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Research Article

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Effects of Treatments on Some Bioactive Components of Selected Lesser Known Legumes Indigenous to Nigeria

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Abstract

The effects of cooking time and roasting temperature on the total phenolics, tannin, anthocyanin, carotenoids and flavonoid contents of cowpea (*Vigna unguiculata* (L.) Walp), bambaranut (*Vigna subterranean* L.), red bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), African breadfruit (*Treculia africana*), African yam bean (*Sphenostylis stenocarpa*) seed, African oil bean (*Pentaclethra mycrophylla* Benth.) and groundnut (*Arachia hypogea*) were evaluated. The results revealed that pressure cooking times exhibited significant ($p<0.05$) reduction in the total phenolic, tannin, anthocyanin, carotenoid and flavonoid contents of all the samples with increasing cooking time. However, there was minimal increase in the total phenolic and carotenoid contents of red bean; total anthocyanin of red bean and African oil bean. Dry heat (roasting) temperatures significantly ($p<0.05$) reduced the phenolic content of the samples except in bambaranut, red bean and African oil bean where there were increases with increasing roasting temperature. The tannin, anthocyanin, carotenoid and flavonoid contents were significantly ($p<0.05$) reduced with increasing roasting temperatures. Therefore, for increase phenolic content in bambaranut, red bean and African oil bean cooking for 40 min and 50 min and roasting bambaranut, African oil bean and groundnut at 140°C should be adopted.

Keyword

Lesser legumes; treatments; bioactive components;

Introduction

Bioactives are compounds that exert physiological effects in a living system when ingested. They must produce physiological benefits related to promoting health and preventing effects of diseases such as blood pressure reduction, blood glucose reduction, anti-cancer, anti-mutagen, anti-inflammatory activity, immuno-stimulatory activity, antioxidant activity among others [1-5]. Singh *et al.* [6] reported that upon the ingestion of bioactives orally, the compound must withstand the effects of digestive enzymes and the conditions therein. Therefore, the bioactive chemical structure must not be altered for it to be physiologically active. In most cases, the compounds are absorbed from the gastrointestinal tract into the blood circulatory system, from where they are carried to target organs. Aluko [4] revealed that in some cases, the inactive part of the compound becomes active due to the activities of digestive enzymes present in the gastrointestinal tract once consumed. Furthermore, bioactive compounds may exert their physiological effect within the digestive tract and may not be absorbed.

Foods that contain bioactives which are consumed as part of normal diet are called functional foods [7]. Plants such as fruits, vegetables, cereals, legumes, nuts and spices are rich sources of bioactive compounds. They are becoming very popular because of their abundance, low cost compared to animal products and their wider acceptability cutting across religious, social or moral divide [4, 8, 9]. Legumes serve as a large reservoir of bioactive compounds most especially the phenolics and these bioactives have been positively implicated in the management of degenerative diseases [6, 10]. This has led to increased research efforts on the possibilities of exploiting locally available and natural sources of bioactives for the dietary management of those diseases.

Food treatments such as fermentation, germination, cooking etc. have shown to affect both the nutritional composition and phytochemical profile. The main cause of phytochemical loss in food is high temperature degradation, however, for lipophilic ones such as carotenoids found in tomatoes they might remain stable or increase in content upon application of high temperature [11, 12]. Other processing techniques like mechanical processing can also liberate carotenoids and other phytochemicals from the food matrix thereby increasing their bioavailability [12, 13]. In some cases, food processing is important in the elimination/reduction of phytotoxins or the so-called antinutrients. A typical example is the application of local processing such as soaking, cooking, fermentation, etc. which are necessary to avert poisoning from cyanogenic glycosides present in raw cassava [14].

In that regard, research studies have been ongoing in presenting lesser known legumes; their suitability in different food applications as well as their bioactive potentials. More studies have been conducted on the bioactive components of conventional legumes, however, there is dearth of information on the non-conventional legumes. To present lesser known legumes, Oboh [15] evaluated the antioxidant properties of some commonly consumed and underutilised legumes in Nigeria. In the same vein, James *et al.* [16] assessed the potentials of protein concentrates from seven legumes indigenous to northern Nigeria for different food applications. Also, Ade-Omowaye *et al.* [17] profiled the nutritional composition of nine underexploited legumes indigenous to Southwest Nigeria. It is therefore important to assess the effects of different treatments on some bioactive compounds in lesser known legumes and evaluate their antioxidant potentials. This will establish their bioactive potentials as alternative food sources to be exploited.

Materials and Methods

Materials

Indigenous and underutilised legumes for this study included cowpea (*Vigna unguiculata* L.), bambaranut (*Vigna subterranean* L.), red bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), African breadfruit (*Treculia africana*) seeds, African yam bean (*Sphenostylis stenocarpa*) seed, African oil bean (*Pentaclethra mycrophylla* Benth.) seed and groundnut (*Arachis hypogea* L.).

Source of raw materials

The samples were procured in the month of January, 2018 from Umuahia Local Market, Abia State, Southeastern Nigeria. The seeds were botanically identified by the Department of Crop Production, Federal University of Technology, Minna, Nigeria. Extraneous matters such as insect infected seed, sand and chaff were manually removed from the samples.

