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# A BIOCHEMIST'S ADVENTURE INTO THE PROCESSING AND PRESERVATION OF FOODS

BY

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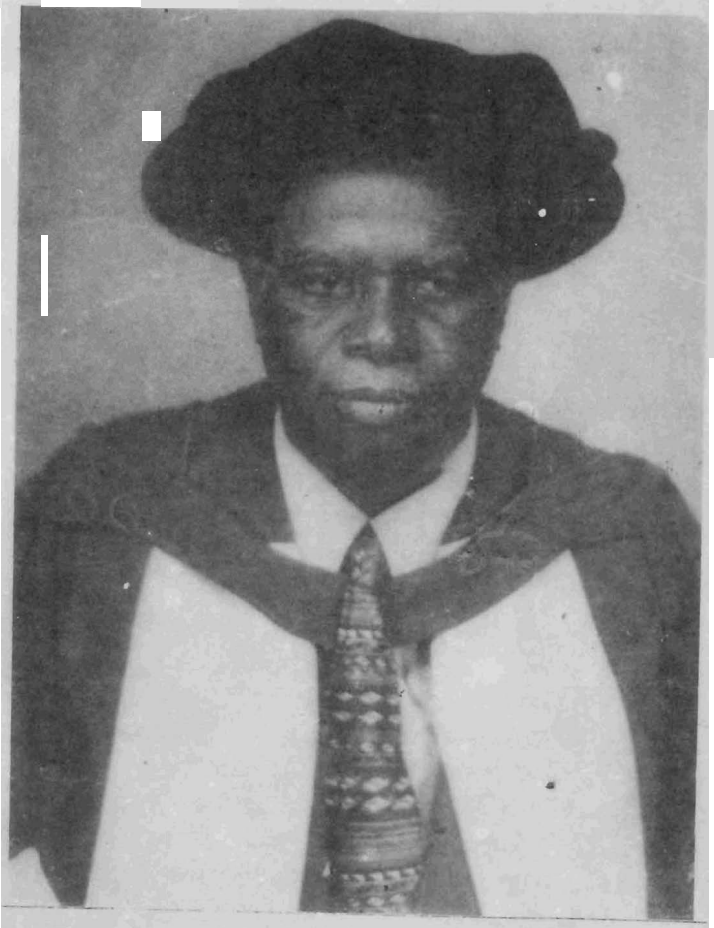

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## INTRODUCTION

The Vice-Chancellor, distinguished ladies and gentlemen,

My entry into food science and technology is fortuitous. Having obtained B.Sc. degree in Agricultural Chemistry (Animal Biochemistry group) at MacDonald College of McGill University, I proceeded to McGill University, Montreal Canada for the M.Sc. degree in Biochemistry. I arrived Nigeria in 1968. At McGill, my research efforts were in the field of Steroid Biochemistry. Having done my research under Prof. M.K. Birmingham the discoverer of the Hormone 18-hydroxy corticosterone, a mineralocorticoid. That work resulted in my first paper - "Adrenal Steroid biogenesis in different species of mouse like rodents." On arrival in Nigeria, I applied to Ife to work as a Biochemistry lecturer in the Dept of Animal Science I went to Professor Adegbola (then Dr Adegbola) who was the acting Head of the department to ask for the status of my application. He told me that Prof. H.A Oluwasanmi was planning to establish a Department of Food Science and Technology, the first of such department in Nigeria. He asked if I would take an appointment as an Assistant Lecturer in Animal Science, pending my transfer to the proposed department. I agreed and taught Biochemistry for two years in the department before I went to University of Reading on study leave in 1970, after being promoted a lecturer.

## WORK ON COMPOSITE FLOURS

In 1970, or thereabout, Nigeria and several other developing countries were importing a lot of wheat, draining a lot of scarce foreign exchange. Although, at this time nobody thought of banning the importation of wheat, in the scientific community especially outside the country, the trend was viewed with alarm. The World Health Organisation turned to two institutions to look into the problem, TPI in United Kingdom and TNO in the Netherlands.

Several workers in these institutions carried out investigations aimed at incorporating cassava starch into bread (Dendy, Clark James,1970). With cassava starch this does not

present a serious problem, but bread qualities deteriorate with the addition of about 10% cassava flour. Cassava flour differs from cassava starch in that it contains other constituents besides starch. We therefore, studied the factor affecting the use of cassava flour in bread (Hudson and Ogunsua, 1974). There are several differents methods of bread making but three of these are mainly used throughout the world, Bulk Fermentation Process (BFP), Mechanical Dough Development (MDD) process and Activated Dough Development (ADD) process. Wheat flour contains an unusual protein called gluten. On hydration it becomes elastic during dough development and that is why it retains gas during fermentation and baking. In the bulk, fermentation process, it is carbon dioxide generated during fermentation that develops the dough. In MDD, an intense mixing energy is put into the dough - 5 WH/lb of dough (11 WH/kg of dough). This develops the dough by breaking S-S-bonds and repositioning them to form new bonds. Glutathione (GSH) present in the flour takes part in the exchange of SH bonds for S-S-bonds (sulphurhydryl-disufide interchange). The mixing takes only five minutes. In the ADD a chemical, a naturally occuring amino acid L-cysteine hydrochloride is added at 35 ppm. The mixing is done by slow mixers used for BFP and mixing takes 30 min, but the dough is fully developed. In both MDD and ADD, ascorbic acid is added as oxidising agent (actually dehydroascorbic acid is the oxidising agent). Azodicarbonamide can also be added to develop the dough. In Nigeria, BFP is used but it is usually coupled with sheeting by the dough break ('Milling') a form of mechanical dough development. Both MDD and ADD shorten bread making time considerably and they save space and increase bread yield. The use of the Bulk Development Process for making wheat cassava bread is not feasible as the bread obtained is of very open structure and very poor volume. In our work, we sieved cassava flour milled to two degrees of starch damage (3%, 12%) through a bank of sieves. The finest sieve pass 12xx (Mesh 49.5 per mm) gave the best baking result. In the ADD process wheat cassava (70/30) gave a loaf



volume of 1390 cc vs 1252 cc for the unfractionated cassava flour. In optimising loaf volume and loaf score (Overall quality), we optimised improvers and found that calcium stearoyl lactylate improved the volume. Similar results but higher loaf volumes were obtained with MDD (Table 1).

**Table 1: Effect of added surfactants made from composite flours**

| Effect of calcium stearyl lactylate (CSL) on loaf volumes | Shortening/ Emusifier | Loaf vol. (cc) | Loaf sp. Vol, | % of control |
|---|---|---|---|---|
| CBP process | | | | |
| Wheat (control) | 8.6g fat | 1388 | 3.68 | 100 |
| Wheat/CF (70:30) | 8.6g fat | 1254 | 3.24 | 88.0 |
| Wheat/CF (70:30) | 7.5g fat + 1.1g CSL | 1313 | 3.49 | 94.8 |
| Wheat/CF (70:30) | 6.4g fat + 2.2g CSL | 1318 | 3.44 | 93.5 |
| Wheat/CF (70:30) | 4.3g fat + 4.3g CSL | 1333 | 3.44 | 93.5 |
| ADD process | | | | |
| Wheat (control) | 8.6g fat | 1379 | 3.69 | 100 |
| Wheat/CF (70:30) | 8.6g fat | 1160 | 3.00 | 81.3 |
| Wheat/CF (70:30) | 7.5g fat + 1.1g CSL | 1175 | 3.06 | 82.1 |
| Wheat/CF (70:30) | 6.4g fat + 2.2g CSL | 1125 | 2.94 | 79.7 |

As cassava flour lacked diastatic enzyme, we added 2% malt to correct for defficiency of diastatic enzyme. Examination of the fractions under the microscopes showed that the fraction passing 5xx but retained on 10xx consisted of starch granules embedded in fibrous material. The finest material (passed 12xx) was virtually free of fibrious materials.

The loaf volumes of breads made from composite flours are less than those from control wheat. The difference can be attributed mainly to differences in the gluten content. The greater the level of cassava product in the composite mixture the less that of gluten. In the case of 30% addition, the gluten content drops from 14% in the control to 9.8% in the wheat/cassava composite mixture. The inferior baking performance of the wheat/cassava composite flour may be

explained in part by the role of wheat gluten in bread making. Wheat proteins form a network of films, presumably complexes in which starch granules are embedded. These films constitute walls of gas vesicles which maintain their integrity in the face of carbon dioxide and moisture vapour diffusion. The present of fibres will interfere with the formation of these films and form weak points in the continuous matrices of the gas vesicles. As most Nigerian bakers use BPP, if composite flour has to be used in Nigeria, the ADD process will be the most promising process. It should also have a place in the 100% wheat bread making as it saves time like the MDD but does not require the replacement of the existing machine with high energy mixer required for the MDD process. We also found that starch damage had no effect on loaf characteristcs.

## FURTHER WORK ON CASSAVA TUBERS

My work on wheat/cassava composite led to more basic work on cassava tubers. It has been found that extraction of lipids from wheat flour led to poor loaves indicating that, although the lipid in wheat is very low (0.6%), it is essential to bread making. It is well known that the lipids of wheat flour, in particular the glycolipids assume an important role in breadmaking, for comparative purpose it seemed important to ascertain the nature and quantities of various lipid classes present in cassava flour. The major classes of lipid present in cassava tubers were quantitated and the fatty acid compositions were established. This was compared with that of potato tubers. Non-polar lipids were extracted with petroleum ether and polar lipids were extracted with water saturated butanol. Thin layer chromatography (TLC) was used to separate the lipids into various classes. The lipids were quantitated by densitometry. The glycerols in individual lipids were quantitated by spectrophotometry (Clark, 1968) and hexoses were quantitated by spectrophotometry (Dubois, 1968). The component fatty acids of each lipid was quantitated by GLC.



