STUDIES ON THE ANTIMICROBIAL, NUTRITIONAL AND OTHER POTENTIAL USES OF *CANARIUM SCHWEINFURTHII (Úbe Okpoko)*

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A THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING, AS PART OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE (Ph.D) IN INDUSTRIAL/FOOD MICROBIOLOGY, OF THE NNAMDI AZIKIWE UNIVERSITY, AWKA, NIGERIA.

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CERTIFICATION

Mr. Orji, Jerry O., a postgraduate student in the Department of Applied Microbiology and Brewing majoring in Industrial/Food Microbiology has satisfactorily completed the requirements for course and research work for the degree of Doctor of Philosophy (Ph.D.) in Industrial/Food Microbiology. The work embodied in this thesis is original and has not been submitted in part or full for any other diploma or degree of this or other University.

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DEDICATION

This thesis is dedicated to God Almighty , for his gifts of life, strength, wisdom and finances at all times during the research.

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Abstract

Microbiological studies of *Canarium schweinfurthii* pear fruit obtained from four markets in four (4) South-Eastern State of Nigeria were carried out. The results showed that five bacterial and thirteen fungal species are associated with the spoilage of the pear fruit. The bacterial genera include *Klebsiella aerogenes, Streptococcus pneumoniae, Erwinia carotovora,*

Pseudomonas putida, and Flavobacterium spiritavorum. The thirteen fungal isolates were *Aspergillus glaucus, Alternaria alternate, Sacharomyces cerevisiae, Cladosporium herbarum, Geotrichum candidum, Aspergillus terrreus, Aspergillus versicolor, Penicillium citrinium, Aspergillus niger, e.t.c.* Reports of the analysis carried out in the four states showed that bacterial and fungal isolates associated with pear spoilage are evenly distributed, and not limited to a specific area. Of the thirteen fungal isolates, *Fusarium monoliforme* had the highest frequency of occurrence (7.4%) while *Aspergillus glaucus, Geotrichum candidum, Aspergillus terreus, Aspergillus versicolor, Candida tropicalis and Saccharomyces chevalieri,* each had percentage frequency of occurrence of 1.23%. Of the five bacterial isolates, *Erwinia carotovora* was the most widely distributed with 22.2% frequency of occurrence while *Flavobacterium spiritivorum* had a frequency of occurrence of 2.5%. The microbial counts of the pear fruits was found to increase with the time of storage, rising to the optimum count on the fourth day, dropping from the time the nutrients in the pears could no longer sustain the microbial load. The nutritional contents of the pear with the exception of protein were found to decrease with the time of storage/biodegradation. The wine produced from the pear had the following characteristics; pH 3.6, titratable acidity 2.6, specific gravity 1.071 and alcoholic content 6% (v/v). The results of antimicrobial activities of aqueous and ethanolic leaf and bark extracts showed that the extracts had antimicrobial activities on the test organisms at varying concentrations. The highest inhibition zone diameter of 20mm at 0.8gm/l was recorded for the ethanolic extracts against *Klebsiella pneumoniae.* The highest inhibition zone diameter of 15mm at 0.8gm/l was recorded for the ethanolic bark extracts against *Candida albicans*. The result of haemagglutination test of *NDV-Lasota virus* revealed that bark and leaf hot water extracts against *Candida albicans.* The result of haemagglutination test of *NDV-Lasota virus* revealed that bark and leaf hot water extracts and the control experiment showed haemagglutinin titre ratio of 1:1024. Also, the result of antibacterial activities of pulp oil extracts showed highest antibacterial activity on *Salmonella typhi* with mean inhibition zone diameter of 27mm at 4.0ml/ml concentration. The result of the phytochemical analysis showed the presence of alkaloids, saponins, tannins and flavonoids. This study shows that lower storage temperature of pear fruits slow down the rate of spoilage and that the pear fruit will be a good source of wine production. Further exploitation of this plant material for antimicrobial activity will possibly unveil the potential use in the treatment of diseases caused by the test organisms.

LIST OF APPENDIX

1.0 INTRODUCTION

1.1 General

Rich store houses of medicinal plants exist every where especially in Africa which offers a vast reservoir of plants that has been categorized *(*Aluyi *et al.,* 2003). The medicinal properties of various plant materials and extracts have been recognized since the beginning of the 5th Century (Kay, 1986).

The use of plant whether herbs, shrubs or tree, in parts or whole in the treatment of and management of diseases dated back to pre-historic times. Plants extracts have been used in folk medicinal practices for the treatment of different types of ailments since antiquity (Okanla *et al*., 1990). During the 5th century, the practice of herbalism became mainstream throughout the world. In spite of the great advances achieved in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medicinal systems. Medicinal plant, has been defined by WHO consultative group as any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Andrews, 1982).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005). The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Sofowora, 1993).

Canarium schweinfurthii Engl. (Burceracea) is known by different tribes in Nigeria by various names such as “ube” (Igbo), ‘atile’ (Hausa), ‘origbo’ (Yoruba), ‘njasun’ (Ekoi), ebenetridon (Efik)(Keay,1964). They are large evergreen trees up to 40 - 50m tall, with alternate pinnate leaves (Chudnoff, 1984). *Canarium schweinfurthii* grows well in savanna areas (grassland) and sandy soil. It is a deciduous and perennial plant tree mainly found in savanna regions of the world.

Despite the utilization of the fruit as food source for rural producers and consumers, it also

generates a large source of income for people in the developing countries when sold. It therefore produces the raw materials for wood industries (Farmer, 1977).

A bark decoction is used against dysentery, gonorrhea, coughs, chest pains, pulmonary affections, stomach complaints, food poisoning, and it is purgative. The pounded bark is used against leprosy and ulcers. The root is used against adenites whereas root scrapings are made into a poultice (Chudnoff, 1984).

The rhizomes and leaves are used as stimulant and against fever, constipation, malaria, diarrhea, sexually transmitted diseases, post partum pain and rheumatism (Koudou *et al.*, 2005).

The resin is used as a fumigant against mosquitoes, round worm infections and other intestinal parasites (Chudnoff, 1984).

1.2 Aims of the Study

The aims of this study are to isolate microorganisms associated with the spoilage of *Canarium schweinfurthii* fruit, to investigate the antimicrobial and preliminary phytochemical analysis of the leaf and bark extracts of *Canarium schweinfurthii*, and to unveil other potential use of the fruit.

2.0 LITERATURE REVIEW

2.1 Origin, Morphology, Varieties, Propagation and Management

Canarium schweinfurthii locally called abel (Cameroon), aiele (Ivory Coast), elemi (Nigeria), eyere (Ghana) and mwafu (Uganda) belongs to kingdom Plantae, division Magnoliophyta, class Magnoliopsida, family Burseraceae, order Sapindales, genus Canarium and species schweinfurthii. It is native to tropical Africa, southern Asia, Madagascar, Mauritius, India, southern China and Philippines (Chudnoff, 1984).

Canarium schweinfurthii is known by different tribes in Nigeria by various names such as ‘ube’

(Igbo), ‘atile’ (Hausa), ‘origbo’ (Yoruba), ‘njasun’ (Ekoi), ‘eben etridon’ (Efik) Keay, 1964. They are large evergreen trees up to 40 50m tall, with alternate pinnate leaves (Chudnoff, 1984). *Canarium schweinfurthii* grows well in savanna areas (grass land) and sandy soil. It is a deciduous and perennial plant tree, mainly found in savanna regions of the world. It has varieties which include *Canarium schweinfurthii* England and *Canarium schweinfurthii* Africa. *Canarium* itself has many species which encompass *C. indicum, C. ovatum* and *C. luzonicum. C. indicum* and *C. ovatum* are among the most important nut bearing trees in India and Philippines, and *C. luzonicum* produces the resin ‘elemi’. These species produce edible nuts known as *Canarium nut*, pili nut or Galip nut (Chudnoff, 1984).

Canarium schweinfurthii is a large forest three with its crown reaching to the upper canopy of the forest, with a long clean, straight and cylindrical hole exceeding 50m. Diameter above the heavy root swellings can be up to 4.5m. Bark thick, on young tree fairly smooth, becoming increasingly scaly and fissured with age. The slash is reddish or light brown with turpentine- like odour, exuding a heavy, sticky oleoresin that colours to sulphur yellow and becomes solid. Leaves are pinnate, clustered at the end of the branches, and may be 5-15cm long with 8-12 pairs of leaflets, mostly opposite, oblong, cordate at base, 5-20cm long and 3-6cm broad, with 12-24 main lateral nerves on each side of the midrib, prominent and pubescent beneath. The lower leaflets are bigger than the upper ones. The lower part of the petiole is winged on the upper side. The creamy white unisexual flowers about 1cm long grow in inflorescences that stand in the axis of the leaves and may be up to 28cm long. The fruit is a small drupe, bluish-purple, glabrous, 3-4cm long and 1-2cm thick. The calyx is persistent and remains attached to the fruit. The fruit contains a hard spindle-shaped; trigonous stone that eventually splits releasing 3 seeds. The seeds are mainly dispersed by hornbills and elephants.

The tree is propagated through seedlings, wildings and direct sowing on sight. The seeds should be immersed in hot water and allowed to cool then soaked for 24 h before sowing.

The tree is amenable to mixed culture plantation husbandry. The ripe fruits should be collected when they fall to the ground and allowed to decompose, the stones should then be separated from the outer fruit coats. Seeds can be stored for a long time (Chudnoff, 1984).

The domestication of *Canarium schweinfurthii* was first reported in the west of Cameroon by Chudnoff (1984). It began with the development of a seed germination technique which finally made it possible to obtain an average rate of seedling emergence of 95% at the end of 3 months period. Plantations then stayed in a tree nursery for 6 months before being transplanted to an experimental plot. Growth, flowering and fruit formation were then followed. The result showed that in the *Canarium schweinfurthii* stand, the average survival rate decreased year after year and was stabilized at 84% from 1992 to 1997. It was only 42% in 2000 as a result of the damage caused by a bush fire in March 1998. In 2000, the average total height of the 1yr-old trees reach 6.8m, and their trunks had a mean diameter of about 8.6cm. The dorminant height and diameter were 10.8cm and 14.3cm, respectively. *C. schweinfurthii* is a dioecious species.

The formation of the first fruits happened with 8yr-old trees. From 1996 to 2001, 19% of the studied trees within the plot entered blooming and fruiting. Floral analyses showed that, during the same period, the experimental *C. schweinfurthii* stand presented a sex ratio of 38% of male individuals for 62% of female trees.

Fig. 1: *C. Schweinfurthii* Tree during dry season

Fig. 2: *C. schweinfurthii* Leaf

2.1.1 Chemical and Nutritional Composition of African Pear Fruit

Fruits and vegetables contribute substantial nutrients to the diet of the people of the tropics. Most fruits and vegetables are high in water and carbohydrates but are low in protein and fat. The water content is frequently greater than 70%. Generally, the protein content of fruits is not greater than 3.5% and fat content is usually lower than 0.5% (Kordylas, 1980; Onimawo and Egbekun, 1998). There are exceptions to the above generalization on the nutritional contents of fruits. Pears, most especially African pear fruit has low moisture differently reported as 9% (Onuorah *et al.*, 2001) and 12% (Omoti and Okiy, 1987). Its protein content was respectively reported to be equal to 6.39% (Onuorah *et al.*, 2001).

Fruits are also good source of organic acids and fair to good source of ascorbic acid (Okaka *et al*., 2002). They are rich in vitamins A, B, C and E and are source of micronutrients. The carbohydrates of fruits are both digestible and indigestible. The digestible carbohydrates

according to Enwere (1998) provide sugar and starch while the indigestible carbohydrates are cellulose, pectin materials and fibre, which are important in normal digestion.

The composition of fruits depends not only on botanical variety, cultivation practices and weather but also in the degree of maturity prior to harvest and condition of ripeness, which continues after harvest (Potter and Hotchkiss, 1998). The post-harvest role of ripening depends on storage method. The nutritional composition of fruits had been found to differ significantly depending upon variety, geographical origin, methods of processing and chemical analysis.

Canarium schweinfurthii fruit is vital due to its nutritional composition and value as a major and cheap source of minerals, vitamins, carbohydrates and fats. It can yield a notable dietary contribution which could ameliorate the food problems in the developing countries since it is not available at most seasons including strategic period of the year when the conventional staples that are difficult to store are scarce (Getahun, 1974; Roche, 1975; Okafor, 1975a; Okigbo, 1977).

The proximate composition and selected physiochemical properties of the fruits of *Canarium schweinfurthii* have many variations, which exist across tree locations (countries), seasons (time of harvest) and degree of maturity.

Most of earlier data on nutritional composition of *C. schweinfurthii* fruit showed that the pulp contained 32.25% moisture, 3.25% ash, 23% crude fat, 0.75% crude fibre, 20.43% crude protein and 20.10% carbohydrate. It also contained 0.21% water soluble ash, 1.75% titrable acidity and 98.45% mg/100g calcium.

The pulp was found to be slightly acidic with a pH value of 5.4. Other physical characters include specific gravity of 1.0012, total soluble solids of 8.2 (Brix) and refractive index of

1.3568 (Onimawo and Adukwu, 2003). On a dry matter basis, the mesocarp contained 68.3% free flowing lipid (FFL) and 13.7% bound lipid (BL) while the endocarp contained 67.0% FFL and 13.0% BL.

The quality characteristics of the mesocarp oil extracts were 151.9 – 195.3mg KOH/g fat saponification value (SV), 20 – 40mEq peroxide /kg fat peroxide value (PV), 71.1 – 94.9g iodine/100g fat iodine value (IV) and 1.33 – 8.30mg KOH acid value (AV). Characteristics for the endocarp oil extracts were 95.4 – 184.3 mg KOH /g fat SV, 4.0 – 8.0m Eq peroxide /kg fat PV, 100.1 – 118.3g iodine/100g fat IV, and AV of 0.48 – 8.70 mg KOH. The fatty acid composition of the first hexane extracts indicated that oils were primarily C16 and C18s. The mesocarp contained 31.7% hexadecanoic acid, 30.0% 9 – octadecanoic acid, 30.1% 6, 9 – octade cadienoic acid and 8.2% 9, 12, 15 octadeca trienoic acid, while the endocarp, contained 31.2% hexadecanoic acid, 28.9% 9 – octadecanoic acid and 31.3% 6, 9 – octadecadienoic acid (Abayeh *et al*., 1999).

Pears generally have the highest energy value than other fruits because of its high oil/fat contents. The energy content ranges from 275kcal/100g in avocado pear (Potter and Hotchkits, 1998) to 545kcal/100g in African pear fruit (Onuorah *et al*., 2001).

2.1.2 Oil

The oil of the African pear fruit is rich in unsaturated fatty acid, which are relatively stable, satisfactory nutritionally and comparable to other currently used vegetable oils (Osagie and Odutuga, 1986). The main fatty acids of the lipid fraction of African pear fruit are palmitic acid, oleic acid and linoleic acid giving a profile similar to palm oil (*Elaesis guineensis*) (Leakey, 1999). Linoleic acid found in the African pear fruit oil is an essential fatty acid for which the

recommended minimum dietary level is about 3% of the Calorie content of the diet (FAO, 1977). The fatty acid contents of African pear fruit oils from different agro-climatic areas are similar as shown by chromatographic determinations carried out by Kapseu *et al.* (1999). The triglycerides of the fruit oil are composed mainly of dipalmitolein, palmitodiolein and palmitolino-olein (Kapseu and Tchiegang, 1996).

African pear fruit is a highly oil fruit (33.3% oil in the pulp) with physical and chemical characteristics that agree with specification (20% content) of Codex Alimentarus Commission for an edible oil (FAO/WHO, 1993). The chemical and physical characteristics of African pear oils are similar to those of palm oil in terms of the saponification value, iodine number, specific gravity, refractive index and titrable acidity values.

The fruit protein had all the essential amino acids required by man with exception of tryptophan (Omoti and Okiy, 1987). Tryptophan is lacking in the fruit. Most oil bearing seeds like soybeans, cotton seed, peanut and sunflower are rich in protein with appreciable presence of some essential amino acids (Salunkhe and Desai, 1986) can be consumed directly by poor people to overcome the problem of malnutrition in place of the expensive animal protein. The concentration of leucine and threonine in African pear fruit are comparable to those found in protein of hen's egg, cow's milk and beef muscle (Davidson *et al*., 1972).

The crude fibre of African pear fruit is 17.9% (Omoti and Okiy, 1987) or 10.67% (Onuorah *et al*., 2001) and this is high. Fibre constitutes primarily cellulose, hemicellulose, lignin, gums and other mucilages. These are not hydrolysed by enzymes secreted into the digestive tract (Trowell, 1974). Fibres when taken may also aid the peristaltic movement of the intestinal tract (Omoti and Okiy, 1987).

The ash content of 10.80% for African pear fruit as reported by Omoti and Okiy (1987) is

three times higher than 3.33% recorded by Onuorah *et al*. (2001)and is far much smaller than the figure given by Omoti and Okiy (1987). These differences are equally reflected in the respective ash contents.

Vitamins A and C content of the fruit is reported to be equal to 0.65kg/100g and 209mg /100g by Okafor *et al.* (1996). However, Achinewhu (1983) reported a lower vitamin C content of 24.5mg/100g for African pear fruit and disclosed that most of this vitamin is lost by some forms of cooking, at times the skin (epicarp) of the fruit is discarded before consumption. It has been discovered that this discarded skin contains some vital vitamins and minerals required by consumers.

The oils have important role in health maintenance. Thus not only in a fat/oil a concentrated source of calories or of unsaturated fatty acids for optimum physical integrity of membranes, but it also provides precursors of physiologically active compounds with potent regulatory functions (Kinsella, 1981). Small amount of dietary linoleic acid (1-2%) of dietary calories (approx 6-7g/day) is reported to relieve essential fatty acid deficiency. Essential fatty acid deficiency symptoms include poor growth, scaly skins (dermatosis), erythrocyte haemolysis, impaired reproduction, mitochondrial swelling, poor energy deficiency and internal hemorrhaging (Holman, 1978). Linoleic acid, which occurs abundantly in most vegetable oil is considered principal essential fatty acids (Kinsella, 1981).

African pear fruit cake has quite a high content of cellulose (18%) on dry weight basis, low forage value and digestible protein content of 138g/kg of dry matter (Kilekoung *et al*., 1998). It is also fairly rich in minerals and organic matter with high total energy giving a similar grade to those of groundnut and cotton seed cakes. This confirms its suitability in livestock feed supplementation. The amino acid profile and oil composition of the feeds justify its

recommendation as a supplement in cereal and starch livestock feed (Omoti and Okiy, 1987; Obasi and Okolie, 1993).

