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Fungal Spoilage of Coconut (*Cocos nucifera* L.) Fruits During Storage and the Growth Differential of Isolates on Selected Amino Acids and Carbohydrates

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Abstract: Husked and dehusked coconut fruits were stored at 10°C and 30°C for three months. The husked coconut fruits stored at both 10°C and 30°C and the dehusked coconut fruits stored at 10°C showed no evidence of microbial spoilage at the end of the three months storage period. However, dehusked coconut fruits stored at 30°C deteriorated. *Aspergillus flavus* and *Aspergillus niger* were the principal fungal agents associated with the spoilage. An investigation of the proximate composition of the dehusked fruits stored at 30°C indicated a marked significant difference in the percentage composition of moisture, protein, ascorbic acid and carbohydrate content of 3.97±0.28, 3.98±0.07, 0.01±0.002 and 9.27±1.02 respectively as against 46.82±0.43, 3.77±0.05, 2.48±0.15 and 11.89±0.22 obtained for dehusked coconut fruits prior to storage. These results suggest that the deterioration in nutritional composition was due to breakdown of protein and carbohydrate by the spoilage fungi. Further tests confirmed the ability of the isolated spoilage fungi to utilize the different carbohydrate and nitrogen sources as source of carbon and energy. *Aspergillus flavus* showed the ability to grow and utilize more of the various carbohydrate sources than *Aspergillus niger*, although the latter utilized lactose better. Both fungi showed evidence of growth and complete utilization of nearly all the nitrogen sources, except cysteine and L-glutamine, which could not support the growth *Aspergillus niger*. Likewise, cysteine and L-glutamine, in addition to D-β-phenylalanine could not support the growth of *Aspergillus flavus*.

Key words: Carbohydrate, dehusked, deterioration, husked, moisture, protein

INTRODUCTION

Cocos nucifera is a tree of the palmaceae family and is indigenous and cultivated in nearly all tropical countries (Gonealves and Teixeira, 1982). Coconuts are referred to as "nuts of India" and has been designated several other names.

Oil from coconut is used in making candles, soaps, perfumes, cosmetics and margarine (Gonealves and Teixeira, 1982). With a total production of 9,200 million nuts during 1989-90, India is the world's third largest producer of coconut (George *et al*., 1991). Coconut culture and processing play a dominant role in the agricultural economy of the Southern States of India, USA and Nigeria (Asiedu, 1989). More than 50% of the nuts are consumed raw in the household sector and some in form of ready-to-eat sweet meats using sugar and jiggery (Satyanarayana Rao *et al*., 1990a,b). New products such as processed coconut milk, coconut water and many other food products including infant foods have been developed and marketed (Prasanna *et al*., 1969; Timmins and Kramer, 1977; Hagenmaier, 1977; Husin and Hassan, 1978; Lupke, 1979; Gonealves and Teixeira, 1982).

In India, coconut economy depends mainly on a single commercial product, which is the coconut oil. Processed coconut cream is also a product that has good market potential. Gwee (1988) reported that 25% of the world's output of coconut is consumed as coconut milk. In Nigeria, coconut is grown mainly for food and wholly eaten raw, until recently when it is being processed on a small scale into candies, chips etc. (Asiedu, 1989).

Despite the fact that coconut has been processed into so many food products, some of which, like coconut milk is an indispensable ingredient in many of the traditional cuisine of South East Asia countries, information on the spoilage pattern of coconut, organisms that cause spoilage and more importantly, the effect of spoilage on the nutritional content of this produce is very sparse. Asiedu (1989) asserted that of the more than 100 products made directly or indirectly from coconuts, seven are of vital importance to world trade-whole coconut, copra, coconut oil, coconut oil cake, coir, desiccated

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shredded coconut and coconut skin milk and protein. Asiedu (1989) in his study observed that well-aired copra has its moisture reduced from 45 or 50% to 3-5%; oil content is increased from 35% to 60-65%. He further observed that when the moisture content is about 4%, putrefaction may likely occur and the oil may become rancid. Asiedu (1989) noted that although the meat of coconut contains 20-25% protein of reasonably good nutritional quality, the crude fibre levels of 10-12% reduce its usefulness in infant food formula.

The storage of coconuts has been a subject of concern for producers and shippers of coconuts for a long time (Aten *et al*., 1958). Aten *et al*. (1958) proposed that coconuts should be piled above the floor on flat surfaces or pallets, a method which keep down insect infestation, provides better air circulation and helps in rodent control. In addition, the group of researchers recommended that coconut should be stored at low temperature and humidity at 2°C and 50% relative humidity and that the lower the temperature as low as 2.2°C the longer the keeping quality of coconut.

