NIGERIAN JOURNAL OF MICROBIOLOGY

ISSN 0794 - 1293
www.nsmjournal.org

Editor-in-Chief
Prof Victor Oluoha Nwaugo

Published by the Nigerian Society for Microbiology

Supported by tetfund

Nigerian Journal of Microbiology, Vol. 26: 2498– 2506, 2012
www.nsmjournal.org

Effects of Spoilage on Microbial Loads and Nutritional Quality of *Dacryodes Edulis* Fruits (African Pear)

[1]Etok,c.a, [2]Odu, N.N. and [3]Nwaugo, V.C.
[1]Dept. of Microbiology, University of Uyo, Uyo
[2]Dept. of Microbiology, University of Port Harcourt
[3]Dept. of Microbiology, Abia State University, Uturu

Abstract
Effects of spoilage on nutritional quality and microbial loads of *Dacryodes edulis* fruits was investigated. Observations showed that the most prevalent bacterial species from farm and market fruit samples was *Bacillus* species with 36% and 86% respectively. While *Staphylococcus epidemidis* was absent in the farm fruits, *Xanthomonas* species (2%) was the least. Other bacterial species observed were *Erwinia, Pseudomonas, Escherichia* and *Lactobacillus* species with all having significantly higher prevalence in market fruits (P = 0.05). The fungal species isolated were *Alternaria, Trichoderma, Geotrichum, Aspergillus* and *Fusarium* species. Others were *Clodosporium, Penicillum* and *Saccharomyces* species. All of them had higher prevalence in the market samples. Moisture content ranged from 12.3% to 18.3% from 0 to 7th day, crude fribre (13.1% to 8.5%). Ash content (2.81% to 1.49%) and fat content was from 53.21 to 38.32% within the same period. Carbohydrate content decreased from 13.13% to 6.99% from 0 to 7th day. However, crude protein content showed a different trend as it increased from 4.41% in the 0 day to 7.93% on Day 3 but thereafter began to decrease till 4.02% on Day 7. Statistical analysis showed that all the nutritional parameters changed significantly after Day 3 of the experiment. Pathogenicity tests showed that only *Erwinia carotovora* and *Pseudomonas putida* caused *Dacryodes edulis* fruit spoilage by the 2nd Day of inoculation. Other bacterial species caused spoilage by the 3rd day except *E. coli* and *Staphylococcus epidemdis*. Among the fungal species, *Alternaria, Geotrichum, Cladosporum* and *Rhizopus* species caused African pear spoilage by the 2nd day while others did same on the 3rd day. Results showed that *E. coli* and *Staphylococcus epidemides* only showed spoilage signs when contaminated with other organisms.

Key words: Spoilage bacteria, Fungi, Nutrition, Fruit Mesocarp
Corresponding Author: Dr. Etok, C.A.

Introduction

Dacryodes edulis (African Pear) is an important fruit in tropical Africa. The tree is common in the region and belongs to the family Burseracec which are mainly shrubs and trees with resinous aromatic gum on their bark. The fruit has a central core with fleshy edible layers as eicarp and mesocap. It is found in the rain forest zone of Africa ranging from Serria Leone, Ghana, Nigeria, Equtorial Guinea, Cameroun and other such areas (Ejiofor and Okafor, 1997, Onuorah, *et al.*, 2001). It is called by different names among the Nigerian tribes, Ube (Igbo), Eben (Efik) and Elemi (Yoruba).

Dacryodes edulis fruit has high dietary contributions as it is softened in various ways, (hot water, hot-ash, or grilled) and eaten with maize and yam (prepared in various ways) or bread (boiled, roosted or fried) (Okigbo, 1977; Nwanekezi, 2005). It has also been reported as a source of oil for both domestic and industrial uses (Okorie *et al.*, 2000; Osagie and Odutuga, 1980). Though African pear has high nutritional values and said to be rich in proteins, fats, carbohydrates, high energy value, its keeping qualities are quite low due to microbial deterioration (Leakey, 1991, Yumbi *et al.*, 1989). This means therefore that much of the products are lost following microbial attacks and spoilage.

This work was therefore designed to access the type of microorganisms

associated with *Dacryodes edulis* fruit spoilage, the effects on nutritional quality and the spoilage potentials of the isolated organisms in Ogba/Egbema L.G.A of Rivers State.

Materials and Methods

Ogba/Egbema L.G.A is located at the northern apex of Rivers State at the boundary with Imo State, Nigeria. It is located within latitudes and longitude in the tropical rain forest zone. The area has high production of African pear but much of the products is lost annually due to microbial spoilage.