Studies on the textural and biochemical changes occurring in *Canarium schweinfurthii* fruits, after harvest was by Virginie e*t al*.(2005).

The fruits were harvested and stored for 7 days (stored hardened fruits), heat-treated at 45^{o}C for 40min (heat softened fruits), and heat-treated at 70^{o}C for 40min (heat hardened fruit). The samples were evaluated for their textural properties, moisture, reducing sugars, total soluble sugars, ash, protein, starch, cellulose, lignin and uronic acids. The hardness of the fruits increased with storage (15.83 to 20.71 N respectively for raw fruits and stored hardened fruits), while the shear force varied from 0.77 to 5.41 N (respectively for heat softened fruits and heat hardened fruits) making the consumption of heat hardened fruits impossible. Chemical analysis showed no significant differences ($P<0.05$) between the values obtained for ash, protein, minerals (Na, K, Mg). The moisture of the pulp decreased from 49.32 to 39.90% in a period of 7 days of storage, suggesting that there was dehydration after harvest. Uronic acid, cellulose and lignin increased during storage. Heat hardened pulp showed different changes in the chemical indices than those stored at 22^{o}C for 7 days, suggesting another mechanism of hardening.

The antioxidant and antimicrobial activities of *Canaruim schweinfurthii* essential oil from Central African Republic were investigated by Obame *et al*. (2007) using 2, 2 – diphenylpicrylhydrazyl (DPPH) radical scavenging assay and the ß – carotene bleaching test. Butylated hydroxytoluene (BHT) was employed as a positive control. The essential oil showed antioxidant and DPPH radical scavenging activities, and it displayed the inhibition of lipid peroxidation.

The antibacterial and antifungal activities of the essential oil of *C. schweinfurthii* from

Central African Republic were also evaluated against twelve strains of bacteria and three strains of fungi using agar diffusion and broth microdilution methods. The essential oil showed antimicrobial activity against almost all the strains studied. The results suggest that *C. schweinfurthii* essential oil could be a natural antimicrobial and antioxidant agent for human and infectious diseases and in food preservation. Furthermore, the development of natural antimicrobial agents will help to decrease negative effects (pollution of environment, resistance) of synthetic chemicals and drugs.

In recent times, the desire to conserve resources spent on importation of oil for domestic and industrial use gave renewed impetus in the search for novel sources to complement the traditional ones. Attention has therefore, been focused on under utilised local seeds for possible development and use (Burkill, 1985).

The physicochemical studies on oils from five selected Nigerian plant seeds was conducted by Akubugwo *et al*. (2007). Oils were extracted from four underutilized seeds of the Nigerian plants *Chrysophylum albidum, Dacryoides edulis, Landolphia owariensis, Napoleona imperialis* using n-hexane and their physicochemical properties compared with oils from seeds of *Elaeis guineensis.* Percent oil yield were 12.00, 15.80, 6.40 and 8.00 for *C. albidum, D. edulis, L. owariensis, N. imperialis* respectively while the value for *E. guineensis* seed is 28.00. The four seed oils were odourless and at room temperature liquids as against *E. guineensis* seed oil that were semi-solid under the same condition. Specific gravity of the seed oils ranged from 0.82-0.94 while peroxide value for all the oil extracts except that from *D. edulis* seed were less than three. Saponification values were as low as 42.40 in *L. owariensis* and as high as 246.60 in *E. guineensis* seed oils. Iodine values were between 15.10 and 45.00 in the extracts. These results suggest that *C. albidum* and *D. edulis* seeds may be viable sources of oil going by their oil yield.

However the studied characteristics of all oils extracts in most cases compared favourably with *E. guineensis* seed oil which is presently used for many domestic and industrial purposes in Nigeria.

The antifungal properties of the aqueous and ethanolic extracts of *C. schweinfurthii* plant leaf and bark using the agar-well diffusion method was investigated by Orji *et al.* (2008). The fungal isolates employed were *Candida albicans, Tinea rodensis, Penicillium marneffei.* The phytochemical analyses of the *C. schweinfurthii* leaf and bark extract were also determined. The result of the study showed that the aqueous and ethanolic leaf extracts of the plant materials produced inhibitory activity against all the tested fungal isolates. However, while the bark aqueous and ethanolic extracts produced inhibitory activity against *Candida albicans* and *Tinea rodenses,* the aqueous extract could not exert any inhibitory activity against *Penicillium marneffei.* The highest inhibition zone diameter of 15mm at 8 gml^{-1} was observed for the ethanolic bark extracts against *Candida albicans.* The minimum inhibitory concentration (MIC) ranged from $0.4gml^{-1}$ – $0.8gml^{-1}$. The result is noteworthy especially in recent times when Candidiasis prevalence has escalated following the emergence of immunosuppressive conditions such as HIV/AIDS.

It was further observed in this study that the ethanolic extract of the plant materials was the most effective against all tested fungi. This could be an indication that the active principle in the plant bark and leaf are probably more soluble in ethanol than water.

The phytochemical analysis of the *C. schweinfurthii* bark and leaf extracts revealed the presence of flavonoids, alkaloids, tannic acid, saponins and phenol. The medicinal values of these and other constituents have long been highlighted (Lever *et al*., 1979).

2.1.3 Economic Importance and General Uses

The urgency of the world's food problem, especially in developing countries of Africa and the need for cheaper and economically more friendly raw materials, have in recent years brought to limelight the potentials of tropical fruit species (Okorie, 2001). African pear fruit has good nutritional value as recorded above by the proximate composition of the fruit and its seeds. It is rich in oil, food energy, and mineral elements and in some degree protein and carbohydrates. It could be a good supplement especially in supply of iron (Onuorah *et al*., 2001). Analysis of the fruit, seeds and tree exudates revealed no toxic principles.

The slightly greenish outer pulp of the *Canarium schweinfurthii* fruit is oily and edible. It can be eaten raw or softened in warm water to improve palatability.

The pulp oil is about 71% palmitic acid and 18% oleic acid. The seed – kernel is oily and edible. They are cooked, and in Nigeria, sometimes prepared into a vegetable butter and eaten as a substitute for shea-butter. They contain several fatty acids including oleic (36%), linoleic (28%), palmitic (26%) and stearic (7%) (Chudnoff, 1984). The fruit pulp contains 30 to 50% of oil used for the manufacture of shampoos and biofuel (Tchiegang, 2001; Ajiwe *et al*., 2000).

2.1.4 The Sapwood

The sapwood, often very thick up to 15cm width with pinkish reflections. The wood, slightly coarse in texture, has interlocked grains, thus causing fine striped figures on quarter-sawn boards. It is used as a substitute for the true mahogany, it seasons slowly but fairly well, works easily, stains and polishes well. End splitting may occur during the drying process. The wood is attacked by termites and fungi. Impregnation of the heartwood is difficult. The timber is used as veneer, for decorative paneling, paraquetry, furniture, flooring and for general utility purposes. Locally, the wood is used for mortars, planks, and Canoe (Chudnoff, 1984).

2.1.5 The Bark

The bark of *C. schweinfurthii* exudes a heavy, sticky Oleoresin that smells like turpentine and solidifies to a whitish resin. It is obtained by slashing the bark and allowing the colourless expiation to trickle to the ground where it solidifies into a sulphur yellow opaque resin. The resin is used as primitive illuminant and as incense and releases a lavender-like smell. The flame is very smoky and soot is collected as carbonblack from the outside of pots held over it for use in tattooing and to make ink in Liberia. The resin is also used to repair broken pottery, for caulking boats and as a gum for fastening arrowheads to shafts (Chudnoff, 1984).

The rhizhomes and leaves are used as stimulant and against fever, constipation, malaria, diarrhea, sexual infection, post-partum pain and rheumatism (Kouduo *et al*., 2005; Ake-Assi; and Guinko, 1991).

A bark decoction is used against cough, chest pain, pulmonary infection, food poisoning, and it is purgative. The resin is used as a fumigant against mosquitoes, roundworm infections and other intestinal parasites. It is an emolient, stimulant, diuretic and has action on skin infections and eczema. The seeds are string into necklaces or attached to traditional instruments. The back of young tree is split off in Gabon to make boxes (Burkill, 1994).

The oil extracted from African pear fruits compared with oils conventionally used in manufacture of soaps, bakery production and pharmaceutical products showed higher specification, iodine and acid values making them equally useful for these industries (Ejiofor and Okafor, 1997). The whole fruit oil can be used as eco-fuel in cars and soap industry while the residual cake is used in animal feed (Kalenda *et al*., 2002). The higher iodine value of African pear fruit seeds placed it as semi-drying oil. Ajiwe *et al* (1997) had used the oil to produce alkyd resin, paint, wood varnish, grease, polish, skin cream, which compared favorably with

commercial products. The cake remaining after the production of the pulp oil may be useful in the food industries such as bakeries and baby foods (Kalenda *et al*., 2002).

The fruit is widely marketed at a local scale and are traded between neighboring countries with Cameroon being the main exporting country (Okafor, 1993). A small quantity of fruit is also exported to Europe and America for expatriate African consumers.

The fresh fruits are also exported from Democratic Republic of Congo to certain European countries like France and Belgium where oil is extracted by solvent extraction from the pulp and seed for use in manufacture of margarine, paint, cosmetics, candle and other industrial purposes (Bassey, 1982; Silou, 1991). This regional trade has been monitored and quantified over recent years and is growing substantially. In addition to being almost a staple food during a fruiting season, the fruit has potential to be an oil crop (Leakey and Ladipo, 1996). The high demand for the fruit has however encouraged governments and private investors to establish African pear fruit plantations. The potential for provision of economic product is great (Leakey, 1998).

Fig. 3: *C. schweinfurthii* pear fruits

2.1.6 Microbiology of the *Canarium schweinfurthii* Fruits

Bacteria and fungi have been reported to be associated with spoilage and post harvest disease of *C. schweinfurthii*. Such fungi are *Alternaria, Aspergillus* and *Penicillium.* Among the bacteria are watery soft rot species and *Pseudomonas* species (USDA, 1974). These bacteria and fungi have negative undesirable effect on the fruit quality. This often leads to changes in taste, appearance, texture and smell of *C. schweinfurthii*. The fruit being a good and suitable host for some microorganisms, their nutrients are good substrates for microbial growths and proliferation

(Coronel, 1991).

2.1.7 Harvesting, Transportation and Preservation

The harvesting of *C. schweinfurthii* fruit is done mainly during the rainy season and early dry season usually June – November. The fruits of *C. schweinfurthii* on a tree do not ripen at the same time, and it is best to harvest only well-ripened fruits (which skin is nearly black). The present system of harvesting is a laborious process. The harvester climbs the tree and with a wooden stick, repeatedly thrashes the branching in the process detaching even the immature fruits and severely defoliating the tree. As mentioned earlier, there is a locally held belief (which has a scientific basis) that this is a "punishment" that is beneficial to the tree, as it induces the tree to produce more flowers and fruits the next fruiting season. Ripe fruits may simply be allowed to fall to the ground. It is then collected manually or by machine. The fruits are usually placed in sacks or bags and taken to people's homes or factory for processing (Coronel, 1991).

In south eastern Nigeria, whether for family consumption or for the local market, the African pear is usually harvested when mature and ripe. The decision to harvest is determined by the usual appearance of the fruits. Traditionally African pear fruit is harvested when the fruits have wholly turned bluish – black (Kapseu and Kayem, 1998). However, tree owners are sometimes forced to harvest the fruits when they have not wholly turned bluish – black as a result of any or a combination of the following: financial need, forces of supply and demand (i.e. when selling prices are highest), safeguard against unplanned harvesting by children and unwanted persons.

Packaging provides protection from mechanical damages, undesirable physiological changes and pathological deterioration during storage, transportation and marketing. Series of researches in post-harvest handling of fruits and vegetables had advocated the use of rigid

accelerated plastic crates especially in the packaging of fruits (Olorunda, 1998). The harvested African pear fruits are packaged into raffia baskets, transported home and later taken to the market. Packaging of fresh fruits also has a great significance in reducing the wastages (Nwufo and Ayim, 1998). A perfect and efficient marketing system covers all aspects of handling from the point of harvesting to the stage where the commodity reaches the consumers. Marketing of perishable fruits like African pear fruit presents more problems compared to other durable agricultural commodities (Emebiri and Nwufo, 1990).

Post harvest losses occur at the period between harvesting and consumption. They are primarily due to desiccation, rotting, sprouting, inulin degradation in fruits and vegetables (Peris *et al*., 1997). It has been estimated that between 30 – 60% of fruits and vegetables produced in Nigeria go to waste and they undergo tremendous chemical changes once they are separated from their parent plant and soon start deterioration (Onimawo and Egbekun, 1998).

There are various causes of post-harvest losses in fruits and vegetables. Snowdown (1990) identified bacteria and fungi as the causes of post-harvest deterioration and losses. Losses in quantity and quality of fruit may also be contributed by mechanical injury, which leaves behind a physical effect. Mechanical damage is a very important aspect of post-harvest changes and it is the primary cause of many losses (Coursey and Booth, 1972). During the harvesting and post-harvest handling some fruits are bruised and squashed or crushed thereby facilitating the development and spread of rot diseases and invasion of the interior tissue (Meredith, 1971). Bruises received by African pear fruit during harvesting and handling also results to breakdown at the point of injury and is likely to lead eventually to rotting by microbial action (Kordylas, 1990).

Casual agents such as the weather during growing season, insects, rodents, birds, farm

implements, etc can cause various types of injury on African pear fruit before, during or after harvest (Dennis, 1983). Insects, rodents and birds leave holes or openings, after eating the fruits and these become points of invasion or infection by microbes.

Softening is the main cause of post-harvest losses in African peer fruits. Studies also revealed that fruit injury accelerates pulp softening (Silou *et al*., 1995). Chemical and biochemical processes, which go on after the harvest of fruit are autolytic and also bring about spoilage (Okaka, 1997). The induction of endogenous cell wall degrading enzymes cellulase, pectinesterase, polygalacturonase and proteinase by heat is responsible for heat-dependent softening of the pulp of *Dacryoides edulis* (Okolie and Obasi, 1992).

2.1.8 Post-Harvest Deterioration of African Pear Fruits

Post-harvest deterioration is the spoilage that sets in after harvest of crop produce (Nwufo *et al*., 1987). The deterioration is generally a negative undesirable effect of microorganisms on fruits. This often results to changes in texture, taste, appearance and smell, most important still, poisonous substances may be produced (Muller, 1988). Snowdown (1990) reported that the microorganisms responsible for fruits spoilage are actually present in the fruits and proliferate in a favourable environment.

The disease of fruits results from the growth of an organism that obtains its food from the host (Frazier and Westhof, 1995). A food is said to be spoilt if it has been damaged or injured so as to make it undesirable for human use. Food spoilage may also be caused by insect damage, physical injury of various kinds such as bruising and freezing, enzyme activity or microorganism (Jay, 1986).

Mature fruits are highly susceptible to invasion by specific pathogenic microorganisms because they are high in moisture and nutrient and are no longer protected by the intrinsic factors

which conferred resistance during development on the plant. In addition, many fruits become easily injured as they approach full maturity and therefore become vulnerable to pathogens (Muller, 1988).

Generally, fruits have high moisture content (about 85%) and average carbohydrate (about 13%). Fruits differ from vegetables in having somewhat less water but more carbohydrate. The mean protein, fat and ash contents of fruits are 0.9%, 0.5% and 0.5% respectively and they contain appreciable quantities of vitamins and organic compounds (Potter and Hotchkiss, 1988). On basis of nutrient content these products would appear to be capable of supporting the growth of bacteria, yeasts and moulds. However, when pH alone is considered it is found to be below the level that generally favour bacterial growth and this explains the general absence of bacteria in the incipient spoilage of fruits. The wider pH range of mould and yeast suits them as spoilage agents of fruits. With the exception of pears, which sometimes undergo *Erwinia* rot, bacteria are of no known importance in the initiation of fruit spoilage (Haard and Salunkhe, 1975).

Post-harvest diseases of fruits and vegetables are of two types (Eckert, 1978):

1. Those in which the infection occurs when the produce is still immature and attached to the plant, i.e. pre-harvest infection. Rain and wind transport spores of fungi, which sporulate in lesions on stems, leaves and flower parts of infected plants on to the fruits of such plants. These spores germinate when free water is present and develop to a limited extent before the resistance of the cells of the immature fruits halts growth. This infection is caused by organisms such as: *Monilinia fructicola* (which causes brown rots in mangoes and peaches), *Botrytis cinerea* (gray mould rot in strawberries and grapes), *Phytophthora* (brown rots of citrus), *Diplodia, Phomopsis* and *Alternaria* (stem end rots

of citrus) and *Dothiroella* (rot of avocado).

2. Those in which infection occurs during harvest operation and subsequent handling and marketing, e.g crown rot of banana and pedicel rot of pineapple.

Common disease of apples and pears include the black spot or scab, caused by the *Venturia ina equalis* (Adams and Moss, 1995).

The anthracnose of avocados cause the microbial deterioration of fruits especially in avocado growing countries like USA and Puerto Rico (Rowell, 1983).

The most important microorganisms in decay of fruits and vegetables as reported by Haard and Salunkhe (1975) are fungi and bacteria. In fungal decay, a variety of genera attack the sugars found in fruits and bringing about fermentation with the production of alcohol and carbon dioxide and they often preceed the mould in the spoilage process of fruits and vegetables are *Rhizopus, Aspergillus, Penicillium, Sclerotina* and *Colletotrichum*. *Rhizopus stolonifer* causes rapid (soft) decay of the sweet potato. The stringy and the watery exudates often wet adjacent roots (Smith *et al.*, 1966). Most soft rot diseases of fruits and vegetables are caused by bacteria and this may occur in the field or on the commodity after harvest. Examples of such bacteria are *Erwinia, Pseudomonas, Clostridium* and *Bacillus*. Most of these pathogens are weak and can only invade damaged produce.

Erwinia carotovora and *Pseudomonas marginalis* are the causative organisms for bacterial soft rots. They break down pectins giving rise to a soft, mushy consistency, sometimes a bad odour and water-soaked appearance. It is likely that the organisms are present on the susceptible fruits and vegetables at the time of harvest (Mount *et al.*, 1987). The cementing substance of the fruit and vegetable body then induces the formation of pectinases, which act by

hydrolyzing pectin thereby producing mushy consistency once the outer plant barrier has been destroyed by these pectinase producers. Non-pectinase producers no doubt enter the plant tissue and help bring about fermentation of the simple carbohydrates that are present. Also fruits like mangoes, avocados, pears, guava and some vegetables are firm at harvest time because they contain protopectin. During storage the protopectin breaks down into soluble pectins with the attendant loss of containing properties, which results to softness (Muller, 1988).

