A NEW POLYSACCHARIDE, *Detarium microcarpium* FROM TRADITIONAL NIGERIAN PLANT FOOD: ITS PHYSIOLOGICAL EFFECTS ON RATS

ONYECHI, Uchenna Agatha ELLIS, Peter and JUDD, Patricia Ann
Department of Home Science, Nutrition and Dietetics, University of Nigeria Nsukka, Enugu State.
Division of Life and Health Sciences, King's College, University of London, London.

Corresponding Author: Onyechi, U. A. Department of Home Science, Nutrition and Dietetics, University of Nigeria Nsukka, Enugu State. Email uche.onyechi@yahoo.com, Phone +2348066794874

ABSTRACT

Detarium microcarpium is a leguminous plant food used traditionally among the Ibos in the South Eastern part of Nigeria as a thickening agent in vegetable soups. Detarium is largely uncharacterised and under exploited. There is a dearth of information in the literature on this plant food. The aim of the study is to process, analyze and characterise detarium flour; screen detarium using rats to investigate it's physiological effect on the general metabolism of rats, compare detarium to guar gum (GG) as a positive control, to determine the effects of the two foods on the plasma cholesterol level of rats. The result of the analysis showed that powdered detarium has a mean particle size of 464µm. The SNSP content per 100 g food sample was 59.8 g. The viscosity of 1% aqueous dispersion of the powdered detarium food sample obtained using the U tube capillary viscometer was 4000 – 24000 cp. The main SNSP fraction of detarium was identified to be a high molecular weight xyloglucan. In the rat study, the experimental diet contained detarium or guar gum, as positive control, at a level providing 80g soluble NSP/kg diet. Food intake, faecal output, weight gain, digestibility, food efficiency ratio and plasma cholesterol (after overnight fasting) were measured. The result showed that the cholesterol levels of rats fed detarium and guar gum diets were significantly lower than the control (P < 0.05) using the analysis of variance. Detarium and guar gum covariates such as weight gain, food intake and faecal output. The results obtained indicate that detarium may possess properties as guar gum which maybe useful in the management of diabetes and disorders of lipid metabolism in humans.

Keywords: *Detarium,* Guar Gum, Soluble Non starch polysaccharides, General rat metabolism

INTRODUCTION

Water-soluble non starch polysaccharides (SNSP) such as guar gum, a glactomannan from locust bean, have received widespread attention as dietary agents that modulate gastrointestinal function as well as lipid and carbohydrate metabolism. Many studies have demonstrated that this polysaccharide when incorporated into starchy foods and glucose drinks, attenuate the postprandial rise in blood glucose and insulin concentrations in healthy and diabetic subjects (Jenkins *et al.,* 1976; Ellis *et al.,* 1981, 1991; Morgan *et al.,* 1990; Fairchild *et al.,* 1996). Animal studies have shown that the postprandial effects of SNSP depend mainly on their capacity to increase the viscosity of the digesta in the upper part of the gastrointestinal tract (Cherbut *et al*., 1990; Ellis *et al*., 1995, 1996; Johansen *et al.*, 1996). In vitro and animal experiments indicate that an increase in intraluminal viscosity of digesta is a major factor in inhibiting the rate of digestion and absorption of available carbohydrate and improved glycemic index (Blackburn *et al.,* 1984; Mann, 1985, 1995; Edwards *et al*., 1988; Ellis *et al.,* 1995).

Soluble NSP has also been shown to modify plasma lipids by reducing the availability of bile acids which interferes with the absorption of fat (Kelly and Tsai, 1978; Gee *et al.,* 1983). The binding of bile acid and cholesterol is the basic mechanism for the hypocholesterol effect of dietary fibre (Kay and Truswell, 1977). This has been shown in animals (Judd and Truswell, 1976; Judd, 1980; Judd and Truswell, 1985) and in humans (Judd and Truswell, 1981; 1982; Anderson and Chen, 1983; Anderson *et al.,* 1984).

In the Eastern part of Nigeria numerous plant food preparations are used as food condiments. Flour produced from these plant foods are traditionally used to thicken vegetable soups and liquid foods. The thickening property is due to the presence of starch and/or SNSP. On the basis of their ability to thicken soups and liquid foods, it was suggested that these plant materials may have properties similar to guar gum and could be useful sources of water-soluble dietary fibre (SNSP) in glucose control and lipid metabolism in humans. Some of the plant foods are legumes; others are derived from different parts of the plant. It was therefore necessary to undertake a detailed investigation of the physicochemical and nutritional properties of one of these plants foods. The food selected in this study is *Detarium senegalensis*, leguminous plant. The local name for *Detarium* is 'Offor'. *Detarium* is unexploited and largely uncharacterized, commonly used as condiment in the Eastern part of Nigeria. There is a dearth of information in the literature regarding this food.