It was found that non-polar lipids include sterols, triglycerides, diglycerides, and fatty acids. Of the polar lipids, the group of glycolipids was clearly demonstrated in both cassava and potato. In the case of potato lipid, the mono and digalactosyl diglyceride reported by Lepage (1968) and trigalactosyl diglyceride reported by Galliard (1969) were estimated (Table 1). Cassava lipids were seen to contain the same three glylolipids with additionally tetragalactosyl diglyceride. This last component was confirmed by the determination of hexose fatty acid molar ratio after hydrolysis (tetragactosyl diglyceride requires 2:1, found 2.01:1.00) also by plotting, along with other glycolipids, the log RF against the number of galactose units, all four glycolipids fall on a straight line in cases of both TLC solvent systems. In these circumstances as far as in known, this is the first time that a tetragalactosyl diglyceride has been formally reported though, Webster and Chang (1962) have reported its propable presence in spinach chloroplasts, though at a very low level relative to the three simpler glycolipids (Table 2).

Table 2: Component lipid classes of cassava and potato tubers

| Lipid Class | Code | $R_F$ Value in t..lc. | | | % of total lipid[a] | |
|---|---|---|---|---|---|---|
| | | System A | System B | System C | Cassava | Potatoes |
| Non-polar lipids | | 0.96 | 0.96 | 0.89 | 44.9 | 54.1 |
| Monogalactosyl-Diglyceride | MGDS | 0.78 | 0.89 | 0.70 | 6.0 | 7.2 |
| Steryl glycoside | | 0.77 | 0.82 | 0.58 | 12.8 | - |
| Unidentified glycolipid | | 0.68 | 0.70 | | 3.8 | - |
| Digalactosyl diglyceride | DGDG | 0.54 | 0.63 | | 11.9 | 5.2 |
| Unidentified Phospholipid | | | 0.58 | | - | 12.9 |
| Unidentified Phospholipid | | 0.40 | 0.45 | | 3.1 | 0.8 |
| Unidentified Phospholipid | | | 0.29 | | - | 8.3 |
| Trigalactosyl diglyceride | TGDG | 0.21 | 0.16 | | 10.2 | 11.5 |
| Tetragactosyl diglyceride | TetGDG | 0.04 | 0.08 | | 7.3 | - |



It was also noted in this work that although 0.6% free lipid is present in cassava, a total 1.34% of lipid is extractable by solvent but a further 1.23% can only be recovered by acid hydrolysis or in practice by trans-methylation of surviving lipid to yield fatty acid methyl esters.

In contemplating the use of cassava flour rather than cassava starch in bread one has to take into consideration the levels of cyanide in bread baked from wheat/cassava flour. Chronic neurological disorders e.g. ataxic neuropathy have been linked to dietary consumption of cassava products, especially in people whose diet is low in protein (Osuntokun, 1969). The levels of cyanide in the root range from low levels (10-20 mg kg-1 root) in the sweet varieties to high levels (60-200 mg kg-1 root) in bitter varieties. I developed a cheap method (Ogunsua, 1989) to prepare linamarase from cassava tubers and a simple method to extract HCN from cassava flour or from bread containing composite flour followed by determination by one of the established methods.

The simple apparatus is shown in Fig. II. After the purification of the enzyme, cassava flour was placed in Thunberg tube with water or buffer (2 ml) and the tube was closed with side arm containing 1.5 ml 0.277 M NaOH. The effects of three incubation periods media (water, acetate buffer, phosphate buffer) were compared. After incubation (15 h) the side arm was removed and the tube covered with a stopper. The NaOH was transferred to a test tube. A simple apparatus (Fig. 1) was used to purge the HCN produced during the incubation into the tube containing the NaOH solution. I found that bread from low cyanide flours sieved or not contained no detectable cyanide in either crust or crumb (table) the crust from bread baked from high cyanide flours did not contain detectable HCN, the crumb did contain significant amounts. Cassava cultivars with low cyanide levels have been developed (Almazan, 1968). This would be suitable for making cyanide free bread.

## FORTIFICATION OF WHEAT-CASSAVA FLOURS

As cassava flour contains little protein, and cassava starch contains none, some protein sources have to be added to composite flours. It is attractive to add oilseed flours to wheat flours so that their protein may complement that of wheat flour limiting in lysine so that overall biological value may be improved. Some seeds generally available in Nigeria are peanut flour (*Arachis*) melon flour (*Citrullus vulgaris*), *Cucumeropsis edulis* and conophor nut flour (*Tetracarpidium conophorum*). In adding cassava flour to wheat, it was found that the preparation of the flour had to be carried out carefully. The particle size must be close to that of the commercial flour. Fresh peanut was shelled by hand. The dehulled nut was dried in the oven at 50°C for one day and the skins were removed by rubbing and were blown away by air jet. The peanut was then milled in disc mill and the oil was pressed out by a hydraulic press. The partially defatted groundnut was then milled in small hammer mill and sieved through 10xx nylon (aperture 0.129 mm). Melon seeds, were sun dried and shelled by hand. It was then milled in a disc mill and the oil was removed by extraction with hexane. The residue was steamed to remove traces of hexane and then dried in the oven at 50°C. The defatted material was then milled and sieved through 10xx nylon sieve. While conophor nuts were dried at 70°C for five hours. The nuts were then shelled in a conophor shelling machine. The dehulled nuts were milled in a disc mill and defatted with hexane. Traces of hexane were removed with steam. The residue was dried, milled in a small hammer mill and sieved through nylon sieve.

Addition of water to the composite flour is very critical and to complicate matters, different brands of flours behave very differently. For instance Life Flour had higher absorption than Golden Penny Flour. The addition of cassava starch led to a slightly less absorption in Life Flour. With Golden Penny Flour, there was a progressive increase in water absorption on addition of cassava starch at 600 B.U. in the Farinograph.



There was no significant increase at 500 BU. Addition of oilseeds flour, to wheat/cassava starch (70:30) composite led to a significant drop in water absorption. Melon seed flour/wheat flour (5.95) gave a water absorption of 60.6% compared with 100% wheat flour which gave a water absorption of 59.4. Baking trials showed that the dough mixed with water absorption at 500 BU was too sticky at dough moulding in CBP process, therefore the 600 BU water absorption was used for the baking tests.

The Farinogram characteristics were studied. The arrival time (I) decreased with increase in percentage of cassava starch in the wheat flour. The mixing time also decreased with higher levels of cassava starch but the addition of 5-10% conophor nut flour had no effect on mixing time. The mixing tolerance of wheat/cassava composite flour dropped at 10% level. The addition of conophor flour resulted in no change in mixing tolerance. In general, the addition of 10% starch showed a drastic change in the Farinogram characteristics of the composite flour. From the Farinograms it appears that conophor nut flour reacts with wheat gluten and did not behave like an ordinary diluent like cassava starch which gave predictable results for mixing time, consistency drop, etc. The dough stability of the melon seed flour/wheat flour composite was the highest among the oilseeds studied. The mixing time dropped from 7 to 4.5 minutes. The addition of peanut flour led to a drastic reduction in the rheological characteristics. The mixing time was only 1 min. The resistance dropped very sharply. Consistency also dropped very sharply.

## OTHER WORK ON CASSAVA TUBER AND CASSAVA PRODUCTS

Apart from the proposed use of cassava in a new product - wheat cassava bread, in Nigeria, there are three main ways in which cassava is eaten. The sweet variety only is boiled and eaten as a vegetable. Both sweet and bitter varieties may be processed into gari or cassava flour (Lafun).

In my work (Ogunsua, 1980), Gari was processed in the usual way - Gari when subjected to microscopic examination show that each grain is composed of thousands of gelatinised and ungelatinized granules bound together. Cassava flour (Lafun) was also produced in the usual way. During the fermentation of cassava mash samples were drawn for analysis every 24 hours, the pH, titratable acidity were determined. Gari was also prepared from cassava mash, allowed to undergo natural fermentation and processed into gari at the end of 1, 2, 3 and 4 days respectively. The pH, reducing sugar content and titratable acidity were determined in all the prepared gari samples made from the portion fermented for four days. The HCN is a hydrolysis product one of the glycosides in cassava tubers. These are linamarin and lotaustralin. Hydrocyanic acid was determined in gari samples made from the portion fermented for four days. Gari and Lafun samples purchased from Ile-Ife market were also analysed. HCN was released as described earlier and was reacted with pyridoxal HCl and measured in a fluorimeter with excitation wavelength at 365 nm and the secondary wavelength at 434 nm (Shigeru and Zengo, 1970). Standard curves were prepared with HCN dissolved in sodium hydroxide.

There has been a lot of controversy in the literature about HCN in cassava products especially gari. This is due to various methods used in the analysis. This is complicated by the fact that gari a product of severe heat treatment has no surviving enzyme, linamarase. Exogeneous linamarase has to be added to break down the cyanogenetic glycoside which may have survived the processing - washing, grating, pressing, fermentation and "garifying" (Akinrele, 1964). To lay all the argument to rest as to whether any cyanogenetic glycoside survived, thin layer chromatography (TLC) of cyanogenetic glycosides, was carried out. Gari or lafun was extracted by refluxing with 80% ethanol for 1 hr. Eighty percent ethanolic extracts from cassava peel were added as standard.