A lot of factors facilitate the infection process of fruits and vegetables but for convenience may be classified into three namely; mechanical damage, insects damage or environmental damage. The physical damage goes a long way to facilitate the invasion of the fruits by microorganisms, resulting in a progressive decay, which may affect the entire organ (Coursey and Booth, 1972).

Insects are also involved in spreading of diseases. Fruit flies attracted to fruits and vegetables infected with soft rot carry spores of the fungus *Geotrichum* from infected produce to healthy produce (Ihekoronye and Ngoddy, 1985).

Environmental conditions that are conducive for soft rot development include abundant rainfall or irrigation; poor drying conditions, high temperatures of 22^{o}C to 35^{o}C and relative humidity greater than 90% are best for soft rot development. Also fruits and vegetables not protected from the heat of the sun after harvest or piled up in sacks where there is no adequate air flow and build up of heat. These factors accelerate the biodeterioration of the fruits. Some microorganisms continue to grow slowly even at temperatures near freezing such as *Penicillium* (Rosen and Kadir, 1989; Anon, 1979).

In growing plants, the carbohydrates, which are broken down during respiration are replaced by products of photosynthesis produced by the leaves. In harvested plant parts, only the

existing carbohydrates are available and so ultimately there are only two alternatives, cell death from natural causes or colonization by microorganisms (Sherman, 1987).

APF changes from purple black or blue-black to dirty brown or grey, soft and distasteful few days after harvest. The major causes of spoilage according to Kordylas (1990) are directly related to chemical, physiological and microbiological or insect attack as noted in fruits and vegetables. All these factors are inter-related.

Harvesting and post-harvest practices (such as bulking in baskets or containers during transportation and storage) results to some fruits being bruised thereby facilitating the development and spread of rot diseases (Nwufo *et. al.*, 1989). Survey carried out in six locations in south eastern Nigeria to assess the incidence of rots by Nwufo *et al*. (1989) showed that four rot disease microorganisms are responsible for rot diseases in *Dacryodes edulis*. These rot disease microorganisms are fungi, namely *Botryodiplodia theobromae, Rhizopus stolonifer* and *Aspergillus niger*. The fourth microorganism is a bacterum (*Erwinia*). When African pear fruits are infected with these microorganisms, they cause soft rots characterized by softening of the mesocarp with accompanying repulsive odour and discolouration.

Discolouration produced by these rot disease micro organisms when inoculated in the African pear fruits are brown by *Aspergillus niger* and *Erwinia*., light brown by *Rhizopus stolonifer* and greyish by *Botryodiplodia theobromae* (Nwufo *et al*., 1989). *Botryodiplodia theobromae* and *Rhizopus stolonifer* are found to be most important soft rot organisms on the fruits accounting for about 80% of the total loss of the fruit while *Aspergillus niger* and *Erwinia* account for the remainder (Nwufo and Anyim, 1998). African pear fruit infected by *Botryodiplodia theobromae* caused a loss of 61% starch and 2% of the total nitrogen while lipid content increased by 19.6%. Infection by *Aspergillus niger* caused a starch loss of 71% and a

loss of 4.6 % of the total lipids and increased content of total nitrogen was 89.6% (Nwufo *et al*., 1989) *Aspergillus niger* in APFs causes soft rot spoilage by production of large amount of amylases which convert the starch of the pear into amylose, thereby altering the fruit nutrient content (Berg, 1993). *Rhizopus stolonifer* caused a starch loss of 81% and a loss of 7.3% total nitrogen while the total lipid content increased by 0.9%. *Rhizopus stolonifer* caused a starch loss of 81% and a loss of 7.3% total nitrogen while the total lipid content increased by 0.9%. *Rhizopus stolonifer* also isolated from avocado pear (*Persea americana*) is characterized by rapid decay of ripe avocados and the formation of a coarse white mould with black spore heads (Darvas and Kotze, 1987).

2.1.9 Shelf- life and storage potential of African pear fruits

Shelf- life is defined as the period between packaging a product and consuming it during which the quality of the produce remains acceptable to the user (Onuegbu, 2004). Normah and James (1977) defined shelf life as the period between processing and retail purchase and use of a food produce. During this finite period of time the product is in a state of satisfactory quality in terms of nutritional value, texture, taste, appearance and use.

Most fruits and vegetables have peak season and off–seasons and economic implication of this seasonal availability leads to price differentials and instability (Onimawo and Egbekun, 1998). Proper post- harvest handling of fresh fruits will prevent seasonal glut and preserve the quality of fruits. African pear fruit is highly perishable and due to poor keeping quality is susceptible to heavy storage losses (Okorie, 2001). Much research work has been directed towards the determination of optimum storage conditions for temperate fruits and vegetables as well as those of the tropical fruits and vegetables. Storage life of most fruits and vegetables are prolonged at low temperature (Hakin *et al*., 1997). The shelf life of fruits and vegetables can also

be extended using waxing, protective packaging, and controlled atmosphere storage. Post–harvest deterioration can be reduced using ethylene addition or removal, fungicide dips as well as the above methods (Okorie, 2001; Saltveit and Mencarelli, 1988). Prolonged storage ability is a particularly very useful characteristic especially under the African marketing system where refrigeration facilities are presently scarce and transportation networks, mostly very poorly developed (Okorie, 2001).

Temperature has a marked effect on the storage duration of pear. Temperature is one of the important factors that determines the rate at which microorganisms develop in fruits. The effect of a particular temperature varies with the product but in general, the higher the temperature, the faster the enzymatic action (Muller, 1988). Warmer temperature can severely limit pear storage life (Kupferman, 1995). At ambient temperature fruits show an exceptionally rapid softening (Kalerda *et al*., 2002).

Treatment of the African pear fruits with ethylene oxidants such as potassium permanganate ($KMn0_4$) instead of prolonging the storage life like bananas, plantains and tomatoes (climacteric fruits), shortened the post- harvest life of the fruit from 6 - 8 days to 2 – 3 days even when stored at 10^oC (Okonkwo, 1984). Onuegbu (1995) disclosed that the wholesomeness of African pear fruit subjected to different storage treatments with wood ash, dry local pepper and lime was maintained for 27 days at 10^oC. African pear fruit mesocarps are traditionally preserved in Cameroon by drying which reduces the moisture content to very low level and this prevents microbial deterioration. This traditional method no doubt preserves the pear but brings about the loss of cherished natural flavour and texture. However, the yields of oil extracted from the dried fruit mesocarps are higher than that obtained from fresh fruit mesocarps (Ali *et al*., 1997).

Refrigeration is a technique used to keep fruits and vegetables in the freshest possible state for as long as required after harvest (Okaka, 1997). Each of their qualities such as appearance, aroma, colour, texture, acidity, drained weight and vitamins respond favourably to refrigeration. Low temperature is known to reduce respiration; enzyme initiated deterioration, and growth of pathogens as well as the extension of the shelf life of pear (Nwufo *et al.*, 1989). Temperature effect on rot disease development in African pear fruits showed that storage under low temperature conditions could reduce the incidence of fruit rot. Temperature is an important component of the environment, which affects the life of fruits and vegetables and especially their rate of deterioration by microbes (Nwufo *et al.*, 1989).

Leakey (1999) reported that the storage life of African pear fruit could be prolonged beyond 8 days by refrigeration. At 15^{o}C storage life was 2 weeks, although some fruit types did not deteriorate over this period. Pathogens that are responsible for African pear fruit spoilage are found not to cause the rot of the fruits after four days of storing at 0 – 15^{o}C. *Rhizopus stolonifer* was observed to be pathogenic at temperatures above 15^{o}C. Pierson (1966) reported the susceptibility of *Rhizopus stolonifer* to chilling at low temperature.

It is also noticed that the shelf life of fruits can be prolonged by storing at temperatures lower than 15^{o}C. African pear fruits stored at 5^{o}C by Emebiri and Nwufo (1990) were found to remain firm for about 25 days before deterioration set in due to chilling injury. Okaka *et al*. (2002) reported that holding of fruits at refrigeration temperature extends their shelf life but leads to loss of colour and nutritional content. The softening of fruits, observed at 40^{o}C was due to adverse response reactions to the high temperature and not to the pathogens.

Okorie (2001) stored African pear fruits at O^{o}C and the fruits remain firm after 28 days when the experiment was terminated due to loss of colour and natural appearance. The fruits

became very soft showing disproportionate increase in volume after thawing with corresponding loss of weight and consequent decrease in fruit density.

Storage of African pear fruits at frozen temperature of – 20^{o}C extended the shelf life to six months when the experiment was terminated. The fruits maintained their color and stability within this period of storage (Okorie, 2001). Storage temperature has special significance for frozen foods because they are defined as foods stored at temperature below -18^{o}C . At such temperatures, microbial growth is completely arrested. However, even at such low temperatures, certain enzymatic and non–enzymatic changes continue, but at a much slower rate, to limit storage life of frozen foods (Krammer, 1979).

2.2 Fruits and Vegetables Shelf-life: Relation to Storage Humidity

The relative humidity of the air in storage room directly affects the keeping quality of the produce held in them (Frazier and Westhof, 1995). It can influence the severity of physiological disorders especially flesh break in apples and skin pitting in citrus (Kupferman, 1997).

The two environmental factors, which affect the shelf life of fruits and vegetables and more especially their rate of deterioration by microorganisms, are relative humidity and temperature (Nwufo *et al*., 1989). High humidity reduces loss of water from plant tissues and therefore reduces wilting or desiccation (Onimawo and Egbekin, 1998). However, this encourages the growth of microorganisms on plant surface due to condensation of moisture on the produce (Wells, 1962; Onimawo and Egbekin, 1998). Low humidity on its part causes shriveling and softening of fruits (Wells, 1962). It has been reported that low relative humidity (0-43%) and high relative humidity (80 – 100% support rot developments or growth on African pear fruits (Nwufo *et al*., 1989).

The control of relative humidity and temperature of storage environment of fruits and vegetables can prolong shelf life by enhancing preservation efficiency of agricultural products (Guynot *et al*., 2003). Low temperature and medium relative humidity 62.5 – 71.5% is known to favour longer storage life of fruits. At this condition respiration rate is reduced, enzyme mediated deterioration as well as the growth of disease-causing microbes are checked (Hall, 1973; Nwufo *et al*., 1989).

High relative humidity of 85 – 95% is recommended for most fruits and vegetables as it retards softening and wilting from moisture loss (USDA, 1974; Lipton and Harvey, 1977). Horticulture products such as apples, pears, beets (topped), cabbage, carrots (topped mature), Irish potatoes (late) and sweet potatoes have been stored for more than 3 months at optimum chilling temperature under a relative humidity range of 90 – 95% (Charalambus, 1993).

2.2.1 Modified atmosphere storage of fruits and vegetables

Modified atmosphere storage is a well–known procedure for reducing decay of fruits and vegetables (Onimawo and Egbekun, 1998). The composition of gases in storage environment can affect the storage life of fruits and vegetables and this gaseous environment can be manipulated to control the rate of respiration of fruits and vegetables. Low oxygen high carbon dioxide atmosphere has been successfully used to extend the shelf life of certain fruits and vegetables. Reduced oxygen levels and / or increased carbon dioxide levels, have been shown to extend shelf life of fresh cut mangoes (Izumi *et al*., 2003). Storage of fruits in modified atmosphere for a week at $12^{o}C$, $15^{o}C$ and $18^{o}C$ did not cause any damage in the final quality parameter of fruits (Marrero *et al*., 2003). Favourable atmosphere shows the metabolic activity of the produce to reduce to a very low level and thus, enables the storage of highly perishable produce for prolonged periods (Okaka, 1977).

The basic difference between the more widely utilized controlled atmosphere systems and modified atmosphere systems is that in controlled atmosphere system the mixture of gases is strictly maintained at all times, whereas in modified atmosphere system the gas mixture is pumped into the system once and changes with time (Brecht, 1980). Elevating the carbon dioxide level of the atmosphere reduces decay by retarding the physiological deterioration of fruits (Frazier and Westhoof, 1995) and their respiratory rates (Onimawo and Egbekun, 1998). The respiration rate and partial pressure difference of the water vapour between the interior of the fruits and its surroundings are the primary factors that influence the fruit mass loss. In the store room, the products of respiration are carbon dioxide, water and heat (Helleckson and Basken, 2000). The rate of respiratory activity of apples and pears and consequently rapidity at which they first become ripe and old is controlled by altering the concentration of oxygen and carbon-dioxide in the atmosphere in which they are stored (Herregods, 1972).

High carbon dioxide has been reported to reduce enzymatic browning in lettuce and its seems to be beneficial in controlling microbial growth. Low oxygen atmosphere alone does not affect the counts of total bacteria and lactic acid bacteria on 'Nam Dokmai mango cubes stored at 1^{o}C, 5^{o}C and 13^{o}C (Poubol *et al.*, 2005). Oxygen concentration alone did not affect growth of vegetable-associated microorganisms, whereas high carbon-dioxide reduced growth in most cases (Bennik *et al.*, 1995; Amanotidou *et al.*, 1999). Inhibition by carbon dioxide may result from dissolved carbon dioxide in aqueous phase of food products, which causes a decrease in the intracellular pH; inhibits enzymatic catalyzed reactions and enzyme synthesis, and "interacts with the cell membrane (Molin, 2000).

There is however a potential for injury to fruits if oxygen levels are low and carbon dioxide levels are high (Sugar, 2001). If the level of oxygen falls too low or is used up, the fruits

will suffocate and develop an alcoholic off flavour in a few days (Okaka, 1997).

2.2.2 Coating and packaging as means of extending shelf- life of African pear fruits

Although refrigeration is considered the most effected method of retarding spoilage of most fruits and vegetables, supplement treatments such as coating and protective packaging can be used to control decay. These supplement treatments also have been found to lengthen the storage life, retard respiration particularly when the produce has to be stored at moderate temperature, control physiological disorder or control moisture loss (Okaka, 1997, Saltviet and Mencarelli, 1988). Coatings are suitable for improving the appearance of fruits by offering glossy sheen and can thus delay ripening, cover tiny injuries and scratches (Onimawo and Egbekun, 1998; Farkas, 1997). Coating has also been said to increase moisture, retain vitamin C content and reduce physiological losses in weight (Cisneros and Krochta, 2002).

Coating applied to the surface of fruits and vegetables can be formed from one or more components and are commonly called “waxes”. However, coating materials used are actually of various mixtures of lipids, proteins, carbohydrates, plasticizers, surfactants, additives and solvents like water and alcohol (Cisneros and Krochta, 2002). Paraffin wax, vegetable oils, natural seal, bee wax have been used for fruits and vegetable coatings (Farkas, 1997). Sheanut butter, which is often used as coating material is solid at room temperature. It is edible and used in cooking, preparation of ointments, water proofing the wall of house and used as illuminants (Kordylas, 1990).

Waxing is an age long practice extensively used in citrus, cucumbers, to a limited extent on crops such as peppers, mature green tomatoes, apples and root crops to reduce moisture,. Orange and cucumbers are often given wax coatings as well as turnips, egg plants, tomatoes, peppers, etc (Norman and James, 1977). A palm oil dip of African pear fruit by Emebiri and

Nwufo (1990) enhanced the storage life at 15^{o}C for 14 days. Waxing formulations are sometimes used as carriers for sprout inhibitors and other growth regulators, fungicides and other preservative (Okaka, 1997). Waxes are applied either by dipping in or spraying an emulsion. The thickness of the wax layer is however often critical since too thick a layer increases decay while too thin a coating may give little or no protection against moisture loss (Okaka, 1997).

Moisture is one of the important factors responsible for fruit spoilage. Most microorganisms require moisture for growth. The texture of the infected produce tissue changes because of the nature of its fibre and pectin and also because of the high water content (Muller, 1988). Loss of moisture in fruits is detrimental to its long storage. Post-harvest life of avocado pear (*Persea americana*) is longer when water is reduced (Adato and Gazil, 1984). Again, reduced water loss has been associated with shelf life extension in ripening pear and banana as well as avocado pear (Littmann, 1972). Beneficial modification of internal atmosphere of fruits and vegetables can also be expected particularly as a consequence of waxing (Ben-Yehoshua and Cameroun, 1989).

Food packaging is a total system which assures separation of the contained product from the environment and protection against external chemical, physical and biological influence (Fellow, 1993). It also serves as containers and can prevent bruising, insect attack and entry of microorganisms especially if hermetically sealed, thus contributing to the shelf life extension of some fresh plant perishables (Okaka, 1997). Plastics are widely used packaging materials for food products due to their numerous advantages, which include low cost, lightness, resistance to impact damage and flexibility (Griffin and Sacharrow, 1972). Plastic film wraps are very useful in reducing water loss in fruits (Ben-Yehoshua and Cameron, 1989). Reduced water loss has been associated with shelf life extension of fruits.

Packaging plastics that are in use today are dominated by polyolefin types such as polyethylene and polypropylene, which are excellent moisture barrier but poor barrier to oxygen and other gases (Omole, 2001). Polyethylene is characterized as having a low permeability to oxygen, carbon dioxide and other gases and is classified as low density or high density polyethylene. Low density polyethylene is classified by types. Type III having a density between 0.941 – 0.959 g/cc, type IV having a density of 0.960 and above (Mizikowski, 1982). However, polypropylenes are one of the highest weight thermoplastics having a density of around 0.905g/cc (Miller, 1982). It is noted for its moisture barrier properties, low temperature durability, dimensional stability, grease and scuff resistance, non-yellowing, non bruizing and relatively low cost (Ayo, 2003).

Enclosure of African pear fruit in polyethylene enhanced the storage life at 15^{o}C for 14 days (Emebiri and Nwufo, 1990). Chaplin and Hawson (1981) have shown that modified atmosphere packaging extends shelf-life of avocado. Film packaging provides a more effective moisture barrier than waxes (Ben-Yehoshua and Cameroon, 1989).

3.0 MATERIALS AND METHODS

3.1 Sample Collection

The samples were ripe pear fruits of *Canarium schweinfurthii* obtained from markets in Enugu, Anambra, Abia and Ebonyi States. The botanical identification was confirmed by Prof. S. Onyekwelu, Applied Microbiology Department, Ebonyi State University, Abakaliki. Samples were kept in the Departmental herbarium.

