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Microbiological Assay of Vitamin B and Biotin in some Nigerian Fermented Foods

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ABSTRACT

The B vitamins and biotin contents of raw seeds, cooked unfermented seeds and the fermented products of castor oil (Ricinus communis) *and African oil bean* (Pentaclethra macrophylla) *were analysed using microbiological assay. Generally, there were decreases in vitamin values, especially in those of the B vitamins, when the raw seeds were cooked in water. Of the vitamins in castor oil bean, fermentation accounted for a significant increase only in riboflavin—about threefold that of the cooked unfermented beans. The niacin and thiamine values were, in fact, less than in the raw seed. In the fermentation of African oil bean seeds, all the B vitamins and biotin increased with fermentation. The most significant increase is also in riboflavin, about fourfold of that of the cooked unfermented beans. Generally, the biotin contents were only slightly higher than in the cooked unfermented beans. The marked increases in the riboflavin were ascribed to the activity of* Bacillus subtilis *in the fermentation. The patterns of results obtained were compared with those of other fermented vegetable protein foods.*

INTRODUCTION

Fermented vegetable proteins contribute significantly to the protein and vitamin intake of the majority of the rural population in Nigeria. Two of

these important, but less known, fermented foods are *ogiri* and *ugba*; they are produced from shelled seeds of castor oil bean (*Ricinus communis*) and African oil bean (*Pentaclethra macrophylla*), respectively. The castor oil seed contains 44%–46% oil and 20% protein while the African oil bean contains 26% protein and 40% oil. In the production of the fermented foods, the seeds are cooked for several hours, dehulled, drained and fermented for two days to produce *ugba* and for 4–5 days to produce *ogiri*.

A consistent biochemical change during the fermentation of vegetable protein seeds is the marked increase in soluble products, particularly nitrogen (Steinkraus, 1983). However, the vitamin changes do not follow a consistent pattern. Increases, decreases or lack of change in vitamin contents have been reported during fermentation, depending on the pre-fermentation processing, conditions of fermentation and the types of microorganism involved (Jones, 1975).

Deficiencies of vitamins—especially of B vitamins—are widespread in tropical Africa (Atinmo, 1982). Riboflavin deficiency has been reported in several areas (May, 1965). The significance of these lesser known fermented food supplements in meeting the vitamin needs of the African people is still unknown. Moreover, since *ugba* is often used as a low-cost meat substitute, vitamins—especially thiamine—are significant, being important in the evolution of meat-like flavour (Manley *et al.*, 1981). It is therefore necessary to obtain information about the vitamin changes during fermentation so as to optimize the use of the beans.

MATERIALS AND METHODS

Niacin, thiamine, riboflavin and biotin were determined for the raw seeds and for fermented and unfermented samples. Vitamin analysis was by microbiological assay.

Sample materials

The raw seeds and fermented samples were obtained from a local producer and from a market in Agulu Village, Anambra State of Eastern Nigeria. The cooked unfermented and fermented beans were also produced in the laboratory from shelled beans. Samples were kept in a deep freezer (−21 °C) prior to analysis.

Assay organisms

The assay organisms used were *Lactobacillus plantarum* ATCC 8014 for niacin and biotin, *L. casei* ATCC 7469 for riboflavin and *L. fermenti* ATCC 9338 for thiamine. Freeze-dried cultures of these organisms were purchased from American Type Culture Collection, Beltsville, USA.

Traditional method of preparing *ugba* and *ogiri*

Ogiri

The castor oil seeds were dehulled by breaking the shells lightly with stones on a clean concrete pavement. The cotyledons were then collected and wrapped in small packets (about 10 cm diameter) with banana leaves. Holes were punched through the leaves to allow water to penetrate into the cotyledons while boiling and the packets were put into a pot and boiled for 6 h. A local rock salt, *kaun* (containing K_2CO_3 and $KHCO_3$) was added to aid softening. After boiling, the packets were removed and put in a warm place (about 32 °C) for 4 days to ferment. The fermented seeds were removed from the leaves. The seeds were then ground in a mortar (or a grinding stone) to a fine paste called *ogiri*. Salt (about 5 % w/w) was added as a preservative.

Ugba

Oil bean seeds were boiled in water in pots for 6 h. The cotyledons (kernels) were then separated from the cooked seeds by removing the seed coats and washing. The kernels were boiled again in water, overnight, over smouldering wood, allowed to cool, drained and washed many times with water to remove their bitter components. The washed cotyledons were cut into long, thin slices. These slices were mixed with salt, put into a clean pot without water, covered and fermented for 24 h at about room temperature (30 °C). The slightly fermented beans were then packaged in clean banana leaves and tied tightly. Each package weighed about 100 g. The packages were then packed together in a basket lined with banana leaves and left to ferment for another 3 days at 32° + 2 °C.

Vitamin assay

Thiamine and riboflavin were extracted following the methods described by Osborne & Voogt (1978). The procedures used for the extraction of

niacin and biotin were those of Gyorgy & Pearson (1967) and Skeggs (1963), respectively.

The procedures followed for the preparation of working standard solutions, stock cultures, inoculum and assay solution were those of DIFCO (1977). The assay sample extracts in tubes were inoculated with one drop of the inoculum and incubated at 35 °C for 72 h. Every 24 h acidimetric determinations were carried out for each tube. The vitamin value in each tube was read from their respective standard curves. Subsequently, the average value per millilitre of the sample extract and the total vitamin content of each sample were calculated using the formula described by Gyorgy & Pearson (1967). Vitamin values were expressed as micrograms of vitamin per gram dry weight of sample.