The extracts were separated on TLC coated with silica Gel G. On spraying with specific reagents, sugars yield purple



spots cyanogenetic glycosides fluoresce with green colour. The results showed that lafun prepared from cassava tubers cubed, dried slowly and ground into flour, had a low cyanide content, but that gari obtained from tubers fermented for four days was free of cyanide (Table 3). Gari purchased at random from Ile-Ife market had very negligible cyanide. Lafun from the market however, contained about 0.5 mg HCN per kg. The results of TLC showed that cyanogenetic glycosides were not present in gari. A very faint spot having the mobility of linamarin was obtained from extracts of Lafun. The peel extract contained a high level of linamarin (Rf 0.32) as judged by the intensity of the spot. All the extracts have compounds having the mobilities of standard glucose, fructose and maltose. Our study showed that gari, if processed properly by fermenting the mashed tubers for four days or longer, is completely free from cyanide or any cyanide - yielding glycoside in cassava tubers and cassava products.

Cassava supplies much of the carbohydrate in the Nigerian diet. It is estimated that it supplies 14% of the total calories. Cassava is rich in Vitamin C (Jones, 1959). Loss of Vitamin C has been associated with many vegetables in processing. In view of the high level of vitamin C in cassava tuber, we undertook study to determine how much of this vitamin is available the diet after processing. In our study (Ogunsua and Adedeji, 1979), we carried out experiments on cassava tubers processed in several ways: cooking studies; sliced cassava tubers were subjected to the following treatments, boiling, steaming and pressure cooking in small pressure cooker at 15 psi and 121°C. We also produced gari and cassava flour (lafun). Storage studies were carried out on lafun and gari stored in polythene bags at room temperature or in the refrigerator. Samples were taken for analysis at the beginning, after one month and after two months of storage. Moisture content, pH ascorbic acid (AA) and dehydroascorbic acid (DAA) were determined. The results presented in Table 1 showed that, in fresh tubers, ascorbic acid contents ranged from 120-150 mg/100 g moisture. There was no significant

difference between the varieties.

**Table 3: Levels of HCN Residue in Cassava Products**

| | | *Mg HCN/kg* | *Mg HCN/kg (mfb)*[a] |
|---|---|---|---|
| Fresh cassava tuber | Mean | 78 | 130 |
| Flour (from unfermented cassava tuber) | SD | 1.5 | |
| | Mean | 13.4 | 15.2 |
| Flour (from tuber fermented for 4 days, i.e. lafun) | SD | 0.6 | |
| Gari sample prepared experimentally (four days of fermentation) | | | - |
| Gari samples obtained from the local market | | | |
| A | | 0.13 | 0.15 |
| B | | | - |
| C | | | |
| D | | 0.04 | 0.04 |
| E | | - | |
| Lafun samples obtained from the local market | | | |
| F | | 0.48 | 0.55 |
| G | | 0.50 | 0.57 |
| H | | 0.46 | 0.52 |

[a]*mfb* = Moisture-free basis
[b]SD = Standard deviation

The pH values were similar in all the varieties stored at room temperature. There was a drastic reduction at room temperature, there was a drastatic reduction in the ascorbic acid to about 25-30% of the original value in 5 days. After 8 days of storage, the ascorbic acid dropped to insignificant levels. There was a greater retention of ascorbic acid in the refrigerated samples, although the level of ascorbic acid decreased by about 50% in 5 days to about 20% of the original value after 8 days. There was a general increase in the level of DAA in the stored samples indicating that some of the AA was converted to DAA.



In the fermented sample (Table 4) only about 5 % of the ascorbic acid is retained whereas up to 50% of the original DAA was present at the end of fermentation. There was no loss of ascorbic acid in the gari and lafun after two months of storage.

**Table 4: Retention of adscorbic and dehydroascobic acids in ferm nted cassava products**

| Variety | Initial levels (mg/100g-mfb) | | Gari | | | Lafun | | |
|---|---|---|---|---|---|---|---|---|
| | AA | DAA | PH | AA Retention (%) | DAA Retention (%) | PH | AA Retention (%) | DAA Retention (%) |
| 60444 | 165 | 96 | 4.0 | 5.1 | 45 | 6.0 | 4.2 | 41 |
| 5311 | 122 | 67 | 4.3 | 3.8 | 54 | 5.8 | 4.9 | 57 |
| Local red | 156 | 67 | 4.1 | 6.0 | 70 | 5.9 | 4.9 | 66 |
| Local white | 150 | 68 | 4.2 | 5.2 | 57 | 6.2 | 4.1 | 62 |

*mfb* = moisture-free basis

The results showed tl at cooking, especially pressure cooking, leads to a high retention of ascrobic acid and dehydroascorbic acid. For maximum retention of ascorbic acid cassava tubers should be processed immediately after harvesting, as the ascorbic acid decreased rapidly during storage. A large percentage of ascorbic acid is lost during the production of gari or lafun. However, adequate levels of total vitamin C may be available in gari or lafun because a large amount of dehydroascorbic acid was retained. Dehydroascorbic acid retains about 70% of vitamin C activity. An adult eating about 100 g of gari (which is not unusual) meets at least half of his vitamin requirement by so doing.

As earlier mentioned in this study, 75 ppm of ascorbic acid/kg flour is required in bread-making processes, MDD and ADD. By calculation, cassava flour introduced into composite flour at 30% will furnish up to 130 ppm of DAA per kg flour in the composite flour. Could it mean that addition of ascorbic acid to wheat cassava composite is unnecessary?

The changes in lipids during fermentation of cassava

flour were investigated. The most obvious reactions occuring are fermentation of sugars as evidenced by gas production. The fate of sugars during fermentation has received substantial research attention. The fate of lipids during the period of fermentation was studied by me (Ogunsua, 1984). Pressed bakers yeast, a strain of *Saccharomyces cerevisiae* was extracted with chloroform - methanol (2:1). The extract was dried with vacuum rotatory evaporation and the residue was extracted with chloroform. TLC was used to separate neutral and polar lipids, and the neutral lipids were quantitated. Gas Liquic Chromatography (GLC) of the fatty acid methyl esters were carried out. Cassava flour was incubated with yeast. The total lipid, fatty acid methyl esters and hexose were determined.

The oil in the the yeast was 1.3%. Free fatty acids were not detected in yeast. A major feature of the fatty acids was the presence of a very high level (48%) of hexadecenoic acid (palmitoleic acid). Linolenic acid was absent. During fermentation, there was a significant increase in the amount of total lipid recovered from cassava flour uncubated with yeast. There was a loss of hexose during incubation. During incubation, there was a decrease in the proportion of fatty acid in both cassava flour incubated alone and fermented cassava flour. There was also an increase in the lipid with the mobility of diglyceride. The proportion of triglyceride fell in the fermented flour.

Among the fatty acids, linolenic fell from 11% in unincubated flour to 6.7% in the fermented flour. There was no evidence for the presence of palmitoleic acid either in fermented flour or in the controls. There was an evidence that the glycolipids were metabolised during the fermentation as evidenced by 17% loss of hexose in the lipids. The absence of palmitoleic acid which was the major yeast fatty acid meant that the ascorbic acid was saturated by cassava enzymes. A decrease in the level of linolenic acid could be due to the saturation of polyunsaturated acids. It was postulated that linamarase a β-glucosidase also had some β-galactosidase action, hence there was the hydrolysis of the galactolipids.



The slight increase in the short chain fatty acids were probably due to synthesis by yeast or some microorganisms present in the fermenting cassava flour.

## WORK ON SORGHUM MALT

Sorghum is Nigeria most widely grown cereal and it is employed in both malted and unmalted forms in a wide variety of porridges and beverages. Malted sorghum is used in the production of opaque beer called *Otika*. *Kaffir* beer made from sorghum malt is widely consumed in South Africa. The diastatic activity of malted sorghum is lower than that of barley due primarily to low concentration of beta-amylase in the sorghum malt, and the higher proprotions of insoluble alpha and beta amylase. Most sorghum varieties are known to have higher malting losses than barley. Intensive breeding studies have resulted in the release of new sorghum varieties. The malting characteristics of two new varieties L243 and SK 5912 were compared with those of two wild varieties (LR and LV) (Okoli and Ogunsua, 1987). The characteristics examined include the effects of malting temperature, bromate and ammonia treatments on malting loss, diastatic and liquefaction powers of the malts produced from these varieties α-amylase activity was determined by two methods (Harberg, 1959 and Perten, 1964).

### Perten Liquefaction Number (LN)

The average falling number values were converted to Perten liquefaction number which is directly proportional to α-amylase activity by the following emperical relationship:

$$\text{Perten liquefactiion No. (LN)} = \frac{6000}{\text{Falling Number} - 50}$$

α-amylase were also determined by colorimetric method (ICC, 1968).

It was found that all the varieties germinated optimally at

30°C. L243 showed a high diastatic activity of 106 KDU/g (kaffir diastatic unit per g. of dry wt of malt. vs about 85 KDU/g for other varieties. During germination at optimum temperature, L243 developed the highest liquefaction power of 500 Perten liquefaction number of 134 SKB units. SK 5912 LR and LW gave PLN of 463 (1055 KB), 146 (343 KB) and 82 (15 SK) units respectively. Malting loss was found to increase in all varieties with the temperature of malting. At the temperature of optimum amylase activity, malting losses were 36, 15, 24 and 33% for LR, SK5912, L243 and LW respectively. Bromate treatment had no significant effect on malting loss, liquefaction power and diastatic activity. Treatment with 0.3% ammonia reduced malting loss significantly and also reduced liquefaction power and diastatic activity.

The improved varieties L243 and SK5912 showed satisfactory alpha-amylase and diastatic activities. Although L243 appears to have the highest potential for commercial malting because of its very high diastatic activity, SK5912 variety, having low protein content (9.3%) low malting loss (15%) and short malting period, may be considered more suitable for commercial malting for brewing industry, while L243 malt may be utilised for purposes such as bread-making supplement and chocolate flavour type beverage production.

