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Effect of Fermentation on the Thiamin, Riboflavin and Niacin Contents of Melon Seed (*Citrullus vulgaris*) and African Oil Bean Seed (*Pentaclethra macrophylla*)

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ABSTRACT

An automated thiochrome method and high performance liquid chromatography (HPLC) with fluorescence detection were used to determine thiamin and riboflavin, respectively, in unfermented and fermented melon seeds (Citrullus vulgaris) *and African oil bean seeds* (Pentaclethra macrophylla). *Niacin was determined microbiologically. In all the methods used, fermentation significantly increased the content of thiamin, riboflavin and niacin in African oil bean seeds and thiamin and riboflavin in melon seeds. There was no change in the niacin content of melon seeds.*

INTRODUCTION

Melon seeds (*Citrullus vulgaris*) and African oil bean seeds (*Pentaclethra macrophylla*) are important basic foods in Nigeria and are consumed in

various ways, either before or after fermentation. African oil bean and melon seeds belong to the families leguminosae and curcubitaceae, respectively, and have a high content of essential amino acids (Achinewhu, 1982 *a*, *b*; 1983 *a*, *b*). The effect of fermentation of the seeds on the B vitamin content is not known and the present study is aimed at investigating some of the B vitamins of the fermented seeds, especially thiamin (B_1), riboflavin (B_2) and niacin. The study is part of an investigation into the potential use of the fermented seeds as a possible weaning food supplement.

Microbiological assay procedures have been used to assay some B vitamins in fermented cereals and legumes (Tongual & Fields, 1979; Zamora & Fields, 1979; Au & Fields, 1981; Kazanas & Fields, 1981; Murdock & Fields, 1984). Conflicting results were obtained concerning the effect of fermentation. In the present study, the thiochrome (automated) method was used to determine thiamin and the fluorometric method, riboflavin, while microbiological assay was used to determine niacin. An automated fluorometric method has been used to determine thiamin by thiochrome and riboflavin, singly or simultaneously, in foodstuffs by Kirk (1974, *a*, *b*), Pelletier & Madere (1975), Jacobson (1977), Dunbar & Stevenson (1979) and Kamman *et al.* (1980). Kamman *et al.* (1980) showed no significant difference between results obtained using HPLC and the automated system in the determination of thiamin and riboflavin while Kirk (1974 *a*, *b*) and Dunbar & Stevenson (1979) showed that results for thiamin and riboflavin from the automated method were similar to, or better than, those achieved by the AOAC (1980) manual method. HPLC has been used to determine thiamin and riboflavin in foodstuffs (Ang & Moseley, 1980; Kamman *et al.*, 1980; Skurray, 1981; Ashoor *et al.*, 1983; Wehling & Wetzel, 1984; Finglas & Faulks, 1984). They all showed that fluorescence detection was highly specific and sensitive and minimised the number of interfering peaks. Their results compared well with the AOAC (1980) manual method.

MATERIALS AND METHODS

Materials

The melon seeds were of the creeping variety. Freshly prepared sun-dried seeds were purchased from a local market in Nigeria. Freshly picked African oil bean seeds were also purchased from the local market.

Methods

Preparation and fermentation of samples

The African oil bean seeds were boiled for 7 h, after which the seed coats were removed and the seeds sliced into small pieces with a sharp knife. The melon seeds were boiled for 2 h and wrapped in aluminium foil after the seed coats had been removed by hand pressure. The boiled melon seeds were ground in a mortar and sodium chloride was added (1 g per kilogram of sample). The samples were inoculated with a previously fermented dried sample (3 g per kilogram of sample). The inoculum was suspended in 5 ml of water and thoroughly mixed with the samples. The inoculated samples were wrapped in aluminium foil and incubated at 31 °C for 4 days (African oil bean seeds) or 5 days (melon seeds).

Analysis of thiamin

Apparatus: An automated method was used for the determination of thiamin which involved the oxidation of thiamin to thiochrome (extraction of the thiochrome was by 2-methyl propanol) and fluorescence measurement. A Chem. Lab. autoanalyser (Chem. Lab. Instrument Ltd., Essex, Great Britain) was used with an AMINCO fluoro-colorimeter (American Instrument Company, Maryland, USA). Fluorescence filters were selected to provide excitation and emission wavelengths of 366 nm and 464 nm, respectively. The procedure was based on the method of Kirk (1974*a*).