Preparation of raw materials

African breadfruit and African oil bean seeds were treated differently from other legumes due to their peculiarities before cooking and roasting. For African breadfruit, the seed coats were removed using the method developed by Nwabueze *et al.* [18] and adopted with some modifications [19]. The seeds were washed in a cold potable water and drained through a local perforated basket. The drained seeds were partially cooked in boiling water for 15 min to facilitate the separation of the seed coats from the endosperm. Partially cooked seeds were drained and allowed to stand for 20 min to further soften the seed coat and to effect cooling. Softened seeds were then decoated in an adjustable disc attrition mill and the endosperm was manually separated from the coat on a tray. The dehulled seeds were stored under refrigeration temperature (4 ± 2 °C) until needed for cooking and roasting. For African oil bean seeds, the hard seed coats were manually removed using a kitchen knife and the endosperm was diced into approximately uniform cubes of 1.0 cm length by 0.5 cm diameter. The cubes were refrigerated until needed for cooking and roasting. While, other legumes namely cowpea, red bean, bambaranut, pigeonpea, African yam bean seed and groundnut were manually sorted to remove cracked and insect infested ones and winnowed to get rid of dust, chaff, stalk and other physical contaminants prior to cooking and roasting experiments.

Treatments

Fermentation

Sorted seeds were washed and fermented in tap water in a ratio of 1:3 (w/v) for 2, 3 and 4 days at room temperature (28 ± 2°C) in an enclosed laboratory beaker. After fermentation, the seeds were drained and oven dried at 80°C for 24 h to a constant weight, milled into a powder of 0.5 mm size, kept in plastic bags and then stored at 4°C for further analysis.

Germination

The intact and viable seeds were germinated in the dark at room temperature 28 ± 2°C for 2, 3 and 4 days after sterilizing in ethanol for 1 min and soaking in distilled water (1:3 w/v) for 12 h. Germinated grains were oven-dried at 80°C for 24 h to a constant weight, milled into a powder of 0.5 mm size, kept in plastic bags and then stored at 4°C for further analysis [20].

Determination of some bioactive compounds

The sample extract was weighed (0.2 g) and dissolved in 25 ml hexane and filtered. Prepared sample was then injected into a Buck Scientific (USA) BLC10/11 High Performance Liquid Chromatography (HPLC) System with a Fluorescence Detector (with excitation at 295 nm and emission at 325 nm) and an analytical silica column (25 cm x 4.6 mm ID, stainless steel,5 µm). The mobile phase used was hexane:

Table 1: Effect of cooking time and roasting temperature on total phenolic content (mg/100 g).

T.C (min)	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
Raw	192.43^{a} ± 0.00	225.63^{a} ± 0.01	221.03^{c} ± 0.01	196.33^{a} ± 0.01	221.36^{a} ± 0.01	196.35^{a} ± 0.00	314.26^{a} ± 0.00	225.26^{a} ± 0.01
30	172.14^{b} ± 0.01 (-10.54)	218.55^{b} ± 0.01 (-3.14)	225.16^{b} ± 0.01 (+1.82)	181.56^{b} ± 0.01	219.36^{b} ± 0.01 (-0.9)	193.16^{b} ± 0.01 (-1.62)	313.65^{b} ± 0.00 (-0.19)	213.16^{b} ± 0.01 (-5.37)
40	162.46^{c} ± 0.01 (-15.57)	216.69^{c} ± 0.01 (-3.96)	225.16^{b} ± 0.01 (+1.82)	181.65^{b} ± 0.00	214.16^{c} ± 0.01 (-3.25)	192.62^{c} ± 0.00 (-1.90)	313.65^{b} ± 0.01 (-0.19)	212.46^{c} ± 0.01 (-5.68)
50	132.46d ± 0.01 (-31.16)	216.55^{d} ± 0.01 (-4.02)	227.57^{a} ± 0.02 (+2.87)	181.45^{b} ± 0.00	212.13^{d} ± 0.01 (-4.17)	192.21^{d} ± 0.01 (-2.11)	312.26^{c} ± 0.00 (-0.64)	212.14^{c} ± 0.01 (-5.82)
R (°C)								
Raw	192.43^{a} ± 0.00	225.63^{b} ± 0.01	212.03^{d} ± 0.01	196.33^{a} ± 0.01	221.36^{a} ± 0.01	196.35^{a} ± 0.00	314.26^{c} ± 0.00	225.26^{b} ± 0.01
120	172.65^{b} ± 0.00 (-3.17)	217.26^{c} ± 0.01 (+2.94)	218.46^{c} ± 0.01 (-7.42)	181.76^{b} ± 0.01 (-2.58)	215.65^{b} ± 0.00 (-0.94)	194.51^{b} ± 0.01 (-1.31)	310.12^{d} ± 0.03 (-3.74)	215.65^{c} ± 0.01
130	172.57^{b} ± 0.01 (-3.77)	217.13^{c} ± 0.00 (+3.00)	218.59^{b} ± 0.01 (-7.73)	181.16^{c} ± 0.01 (-3.75)	213.06^{c} ± 0.01 (-1.54)	193.33^{c} ± 0.01 (+0.29)	315.16^{b} ± 0.01 (-4.50)	215.13^{d} ± 0.02
140	172.66^{b} ± 0.01 (+0.23)	226.15^{a} ± 0.00 (+3.03)	218.65^{a} ± 0.00 (-7.64)	181.33^{c} ± 001 (-4.38)	211.67^{d} ± 0.02 (-1.99)	192.45^{d} ± 0.00 (+0.48)	315.76^{a} ± 0.01 (+0.39)	226.15^{a} ± 0.00

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).

Key: CPB = Cowpea, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, ABF = African breadfruit, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, C = Cooking, R = Roasting and (-/+) = % decrease/increase.

tetrahydrofuran: isopropanol (1000:60:4 v/v/v) at a flow rate of 1.0 ml/min. The standard for each bioactive compound was also prepared and ran using similar method. The data obtained from each chromatograph developed by the peak sample data processor was used. The following formula was used to obtain the concentration of each bioactive compound in the sample.

[Bioactive compound] = [A.Sample x [STD] (ppm) x Vhex (ml)]/[A.STD x Wt. Sample (g)]

Where; [STD] = concentration of standard, A.Sample = area of sample, A.STD = area of standard, Vhex = volume of hexane, Wt. Sample = weight of sample. This method was used for the determination of total flavonoid, carotenoid, tannin and anthocyanin.