3.2 Isolation and Identification

Parts of *C. schweinfurthii* pear fruit mesocarp (2g) were obtained from the tail, medium and head portions. A quantity (1g) from each of the cut portions was separately homogenized in 10 ml sterile 0.1% peptone water. From the broth, 1ml was serially diluted to 10-folds and later cultured on sterile Petri dishes by the pour plate method (Cruickshank *et al.*, 1980). Nutrient agar was used for bacterial isolation while Sabouraud dextrose agar was used for fungal isolation. MacConkey agar was used for specific bacterial isolation. The nutrient agar and MacConkey agar were incubated at 37^{o}C for 48- h while Sabouraud dextrose agar medium was incubated at 37^{o}C for 5 days under aseptic conditions. The plate counts were done in triplicates. Discrete bacterial colonies were aseptically transferred into test tubes, while colonies of fungi appearing in cultures were sub-cultured on fresh media to obtain pure cultures.

3.2.1 Identification of bacterial isolates

The purity and identification of each of the bacterial isolates were confirmed according to standard procedures of biochemical and physiological examinations (Holt *et al*; 1994, Cruickshank *et al.,* 1980).

3.2.2 Identification of fungal isolates

Identification was done based on morphological characteristics and physiological attributes. Colony morphology was studied in subcultures on Sabouraud dextrose agar and microscopic characters, i.e. hyphal morphology and sporulation pattern were studied in lactophenol mounts prepared from slide cultures (Cruickshank *et al*., 1980, Kreger Van Rij *et al*.,1984).

3.2.3 Biochemical tests for bacteria identification

The methods described by (Cruickshank *et al.*, 1980, Holt *et al*.,1994) were used to identify the bacterial isolates biochemically.

(a) **Oxidase test:-** Two or three drops of 1% aqueous solution of tetramethyl para phenylene diamine hydrochloride (T_4M_3-p-Ph-$2NH_2$) reagent was added to a piece of filter paper in a Petri dish. Then, the test organism was smeared onto the impregnated filter paper using sterile platinum loop or glass rod. A purple colouration produced within 10 minutes indicated positive cultures. This test was used to identify oxidase producers.

(b) **Catalase test:-** A small amount of pure culture from a nutrient agar slant was picked using a sterile clean, thin glass rod and inserted into hydrogen peroxide solution (3ml) held in a small clean tube. Production of gas bubbles in the tube indicated a positive reaction. The test was used to distinguish catalase enzyme producers from non catalase producers.

(c) **Coagulase Test**: The slide test method described by Cheesbrough (2004) was used in this test. In this, a grease pencil was used to mark a glass slide into two sections, after which a loopful of normal saline was placed on each of the two sections. A colony of the test organism (previously checked by Gram's staining) was collected using a sterile wire loop and emulsified homogenously on each drop of the normal saline on the slide. After the above step, a drop of human plasma was added to only one of the suspensions and

mixed gently for 5 seconds. The observation of clumping after few seconds indicated a positive result (that is, the presence of coagulase).

(d) **Indole test:** Peptone (tryptone) water was dispensed in 5ml amounts in test tubes and sterilized by autoclaving for 15 mins at 121^{o}C and 1.5kgcm^{-2}. Then, a loopful of the stock culture of the isolates was inoculated into sterile bottles containing tryptone water and incubated for 48h at 37^{o}C., to this culture, 0.5ml Para-dimethylamino benzaldehyde reagent was added and allowed to stand for 30 min. Reddish colour production observed at the surface layer indicated a positive test.

(e) **Voges–Proskauer (V-P) test:** Exactly 3ml of 5% alpha naphthol in absolute alcohol (alcoholic naphthol) and 1ml of 40% potassium hydroxide were added to 5 day old culture of the isolate in glucose phosphate medium. Red colour develops within 1 hr.

(f) **Motility test:** The hanging drop method was used for the test. A drop of the specimen was placed on the center of glass slide. Then, a cover slide was gently placed on it. The edges of the slide were sealed with paraffin wax. This was quickly inverted and observed under microscope using 10x and 40x objectives lenses. Any observation of movement by the test organism indicated a positive result.

(g) **Sugar Fermentation test:** The medium was prepared using 1% sugar (sucrose, glucose etc) in peptone water and two drops of 0.01% phenol red indicator solution. After thorough mixing, it was dispensed into Bijou bottles. Durham tubes were inverted into the tubes. The bottles were then corked and sterilized by autoclaving at 121^{o}C and 1.5kgcm^{-2} for 15 minutes. Each test organism was inoculated into each sugar in duplicates. It was then incubated at 37^{o}C for 24h. It was observed for a colour change to

yellow.

(h) **Gram stain:** Gram staining is of great importance in the recognition and identification of bacteria. Gram-staining reaction has the widest application, distinguishing nearly all bacteria as Gram-positive or Gram-negative; according to the resistance to decolorisation of gentian violet and treatment with carbol fushsin. Using the method employed by Koneman *et al.* (1979), a smear of the bacterial isolate was made on a clear-grease free slide, air-dried and fixed by passing through the Bunsen flame about three times. The smear covered with crystal violet for one minute and washed away with distilled water. It was followed by application of Lugol's iodine to mordant it for about one minute. The smear was decolorized by acetone, washed with distilled water and finally covered with dilute carbon fuchsim for another one minute. The stain was then washed off with distilled water; allow to dry before examination in the microscope.

3.2.4 Frequency of occurrence of the microbial isolates

Isolation of microbes was done on the three parts of the pear fruits, the head, the middle and the tail in triplicates. This means that for a particular sample of fruit from one market a total of nine isolation experiments were carried out. The frequency or number of times one particular organism occurred was recorded in a Table. The analysis of frequency of occurrence of microbial isolates was carried out. The frequency of occurrence of the isolates was carried out separately for *Canarium schweinfurthii* pears collected from the markets. The percentage distribution of the isolates in different regions (head, middle and tail) was also determined.

3.2.5 Changes in the microbial counts and nutritional contents of pear fruit sample preparation

The pear fruit samples were divided into five (5) portions. One portion was withdrawn within two (2h) hours of harvest for zero day microbial load determination and nutritional analysis. This portion served as a control sample. Every 24h interval, a portion from the remaining four (4) portions was used to determine microbial load and nutritional content of the pear fruits. These experiments continued every 24h until the last portion was used up on the forth(4) day. This was done by scrapping out the mesocarp of the pears in each portion with sterilized sharp knife. The mesocarp was then ground with sterilized porcelain pestle and mortar (sterilized with 95% ethanol). Measured samples of the ground pulp were withdrawn and used for direct microbial count determination as described by Okpokwasili and Ogbulie (1993), and nutritional content analysis described in AOCS's Official Methods of Analysis (1990) and in AOCS's Official Methods of Analysis (1997).

3.2.6 Determination of the microbial counts of the I solates

The mesocarp of each of the pear fruits was scrapped out with sterilized sharp knife. It was pulped using sterilized porcelain mortar and pestle (sterilized with 75% ethanol). One (1) gram of the pulped mesocarp was serially diluted using deionized water to 10^{-3} and 10^{-4} pour plate method described by Okpokwasili and Ogbulie (1993).From each dilution, 0.1ml was plated in corresponding nutrient agar and MacConkey agar for bacterial counts. Also, 0.1ml of dilution was plated on Sabouraud's dextrose agar for fungal count.

The total (cell/hyphal) count of either bacteria or fungal isolates in each plate was conducted using microscopic count method described by Isu and Onyeagba (2002). The diluted broth of pear fruit mesocarp of volume 0.01ml was introduced on a $1cm^2$ delineated slide using

Pasteur pipette. The sample was spread out and dried while avoiding the introduction of dust into the culture. It was subsequently stained and viewed using oil immersion objective lens microscope. The value obtained served as the initial load count of isolates on the zero day after harvest. The experiment was repeated for the remaining three portions within 2-days interval.

3.2.7 Determination of moisture content

The oven method as described in AOAC's Official Methods of Analysis (1990) was used to determine the moisture content of pear fruit mesocarp. Three (3) aluminum dishes had their lids dried in Gallenkamp (England) hot box oven set to 105^{o}C for 2h. The dishes were cooled in desiccators containing silica gel granules after which their weights were taken with standard analytical weighing balance. Into each of the dishes 5.0g of ground mesocarp of pear fruit was weighed (for triplicate determinations). The samples were dried without their lids in the Gallenkamp hot box oven maintained at 105^{o}C for 8h. The samples were transferred to the desiccators with their lids replaced and allowed to cool to room temperature (32^{o}C). They were returned to the oven and dried without their lids for two more hours, and were cooled in the desiccators and weighed again. The drying, cooling, and weighing were repeated until a constant weight was obtained.

Calculation:

Weight of dish = X_1

Weight of dish + mesocarp = X_2

Weight of dish + dried mesocarp = X_3

Weight of sample = $X_2 - X_1$

% Moisture content = $(X_3 - X_1)/(X_2 - X_1) \times 100/1$

The experiments were carried out in three replicates after which the mean was calculated. Also the standard deviation from the mean was calculated.

3.2.8 Determination of the total ash content

Ash content is a measure of inorganic salts or components of the samples. The ash content of pear fruit mesocarp was determined using the method described in AOAC's Official Methods of Analysis (1990). This was carried out in triplicate Dry porcelain dish and lid was weighed using an analytical balance. The pulped mesocarp of pear fruit weighing 5 g was put into the porcelain dish. Dilute sulphuric acid of volume 3ml was added to the sample to facilitate the destruction of organic matters. The dish and its content were heated gently in open Bunsen burner flame until the content turned black. The porcelain dish and content was placed into muffle furnace (Gallenkamp hot spot furnace) set at 200^{o}C and the lid partially covering the sample. The temperature of the furnace was gradually increased to 600^{o}C. The sample was incinerated for about 6h until gray-white residue is obtained. Using tongs, the hot porcelain dish was removed, covered with lid and put into the desiccators where it was allowed to cool. After attaining the room temperature, the dish and its content was weighed with standard analytical balance.

The incineration, cooling and re-weighing were repeated at 1h intervals until constant weight was obtained and the ash content in percentage was calculated.

Weight of dish/lid = X

Weight of dish/lid + sample = Y

Weight of dish/lid + Ash = Z

Weight of sample = Y – X

Weight of Ash = Z - X

% Ash content = $(Z - X)/(Y - X) \times 100/1$

The mean ash content and standard deviation from the mean were calculated from the percentage of the triplicate determinations.

3.2.9 Determination of crude fibre content

Crude fibre is loss in weight on incineration of dried residue remaining after digestion of a sample. The crude fibre content of macerated pear fruit was determined using the method adopted in AOAC's Official Methods of Analysis (1990). Ground pear fruit mesocarp weighing 2g was put into1-liter conical flask containing 200ml of previously boiling 1.25% sulphuric acid. The mixture was boiled for 30 minutes. The hot acid solution containing the sample was filtered through a muslin cloth fastened onto a Buchner funnel by means of an elastic band. The residue was thoroughly washed with boiling distilled water to free it from the acid. It was returned to the 1-litre conical flask, filled with boiling 200 ml 1.25% sodium hydroxide solution and this was heated to boil for 30 minutes. The residue was filtered and washed with boiling water followed by 1% hydrochloric acid. The insoluble matter was free of alkali by further washing with boiling hot water.

The residue or insoluble matter was again washed twice with 75% alcohol and then thrice with petroleum ether. Lastly the residue was drained, transferred completely to porcelain dish and dried in a Gallenkamp even at 105^{o}C to a constant weight. It was cooled in a desiccator and weighed. The dried sample was incinerated at 600^{o}C for 2h in a muffle furnace (Gallenkamp hotspot furnace). The porcelain dish and ash was cooled in a dessicator to room temperature

(32^oC) and weighed.

Loss of weight on incineration is the weight of crude fibre. This was carried out in triplicates and the mean value was calculated as well as standard deviation from the mean.

Calculation:

Weight of sample = X

Weight of the sample after extraction = Y

Weight of the ash = Z

Percentage crude fibre = $(y - Z)/X \times 100/1$

3.3 Determination of crude fat / oil content of *Canarium schweinfurthii* pear fruit

The crude fat/oil content of pear fruit was determined using the method as described in AOAC's Official Methods of Analysis (1997). Ground mesocarp of pear fruit weighing 2g was wrapped in fat-free Whatman No. 542 hardened ashless filter paper and placed into an extraction thimble. The thimble was put in soxhlet apparatus fixed to round bottom flask containing 150ml of petroleum ether. The flask was placed on heating mantle and heated at 60^oC. The petroleum ether boiled releasing vapours and is refluxed for 7h. At the end of refluxing, the soxhlet apparatus (Electro-mantle ET 2200) was disconnected and the fat-free sample wrapped in the filter paper was dried in an oven (Gallenkamp hot box) for 10 minutes. The defatted sample was reweighed and the fat content calculated as weight loss by the sample. This was carried out in triplicates and the mean value taken.

$$\text{Percentage fat content} = \frac{\text{Loss in weight of the sample}}{\text{Original weight of the samples}} \times \frac{100}{1}$$

3.3.1 Determination of crude protein content

The micro Kjeldahl method as described in AOAC's Official Methods of Analysis (1990)

was used to determine the protein content of pear fruit. A sample of *C. schweinfurthii* pear fruit mesocarp measured with standard analytical chemical balance and weighing 0.2g was put in a nitrogen free Whatman filter paper No. 1. The paper and its content were dropped into a kjeldahl digestion flask (Eclipse scientific model K260).

Few bumping chips, spatula full of catalyst (Copper sulphate + sodium sulphate) and 5ml of concentrated sulphuric acid were added into the flask to assist oxidation. The flask was placed on an electrothermal heater clamped on a retort stand on slanting position. The mixture was heated for about 2h at 120^{o}C until a light green colour which turned colourless on standing was obtained. The flask was cooled in a fume chamber to room temperature (32^{o}C). The digest was diluted with distilled water to 100ml level to obtain the aliquot. A blank was also set up involving the digestion of all the materials except any of the samples.

With a pipette, 10ml of the digest aliquot was introduced into the kjeldahl distillation apparatus followed by10ml of 40% sodium hydroxide through a funnel and plugs quickly replaced. A receiving 100ml conical flask containing 5ml of 4% boric acid and 3 drops of methyl indicator was set under the condenser connected to the Kjeldahl distillation apparatus. The tip of the delivery tube was extended below the surface of the boric acid solution. The mixture was distilled into the conical flask and distillate collected for about 15 minutes. This was carried out in triplicate.

The distillates were respectively titrated with 0.1NHCl solution using automatic burette. The titre value was taken when the colour changes from green to pink (i.e the end point). The difference between the sample and the blank titre value was multiplied by the general protein factor (Nx6.25) to give the protein content of the samples.

Percentage crude protein =

$$\frac{(T - B) \times NHCl \times 0.00014 \times \text{Made up volume} \times 6.25}{\text{Aliquot volume} \times \text{Weight of sample}} \times \frac{100}{1}$$

Where

T = Titre value of sample

B = Titre value of blank

NHCI = Normality of N HCl used = 0. 1 N

Aliquot volume = 10ml

Made up volume = 100ml

The mean crude protein percentage and standard deviation from the mean were calculated from the values obtained from the three replicate determinations.

3.3.2 Determination of carbohydrate and energy contents

The carbohydrate content was determined by difference i.e 100% - (% protein + % fat + % moisture + % Ash + % crude fibre) as described by Bryant *et al*. (1988). Food energy was calculated using the factor 4 x % protein + 4x % carbohydrate + 9x % fat (Marero *et al.*, 1988).

3.3.3 Wine production using *Canarium schweinfurthii* pear fruit

test organisms

The test organism used for this study was a pure culture of *Saccharomyces cereviseae ellipsoideus* collected from Dr. Lawrence O. Eneje of Applied Microbiology and Brewing Department, Enugu State University of Technology, Agbani.

3.3.4 Preparation of yeast inocula build up

Distilled water (300ml) was used to dissolve the following substances in a conical flask: 20 g of glucose, 0.5 g each for sodium chloride, sodium sulphate, magnesium sulphate and dipotassium hydrogen phosphate. The mixture was sterilized at 121^{o}C for 15 mins at 15psi and

allowed to cool to 20^{o}C in a refrigerator before adding the yeast *S. cereviseae ellipsoideus* (1g). The preparation was allowed to stand on the electric rotary shaker at 32^{o}C for 48 h.

3.3.5 Preparation of the juice 'must"

Large sized ripe pear fruits weighing 2 kg were washed with water before peeling to reduce their microbial load. The pear was peeled to collect only the freshly mesocarp which is blended with little addition of distilled water to form a paste so that the juice can easily be extracted by filtration using the sterilized siever. About 800 ml of pear fruit must was collected and heated to about 80^{o}C to destroy any wild yeast. It was then poured into a clean sterilized container.

3.3.6 Standard syrup preparation

A saturated solution of commercial sugar "St. Louis" known as a syrup was prepared by dissolving 980 g of cubed sugar bit by bit into two litres of distilled water and constantly stirred, until a saturated solution was obtained. The prepared syrup was stored in the refrigerator at 4^{o}C for further use.

3.3.7 Preparation of the starter culture

About 250 ml of the juice was collected in a conical flask and the yeast was transferred into the juice. The flask was corked immediately and then put into an electric shaker to be shaken for 24h. The agitation / aeration helps in the multiplication of the yeast.

3.3.8 Sterilization of the "must"

The 'must' was sterilized with 0.8 g of sodium metabisulphite. It was then fortified by

adding the prepared sugar solution. This is done to raise the sugar content of the 'must' so that the wine will have enough alcohol. Nitrogenous yeast food (ammonium sulphate 0.88%) was added to the 'must' to enrich it and to encourage rapid yeast growth.

3.3.9 Analysis of the 'must'

pH determination: The pH meter electrode was first dipped into a buffer solution and then allowed to stabilize at 7. The electrode was then removed and dipped into the conical flask containing the sample. The deflection of the needle on the meter to a steady state was read as the pH value (Akande *et al.*, 2004).

Temperature determination: The thermometer was placed in the conical flask or bottle containing the 'must' for some time and when a steady state was reached, the reading on the thermometer was read as the temperature value.

3.4 Determination of titrable acidity

About 200 ml distilled water was poured in a conical flask and allowed to boil. Then 10 ml of the sample and 1ml of 1% phenolphthalein indicator were added. The mixture is mixed vigorously and titrated with 0.1m NaOH solution. The end point was reached when the first permanent pale-pink colour appeared. This method was described by Kirk and Ronald (1991). Mitchell (1980) recommends that the average titre be multiplied by 0.15 to obtain the acidity value of the juice expressed in percentage.

