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The hypolipidemic effect of black eyed pea (*Vigna unguiculata*) is influenced by the extent of seed boiling

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Abstract
This study investigated the effect of degree of boiling or cooking of the bean seed on lipid profile in Wistar rats. Eighteen Wistar rats weighing 82g-166g, were randomly assigned into 3 groups (1-3). Group 1 (control) was fed with normal rat feeds, group 2 was fed with moderately cooked/soft beans diet while group 3 was fed with highly boiled\very soft beans diet. Duration of feeding was 21 days after which animals were anesthetized and blood samples taken for determination of lipid profile. From the result, total triglyceride (TG) levels were significantly decreased in groups 2 and 3 ($p<0.001$ each) compared with control and also significantly decreased in group 3 compared with group 2 ($p<0.001$). Total cholesterol (TC) was significantly decreased in groups 2 and 3 ($p<0.001$ each) compared with control while it was significantly decreased in group 3 ($p < 0.001$) compared with group 2. There was significantly decreased HDL-c in groups 2 and 3 ($p<0.001$ each) compared with control and in group 3 ($p<0.001$) compared with group 2. LDL-c was significantly decreased in group 3 compared with control ($p<0.001$) and group 2 ($p<0.001$). VLDL-c was decreased in groups 2 and 3 compared with control ($p<0.001$ each) and decreased in group 3 compared with group 2 ($p<0.001$). The TG\HDL-c ratios in groups 2 and 3 were significantly decreased ($p<0.001$ each) compared with control. Consumption of black eyed pea diets cause reduction in serum TG, TC, HDL-c, LDL-c and TG\HDL-c ratio, effects which are more with consumption of highly boiled pea.

Keywords: Hypolipidemic, boiled, black eyed pea, consumption

1. Introduction
The black eyed beans or pea (*Vigna unguiculata*) is a stable food in most homes in Nigeria and West African subregion [1], Nigeria been the largest producer and consumer [2]. The growing popularity of black eyed pea is due to its high protein content which makes it a ready and available alternative to the animal protein, which many families may not be able to afford [3]. It is consumed in the baked, boiled, cooked, or fried forms depending on the meal desired. It has therefore proven to make up for lost dietary requirements in some stable foods [4].

The black eyed pea is rich in proteins, carbohydrate and fibre but no cholesterol. It also contains calcium, zinc, iron, magnesium, niacin, folate, vitamins B6, B12, E, and little fats [5]. It also has alkaloids, flavonoids, saponins, tannins and phenols [6].

Black eyed pea has several nutritional benefits. It is used as a supplement in improving wearing foods in Nigeria [7] and as food supplement for livestock. It possesses antibacterial [8] antifungal [9] and antilipidemic properties [10]. It has a low glycemic index as a result of which it is an important component of antidiabetic diets [11].

Lipid profile is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids such as cholesterol and triglycerides, the results of which are valuable for evaluating diabetic complications, coronary heart disease risk etc. [12]. This panel typically includes triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), very low density lipoprotein-cholesterol (VLDL-c) and their computed ratios like TC\HDL-c, TG/HDL-c and LDL-c/HDL-c.

Triglycerides are esters of glycerol and free fatty acids. They enable the bidirectional transfer of adipose fats and blood glucose from the liver and may be saturated or unsaturated. Higher levels of serum triglycerides are said to be associated with risk of developing atherosclerosis and by extension coronary heart disease [13].

Cholesterol is a major component of cell membrane, steroids, bile acid and signaling molecules. Hypercholesterolemia could be as a result of increase in HDL-c, LDL-c, VLDL-c or combination of these [14]. Generally, a decrease in total cholesterol corresponds with low rate of lipid-associated vascular diseases [15]. HDL-c is about 30 percent of total cholesterol. The HDL-c functions primarily to remove fats including cholesterol from cells within arterial wall atheromas transporting them back to the liver for excretion or re-utilization. This is why HDL-c is termed “good” cholesterol and so have negative risk factor for coronary heart disease (CHD) [12].

Low density lipoprotein carries about 60 percent of the total cholesterol and is the primary antherogenic class of lipoproteins and mobilizes cholesterol from cells into extracellular tissue like vascular endothelium to form vascular plague, a potential risk to coronary artery disease [16].