Okoli and Ogunsua (1989) used enzyme active Nigerian sorghum (L243) malt flour as source of sugar and quality improver in bread. Adequate amount of enzyme active malt in the dough resulted in higher loaf volume, better crumb softness, and stability, better eating and keeping qualities and increased residual sugars in bread. However, excessive amounts may cause extensive deterioration in these qualities. Bread was made from Bakers grade wheat flour obtained from Flour Mills of Nigeria, Lagos. A new variety of sorghum (L243) was obtained from Institute of Agricultural Research (IAR) Samaru, Zaria. The sorghum was malted for eight days as described by Novellie (1959). The malt had diastatic activity of 40°L per gramme dry weight of original grain and 134 SKB units of alpha amylase activity. Kilned grains were milled to



pass through 0.8 mm sieve in brabender Quadromat Junior Experimental Mill. Malt flour was introduced into wheat flour at various levels and baking was carried out using Chorleywood Bread Process using a formula containing 6% sugar and no malt as control. It was found that increasing the malt from 0 to 1.5% in sugar free formula reduced proofing time from 65 minutes to 39 minutes. This effect became less significant as the sugar level increased from 2 to 5%. Malt increment up to 0.75% improved loaf specific volume significantly in recipes containing between 0 to 3% sugar. Increasing malt level from 0 to 1% in all sugar formulas improved overall bread qualities particularly crumbs softness, resillence and eating qualities and overall acceptability. Using up to 0.75% malt, it was possible to save about 2% added sugar in the control without adversely affecting the overall acceptability or quality of bread. Thus sorghum malt could serve as a means of reducing large quantities of sugar used in breadmaking. At 1989 prices, in our investigations we found that the cost of producing 1 kg of sorghum (L243) malt in the laboratory was about 70k (this cost may even be lower in large scale production) whereas the cost of sugar at that time was N2.00 per kg on the average.

**Fate of Cyanogenetic glycoside residue during the production of beverages from sorghum malt**

Sorghum grain is free from cyanogenetic glycoside and therefore does not contain hydrocyanic acid residue. However, in the production of sorghum malt, the sorghum grain is sprouted and in this form elaborates the cyanogenetic glycoside dhurrin. This dhurrin is broken down to p-hydroxy benzaldehyde, Hydrogen cyanide and glucose. The question arises, "What happens to the p-hydroxybenzaldehyde in the different stages of beer production from sorghum malt?" I gave this problem as a closely supervised undergraduate project to Miss Adio, I. B. (Adio, 1990). the hypotheses are: (1) it is oxidised to p-hydroxybenzoic acid, (2) it is reduced to hydroxybenzyl alcohol (3) it remains unchanged in the beer. Beer was produced from sorghum malt produce closely

following procedure developed by Nout (1981). The sorghum variety used was a cross between Local White (LW and Local Red (LR) and it was obtained from University of Agriculture, Makurdi. Cyanide contents were monitored in wort and beer. The p-hydroxybenzadehye and p-hydroxbenzoic acid were determined by High Performance Liquid Chromatography (HPLC). Commercial beer samples were also analysed. Standard curves were prepared by plotting the height of different amounts of para-hydrox benzaldehyde and para-hydroxybenzoic acid respectively. The resuls showed that p-hydroxybenzaldehyde was found to have been oxidised to para-hydroxybenzoic acid. In the wort, a little quantity of p-hydroxybenzaldehyde was still present but it was found to have disappeared in beer. 1.3 mg/100 ml of parahydroxybenzoic acid (P-HBA) was found in the beer. Para-hydroxybenzoic acid content of four commercially available beers as well as Maltina (a malt non-alcoholic beverage)were determined. Two of the beers contained 0.78 and 0.68 mg/100 ml p-HBA whereas Guiness Stout and Maltina contained neither parahydroxybenzoic nor para-hydroxybenzaldehyde. The beer prepared by us contained 1.3 mg/100 ml. The lower values contained in these beers may have been due to adjuncts diluting the sorghum malt. The presence of parahydroxybenzaldehyde or parahydroxybenzoic acid is suggested as a quality control measure for beer or malt drink prepared from sorghum malt as distinct from those prepared from barley malt. There was no detectable cyanide (HCN) in the beer prepared in the laboratory or in the commercial samples. All the hydrogen cyanide must have been lost during wort boiling and subsequent fermentation.



## PROCESSING OF OILSEEDS

My studies on composite flours led me to work on oilseeds. Many oilseeds are rich in proteins. I made a survey of various possible diluents to wheat flour-cereals, pulses, peanut, etc. One nut that fascinated me was conophor nut (Asala) (*Tetracarpidium conophorum*). At that time Mr. Adebona of blessed memory was also looking for cereals, etc he could incorporate to instant foods like cowpea flakes, moinmoin mix flakes, biscuits, etc. We decided to work together on conophor nut which was mainly used as a snack. A cursory look at the nut did not strike one as its being an oilseed. We dried some of the nuts and decided to mill them with an attrition mill. We were surprised that what came out was a very oily brownish yellow material with the consistency of peanut butter. We were expecting something like cowpea flour! It dawned on us that it contained a lot of oil. We decided to take a closer look at the oil. This resulted in our paper on chemical composition of conophor nut (Ogunsua & Adebona, 1982). The iodine value of the oil was very high (204). We carried out the analysis of the fatty acid ester by Gas Liquid Chromatography. The oil contained 66% linolenic acid (another great surprise). This was very high, the highest linolenic containing plant oil, as high as that of linseed oil. The dried seed contained 40% free lipid and 50% total lipid. The protein content of the defatted flour was 38%. Linolenic acid is a polyunsaturated oil - an *w*-3 polyunsaturated acid. This has been known to be beneficial in humans. Conophor oil is a very prolific climbing plant.

I put my M.Sc. student, Miss Babalola to work on the manufacture of peanut butter-like commodity from conophor nut. In our study we found that the best quality oil was obtained from boiled seeds. We therefore subjected the nuts to boiling before drying. Mr. Adebona's M.Sc. student worked on the manufacture of biscuits from conophor nut. In doing large scale processing of the nuts, the removal of the shell would create a bottleneck. Fortunately, Prof. G. A. Makanjuola of the Department of Agricultural Engineering had earlier designed

and built a conophor nut shelling machine. This we used in the large scale processing used in the research.

Babalola (1983), working under my supervision for M.Sc. degree pursued a project with the title "Development of Conophor nut butter (peanut butter-like paste)". In this work the oilseeds were processed into a peanut butter-like food, following the standard steps of peanut butter manufacture with some modifications. The seeds were cooked in the shell, shelled, whitened (skin coat removed), dried, roasted, and ground to butter of medium texture. The optimum roasting temperature was at 200°C for 15 min. It was necessary to incorporate 0.02% butylated hydroxyanisole (BHA) to slow down peroxidation in conophor nut butter. The exclusion of oxygen also slowed down peroxidation in the product without added BHA. Incorporation of glyceryl monostearate (GMS) into conophor butter improved gloss and palatability of the butter but did not prevent bitter after-taste which is felt if water is taken after consuming the butter. The addition of some flavourants did not increase its acceptability when compared to the unflavoured butter.

In carrying out the Taste Panel Assessment, warm water was used as mouth rinsing agent instead of the usual chilled water because of the bitter after taste which is peculiar to conophor. Milk with a pH of about pH 6.3 masks the after taste bitterness. It was observed that corn on the cob with pH of about 6.2 also masked the bitterness. Addition of GMS reduced phase separation of the conophor butter considerably. Conophor butter stored in the refrigerator did not exhibit phase separation. The spreadability of the butter was also measured. Penetrometer readings varied for the conophor butters, ranging from 33 in the butter without GMS, to 20 mm for the butter with 2.5% GMS both under a load of
130 gm/cm$^2$/10 secs. Purchased peanut butter had 21 mm penetration under the same load. Conophor butter without GMS addition was not spreadable. It merely thinned out when spread on bread. All the butters with GMS addition were spreadable; being neither two thin nor too stiff. The purchased



peanut butter used as a control was equally spreadable.

When conophor butters with different emulsifier levels were subjected to color test by a group of twenty panelists, it was found that there was more gloss in the butters compared to that without. With a 7-point hedonic scale rating: 1 - unattractive; 7 - extremely glossy. The butter without GMS had an average score of 1.5 whereas those with added GMS had scores between 4.6 and 5. The reason for the improvement of gloss in conophor butter is not known. Griffin and Lynch (1975) have also found that emulsifier improved gloss in peanut butter. It was found that crystal structure of tristearin was modified by added emulsifier. Since the added GMS stiffened conophor butter, it is possible that a re-structuring of fats relative to the particles occured, leading to an increase in gloss. The proximate analysis of the developed conophor butter was very similar to that of the purchased butter.

