

EFFECT OF AFRICAN OIL BEAN SEED (*PENTACLETHRA MACROPHYLLIA*) ON BLOOD CHOLESTEROL LEVEL IN RATS.

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ABSTRACT

The effects of fermented and unfermented African oil bean seed on plasma cholesterol levels were studied in rats. The sample was treated as unfermented (Fu), fermented for one day (F1), two days (F2), three days (F3) and four days (F4). The lipid, crude protein and carbohydrate contents of these samples were analysed. The carbohydrate content decreased significantly $P < 0.001$ as fermentation time increased. The protein and the fat initially increased after one-day fermentation and continued to decrease till the fourth day of fermentation. When F1, F2, F3 and F4 protein contents were compared with that of unfermented (Fu), the protein contents were significantly decreased at $P < 0.05$. The plasma cholesterol level of rats fed with diets composed with Fu, F1, F2, F3 and F4 increased initially and decreased with the time of fermentation. Degree of fermentation of the African oil bean seed therefore affected the plasma cholesterol.

KEY WORDS. Cholesterol, African bean seed, *Pentaclethra macrophyllia*, Protein, carbohydrate, fat.

INTRODUCTION

Major risk factors associated with atherosclerosis and coronary heart disease are elevated serum lipids particularly cholesterol i.e. hypercholesterolemia, abnormal glucose tolerance and hypertension. Consequently cholesterol obtained in the diet is not cleared from the blood; it accumulates and contributes to the formation of atherosclerotic plaques. (Zilversmit, 1979).

Almost 30 % of the two million deaths in U.S.A. each year are the result of coronary heart disease (Willett et al., 1993).

Investigations show that cholesterol level has been found to change as a result of different types of fats fed into the animal. Investigations show that in animals, the type of fat from which chylomicrons remnants are derived can influence the uptake and subsequent metabolism of their cholesterol by the liver (Lambert et al, 2000). The level of cholesterol carried in the chylomicrons derived from maize oil or fish oil which contain polyunsaturated fatty acid is cleared from the blood and excreted into the bile, more rapidly than that of palm oil which contain long-chain saturated fatty acids (Bravo et al., 1996). Fat from the diet therefore enter the blood in triacylglycerol rich chylomicrons. Lipoprotein lipase in the extrahepatic capillary beds, hydrolyses most of the chylomicron triacylglycerol

and generates smaller cholesterol enriched chylomicron remnant particles, which are removed from the circulation by the liver (Redgrave, 1970). It was shown that rats fed on certain highly - fermentable carbohydrates especially oligosaccharides exhibit high-propionic acid fermentation together with an induction of hydroxymethylglutaryl - COA (HMG -COA) reductase (EC1.1.1.88). This is the rate-limiting enzyme of cholesterol synthesis [Levrat et al., 1994]. Feeding of animals with such carbohydrates was reported to decrease blood cholesterol (Demigne et al. 1995). The mechanism was attributed to the fact that these carbohydrates increase faecal excretion of neutral steroids and bile acids (Verbereek et al. 1995). These causes a drain on the whole -body cholesterol pool, which in turn may lead to a fall in the serum cholesterol concentration.

Technologies have been developed to reduce the cholesterol content of animal foods (Labat et al. 1997). One of such technologies is fermentation. Fermentation is one of the oldest methods of processing legumes. Fermentation has been reported to enhance nutritional volume, texture, shelf life, aroma and taste of food products (Yadan and Kheterpaul, 1994). Fermentation was observed to decrease levels of crude protein, fat and carbohydrate in the seeds of *Prosopis africana* (Obeta and Ugwanyi, 1998). In castor oil

seed, similar decrease in protein, fats and carbohydrate was observed (Anosike and Egwuatu, 1981) *Pentaclethra macrophylla* (the African oil bean) is widely distributed in Nigeria. The seeds are boiled and fermented before eating. The seed contain high polyphenols, phytic acid, high in-vitro starch and protein digestibility (Enomfon and Nkereuwem, 1999). The proximate composition of African oil bean was reported by Achinewhu (1983).

MATERIALS AND METHODS

Preparation of Samples

Dry and mature oil bean seed was collected fresh from the tree, randomly. Boiled seeds were dehulled to remove the brown coat. The seed was boiled, sliced and fermented for 1,2,3 and 4 days. Fermentation was done by rapping the boiled seed in clean banana leave and left in a closed container to generate enough heat for fermentation. The unfermented seed was used immediately.