Kuku and Agboola (1984), Okpokwasili and Molokwu (1996) observed that an important prerequisite for mould development in vegetable oils is high moisture content. Kuku and Adeniji (1976), Kuku and Broadbent (1979) also reported that inadequate drying or accidental wetting of oil seeds usually leads to mould development in seeds with subsequent development of high free-fatty acid in oil extracted from such mould-infected seeds.

The effects of lipolysis by moulds associated with some vegetable oils (groundnut oil, palm oil, palm kernel oil) on the free-fatty acid contents of the oils have been reported (Ward and Diener, 1961; Kuku, 1976; Sowunmi and Adesuyi, 1981; Okpokwasili and Molokwu, 1996). The ability of bacteria to use the organic compounds in vegetable oil as sources of cell carbon and energy has also been reported (Okpokwasili and Williams, 1991). Okpokwasili and Molokwu (1996) in their study of different vegetable oils (groundnut, palm oil and palm kernel oil) isolated various moulds and yeasts genera-*Saccharomyces*, *Candida*, *Debaromyces*, *Hansenula*, *Trichosporon*, *Torulopsis*, *Pichia* (all yeasts) and *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Geotrichum*, *Cladosporium* for moulds.

Coconut and its extracted oil from copra have served man as important foods for thousands of years. Since it contains mostly saturated fatty acids it is believed to be hypercholestrolemic in action. Presently, virgin coconut oil (vco) is gaining wide polarity in the scientific field and among the public. It is believed that VCO is more beneficial than easily obtained copra oil sine the mode of extraction retains more biologically active components such as vitamin E and polyphenols (Nevin and Rajamohan, 2004). Several nutritional factors such as intake of vitamin E as an antioxidant or the nature and amount of dietary fatty acids have been shown to reduce the susceptibility of Low Density Lipoproteins (LDC) to lipid peroxidation in humans and animals (Nicolosi *et al*., 2001; Stephens *et al*., 1996; Nevin and Rajamohan, 2004).

Various studies have shown that vegetable oil affect lipid perioxidation and antioxidant parameters, and lead to favourable changes in the plasma lipid status. Ip *et al*. (1999) and Liu *et al*. (2002) reported in their studies that Conjugated Linoleic Acid (CLA) has anti-carcinogen, anti-antherogen. Dietary Conjugated Linoleic Acid (CLA) has anti-carcinogenic (Ip *et al*., 1999; Liu *et al*., 2002; Hargrave *et al*., 2005), anti-antherogenic (Kritchevsky *et al*., 2002; Lee *et al*., 1994; Munday *et al*., 1999) and antiobesity (Hargrave *et al*., 2002; Park *et al*., 1999) effects. Linoleic and linolenic acids are dietary essential fatty acids.

Copra Oil (CO) processed by exposing acids are dietary essential fatty acids or high temperatures which may inactivate the major components like tocopherols, tocotrienols and polyphenols (Wyatt *et al*., 1998). Contrariwise, Virgin Coconut Oil (VCO) extracted by wet process directly from coconut milk under a controlled temperature may have more beneficial effects than Copra Oil (CO) since it retains most of the unsaponifiabe components (Nevin and Rajamohan, 2004). Nevin and Rajamohan (2004) in their study on effect of VCO and CO on the weight of animals observed that there was no significant change. They also observed that the concentration of cholesterol in serum, liver and heart of the VCO-treated group was significantly lower compared to CO fed and controlled animals. In addition, that triglycerides in serum and tissues were significantly lower in VCO-treated animals compared to CO and control animals and phospholipid levels also showed the same pattern among the three groups.

Nevin and Rajamohan (2004) observed that feeding of the animals with VCO resulted in decreased concentrations of all the lipids tested (cholesterol, triglycerides and phopsholipids) when compared with CO. They adduced that for the lower lipid levels in serum and tissues (liver, kidney and heart) accompanying VCO feeding may be due to the relative rate of synthesis and catabolism of these lipids. They also explained that the minor and/or unsaponifiable components in VCO may be influencing the rate of synthesis and oxidation of fatty acids in the liver.

Muller *et al*. (2003) observed in their studies that a diet rich in coconut oil reduces diurnal postprandial variations in circulating tissue plasminogen activator antigen and fasting lipoprotein compared with a diet rich in unsaturated fat in women. Findings by researchers also demonstrate that the fatty acids in coconut oil oxidation of cholesterol, which initiate the process was prevented by the biologically active polyphenol components present in coconut oil. Hence coconut oil is

beneficial for heart disease, lowers lipoproteins, it acts as a poison antidote especially against acute aluminium phosphide poisoning (Shalini *et al*., 2007), the lauric acid and monolaurin components of coconut oil are safe antimicrobial agents that can kill or stop completely the growth of the most dangerous viruses and bacteria (Preuss *et al*., 2005).