The present investigation was designed to process, analyze and characterise and determine the polysaccharide composition of detarium flour using a

range of chemical and physical techniques; screen *Detarium* using rats to establish it's physiological effect on the general metabolism of rats by comparing it to Guar gum, as a positive control, to determine it's effect on the plasma cholesterol level of rats.

MATERIALS AND METHODS

Preparation and Processing of Plant Food Extracts: *Detarium senegalensis* Gmelin is a leguminous plant belonging to the subdivision Caesalpinoideae (Balogun and Fetuga, 1986) and is considered to be synonymous with *Detarium microcarpium* (FAO, 1988). Each pod produced by the plant contains one seed, which is usually rounded, oval or flattened and about 40 mm in diameter (FAO, 1988). The legumes grow predominantly in West Africa, Chad and Sudan. The seed samples were purchased in Nsukka and transported to the UK for processing into flour.

The processing method involved boiling the seed for 45 – 60 minutes until the deep brown-purple seed coats (testae) were peeled off easily when touched. The testae were then removed and the white cotyledon was soaked in water for 60 min. The cotyledons were washed three times with cold tap water and discarded after each washing. The cotyledon was then soaked in water overnight to wash away some of the gummy exudates. The washed cotyledons were sun-dried for 24h and ground into fine powder that passes through a 1 mm screen using a coffee grinder (Moulinex blender/mill). The powdered material was air-dried at room temperature for 24h until the powder did not form lumps when touched. The detarium powder (Table 1) was yellowish-white in colour and possessed strong characteristic odour (Onyechi, 1995).

Chemical and Physical Methods of Analysis of the Plant Food Extract: The test food was analysed using standard methods (Kirk and Sawyer, 1991) for moisture (104^{o}C for 16h); ash (Total minerals; 525 ^{o}C for 12h); fat (Soxhlet; light petroleum-diethyl ether extraction) and protein (micro-Kjeldahl method; N x 5.7). The starch content of the flour was determined by an enzymatic method (Englyst *et al.,* 1992a.).

The Englyst method (Englyst *et al.,* 1992b) was used to determine total NSP and the water-insoluble fraction of the NSP; the water-soluble fraction of the NSP was determined as the difference. The procedure involves acid hydrolysis of the NSP followed by gas chromatography of the alditol acetate derivatives of the neutral sugars. The test food sample was boiled with 80% ethanol for 1 hour under reflux. The residue obtained by filtration was washed with 95% ethanol and air dried at room temperature. The dried residue was extracted with 7 ml of distilled water then followed by centrifugation. The supernatant was collected, pH adjusted and centrifuged. The SNSP in the supernatant was precipitated by addition of absolute ethanol. The precipitate was collected by filtration and stored at 4 ^{o}C

The particle size distributions of the test foods were determined by a standard laboratory mechanical sieve analysis method (Lauer, 1966); water binding, by the method described by Quin and Paton (1978) and the viscosity of 1% aqueous dispersion of the test foods obtained by the U tube capillary viscometer.

Rats: Six litters, each containing four male Sprague Dawley rats weighing between 86 g and 141 g were used. The rats were supplied by A. Juck and Sons, London. Each litter of rats was placed in a cage. The rats were fed stock diet (CRM Labsure, Christopher Hill, London), for the first two days and then placed on ground stock diet for a further five days to acclimatise them to eating a ground diet.

Formulation of the Control Diet: The batch size of diet prepared was 5 kg. The calculated quantities of casein (New Zealand Milk Products UK Ltd), vitamin mix and mineral mix (King's College, London mix), sucrose (Booker Fitch Food Services), solka floc (Jordensen and Wettre Limited) and corn starch (Cerestar, Manchester HHIPA), were each weighed and transferred to Hobart mixer and blended for 15 minutes. Sufficient corn oil was heated in beaker to approximately 80 ^{o}C and calculated amount of cholesterol (BDH Chemicals Limited) was weighed and stirred into the corn oil and mixed well to dissolve. This mixture of cholesterol and corn was added to the dry ingredients and blending continued for another 30 min until well distributed. The mixture was passed through a 1/8 inch mesh size. Homogenization of the total mixture was ensured by mixing for a further 30 minutes in the Hobart mixer. The diet was stored at -20^{0}C in self- sealed freezer bags. The proximate composition of the diet is shown on Table 1.

