic acid as presented in Table 3. It was observed that absorbance for ascorbic, blank, samples and control all increased till the fourth day then declined (Jayaprakasha et al., 2001; Pownall et al., 2010). Blank had the highest inhibitory abilities, this is in accordance to published works (Girgih et al., 2013; Girgih et al., 2015). Control had an initial absorbance value of 1.9920 nm, increased to 2.6540 nm on the fourth day with a fall absorbance value to 1.2485 nm on the sixth day. According to Jayaprakasha et al. (2001) and Pownall et al. (2010), increase in the inhibitory ability of the control up to the fourth day of incubation has been reported. Its depletion afterwards might be due to the limited reactive oxidation products like hydroperoxides produced. Reduction in the production of hydroperoxides has been associated to decrease in absorbance values (Jayaprakasha et al., 2001).

Netlespurge vegetable had an initial absorbance values of 2.3220 nm, increased to 2.6845 nm on the fourth day with a fall in absorbance value to 1.1845 nm on the sixth day while Black Nightshade vegetable had an initial absorbance values of 2.3670 nm, increased to 2.7490 nm on the fourth day with a fall absorbance value to 1.2355 nm on the sixth day. The vegetables were able to inhibit the oxidation of linoleic acid consistently for four days. The fall in the absorbance value between the fifth and sixth day could be caused by the formation of peroxides. Peroxides have been reported to increase with storage (Yadav et al., 2014; Akindele et al., 2017).

The inhibitory abilities of veggie peanut burgers from unblanched vegetables were higher than those of blanched vegetables complementing the results of the antioxidative activities presented in Table 2. Gonçalves et al. (2010) reported that reduction in the inhibitory abilities of blanched samples could be due to the thermal degradation and leaching. Schneider (2009) reported that the role of antioxidants during lipid oxidation was to reduce peroxy radical to hydroperoxide. This reduction inhibits the radical chain propagation.

Conclusion

Blanching lowered but does not eradicate the TPC, DPPH, FRAP, MCA and Ascorbic acid content of the vegetables. Extreme harsh drying condition of the sun and 60 °C oven-drying temperature had lowered values for the TPC, DPPH, FRAP, MCA and Ascorbic acid content of the vegetables as compared to the 50 °C oven-drying temperature. Inclusion of vegetables to the peanut burger increased the TPC, DPPH, FRAP and MCA of the peanut burger (control sample). Inhibition of linoleic acid oxidation of all the veggie peanut burger samples rises till the fourth day and declined from the fifth day. The inhibitory abilities of veggie peanut burgers from unblanched vegetables were higher than those from blanched vegetables.

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