Dyslipidemia, defined as the presence of high total cholesterol, increased low density lipoprotein cholesterol, low HDL-c or high TG is recognized as he major coronary heart disease risk factor [13].

Studies have attempted to grade the risk of coronary heart diseases using ratio of the various lipid fractions like TG/HDL-c, TC/HDL-c and LDL-c/HDL-c These indices or ratio have been used successfully to access the risk of coronary heart disease. However, the TG/HDL-c ratio appears to show the strongest association with extent of coronary heart disease [17] indicating the TG/HDL-c was the

single most powerful predictor to the extensive coronary heart disease.

The consumption of the beans is said to impart positively to lipid parameters. But information on the effects of extended boiling on the parameters is not available and hence this study.

2. Materials and methods

2.1 Preparation of black eyed pea diets

The pea or beans was purchased from a local market, washed and boiled without additives in an aluminium pot with a lid. After 35 minutes of boiling when the pea was just soft enough for consumption, part of it was scooped out, dried, mashed and mixed with rat feed in the ratio 40g:60g respectively to form the moderately soft pea diet for group 2 rats. The remaining pea in the pot was subjected to extra 10 minutes of boiling to make the pea very soft. A portion of the very soft pea was then mashed and mixed with rats feed in the ratio of 40g:60g respectively to form the very soft pea diet for group 3 rats.

2.2 Experimental animals:

Eighteen Wistar rats weighing 82g-166g used for the study were housed in metal cages in the animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria and were given standard care.

2.3 Experimental design:

The rats were randomly divided into 3 groups of six rats each. Group 1 served as the control and was fed on normal rat feeds. Group 2 served as the moderately boiled soft pea diet-fed group while group 3 served as the highly boiled very soft pea diet-fed group. All rats had free access to potable water and their respective food for 21 days after which they were anaesthetized and their blood collected for determination of lipid parameters, TG, TC, HDL-c, LDL-c and VLDL-c.

2.4 Determination of serum lipid parameters

2.4.1 Total cholesterol (TC)

This was done by the enzymatic colorimetric method using Giesse Diagnostic Kit [18]. In brief,1000µL of the cholesterol agent was added into each of 3 test tubes containing 10µL of distilled water, 10µL of sample (serum) and 10µL of standard for the blank, test and standard tubes respectively. Each tube was then mixed and incubated for 5 minutes and their absorbance read at 520nm.

2.4.2 Triglycerides (TG)

Triglycerides were measured according to the method by Allain *et al.* [19] which involves enzymatically using a series of coupled reactions in which triglycerides are hydrolysed to produce glycerol. Absorbance was measured at 520nm.

2.4.3 High density lipoprotein-cholesterol (HDL-c)

This was determined using the method described by Allain *et al.* [19] in which non-HDL-c fractions of cholesterol were first made to form complexes with magnesium\dextran complexeswhich are water-soluble and the HDL-c fraction forming the supernatant. The supernatant was incubated for 5 minutes at 37^0C and the absorbance read at 520nm.

2.4.4 VLDL-c, LDL-c, TG\HDL-c, TC\HDL-c and LDL-c\HDL-c

These were computed from the values of TC, TG and HDL-c obtained from the experiments.

2.5 Statistical analysis

Results were expressed as mean±SEM and analyzed by One-way analysis of variance (ANOVA) followed with a post hoc test of least significant difference. A p-value of $p<0.05$ was accepted as significantly different.

3. Results & Discussion

3.1 Total cholesterol, TC (mmol\L)

Serum cholesterol levels in control (group 1), moderately boiled\soft pea diet (group 2) and highly boiled\very soft pea diet (group 3) were 3.65±0.04, 3.18±0.05 and 2.50±0.04 respectively. This showed a significantly decreased TC in both groups 2 and 3 ($p<0.001$ and $p<0.001$ respectively) compared with control and significantly lower in group 3 compared with group 2 ($p<0.001$) as in table 1.

3.2 Triglycerides, TG (mmol\L)

Triglyceride levels in groups 2 (1.25±0.05) and 3 (0.93±0.02) were significantly reduced ($p<0.001$ and $p<0.001$ respectively) compared with control (1.63±0.05) and also significantly lower in group 3 compared with group 2 ($p<0.001$) as in table 1.