Table 1: Composition of the control diet, positive control diet, containing guar gum and the test diet containing detarium

Ingredients (g/kg).	CD	GGD	DTD
Casein	150	150	129
Fat (corn oil)	100	100	90
Vitamin mix	40	40	40
Sucrose	100	100	100
Cholesterol	10	10	10
Solka floc	50	50	50
Guar gum (M90)	-	100	-
Detarium	-	-	180
Starch	550	430	381

Note: The starch, casein and corn oil content of each was corrected so that all diets had equivalent nutritional content. Each test diet contained approximately 80g/kg total NSP. The vitamin and mineral mix were King's College London mix.

Formulation of the Test Diets: The test diets contained 80g NSP/kg. Corn starch was added to substitute the test flour, in order to supply 80 g NSP/kg diet. The NSP content of the test flour was obtained from the analysis of the foods (Onyechi,

1995). The protein and fat contents were also adjusted as required to accommodate the protein and fat already in the foods. The positive control diet GGD, contained medium grade guar gum, (M90) to provide 80g NSP/kg diet (Meyhall Chemical Company Ltd, Switzerland). The proximate composition of the diet is shown on Table 1.

Feeding of the Rats: On arrival the rats were fed stock diet for 2 days and placed on ground stock diet for a further 5 days to acclimatise them to eating a ground diet. After one week of acclimatization the rats were assigned into groups so that one rat from each litter went into each experimental group. The groups were therefore assumed to be genetically similar and fed for 14 days. The rats were individually housed in stainless steel cages with suspended trays containing filter paper linings for collection of spill and faeces. The rats were weighed daily for the first two days and then on alternate days. Weight was determined by difference from week to week.

Food intake was recorded by providing each rat with an individual weighed pot of food, weighed on alternate days before topping up the food supply and reweighed. At the end of each week of the two experimental periods, the spillage was collected by sifting the faeces from the spilt food.

The rats were pair fed. Each rat was provided with individual weighed pot of food. The daily food intake for the poorest feeders was calculated. This amount plus additional 2-3g of food was fed to each rat. On subsequent days each litter mate across the groups was given an amount of food equal to the previous day's intake of the poorest feeder. If all the food provided was eaten by the rats in a litter-group, the food available for intake was increased by 3-5g on subsequent days.

The faeces samples were collected separately from each animal and stored in self-sealed bags at -20^{o}C until analysis. Dry weight (DW) of spilt food was determined by drying spilt food together with the cage lining paper and food adhering to it, in an oven at 105^{o}C for 48 hours. The dry weight of the paper was subtracted from the total to give dry weight of spillage. Dry weight of the remaining food in the pot was similarly determined after drying for 48 hours at 105^{o}C. Food intake was calculated.

Food intake, faecal output, weight gain, energy digestibility and plasma cholesterol were the parameters that were assessed in the rats.

Bleeding of the Rats and Plasma Cholesterol Analysis: At the end of 14 days of experimental feeding, the rats fed the experimental diets were anaesthetized and bled from the heart using a heparinized needle and syringe to prevent the blood from clotting. The blood was collected in a centrifuge tube and centrifuged at 2,500 rpm for 15 minutes. The plasma was separated from the cells and stored in LP tubes at a temperature of -20^{o}C until analysis. The fasting plasma cholesterol levels were determined by enzymatic method (Roschkur, 1975; Sidel *et al*., 1981) using Boehringer-Mannheim kit method.

Statistical Analysis: The differences between the effects of the types of diets on the rats were analysed by analysis of variance, ANOVA (Statistical analysis system package, SAS Institute, 1995) The level of significance was fixed at $p<0.05$. The results were further investigated using Tukey's method and Least Square Means. Tukey's method compared the means of groups by modified t-test which takes into account the multiplicity of comparison of adjusted means which would have resulted if all experimental units were identical with regard to covariates. Thus the statistical analysis model examined not only the effect of diet on rat plasma cholesterol level but also the effect of a number of additional covariates such as weight gain, food intake, faecal output and diet digestibility.

RESULTS

Chemical and Physical Characteristics of Plant Food Extract: The analysis of detarium flour was based on 100g flour of the food sample (dry weight). The results indicated that detarium contained 5.9g fat, 12.1g protein, 0.4g starch and 1.9g ash (Table 2). The total NSP determined by the Englyst method (Englyst *et al.,* 1992), was 63.8g/100g of which 59.8 g/100g was the SNSP fraction and 4.0g/100g was the water insoluble NSP (Table 3). The sugar composition of the SNSP fraction indicated a high proportion of glucose, xylose and galactose (Table 4). The mean particle size of the detarium flour was 464µm and the viscosity of a 1% aqueous dispersion of the flour was between 4,000 and 40,000 cps ().