Sample Collection

Dacryodes edulis fruits were collected both from farms (from trees directly clean catch) and marekts in the study area and taken to the laboratory within 2 hours for analysis. Only mature deep/dark blue healthy fruits were used in the research.

The fleshy mesocarp of the fruits were cut out from randomly selected (from five fruits in a pool of 100) and ground into a pulp and 1.0g of which was used taken to make the decimal dilution (sterile scapel was used in the cutting). 0.2ml of 10^{-2} and 10^{-3} dilutions were inoculated on Nutrient Agar and Sabourand Dextrose Agar Plate using the spread plate technique. This was done for each day of the work and the number of colonies observed recorded as colony forming units per gram (cfu/g). The bacterial isolates were characterized and identified according to Chessbrough (2003) while the fungal isolates were characterized and identified according to Banneth and Hunter (2000) and Larane (1979).

Determination of the nutritional properties of the *Dacryodes edulis* (African pear) fruits was done according to AOAC (2000) methods. The moisture content was determined using the weight loss technique after drying of the mashed fruit mesocarp in a porcelain dish at 105°C for 8hr. The ash content of the fruit was determined by heating in a porcelain boat till the mesocarp turned gray-while at 600°C in muffle furnace (Gallenkemp, Hotspoot). The difference in weight was determined after cooling to room temperature.

Determination of crude fat/oil content was carried out as described by AOAC (2000). 2.0g of the pulped African Pear was wrapped in fat-free Watman (Ashless) No. 54 paper and placed in soxhlet extraction apparatus. Petroleum ether was used as the extractant while heating at 60°C for 7hr. After the heating and extraction, the sample was dried in the hot air oven (Gallenkemp) to dryness and re-weighed after cooling to obtain the fat content by loss of weight method.

Crude fibre was determined according to AOAC (2000). The macerated fruit mesocarp was boiled in 1.25% Sulphuric acid for 30 min before washing in distilled water after filtration in muslin cloth. It was then boiled in 1.25% NaOH for 30
min. The process of filtration and washing in distilled water was repeated. The insoluble portion of the mescocarp was then washed in ethanol and later petroleum ether before heating at 105°C for 10 min to arrive at a constant weight. It was then incinerated at 800°C for 2hr and allowed to cool. The crude fibre was obtained by the difference after the H_2SO_4 and NaOH treatments from the final weight.

Similarly crude protein content was obtained by the AOAC (2000) method using the Kjeldahl digestion apparatus (Eclipse Scientific Model K 285). The distillate obtained was titrated against 0.1N HCL. Change of color from green to pink was taken as the end point. The difference between sample and blank (N) was multiplied by the constant 6.25 to give the protein content of the fruit i.e (N x 6.25).

The carbohydrate content of the fruit was obtained by inference through the formular stated by Bryant *et al.* (1988) as follows:

Carbohydrate % =

100 – (Protein % + fat % + moisture content % + Ash % + Crude fibre %)

All the experimental processes above were repeated daily in duplicates for seven days. The results obtained were subjected to standard deviation analytical tool for test of significance.

Results

Table 1 shows the prevalence of each of the microorganisms isolated in the work. Among the bacterial species, the *Dacryodes edulis* fruits from farm, *Bacillus* species had the highest frequency (36%) followed by *Erwinia caratovora (7%) while Lactobacillus* species had the least (2%). *Staphylococcus* and *Escherichia* species were not observed. However, in the market samples, *Bacillus* species was the highest (86%), followed by *Staphylococcus* species (36%) while *Pseudomonas putida* (12%) was the least. All the seven bacterial isolates reported in the work were found in the market samples of the fruits. Results obtained showed significant difference between the sources of the fruits farm and market. In fungal organisms, *Aspergillus* species (14%) had the highest prevalence, followed by *Fusarim* species while *Gostrichum* species was the least (3%) but *Saccharomyces cereviasiae* was not observed in the farm samples. On the other hand, *Aspergillus* species with 43% was the most prevalent, followed by *Fusarim* (36%) with *Saccharomyces* species being the least (21%) in the market fruits. All the fungal isolates here had over 20% prevalence. The variation between farm and market samples in frequency of fungal species was quite significant (P = 0.05). Other values are as shown in the Table 1.