Reagents used

The reagents used for the study were of analytical grade. Total phenolics standards of gallic acid was from Sigma Chemical Co. (St. Louis, MO, USA). The solvents employed for the extraction of the samples were pure water; and HPLC grades of acetone manufactured by Lobal Chemie Pvt. Ltd., India with CAS No. (64-17-5), ethanol manufactured by Guangdong Guanghua Sci-Tech. Co. Ltd. India with CAS No. (67-64-1) and methanol manufactured by Lobal Chemie Pvt. Ltd., India with CAS No. (67-56-1). The extraction solvents were procured from Finlab Abuja, Nigeria. The Folin reagent (Sigma Chemical Co., St. Louis, MO, USA) and sodium carbonate (Fluka, Buchs, Switzerland) were employed for the measurement of the total phenolic and tannin using the Folin-Ciocalteu method. The calibration curve was constructed with gallic acid (Sigma Chemical Co., St. Louis, MO, USA). Potassium chloride (Fluka, Buchs, Switzerland) and sodium acetate were used for total monomeric anthocyanin determination by the pH-differential method. The reagents were procured from Finlab Abuja, Nigeria.

Statistical analysis

The data obtained were in triplicates and the results were subjected to one-way analysis of variance and expressed as mean with standard deviation. The differences between means were separated by Duncan's Multiple Range Test using IBM SPSS Statistics Programme, Version 19.0 (Illinois, USA). Significant differences were expressed at 5% level.

Results and Discussion

Effects of cooking time and roasting temperature on total phenolic content

The dietary intake of phenolics differ considerably among countries and regions. It is estimated that the daily intake of total free phenolics ranged from 20 mg – 1 g [21]. The result of the evaluation of cooking time on total phenolics is shown in Table (1). The TPC were found to be 192.43 mg/100 g, 225.63 mg/100 g, 221.03 mg/100 g, 196.33 mg/100 g, 221.36 mg/100 g, 196.35 mg/100 g, 314.26 mg/100 g and 225.26 mg/100 g in cowpea (CPB), bambaranut (BBN), red bean (RBS), pigeon pea (PGP), African breadfruit (ABF), African yam bean seed (AYB), African oil bean (AOB) and groundnut (GGN),

Table 2: Effect of cooking time and roasting temperature on total tannin content (mg/100 g).

T. C(min)	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
Raw	6.11^{a} ± 0.01	7.96^{a} ± 0.00	6.94^{a} ± 0.01	7.01^{a} ± 0.01	6.86^{a} ± 0.01	5.98^{a} ± 0.01	6.25^{a} ± 0.00	9.34^{a} ± 0.01
30	6.11^{a} ± 0.00	5.85^{b} ± 0.01 (-26.51)	6.30^{b} ± 0.00 (-9.22)	6.01^{b} ± 0.01 (-14.27)	3.45^{b} ± 0.00 (-49.71)	4.00^{b} ± 0.00 (-33.11)	5.75^{b} ± 0.00 (-8)	6.43^{b} ± 0.01 (-31.16)
40	4.12^{b} ± 0.00 (-32.56)	3.56^{c} ± 0.01 (-55.28)	5.96^{c} ± 0.01 (-14.12)	5.20^{c} ± 0.01 (-25.82)	3.45^{b} ± 0.02 (-49.71)	3.16^{c} ± 0.01 (-47.16)	4.96^{c} ± 0.01 (-20.64)	3.44^{c} ± 0.01 (-63.17)
50	3.16^{c} ± 0.01 (-48.28)	2.96^{d} ± 0.01 (-62.81)	5.99^{d} ± 0.01 (-13.69)	3.11^{d} ± 0.00 (-55.63)	3.17^{c} ± 0.02 (-53.79)	2.97^{d} ± 0.02 (-50.33)	4.25^{d} ± 0.00 (-32)	3.12^{d} ± 0.02 (-66.60)
R (°C)								
Raw	6.11^{a} ± 0.01	7.96^{a} ± 0.00	6.94^{a} ± 0.01	7.01^{a} ± 0.01	6.86^{a} ± 0.01	5.98^{a} ± 0.01	6.25^{a} ± 0.00	9.34^{a} ± 0.01
120	5.96^{b} ± 0.01 (-2.45)	5.96^{b} ± 0.01 (-25.13)	5.79^{b} ± 0.01 (-16.57)	5.95^{b} ± 0.01 (-15.12)	4.00^{b} ± 0.00 (-41.69)	3.75^{b} ± 0.00 (-37.29)	5.77^{b} ± 0.01 (-7.68)	5.98^{b} ± 0.00 (-35.97)
130	3.80^{c} ± 0.01 (-37.81)	4.14^{c} ± 0.01 (-47.99)	5.46^{c} ± 0.01 (-21.33)	5.27^{c} ± 0.00 (-24.82)	3.69^{c} ± 0.01 (-46.21)	3.12^{c} ± 0.02 (-47.83)	3.15^{c} ± 0.00 (-49.60)	4.00^{c} ± 0.00 (-57.17)
140	3.15^{d} ± 0.01 (-48.45)	4.13^{c} ± 0.01 (-47.74)	5.11^{d} ± 0.01 (-26.37)	3.79^{d} ± 001 (-45.93)	3.25^{d} ± 0.01 (-52.62)	3.12^{c} ± 0.00 (-47.83)	3.01^{d} ± 0.00 (-51.84)	2.99^{d} ± 0.01 (-67.98)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).

Key: CPB = Cowpea, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, ABF = African breadfruit, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, C = Cooking, R = Roasting and (-/+) = % decrease/increase.

Table 3: Effect of cooking time and roasting temperature on total anthocyanin content (mg/100 g).