The current studies were designed to determine the storage quality of coconut fruit (dehusked and husked) stored at 10°C and 30°C, spoilage and microbes associated with spoilage and proximate composition of possibly deteriorating and health nuts, perform a comparative study of the proximate value of dehusked and husked coconut at 10°C and 30°C and determine the microbiological quality of the set of coconut at the different temperatures.

MATERIALS AND METHODS

Source and treatment of fruits: The coconut fruits used in this study were obtained from coconut market in Badagry, Lagos State. In all, ninety (90) healthy, husked coconut fruits were divided into three groups of thirty each prior to storage. Group A represent samples of healthy, unbroken dehusked coconut fruits stored at 30°C. Five out of this were selected at random to determine the proximate composition of the endosperm and the remaining twenty-five stored at the same temperature for three months. Group B samples were also healthy, unbroken, husked coconut fruits. Fifteen fruits each were stored at 30°C in the laboratory bench and 10°C in a regulated refrigerator respectively for three months. All the coconut fruits used in this study were harvested at their best state of maturity.

Microbiological analysis: In order to access the microbiological quality of the fruits Czapek dox agar containing 50 mg/ml streptomycin was used for the isolation of fungi from spoilt fruits. A section of the spoilt fruit was picked aseptically with sterile inoculating needle and transferred onto the surface of Czapek dox agar plates and incubated at 28±2°C for seven days until defined fungal colonies emerged. The emerging colonies were transferred onto Czapek dox agar slants as stock cultures after purification for further studies. The identification of the fungal isolates was based on their cultural and microscopic features as described by Bessey (1950).

Preparation of Spore suspension: The fungal spores of each fungus were harvested on Czapek dox agar slant and introduced into fifty milliliters of sterile distilled water in a capped test tube and homogenized.

Growth studies on isolates

Effect of different carbon sources on growth of isolates: A modified replacement culture medium after Winsted and Walker (1954) was employed as basal medium. The medium consists of 2g sodium nitrate, 1g potassium dihydrogen phosphate, 0.5g potassium chloride, 0.5 g $MgSO_4.7H_2O$; 0.01 g Fe $(SO_4)_2.7H_2O$ per litre of water. About 100 milliliters of the basal medium was transferred into 250 ml conical flask. Two percent of each of the following carbon sources-fructose, galactose, glucose, lactose, maltose, mannitol, pectin, raffinose, rhamnose and xylose were filter-sterilized using 0.45 µm Millipore membrane (Schleicher and Schuell, Dassel, Fed. Rep. of Germany) into sterile separate flasks. Thereafter, one milliliter of the spore suspension was then inoculated into each flask and incubated at 28±2°C for 14 days. The control contained only the basal medium without any carbon source, but sugars was replaced with sterile distilled water. The resulting mycelial masses were aseptically filtered through a pre-weighed and sterile filter paper, dried in an oven at 80°C to constant weight and then placed in a desiccator to cool. Thereafter, they were accurately weighed using a digital weighing balance (Model, Kern 440-43N) to determine the dry weight of the mycelia.

Effects of different nitrogen sources on the growth of isolates: The effect of ten different N-sources on growth of the mould isolates was investigated. In the study a modified Czapek dox liquid medium was employed as basal medium. The recipe of the medium was 30 g sucrose; 1 g K_2HPO_4; 0.5 g $MgSO_4.7H_2O$ and 0.01 g yeast extract per litre of water. Hundred milliliters of the basal medium was transferred into 250 ml conical flask. Two percent of each of the following nitrogen sources ammonium nitrate, ammonium sulphate, D-β-phyenyalanine, tryphtophan, glycine, threonine, cysteine, L-glutamine, methionine and Sodium nitrate were incorporated into each flask containing the basal medium in triplicate after filter-sterilizing. This was followed by inoculation of one milliliter of the spore suspension into each of the flasks and incubated at 28±2°C for 14 days. The control contained only the basal medium and water without nitrogen source. The mycelia mass were aseptically filtered through pre-weighed filer paper, dried in an oven at 80°C and placed in a desiccator to cool. Thereafter, it was accurately weighed to determine the dry weight of the mycelia.

Proximate analysis: This was done only on the fleshy endosperm (meat) of the fruit. The parameter determined were moisture, protein, fat, ash, carbohydrate, crude fibre, ascorbic acid, phosphorus, potassium, calcium, magnesium and iron. Analyses were done according to methods described by AOAC (2002). Results obtained are as presented in the result session.

RESULTS

Fungal isolates: After analysis moulds isolated were identified as *Aspergillus niger* and *Aspergillus flavus* using morphological and colonial characteristics as described by (Barrett and Cheek, 1962).