Feeding of the Animal. Male Wistar albino rats weighing 250 ± 6.00 g were used in this experiment. The reference diet contained corn flour (800g/kg), vitamin/mineral mix (KQC) comprising of ascorbic acid (2.1), nicotinic acid (1.8), panthothenic acid (1.4), riboflavin (0.6), folic acid (0.83), biotin (0.25), cobalamin (0.80), vitamin k_3 (.020), vitamin A (0.60), vitamin E (0.60), vitamin D (0.90), magnesium sulphate (8.50), calcium carbonate (9.80), chloride (8.20), Iron (3.50), manganese (2.40), zinc (3.0), copper (0.50), cobalt (0.10), potassium iodate (3.5), selenium (0.5), non-nutritive cellulose (117) and oil from each text sample (10g/kg) of the feed. The reference diet was mixed with 23g of Fu, F1, F2, F3 and F4 representing test diets for unfermented, fermented for 1 (f1), 2(f2) 3(f3) and 4(f4) days. The rats were fed for six weeks with the diet and water, ad libitum, at ambient temperature and humidity. At the end of six weeks, the rats were killed and blood samples were collected for cholesterol assay.

Determination of Proximate Composition.

The crude protein of the oil bean seed was determined using the Micro-Kjeldahl nitrogen method (AOAC, 1984). One gram of the dried sample was digested with conc. sulphuric acid and a mixture of 96% anhydrous sodium sulphate and 3.5% copper sulphate as catalyst. The clear digests were distilled into 40% boric acid double

indicator and trapped ammonia was titrated with 0.1N HCL. The values obtained for the nitrogen were multiplied by the nitrogen conversion factor of 6.25 to give the protein content of each sample. Water-soluble carbohydrate (%WM) in unfermented and fermented seeds was determined in the protein-free solutions by the Anthrone method as described by Plummer (1971). The crude fat was determined using soxhlet extraction method of (AOAC, 1984). Two grams of each oven-dried samples was placed inside soxhlet extraction thimbles and extracted with petroleum ether (b.p $40^\circ\text{C} - 60^\circ\text{C}$). Extraction was carried out for 4 hours and the percentage crude fat was calculated relative to raw samples.

Determination of Plasma Cholesterol Level

The cholesterol level was determined by the method of Allain(1970). The blood samples were collected and analysed against a blank and standards provided in the kit. The absorbance was read at 520nm after incubating at 37°C for 10 minutes.

RESULTS

Table 1.0 shows the results of proximate composition of the fermented and unfermented oil bean seed. The crude protein and fat significantly increased after the first day of fermentation. The crude protein significantly ($P < 0.05$) increased from 23.70 ± 0.75 to $24.30 \pm 0.54\%$ (Mean \pm SEM). While the crude fat significantly increased ($P < 0.001$) from 38.44 ± 0.11 to $38.70 \pm 0.01\%$ (Mean \pm SEM). The increase in crude protein and fat after one-day fermentation was interrupted by a decrease in crude protein after the second-day fermentation. This was followed by a continued decrease in crude protein and fat until the fourth day of fermentation. Carbohydrate, on the other hand decreased from the first day of fermentation to the last day of fermentation. The decrease was significant at $P < 0.01$ for F1; $P < 0.05$ for F3; $P < 0.01$ for F3 and F4.

The plasma cholesterol level after feeding the rats for six weeks showed a significant decrease in cholesterol level. The cholesterol level increased significantly ($P < 0.01$) after the first day of fermentation. However a decrease in plasma cholesterol followed immediately after feeding the rats with oil bean seed fermented for two, three and four days.

TABLE 1.0: PROXIMATE COMPOSITION AND PLASMA CHOLESTEROL LEVEL OF UNFERMENTED AND FERMENTED OIL BEAN SEED IN RATS.