T.C (min)	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
Raw	6.45^{a} ± 0.00	8.94^{a} ± 0.01	2.41^{d} ± 0.01	2.52^{a} ± 0.01	2.35^{a} ± 0.00	2.30^{a} ± 0.00	2.16^{c} ± 0.00	12.42^{a} ± 0.01
30	6.45^{a} ± 0.01	1.34^{b} ± 0.01 (-85.01)	4.33^{c} ± 0.01 (+44.34)	1.45^{b} ± 0.00 (-42.46)	1.10^{b} ± 0.14 (-53.19)	1.06^{b} ± 0.01 (-53.91)	3.05^{b} ± 0.02 (+29.80)	1.87^{b} ± 0.02 (-84.94)
40	1.06^{b} ± 0.00 (-83.57)	1.16^{c} ± 0.02 (-87.02)	6.55^{b} ± 0.01 (+63.21)	1.24^{c} ± 0.00 (-50.79)	1.10^{b} ± 0.14 (-53.19)	0.98^{c} ± 0.01 (-57.39)	3.88^{a} ± 0.01 (+44.33)	1.21^{c} ± 0.01 (-90.26)
50	1.03^{c} ± 0.00 (-84.03)	1.06^{d} ± 0.01 (-88.14)	6.72^{a} ± 0.00 (+64.14)	0.96^{d} ± 0.01 (-61.90)	1.05^{b} ± 0.00 (-55.32)	0.97^{c} ± 0.00 (-57.83)	3.88^{a} ± 0.01 (+44.33)	0.96^{d} ± 0.01 (-92.27)
R (°C)								
Raw	6.45^{a} ± 0.00	8.94^{a} ± 0.01	2.41^{a} ± 0.01	2.52^{a} ± 0.01	2.35^{a} ± 0.00	2.30^{a} ± 0.00	2.16^{b} ± 0.00	12.42^{a} ± 0.01
120	4.06^{b} ± 0.01 (-37.05)	1.65^{b} ± 0.02 (-81.54)	1.45^{b} ± 0.01 (-39.83)	1.16^{b} ± 0.01 (-54.89)	1.16^{b} ± 0.01 (-50.64)	1.06^{b} ± 0.01 (-57.91)	3.87^{a} ± 0.01 (+44.19)	1.63^{b} ± 0.01 (-86.88)
130	1.06^{c} ± 0.02 (-83.57)	1.33^{c} ± 0.00 (-85.12)	1.26^{c} ± 0.01 (-47.72)	0.96^{c} ± 0.01 (-61.90)	1.06^{c} ± 0.01 (-54.89)	1.06^{b} ± 0.02 (-57.91)	3.88^{a} ± 0.02 (+44.33)	1.01^{c} ± 0.01 (-91.87)
140	0.96^{d} ± 0.01 (-85.12)	1.05^{d} ± 0.01 (-88.26)	1.06^{d} ± 0.01 (-56.02)	0.97^{c} ± 000 (-61.51)	0.76^{d} ± 0.01 (-67.66)	1.06^{b} ± 0.01 (-57.91)	3.96^{a} ± 0.01 (+45.45)	1.00^{c} ± 0.00 (-91.95)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).

Key: CPB = Cowpea, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, ABF = African breadfruit, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, C = Cooking, R = Roasting and (-/+) = % decrease/increase.

respectively. Cooking time significantly ($p< 0.05$) affected the total phenolic content of all the legumes. There was significant ($p < 005$) reduction in the total phenolic content of cowpea, bambaranut, African breadfruit, African yam bean, African oil

Table 4: Effect of cooking time and roasting temperature on total carotenoid content (mg/100 g).

T.C (min)	CPB	BBN	RBS	PGP	ABF	AY	AOB	GGN
Raw	0.95^{a} ± 0.00	1.94^{a} ± 0.01	0.73^{a} ± 0.01	0.82^{a} ± 0.01	0.72^{a} ± 0.00	0.67^{a} ± 0.01	0.71 ± 0.01	2.46^{a} ± 0.01
30	0.95^{a} ± 0.00 (-67.01)	0.64^{b} ± 0.01 (+30.48)	1.05^{b} ± 0.00 (-20.73)	0.65^{b} ± 0.01 (-6.94)	0.67^{b} ± 0.00 (-31.34)	0.46^{b} ± 0.00	0.70 ± 0.02	0.64^{b} ± 0.01 (-73.98)
40	0.40^{b} ± 0.00 (-57.89)	0.39^{c} ± 0.01 (-79.90)	1.12^{b} ± 0.00 (+34.82)	0.64^{b} ± 0.02 (-21.95)	0.36^{c} ± 0.00 (-50)	0.43^{c} ± 0.01 (-35.82)	0.71 ± 0.01	0.48^{c} ± 0.01 (-80.49)
50	0.36^{c} ± 0.01 (-62.11)	0.38^{c} ± 0.01 (-80.41)	1.18^{a} ± 0.01 (+38.14)	0.45^{c} ± 0.00 (-45.12)	0.36^{c} ± 0.00 (-50)	0.39^{d} ± 0.01 (-41.79)	0.67 ± 0.01	0.45^{d} ± 0.00 (-81.71)
R (°C)								
Raw	0.95^{a} ± 0.00	1.94^{a} ± 0.01	0.73^{a} ± 0.01	0.82^{a} ± 0.01	0.72^{a} ± 0.00	0.67^{a} ± 0.01	0.71^{a} ± 0.01	2.46^{a} ± 0.01
120	0.63^{b} ± 0.01 (-33.68)	1.16^{b} ± 0.01 (-40.21)	0.66^{b} ± 0.01 (-9.59)	0.67^{b} ± 0.01 (-18.29)	0.37^{b} ± 0.01 (-48.61)	0.45^{b} ± 0.01 (-32.84)	0.64^{b} ± 0.01 (9.86)	1.15^{b} ± 0.00 (-52.85)
130	0.41^{c} ± 0.01 (-56.84)	0.41^{c} ± 0.01 (-78.87)	0.65^{bc} ± 0.01 (-10.96)	0.59^{c} ± 0.00 (-28.05)	0.37^{b} ± 0.01 (-48.61)	0.40^{c} ± 0.00 (-40.30)	0.41^{c} ± 0.01 (-42.2)	0.41^{c} ± 0.00 (-83.33)
140	0.36^{d} ± 0.01 (-62.11)	0.34^{d} ± 0.01 (-82.47)	0.63^{c} ± 0.01 (-13.70)	0.38^{d} ± 000 (-53.66)	0.39^{b} ± 0.01 (-41.46)	0.36^{d} ± 0.00 (-46.27)	0.37^{d} ± 0.02 (-47.89)	0.42^{c} ± 0.01 (-82.93)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).