Table 1: Proximate composition of healthy coconut endosperm

Samples	
Parameter	Content (%)
Moisture	46.82±0.43
Protein	3.77±0.05
Fat	36.49±0.25
Ash	0.98±0.01
Carbohydrate	11.89±0.22
Crude fibre	4.84±0.04
Nutritional components	Content (mg/100 g)
Ascorbic acid	2.48±0.15
Calcium	13.77±0.12
Potassium	180.00±1.31
Phosphorus	95.96±0.13
Magnesium	48.90±0.35
Iron	1.89±0.01

Proximate analysis: Table 1 shows the percentage composition of parameters used in proximate analyses for unbroken healthy coconut fruits prior to storage at temperatures of 30°C and 10°C. The values displayed are the average estimate for each parameter and it lies between the sample means plus or minus the standard error. The results revealed the true compositions of the fresh coconut endosperm before storage.

Table 2 and 3 represent the proximate compositions of the healthy, unbroken dehusked coconut fruits stored for 3 months at temperatures of 30°C and 10°C. Microbial analysis revealed that the coconut endosperm for this group was void of spoilage in spite of the storage duration and temperature. However, some of the dehusked coconuts were attacked by spoilage organisms as shown in Table 4.

We also noted changes in the proximate compositions of the coconut endosperm when compared with those obtained before storage. There was a fall in the percentage moisture from 46.82±0.43 for sample of fresh coconut endosperm prior to storage to 5.86±0.09 and 6.04±018 for the endosperm of the dehusked coconut fruits stored at the temperatures of 30°C and 10°C respectively for 3 months.

Table 1 shows the mean proximate composition of the endosperm of fresh healthy coconut fruits prior to storage at temperatures of 30°C and 10°C. The values obtained for each parameter lies between the sample means plus or minus the standard error. The percentage protein, fat, carbohydrate, ash and crude fibre were 3.77±0.05, 36.49±0.25, 11.89±0.22, 0.98±0.01 and 4.84±0.04 as represented in the table. Table 2 and 3 represents the mean proximate composition of the healthy, unbroken dehusked coconut fruits stored for 3 months at temperatures of 30°C and 0°C. After series of microbiological analysis, the endosperm appeared free of spoilage microbes. However, few of the coconut fruits were attacked by spoilage organisms as represented in Table 4. With this attack there was a tremendous change in the proximate composition of this group of coconut fruits compared with those in Table 1 (before storage). This can be seen in the drastic reduction in the moisture content from 46.82±0.43 (Table 1) for fresh coconut endosperm prior to storage to 5.86±0.09 and 6.04±0.18 (Table 2) for the endosperm of the dehusked coconut fruits stored at 30° and 0°C respectively. Also noticeable, is the depletion of the nutrient levels in the stored coconut fruits as evident in stored coconut fruit compare with fresh coconut before storage.

An increase was also recorded for the % protein, fat, ash carbohydrate, calcium, potassium, phosphorus, magnesium and iron. For instance, in Table 4 where the result before storage of the healthy, fresh coconut endosperm, at both temperatures for protein was 3.77±0.05 as against 8.46±0.07 and 8.68±0.08 after storage at 30°C and 10°C respectively for three months. Also % fat was 36.49±0.25 as against 66.82±0.49 and 67.58±0.28; % ash was 0.98±0.01 as against 1.42±0.07 and 1.49±0.08; % carbohydrate was 11.89±0.22 as against 17.26±0.56 and 16.38±0.28. The calcium content increased as well from 13.77±0.12 mg to 26.22±0.34 mg and 27.49±0.45 mg. Potassium content increased from 180.00±1.31 mg to 395.31±1.71 mg and 421.19±1.32 mg. Phosphorus content increased from 95.96±0.13 mg to 147.31±0.73 mg and 149.12±0.71 mg. Magnesium content increased from 48.90±0.35 mg to 77.49±1.11 mg and 79.35±0.62 mg and iron content from 1.89±0.01 mg to 3.06±0.12 mg and 3.32±0.09 mg. The crude fibre and ascorbic acid, which decreased from 4.84±0.04 mg and 2.48±0.15 mg (Table 1) to 3.89±0.08 mg and 0.07±0.004 mg for crude fibre and ascorbic acid respectively for the meat of husked coconut fruits stored at 30°C for 3 months (Table 2). In the same manner the result shows further decrease in crude fibre and ascorbic acid with 3.79±0.09 mg and 0.07±0.003 mg respectively, for the meat of the husked coconut fruits stored at 10°C for 3 months (Table 2). Table 4 also shows the results of the proximate composition of the endosperm of the dehusked coconut fruits after storage for 3 months at 30°C and 10°C respectively. These results revealed changes in the nutritional compositions of the coconut fruit when compared with the results obtained for the same fruits before storage (Table 2). While there were increases in moisture, protein, fat, ash, carbohydrate, calcium, potassium, phosphorus, magnesium and iron content, there were actual noticeable decreases in the crude fibre and the ascorbic acid content. The husked coconut fruits retained more moisture of 8.60±0.01 and 10.61±0.01 at 30°C and 10°C when compared with the dehusked coconut fruits with values of 5.86±0.09 and 6.04±0.18 in Table 2. This may be attributed to the husk still in place. It also retained more ascorbic acid, but there was however reduction in other nutrients.