Table 2 shows the variation in the nutritional properties of the fruits with microbial deterioration. Major variations in all the parameters measured only occurred significantly (P = 0.05) from the 3rd day after harvesting. The most significant were crude fibre that changed from 13.1 (0 day) to 8.5 (7th day), followed by the fat/oil content from 53,21 (0 day) to 38.31 (7th day). Results obtained in this section were of three patterns. Crude fibre, crude ash, carbohydrate and fat contents decreased significantly within the experimental time while only moisture content increased from 12.3% (0 day) to 18.0% (7th day). Observations showed that only the crude protein values increased from 4.41% (0 day) to 7.93%, (3rd day), before decreasing to 4.02% (7th day). Variations with days of experimentation showed significant variations (P = 0.05).

The pathogenicity test carried out showed that the most pathogenic bacteria in the fruits were *Erwinia* and *Pseudomonas* species as they caused spoilage of the fruit by the second day of inoculation. Except *Staphylococcus* and *Escherichia spices*, other bacterial isolates caused spoilage by the 4th day of inoculation. Among the fungal species, 4 (*Alternaria, Geotrichum, Cladosporum and Rhizopus* species) caused fruit spoilage by the 2nd day. All other fungal species caused spoilage by the 3rd day.

Discussion

The assessment of microbial spoilage of *Dacryodes edulis* (African pear) fruits showed the presence of seven bacterial and eight fungal genera. Most of the fungal genera have been implicated in fruits spoilage. Mahovic *et al*., (2004) and Agrios, (1997) reported that *Rhizopus* and *Aspergillus* and *Penicillum* species spoil most tropical fruits. *Alternaria, Trichoderma, Geotrichum,* and *Cladosporum* species have individually and collectively been associated with fungal deterioration of fruit and plant oil. Their involvement in the spoilage of *Dacryodes edulis* followed the same level. On the other hand, *Erwinia caratovora, Xanthamonas, Pseudomonas* and *Bacillus* species have also been implicated in many plant diseases (Adams and Moss 1995; Onuorah *et al*., 2004; Mahovic *et al*., 2004). *Lactobacillus* species is well-known fermenter of plant materials and

could therefore cause its own spoilage by fermenting the pear materials.

Generally, more of the fungi caused spoilage of African pear than bacteria. Angie (2001) and Onuorah *et al.*, (2001) reported that African pear has acidic mesocarp. Similarly, Alexopolous and Mims (2000) stated that fungi grow well in acidic media. Only a few bacterial species are known to grow in the acidic pH range. *Erwinia, Xanthamonos* and *Pseudomonas* species which can survive in the acidic pH, were able to cause spoilage with the fungi in the *Decryodes edulis fruit.*

Analysis of the results obtained in this work showed that market samples of the fruit had significantly more bacterial and fungal species than the clean-catch farm samples. This observation tallies with the observation of Dawas and Kotze (1989) reported similar observation. The market samples were conveyed to the market in non-sterile containers which can then contaminate the fruits. In addition, when displayed for sale, the fruits were exposed to various contamination sources including human begins (in the process of selection to buy). The market environment, itself could also have added to the contamination as the surrounding were of low hygienic standard. Most of the organisms found in the market samples were spore-formers, whose spores could easily survive harsh conditions till they find their way to the fruits by various methods.

Assessment of the effects of microbial deterioration of the *Decryodes edulis* fruits showed lowering of nutritional parameters with time of microbial growth. This agrees with the principle of microbial utilization of nutrients in a batch culture. The fruit carbohydrates, proteins, fibre and fats/oil served as nutrient for the various organisms. Continuous metabolism of these nutritional components caused the decrease in their quantity. However, the initial increase in protein content experienced in the first two days is similar to the observation of Isu (2004) who observed a similar crude protein increase in African oil bean (Ugba). Fermentation of the fruit caused the release of some solid protein materials thereby making them available for assessment. However, when the micro-organisms continued to utilize them for growth,the protein content began to decrease as their was no more replacements.

Further observation showed that moisture content of the fruits increased till the end of the experiment. This is similar to the observation of Isu (2004) working on African oil been seed. Fermentation caused the breakdown of solid plant materials with consequent release of liquid components (Pelczar *et al,* 2004). The microbial spoilage of *Dacryodes edulis* also included the fermentation of the fruits hence the increase in the moisture content.

Generally, the microbial spoilage of the fruits caused decreased nutritional value. Pathogenicity tests carried out showed that the major bacterial pathogens of African pear were *Erwinia carotovora* and *Xanthomonas* species while *Staphylococcus epidemidis* and *E coli* were contaminants. The case was also similar with fungal species were *Alternaria, Geotrichum, Cladosporum* and *Rhizopus* species were the major pathogens. However unlike the case of bacteria where only two organisms could cause spoilage by day 2, all the other fungal species caused spoilage by the 3rd day. This shows that while few bacterial species are disease-causing in African pear, many fungi cause plant diseases.