Sample preparation: The sample preparation was based on that of Osborne & Voogt (1978). Six grams of the samples were homogenised with 38 ml 0·1 M HCl for 2 min followed by the addition of 5 ml of enzyme solution (6 g papain + 6 g takadiastase in 100 ml of 2·5 M sodium acetate) and the mixture was incubated in a shaker water bath at 48 °C overnight. The extract was applied to the auto-analyser sampler and thiamin oxidation was achieved by alkaline potassium ferricyanide solution (0·6 g potassium ferricyanide in 100 ml 15% NaOH). Thiochrome was extracted by 2-methyl propanol. After correction for blank readings, a standard curve of thiamin concentration (μg ml) was plotted against peak height (mm) and the thiamin concentration of the sample was determined from this curve.

For the recovery studies 0·1 μg ml was prepared from the standard solution and 2 ml of this was added to the sample solution before the

enzyme hydrolysis step. Blank values were determined by omitting potassium ferricyanide from the oxidation step.

Analysis of riboflavin

Apparatus: Modular liquid chromatograph LC 750 (Applied Chromatography System Ltd., Luton, Bedfordshire, Great Britain) was used with a pump and a Rheodine injection valve, an AMINCO J4–7440 fluorescence detector with a 435 nm excitation filter and a 545 nm emission filter.

Sample preparation: The method was that of Osborne & Voogt (1978). After extraction, the supernatant (100 μl), was injected onto the HPLC (column, 25 cm × 4·5 mm inside diameter, stainless steel packed with Spherisorb 10 μm silica) and eluted with a solution of 2·72 g of sodium acetate trihydrate and 1·2 g of glacial acetic acid per litre at a flow rate of 1 ml/min. Riboflavin concentration of the samples was quantified by measuring chromatographic peak heights of the samples and standards.

The recovery study was similar to that described for thiamin.

Microbiological assay of niacin

The microorganism used was *Lactobacillus plantarum* ATCC 8014. The materials used and the procedure for the preparation of the standard solution, standard curves, assay tubes, inoculum and culture maintenance were according to Bell (1974), but with minor modifications. The stab culture used for the inoculum was prepared freshly from the stock culture stored in liquid nitrogen and the inoculum, after incubation for 6 h in nicotinic acid broth, was washed with saline and resuspended in 5 ml of single strength niacin assay medium.

RESULTS AND DISCUSSION

Figures 1 and 2 show an example of the separated riboflavin peaks. Riboflavin appeared as a single peak in the standard and test samples. Each sample was chromatographed with standard riboflavin and the samples were identified by comparing their retention times with that of the corresponding standard. Four duplicate preparations were made of the standard and the samples and, in each case, the retention times were

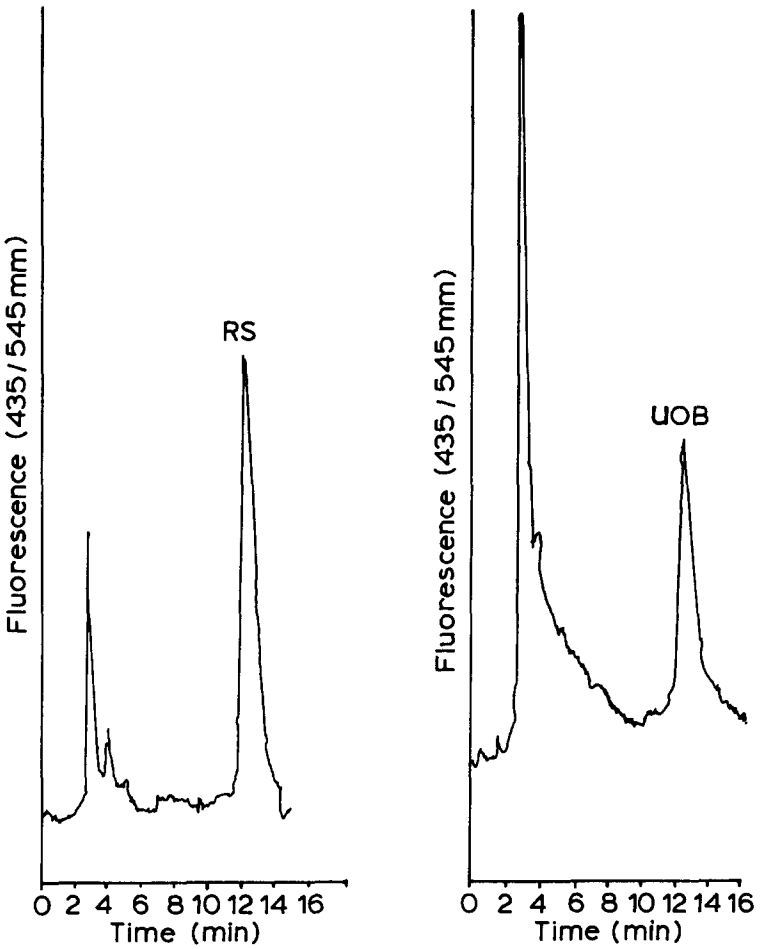

Fig. 1. Chromatogram of riboflavin standard (RS) and extracts from unfermented African oil bean seed.