3.3 High density lipoprotein-cholesterol, HDL-c (mmol\L)

From table 1, the HDL-c in both groups 2 (0.80±0.01) and 3 (0.63±0.01) were significantly decreased ($p<0.001$ and $p<0.001$ respectively) compared with control (0.92±0.01) and also significantly reduced in group 3 compared with group 2 ($p<0.001$).

3.4 Very low density lipoprotein-cholesterol, VLDL-c (mmol\L)

The VLDL-c levels in groups 2 (0.57±0.02) and 3 (0.42±0.0) were significantly low ($p<0.001$ and $p<0.001$ respectively) compared with control ($p<0.74$±0.02) and also significantly decreased in group 3 compared with group 2 as in table 1.

3.5 LDL-c\HDL-c, TG\HDL-c and TC\HDL-c

The TG\HDL-c ratio in both groups 2 (1.56±0.05) and 3 (1.49±0.03) was significantly decreased ($p<0.001$ and $p<0.001$ respectively) compared to control (1.78±0.01). There were no significant differences in the ratios TC\HDL-c and LDL-c\HDL-c among the experimental groups as shown in table 1.

3.6 Total cholesterol, TC (mmol\L)

Serum cholesterol levels in control (group 1), moderately boiled\soft pea diet (group 2) and highlyly boiled\very soft pea diet (group 3) were 3.65±0.04, 3.18±0.05 and 2.50±0.04 respectively. This showed a significantly decreased TC in both groups 2 and 3 ($p<0.001$ and $p<0.001$ respectively) compared with control and significantly lower in group 3 compared with group 2 ($p<0.001$) as in table 1.

3.7 Triglycerides, TG (mmol\L)

Triglyceride levels in groups 2 (1.25±0.05) and 3 (0.93±0.02) were significantly reduced ($p<0.001$ and $p<0.001$ respectively) compared with control (1.63±0.05) and also significantly lower in group 3 compared with group 2 ($p<0.001$) as in table 1.

3.8 High density lipoprotein-cholesterol, HDL-c (mmol\L)
From table 1, the HDL-c in both groups 2 (0.80±0.01) and 3 (0.63±0.01) were significantly decreased (p<0.001 and p<0.001 respectively) compared with control (0.92±0.01) and also significantly reduced in group 3 compared with group 2 (p<0.001).

3.9 Very low density lipoprotein-cholesterol, VLDL-c (mmol\L)
The VLDL-c levels in groups 2 (0.57±0.02) and 3 (0.42±0.0) were significantly low (p<0.001 and p<0.001 respectively) compared with control (p<0.74±0.02) and also significantly decreased in group 3 compared with group 2 as in table 1.

3.10 LDL-c\HDL-c, TG\HDL-c and TC\HDL-c
The TG\HDL-c ratio in both groups 2 (1.56±0.05) and 3 (1.49±0.03) was significantly decreased (p<0.001 and p<0.001 respectively) compared to control (1.78±0.01). There were no significant differences in the ratios TC\HDL-c and LDL-c\HDL-c among the experimental groups as shown in table 1.

Table 1: Comparison of lipid profile in the different experimental groups

Parameter	Control (group 1)	Group 2	Group 3
TC	3.65±0.04	3.18±0.05***	2.5±0.04^{***,c}
TG	1.63±0.05	1.25±0.06***	0.93±0.02^{***,c}
HDL-c	0.92±0.01	0.80±0.01***	0.63±0.01^{***,c}
VLDL-c	0.74±0.02	0.55±0.02***	0.42±0.01^{***,c}
LDL-c	1.98±0.03	1.92±0.09	1.46±0.03^{***,c}
LDL/HDL	2.17±0.02	2.42±0.16^{ns}	2.32±0.02^{ns}
TG/HDL	1.78±0.04	1.56±0.05**	1.49±0.03***
TC/HDL	3.99±0.01	3.99±0.01^{ns}	3.99±0.04^{ns}

Values are expressed as mean ± SEM, n = 6. Ns = not significantly different among the groups **p<0.01, ***p<0.001 vs control c = p<0.001 vs Group 2