In conclusion, microbial contamination and spoilage of African Pear caused decreased nutritional value.

Table 1: Prevalence bacterial and fungal isolates from *Dacryodes edulis* fruits from farm and market

Organism	Farm			Market		
	NFE	NFI	%	NFE	NFI	%
Bacteria				100	86	86
Bacillus species	100	36	36	100	24	24
Erwinia carotovora	100	7	7	100	15	15
Xanthomonas species	100	5	5	100	12	12
Pseudomonas putida	100	3	3	100	36	36
Staphylococcus epidemides	100	-	-	100	40	40
E. coli	100	-	-	100	27	27
Lactobacillus species	100	2	2			
Fungi						
Alternaria species	100	9	9	100	23	26
Trichoderna species	100	11	11	100	36	36
Geotrichum species	100	3	3	100	28	28
Aspergillus species	100	14	14	100	43	43
Fusairum species	100	12	12	100	36	36
Cladosporium species	100	10	10	100	29	29
Saccharomyces species	100	-	-	100	21	21
Penicillum species	100	9	9	100	26	26

Table 2: Variations in nutritional contents and microbial loads with storage time

Days	MC	CF	ASC	CP	CHOc	FAT	Microbial content	
							Bacterial count	Fungal count
0	12.3±0.03[a]	13.1±0.03[a]	2.81±0.05[a]	4.41±0.04[d]	13.13±0.02[a]	53.21±0.23[a]	1.0 x 10	1.3 x 10^1
1	12.3±0.03[a]	13.1±0.03[a]	2.80±0.05[a]	4.46±0.04[a]	13.10±0.02[a]	53.19±0.21[a]	1.2 x 10^1	2.2 x 10^1
2	13.9±0.63[a]	11.4±0.42[b]	2.61±0.11[a]	5.82±0.06[b]	12.41±0.21[a]	51.31±0.41[a]	1.5 x 10^2	2.1 x 10^3
3	14.8±0.72[b]	10.2±0.62[b]	2.22±0.15[b]	7.93±0.09[c]	10.32±0.42[b]	49.38±0.42[b]	2.1 x 10^4	4.7 x 10^3
4	16.1±1.03[c]	9.8±0.91[c]	2.13±0.26[b]	7.03±0.08[c]	8.91±0.62[c]	42.11±0.31[c]	2.7 x 10^4	1.3 x 10^4
5	17.4±1.08[c]	9.1±1.10[d]	1.81±0.39[c]	4.74±0.11[d]	7.11±0.94[d]	40.31±0.54[d]	1.3 x 10^4	3.1 x 10^4
6	18.2±1.10[d]	8.7±1.34[e]	1.62±0.41[c]	4.61±0.14[d]	7.02±0.91[d]	39.21±0.51[a]	1.2 x 10^4	2.1 x 10^4
7	18.0±1.10[d]	8.5±34[e]	1.40±0.91[d]	4.02±0.20[e]	6.99±0.96[d]	38.31±0.81[d]	1.2 x 10^3	7.1 x 10^4

* Figures followed by the same alphabets are not significantly different
* Figures followed by the different alphabets are significantly different

MC = Moisture content
CF = Crude fibre
ASC = Ash content
CP = Crude protein
CHOc = Carbohydrate content
Fat = Fat/oil content

Table 3: Spoilage potentials of the isolated micro-organisms in APF

Organism	**No of days for spoilage signs to appear**						
Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Bacillus species	-	-	X	X	X	X	X
Erwinia caratovora	-	X	X	X	X	X	X
Xanthomonas species	-	-	X	X	X	X	X
Pseudomonas putida	-	X	X	X	X	X	X
Staphylococcus epidemidis	-	-	-	X	X	X	X
E.coli	-	-	-	X	X	X	X
Lactobacillus species	-	-	X	X	X	X	X
Fungi							
Altennaria species	-	X	X	X	X	X	X
Geotrichum species	-	X	X	X	X	X	X
Aspergillus species	-	-	X	X	X	X	X
Fusarium species	-	-	X	X	X	X	X
Clodosporium species	-	X	X	X	X	X	X
Saccaromyces cerevislae	-	-	X	X	X	X	X
Penicillum species	-	-	X	X	X	X	X
Rhizopus species	-	x	X	X	X	X	X

X = Spoilage signs observed

References

Adams, M.R. and Moss,M.O. (1995). Food Microbiology. The Royal Society of Chemistry. Thomas Graham House, Science Park, Cambridge UK, 3041, 130.