Key: CPB = Cowpea, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, ABF = African breadfruit, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, C = Cooking, R = Roasting and (-/+) = % decrease/increase.

Table 5: Effect of cooking time and roasting temperature on total flavonoid content (mg/100 g).

T.C (min)	CPB	BBN	RBS	PGP	ABF	AYB	AOB	GGN
Raw	5.21^{a} ± 0.01	14.97^{a} ± 0.01	11.50^{a} ± 0.01	11.51^{a} ± 0.02	11.32^{a} ± 0.00	11.22^{a} ± 0.01	3.60^{b} ± 0.00	27.16^{a} ± 0.01
30	5.21^{a} ± 0.01	3.56 ± 0.01 (-76.22)	6.30^{b} ± 0.07 (-45.22)	3.93^{b} ± 0.01 (-65.86)	3.65^{b} ± 0.00 (-67.76)	3.02^{b} ± 0.01 (-73.08)	3.60^{b} ± 0.00 (+1.37)	3.55^{b} ± 0.00 (-86.93)
40	2.87^{b} ± 0.01 (-44.91)	3.16^{c} ± 0.02 (-78.89)	6.13^{c} ± 0.04 (-46.78)	3.56^{c} ± 0.01 (-69.07)	2.65^{c} ± 0.00 (76.59)	2.97^{c} ± 0.01 (-73.53)	3.63^{a} ± 0.04 (+0.83)	3.10^{c} ± 0.01 (-88.59)
50	2.80^{c} ± 0.00 (-46.26)	2.99^{d} ± 0.01 (-80.03)	3.45^{d} ± 0.04 (-70.00)	2.17^{d} ± 0.01 (-81.15)	2.55^{d} ± 0.00 (-77.47)	2.80^{d} ± 0.00 (-75.04)	3.65^{a} ± 0.14 (+1.37)	2.96^{d} ± 0.00 (-89.10)
R (°C)								
Raw	5.21^{a} ± 0.01	14.97^{a} ± 0.01	11.50^{a} ± 0.01	11.51^{a} ± 0.02	11.32^{a} ± 0.00	11.22^{a} ± 0.01	3.60^{a} ± 0.00	27.16^{a} ± 0.01
120	3.21^{b} ± 0.01 (-38.89)	6.35^{b} ± 0.02 (-57.58)	3.75^{b} ± 0.02 (-67.39)	3.57^{b} ± 0.00 (-68.98)	3.12^{b} ± 0.01 (-72.44)	3.03^{b} ± 0.01 (-72.10)	3.34b ± 0.01 (-7.22)	6.20^{b} ± 0.00 (-77.17)
130	2.80^{c} ± 0.00 (-48.53)	2.82^{c} ± 0.00 (-81.16)	3.56^{c} ± 0.01 (-69.04)	3.41^{c} ± 0.01 (-70.37)	2.96^{c} ± 0.01 (-73.85)	2.85^{c} ± 0.00 (-74.60)	3.16^{c} ± 0.00 (-12.22)	3.15^{c} ± 0.00 (-88.40)
140	2.75^{d} ± 0.00 (-47.57)	2.65^{d} ± 0.00 (-82.30)	3.52^{c} ± 0.01 (-69.39)	2.46^{d} ± 001 (-78.63)	2.80^{d} ± 0.00 (-75.27)	2.80^{d} ± 0.00 (-75.04)	2.81d ± 0.01 (-21.94)	3.00^{d} ± 0.00 (-88.95)

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same column are significantly different ($p < 0.05$).

Key: CPB = Cowpea, BBN = Bambaranut, RBS = Red bean, PGP = Pigeon pea, ABF = African breadfruit, AYB = African yam bean seed, AOB = African oil bean and GGN = Groundnut, T = Treatment, C = Cooking, R = Roasting and (-/+) = % decrease/increase.

bean and groundnut with increasing cooking time. Cooking time had no effect on the total phenolics of pigeonpea; while, red bean phenolics showed increase with increasing cooking time. Reduction in the TPC of cooked samples indicates

it breakdown/oxidation during cooking. Boori *et al.* [22] revealed that loss in the total phenolic content of food samples depends on plant species and cooking method adopted. For example they reported that steaming of banana blossom and cauliflower floret caused an increase in TPC while, microwaving and boiling resulted in to significant reduction. Saikia and Mahanta [23] indicated that application of heat during cooking involves changes in the structural integrity and food cellular matric. These result into both positive and negative effect on the phytochemical profiles. Furthermore, high temperature treatments have destructive effect on TPC with resultant reduction in their physiological benefits.