In this study, the effects of consumption of two forms of black eyed pea or beans diets (moderately boiled\soft and highly boiled\very soft pea) on serum lipid profile in wistar rats were studied. The results show that the diets reduce lipid parameters in a manner dependent on the extent of pea boiling.
The TC was significantly decreased in the two pea diet-fed groups which is in line with the findings of Hu *et al.* [10] and Anderson *et al.* [20]. This might be due to the presence of soluble fibres in pea which could prolong the post-prandial intestinally-derived lipoproteins and augment the cholecystokinin response to the meal. This reduction in TC could equally have been due to the presence in the pea of high quantity of saponins and phytates [21] which are said to form insoluble complexes with cholesterol and bile, thus making them unavailable for absorption [22]. Serum TC was also significantly reduced in group 3 that was fed with very soft pea that had undergone extra-boiling. Black eyed pea contains anti-nutrients like phytates and polyphenols which help to modulate physiological lipid concentration [23] as well as saponins and oligosaccharides which are heat-stable [24]. Extended cooking or boiling rather may concentrate these factors making them to contribute more to their cholesterol-loering effect in group 3 rats.
The observed decrease in TG concentration in the pea-fed groups agrees with the report of Carew *et al.* [25] who attributed it to the effect of saponins in velvet beans. Also, antinutrients like phytates present in pea are said to lower plasma cholesterol and triglycerides [23] levels by forming insoluble salts that are not absorbable. The observed greater reduction in TG in group 3 fed with pea that underwent extra boiling could be the result of concentration of heat-stable factors which might have affected the digestion and absorption of lipids.
Our results show a reduction in LDL-c and HDL-c in both groups fed with black pea diets, a finding which is in consonant with the report of the works of Khosla *et al.* [26] and Macarulla *et al*. [27] who attributed the observation to an increased removal of HDL-c and LDL-c by hepatocytes. The reason for the greater reduction in HDL-c and LDL-c levels in group 3 compared with group 2 which was fed with moderately boiled pea is not understood. However, it might be the result of concentration of heat-stable substances which could affect the digestion or absorption of lipids.
Our results also show a significantly reduced TG\HDL-c ratio in both pea diets-fed groups. Although TG and TC are important in assessing cardiovascular risk from dyslipidemia, the levels of the individual fractions are more important [12]. More so, the ratios of these fractions appear to be most reliable in assessing cardiovascular risk from dyslipidemia [28]. Of the variables TC, TG, TG\HDL-c, TC\HDL-c, and LDL-c\HDL-c, the TG\HDL-c appear to be the most single most powerful predictor of coronary heart disease [17]. The TC\HDL-c and LDL-c\HDL-c were not significantly different among the different experimental groups.

4. Conclusions
We therefore conclude that consumption of black eyed pea diets causes reduction in serum TG, TC, HDL-c, LDL-c and TG\HDL-c ratio, effects which are more with consumption of highly boiled pea.

5. References

1. King J, Nnanyelugo DO, Ene-obong H, Ngoddy OP. House-hold consumption profile of cowpea (Vigna unguiculata) among low-income families in Nigeria. Ecology of Food and Nigeria. 1984; 16:209-221.
2. 1IITA. Black eyed peas. IITA Recharch for Development Review, 2013. www.r4dreview.iita.org
3. Egba SI, Uzoegwu PN, Emmanuel TN, Joshua PE. Amino acid content and proximate analysis of the seed extract of Vigna unguiculata used in the management of sickle cell disease. J Chemical and Pharmaceut Res. 2011; 3(4):538-541.
4. Rangel A, Domont GB, Pedrosa G, Ferriera ST. Functional properties of purified vicilins from cowpea (Vigna unguiculata) and pea (Pisum sativum) and cowpea protein isolate. J Agric and Food Chemistry. 2003; 51:5792-5797.
5. United States Department of Agriculture. Natural Resources Conservation Service. The Plants database, National Plants data team, Greensboro, NC, 2012. https://plants.usda.gov/documents.html
6. Mbagwu FN, Okafor VN, Ekeanyanwu J. Phytochemical screening of four edible legumes found in Eastern Nigeria. Afr J Plants Science. 2011; 5(6):370-372.
7. Jirapa P, Nornah H, Zamallia MM, Asma R, Mohamed K. Nutritional quality of germinated cowpea flour (Vigna unguiculata) and its application in home prepared powdered weaning foods. Plant Foods for Human Nutrition. 2001; 56(3):203-216.
8. Sandeep D. Evaluation of antibacterial activity of seed