Table 5 shows the proximate composition of the endosperm of the dehusked coconut fruits that showed

Table 2: Mean proximate composition of dehusked coconut endosperm after 3 months storage (at 30°C)

Group A samples (30°C)				Group B samples (0°C)			
Parameter	Comp. (%)	NC	mg/100 gm	Parameter	Comp. (%)	NC	mg/100 gm
Moisture	5.86±0.09	Ascorbic acid	0.07±0.004	Moisture	6.04±0.18	Ascorbic acid	0.07±0.003
Protein	8.46±0.07	Calcium	26.22±0.340	Protein	8.68±0.08	Calcium	27.49±0.450
Fat	66.82±0.49	Potassium	395.31±1.710	Fat	67.58±0.28	Potassium	421.19±1.320
Ash	1.42±0.07	Phosphorus	147.31±0.730	Ash	1.49±0.08	Phosphorus	149.12±0.710
Carbohydrate	17.26±0.56	Magnesium	77.49±1.110	Carbohydrate	16.38±0.29	Magnesium	79.35±0.620
Crude fibre	3.89±0.08	Iron	3.04±0.120	Crude fibre	3.79±0.09	Iron	3.32±0.090

Comp. = Composition (%), NC = Nutrient Composition

Table 3: Mean proximate composition of husked coconut endosperm after 3 months storage (at 30°C)

Group C samples (30°C)				Group D samples (0°C)			
Parameter	Comp. (%)	NC	mg/100 gm	Parameter	Comp. (%)	NC	mg/100 gm
Moisture	8.60±0.01	Ascorbic acid	1.26±0.01	Moisture	10.61±0.01	Ascorbic acid	1.50±0.01
Protein	6.93±0.03	Calcium	23.65±0.09	Protein	6.97±0.02	Calcium	20.53±0.03
Fat	55.96±0.03	Potassium	345.03±0.08	Fat	49.15±0.22	Potassium	256.80±0.11
Ash	1.38±0.01	Phosphorus	130.74±0.16	Ash	1.01±0.01	Phosphorus	102.07±0.02
Carbohydrate	27.13±0.01	Magnesium	70.94±0.04	Carbohydrate	32.25±0.19	Magnesium	56.95±0.04
Crude fibre	3.80±0.01	Iron	2.92±0.09	Crudefibre	4.49±0.01	Iron	2.41±0.01

Comp. = Composition (%), NC = Nutrient Composition

Table 4: Mean proximate composition of spoilt dehusked coconut endosperm after 3 months storage (at 30°C)

Group A samples (30°C)				Group B samples (10°C)			
Parameter	Comp. (%)	NC	mg/100 gm	Parameter	Comp. (%)	NC	mg/100 gm
Moisture	3.97±0.28	Ascorbicacid	0.01±0.002	Moisture	6.04±0.18	Ascorbic acid	0.07±0.003
Protein	3.98±0.07	Calcium	27.69±0.890	Protein	8.68±0.08	Calcium	27.49±0.450
Fat	80.78±1.16	Potassium	407.39±1.340	Fat	67.58±0.28	Potassium	421.19±1.320
Ash	1.48±0.03	Phosphorus	153.23±3.900	Ash	1.49±0.08	Phosphorus	149.12±0.710
Carbohydrate	9.79±1.02	Magnesium	71.15±2.750	Carbohydrate	16.38±0.29	Magnesium	79.35±0.620
Crude Fibre	3.59±0.18	Iron	3.36±0.110	Crudefibre	3.79±0.09	Iron	3.32±0.090

Comp. = Composition (%), NC = Nutrient Composition

Table 5: Mean proximate composition of spoilt dehusked coconut endosperm after 3 months storage (at 30°C)

Group A samples			
Nutrient	Content (%)	Nutrient	C (mg/100 g)
Moisture	3.97±0.28	Ascorbic acid	0.01±0.002
Protein	3.98±0.07	Calcium	27.69±0.890
Fat	80.78±1.16	Potassium	407.39±1.340
Ash	1.48±0.03	Phosphorus	153.23±3.900
Carbohydrate	9.79±1.02	Magnesium	71.15±2.750
Crude fibre	3.59±0.18	Iron	3.36±0.110