Adebona, Ogunsua and Ologunde (1988) developed conophor nut-based biscuit. Conophor nut was processed into paste. The unshelled nuts were washed and placed in a pressure cooker and cooked at 1.36 atm. pressure and 121°C for 15 min. It was dried for 12 hrs in an oven at 60°C to a moisture content of 20%. Shelling of fruit was by mechanical sheller developed by Prof Makanjuola (Makanjuola, 1978). The shelled fruits were further dried at 50°C for 48 hrs to a moisture content of 5%. Dried fruits were milled first in a hammer mill followed by milling in pin-disc mill and finally in a disc mill for fine particle size reduction. The paste obtained was put in polythene bags and stored in a deep freezer (-20°C) until required for use. Corn milled to a size less than 300 um was prepared. The baking formula used for the production of biscuit was that of Whiteley (1971). Four types of biscuits were produced and conophor paste was varied from 6 to 32% based on soft wheat flour weight. Corn flour was also varied from 6 to 24%. The control had the following formula. Wheat flour 100g, and the additives common to all baking formula were 20 g sugar, 1.0 g salt, 1.6 g cream of tartar, 10g egg powdered milk and vegetable shortening, 20 g. In general all formula having

less than 64% wheat flour were poor in quality. Optimum biscuit formula was obtained with 12 and 18% conophor paste, with 10 and 18% corn flour respectively. Shelf life study was carried out using the parameters: peroxide value; moisture content, pH, free fatty acid levels. There was no significant difference in the values of the parameters over twelve months storage which is more than the average shelf life of common snack food. The peroxide value and the free fatty acid levels were constant, indicating neither oxidative nor hydrolytic rancidity. The result of the preliminary test on consumer survey on snack containing, 10% corn flour, 12% conophor nut paste and 78% showed bright prospects for the newly developed product.

Ogunsua (1988) determined the amino acid in conophor nut by Gas-Liquid Chromatography. This study was carried out in the laboratories of Prof Pilnick, of Agricultural University, Wageningen, The Netherlands. The aim of the study was two fold. (1) To develop further the new method of determining amino acids by Gas Liquid Chromatography. Normally amino acids are determined by ion exchange followed by colorimetric determination as post coloumn reaction with ninhydrin. At that time amino acids were determined by precolumn formation of the o-phthaledehyde (OPA) derivatives; followed by reverse phase HPLC separation and fluorimetric detection. The gas chromatograph compares favourably with HPLC method in rapidity. Both separation are much faster than the ion exchange method, both taking between 30 min and 60 min. The sensitivity of the HPLC, however is greater, than that of GLC. I therefore carried out the work to use GLC to determine amino acid composition of *Tetracarpidium conophorum* (Conophor nut) (and as a second objective) to compare the results with those obtained by ion-exchange chromatography. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of the amino acid derivatives were carried out to confirm the eluting order of the derivatives and to rule out the presence of artifact during derivative formation. The derivatives were heptafluorobutyryl isobutyl esters which were separated by



GLC on 3% SE 30 on Chrom. Q column. Retention times, peak areas and relative areas compared with area of norleucine (internal standard) were obtained by an electronic integrator for GLC, the conophor samples were defatted with petroleum ether. The dried sample was subjected to hydrolysis and an ion-exchange clean up. In this work, although different column and programmes were used for GC and GC-MS, the orders of elution of the derivatives are the same. Each amino acid was distinctly separated. The fragmentation patterns of the amino acids agree with those of other workers (Makenzie *et al*; 1974). The amino acids are produced without the formation of artefacts. The identity of each of the amino acid derivative of the sample was established by comparison with the mass spectre of authentic amino acids. For ion exchange analysis, the hydrolysed sample after the removal of HCl, was dissolved in lithium citrate/buffer and it was filtered on a membrane filter prior to analysis. In this work, the relative amounts in millimoles of amino acids were determined and there was a general agreement between GLC and Ion exchange analysis. The essential amino acid composition of the flour (ion-exchange values was compared with FAO/WHO reference pattern for essential amino acid). In Conophor nut all amino acids except methionine (68 mg/gN vs 140 mg/gN) are adequate for human nutrition (Table 5).

Two minor unusual amino acids, α-aminobutyric acid (α-ABA) and γ-aminobutyric acid (γ-ABA) were determined by ion exchange. These were present at 0.6 and 0.5 mmoles respectively. Two unidentified minor peaks were present In the GLC, these may be α-ABA and γ-ABA.

**Table 5: Essential Amino Acid Analysis of Corophor Flour (mg/gN)**

| Amino Acid | FAO/WHO Reference | Conophor Nut Flour |
|---|---|---|
| Try | 90 | 184 |
| Phe | 180 | 194 |
| Met | 140 | 68+ |
| Lys | 270 | 354 |
| Thr | 180 | 343 |
| Leu | 300 | 441 |
| Isoleu | 270 | 304 |
| Val | 270 | 345 |

Another oilseed that has engaged my attention is the melon seed. A substantial amount of nutritional research has been carried out on melon seeds mostly under Prof. Oyenuga and Fetuga (1972) and Oke (1965). The processing aspect had not been much researched upon. I believe the very first research carried out on the processing of melon into 'Ogiri' a fermented condiment' was carried out by Akindele, A. M. for her M.Sc. degree in Microbiology under my supervision (Akindele, 1978). Melon seed contains about 44% oil. Ogunsua and Badifu (1989) carried out research on the stability of purified melon seed oil obtained by solvent extraction. We purified crude melon oil from *C. vulgaris* using a laboratory scale method. We also determined the effect of this purification on some of the chemical components of the oil and assessed the stability of the purified oil with or without added butylated hydroxyanisole (BHA) under selected storage conditions. Melon seeds were cleaned and shelled by hand. The seed coats were separated by winnowing unroasted melon seeds were flaked in a roller mill and extracted with petroleum ether



(60-80°C), the solvent was removed by rotary evaporation. Oil obtained was dried in oven at 90°C for 1 hour. Another set of seeds was roasted at 125°C for 15 min, the seeds were flaked and extracted as described for the unroasted kernels. Laboratory purification of the oil was carried out. Degumming and neutralisation were carried out. The purified oil was washed with water and was bleached with Fuller's earth technical powder. Storage studies were carried out on the purified, purified and bleached and crude oil with or without added Butylated hydroxyanisole (BHA). Storage was carried out 5°C, 25°C to 30°C and 100°C. Some oil samples were at ambient temperature (25-30°C) were stored in the dark. The refrigerated (5°C) samples, stored in the dark, were monitored monthly for a period of 6 months. Data generated were analysed (analysis of variance and correlation matrix) on a microcomputer model II. Peroxide values, thiobarbituric acid (TBA) number, α-tocopherol and β-carotene were determined. Fatty acid analysis were also carried out. The fatty acid profile showed a very simple pattern consisting of only palmitic, stearic, oleic and linoleic acids. Oleic acid increased with decrease in linoleic acid and unsaturation during roasting. There was no change in the fatty acid pattern during purification and bleaching of the oil from roasted kernels. The α-tocopherol retention in the purified oil was high (90%). Bleaching led to a further loss (52% retention) of tocopherol. The retention of beta-carotene in purified oil was 64% but decreased to 32% in the purified and bleached oils. These results indicated that it was advisable to exclude the bleaching stage in the purification of melon oil intended for domestic use, particularly, in Nigeria where the characteristic colour of melon oil is a major criterion for use in cooking. Bleaching makes melon oil appear too pale. There was a loss in aesthetic appeal due to the high depletion (about 65%) in beta-carotene. Free fatty acid which were 2% (as oleic acid) decreased to 0.2% to 0.3% after purification. Oil recovery was about 78%. In general, peroxide values were higher in purified oil than crude oil.

BHA was effective in lowering peroxide value in crude oil. BHA gave lower values in purified oil but at much lower levels. This is probably due to the fact that some substances which may be synergistic with BHA, e.g. lecithin or carotene may have been removed during purification. There was no statistically significant difference in the TBA values for purified and crude sample both with or without added BHA during the first 18 hr of storage at elevated temperature. At 24-hour storage, there was a significant increase in the TBA values over those in purified samples indicating probable protective action of the pigments e.g. β-Carotene. Bleaching reduced the β-carotene from 17 ppm to 6 ppm. There was no difference in TBA value of the 24 hr samples. (Table 6)

**Table 6: Effect of Laboratory Purification of *C. vulgaris* seed on some of the chemical components**

| Fatty acid methyl Esters (% of total Methyi esters) | Unroasted seeds | Seeds roasted at 125°C for 15min | | |
|---|---|---|---|---|
| | Crude Oil | Crude Oil | Purified Oil | Purified and Bleached Oil |
| Palmtic acid | 10.06 | 10.66 | 10.59 | 10.47 |
| Stearic acid | 9.37 | 9.64 | 9.49 | 9.43 |
| Oleic acid | 15.55 | 18.30 | 17.98 | 17.91 |
| Linoleic acid | 55.03 | 61.40 | 61.30 | 61.25 |
| Saturation/ unsaturated Ratio | 0.24 | 0.25 | 0.25 | 0.25 |
| α-Tocopherol (mg/100g oil) | 19.05 | 19.21 | 17.25 | 9.84 |
| β-Carotene (ppm) | 17.84 | 17.93 | 11.46 | 5.68 |



Acid values (3.9 to 4.8 mg KOH/g oil) for crude melon oil with or without added BHA were obtained through the storage period (24 hr) at oven temperature (100°C). For the samples stored in the dark at 25°C to 30°C for six months, there was an increase in peroxide value with time of storage. The peroxide values (PV) in the purified samples were higher than those for the crude oil samples both in the presence and absence of added BHA even at the highest recommended BHA level. In the crude oil, addition of BHA led to a decrease in the PV. In the purified oil, there was an increase in PV with storage time. But the values were still higher than those in crude oil to which BHA was added. Purified and bleached oil had the highest PV values which were only very slightly reduced by BHA. The results may be related to the levels of tocopherol and β-carotene which are reduced in the purified oil and the bleached oil β-carotene is thought to have a difference mechanism as an antioxidant which might make its action synergestic with other antioxidants.

In purified oil stored in the dark at ambient temperature (25-30°C) for a period of six months TBA values also increased after six months of storage reaching a much higher level in the purified and bleached oil than in the crude oils. BHA lowered TBA values in crude oils in two and four months samples. There were no differences between sample with BHA in crude oil and purified oils after two months. With the removal of some tocopherol during purification, the BHA antioxidant effect was noticeable. The synergistic effect of β-carotene in the bleached oil was no longer present, hence BHA has no effect. It appears for BHA to exert its full antioxidant effect at the level used, β-carotene must be present.