identical. Table 1 shows the composition of thiamin, riboflavin and niacin of the test samples. Fermentation increased the composition of thiamin and riboflavin in African oil bean and melon seeds. These increases were significant ($P < 0{\cdot}05$). There was no difference between the niacin content of the unfermented and fermented melon seeds but fermented African oil bean seeds had significantly ($P < 0{\cdot}05$) higher niacin than the unfermented sample. The figures for the unfermented products were fairly similar to figures published for soya bean (a legume) and melon

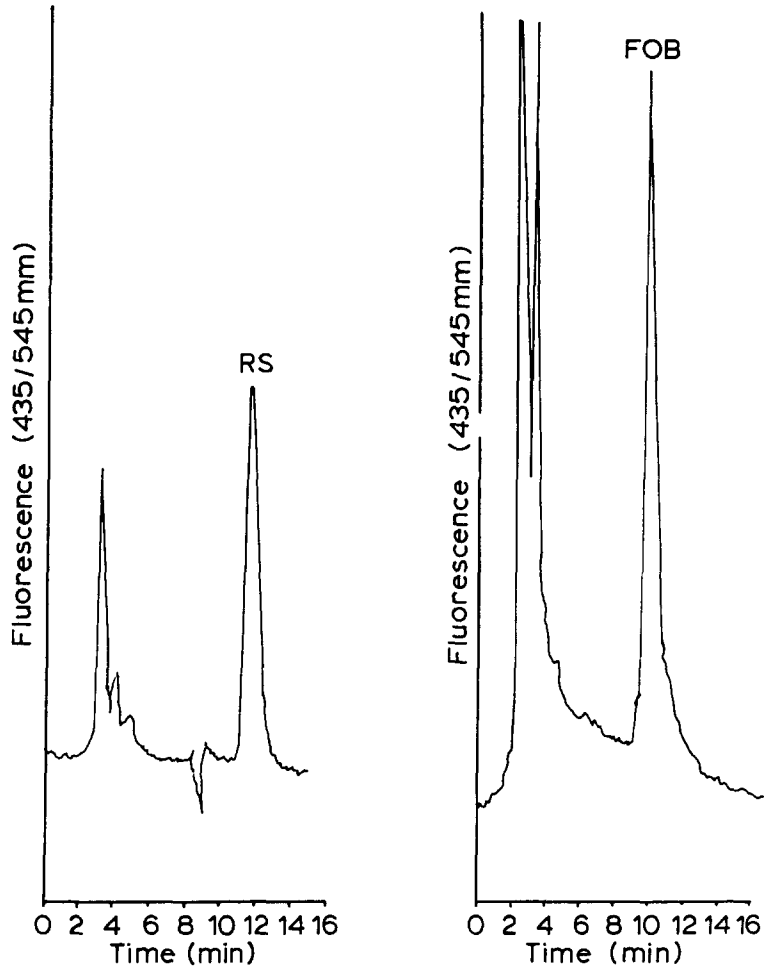

Fig. 2. Chromatogram of riboflavin standard (RS) and extracts from fermented African oil bean seed (FOB).

seed. Watt & Merril (1975) showed that raw soya bean contained 1·1 mg/100 g thiamin, 0·31 mg/100 g riboflavin and 2·2 mg/100 g niacin, while Platt (1962) showed that melon seed contained 0·1 mg/100 g thiamin, 0·15 mg/100 g riboflavin and 1·5 mg/100 g niacin. Thiamin, riboflavin and niacin had been determined for other foodstuffs. Kamman *et al.*, (1980) showed riboflavin and thiamin contents of wheat ranging from 1·65 to 1·85 mg/100 g and 1·73 to 1·88 mg/100 g, respectively, and 1·57 and 1·25 mg/100 g, respectively, in corn using the auto-analyser and

TABLE 1
Thiamin, Riboflavin and Niacin Composition of Unfermented and Fermented African Oil Bean Seeds and Melon Seeds (mg/100 g dry weight) ± SE Mean of Four Determinations