C = Content (mg/100 g)

evidence of microbial spoilage after 3 months of storage at 30°C. The results show a remarkable reduction in the percentage moisture, protein, ascorbic acid and carbohydrate content to 3.97±0.28, 3.98±0.07, 0.01±0.002 and 9.79±1.02 respectively as against 5.86±0.09, 8.46±0.07, 0.07±0.004 and 17.26±0.56 obtained for the same dehusked coconut fruits stored at the same temperature but without evidence of microbial spoilage.

The percentage fat increased probably as a result of the reduction in moisture content while the reduction in percentage protein and carbohydrate was attributed to the breakdown of the amino acid components by the spoilage fungi isolates which also reduced the available carbohydrate used incidentally for respiration by the fungus. It was observed also that of the twenty-five coconut fruits stored under this condition, twenty showed evidence of spoilage. This represents 80% of the total coconut understudy. Secondly, the dehusked coconuts fruits stored at 10°C and the husked coconut fruits stored at both 30°C and 10°C did not show any evidence of spoilage and as a result were not analyzed further.

Table 6 contain the results of the extent of utilization of nitrogen and carbohydrate nutrients by fungal isolated from deteriorating coconut endosperm in growth experiments. The results obtained demonstrates the ability of these fungal isolates to utilize the nitrogen and carbohydrate substrates as sources of energy. Fructose, galactose, glucose, lactose, maltose, mannitol, pectin, raffinose, rhamnose and xylose were the carbohydrate sources while the nitrogen sources include ammonium nitrate, ammonium sulphate, di-β-phenylalanine, tryptophan, glycine, threonine, cysteine, L-glutamine, methionine and sodium nitrate.

The results further confirm that the decrease in the percentage of protein and carbohydrate obtained from

Table 6: Test for the utilization of carbohydrate and nitrogen sources by Fungal isolates (*A. niger* and *A. flavus*) incubated at 30°C for 14 days

	Carbohydrate		Nitrogen	
	Dry weight of fungal mycelia (mg)		Dry fungal mycelia (mg)	
Carbohydrate sources	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus flavus*
Basal medium	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Fructose	131.00±0.58	679.67±0.88	1041.00±0.58	1041.00±0.88
Galactose	57.00±1.53	721.00±1.15	1262.00±1.15	1002.67±0.88
Glucose	101.67±0.67	712.67±0.88	0.00±0.00	0.00±0.00
Lactose	124.00±1.15	71.33±0.88	1162.33±0.88	841.67±0.88
Maltose	0.00±0.00	685.67±3.69	0.00±0.00	0.00±0.00
Mannitol	0.00±0.00	715.00±0.58	849.67±0.88	466.67±0.88
Pectin	0.00±0.00	0.00±0.00	1021.33±0.88	866.67±0.88
Raffinose	0.00±0.00	572.00±1.15	86.33±2.73	0.00±0.00
Rhamnose	0.00±0.00	0.00±0.00	850.67±1.201	1053.67±1.45
Xylose	1.00±0.58	633.00±1.73	659.00±1.53	896.67±0.67

the analyses of the deteriorated endosperm of the dehusked coconut fruits was an evidence of microbial deterioration, degradation and utilization. However, it was observed that while these isolates utilized most of the nitrogen sources except cysteine, it was not so for the carbohydrate sources.

The *Aspergillus flavus* utilized all the carbohydrate sources except pectin and rhamnose while *Aspergillus niger* utilized fructose, galactose, glucose and lactose and partially xylose. The mean dry weight of the mycelia of *Aspergillus niger* on the fructose, galactose, glucose and lactose in milligram were 131.00±0.58, 57.00±1.5, 101.67±0.67 and 124.00±1.15 while it was 679.67±0.88, 721.00±1.15, 712.67±0.88 and 71.33±0.88 respectively for *Aspergillus flavus* on the same carbohydrate sources.

Aspergillus flavus grew well and utilize more of the carbohydrate sources than *Aspergillus niger*, though *Aspergillus niger* showed preference for lactose utilization with a mean dry mycelia weight of 124.00±1.15 mg as against 71.33±0.88 mg for *Aspergillus flavus*. However, both fungi grew and fully utilized most of the nitrogen sources, except cysteine and L-glutamine, substrates where *Aspergillus niger* never showed any evidence of growth. However, *Aspergillus flavus* could not utilize three amino acid sources-cysteine, L-glutamine and D-β-phenylalanine.