In general, there was positive association between peroxide values obtained during accelerated and ambient storage conditions respectively. The correlation coefficient of the peroxide values under accelerated and ambient storage was r = 0.934 at the 5% level of significance which suggested that data from accelerated storage could be used to predict the

deterioration of melon under ambient storage condition. Similar high correlation (r) values in the range of 0.835 to 0.979 were obtained for purified and purified and bleached oils with and without added BHA by the indices of (PV and TBA) of oxidative assessment.

Under refrigerated storage (5°C), there was no detectable changes in peroxide value (PV) acid value (AV) and TBA value of crude and purified oils and 6 months of storage. Refrigeration is a very effective way of storing melon seed oil but it may not be economical for developing countries such as Nigeria where most of the household do not have refrigeration.

In another study, Badifu and Ogunsua (1991) examined the chemical composition of some seed of lesser known species of *Cucurbitaceae* family: *Cucumeropsis manni* (white melon), *Lagenaria sicceraria (*gourd) variety 1 and variety 2, *Telfairia occidentalis* (fluted pumpkin). The seed of flutted pumpkin is boiled and the kernel eaten as such or with other condiments. The cooked kernel of fluted pumpkin is used in making OGILI - a fermented product in the southern part of Nigeria. It is similar in preparation to 'Ogiri' popular in Yoruba-speaking areas of Nigeria. Kernels of *Lagenaria* is used to make 'Oseani' which is popular among some Ibo-speaking people of Delta State of Nigeria from where the name is derived. It is a sort of instantised soup that can be used in emergency situation. Traditionally, it is prepared by roasting the kernel of *Lagenaria sicceria* to light brown colour and grinding, with some condiments mainly crayfish, fish smoked to brittleness, pepper, fermented melon kernel, in wooden mortal with pestle. Under these conditions it can be packaged in powered form after the addition of some quantity of salt to taste. When needed for consumption, cold or warm water is added and mixed to desired soup thickness. In spite of the food potentials of the kernels of Lagenaria sp, *Telfaria occidentalis* and *Cucumeropsis manii* as of 1990 not much was known about their chemical constituents. The fruits of the four plants were harvested and heaped together, covered with leaves and allowed to ferment for 4-5 days after which the



seeds were scooped out, washed with water and spread on concrete platform. The seeds were dried in the sun for two weeks. Specimens of the fruits and the seeds were authenticated at the forest harbarium Ibadan, Forestry Research Institute of Nigeria, Ibadan. Measurements of the dried seeds were taken with a caliper. Sodium and potassium were determined by flame photometry. All other metals were determined by atomic absorption spectrophotometlry. The lipase activity of the kernels was determined as described by Fais (1972).

In general, the seeds of these species fell into three groups on the basis of their kernel/seed ratio. *Lignecaria* (0.29 to 0.32); *C. manii* (0.4 group) and *Telfaria occidentalis* (0.78 group). These values are important to the processor who wishes to buy the seeds for the production of vegetable oil. The values estimate the quantity of kernel expected from a given quantity of seed. This in turn, would influence the amount of vegetable oil that would be obtained. The cotyledon (kernel) of dry *Occidentalis* stuck to the shell unlike the other species and this made shelling difficult. *Cucumeropsis manii* (white melon) had 36.1% protein content, the gourds and the fluted pumpkin had 33% protein content. *Cucumeropsis manni* had ether extract of 44%, the gourd had 46% whereas *Telfaria* had somewhat lower percent of ether extract (42%). The ash content of fluted pumpkin was 5.5% and the Ash was 4.66%. The crude fiber of white melon was 2.5% whereas the gourds had 3.6% crude fiber. The ash of melon, and the gourds were 3.8%. Lignecaria had 16% NFE.

The mineral compositions of the kernels are shown in Table 3. The species are very low in minerals but relatively rich in potassium and magnesium.

All the kernels of the species exhibited lipase activity. Lipase hydrolyses triglycerides of the oil to glycerol and fatty acid. The liberated fatty acid resulting from lipase activity present in the oil caused pH changes with time in the medium. The pH changes with time which were monitored served as an index of lipase activity. Lipase activity however occurs only

when the natural compartmentalization of oil cell is ruptured. This rupture allows contact of the oil with lipase present in the oilseed. The presence of lipase in oilseed could affect the storage stability of the oil extracted from such kernel, and hence the need of roasting melon kernel (Ogunsua and Badifu, 1989) before the extraction of its oil. Ever since the detection of lipase in castor seed, there have been conflicting reports in the literature on lipase activity in oilseeds. It has been reported that lipase was absent in peanut, soyabean and corn germ, but lipases do occur in these seeds, during their germination.

Ige, Ogunsua and Oke (1984) examined the functional properties of the proteins of some Nigeria oilseeds; conophor seeds and three varieties of melon seeds. There is a demand for food with aesthetic and organoleptic appeal. These are conferred by components such as carbohydrates, proteins, etc. Plant proteins with relevant functional properties are therefore essential in some food systems. Some of the functional properties include water holding capacity, increase in viscosity, gelling properties and emulsifying properties. Soluble proteins are easier to incorporate into foods. Soyabean, sunflower, sesame, cotton and castor are some of the few oilseeds whose protein products have been extensively used to fortify bakery products, cereal products, dairy products, and communited processed meats such as sausages. Among the most commonly eaten oilseeds in Nigeria are melon seeds, conophor and peanuts. Only the functional properties of peanuts have been thoroughly studied. Defatted conophor seeds, *Tetracarpidium conophorum* and three defatted melon seeds, viz: *Cucumeropsis edulis* and two varieties of *Citrullus vulgaris*, were prepared as previously described.

Oilseed protein isolates were also prepared. The defatted meal were dispersed in distilled water and the pH of the slurries was adjusted to values between 1 and 12 by using either 0.01 NHCl or 0.01N NaOH. Insoluble material were removed by centrifugation and the nitrogen content were determined. The extractibilities of the protein at optimum pH (10-12) for extraction were *T. conophorum* 85%, *C. edulis* 94%,



*C. vulgaris* (var 1) 83% and *C. vulgaris* (var 2) 70%. The emulsion capacity of the defatted oilseed meals were determined their water holding capacity, foaming capacity and foaming stabilities of the defatted flours were determined.

The results showed that Conophor flour showed more solubility at acid pH. 50% is soluble at pH 2.0 whereas the other oilseeds show a solubility lower than 20%. All the proteins show high solubility at pH 9.0. Minimum solubilities of the N is at pH 5.5 and pH 8.0 for conophor. For the melons, however, the minimum solubility is at low pH about pH 3.0 and 5.5 respectively. That there are two points of minimum solubility suggests that there may be two major proteins in these oilseeds. The higher solubility of conophor at acid pH suggest that the proteins of this nut may be used in acid foods e.g. beverages. To do the same for melon seeds, one may have to hydrolyze the proteins to small peptides - a process that has been known to give rise to bitter peptides.

The solubility profile of the protein isolates show that there is virtual elimination of the double minimum solubilities observed for the flours. the lower pH (5.5) for minimum solubility for conophor was retained with a slight shoulder at higher solubility at pH 7.5. The minimum solubilities for *Citrullus vulgaris* 1 and 2 was at pH 4.5 and pH 5.0 respectively and for *Cucumeropsis* was at a very low pH of 3.5. *Tetracarpidium* showed a very high solubility of 80% at pH 2.0 whereas Citrullus showed lower than 25% solubility at this pH. This suggests that the isolate of *Cucumeropsis* will find a very good use in acid beverages and foods. That the double minimum points found in the flours are eliminated in the isolate is probably due to the absence of some interfering substances eliminated during the preparation of the isolates. The substances may affect the charges on the proteins and as such may modify their solubility behaviour near the isoelectric points (or that a second protein may not be isolated).

The results showed that the water holding capacity (WHC) of the isolates are higher than that of the flours. The WHC of *T. conophorum* is significantly lower than those of the

melon seeds. Compared with soy isolates, the WHC's of the isolates of the seeds studied are low with the exception of *C. vulgaris* variety 2, which has a WHC comparable to that of soyabean.

Emulsification capacity also follows the same trend, being very high in *C. vulgaris* varieties. An important property of protein intended as a meat additive is the ability to bind fat. The high values shown by the oilseeds under study indicated that they may be useful in meat additive and as meat extenders. The foaming capacity of the flour was inferior to that of soy flour. The isolate of the studied oilseeds showed no little or no foaming power. However, *Citrullus* and *Cucumeropsis* showed very good foam stability comparable to that of soyaflour and soy isolate after 2 h. *Tetracarpidium* showed very little foaming capacity and stability.

Food Science and Technology involves processing of animal and plant products. So far in this lecture, I have dealt with our work on processing of plant products. I will now deal with the processing of animal products.

Recently there has been an increase in the harvesting and processing of less exploited and 'unconventional' species such as the periwinkle. This is due overexploitation and depletion of `conventional' species such as shrimps, mackerel and sardine. The periwinkle (*Tympanostomus fuscatus*) is a common component in the diets of people in some coastal regions of Nigeria and is or potential source of protein (Mba, 1980). Attempts are being made to harvest and process these marine moluscs for domestic and export markets. Therefore, the raw material must be of very good quality. Due to high perishability of the shucked periwinkle meat and inadequate cold storage facilities the usual practice is to keep the molluscs in the shells at ambient conditions until ready for consumption. If they have been unknowingly harvested from a polluted area, they might pose a potential health hazard.