Assays were carried out on duplicate fermentations and, for each analysis, three determinations were made.

RESULTS AND DISCUSSION

A general decrease in vitamin content results from the cooking of the raw seeds (Tables 1 and 2). This is due to the fact that most of the vitamins are water-soluble and hence are leached into the cooking water or washing water during the dehulling process.

In the *ogiri* fermentation the increases in niacin and thiamine contents, compared with those of the cooked beans, were slight. The contents of these vitamins in *ogiri* were even less than in the raw seeds. The only significant increase was in the riboflavin content which rose threefold over

TABLE 1
Niacin, Thiamine, Riboflavin and Biotin Contents of Raw, Cooked and Fermented (*Ogiri*) Castor Oil Bean

Vitamins	*Raw seed*	*Concentration per gram*	
		Cooked unfermented	*Fermented*
Niacin (μg)	5·2[a]	3·3[b]	3·1[b]
Thiamine (μg)	3·2[a]	1·8[a]	2·3[a]
Riboflavin (μg)	10·5[a]	8·4[a]	26·1[a]
Biotin (ng)	8·9[a]	7·8[a]	10·8[b]

[a,b] Where superscripts differ within a vitamin, results differ significantly ($p < 0·05$).

TABLE 2
Niacin, Thiamine, Riboflavin and Biotin Contents of the Raw, Cooked and Fermented (*Ugba*) African Oil Bean

Vitamin	*Raw seed*	*Concentration per gram*	
		Cooked unfermented	*Fermented*
Niacin (μg)	2·5[a]	1·8[a]	2·5[a]
Thiamine (μg)	3·5[a]	2·1[a]	8·8[b]
Riboflavin (μg)	9·2[a]	8·5[a]	30·3[b]
Biotin (ng)	7·4[a]	6·4[a]	7·9[a]

[a,b] Where superscripts differ within a vitamin, results differ significantly ($p < 0·05$).

the cooked unfermented beans. The biotin content also increased in *ogiri* from 7·8 ng to 10·85 ng (Table 1).

In the fermentation of African oil bean to make *ugba*, all the B vitamins and biotin increased with fermentation (Table 2). The vitamin values in *ugba* were even greater than those of the parent seed. The greatest increases were up to fourfold that of the cooked beans.

Generally, the niacin contents of both the cooked and fermented products were low. Except in *tempeh*, an Indonesian mould-fermented soy product (Steinkraus *et al.*, 1961), the values of niacin have not been found to increase with fermentation. The decrease in *ogiri* may be ascribed to its utilization by bacteria for growth.

The results for thiamine are variable. The value increased in *ugba* but remained almost unchanged in *ogiri*. Similarly, observations on other fermented foods have not shown a consistent pattern. In *tempeh*, the thiamine content decreased with fermentation (Steinkraus *et al.*, 1961) whilst, in *natto*, a Japanese bacterial fermented soybean food, the thiamine content remained the same (Arimoto, 1961). In some other fermented foods, slight increases in thiamine were also recorded (Steinkraus, 1983). The increased thiamine content in *ugba* would increase its meat-like flavour (Manley *et al.*, 1981), as well as enhancing its nutritional value.

A significantly increased biotin content was seen in *ugba* and a slight increase in *ogiri*. Biotin was found to increase four to fivefold in *tempeh* compared with unfermented soybeans (Murata *et al.*, 1970). Biotin showed only a slight loss during cooking, possibly because it is water stable and not easily leached during cooking and washing.

The increases in the riboflavin contents of both foods are significant, especially as riboflavin is the most limiting nutrient in the diet of many West Africans (Whitby, 1968). Increases in riboflavin content have been observed during the fermentation of many vegetable proteins. Riboflavin increased during the fermentation of African locust bean (*Parkia biglobosa*) to produce *iru* (*dawadawa*) (Platt, 1964; Eka, 1980). Increased riboflavin contents were also reported during the fermentation of soybean to produce *tempeh* (DIFCO, 1977), *natto*, *soyidli* and *miso* (Jones 1975; Steinkraus, 1983).

The more pronounced increases in riboflavin have been in vegetable protein foods partially or wholly fermented by *Bacillus subtilis*. No increase in riboflavin was observed in several fermented starchy foods of West Africa (Ankrah, 1972). *Bacillus subtilis* is the main organism involved in the fermentation of *natto*, *iru* (fermented African locust bean), *ugba* and *ogiri* (Odunfa, 1981, in press; Odunfa & Oyeyiola, 1985). The increase in riboflavin is presumably due to *B. subtilis*, many strains of which are unique in producing high levels of riboflavin synthetase (Bacher *et al.*, 1980). In fact, this product has been exploited to produce riboflavin commercially (Ajinomoto Co. Inc., 1976).

The quantity of *ugba* normally used as soup condiments is enough to meet the recommended daily allowance of riboflavin (Anon, 1974). However, the riboflavin in the fermented product needs to be conserved if it is not to be lost by improper post-fermentation handling. The present traditional practice of wrapping the fermented products in leaves conserves the riboflavin since it is sensitive to light (Woodcock *et al.*, 1982).

Ugba may be either consumed directly as hors d'oeuvres or added to soup as a flavouring condiment. The former practice has been recommended since prolonged cooking destroys riboflavin. However, *ogiri* is not normally consumed directly because of its strong putrefying odour; moreover, direct consumption is not advisable because of its residual toxicity (Odunfa, in press). Hence, some of the riboflavin content of *ogiri* will be lost.

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