DISCUSSION

This study was designed to investigate the effect of storage temperatures on the proximate and nutritional composition of the endosperm of the fresh, healthy, coconut fruits stored at 10°C and 30°C. The proximate composition of fresh, healthy, husked and dehusked coconut fruits as well as varieties of coconut fruits stored at different temperatures were studied in order to make a correlation between them. We observed that the percentage moisture content was 46.82% for fresh and healthy coconut endosperms a result that is line with the observation of Jarman and Jayasundara (1975) who noted that well aired copra has an initial moisture content of between 45-50%.

Anonymous (1993) showed the percentage moisture content of fresh, healthy nut to be 46.30, protein 4.08, fat 37.29, carbohydrate 11.29, crude fibre 3.39 and ash 1.03 as against 46.82±0.43, 3.77±0.05, 36.49±0.25, 11.89±0.22, 4.84±0.04, 0.98±0.01 obtained for % moisture, protein, fat, carbohydrate, crude fibre and ash respectively in this study. These results serve as standard to establish the initial nutritional state of the coconut fruits before they were subjected to the various treatments. The nutrients determined are the essential classes of food nutritional requirement of human, which includes protein, carbohydrate, fat, vitamins and minerals. It was established that the coconut fruits showed no evidence of spoilage before storage.

The result of proximate composition of the endosperm of dehusked fruits stored at 30°C for three months showed a decrease in the percentage moisture, crude fibre and ascorbic acid content from 46.82±0.09, 4.84±0.04 and 2.48±0.15 before storage to 5.86±0.09, 3.89±0.08 and 0.0714±0.0042 after storage respectively. At 10°C the values were 6.04±0.18, 3.79±0.09 and 0.07±0.003 as shown in Table 2. There was an increase in the percentage protein, fat, ash, carbohydrate, calcium, potassium, phosphorus, magnesium and iron. Kuku and Agboola (1984), Okpokwasili and Molokwu (1996) observed that an important prerequisite for mould development in vegetable oils is high moisture content. Okpokwasili and Molokwu (1996) in their study of different vegetable oils (groundnut, palm oil and palm kernel oil) isolated various moulds and yeasts genera-*Saccharomyces*, *Candida*, *Debaromyces*, *Hansenula*, *Trichosporon*, *Torulopsis*, *Pichia* (all yeasts) and *Aspergillus*, *Fusarium*, *Penicillium*, *Mucor*, *Geotrichum*, *Cladosporium* for moulds. However, only the genus *Aspergillus* was isolated from spoilt dehusked coconut stored at 30°C for 3 months.

Anonymous (1993) stated that the decrease in moisture results in a compensatory increase in none water-soluble nutrients of the coconut endosperm and that the coconut water that dried up during storage has it nutrients absorbed into the endosperm. Moisture and ascorbic acid are usually affected during storage. Fox and Cameron (1982) observed that storage decrease the ascorbic acid content of fruits and concentrate the quantity of other nutrients that are not affected by moisture loss.

For husked coconut fruits, there were increases in moisture, protein, fat, ash, carbohydrate, calcium, potassium, phosphorus, magnesium and iron content at the end of storage for three months. However, the crude fibre and ascorbic acid content decrease. This finding is in line with the observation of Fox and Cameron (1982) that ascorbic acid is lost during prolonged storage. The husked coconut fruits retained more moisture with 8.60±0.01 at 30°C and 10.61±0.01 at 10°C storage when compared with the dehusked coconut fruits which had percentage moisture content of 5.86±0.09, at 30°C and 6.04±0.18, at 10°C storage. This may be attributed to the husk still in place, which reduces evaporation of water from the fruit because of its thick fibrous mesocarp. The husked coconut fruit also retained more ascorbic acid.

It was observed that the dehusked coconuts fruits stored at 10°C and the husked coconut fruits stored at both 30°C and 10°C did not show any evidence of spoilage. Fox and Cameron (1982) stated that storage decreases the ascorbic acid content of fruits and concentrated the quantity of the other nutrients that are not affected by moisture loss, which may include the fat content. Labuza and Erdman (1993) emphasized that temperature higher than 10°C can increase the deterioration of fatty foods and went further to complement that lower temperature would preserve the product, nutrient and extent shelf-life. The proximate compositions of the endosperm of the dehusked coconut fruits that showed evidence of microbial spoilage after 3 months of storage at 30°C revealed a remarkable reduction in the percentage moisture, protein, ascorbic acid and carbohydrate content when compared with those obtained for same dehusked coconut fruits but without evidence of microbial spoilage.