For processing, for export and domestic markets, the raw periwinkle must meet the international microbiological standards with respect to indicator and toxigenic



microorganisms but data on the microflora of *Tympanostomus fuscatus* were lacking. Also, shellfish are capable of accumulating various heavy metals at unacceptable levels. Adebona, Ariahu and Ogunsua (1990a) investigated the effects of season on the bacteria flora, proximate compositions and mineral constituents of the Tropical Periwinkle (*Tympanostomus fuscatus*). For the microbiological analysis, enough shellfish were aspectically shucked immediately at harvest, homogenized and appropriate dilutions distributed on to sterile plates of non-selective media. The rest of the samples were transported to the laboratory in jute bags and analyzed for proximate and mineral composition within 8 hrs of catch. In order to investigate seasonal variations, the study was carried out at quarterly intervals to cover both the dry (February-March, November-December) and rainy (May-June, August-September) seasons.

The indicator organisms and toxicogenic microorganisms were enumerated. the indicator organisms are coliforms (e.g. *E. coli* and Faecal streptococci. Coagulase positive *Staphylococci, Salmonella, Vibrio parahemolytius* and *Clostridium perfringens*, which are of public health significance were also established in *Tympanostomus fuscatus*. The counts showed significant seasonal variations. The proximate composition was moisture 79%, on a dry weight basis, ether extract, crude protein, ash, and NFE values were 6.2, 61.5, 12.4 and 19.9 respectively. Shellfish is rich in essential elements and on a dry weight basis is a potential source of Ca 55275 ppm, Fe 338 ppm Cu 195 ppm and P 3682.5ppm, Pb 0.8 ppm. The minerals showed significant seasonal variations with peaks in the rainy sampling season (May-June and August-September), Enterobacteriaceae are used as indicator of faecal pollution. The highest values for the periwinkle samples were 95.4 cfu/g (May-June) 240 cfu/100 g, $0.8 \times 10^3$ cfu/g (August-September) and $0.54 \times 10^3$ cfu/g (May-June for coliforms, *E. coli, Staphylococcia* and *Vibrio parahemolyticus* respectively. The International Commission on Microbiological Specifications for Foods (ICMSF) recommends a limit of $4 \times 10^2$

cfu/g, $10^3$ cfu/g and $10^2$ cfu/g for *E. coli*, *Staphylococcus aereus* and *Vibrio parahaemolyticus*, respectively for sea foods. The hygenic levels of the periwinkle samples were, therefore, within these standards. By the United States Food and Drugs Administration (USDFA) standards for Salmonella in seafoods of "absence in 50 g or 25 g when analysed by standard methods", the raw periwinkle harvested in the May-June period would be rejected. Salmonella are heat-sensitive organisms which do not survive normal shellfish cooking temperatures. Therefore, a precooking step prior to shucking of periwinkle meat is the most likely to be an effective way of killing Salmonella present in the gut.

The minerals and proximate compositions are affected by season. The periwinkle is a good source of animal protein as well as essential minerals such as Ca, Mg, Fe and Cu. Pb was found at low levels.

Ogunsua, Ariahu and Adebona (1990b) investigated the quality changes in the *T. fuscatus* with the purpose of establishing quality indices for grading prior to processing

We investigated post-harvest changes in periwinkle at ambient storage conditions. The periwinkles (*T. fuscatus*) were obtained fresh from the lower Benin/Sapele river mangroves in Nigeria. The harvested shell fish landings were washed with seawater to remove mud and other adhering materials. The samples were transported to the laboratory in jute bags and control ('zero time') samples withdrawn for analysis within 8 hours of landing. The lots were transferred into plastic tubs and kept under shade at ambient conditions ($20^{\circ} \pm 1^{\circ}C$; RH 65-86%). The shellfish were mixed at regular intervals every morning, afternoon and evening in order to randomly redistribute them. The samples were subjected to chemical, microbiological and organoleptic assessments at weekly intervals over a period of 6 weeks. In order to investigate seasonal variation, the study was carried out at quarterly intervals (February/March, November/December) to cover dry and (May/June; August/September) to cover rainy seasons.

The organoleptic studies showed that the periwinkle



undergoes reduction in overall quality during the storage. The shellfish, for further processing and export must be of very good quality scores 6-7 (1 = extremely poor; 7 = excellent). Hence the limit of suitability is reached between 2-3 weeks after harvest. The sweetness detected during the first 3 weeks of storage could be due in part to sugar arising from the breakdown of glycogen, which was initially present in high concentrations.

The total viable count revealed an initial lag lasting for about 2 weeks, followed by a significant increase on further storage. The bacterial counts were significantly affected by season with higher counts in the rainy season (May/June and August/September).

The non-protein nitrogen (NPN) ranged from 40.7-45.3 N/100 g flesh. The changes during storage showed fluctuations within the initial range. The initial tyrosine values ranged from 114 to 115.9 mg/100 g flesh and there were values of 135.5 to 139 mg tyrosine after six weeks of storage with little seasonal variations but significant storage differences. The FFA values ranged from 0.6 to 0.66% oleic acid of extracted fat. The values did not show significant changes during storage. The glycogen level ranged from a initial level of 2.84 to 3.15 g/100 g flesh for the four sampling periods with steady but gradual decline to values of 1.41 to 1.84 g/100 g flesh. At the end of 6 weeks of storage, the initial pH value was 6.5 for the four seasonal sampling periods. This was maintained for 2 weeks before changes to the final values of 6.7 to 7.1 at the end of six weeks. The log bacterial counts showed initial values of 3.34 to 3.95 with significant changes to the values of 5.08 to 5.54 at the end of six weeks. The system of quality grading of the periwinkle established from organoleptic assessments of cooked flavour, odour, mouthfeel and general appearance, predicted a maximum shelf life of 2-3 weeks for the shellfish intended for further processing and export. Trimethylamine (TMA) with high within sample variations, as measured by the picric acid method, as well as non-protein nitrogen (NPN) and free fatty acids (FFA) which varied a little

during storage and also TVB (total volatile bases) and bacteria counts with wide seasonal variations are not suitable indices for the grading of periwinkle. However, tyrosine value (TV), pH and glycogen gave little seasonal variations, showed definite trends and gave significant correlations with organoleptic attributes, thereby indicating that they are reliable indices for the grading of periwinkle prior to processing.

It has been shown in our research presented in this lecture that toxigenic organism are present in periwinkle (*Tympanostomus fuscatus*). For export purposes and domestic consumption far into the hinterland, it will be much safer for these products to be canned. The high temperature in canning will kill all the toxicogenic and spoilage organisms in the product.

The periwinkle has a pH of about 6.5. We carried out the canning of three products, periwinkle in brine, periwinkle-in-sauce and periwinkle-in-egusi soup (Ogunsua, Ariahu and Adebona (1993). Essential to the canning of low foods ( > pH 4.5) is the destruction of *Clostridium botulinum* spores. The determination of such a safe process requires three types of data (i) thermal resistance of the most heat resistant organism that is capable of growing and causing spoilage as well as those of public health significance; (ii) initial contamination (number and kind) in the raw product, in order to establish an accurate decimal reduction factor and (iii) heat penetration at the slowest heating point (SHP) in the canned food (Rodrigo *et al*, 1988). The process time calculated from these three parameters should be confirmed by an innouclated experimental pack test. The test organism most frequently used for determination of process time for canned low acid foods is *clostridium sporogenes*. The spores of this organism have a similar Z-value (slope of the thermal death time curve) to that of *C. botulinum* and are more heat resistant. A thermal process that produces a five log (5 $D_T$) reduction of *C. sporogenes* spores produces more than a twelve log (12 $D_T$) reduction in the spores of *C. botulinum* (Grischy *et al.* 1983). The higher heat resistance of *C. sporogenes* over that of *C.*



*botulinum* provides a limit in safety factor when *C. sporogenes* are used for process time determinations. Also, *C. sporogenes* is non pathogenic and can be used safely for thermal process evaluation in canneries. Among the common strains of *C. sporogenes* are Putrefactive Anaerobe (PA) 3679 and ATCC 19404 isolated by the National Food Processor Association (NFPA) and the American Type Culture Collection (ATCC) Rockville, USA respectively. The thermal resistance of *C. sporogenes* spores in periwinkles products with a PH > 4.5 are not known although the influence of substrate and pH on thermal resistance in other products has been studied in artichoke hearts, meat balls-in-tomato sauce, low and acid medium vegetable as well as in phosphate buffer and pea puree. These studies showed that as pH fell, the heat resistance generally decreased. We established thermal process times for the production of periwinkle-brine, periwinkle in sauce and periwinkle-egusi-soup with the purpose of presenting this localised species in the forms that will enjoy wider geographical distribution and greater protection against degradative agents such as microorganisms, vermins, sunlight, oxygen and undesirable gains or loss of moisture. The critical point and the 'j' and 'f' values of heat penetration and the necessary time to reach an Fo target for each canned product was deduced. The process times were finally confirmed by inoculated experimental pack tests with *C. sporogenes* as reference microorganism.

The brines, sauces, and egusi soups treated with citric acid, and sodium tripolyphosphate (STPP) gave periwinkle products with good appearance and flavour for all pH values studied. The periwinkle products treated with 0.2% citric acid and 0.4% STPP and 4% salt had an equilibrium pH of 5.8 and had acceptable flavour and appearance that persisted after opening the cans. Therefore, to enable comparison of the products, the thermal of resistance of ATCC 19404 and innoculated experimental packs were carried out at pH 5.8 for all products using 0.2% citric acid, 0.4% STPP and 4% salt. Increase in product temperature to above 100°C caused a pH

increase of 0.1 pH unit. After the formulation of the products, the cans were autoclaved in a pilot scale batch retort equipped with an atuomatic temperature control.