Cooking time produced minimal loss in TPC from 10.54 - 31.16%, 3.14 - 4.02%, 0.9 – 4.17%, 1.62 - 2.11%, 0.19 - 0.64% and 5.37 - 5.82% in cowpea, bambaranut, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The percentages recorded in this study is low compared with 55.9%, 83.5% and 47.6% loss in *P. eduli*, *S. grandiflora* and *O. zeylanica*, respectively. The result implies that pressure cooking applied for this study produced minimal losses despite the instability of TPC to high temperature [23, 24]. There was minimal increase in red bean TPC with increasing cooking time. Pressure cooking for 30 min and 40 min gave 1.82% increase in TPC, while 50 min cooking gave 2.87% increase. The increase in TPC agrees with [23, 25, 26] who showed that application of heat cleaves the phenolic-sugar glycosidic bond resulting in the formation of phenolic aglycon, which has high affinity to react with Folin-Ciocalteu regents, hence, increasing total phenolic recovery. Also, cooking leads to the decomposition of some polyphenols bound to dietary fibre in the food matrix, thereby releasing free phenolic compounds with resultant availability for detection. Ferracane *et al.* [27]; Gunathilake and Rupa [28] revealed that increase in total phenolics during thermal processing might have been due to the liberation of polyphenols embedded in the plant matrix, disruption of protein-polyphenol complexes, changes in plant cell structure, matrix modification or the inactivation of endogenous polyphenol oxidase which favour efficient recovery of TPC from plant materials.

There was significant ($p < 0.05$) reduction in TPC of pigeonpea, African breadfruit and African yam bean seed with increasing roasting temperatures but, roasting temperatures had no effect on the TPC of cowpea bean. The loss in the TPC is minimal ranging from 7.42 - 7.73%, 2.58 - 4.38%, 0.94 - 1.99% in pigeonpea, African breadfruit and African yam bean seed, respectively. The decrease in the TCP agrees with the report of Larrauri *et al.* [29]; Katsube *et al.* [30] and Hecimovic *et al.* [31] who revealed that phenolic compounds are highly thermo-labile and are early decomposed with high temperature (above 80°C). However, bambaranut, red bean, African oil bean and groundnut exhibited minimal increase in TPC at high roasting temperature (140 °C). Roasting at 120 °C and 130 °C significantly ($p < 0.05$) reduced the TPC in bambaranut and groundnut, however, at 140 °C roasting temperature there was an increase in the TPC by 0.23% and 0.39%, respectively. In red bean, there was increase in TPC with increasing roasting temperature from 2.94 - 3.03%. African oil bean showed a decrease in TPC at 120°C roasting temperature (-1.31%), however, at 130 °C and 140 °C there was increase of 0.29% and 0.48%, respectively. The increase in TPC is in line with the findings of Sadeghi *et al.* [32] who showed high TCP in sesame roasted up to 200 °C for 20 mins, however, at 220 °C the TPC reduced. Also Jeong *et al.* [33] reported high TPC with increasing roasted temperature in sesame. The increase in TPC is attributed to the formation of several low-molecular phenolic compounds after roasting [33]. Also, Bunea *et al.* [34] asserted that the increase in the concentration of certain phenolic compounds after thermal treatment might be explained by their better release from the food matrix as a result of breakdown of supramolecular structures containing phenolic groups or because of their thermal stability.

Effects of cooking times and roasting temperatures on total tannin content

According to Serrano *et al.* [35], the mean daily intake of condensed tannin among the US population was extracted to be 53.6 mg/person/day, whereas, among the Spanish is put at 450 mg/person/day. Vadivel and Biesalski [21] reported that there are epidemiological data which strongly suggested that tannin intake may prevent the onset of chronic diseases. The tannin content of the legumes samples (Table 2) were found to be 6.11 mg/100 g, 7.96 mg/100 g, 6.94 mg/100 g, 7.01 mg/100 g, 6.86 mg/100 g, 5.98 mg/100 g, 6.25 mg/100 g and 9.34 mg/100 g in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Groundnut had the highest tannin content (9.34 mg/100 g) while, Africa yam been seed had the lowest value (5.98 mg/100 g). Treatments applied cooking times, roasting temperatures, fermentation times and germination times significantly ($p < 0.05$) influenced the tannin content of the legumes.

Cooking times significantly ($p < 0.05$) decreased the tannin content of all the legumes. The percentage decrease with increasing temperature ranged from 32.56 - 48.28%, 26.51 - 62.81%, 9.22 - 14.12%, 14.27 -55.63%, 49.71 - 53.79%, 33.11 - 50.33%, 8 - 32% and 31.16 - 66.60%, in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The lowest reduction in tannin was observed in red bean (16.30 mg/100 g) (9.22%) at 30 min of cooking while, highest reduction was observed in groundnut (3.12 mg/100 g) (66.60%) at 50 min. cooking time. The results agree with the findings of Reddy and Pierson [36] and Osunbitan *et al.* [37] who reported that high temperature treatment exhibits significant reduction in the tannin content of plant materials. The reduction may be attributed to tannin solubility and subsequent leaching into the cooking medium [36, 37].

Dry heat treatment (roasting) has been reported to significantly reduce the anti-nutrient content of food samples, however, the level of reduction is low compared with cooking treatment [37]. Roasting temperatures applied in this study decreased the tannin content with increasing roasting temperatures. The decrease in tannin upon roasting ranged

from 2.45 - 48.45%, 25.13 - 47.99%, 16.57 - 26.37%, 15.12 - 45.93%, 46.21 - 52.62%, 37.29 - 47.83%, 7.68 - 51.84% and 35.97 - 67.98% in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The highest reduction was observed in groundnut (67.98%) (2.99 mg/100 g), while, red bean had the lowest reduction (16.57%) (5.11 mg/100 g). The reduction in tannin with increasing roasting temperatures is in line with the findings of Khandelwal *et al.* [38] and Rusydi and Azrina [39]. Vadivel and Biesalski [40] reported a reduction in the range of 26 - 52% in roasted ten wild legumes. This is also in agreement with the earlier report of Siddhuraju and Manian [41] who reported a loss of 54 - 72% in light brown colour seed coated *Vigna unguiculata*. The reduction upon roasting is attributed to the fact that some of the polyphenol compounds like tannins are known to accumulate in the cellular vacuoles and direct heat application might denature them. Also, it could be attributed to Mallard reaction, caramelization and chemical oxidation [40].