Table 2: Constituents of Detarium food samples g/100g

Parameter (g/100g)	Detarium
Moisture	6.4
Fat	5.9
Protein	12.1
Ash	1.9
Total CHO	73.8
Available CHO	18.3
Dietary fibre (by difference)	55.5
NSP	59.7
Kcal (by bombing)	369.0
Available energy	179.0
Starch	0.4

Onyechi 1995.

Table 3: Soluble, insoluble and total NSP content (g/100g) of wet and dry detarium flour

Food sample [Detarium]	Wet powder (g/100g)	Dried powder (g/100g)
Soluble NSP	55.9	55.8
Insoluble NSP	3.7	4.0
Total NSP	59.7	63.8

Onyechi, 1995.

The food intakes across the groups of rats were similar. There was no significant difference in weight gain of the groups of rats fed the Control, Guar and Detarium diets. However, rats on the control diet had higher weight gain.

The mean faecal output of the groups of rats fed control (25.9g); guar (25.3g) and detarium diets (27.7g) were similar with detarium having the highest faecal output. The mean plasma cholesterol levels of groups of rats fed the control diet (3.38 mmol/L) was significantly higher than the plasma cholesterol levels of the groups of rats fed the two test diets detarium diet (2.25 mmol/L); guar gum diet (2.14 mmol/L) at $p<0.05$. The percentage reduction was 34% for detarium as shown in Table 5.

Table 4: Non-cellulosic polysaccharide composition (g/100g) of Detarium flour

Detarium sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Uac
Soluble NSP	t	t	2.3	18.0	0.3	10.0	26.7	2.5
Insoluble NSP	t	t	0.5	0.1	0.1	0.3	t	0.3
Total NSP	t	t	2.8	18.5	0.4	10.3	2.6	2.8

Rha= rhamnose; Fuc= fructose; Ara= arabinose; Xyl= xylose; Man= mannose; Gal = Galactose; Glu= glucose; Uac = uronic acid (Onyechi, 1995)

Table 5: Mean plasma cholesterol levels (mmol/L); food intake (g/14d); weight gain (g/14d); food efficiency ratio (g wt gain/g food eaten); faecal weight (g/dw/14d) and diet digestibility for rats CD, GGD and DT treatments

Parameters	CD	GGD	DTD
Plasma cholesterol	3.38 ± 0.19^{abc}	2.14 ± 0.21^{a}	2.25 ± 0.14^{c}
Food intake(g)	169 ± 4.0	144 ± 9.0	157 ± 8.0
Weight gain (g)	42.8 ± 3.30^{a}	30.2 ± 2.80^{b}	37.2 ± 3.20^{c}
Food efficiency ratio	0.25 ± 0.02	0.20 ± 0.02	0.24 ± 0.01
Faecal output (g)	25.9 ± 0.05	25.3 ± 1.50	27.7 ± 1.60
Digestibility (%)	84.7 ± 0.30	81.9 ± 0.70	82.1 ± 1.30

Values in the same row with same superscript are significantly different $p<0.05$. Note: CD= Control diet; GGD= Guar gum diet; DTD=Detarium microcarpium diet (Onyechi, 1995)

ANOVA was also used to examine the effect of all the variables interacting with the type of diets to determine their effect on plasma cholesterol levels. Only the type of diet had a significant effect on cholesterol level. This is probably because the group of rats fed guar diet ate slightly less of the diet (144 g) than the rats fed on the control diet (169 g) and detarium (157 g). However, the effect of the food intake was less significant than the effect of the type of diet in affecting the plasma cholesterol level of the rats. ANOVA followed by the LSM test did not reveal any significant effect by such variables as food intake, weight gain, FER, faecal output and digestibility. The mean plasma cholesterol levels obtained after adjustment for effects of different co-variants is highlighted in Table 6.

Table 6: Mean plasma cholesterol levels (mmol/L) of rats unadjusted and adjusted for co-variates fed control and positive control diet (Guar gum diet) and test diet containing detarium

Diet	A	B	C
Control diet	3.18	2.70	2.68
Guar gum diet	2.14	2.05	1.78
Detarium diet	2.25	1.99	1.89

Note: A - Unadjusted means; B - Cholesterol levels adjusted for faecal output and weight gain; C - Cholesterol levels adjusted for weight gain and digestibility.