Agrios, G.N. (1997). Plant Pathology, 4th Edition. Academic Press, San Diego.

Aiyelaagbe, T.O.O; Adela, A.O. and Popoola, L. (1998). Agro-forestry Potential of Dacryodes edulis in the Oil Palm-Cassava belt of Southeastern Nigeria. *Journal of Agroforestry System, 40(3)*:263-274.

Ajiwe, V.I.E.; Okeke, C.A.; Nnabuike, B.; Ogunleye, G.A. and Elebo, E. (1997). Application of oils extracted from African starapple (Chrysophyllum Africana), horse eye bean (*Mucuna sloana*) and African pear (*Dacryodes edulis)* seeds. *Bioresource Technology 59*:259-261.

Angie, P. (2001). Introduction to Pathogenic Organisms: In Microbiology, 12th Edition. City College of San Francisco, 50.

A.O.A.C (1990). Official Methods of Analysis 13th Edition. Association of Official Analytical Chemists. Wahsington DC. USA.

Bryant, L.A; Montecalw, J.J.R.; Morey, K.S. and Lay B. (1988). Processing, Functional and Nutritional Properties of Okoro Seed Products. *Journal of Food Sciences, 53(3):*810-816.

Cheesebrough, M. (2000). District Laboratory Practice in Tropical Countries, Low Price Ed. Cambridge University Press, United Kingdom.

Cowan, S.T. and Steel, K.J. (1982). Manual for the Identification of Medical Bacteria. Cambridge University Press, London Pp. 5582.

Dawas, J.M. and Kotze, J.M. (1987). Phytopathology of Fungi causing pre-and post-harvest diseases of avocado fruit. *Phytophylactica 19*,489-493.

Ejiofor, M.A.N. and Okafor, J.C. (1997). Prospects for commercial exploitation of Nigeria indigenous trees, vegetables, fruits and seeds through Food and Industrial Products Formulation. *Journal of. International Tree Crops 9(4):*125.

Frazier, W.E. and Westhoof, D.C. (1995). Food Microbiology, 4th Edition. Tata McGraw-Hill Publishing Co. Ltd. New Delhi, PP. 204-209.

Harrigan, W.F. (1998). Laboratory Methods in Food Microbiology, 3rd Edition. Academic Press San. Diego, California, 52, 162,350-378.

Mahoric, M.; Sargent, S.A. and Bartz, J.A. (2004). Identifying and Controlling Post Harvest Tomato Diseases in Florida. Florida Cooperation Extension Services Dept; Institute of Food and Agricultural Service. *HS 866.*

Nwufo, M.I. and Anyim, C.O. (1998). Post-harvest handing and storage of Africa pear (Sacryodes edulis) in Southeastern Nigeria. proceedings of second international workshop of African pear improvement and other sources of vegetable oils. Eds Kapseu, C. and Kayem,G.T. Presses Universitaries de Yaounde, Pp. 140-142.

Obasi, N.B.B and Okolie, N.P. (1993). Nutritional constituents of the seeds of the Africa pear (*Dacryodes edulis*). *Food Chemistry.* 46, 297-299.

Okafor, J.C.; Okolo, H.C. and Ejiofor, M.A.N. (1996). Strategies for enhancement and utilization potentials of edible woody forest species of Southeastern Nigeria. Proceedings of fifth AETEAT Congress, August 22-27, 1994. Kageninger, the Netherlands. Eds. Maesen, L.J.G; Burgt, X3 M.V and

DeRoy, J.M. Kluwer Academic Publishers, Onatorio, Pp. 684-695.

Okorie, H.A. and Ndubizu, T.O.C.; Janssens, M.J.J. (2000). Studies on the pomology of the African pear (*Dacryodes edulis* (G. Don) H.J.. Lam) *Nigerian Act of Horticulture* 531:207-211.

Onuorah, C.E.; Nzewi, D.C. and Abiodun, O. (2001). Proximate composition, mineral content and physical/chemical characteristics of fresh, cooked and roasted local Nigeria peeaar (Dacryodes edulis). *Nigerian Food Journal 19,*120-124.

Osagie, A.U. and Odutuga, A.A. (1986). Chemical characteristics and edibility of oils extracted from four Nigeria oil seeds. *Nigerian Journal of Pure and Applied Science 1*:15-25