3.4.1 Specific gravity determination

A clean sterile hydrometer was used to measure the sugar concentration of the 'must' and wine. The hydrometer is gently inserted into a measuring cylinder containing about 100 ml of the sample and then allowed to float freely. The value of the hydrometer that corresponded with the

lower meniscus of the 'must' was recorded as the concentration of the sugar (Amerine *et al*., 1988).

3.4.2 Quantitative test for reducing sugars

The method employed was that outlined by Plummer (1971). Here 25 ml of Benedict's quantitative reagent was poured into a 250 ml conical flask and 1.0 g of anhydrous sodium carbonate added. The mixture was heated over a burnsen flame to boil and was titrated against 20 ml of the sample diluted 1:1 with distilled water, all contained in a burette. The end point was reached when the last traces of the blue colour of the reagent disappeared leaving a dirty brown precipitate. The weight in gram of reducing sugar per 100 ml of sample was determined.

3.4.3 Alcohol content determination

The alcoholic content of the must was determined before and after secondary fermentation by the use of distillation apparatus described by Amerine *et al,* (1988). About 100 ml of the sample was poured into the distillation flask. Seventy-five (75) ml of the sample was distilled out and distilled water poured into the distillate to make up to 100 ml. The mixture was allowed to cool to room temperature and the specific gravity was measured using the hydrometer. The corresponding alcohol content was read from the potential alcoholic scale.

3.4.4 Juice fermentation

Primary fermentation

The juice was pitched for primary fermentation and about half a litre of it was collected and allowed to undergo natural fermentation as a control sample. Primary fermentation lasted for 11 days. At each day, samples were collected and tested for pH, specific gravity, titrable acidity and reducing sugar. Towards the end of primary fermentation, yeast cells started flocculating at the bottom of the container as described by Fugelsang (1997). Tests mentioned above were also repeated at the end of primary fermentation after racking.

First racking: At the end of the primary fermentation, the young wine was full of 'lees' (countless unstable particles). Effective racking of the wine of their lees was achieved by using a sterile white cloth. This was carefully done not to rack the flocculated yeast sediments along with the wine.

Secondary fermentation

This started immediately after the racking and it lasted for 14 days. The wine was fortified with sugar syrup and left at room temperature (32^{o}C). Each day samples were withdrawn and analysed for pH, specific gravity and other parameters as described by Fugelsang (1997).Slight yeast sedimentation occurred at the end of this period, with the wine becoming more mature with more detectable alcohol. 0.75 g/l sodium benzoate was added to stop the activity of every other organism including yeast.

Second racking: This was carried out as in first racking and the wine transferred into sterile bottle, placed in the refrigerator to age for 3 days.

Primary ageing: This ageing lasted for 7 days due to time constraint, by leaving the well-closed

jar in a fermentation room (11-15^{o}C) to help in the maturation of the wine.

Clarification: About 0.5 g/l of bentonite powder was added to the wine and mixed vigorously, pasteurized at 60^{o}C for 30 mins as described by Fugelsang (1997). This bentonite aided the precipitation. The wine was kept in the refrigerator for further ageing.

Third racking: Using the same methods employed in the first and second racking, but in this case a clearer wine was obtained and they were transferred into sterile bottles.

Secondary ageing and pasteurization

The wine was first pasteurized at 65^{o}C for 30 mins using Holder Method described by Amerine and Joslyn (1970). This is achieved by immersing them in a water bath preset at 65^{o}C and allowed for 30 mins period. The pasteurized bottle is then stored in the refrigerator and left for secondary ageing when it acquires its aroma and other characteristics.

Control sample: This control sample is part of the juice that was left to ferment (i.e during primary fermentation). Unlike the sample pitched with yeast, no yeast was added in the control. This control sample was allowed to ferment for 11 days as in the pitched sample.

3.4.5 Sensory Evaluation of Wine

Pear wine samples were assessed by a ten member panel described by Ihekoronye and Ngoddy (1985). The panel was untrained but consisted of wine consumers who are familiar with wine quality. The wine was served on a randomly coded glass cup containing 50 ml sample. The panel in a well-illuminated area rated the pear wine colour, taste, and the overall acceptability of the samples using a four-point Hedonic scales shown in the Appendix5.

3.4.6 Statistical analysis

The Kruskal-Wallis test was employed at 5% significant level to determine if there was any statistically significant difference between average rate of occurrence and distribution of some members of bacterial and that of fungal isolates. Spearman Rank Correlation test was also employed to determine the significant difference in the wine attributes. (Oyeka, 1990)

3.4.7 Antimicrobial Activities of *Canarium schweinfurthii* Bark and Leaf Extracts

The samples were bark and leaf of *C. schweinfurthii* plant.

Culture media: The culture media used were nutrient agar, and sabouraud's dextrose agar prepared according to the manufacturer's specifications in 100 ml flask and sterilized by autoclaving at 121^{o}C for 15 min.

Test microorganisms: Clinical isolates of *Candida albicans, Penicillium marneffei, Tinea rodenses, Klebsiella pneumoniae, Esherichia coli* and *Staphylococcus aureus* were obtained from the Medical Microbiology Laboratory Unit of University of Nigeria Teaching Hospital, Ituku – Ozalla, Enugu. The isolates were identified using standard methods (Cruickshank *et al* ,1980; Holt *et al.*, 1994).

3.4.8 Preparation of materials

The fresh leaves and bark of *Canarium schweinfurthii* were harvested and carefully washed in running tap water and then rinsed in sterile distilled water. The leaves and bark were dried separately in oven at 65^{o}C till the moisture content reduced to between 8% and 13%. It was then pulverized to fine powder and sieved through Muslim cloth. The fine powder were stored in

airtight glass containers protected from sunlight. (Kudi *et al*., 1999).

3.4.9 Preparation of crude aqueous extracts

Exactly 50 g of the pulverized bark each, was respectively introduced into 250 ml of cold distilled water and hot water. The same quantities of the ground leaves were similarly introduced into the solvents. Each mixture was allowed to stand for 24 h with constant agitation and filtered with Whatman No 1 Filter paper and subsequently evapourated to dryness in a rotary evaporator. The dried extracts were each transferred into sterile bijoux bottles and kept at room temperature (32^{o}C) until used. The extracts were later reconstituted using distilled water in the following concentrations 10mg m^{-1}, 20mg m^{-1}, 40mg m^{-1}, 60mg m^{-1} and 80mg m^{-1} (Ibrahim *et al*., 1997).

3.5 Preparation of crude ethanolic extracts

Pulverized bark (50g) each, was respectively introduced into 250 ml of 95% ethanol. The same quantities of the ground leaves were similarly introduced into the solvents. Each mixture was allowed to stand for 24 h with constant agitation and filtered with Whatman No. 1 Filter paper and subsequently evaporated to dryness in a rotary evaporator. The dried extracts were each transferred into sterile bijoux bottles and kept at room temperature (32^{o}C) until use. The extracts were later reconstituted using distilled water in the following concentrations 10mg m^{-1}, 20mg m^{-1}, 40mg m^{-1}, 60mg m^{-1} and 80mg m^{-1} (Ibrahim *et al*., 1997).

3.5.1 Antimicrobial susceptibility test

This was used to assess the antibiotic susceptibility patterns of the various isolates used in this study. This was carried out using the procedure described by Perez *et al.* (1990) with modification after the organisms had been standardized to 0.5 McFarland.

3.5.2 Preparation of turbidity standard

The turbidity standard was a barium sulphate standard against which the turbidity of the test inocula was compared. In this, a 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99 ml of distilled water and mixed thoroughly, also, a 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dehydrate barium ($BaCl_2.2H_2O$) in 50ml of distilled water. After the preparations, a 0.6ml of the prepared barium chloride solution was added to 99.4ml of the sulphuric acid solution and thoroughly mixed. A small volume of the turbid solution was transferred to a test tube and covered with sterile cotton wool and aluminum foil.

3.5.3 Preliminary screening of extracts

The extracts were screened for antimicrobial activity against test organisms using agar well diffusion technique (Perez *et al*., 1990) with modification. The test organisms were respectively diluted to 0.5 MacFarland equivalent standard. The tests were performed using nutrient agar medium for bacterial strains and sabouraud's dextrose agar for fungal strains. Wells were bored on the agar media with sterile No. 4 cork borer of 8mm. One drop of each extracts prepared as described above were dispensed accordingly into the wells and then allowed to stand for 30 minutes for pre-diffusion. Each extract of ethanolic, cold water and hot water were respectively screened against each of the bacterial and, fungal isolates. Ofloxacine and Fluconazole served as the control antibiotics for bacterial and fungal isolates respectively. The plates were then incubated at 37^oC for 24h and 37^oC for 7 days respectively for bacteria and fungi growth.

3.5.4 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the extracts of *C. schweinfurthii* leaves and bark was determined using agar well diffusion technique. The following concentrations of the respective plant extracts: 10mg m^{-1}, 20mg m^{-1}, 40mg m^{-1}, 60mg m^{-1} and 80mg m^{-1} (Perez *et al*., 1990) were used.

Control experiment of plates containing the growth media and each of the test organisms, excluding the extracts were also set up. The plates were then incubated at 37^{o}C for 24h and were performed in triplicate for each bacteria and fungi isolates. The least concentration of the extract that did not allow growth within the incubation period was taken to be the minimum inhibitory concentration.

3.5.5 Measurement of inhibition zone diameter

This was carried out bearing in mind of the diameter of the wells (8mm). Using a metre-rule on the underside of each plate, the inhibition zone diameter of each extracts (marked by clear zones around each well) was observed, measured and recorded after overnight incubation. The diameter of the well (8mm) was subtracted from the total inhibition zone diameter of each well and accurately recorded (Perez *et al*., 1990).

3.5.6 Phytochemical Analysis

Phytochemical analysis of *Canarium schweinfurthii* dried leaves and bark extracts was carried out as described by Harbone (1973) to identify their active constituents. The preparation of the reagents used were explained on the appendices of this work.

Test for Alkaloids

About 0.2g of the samples was each boiled with 5ml of 2% hydrochloride on a steam bath. The

mixture was filtered and 1ml of the filtrate was treated with 2 drops of Wagner's reagent. A reddish brown precipitate indicates the presence of alkaloids.

Test for flavonoids

About 0.2 g of the samples was each heated with 10ml of ethylacetate in boiling water for 3 mins. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of NaOH solution and observed for light yellow precipitate which indicate the presence of flavonoids.

Test for glycosides

Exactly 0.29 of each sample was mixed with 30ml of water. The mixture was heated on a water bath for 5 minutes, filtered and used for the following test. The filtrate (5ml) was added to 0.3ml of Fehling solution A and B until it turned alkali (tested with litmus paper) and heated on a water bath for 2 minutes. A brick red precipitate indicates the presence of glycosides.

Test for saponins

About 0.1g of the sample was boiled in 5 ml distilled water for 5 mins. The mixture was filtered while still hot. One millilitre of the filtrate was diluted with 4ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth.

Test for Tannins

About 2 g of each sample was boiled with 5 ml of 45% ethanol for 5 mins. The mixture was cooled and filtered. The filtrate was then used for lead acetate test. 1ml of the filtrate was added to 3 drops of lead acetate solution. A gelatinous precipitate indicates the presence of tannins

3.5.7 Antiviral Activity of *Canarium schweinfurthii* Leaf and Bark Extracts

Materials: The samples were dried filtrates of aqueous and ethanol extracts of *Canarium schweinfurthii* prepared at Applied Microbiology Laboratory Unit of Ebonyi State University, Abakaliki. Newcastle disease virus (*NDV–Lasota*) and embryonated chicken eggs were obtained from the Department of Virology, Vertinary Research Institute Vom, Plateau State, Nigeria.

3.5.8 Preparation of 20% extract

Dried filtrates of aqueous and ethanolic extracts of *Canarium schweinfurthii (2g each)* was weighed with a metler balance and dissolved in 8ml of phosphate buffer saline (PBS). The dissolved extract was clarified by centrifugation at 200 rpm. The supernatant was decanted and further clarified to remove debris. It was filtered by passing it through a millipore membrane $(22\mu m)$ to remove any contaminant. The filtered extracts were stored at 4^oC for further use (Bachrach and Don, 1970).

Toxicity test of extract

This was done to check for likely toxic effect of the extract on the test host (eggs). Parameters used were mortality and effect on the size of embryo. A total of fifteen embryonated chicken eggs were used (5/sample).

10-day-old chick embryos (5/sample) were candle marked and swabbed using cotton wool and alcohol. Holes were punched on the air sac region and an inch below the air sac. 0.1ml of the extract was inoculated into the allantoic cavity of the eggs and sealed using molten wax before incubation at 37^oC for 96 h (Bachrach and Don, 1970).

3.5.9 Anti-viral test

Extract (1ml) was mixed with 1ml of virus (*NDV-lasota*), the mixture was shaken and incubated at 4^oC for 1hr. the embryonated chicken eggs were punched and 0.1ml mixture of virus and

extract was inoculated into the allantoic cavity of the embryonated eggs. The eggs were sealed with molten wax and incubated at 37^{o}C for 48 h. Embryonated eggs were candled daily to remove dead ones and chilled for 24 h to kill the living embryo, and clot the red blood cells prior to spot testing. The embryonated eggs were removed from the refrigerator and kept at room temperature (32^{o}C) for 30 mins before they were decontaminated using 70% alcohol and cotton wool.

With the aid of a sterile scissors the shell above the air sac was removed exposing the air sac membrane. A drop of 10% chicken red blood cells (C-RBC) was placed on white tile. With the aid of wire loop, a loopful of the allantoic fluid (ALF) was mixed with the C-RBC and checked for agglutination. Allantoic fluids were harvested from each and kept at 4^{o}C for quantitative heamagglutination test described by Sever (1962).

3.6. Heamagglutination (HA) test

Phosphate buffer saline (25ml) was dispensed into each well of the V-bottom microlitre plate and 25ml of the virus suspension (i.e. Infective allantoic fluid) was poured in the first well. Serial two-fold dilution of 25ml volume of the virus suspension was made across the plate. Furthermore, 25ml of phosphate buffer saline and 25ml of 1% chicken red blood cells were poured to each well. The solution was mixed by tapping the plate gently and the red blood cells were allowed to settle for about 40 minutes at room temperature (32^{o}C). Haemagglutination was determined by tilting the plate and observing the presence or absence of tear shaped streaming of the red blood cells.

The titration was read to the highest dilution showing complete Haemagglutination (No streaming).

3.6.1 Anti-Microbial Activities of Pulp Oil Extract of *Canarium schweinfurthii*

Plant material: Sound ripped pear fruits of *Canarium schweinfurthii* was used.

Extraction of Oil: The oil was extracted from the pulp using normal hexane invacou with soxhlet apparatus. The extracting solvent was evaporated leaving the concentrated oil sample for analysis (Koudou *et al*., 2005).

Test organisms: Clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, and Enterococcus faecalis* obtained from the Medical Microbiology Laboratory Unit, University of Nigeria Teaching Hospital, Ituku Ozalla, Enugu. The isolates were identified using standard methods (Cheesebrough, 2004; Onyeagba, 2005).

3.6.2 Preparation of pulp oil concentrations with 20% dimethylsulphoxide (DMSO)

Different concentrations of pulp oil extract was made by diluting the respective volumes: 1ml. 2ml, 4ml, and 8ml of oil extract into 2ml of dimethylsulphoxide to give 0.5ml, 1ml, 2ml and 4ml preparations.

3.6.3 Screening of pulp oil extract

The extract was screened for antimicrobial activity against bacterial strains using modified agar well diffusion technique (Perez *et al.*, 1990). The bacterial strains were diluted to 0.5 MacFarland equivalent standard. The test was performed using nutrient agar medium. Wells were bored on the nutrient agar media with sterile no. 4 cork borer of 8mm. One drop of the prepared extract was dispensed accordingly into the wells and then allowed to stand for 30 minutes for pre-diffusion.

The pulp oil extract was screened against each of the test bacterial strains. Ofloxacin

served as the control antibiotic. The plates were then incubated at 37^{o}C for 24h.

3.6.4 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the pulp oil extract was determined using the Microbroth dilution method described by Irobi *et al.* (1993). The extract was incorporated into nutrient broth in test tubes at varying concentrations: 0.5ml, 1ml, 2ml and 4ml.

Control tubes containing the growth medium and each of the test organisms, excluding the extract were also set up.

The tubes were then incubated at 37^{o}C for 24h and were performed in triplicate for each bacterial strains. The least concentration of pulp oil extract that did not allow growth within the incubation period was taken to be the minimum inhibitory concentration.

4.0 RESULTS

4.1 Isolation and Identification of Microorganisms Associated With *Canarium schweinfurthii* Pear Fruit

The results of this investigation showed that five bacterial and thirteen fungal species are associated with the spoilage of *Canarium schweinfurthii* pear fruits. These include: *Klebsiella aerogenes, Streptococcus pneumoniae, Erwinia carotovora, Pseudomonas putida* and *Flavobacterium spiritavorum* (Table 1).

Four out of the five bacteria are Gram negative and catalase positive. All the isolated and identified bacteria produced acid or acid and gas in glucose utilization test.

Canarium schweinfurthii pear fruits are associated with thirteen fungal species. These fungi are mainly spore-forming types and are found in Table 2.