From the study we noted that 80% of the fruits showed evidence of spoilage. According to Labuza and Erdman (1993), microbial spoilage of foods is accelerated when such foods contained growth-promoting elements that enhance the well-being of the invading microbes. They further stated that fats in foods may undergo quality deterioration during prolonged storage and that vitamins could be lost in fruits and vegetables particularly canned ones when stored at high temperatures of 38°C and above. Protein could as well be denatured and deteriorate in quality by high temperature storage. Certain amino acids such as lysine will chemically bind with simple sugars such as glucose to form brown pigments. This is known as the "Maillard" or browning reaction and it occurs non-enzymatically. This reaction causes the essential amino acids in foods to become physiologically unavailable. These results indicate that the spoilage organisms may be responsible for the decreases observed during deterioration in the nutrient composition of dehusked fruits.

The growth experiment done on the spoilage fungi of coconut shows that these fungi use the nutrient available in the fruits and render them unfit for human consumption. The results obtained *in vitro* are comparable to the deteriorating conditions they make to bear on these fruits on the field. Tests on the ability of these fungi to utilize different carbohydrates showed they can grow on a wide range of carbohydrate and nitrogen with *Aspergillus flavus* having a higher propensity to grow on all sugars compared with *Aspergillus niger*. However, *Aspergillus niger* (mycelia weight 124±1.15 mg) grew better in lactose medium than *A. flavus* (71.33±0.88 mg) with higher mycelia mass. This growth result in increase in mycelia mass with proportional decrease in sugar and nitrogen content of the growth medium. Both fungi showed disproportionate growth in the different sugars used showing that mean mycelia mass increase depending on the preferred carbohydrate and nitrogen sources. Maera *et al*. (1993) reported that the greatest hazard faced by stored coconut is its attack by *Aspergillus*, due to improper and prolonged storage. Both fungi showed evidence of growth and full utilization of almost all the nitrogen sources, except cysteine and L-glutamine in which *Aspergillus niger* did not show evidence of growth. *Aspergillus flavus* also did not grow on cysteine, L-glutamine and D-β-phenylalanine.

The moulds could not grow on certain carbon and nitrogen sources due to their inability to produce the specific enzymes that could degrade the compounds (Pelczar *et al*., 1983). These results show that the nutritional quality of coconut is affected by the growth of microorganisms.

On the other hand, the results showed that storage of coconut fruits at 30°C for long period could affect its nutritional composition, while storage temperature of 10°C will prolong its shelf life. In addition, coconut fruits stored with the husk kept longer than the dehusked coconut fruits. Reports from studies done on coconut have indicated that certain components of coconut causes reduction in diurnal post-prandial variation (Muller *et al*., 2003), prevent heart diseases as well as acting as poison antidote against acute aluminium phosphide poisoning (Becaria *et al*., 2002), lauric and monolaurin components act against dangerous viruses and bacteria (Preuss *et al*., 2005), reduction in cholesterol level (Nevin and Rajamohan, 2004). The

dietary conjugated linoleic acid has anti-carcinogenic (Ip *et al*., 1999; Liu *et al*., 2002), anti-antherogenic (Kritchevsky *et al*., 2002; Lee *et al*., 1994; Munday *et al*., 1999) and antiobesity (Hargrave *et al*., 2002; Park *et al*., 1999). Sequel to the importance that coconut play in human nutrition, prevention of microbial infection and other diseases associated with physiological processes, it is imperative that the plant be adequately preserved and prevented from both physical and microbial spoilage.

Conclusion: The healthy, unbroken, dehusked coconut fruits stored at 10°C and the healthy, unbroken, husked coconut fruits stored at both 10°C and 30°C kept best; because they show not any evidence of microbial spoilage.

We also established that fructose was the carbon source best utilized by the moulds with an average dry weight of the mycelia of 131.00±0.58 mg for *Aspergillus niger* and 679.67±0.88 mg for *Aspergillus flavus*. In addition, the nitrogen source supported luxuriant growth of the moulds was ammonium nitrate with an average dry weight mycelia of 1041.00±0.58 mg for *Aspergillus niger* and 1041.00±0.88 mg for *Aspergillus flavus*. The result established that the nutritional quality of coconut is affected by the growth of microorganisms, as seen in the reduction of the percentage protein and carbohydrate content of the coconut endosperm from 8.46±0.07 and 17.26±0.56 for dehusked coconut fruits stored at 30°C but without evidence of microbial spoilage to 3.98±0.07 and 9.79±1.02 for the endosperm of the dehusked coconut fruits that showed evidence of microbial spoilage after 3 months of storage at 30°C.

This study establishes that storage of coconut at 10°C guarantees longer shelf-life as well as enhances its nutritional and keeping quality. If these conditions are employed in storage process, it will definitely guarantee continuous supply of fresh coconut and ensure its supply all year round.

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