extract of Vigna unguiculata. Intern J Pharm and Pharmaceut sciences. 2013; 6(1):75-77.
9. Ye XY, Wang HX, Ng TB. Structurally dissimilar proteins with antiviral and antifungal potency from cowpea (Vigna unguiculata) seeds. Life Science. 2000; 67(26):3199-3207.
10. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Wyllet WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. The American J Clin Nutr. 2000; 72:912-921.
11. Akinlua O, Sedodo NS, Akinlotan JV. Glycemic index of selected Nigerian Foods for apparently healthy people. Journal of Obesity and Weight Loss Therapy, 2013; 3:1-4. DOI: 10.4172/2165-7904.1000160
12. Sidhu D, Naugler C. Fasting time and lipid levels in a community-based population. Arch Intern Med. 2012; 172(22):1707-1710.
13. Gupta R, Gupta VP, Sarma M, Bhatnagar S, Thanvi J, Sharma V, Singh AK, *et al*. Prevalence of coronary heart disease and risk factors in an urban Indian population. Indian Heart Journal. 2002; 54:59-66.
14. Israel MK, Mckenny JM. Hyperlipidemias: In Carter B, Angaram D, Lake KD (eds) Pharmacotherapy self-assessment program. Am College Clin Pharm, 1995, 65-94.
15. Downs JR, Clearfield M, Weis S. Primary prevention of acute coronary events with levostatin in men and women with average cholesterol levels Results of AFC APS\TexCAPS Airforce\Texas goronary atherosclerosis prevention study. JAMA. 1998; 279:1615-1622.
16. Ginseberg HN. Lipoprotein metabolism and its relationship to atherosclerosis. Medcal Clinics of North America. 1994; 78:1-20.
17. da Luz PL, Favarato D, Faria-Neto JR, Lemos P, Chagas ACP. High ratio of triglycerides to HDL-c predicts extensive coronary disease. Clinics. 2008; 63(4):427-432.
18. Vassault A, Grafmeyer D, Naudin C, Domont G, Bailly M, Henry J. Protocole de validation de techniques. Annual Bio Clin. 1986; 44:686-745.
19. Allain C, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974; 20:470-475.
20. Anderson JW. Cholesterol-lowering effect of can beans for hypocholesterolemics. Medical Clinical Resources. 1985; 33:871-875.
21. Reynold K, Chin A, Lees KA, Nguen A, Bugnowski, He J. Meta-analysis of the effect of soy protein supplementation on serum lipids. American J Cardiology. 2006; 98:7981-7994.
22. Oboh HA, Osagie AU. The saponin content and sapogenol composition in raw processed and germinated lima beans and jack beans. Nigerian J Biochemistry and Mol Biol. 2003; 18(1):1-6.
23. Potter SM. Overview of proposed mechanism for the hypocholesterolemic effect of soy. Journal of Nutrition. 1995; 125:606-611.
24. Tumwebaze, J. Antinutrients in legumes and their removal. 2011. http://nutrientdigester.blogspot.com/2011/08/anti-nutrients-in-legumes-and-their.html
25. Carew LB, Hardy D, Weis J, Alster FA, Mischler SA, Germat AG, *et al*. Eating raw velvet beans reverses some antinutrtion effects on organ growth, blood chemistry and organ histology in growing chickens. Trop. Subtrop. Agroecosystem. 2003; 1:267-275.
26. Khosla P, Sammam S, Carrol K. Decreased receptor-mediated catabolism in casein-fed rats. J Nutrtional Bio chem. 1991; 2:203-209.
27. Macarulla MT, Medina C, de Diego MA, Chavari M, Zulet MA, Martinez IA, *et al*. Effects of the whole seed and a protein isolate of faba beans (Vicia faba) on the cholesterol metabolism of hyper cholesterol mica rats. British J, Nutrition. 2001; 85:607-614.
28. Ballatyne CM, Hoogeveen RC. Role of lipid and lipoprotein profiles in risk assessment and therapy. American Heart Journal. 2003; 146:227-33.