Effects of processing on anthocyanin content

Anthocyanin are known to be unstable compounds and food processing, storage conditions and temperatures are crucial factors that influence their stability and availability [42]. These factors can lead to several chemical and enzymatic reactions. The result of this study (Table 3) showed that cooking times and roasting temperatures significantly ($p < 0.05$) affected the anthocyanin content of the samples. The anthocyanin content of the samples were found to be 6.45 mg/100 g, 8.94 mg/100 g, 2.41 mg/100 g, 2.52 mg/100 g, 2.35 mg/100 g, 2.30 mg/100 g, 2.16 mg/100 g and 12.42 mg /100 g in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. Cooking times and roasting temperatures significantly ($p < 0.05$) reduced the anthocyanin content of cowpea, bambaranut, African breadfruit, African yam bean seed and groundnut. However, there was significant ($p < 0.05$) increase in the anthocyanin content of red bean with increasing cooking times, while, roasting temperatures showed decreasing effect. In African oil bean, cooking times and roasting temperatures significantly ($p < 0.05$) increased the anthocyanin content from 2.16 - 3.88 mg/100 g and 2.16 - 3.96 mg/100 g, respectively. High temperature treatments such as cooking, roasting might lead to enzymes in activation, changes in textural characteristics and leaching of water soluble compound. These alter the entire phytochemical profile of food matrix [43, 44]. The percentage reduction with cooking time ranged from 83.57 - 84.03%, 85.01 - 88.14%, 42.46 - 61.90%, 53.19 - 55.32%, 53.91 - 57.83% and 84.94 - 92.27% in cowpea, bambaranut, pigeonpea, African breadfruit, African yam bean seed and groundnut, respectively. The percentage increases in anthocyanin in red beans and African oil bean were 44.34 - 64.14% and 29.18 - 44.33%, respectively. Increases in the anthocyanin content of red bean and African oil bean could be attributed to the fact that heating process facilitates the extraction of plant secondary metabolites from the plant matrix with resulting increase in their concentrations. Also, high temperatures inactivate indigenous enzymes such as polyphenol oxidase, peroxidase and glycosidase that would have hydrolyzed anthocyanins thereby enhancing their retention [45-47]. The decrease in the anthocyanin content of some of the legumes with increasing cooking times could be attributed to the fact that, the anthocyanins is composed of acylated and non acylated forms. Dyrby *et al.* [48] reported that non acylated anthocyanins are more heat susceptible than acylated forms. Here, it can be suggested that cowpea, bambaranut, pigeonpea, African breadfruit, African yam bean seed and groundnut have more of non acylated anthocyanins, while, red bean and African oil bean are majorly composed of acylated anthocyanins. The same trend of decrease was observed by Kim *et al.* [49] and Phan *et al.* [50] in plant materials.

Roasting temperatures significantly ($p < 0.05$) decreased the anthocyanin content of the samples with increasing roasting temperatures from 37.05 - 85.12%, 81.54 - 88.26%, 39.83 - 56.02%, 61.51 - 65.89%, 50.64 - 67.66%, 57.91 - 58.26% and 86.88 - 91.95% in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed and groundnut, respectively. However, roasting temperatures increased the anthocyanin content of African oil bean in the range of 44.19 - 45.55%. The results of this study agree with the findings of Surh and Koh [51] who reported 94% reduction in anthocyanin content of roasted rice. Xu and Chang [52] showed that roasting reduced the anthocyanin content of yellow and black soy bean. The decrease in the anthocyanin could be attributed to thermal susceptibility of anthocyanin to dry heat.

Effect of treatments on carotenoid content

The carotenoid content of the legume samples were found to be 0.95 mg/100 g, 1.94 mg/100 g, 0.73 mg/100 g, 0.82 mg/100 g, 0.72 mg/100 g, 0.67 mg/100 g, 0.71 mg/100 g and 2.46 mg/100 g in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. The samples under investigation exhibited lower levels of carotenoid with groundnut (2.46 mg/100 g) having appreciable quantity. Carotenoids are lipophilic plant pigments that are present ubiquitously in nature. They are commonly used as natural pigments in foods and have important biological functions related to their pro-vitamin A activity, antioxidant activity, ability to regulate gene transcription enhancement of gap junction communication, phase II enzyme inducing activity and ability to enhance immense function [53].

The carotenoids content of the raw and treated samples is shown in Table 4. Treatment applied significantly ($p < 0.05$) affected the carotenoid content of the samples. Cooking times significantly ($p < 0.05$) reduced the carotenoid content of cowpea, bambaranut, pigeonpea, African breadfruit, African yam bean and groundnut from 57.89 – 62.11%, 67.01 - 80.41%, 20.73 - 45.12%, 6.94 - 50%, 31.34 - 41.79% and 73.98 - 81.71%, respectively. Cooking times did not significantly ($p > 0.05$) affect carotenoid content of African oil bean, however, there was significant ($p < 0.05$) increase in the carotenoid content of red beans with increasing cooking time from 0.73

mg/100 g to 1.18 mg/100 g (38.14%). The reduction in the carotenoids content with increasing cooking time agrees with the findings of Vanjaarsveld *et al.* [54] and Prasanna *et al.* [55] who reported a loss of 20.40 - 59.1% and 43 - 68%, respectively in cooked wild legumes. Rodriguez-Amaya [56] asserted that the lost in carotenoid in cooked food could be attributed to it susceptibility to light, oxygen, heat and acid degradation. Also, cooking can lead to Isomerization of native trans-forms to its cis-isomers [57]. However, from the nutritional view point, its cis-isomers are more bioavailable than the trans-carotenoids in crossing the intestinal wall as they are readily solubilized in micelles [55, 58]. Some research findings also reported increase in carotenoids in some green vegetables [59], pumpkin [60] and artichoke [27] after cooking treatment. To justify the increase in carotenoid after cooking, Khachik *et al.* [61] reported that, thermal processing facilitates the breakdown of the cellular structure of the plant material and denaturation of carotenoids-protein complexes which allow for a more effective and efficient extraction of the carotenoids.