Heat penetration tests: The results indicate that the canned periwinkle-in-brine and periwinkle in sauce heated mainly by convention whereas the periwinkle-in-egusi soup heated mainly by conduction. The canned periwinkle-in-brine (pH 5.8) with a $D_{121.2}$ value of 0.89 min and Z-value of 9.9°C for *C. sporogenes* has a five $D_T$ = 4.5 min equivalent to Fo of 4.5 min. Also the periwinkle in sauce (pH = 5.8) with $D_{121.2}$ value of 1.28 min and Z-value of 10.3°C has a five $D_T$ = 6.4 min equivalent to Fo = 6.3 min while periwinkle in egusi soup (pH 5.8) has a five $D_T$ = 7.3 min equivalent to Fo = 7.7 min. Hence these values have been established as minimum required Fo value for commercial sterilization of the periwinkle products.

To verify the adequacy of the determined thermal processing time for each product, inoculated pack tests were carried out. The results of the pack tests are given in Table 8. Heating times of 7.8, 12.26 and 17.1 mm corresponding to 7.0; 11.0; and 15.0 min at constant temperature of 121.1°C were enough for commercial sterilization of canned periwinkle-in-brine, periwinkle-in-sauce and periwinkle-in-egusi soup respectively. A process time of 9.0, 13.0 and 18.0 min are recommended for thermal processing of periwinkle-in-brine; periwinkle-in-sauce and periwinkle-in-egusi respectively in 300 x 208 cans (76 x 63.5 mm) with a fill in weigh of 225 g, pH 5.7-5.8 at initial temperature of about 68°C.



**Table 7: Thermal Process Evaluation Data Calculated by Stumbo and Patashnik Methods for Periwikle-in-Brine (PIB), Periwinkle-in-Sauce (PIS) and Periwinkle-in-Egusi Soup (PES) respectively in 300/208 cans (76 x 63.5mm) at Thermal Processing Temperature of 21.1°C.**

| Product | Initial Temp (°C) | $F_o$-target $=F^z_{121.1^o}$ | Formula method (Stumbo) Heating Time (min) | Integration Method (Patashnik) Heating time (min) | Process time at Constant temp. (min) | F-delivered Heating | F-delivered Cooling |
|---|---|---|---|---|---|---|---|
| PIB | 48 | 4.5 | 9.45 | 8.84 | 8.0 | 4.031 | 0.430 |
| | 68 | 4.5 | 9.02 | 8.84 | 8.0 | 4.061 | 0.469 |
| | 76 | 4.5 | 7.90 | 7.84 | 7.0 | 4.009 | 0.491 |
| | 78 | 4.5 | 7.71 | 7.84 | 7.0 | 4.023 | 0.482 |
| PIS | 48 | 6.4 | 14.2 | 14.26 | 13.0 | 5.538 | 0.864 |
| | 68 | 6.4 | 13.0 | 12.26 | 11.0 | **5.426** | **0.976** |
| | 76 | 6.4 | 12.37 | 12.26 | 11.0 | **5.438** | **0.968** |
| | 52 | 6.4 | 13.8 | 14.26 | 13.0 | **5.546** | **0.866** |
| PES | 48 | 7.2 | 22.5 | 19.1 | 17.0 | 4.09 | 3.11 |
| | 52 | 7.2 | 22.1 | 19.1 | 17.0 | 4.22 | 2.99 |
| | 66 | 7.2 | 19.72 | 17.1 | 15.0 | 4.36 | 2.84 |
| | 68 | 7.2 | 19.60 | 17.1 | 15.0 | 4.38 | 2.82 |

**Table 8:** Inoculated pack data for periwinkle-in brine (PIB), periwinkle-in sauce and periwinkle-in egusi soup (PES) respectively in 300 x 208 cans (76 x 63.5 mm) inoculated at the level of $2x10^4$

| Product | Heating time (min) | Process time at constant temperature (min) | Initial product temperature | No of positive cans/ No of inoculated cans | |
|---|---|---|---|---|---|
| | | | | Processed cans | Unheated control |
| PIB | 3.84 | 3.0 | 68 | 34/50 | 6/6 |
| | 5.84 | 5.0 | 68 | 38/50 | 6/6 |
| | 7.84 | 7.0 | 68 | 0/50 | 6/6 |
| | 8.84 | 8.0 | 68 | 0/50 | 6/6 |
| | 10.84 | 10.0 | 68 | 0/50 | 6/6 |
| PIS | 5.26 | 4.0 | 68 | 36/50 | 6/6 |
| | 8.26 | 7.0 | 68 | 19/50 | 6/6 |
| | 12.26 | 11.0 | 68 | 0/50 | 6/6 |
| | 13.26 | 12.0 | 68 | 0/50 | 6/6 |
| | 15.26 | 14.0 | 68 | 0/50 | 6/6 |
| PES | 8.1 | 6.0 | 68 | 38/50 | 6/6 |
| | 10.1 | 8.0 | 68 | 16/50 | 6/6 |
| | 17.1 | 15.0 | 68 | 0/50 | 6/6 |
| | 18.1 | 16.0 | 68 | 0/50 | 6/6 |
| | 21.1 | 19.0 | 68 | 0/50 | 6/6 |

## CONCLUSION, REMARKS AND RECOMMENDATIONS

Sir, I like to take the liberty of making some remarks, some impinging of this lecture, others from my experience. From my work, I recommend that the processing of cassava tubers has to be carried out following closely the traditional method of fermenting for at least four days. There is now a tendency of shorten fermentation and claim that fermentation can be shortened to twenty four hours. While this can develop flavour, it may fail to reduce cyanide adequately.



I remember on Christmas day in 1980, the officer in charge of the public health laboratory in Akure came to my house and wanted some food samples analyzed urgently. A family in one town in the Ondo State had eaten some cassava meal and six of them had died. She wanted, as matter of public concern, to know what was the cause. I had to suspend my Xmas celebration, called on our laboratory senior technologist, Mr Adetona and we went to work on the analysis of the left-over meal ('lafun').

The first thing I suspected was the cassava dough (lafun) instead of the soup. We analyzed it and found that it contained 272mg HCN/kg, a very lethal amount! An adult taking 0.2kg of this would have taken a lethal dose which is 60mg.

You would have seen from this lecture how involved it was to can shucked shellfish – periwinkle. Many industrialists who process food, especially those with the small scale and medium scale plants, do not bother to hire a food scientist or food technologist. They engage in canning a whole lot of things, meat, beans, drinks and bottling of palm wine, tomato juice, etc. To cook and eat in the home or even in the restaurant is the right of everybody. But to manufacture food on a large scale like ice cream manufacture, packaging of water, canning, etc is a serious business and it needs a lot of attention from government agencies. Even packaging of cassava flour, water, etc in sealed cellophane bags could be a source of danger. Professor Baker, my chemistry Professor at Macdonald College, McGill said "Once you start a chemical reaction and you put it in a sealed tube or sealed container, you have already prepared a bomb! It is so with food processing. Once you hermetically seal a food in a container, you already have a bomb in your hand, if the packaged food is not properly processed. Apart from the fact that you can end up with blown cans or shattered bottles, literally people who consume such ill-prepared food are consuming bombs! Food poisoning like staphylococcus poisoning, botulism, salmonellosis, listeriosis, etc have wrecked many a life. Food poisons such as HCN,

aflatoxins, carcinogenic food colors can be distributed to unsuspecting consumers.

Another observation I will like to make is the changing pattern of vegetable oil consumption. In the past, we used to consume a lot of groundnut oil, palm oil, etc. Palm oil is still widely available and is good because it is rich in Provitamin A, β-Carotene, although it is rich in saturated fatty acid. Now in our market, we find a lot purified deodorized palm kernel oil which is not good nutritionally as it contains a lot of saturates, mainly lauric and myristic acids. This is not a good trend as in the long run, we may have problems of circulatory system. Our processors should divert palm kernel oil to soap and cosmetics manufacture and other industrial uses.

The life of an academic is not fully devoted to research. It also involves teaching. For the past 32 years, I have taught generations of students in this great University. I have been privileged to train four Ph.D. graduates and several M.Sc. graduates who are now respected researchers, teachers and administrators in their various institutions. One thing I will like to say as the years go by is that, it becomes difficult to teach even undergraduates not to talk of postgraduates because of dwindling facilities – laboratory equipment, processing equipment, chemicals and other materials. One has to support ones own postgraduate students with ones own fund from salaries. Now, I think it is only a bold University teacher, who can take on a new Ph.D. student especially in the Sciences. The government should pour in a lot more resources to make our teaching and research more effective. I could remember my Food Microbiology Professor, Prof. Blackwood, when we had our last lecture in Part IV, he said, "*You are the children of frontier*". I now realize what he meant. We were in the forefront of knowledge in that field because of the up-to-date equipment, books, journals, etc and dedicated teachers.

If we in Nigeria Universities are provided with up-to-date facilities, we can also make our students *children of frontier*.

A lot has happened in the world of biochemistry and food science and technology since I first entered the postgraduate



school. The genetic code had just been cracked. All the enzymes for splicing, cutting DNA were just being discovered and a lot of the enzymes for manipulating the DNA and the RNA were unknown. Now, genetic engineering has profoundly influenced biochemistry and food science. Many of the equipment we used in the studies described in this lecture are new. TLC and GLC were in their infancy, GC-MS, HPLC, Solid Phase Synthesis and Protein and DNA Sequencing were then unknown when I was a graduate student. For me it has been an exciting adventure seeing all these knowledge develop these past four decades.

Mr. Vice-Chancellor, I thank this great Citadel of Learning, Obafemi Awolowo University, for giving me the opportunity to develop myself these past thirty two years.

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