It appeared that rats fed guar and detarium diets had similar covariate levels. The LSM test verified that the cholesterol lowering effect was due to the diet. Also when the cholesterol means were adjusted for covariates, the two diets still had a similar effect.

DISCUSSION

The chemical analysis of detarium showed that it is high in SNSP. On a dry weight basis the SNSP content was 55.8g/100g. *Detarium* was shown to have a significant amount of SNSP and very viscous. The high SNSP fraction of detarium seed extract and the viscous nature could have positive physiological effects. The chemical analysis has shown that the main monosaccharide component of the extracted SNSP fraction of detarium flour consists mainly of xyloglucan. This is structurally similar to the main polymer found in tamarind gum, a seed extract of the plant *Tamarindus indica L*. (Reid, 1985) which is also known to have beneficial effects on lipid metabolism in rats (Yamatoya *et al.,* 1996).

An important determinant of the biological activity of SNSP, which has been found in significant amount in detarium and shown in guar gum studies, is their capacity to generate viscosity in the lumen of the stomach and small intestine. This has been shown to be of primary importance in reducing the rate (and possibly the extent) of digestion and absorption of available carbohydrate (Ellis *et al.,* 1996). An increase in the viscosity of stomach content can impair gastric function, such as sieving and mixing, one consequence of which is an increase in the size of large-sized food particles entering the small intestine (Meyer and Doty, 1998). Furthermore, an increase in digesta viscosity is thought to reduce the rate of emptying from the stomach (Ellis *et al.,* 1996). Increased viscosity in the gut lumen, inhibits the propulsive and mixing effect of intestinal contractions (Blackburn *et al.,* 1984). Under these conditions, SNSP, which are contained in detarium, just as guar gum, may likely impair the rate of digestion of starch as a result of less disruption of food particles, reduced mixing of food with intestinal secretions and decreased transport of hydrolysed products of starch to the mucosal surface. Recent evidence also suggests that such polymers in addition to increasing digesta viscosity, may act as a physical barrier to amylase-starch interaction in the lumen of the small intestine (Brennan *et al*., 1996).

The proximate analysis showed that detarium contains 55.8g/100g of NSP (Onyechi, 1995). Results of the rheological study (Onyechi, 1995) indicated that a dilute solution of detarium when fully hydrated has properties similar to medium grade guar gum (M90) with similar intrinsic viscosity

of 8.9 +/- 0.2 dl/g and 8.7 dl/g respectively. Thus, the NSP extracted from detarium like guar gum appears to be a high molecular weight polymer which when hydrated gives viscous solution. The two diets had similar effects on the rats food intake, faecal output, digestibility, weight gain and food efficiency ratios and resulted in a similar decrease in the level of plasma cholesterol thus apparently confirming the rheological study.

The higher fat excretion of rats fed detarium, seem to indicate an effect of the fibre in reducing fat absorption rather than solely the lower food intake. The effectiveness of dietary fibre in reducing cholesterol levels probably lies in its ability to reduce availability of fatty acids and cholesterol for absorption in the upper intestine. This maybe seen as increased faecal fat; increased cholesterol and its bacterial metabolites, and reduced bile acid re-absorption, which affects cholesterol esterification (Dreher, 1987).

It can therefore be suggested that the mode of action by which detarium reduced plasma cholesterol levels in rats is similar to that of guar gum. The mechanism by which guar gum reduces glucose, insulin and cholesterol levels is not completely understood but several hypotheses have emerged regarding these cholesterol level lowering effects. These hypotheses include alteration in bile acid absorption and metabolism; decreased activity of the digestive enzymes; effect of short chain fatty acids (SCFA); changes in hormone concentrations and reduction in fat absorption (Cummings, 1981; Anderson, 1986 and Anderson *et al*., 1990). Dietary fibre like detarium, may affect lipid absorption by such mechanisms as altered gastric function, decreased availability of bile acids interference with effective micelle formation, altered digestive enzyme activity (Anderson *et al.,* 1990). NSP bind bile acids and decrease their availability for optimal fat digestion absorption. This may be indicated in high faecal fat content of detarium fed rats. *Detarium* contains some carbohydrate (CHO), however, analysis shows that the starch content is very low 0.4g/100g dry weight (Onyechi, 1995). It is therefore likely that the effects of the SNSP in the detarium outweigh the possible effects of CHO digestion and SCFA production in the large gut.

In conclusion, the result of this study suggested that detarium may have beneficial physiological effects and there is need to study it's effect on glucose and insulin profiles in humans.

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