Table 1: Description of Suspected Bacterial Isolates from *Canarium schwienfurthii* fruit

S/N	Colour	Consistency	Other characteristics	Microscopical characteristics	Remarks	Biochemical Characteristics G 1 2 3 4 5 6 7 8 9	Suspected bacteria Isolate
1	White	Dry, smooth and flat surface	Crenate edge	Rods shape in chain form or rods in chain	Fast growth	- + - - - + - AG AG AG	*Klebsiella aerogenes*
2	While	Creamy, smooth surface	Shiny	Non-chained rod cells or rods in scattered form	Erase at edge	+ + + - - + - - AG AG	*Streptococcus pnuemoniae*
3	Dirty white	Mucoid and smooth surface	Spreading and raised	Short rods in shape	Fast growth	- + - + + - - + AG +	*Erwinia carotovora*
4	White	Creamy and raised	Irregular edge	Rod shape in scattered form	Fast growth	- + + - + + - A A -	*Pseudomonas putida*
5	Milky	Creamy and smooth surface	Raised in form	Rod shape in scattered form or single form	Fast growth	- + - - - + - - AG AG	*Flavobacterium spiritivorum*

Keys:

+	=	Positive reactions	-	=	Negative reaction
G	=	Gram reaction	A	=	Acid production
AG	=	Acid and Gas production	1	=	Catalase
2	=	Oxidase	3	=	Indole
4	=	Motility	5	=	Voges
6	=	Starch hydrolysis	7	=	Lactose utilization
8	=	Glucose utilization test	9	=	Fructose utilization test

Table 2: Description of Suspected Fungal Isolates from *Canarium Schweinfurthii* Pear Fruits

S/N	Colour	Consistency	Other characteristics	Remarks	Suspected fungal isolates
1	White – black	Filametous growth	Non powdery and sporulate fast	Fast growth, posses long non- septate hyphae with spherical conidiospore with phialides at the apex.	*Aspergillus niger*
2	Yellow – green	Powdery surface	Sporulate fast	Fast growth, posses septate and branched condiospore with brush like conidial head.	*Penicillum citrinium*
3	White – brown	Powdery in nature	Fast growth	Sporulate fast, round, variate, colourless and smooth conidospore with loosely columnar head	*Aspergillus glaucus*
4	White	Creamy	Rough surface	Rough edge, phase geniculate, conidiospore which appear acropetal in chain like germ tube in appearance. The spore also appear oblong in nature.	*Alterneria alternate*
5	White	Creamy face in texture and butyrous in consistency	Shiny and crenated edge	Smooth surface, Unicellular ovoid cells without mycelium with rounded end	*Saccharomyces cerevisae*
6	White	Dry surface	Smooth surface	Spreading growth posses branched chained conidiospore with one celled conidia	*Cladosporium herbarum*
7	White	Fluffy growth	Raised colony	Fast growth, posses septate hyphae with anthroconidiospores	*Geotrichum candidium*
8	White – brown	Wooly in texture	Undulate colony	Fast growth, branched septate hyphae with sporangiospores	*Aspergillus terreus*
9	White – yellow	Filamentous growth	Uniform and raised	Sporulate fast, presence of septate hyphae with conidiospore at the apex	*Aspergillus versicolor*

10	White – black hyphae growth.	White hyphae formed black spores after 2days incubation.	Filamentous hyphae and wooly in nature.	Inhibited by isolate no 13. Non septate, long branched mycelium with club shaped sporangia on columella	*Rhizopus stolonifer*
11	Dirty white	Creamy / rough surface	Rough edge	Inhibited by no 12 growth .large number of sporangiospore with branched shape in chain and posses blastosphore on hyphae with long pseudomycelium .	*Candida tropicalis*
12	Milky	Creamy / rough surface	Irregular edge	Moderate growth. Septate hyphae with clamydiosphore which appear sickle in shape	*Fusarium monoliforme*
13	White	Creamy yeast structure	Irregular surface	Unicellular, spherial to ovoid cell with small panded and smaller than that of Sacc. cerevisiae	*Saccharomyces chevalieri*

4.1.1 Frequency of Distribution of the Associated Organisms

Table 3 shows the results of the survey carried out in four markets in four south east Nigerian states. Bacteria and fungi associated with the *C. schweinfurthii* pear fruits spoilage are evenly distributed. The microorganisms are not limited to a specific area. Out of the thirteen fungi isolated, *Fusarium monoliforme* occurred in six out of eighteen experiments conducted in all the four states sampled for this report. This represents the highest frequency of occurrence (7.4%). This fungus is most widely distributed in *Canarium schweinfurthii* pear as it was isolated and identified in all the market samples. The second most widely distributed fungus isolated and identified from the market samples was *Aspergillus niger* with total number and percentage frequency of occurrence of 5 and 6.2% respectively.

Of the five bacteria isolated and identified from *C. schweinfurthii* pear obtained from the markets, the most widely distributed was *Erwinia carotovora* with 22.9% frequency of occurrence. *Flavobacterium spiritivorum* had the lowest percentage of 2.5% of spread within the four markets (Table 3).

Table 3: Percentage Frequency of Occurrence of Bacterial Isolates in *Canarium schweinfurthii* Collected from the Markets

Suspected isolates	**Enugu Ogbete Market**	**Ebonyi Onueke Market**	**Abia Eziukwu Market**	**Anambra Eke Obosi Market**	**Total No of species of Bacteria**	**% Frequency**
Klebsiella aerogenes	1	2	1	-	4	4.9
Strep. pnuemoniae	5	3	2	1	11	13.6
Erwinia carotovora	7	3	4	4	18	22.2
Pseudomonas putida	4	6	2	-	12	14.8
Flavobacterium spiritivorum	2	-	-	-	2	2.5

Table 4: Percentage Frequency of Occurrence of Fungal Isolates in *Canarium schweinfurthii* Collected from the Markets

Suspected isolates	**Enugu Ogbete Market**	**Ebonyi Onueke Market**	**Abia Eziukwu Market**	**Anambra Eke Obosi Market**	**Total No of species of Fungi**	**% Frequency**
Aspergillus niger	1	1	1	2	5	6.2
Penicilium citrinium	1	-	1	1	3	3.7
Aspergillus glaucus	-	1	-	-	1	1.2
Alternaria alternate	-	2	1	-	3	3.7
Sacch. cerevisiae	1	1	1	1	4	4.9
Cladosporium herbarium	2	1	-	1	4	4.9
Geotrichum candidium	1	-	-	-	1	1.2
Aspergillus versicolor	-	-	1	-	1	1.2
Fusarium monoliforme	1	2	2	1	6	7.4
Candida tropicalis	1	-	-	-	1	1.2
Rhizopus stolonifer	-	1	2	-	3	3.7
Sacch. chevalieri	-	1	-	-	1	1.2
Aspergillus terreus	1	-	-	-	1	1.2

4.1.2 Microbial Counts and Nutritional Contents of *Canarium schweinfurthii* Undergoing Biodeterioration

The microbial count of freshly harvested *C. schweinfurthii* pear fruits was found to be 0.12 x 10^4 cfu/g. The microbial count rapidly increased from 0.12 x 10^4 cfu/g on the zero day to 15.06 x 10^4 cfu/g on the second day of storage and decreased afterwards to 8.5 x 10^4 cfu/g on the fourth day when the experiment was terminated due to spoilage of the pear (Table 5).

The moisture content of the pears decreased steadily with increase in microbial counts. It decreased from 15.47% moisture content observed on the zero and first days of harvest to 11.40% on the eight day of storage / biodeterioration. There was significant relationship however in the moisture content of the pear in the subsequent days of storage / biodeterioration (Table 5). The crude fibre, fat, carbohydrate, ash, sugar and mineral contents of *C. schweinfurthii* decreased from the second day of harvest as each nutrient content varied significantly with each day of storage while the protein content increased from the zero day to the last day of the experiment.

Table 5: Microbial Counts and Nutritional Contents of the Fruit

Days	Microbial cfu/g x 10^4	Moisture %	Crude fibre %	Fat %	Ash %	Sugar %	CHO %	Protein %	Mineral %
0	0.12	15.47	10.27	49.32	2.73	0.57	14.89	4.88	1.85
2	3.21	14.75	10.00	49.29	2.68	0.47	14.69	6.68	1.69
4	15.06	13.37	9.60	48.96	2.56	0.38	14.60	8.96	1.56
6	12.07	12.10	9.16	48.39	2.15	0.15	13.96	12.80	1.29
8	8.56	11.40	8.70	47.98	1.95	0.05	13.80	14.92	1.19

Values are mean of the triplicate experiment expressed in percentage

4.1.3 Wine Production Using *Canarium Schweinfurthii* Pear Fruit

The results obtained after analyzing the must extracted from freshly harvested ripped *C. schweinfurthii* pears are shown in Table 6. The results showed that the values of pH decreased slightly from 4.1 to 3.6, while temperature values increased significantly from 21^{o}C to 26^{o}C. Moreover, specific gravity of the must decreased from 1.21 to 1.01.

The alcohol content increased slightly from 5% to 6%.

Table 6: Must Characteristics

Parameters	Values	Primary Fermentation	Secondary fermentation	Control
pH	4.1	4.0	3.6	3.2
Temperature ^{O}C	21	25	26	21
Specific gravity	1.21	1.01	1.07	0.89
Titratable acidity %	0.8	1.9	2.6	3.1
Reducing sugar	5.3	1.3	4.1	3.5
Total Ash (g)	2.1	4.1	5.2	3.2
Alcohol Content %	-	5	6	3.5

Sensory Evaluation

Results of the sensory evaluation of *Canarium schweinfurthii* wine and control sample are shown in Tables 7,8, 9, and 10 below.

The colour and body (appearance) of the product were similar to those of control wine sample (Tables 7 and 10).

The flavour/taste of the product was preferred to the control sample (Table 11). The preference in taste/flavour may be attributed to alcohol, and acidity of the must upon which astringency depends.

The *Canarium schweinfurthii* pear wine was significantly more acceptable than the control wine sample (Table 10). The appearance and flavour/taste of the product were the factors that combined to make it more acceptable.

Results of Sensory Evaluation of the Attributes of Products

Table 7: Attribute Colour

Panelists	**A**	**B**	**d**	**d^2**
1	7	7	0	0
2	7	7	0	0
3	6	6	0	0
4	7	7	0	0
5	5	6	-1	1
6	6	5	1	1
7	7	7	0	0
8	7	6	1	1
9	6	5	1	1
10	6	7	-1	1
Total	64	63	1	5
Mean	6.4	6.3	0.1	0.5

A = Control wine sample
B = *C. schweinfurthii* wine
d = Averaged difference between mean A and B

Table 8: Attribute: Flavour / Taste

Panelists	**A**	**B**	**d**	**d^2**
1	7	5	2	4
2	6	7	-1	1
3	6	6	0	0
4	7	7	0	0
5	6	5	1	1
6	5	5	0	0
7	6	7	-1	1
8	6	5	1	1
9	7	6	1	1
10	6	6	0	0
Total	62	59	3	9
Mean	6.2	5.9	0.3	0.9

A = Control wine sample

B = C. schweinfurthii wine

d = Averaged difference between mean A and B

Table 9: Attributes: Body

Panelists	A	B	d	d2
1	5	6	-1	1
2	6	7	-1	1
3	7	7	0	0
4	7	6	1	1
5	6	6	0	0
6	6	5	1	1
7	6	6	0	0
8	7	6	1	1
9	7	7	0	0
10	5	5	0	0
Total	62	61	1	5
Mean	6.2	6.1	0.1	0.5

A = Control wine sample

B = *C. schweinfurthii* wine

d = Averaged difference between mean A and B

Table 10: Attribute: General Acceptability

Panelists	A	B	d	d^2
1	6	5	1	1
2	5	5	0	0
3	5	6	-1	1
4	6	7	-1	1
5	7	7	0	0
6	7	6	1	1
7	7	5	2	4
8	6	6	0	0
9	7	6	1	1
10	6	7	-1	1
Total	62	60	2	10
Mean	6.2	6.0	0.2	1.0

A = Control wine sample

B = *Canarium schweinfurthii* pear wine

d = Average difference between mean A and B

4.1.5 Antifungal Activities of Leaf and Bark Extracts of *C. schweinfurthii*

Table 11 shows that the ethanolic extracts of leaf and bark of *Canarium schweinfurthii* have antifungal activity against the three tested fungal isolates. The highest inhibition zone diameter of 15mm at $8gm^{-1}$ concentration was produced by the bark extract against *Candida albicans* while the least was by the bark extract against *Penicillium marneffei*. The minimum inhibitory concentration (MIC) ranged from $0.4gm^{-1}$ to $0.8gml^{-1}$.

The cold water bark extract of *Canarium schweinfurthii* produced inhibition zone diameter of 14mm at $0.8gml^{-1}$ against *Candida albicans.*

However, the bark extract did not show any inhibitory activity against *Penicillium marneffei*. The MIC ranged from. $0.4gml^{-1}$ - $0.8gml^{-1}$ (Table 12).

Table 13 shows that the hot leaf water extract of *Canarium schweinfurthii* exhibited inhibitory activity against all the fungal isolates tested. Similarly, the bark extract produced inhibitory property against the tested fungal isolates with the exception of *Penicillium marneffei*. The highest inhibition zone diameter of 10mm at $0.8gml^{-1}$ concentration was obtained with the hot water leaf extract against *Tinea rodenses.* The MIC ranged from $0.4gml^{-1}$ - $0.8gml^{-1}$.

Table 11: Inhibition Zone Diameter of Leaf and Bark Ethanolic Extracts of *C. schweinfurthii* on Fungal Isolates

Conc.(g/ml)	*C. albicans*		*T. rodenses*		*P. marneffei*	
	Leaf	**Bark**	**Leaf**	**Bark**	**Leaf**	**Bark**
0.1	-	-	-	-	-	-
0.2	-	-	-	-	-	-
0.4	6	6	-	2	2	-
0.6	10	9	7	4	7	-
0.8	12	15	10	6	9	4

Control antibiotic: Ketoconazole 18mm.

Key

Negative (-)

Table 12: Inhibition Zone Diameter of Leaf and Bark Cold Water Extract of *C. schweinfurthii* on Fungal Isolates.

Conc.(g/ml)	*C. albicans*		*T. rodenses*		P. marneffei	
	Leaf	Bark	Leaf	Bark	Leaf	Bark
0.1	-	-	-	-	-	-
0.2	-	-	-	-	-	-
0.4	6	6	-	2	6	-
0.6	9	13	6	2	5	-
0.8	11	14	10	4	8	-

Control antibiotic: Ketoconazole 18mm.

Key

Negative (-)

Table 13: Inhibitory Zone Diameter of Leaf and Bark Hot Water Extract of *C. schweinfurthii* on Fungal Isolates

Conc.(g/ml)	*C. albicans*		*T. rodenses*		*P. marneffei*	
	Leaf	**Bark**	**Leaf**	**Bark**	**Leaf**	**Bark**
0.1	-	-	-	-	-	-
0.2	-	-	-	-	-	-
0.4	5	-	5	-	-	-
0.6	7	4	7	-	3	-
0.8	8	8	10	4	4	-

Control antibiotic: Ketoconazole 18mm.

Key

Negative (-)

4.1.6 Antibacterial Activities of Leaf and Bark Extracts of *Canarium schweinfurthii*

Tables 14 - 16 summarized the antibacterial properties of aqueous and ethanolic leaf and bark extracts of *Canarium* schweinfurthii on the pure clinical isolates. The extracts showed antibacterial activities against all the test isolates at varying concentrations.

The ethanolic leaf extracts showed highest susceptibility (20mm) for *Klebsiella pneumoniae,* 18mm for *Staphylococcus aureus* and 17mm for *Escherichia coli* while hot aqueous leaf extracts exhibited the least susceptibility of 17mm for *Klebsiella pneumoniae*, 15mm for *Staphylococcus aureus* and 10mm for *Escherichia coli*. However, the ethanolic bark extract showed highest susceptibility of 16mm for *Staphylococcus aureus* and *Escherichia coli* while hot aqueous bark extract showed the least inhibition of 9mm for *Klebsiella pneumoniae* and 14mm for *Staphylococcus aureus* and *Escherichia coli* respectively. The result showed that the different strains were less sensitive to plant extracts than the control antibiotic Orfloxacin (24mm). The MIC ranged from 0.2 to 0.8mg/ml.

Table 14: Inhibition Zone Diameter of Leaf and Bark Ethanolic Extracts of *C. schweinfurthii* on Bacterial Isolates

Conc.(g/ml)	*K. pneumoniae* (mm)		*S. aureus* (mm)		*E. coli* (mm)	
	Leaf	**Bark**	**Leaf**	**Bark**	**Leaf**	**Bark**
0.1	-	-	-	-	-	-
0.2	9	5	7	8	5	9
0.4	10	5	8	10	9	10
0.6	13	8	12	11	15	14
0.8	20	10	18	16	17	16

Control antibiotic: Oxfloxacin (24mm)
Key

Negative (-)

Table 15: Inhibition Zone Diameter of Leaf and Bark Cold Water Extract of *C. schweinfurthii* on Bacterial Isolates.

Conc.(g/ml)	*K. pneumoniae* (mm)		*S. aureus* (mm)		*E. coli* (mm)	
	Leaf	Bark	Leaf	Bark	Leaf	Bark
0.1	-	-	-	-	-	-
0.2	9	3	-	3	-	4
0.4	11	5	11	10	8	6
0.6	13	8	12	11	15	10
0.8	19	11	16	15	15	15

Control antibiotic: Orfloxacin (24mm)

Key

Negative (-)

Table 16: Inhibition Zone Diameter of Leaf and Bark Hot Water Extracts of *C. schweinfurthii* on Bacterial Isolates

Conc. g/ml	*K. pneumonia* (mm)		*S. aureus* (mm)		*E. coli* (mm)	
	Leaf	Bark	Leaf	Bark	Leaf	Bark
0.1	-	-	-	-	-	-
0.2	7	-	-	-	-	5
0.4	11	5	8	10	-	9
0.6	15	7	13	13	7	12
0.8	17	9	15	14	10	14

Control antibiotic: Orfloxacin (24mm)

Key

Negative (-)

Table 17 shows the results of phytochemical analysis of *C. schweinfurthii* bark and leaf extracts. It revealed the presence of saponin (0.01g), flavonoid (0.22g), tannin (0.42g) and alkaloids (4.98g) for bark extracts; saponin (0.03g), flavonoid (0.21g), tannin (0.44g) and alkaloids (4.99g) for leaf extracts.

Table 17: Phytochemical constituents of *C. schweinfurthii*

Constituent	Leaf	Bark
Saponin	0.03g	0.01g
Flavonoid	0.21g	0.22g
Tannin	0.44g	0.42g
Alkaloids	4.99g	4.98g

4.1.7 Antiviral Activity of Aqueous and Ethanolic Leaf and Bark Extracts of *Canarium schweinfurthii.*

Table 18 summarized the results of toxicity test for aqueous and ethanolic leaf and bark extracts of *C. schweinfurthii* on the test host (eggs) and *NDV – Lasota* virus. The extracts were non toxic to the chick embryo. Also, the ethanolic and hot water leaf and bark extracts as well as the control are positively active against the test virus (*NDV – Lasota*) while the cold water leaf and bark extracts are negatively active against the test virus (*NDV – Lasota*).

Moreover, Table 19 shows the results of haemagglutination test (HA). The hot water extracts of leaf, bark with virus and control experiment showed a high significant titre ratio of 1:1024 while the leaf and bark ethanolic extracts with virus showed significant ratio of 1: 512. The leaf cold water extract with virus showed no significant titre ratio.