Roasting temperatures significantly ($p < 0.05$) reduced the carotenoid content of all the samples with increasing roasting temperatures. However, roasting temperatures had no effect on cowpea bean carotenoids. The decrease in carotenoids with increasing roasting temperatures ranged from 33.68 - 62.11%, 40.21 - 82.47%, 9.59 - 13.70%, 18.29 - 53.66%, 32.84 - 46.27%, 9.86 - 47.89% and 52.85 - 83.33% in cowpea, bambaranut, red bean, pigeonpea, African yam bean seed, African oil bean and groundnut, respectively. The reduction in the carotenoid on roasting agrees with the findings of Struetz *et al.* [62] who reported a significant ($p < 0.05$) loss in the carotenoid content of nut roasted at 140°C for 25 min. Also, Prasanna *et al.* [55] reported 55% loss in roasted *C. asiatica*. Barba *et al.* [63] indicated that direct heat application could accelerate the degradation of carotenoid. Groundnut had the highest percentage loss (52.85 - 833%) while, red beans had the least loss (9.59 - 13.70%). Equally, Sinha *et al.* [44] and Stuetz *et al.* [62] reported significant ($p < 0.05$) loss in the carotenoid content of almond, hazelnut, macadamias, pistachios and walnuts roasted at 140°C, 160°C and 170°C. However, roasting temperatures did no significantly ($p > 0.05$) affect the carotenoid level in ABF.

Effect of treatment on flavonoid content

The total flavonoid content of treated samples is shown in the Table 5. Various treatments applied significantly ($p < 0.05$) influenced the total flavonoid. Groundnut had the highest content 27.16 mg/100 g. This was followed by bambaranut, pigeonpea, red bean, African breadfruit and African yam bean which had 14.97 mg/100 g, 11.51 mg/100 g, 11.50 mg/100 g, 11.32 mg/100 g and 11.22 mg/100 g, respectively. However, African oil bean seed (3.60 mg/100 g) and cowpea (5.21 mg/100 g) had the lowest total flavonoid content.

Flavonoids are wide spread in most edible fruits, vegetables and pulses and are heat sensitive phenolic compounds [55]. Pressure cooking for 30, 40 and 50 min. showed a steady decrease in flavonoid content with increasing cooking time in all the legumes except in African oil bean which showed an increase in the flavonoid with increasing cooking time. The decrease in the flavonoid with increasing cooking time ranged from 44.91 - 46.26%, 76.22 - 80.03%, 45.22 - 70%, 65.86 - 81.15%, 67.76 - 77.47%, 73.08 - 75.04% and 86.93 - 89.10% in cowpea, bambaranut, red beans, pigeonpea, African breadfruit, African yam bean seed and groundnut, respectively. The decrease in flavonoid content has been reported in leafy vegetables by Hiemori *et al.* [64] and Prassanna *et al.* [55]. The gain/loss of flavonoid due to cooking treatment could be attributed to cooking types, nature of the food material and forms of the flavonoids present in the plant material [55, 64]. There was minimal increase in the flavonoid content of African oil bean seed, 0.81% and 1.37% at 30 and 40 min cooking time, respectively. Increase in flavonoid content with cooking may be related to its release from intracellular macro molecules such as carbohydrates and protein and altered cell wall structures [55].

Roasting temperatures significantly ($p<0.05$) influenced the total flavonoid content of all the samples. Like in cooking, roasting has been shown to results into either increase or decrease in the flavonoid. Saikia and Mahanta [23] and Ismail *et al.* [24] have reported that high temperature treatment such as cooking and roasting have destructive effect on flavonoids and phenolic compounds due to their high degree of instability. There was a steady decrease in flavonoid content with increasing roasting temperatures. The percentage loss in total flavonoid ranged from 38.89 - 48.53%, 57.58 - 82.30%, 67.39 - 69.39%, 68.98 – 78.63%, 72.44 - 75.27%, 72.10 - 75.04%, 7.22 - 21.94% and 77.17 - 88.95% in cowpea, bambaranut, red bean, pigeonpea, African breadfruit, African yam bean seed, African oil bean and groundnut, respectively. It can be observed that roasting temperatures had less effect on African oil bean seed total flavonoid (7.22 - 21.94%) while, groundnut had the highest loss (77.17 - 88.95%).

Conclusion

Treatments applied cooking time and roasting temperature significantly ($p<0.05$) influenced the total phenolics, tannin, anthocyanin, carotenoid and flavonoid contents of the samples. There was corresponding reduction in total phenolic, tannin, anthocyanin, carotenoid and flavonoid contents of all the samples with increasing cooking time; however, minimal increases in the total phenolic, anthocyanin and carotenoid contents of red bean and anthocyanin of African oil bean were observed. There was decrease in total phenolic with corresponding increase in roasting temperature The tannin, anthocyanin, carotenoid and flavonoid contents were significantly ($p<0.05$) reduced with increasing roasting temperatures.

Conflict of Interest

The authors declared no conflict of interest.

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