Sample	Fu	F1	F2	F3	F4
Carbohydrate (%)	3.67 ± 0.11	2.49 ± 0.10 ^c	2.45 ± 0.40 ^a	2.30 ± 0.17 ^b	2.29 ± 1.16
Crude Protein (%)	23.70 ± 0.75	24.30 ± 0.54 ^a	23.20 ± 0.66 ^a	23.10 ± 0.04 ^a	23.05 ± 0.67 ^a
Crude fat (%)	38.44 ± 0.11	38.70 ± 0.10 ^a	37.10 ± 0.10 ^b	36.10 ± 0.80 ^b	37.01 ± 0.55 ^b
Cholesterol (Mg/dl)	130.02 ± 1.22	140.19 ± 1.57 ^b	118.09 ± 1.23 ^b	106.44 ± 1.60 ^c	95.09 ± 1.59

Values represent Mean + SEM of triplicate analysis.

a = Significant values of P < 0.05 when Fu was compared with each sample.

b = Significant values of P < 0.01 when FU was compared with each sample.

c = Significant values of P < 0.001 when FU was compared with each sample.

DISCUSSION

The proximate composition of oil bean seed found in this study corresponds to other studies done by other workers (Achinewhu, 1983; Onyike and Onwuka, 1999). The decrease in crude protein, fat and carbohydrate as fermentation time increased, was similarly observed during fermentation of *Prosopis africana* (Obeta and Ugwuanyi, 1998) and castor oil seeds (Anosike and Egwuatu, 1981). The decrease in protein, fat and carbohydrate content during fermentation may be as a result of the presence of proteolytic, lipolytic, and amylolytic enzymes that break down protein, lipids and carbohydrates respectively. Achinewhu (1983) reported an increase in crude protein during fermentation of African oil bean seed. He reported an increase in value of 39.90 to 40.70 % of unfermented to fermented seed. The crude fat was reported to be 36.33 and 33.55% for unfermented and fermented respectively.

There has been a direct relationship between the quantity and quality of fat, carbohydrate and protein with the level of plasma cholesterol. Controlling the level of these food components will therefore likely affect the plasma cholesterol. The most important of these three is fat. Consumption of fat-enriched diets alters the uptake of cholesterol by the liver. This is because of the fact that the liver adapts the high fat diets and changes the lipid composition and metabolism leading to increase in cholesterol levels (Lambert et al. 2000).

Consumption of diets enriched in monounsaturated fatty acid and polyunsaturated fatty acid were reported to increase bile acid and cholesterol excretion in rat (McGovern and Quackenbus, 1973). The cholesterol lowering effects found in rats fed with fermented than unfermented oil bean seed, might be as a result of this fact. Fermented African Oil bean seed was

reported to contain unsaturated fatty acids like oleic and linoleic than the unfermented (Achinewhu 1983). The same lowering effects was found in human subjects fed with rapeseed

oil which contains more unsaturated than saturated fatty acids and higher content of polyunsaturated n-6 fatty acids (Yu et al. 1995). Fermentation of oil bean seed is therefore a good source of diet for lowering cholesterol level. Fermentation also decreases the fat content and thus, has a direct effect on cholesterol level. This assumption is also supported by this other fact that more polyunsaturated fatty acids are produced during fermentation.

A recent analysis of dietary intervention programme using the National cholesterol education programme (steps I&II) in America concluded that for every one percent decrease in energy consumed as dietary saturated fatty acids, total cholesterol level decreased by 0.056 mmol/L (Yu-Poth et al. 1999). Another analysis done from pharmacological and dietary intervention programme showed that a reduction of total cholesterol concentrations of one percent (1%) will result in a risk reduction of cardiovascular disease of 2% (Law. et al. 1994). Increase in intake of 0.9% rapeseed oil was observed to have a corresponding increase in plasma cholesterol levels by 0.9%. (Vals et. al. 1996). There is therefore a possibility that fermented oil bean seed can reduce the cardiovascular disease associated with high cholesterol. Carbohydrate also has an indirect effect on cholesterol level by removing the blood cholesterol through the faeces, (Overbreek et al, 1995), thus helping in lowering blood cholesterol. The mechanism of cholesterol lowering effect of carbohydrate, most frequently suggested is interference with lipid digestion or with cholesterol digestion in the small intestine, leading to faecal loss of cholesterol. This in turn leads to the up-regulation of liver

lipoprotein receptors and to depressed cholesterol concentrations. (Levrat et al. 1994).

Finally two to three-day fermentation of oil bean seed should be encouraged before consumption; four -day and one day -fermentation should not be encouraged. The first is likely to increase the cholesterol levels while the other will reduce or decrease the essential food components.

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