Table 18: Toxicity Test for Aqueous and Ethanolic Leaf and Bark Extracts on Chick embryo and *NDV- Lasota virus*

Extracts (0.1ml)	Number Positive	Number Negative
Leaf (hot aqueous extract) + chick embryo.	-	5
Leaf (ethanolic extract) + chick embryo.	-	5
Leaf (cold aqueous extract) + chick embryo.	-	5
Bark (ethanolic extract) + chick embryo.	-	5
Bark (hot aqueous extract) + chick embryo.	-	5
Bark (cold aqueous extract) + chick embryo.	-	5
Leaf (ethanolic extract) + virus.	5	-
Leaf (hot aqueous extract) + virus.	5	-
Leaf (cold aqueous extract) + virus.	-	5
Bark (ethanolic extract) + virus.	5	-
Bark (hot aqueous extract) + virus.	5	-
Bark (cold aqueous extract) + virus.	-	5
NDV – Lasota virus (control).	5	-

Key

Negative (-)

Table 19: Haemagglutinability of *NDV- Lasota Virus*.

Extracts	HA Titre	HA Ratio
Leaf (ethanol extraction) + virus	2^9	1:512
Leaf (cold water extraction) + virus	$< 2^1$	0
Bark (ethanol extraction) + virus	2^9	1:512
Bark (hot water extraction) + virus	2^{10}	1:1024
Bark (cold water extraction) + virus	2^{10}	1: 1024
Leaf (hot water extraction) + virus	2^{10}	1:1024
Virus control	2^{10}	1:1024

4.1.8 Antibacterial Activity of Pulp Oil N-Hexane Extract on Bacterial Isolates.

Table 21 shows that pulp oil n- hexane extract is positively active against the growth of other isolates except *Pseudomonas aeruginosa* and *Enterococcus faecalis* while DMSO had no observable effect on the viability of the test organisms at concentrations similar to those used in dispersion. The inhibition occurs in a concentration-dependent manner with minimum inhibitory concentration ranging from 0.5- 4.0 ml /ml.

The pulp oil n-hexane extract exhibited the highest activity of 27mm on *S. typhi*, and a lowest activity of 12mm on *Staphylococcus aureus* at 4.0ml/ml concentration respectively.

The Ciprofloxacin used as control antibiotic exhibited the highest activity of 26mm on the clinical isolates.

Table 20: Inhibition Zone Diameter of Pulp Oil N-Hexane Extracts on Bacterial Isolate

Oil extracts ml/ml	*S. typhi*	*P. aeruginosa*	*K. pnumoniae*	*E. coli*	*S. aureus*	*E. faecalis*
0.5	24	-	6	6	6	-
1.0	20	-	13	11	7	-
2.0	23	-	16	13	10	-
4.0	27	-	18	14	12	-
DMSO	**-**	**-**	**-**	**-**	**-**	**-**

Control antibiotic: Ciprofloxacin (26mm)

Key

Negative (-)

5.0 DISCUSSIONS

The results obtained from this study showed that five bacterial and thirteen fungal species were found to be associated with the spoilage of *Canarium schweinfurthii* pear viz: *Klebsiella aerogenes, Streptococcus pneumonaie, Erwinia carotovora, Pseudomonas putida* and *Flavobacterium spiritavorum.* The thirteen fungal isolates were *Aspergillus glaucus, Alternaria alternate, Saccharomyces cerevisae, Cladosporum herbarum, Geotrichum candidium, Aspergillus terreus, Aspergillus versicolor, Fusarium monoliforme, Candida tropicalis, Rhizopus stolonifer, Saccharomyces chevalieri, Penicillium citrinium* and *Aspergillus niger.* These fungi are mainly spore forming types and filamentous.

The five isolated bacteria from *Canarium schweinfurthii* pear fruits are mainly opportunistic pathogens that are found in the soil. *Erwinia carotovora* and *Klebsiella aerogenes* are important agents of spoilage in fresh vegetables (Mossel *et al*. 1995). The bacteria contaminate the pears in the field during transportation, washing with dirty or contaminated water. Some of the bacteria such as *Erwinia carotovora* are ubiquitos in the natural environment. These bacteria can be spread by rainstorms, insects, harvest crews, picking containers and packaging equipment (Mahovic *et al*., 2004). Certain species of bacteria such as *Pseudomonas putida, Erwinia carotovora* are readily dispersed in solution and are quickly moved by liquids such as the water dump tanks, flumes or washers, or in the juice from the decayed fruit (Mahovic *et al*., 2004). Therefore, there is no shortage of source of contamination of the pears by the associated microbes.

The dominance of fungi in the *Canarium schweinfurthii* pear fruits is due to the fact that the fruit is acidic. Onuorah *et al* (2004) reported that *Canarium schweinfurthii* pear fruit mesocarp has a pH of 3.85. Fruits being the most acidic of all the plant products are good substrates for fungi (moulds and yeasts). Acidity favours the growth of fungi (Campbell, 1987). The high acid content of *Canarium schweinfurthii* pear fruits make it an ideal substrate for fungi rather than

bacteria (Angie, 2001). However, one of the isolated bacteria from *Canarium schweinfurthii* pear fruits, *Erwinia carotovora* has been noted to cause soft rots of fruits, one of which is reported to be pear (Jay, 1986).

All the isolated microorganisms are not restricted to any particular area of the south east but were isolated generally from areas where these pears were collected (Table 3). The distribution of the microbes within the *Canarium schweinfurthii* pear fruits showed that larger numbers of microbial isolates were found in the middle region of the pears. The number isolated from the middle region was fourteen more than those isolated from the tail region and exceeded those isolated from the head region by three (Table 4). *Canarium schweinfurthii* pear fruit has a sausage shape. This type of shape made the middle region to lie on the objects or containers whenever they are packed together or when being distributed. This exposes the middle region of the pears more to number of isolated microorganisms than the head or tail region.

All the isolated fungi were found to be pathogenic to *Canarium schweinfurthii* pear fruits. Fungi generally grow better in an environment with a pH of 5.0, which is too acidic for most bacteria (Atlas, 1988).

Most moulds being aerobic, can grow on surfaces rather than throughout a substrate (Tortora *et al.*, 1994). Bacterial rots found in fruits gain entry through wounds or natural openings and multiply in the spaces (Walker, 1989). Entry via wounds or natural openings is also characteristic of many fungi (Burchill and Maude, 1986). Certain species of fungi are capable of direct penetration of the intact cuticle. Breach of this barrier is often facilitated by a special procedure following germination of the mould spore on the fruit surface, the fungus produces appressorium (swelling) from the underside of which a thin strand grows through the cuticle and into or between the fruit cells. Penetration is achieved by mechanical pressure and more importantly by an array of enzyme and toxic substances produced by fungi (Collmer and Keen, 1998).

Out of the thirteen fungi Isolated from the *Canaruim schweinfurthii* pear fruits in this study, two (*Rhizopus stolonifer* and *Aspergillus niger*) have been reported by Nwufo *et al.*, (1989) to be pathogenic to *Canarium schweinfurthii* pear fruits. Also *Erwinia carotovora*, one of the four bacteria found to be pathogenic to *Canarium schweinfurthii* pear fruits has also been noted as being pathogenic to it by Nwufo *et al.* (1989).

Mechanical injuries (e.g bruises, cuts, punctures, etc) that occur during harvest and handling are predominant causes of decays by these bacteria, including *Erwinia carotovora* because they provide infection courts (protected sites) for decay pathogens (Mahovic *et al*., 2004).

Fruits are subject to rot because of the microbial degradation of pectin, the biochemical responsible for maintaining firmness and texture of fruits. Microbiologically produced pectinesterases and polygalacturonases hydrolyze pectin, resulting in the formation of soft rots in fruits and vegetables (Atlas, 1988). Three of the isolated fungi from *Canarium schweinfurthii* pear fruits, *Geotrichum candidum*, *Rhizopus stolonifer* and *Fusarium monoliforme* have been reported by Mahovic *et al*. (2004) to cause rot in fruits. Amusa *et al*. (2002) noted that *Rhizopus stolonifer* cause spoilage in freshly harvested bread fruit.

The results in Table 5 revealed a rapid increase in microbial counts between the zero and first days of spoilage. The rapid increase is as a result of abundant nutrients during the logarithmic phase (Ogbulie *et al*., 1998). The gradual decrease in the microbial counts after the fourth day till the eight day is as a result of decrease in the amount of available nutrients and oxygen, excretion of organic acids and other biochemical pollutants into the growth medium, and an increased density of cells. As the limiting factors intensify, cells begin to die in exponential numbers (literally perishing in their own wastes), or they are unable to multiply and to some extent the death of some microbial cell. Nutrition analysis of *Canarium schweinfurthii* pear fruit showed that it contains appreciable amount of nutrients, which are utilizable by fungi. The decline in carbohydrate content of *Canarium schweinfurthii* pear fruit and increase in the number of

microorganism confirmed that the microbes use the carbohydrates for growth. Microorganisms can degrade carbohydrates, which are present in high concentration in fruits resulting in the production of various degradation products (Atlas, 1988). Starch, the principal reserve of polysaccharide is an excellent carbon source for growth of most fungi. The by-products of the metabolized carbohydrates are soluble polysaccharides, carbon dioxide, organic acid and alcohols.

The increase in protein content as the days of storage/biodeterioration was increased depicts that protein is not necessarily the principal element needed for nutrition in fungal growth. Cochrane (1978) reported that the principal element needed for *Aspergillus niger* is carbon. *Aspergillus* secrete copious amounts of homologous proteins into their culture medium (van Brunt, 1986). *Aspergillus* strains possess post-translational mechanism capable of correctly processing proteins that are difficult to express in traditional host organism. The biochemists have been using a variety of *Aspergillus* strains to express heterologous proteins of commercial interest (van Brunt, 1986). Again most of the fungi and bacteria found in the *Canarium schweinfurthii* pear fruits contain high percentage of protein. There is no doubt that their proteins form part of the total calculated protein percentage in the experimental determination. Microorganisms use moisture for metabolic activities therefore there was decrease in the moisture content of the pears as time of storage/biodeteroriation progressed. The moisture in *Canarium schweinfurthii* pear fruits reduced from the initial 15.47% on the zero day to 12.10% on the fourth day of the storage (Table 5). The rate of consumption of the nutrients in the *Canarium schweinfurthii* pear fruits is determined by the rate of respiration (Day, 1990). Also, the formation of sexual structures in ascomycetes and sporangium are believed to proceed more rapidly in moist environments. The spores draw moisture as they grow thereby resulting in loss of moisture (Heintzeler, 1979).

The total fat content also decreased as the time of storage/biodeterioration increased. Many microorganisms according to Horowitz-Wlassova and Livschitz (1985) use fat as source of

carbon. This leads to degradation of fats and oils in the *Canarium schweinfuthii* pear fruits. The by-products of fat hydrolysis are free fatty acid and glycerol (Atlas, 1988). The ash content of *Canarium schweinfurthii* pear fruits decreased as the pears got spoilt.

This was expected in the sense that ash is mainly composed of minerals. Therefore as the deterioration continued, there was a decline in the percentage contents of the ash in the *Canarium schweinfurthii* pear fruits. The microbes use up the minerals for growth (Cochrane, 1979).

The decrease in fibre content (lignin and cellulose) of *Canarium schweinfurthii* pear fruits showed that microorganisms are capable of attacking cellulose and lignin. Most microorganisms are known to derive their foods (carbon) from crude fibre for growth and energy. Fibre being the principal structural element of polysaccharides and its decomposition by microorganisms is important if the required energy is to be released for metabolic activities.

The inoculum of the yeast *Saccharomyces cerevisae elliposideus* used for the inoculation was added in a high volume to ensure that the yeast used superceded any contaminating yeasts. The reducing sugar of the “must” showed that it contained glucose and fructose as reducing sugars. Fortification of the must with sugar syrup was essential in order to sustain the vigorous fermentation that always take place in the first day of fermentation and as well to encourage better yield of alcohol. There is always decrease in the rate of metabolic activity of the wine yeast towards the end of fermentation however not much appreciable decrease was observed.

Before fermentation, there was a colour change to dirty brown in the quantitative (sugar) test. But it was observed that after primary fermentation, there was negligible colour change indicating that most part of the sugar has been converted to alcohol. Also it was noticed that as the fermentation days increased, the pH of the sample kept decreasing and the alcoholic content increased showing that actually fermentation is in progress.

During primary and secondary fermentation, the titrable acidity which was observed during the process was according to Rous and Snow (1983) statement that during fermentation of

the must by wine yeast organic acids such as pyruvic, succinic and acetic acids are produced. The initial decrease, increase and further decrease in specific gravity during the process is accounted for by a decreased density in must (which is due to decreased sugar content), a sudden increased density (which is due to fortification) and finally decreased density due to production of alcohol which is of lower density than that of sugar.

The steady increase in acidity observed during the secondary fermentation may be attributed to revival of metabolic activities of the wine yeast perhaps due to the addition of sugar syrup and aeration made possible during the first racking. The dead yeast cells also settle at the bottom of the fermentor together with the "lees". Bach *et al.* (1978) demonstrated the use of bentonite for clarification and they stated that it is very good because it does not impair taste and it ensures protein stability in wines. It should be noted that the reason for drawing off young wines from the "lees" is to avoid development of unpleasant off flavours and increased susceptibility to wine diseases. Norton and Eposito (1993) stated that the interaction of wine components that occur during ageing helps in the development of bouquet, special odour and flavours owing to a variable mixture of volatile and non-volatile constituents.

The sensory evaluation of pear wine and control showed that colour and body of the product was similar to the control while the flavour/taste of product was preferred to the control. The preference in flavour/taste may be attributed to alcohol acidity of the must upon which astringency depends. The quality of pear wine differs significantly with that of grape wine and other fortified sweet wines commercially produced in terms of ethanolic content, aroma and general acceptability. This could be due to lower quantity of fermentable sugars and high fats/oil contents of *Canarium schweinfurthii* pear according to Onuora *et al.* (2001).

On the antimicrobial activities, the ethanolic and aqueous extracts of leaf and bark of *C. schweinfurthii* exhibited antimicrobial properties, which inhibited the growth of the test organisms to varying degree while the aqueous extracts of the bark did not show any inhibitory

activity against *Penicillium marneffei.* Activities of the various extracts were comparable to those of standard antimicrobial agents Orfloxacin and Ketoconazole. Demonstration of antimicrobial activity against the test organisms is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for development of newer antimicrobial agents.

The ethanolic and hot water leaf and bark extracts of *C. schweinfurthii* showed toxicity against the test virus *(NDV-Lasota)* while the cold leaf and bark extracts did not show toxicity against the test virus *(NDV-Lasota)*. The extracts also did not show toxicity against the test chick embryos.

The hot water and ethanolic extracts of leaf with virus and bark with virus, and control showed haemagglutinin titre ratio of varying degrees while the cold water leaf extract with virus showed no significant haemagglutinin titre ratio. Previous work had shown that antimicrobial activities of plant extracts varies with the solvent used in the extraction (El Astral *et al.*, 2005).

Moreover, the pulp oil extract exhibited antibacterial properties against *Salmonella typhi*, *Klebsiella pnumoniae, Escherichia coli* and *Staphylococcus aureus* to varying degrees while no antibacterial activities were exhibited against *Pseudomonas aeruginosa* and *Enterococcus feacalis.* Thus the crude extract possessed some active ingredients that can inhibit the growth of microorganisms responsible for some common hospital infections. The findings agreed with other researchers, which stated that various extracts of plants exhibit antimicrobial properties against the growth of some common hospital infections (Ekhaise and Okoruwa, 2001; Aluyi *et al*., 2003; Esimone *et al*., 1998). The result obtained from studying the antibacterial properties of pulp oil extract of *Canarium schweinfurthii* is in line with that reported by Obame *et al*. (2007) on antimicrobial activities of *C. schweinfurthii* essential oil and Onawunmi (1987) on effects of dimethylsulphoxide on the antibacterial activity of lemon grass oil. The differing effects observed from the Gram positive and Gram negative organisms, respectively, could be due to differences in the degrees of diffusion of DMSO/LGO complexes into the cells studied. The findings generally

indicate that the methodology of dispersing essential oils in DMSO could be useful in detailed antimicrobial activity studies. Due to high saponification value and antimicrobial activity of the pulp oil extract, it can be used to produce germicidal soap. The presence of antimicrobial properties in *Canarium schweinfurthii* is of great importance in health care delivery system, since it can find use as an alternative to orthodox antibiotics in the treatment of infections due to these microorganisms, especially as they frequently develop resistance to known antibiotics (Singleton, 1999) and will reduce the cost of obtaining health care.

Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponins and flavonoids. These are believed to be responsible for the observed antimicrobial effects of plant extracts to the presence of these secondary plant metabolites (Nweze *et al.*, 2004). Phytoconstituents have been found to inhibit bacteria, fungi, viruses and test (Marjorie, 1999). They were known to show medicinal activity as well as exhibiting physiological activity on the human body (Sofowora, 1993).

Canarium schweinfurthii can be a good source of energy and other important nutrients to the consumers, the fruits high perishability and inability to be preserved naturally are due to invasion by an array of competing microorganisms after harvest. The invasion immediately after harvest by thirteen pathogens no doubt overwhelms the fruits and the consequence is the time-dependent depletion of treasured nutrients and accelerated spoilage of the pears. This implies that substantial quantity of the pear consumed directly by poor people annually to overcome the problem of malnutrition in place of the expensive animal protein. The cash income it generates to rural people will be drastically affected.

The extension of shelf life of *Canarium schweinfurthii* in ambient condition is not encouraging as only storage at modified atmosphere and packaging with low density polyethylene film extended the shelf life by 3 days more than normal storage period of 5 - 8 days. The use of cold refrigeration temperature of 5^{o}C in combination with packaging in low or high density

polypropylene, coating with paraffin wax proved in the previous work to extend the shelf life to 36 days or more.

In view of the fact that a substantial amount of *Canarium schweinfurthii* pear is wasted annually due to only one method of preparation for consumption, it is advisable to process sound ripe pear with other juicy fruits to enhance its initial must composition for the production of a new flavour sweet wine.

For the treatment of several infections in Africa, indigenous medicinal plants are often the only means (Fennell *et al.*, 2002). This highlights the continuous interest in laboratory screening of medicinal plants, not only to determine the scientific rationale for their usage, but also to discover new active principles. African medicinal plants have been screened for their in-vitro antimicrobial activities. It could be inferred that the extracts tested had pronounced inhibitory effect against all test organisms. The test gave validity to the traditional use as a natural antimicrobial agents.