Sample	*Thiamin*	*Riboflavin*	*Niacin*
Unfermented oil bean seed	1·1 ± 0·004	0·11 ± 0·000 2	2·0 ± 0·001
Fermented oil bean seed	2·2 ± 0·003	0·3 ± 0·000 2	3·2 ± 0·001
Significance between means	$P < 0·05$	$P < 0·05$	$P < 0·05$
Per cent increase	100	100	60
Unfermented melon seed	0·08 ± 0·000 2	0·12 ± 0·000 2	3·2 ± 0·003
Fermented melon seed	0·23 ± 0·000 3	0·22 ± 0·000 4	3·2 ± 0·001
Significance between means	$P < 0·05$	$P < 0·05$	NS
Per cent increase	100	83·3	0

NS, Not significant.

the HPLC method. Toma & Tabekia (1979) showed that paddy rice contained 4·6 μg/g thiamin, 0·97 μg/g riboflavin and 61·4 μg/g niacin as determined by the HPLC method. Wehling & Wetzel (1984) and Finglas & Faulks (1984) had used HPLC to determine thiamin and riboflavin in wheat and potato, respectively, while Dunbar & Stevenson (1979) and Kirk (1974 *a*, *b*) used the automated method to determine thiamin and riboflavin in fortified infant food and milk, respectively. They all concluded that the methods were specific, rapid, sensitive and provided good reproducibility of results with less interference from the sample matrix. Table 2 shows the per cent recovery of added thiamin and riboflavin. In all four separate determinations, the recovery was higher

TABLE 2
Recovery of Added Thiamin and Riboflavin from the Test Samples (Mean of Four Determinations)

Sample	*Thiamin (Per cent recovery)*	*CV(%)*[a]	*Riboflavin (Per cent recovery)*	*CV(%)*[a]
Unfermented oil bean	94·3	1·6	93·0	1·8
Fermented oil bean	94·8	1·1	95·0	2·1
Unfermented melon	96·5	1·5	96·8	1·5
Fermented melon	95·8	1·3	97·3	2·1

[a] Per cent coefficient of variation.

than 90%. Other workers (Ashoor *et al.*, 1983; Finglas & Faulks, 1984) similarly achieved recoveries of added thiamin and riboflavin higher than 90% while Ang & Moseley (1980) had recoveries less than 90% but higher than 80%.

Microbiological assay procedures have been used to study the effect of fermentation on B vitamins in other foodstuffs. Murdock & Fields (1984) determined thiamin, riboflavin and niacin in fermented whole corn meal and showed significant increases in riboflavin but no differences between the niacin and thiamin contents of the unfermented and fermented samples. Their findings did not agree with that of Akinrele (1970) who showed that, during the traditional method of *ogi* (fermented corn) preparation, thiamin, niacin and riboflavin increased. Similarly, Kazanas & Fields (1981) showed that fermentation significantly increased the thiamin, riboflavin and niacin contents of sorghum grain. In other studies of fermentation of cowpeas and chickpeas, Zamora & Fields (1979) reported a significant increase in riboflavin and a significant decrease in niacin in both fermented cowpeas and chickpeas. Thiamin did not change significantly in fermented cowpeas but did decrease significantly in chickpeas. Tongual & Fields (1979) also reported a significant increase in riboflavin in fermented rice meal while there was a significant decrease in niacin and thiamin. Van Veen & Steinkraus (1970) showed increases in riboflavin and niacin and a decrease in thiamin in fermented soya bean to produce tempeh, while Eka (1980) showed increases in thiamin and riboflavin in fermented locust bean. In the present study, apart from niacin, which was similar in both unfermented and fermented melon seeds, thiamin and riboflavin were significantly increased by fermentation.

All the fermentations studied by the above workers with cereals and legumes (except tempeh and locust bean) were of the lactic type. In this type of fermentation, some workers showed that the pH of the fermented samples decreased as titratable acidity increased (Zamora & Fields, 1979; Murdock & Fields, 1984). In the present study, the pH of the fermented seeds increased as the titratable acidity increased (Achinewhu, In Press), indicating that the microorganisms involved in their fermentation were probably not the lactic type. Different microflora would result in different metabolites. Differences might also result from the types of product used and the time and temperature of fermentation. Differences in products might result in differences in the availability of substrates naturally present in the seeds before fermentation.

These fermented seeds will be a good source of several B vitamins. The organisms responsible for the fermentation of African oil bean and melon seeds have not been fully characterised and research in this direction is in progress; so also is the determination of the effect of fermentation on other B vitamins.

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