6.0 CONCLUSIONS

C. schweinfurthii in this study exerted antimicrobial activity against bacteria , fungi and virus associated with different types of infections including urinary tract infections (*S. aureus*), gastrointestinal diseases (*E. coli*), typhoid fever (*S. typhi*), candidiasis (*C. albicans*), Miliaria rubra (*Tinea rodenses*) and Newcastle disease (*NDV-Lasota*). The study therefore provides the scientific basis for its traditional application as an ethnomedicine. The demonstration of activity against these clinical isolates is an indication of broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these microbial infections. The study also shows that it has other industrial potentials.

Recommendation

A qualified medical professional should be consulted before using any herbal supplement or self-treating any condition as the Food and Drug Administration (FDA) does not regulate herbal products fully.

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Appendix I

Materials.

1. Weighing balance (salter model-250).
2. Incubator (precision scientific model INA 305)
3. Gallenkamp hotspot furnace.
4. Soxhlet apparus (Electro-mantle Et 2200)
5. Kjeldahl digestion flask (Eclipse scientific model K 260).
6. Porcelain mortar and pestle.
7. Electric shaker.
8. Optical microscope.
9. Autoclave
10. Refrigerator Haier thermocool.
11. Hot air oven (isotemp oven model 801)
12. Analytical balance (mettle balance 480).
13. pH Meter (Jenway 3510)
14. Conical flasks.

Media

1. Nutrient agar (Difco).
2. MacConkey agar (Difco)
3. Sabouraud dextrose agar
4. Peptone Water (Biotech Laboratory United Kingdom)

Media Preparation

Nutrient Agar

Dehydrated commercial nutrient agar powder (TM 7145A) weighing 13g was dispersed in one litre of deionised water and allowed to soak for 10 minutes. It was swirled to mix and disperse properly into the solution. The dissolved nutrient agar is then sterilized in a laboratory autoclave at 121^{o}C for 15 minutes.

Sabouraud's Dextrose Agar

This was prepared according to the manufacturer's specification. Sabouraud's dextrose agar powder (TM 7150A Tekia) weighing 16.5g was dispersed in 250ml of deionised water, allowed to soak for 10 minutes and swirled to mix uniformly. It was then autoclaved at 121^{o}C for 15 minutes. The medium was left to cool to about 50^{o}C before 5ml of sterile chloromphenicol solution was aseptically added before pouring the plates.

Preparation of Liquid Media (Peptone Water)

This was prepared by dissolving 10g of commercial peptone powder and 5g of sodium chloride in one litre of distilled water. It was then sterilized in laboratory autoclave at 121^{o}C for 15 minutes in conical flask.

Preparation of Agar Slants

Agar slants of nutrient agar and sabouraud's dextrose agar were prepared by dissolving 7g of commercial nutrient powder and 16.5g of SDA in 250ml of deionised water respectively. 10ml volume of the dissolved agar were poured into bijou bottles and sterilized at 121^{o}C for 15 minutes. While the agar was still in molten form, it was dispensed into test tubes and kept in slanted forms to solidify.

Appendix 2

Preparation of Reagent for Phytochemical Analysis

Ammonium Solution

Stock concentrated ammonium solution, 37.5ml was diluted in 62.5ml of distilled water and made up to 100ml.

- 45% ethanol: Absolute ethanol (45ml) was made up to 100ml with distilled water.
- Aluminium chloride solution: A 0.5g of aluminium chloride was dissolved and made up to 100ml with distilled water.
- Dilute sulfuric acid solution: Concentrated sulfuric acid (10.4ml) was poured into 5ml of distilled water and made up to 100ml with distilled water.
- Lead Acetate solution: A 45ml of 15% lead acetate was diluted in a mixture of 20ml of absolute ethanol and 35ml of distilled water.
- Wagner's Reagent: Potasium iodide (3g) was dissolved in 10ml of distilled water and pure sublimation iodide (2g) was dissolved into this solution and was made up to 100ml with distilled water.
- Mayer's Reagent: A 1.55g of mercuric chloride was dissolved in 60ml of distilled water. Also 5g of potassium iodide was dissolved in 20ml of distilled water.
- Dragendorff's Reagent: A 0.85g of bismuth carbonate was dissolved in 100ml glacial acetic acid and 40ml of distilled water to give solution A. Another solution called solution B was prepared by dissolving 8.0g of potassium iodide in 20ml of distilled water. Both solution were then mixed to give a stock solution of Dragendorff's reagent.
- Molish Reagent: A 0.1g of Naphthol was dissolved in ethanol and made up to 100ml with ethanol.
- Alkaline phosphatase reagent: Buffer substrate .

- Alkaline phosphate standard curve: 6 test tube was added 0ml, 1ml. 2ml, 4ml, 6ml and 8ml of the 4-nitrophenol standard solution and 12.05, 11.05,10.05, 8.05, 6.05 and 4.05ml of 0.1N NaOH was added respectively to make a total volume of 12.05ml of the mixture. The optical densities were plotted against their respective activities to generate a standard curve.

Calculation of The Phytochemical Analysis

Test for saponins.

- Weight (wt) of empty beaker for 20% ethanol extract of leaf = 59.424g
- Weight of empty beaker + sample for 20% ethanol extract of leaf = 59.454g.
- Weight of residue – (wt of empty beaker + sample) – (wt of empty beaker alone).

∴ Wt of residue for 20% ethanol extract of leaf = (59.454 – 59.424)g = 0.03g.

Test for Flavinoids

- Weight (wt) of empty beaker for 10% diethyl ether in ethanol extract of leaf = 59.10g
- Weight (Wt) of empty beaker + sample 10% diethyl ether in ethanol extract of leaf = 59.1316g.
- Weight (Wt) of residue = (Wt of empty beaker + sample).
- (Weight of empty beaker alone).
- Weight of residue for 10% diethyl ether in ethanol of leaf = (59.316 – 59.107)g = 0.209g.

Test for Phenol

- Weight of empty beaker for 2MHCL extract of leaf =59.27g
- Weight of empty beaker + sample for 2MHCL extract of leaf = 59.31g.
- Weight of leaf = (Wt of empty beaker + sample) – (Wt of empty beaker alone).

- Weight of residue for 2MHCL extract of leaf = (59.318-59.274)g = 0.4g.

Test for Alkaloids

- Weight of filter paper for 10% acetic acid in ethanol extract of leaf = 0.698g.
- weight of filter paper + sample for 10% acetic acid in ethanol extract of leaf = 5.692g
- Weight of residue = (Wt of filter paper + sample) – (Wt of filter paper alone)g = 4.994g.

Appendix 3

Calculation associated with Characterization of *Canarium Schweinfurthii* Oil Extracts

Specific Gravity Determination.

$$\text{Specific Gravity (SG)} = \frac{\text{Weight of the oil}}{\text{Weight of distilled } H_20}$$

Weight (wt) of oil = (Wt of oil and bottle) - weight of specific G empty bottle.

$$\text{Therefore, S.G} = \frac{\text{(wt of oil and bottle)} - \text{(Wt of empty bottle)}}{\text{Weight of S.G. empty bottle}}$$

$$\frac{69.037 - 24.686}{74.233 - 24.686} = \frac{44.684}{49.547} = 0.9019$$

Saponification Value Determination

$$\textbf{Saponification Value (SV)} = \frac{56.1 \times (V_2 - V_1)}{VO} N$$

Where V_1 = Vol. of hydrochloric acid in blank.

V_2 = Vol. of hydrochloric acid in test

N = Normality of hydrochloric acid used

Vo = Vol. of oil used.

$$\text{Therefore, SV} = \frac{(63.70 - 49.10)0.5}{2}$$

$$= 204.765$$

Acid value Determination

$$\text{Acid Value (AV)} = \frac{56.1 \times T \times N}{Vo}$$

Where 56.1 = molecular weight of NaOH

T = Titer vol. of NaOH

Vo = Vol. of oil used

$$\text{AV} = \frac{56.1 \times 3.8 \times 0.1}{2} = 10.659$$

Free fatty Acid Determination

Percentage free fatty acid (FFA) = 0.503 x Acid value

= 0.503 x 10.059 = 5.3618

Iodine Value Determination

Iodine value (iv) = $\frac{12.6N \times (V_2 - V_1)}{Vo}$

Where N = Normality of thiosulphate (0.1)

V_1 = Vol. of thiosulphate in test.

V_2 = Vol. of thiosulphate in blank

Vo = Vol. of oil used.

Iv = $\frac{12.6 \times (2.7 - 1.7) \times \quad 0.1 \quad = \quad 0.6345}{2}$

Peroxide Number Determination

Peroxide Value = $\frac{100(V_1 - V_2)}{Vo} N$

Where V_1 = Vol. of thiosulphate used in test.

V_2 = Vol. of thiosulphate used in blank

N = Normality of thiosulphate

Pv = $100 \frac{(59.2 - 0.10) \times \quad 0.02 \quad = \quad 59.1}{2}$

Appendix 4

Statistical Analysis

To determine the significant difference between the average rate of occurrence and distribution of some members of bacteria and fungi isolates using data generated from Table 4.

Solution:

Apply Kruskal-Wallis test statistic, H

Recall $H = \frac{12}{n(n+1)} \sum_{i=1}^{K} \frac{Ri^2}{ni} = 3(n+1)$

$= \frac{12}{33(34)}\left[\frac{(29)^2}{10} + \frac{(32)^2}{14} + \frac{(18)^2}{9}\right] - 3(34)$

$= \frac{12}{1122}[84.1 + 73.14 + 36] - 102$

$= 0.01069[193.24] - 102$

$= 0.0106[91.24]$

$H = 0.975$

$P < 0.05$ (given)

Note H is distributed as X^2 with 2d.f and for the above value of H, P is > 0.05 i.e. the differences observed can occur by chance alone more than 5% of the time. The null hypothesis is, therefore, not rejected. So there is no significant difference between average rate of occurrence and distribution of some members of bacteria and that of fungi isolates.

Table 4;

Percentage frequency of occurrence of microbial isolates in the various regions of *Canarium schweinfurthii* pear

	Head	Middle	Tail
1	1	3	-
2	3	5	3
3	6	7	5
4	5	5	2
5	1	1	-
6	1	5	1
7	1	2	1
8	3	-	-
9	-	-	-
10	-	3	-
11	-	1	4
12	3	1	-
13	-	-	1
14	-	-	1
15	6	-	-
17	-	1	-
18	-	3	-
19.	-	1	-
	-	-	-

Total Ri $R_1 = 29$ $R_2 = 32$ $R_3 = 18$

Table 5

	Pear on Zero day	Pear on second day	d	d^2
1	0.12	3.21	-3.09	9.5481
2	15.47	14.75	0.72	0.5184
3	10.27	10.00	0.27	0.0729
4.	49.32	49.29	0.03	0.0009
5.	2.73	2.68	0.05	0.0025
6.	0.57	0.47	0.1	0.01
7.	14.89	14.69	0.2	0.04
8.	4.88	6.68	-1.8	3.24
9.	1.85	1.69	0.16	0.256
				13.4584

Recall P = $1-\frac{1-6\Sigma di^2}{n\left(n^2-1\right)}$

$= 1-\frac{6\times 13.4584}{9\left(9^2-1\right)} = \frac{1-80.7504}{720}$

P = 1 – 0.112 = 0.88

Testing for significance

t = $\frac{P\sqrt{n-2}}{\sqrt{1-p^2}} = 0.888\frac{\sqrt{9-2}}{\sqrt{1-0.888^2}} = \frac{2.3494}{0.4636} = 5.0677$

From Table 5

t 0.995; 9-2 = t 0.995.7 = 3.4995

Since 5.068 is greater than 3.4995 we reject Ho and conclude that there is significant difference in the moisture content of the pear on zero day and second day.

Table 5

	Zero day	4^{th} day	d	d^2
1	0.12	15.06	-14.94	233.20
2	15.47	13.37	2.1	4.41
3	10.27	9.60	0.67	0.4489
4	49.32	48.96	0.36	0.1296
5.	2.73	2.56	0.17	0.0289
6.	0.57	0.38	0.19	0.0361
7	14.89	14.60	0.29	0.0841
8.	4.88	8.96	-4.08	16.6464
9.	1.85	1.56	0.29	0.0841
				245.07

Using P = $\frac{1-6\Sigma di^2}{n(n^2-1)}$

Where Σdi^2 = 245.07

$= 1-\frac{1-6\times 245.07}{9(9^2-1)} = \frac{1-1470.42}{720}$

$= 1 - 2.0423 = -1.0423$

$\therefore P = 1.0423$

This shows inverse relationship between the two things considered.

Testing for significance.

$t = \frac{P\sqrt{n-2}}{\sqrt{1-p^2}} = -1.0423\times 2.6457$

Table 5

	Zero day	6^{th} day	d	d^2
1	0.12	12.07	-11.95	142.80
2	15.47	12.10	3.37	11.36
3.	10.27	9.16	1.11	1.232
4.	49.32	48.39	0.93	0.865
5.	2.73	2.15	0.58	0.34
6.	0.57	0.15	0.42	0.1764
7.	14.89	13.96	0.93	0.865
8.	4.88	12.80	-7.92	62.73
9.	1.85	1.29	0.56	0.314
				220.68

$$P = \frac{1-6\Sigma di^2}{n\left(n^2-1\right)} = \frac{1-6\times 220.68}{720} = 1-1.839$$

$$\therefore P = 0.839$$

Testing for significant difference.

$$t = \frac{P\sqrt{n-2}}{\sqrt{1-p^2}} = \frac{-0.839\times 2.6458}{0.54415} = -4.0794$$

$$t = -4.0794$$

$$\therefore /t/=/-4.0794/=4.0794$$

But t 0.995; 7 = 3.4995. Since 4.0794 > 3.4995. We reject Ho and conclude significant relationship.

Table 5

	Zero day	6^{th} day	d	d^2
1	0.12	8.56	-8.44	71.23
2	15.47	11.40	4.07	16.56
3.	10.27	8.70	1.57	2.46
4.	49.32	47.98	1.34	1.796
5.	2.73	1.95	0.78	0.608
6.	0.57	0.05	0.52	0.2704
7.	14.89	13.81	1.08	1.1664
8.	4.88	14.92	-10.04	100.80
9.	1.85	1.19	0.66	0.436
				195.33

$$P = \frac{1-6\Sigma di^2}{n\left(n^2-1\right)} = \frac{1-6\times 195.33}{9\left(9^2-1\right)} = 1-1.6278 = -0.628$$

$$\therefore P = 0.628$$

Testing for significant difference.

$$t = \frac{P\sqrt{n-2}}{\sqrt{1-p^2}} = \frac{-0.628\times 2.6457}{7.7820} = -0.2135$$

/t/=0.2135. But t 0.995; 7 = 3.4995

Since 3.4995 > 0.2135. We accept Ho and conclude that no significant difference exist between zero day and 8^{th} day.

To determine the significant difference in the colour of pear wine (B) and control wine sample (A) using data generated on Table 10.

Solution

Recall Spearman Rank correlation

$$P = \frac{1-6\Sigma di^2}{n(n^2-1)} = \frac{1-6\times 5}{10(10^2-1)} = \frac{1-30}{10(100-1)}$$

$$P = 1 - \frac{30}{990} = 1-0.0303 = 0.969$$

$$\therefore \sqrt[P]{\frac{n-2}{1-P^2}} = \frac{0.97\sqrt{10-2}}{\sqrt{1-0.97^2}} = \frac{0.97\times 2.83}{0.2431}$$

$\therefore$ t = 11.292

For a two sided test, t 0.995; 8=3.3554. Since 11.292 > 3.3554 we reject the null hypothesis and conclude that there is significant difference in the colour of pear wine (B) and control wine sample (A).

Table 11

Recall that $P = \frac{1-6\Sigma di^2}{n(n^2-1)}$

$$P = \frac{1-6\times 9}{10(10^2-1)} = \frac{1-54}{990} = 1-0.0545 = 0.9455$$

Testing for significance of P,

$$t = \frac{P\sqrt{n-2}}{\sqrt{1-p^2}} = \frac{\sqrt[P]{n-2}}{\sqrt{1-P^2}} = \frac{0.9455\sqrt{10-2}}{\sqrt{1-0.9455^2}}$$

$$\therefore t = \frac{2.674}{\sqrt{1-0.8941}} = \frac{2.674}{0.3256} = 8.213$$

For a two-sided test where df = 10-2 = 8

t 0.995; 8 = 3.3554 from table 11

Since 8.213 > 3.3554 we reject Ho and conclude that there exist a significant difference in the flavour /task of (B) and (A).

From Table 12, P = $\frac{1-6\Sigma di^2}{n(n^2-1)}$

Where Σdi^2 = 5

n = 10

$\therefore$ P = $\frac{1-6\times 5}{990} = 1-0.0303 = 0.9697$

Testing for significance using the t statistic

t = $\frac{P\sqrt{n-2}}{\sqrt{1-P^2}}$ Where n = 10

$\therefore$ $\frac{0.9697\times 2.8284}{0.7171} = \frac{2.743}{0.2449} = 11.20$

From the table t 0.995; 8 = 3.3554. Since 3.3554 > 11.20 we reject Ho and conclude that there is significant difference between the body of pear wine (B) and control sample (A).

Table 13

Given P = $\frac{1-6\Sigma di^2}{n(n^2-1)}$ Where Σdi^2 = 10

P = $\frac{1\times 6}{10(10^2-1)} = \frac{1-60}{990} = 1-0.06061 = 0.93939$

Testing for significance using the 6 statistics

t = $\frac{P\sqrt{n-2}}{\sqrt{1-P^2}} = \frac{0.93939\sqrt{10-2}}{\sqrt{1-0.939392}}$

$\frac{0.93939\times 2.828}{34.2783} = \frac{2.6566}{34.2783} = 7.7501$

From the table, using two tail test t 0.995; 8 = 3.3554.

Since 7.7501 > 3.3554 we reject null hypothesis and conclude that significant difference exist between the general acceptability of pear wine (B) and control sample (A).

APPENDIX 5

Sensory Evaluation of Wine Using 4 Points Hedonic Scale

7 = Highly Acceptable

6 = Acceptable

1 = Highly unacceptable

Questionnaire

1. Determine if the colour of the wine is Red, Rose, Pink or Orange
2. Determine if the clarity of the wine is very clear, clear of unclear.
3. Determine if the wine aroma is Ethereal, camp horoceous, musky, floral, pungent, peppe-minty or putrid.
4. Determine if the wine taste is astringent, smooth, pungent, viscous, palate full, rough or bland.