Microbial load counts of stored *Bombax costatum* calyx

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Abstract

This study was conducted on Bombax costatum calyx obtained from Yandev Community of Gboko Local Government Area of Benue State, Nigeria. Flowers were collected from a chosen tree as they dropped without the influence of any human activity. Calyxes were collected and sun dried to 5.5%wb for the study. The dried samples were milled and sieved using sieve of 0.25mm aperture to achieve uniform particle size. Bombax costatum samples were hermitically stored in different containers namely; plastic, glass, ceramics, metal and control (open calabash). Microbial and bacteria counts were performed before and after storage, to check the level of Microbes infestation and spoilage before and during storage. The mean Total viable counts (TVC) in this study across dilutions and containers showed that Plastic ranged from 3.55logcfu/g^{-1}- 7.12logcfu/g^{-1}, glass ranged from 3.57logcfu/g^{-1} – 6.98logcfu/g$^{-1,}$ metal ranged from 3.60logcfu/g^{-1} to 7.04logcfu/g^{-1}, and ceramic ranged from3.59logcfu/g^{-1} – 7.27logcfu/g^{-1},the control had TVC ranged from 4.15logcfu/g^{-1}-7.95logcfu/g^{-1}. In line with HACCP-TQM technical guidelines, both the sample before storage and samples in containers after storage belong to the category of good food with glass presenting the best protection, followed by metal, ceramic, plastic and lastly control

Introduction

Bombax costatum is a deciduous, open savannah woodland tree; it is a species from the *Bombaceae* family with *bombax* as Genus name. *Bombax costatum* is common in the savanna zones of West Africa and Central Africa Republic. It is 3 – 30m high and up to 1m in girth and does well on stony soils (Gernnah and Gbakaan,2003). It produces flowers from November to February and then fruits from February to June. During Hamattan season (from November to March) when most crops are harvested, the flowers become loosened from the stalk and fall freely with little blow of wind (Tingir, 2003). The petals are detached from the calyx which is then dried and ground into powder and stored for reconstitution into soup. Because of the ability of the powder to form a gel when mixed with water, it can be classified as a food gum. Food gums are high molecular weight polymeric compounds, mostly carbohydrates which are characterized by their ability to give highly viscous solution at low concentration (Muhammad et, al 2017). Traditionally foods such as pounded yam and cereals moulded foods are eaten along with slimming soups such as okra, ewedu, ogbono, okoho, ager and stews that are prepared to facilitate the movement of food along the digestive track. One additional of such very popular soups in Nigeria is Genger which is produced from the flowers of the plant *Bombax costatum* both in fresh and dry form, which is the focus of this study. *Bombax costatum* in Tiv land is a delicacy with high viscosity or gelling capacity from

November to March. During the wet season, from April to October, Genger does not gel at all because it losses its viscosity and when this happens, it becomes inedible and wasteful. Due to wide acceptability and popularity of the soup, the need to produce data that will aid its handling, processing and storage is gaining prominence. Rheological parameter such as viscosity and quality of *Bombax costatum* is of paramount interest. This study is therefore aimed at investigating the microbial load of stored *Bombax costatum* calyx for the purpose of achieving quality of finished and stored product. Measurement of moisture content and microbial load are often very important for quality control, particularly on stored products that we expect to last for months (Mkavga, 2004).

It is no longer news that *Bombax Costatum* calyx which is the target of this study losses its viscosity immediately rain sets in, rendering the stored produce useless. The control of this menace through storage is intended. The broad objective of this study is to control the qualitative and quantitative loss of *Bombax costatum* through hermitic storage, and the study is specifically aimed at checking the effect of varying storage containers on the quality of stored *Bombax costatum* calyx.

Materials and Methods.
Materials and Methods

This study was conducted on *Bombax costatum* calyx obtained from Yandev Community of Gboko Local Government Area of Benue State, Nigeria. Flowers were collected from a chosen tree as they dropped without the influence of any human activity. Petals were manually detached using hands while the calyxes were sun dried to 5.5%wb and reserved for the study. The dried samples were pounded and sieved using sieve of 0.25mm aperture to achieve uniform particle size. Potassium carbonate was obtained from the Gboko main market and ground into powde and mixed with the powdered calyxr. Dosages of the preservative were computed as percentage of *Bombax costatum* sample (0%, 3%, 6%, 9%) in grams and blended with the sample, which was package into ceramic, metal, plastic and glass containers.

Quantities and methods of Determination

(b) Moisture Content of *Bombax costatum* Samples

The moisture content (percent wet basis) was determined by air-oven drying method. Wet basis which is expressed in a lot of literature is mostly used by farmers. Apparatus such as electronic weighing balance, crucibles, oven (gallencarp) and thermometer were used to carry out the experiments, while Equations 1 was used for the calculation of moisture content.

$$Moisture\ content(\%) = \frac{weight\ of\ water}{weight\ of initial\ sample} *100 \qquad (1)$$

Akinremi(1999).

(a) Temperature and relative humidity

Temperature at all experiments was measured using the mercury in glass thermometer graduated in degree celcius ($^{\circ}C$).

The thermometer head was always positioned to keep contact with the body to be measured. Samples were always stirred for even distribution of heat during measurements. The relative humidity of both the storage environment and the ambient environment were measured with a hygrometer graduated in percentage.

3.3.1 Microbial and Bacteria load

Bombax costatum calyx were collected from *Bombax Costatum* tree aseptically in sterile polyethen bags, sun dried, milled and transported to the Benue State University Microbiology Laboratories for analysis and storage. *Bombax costatum* samples were stored in different containers namely; plastic, glass, ceramics, metal and control as shown inplate 3. Microbial and bacteria counts were performed before and after storage, to check the level of Microbes infestation and spoilage before and during storage. Each sample was homogenized with 9ml of sterile normal saline to prepare stock solution; stocks were serially diluted (1:10) to 10^{-5} by adding 1ml of stock solution to 9mls of normal saline in test tubes. 1ml of the diluted sample was inoculated on Nutrient Agar and MaCconlcay Agar following pour plate method and incubated at 37^0C for 18-24 hours (Cheesbrough , 2006). Two media were used to carry out this analysis, these include nutrient and MaConcay agar which were prepared according to manufacturers instruction. Total number of bacteria cfu/g of the sample was calculated and recorded for interpretation of the result. Cfu/g was converted to logcfu/g^{-1} (Azoro, 20003). The sensitivity of the two media (MA and NA) to microbes and bacteria was calculated using equation 36

$$Sensitivity\ (\%) = \frac{value\ after\ storage - value\ before\ storage}{value\ before\ storage} \times 100 \text{.--------------------(2)}$$

(Azoro, 20003).

Results

Table 1: Average Moisture Content of *Bombax Costatum* calyx stored with 0% of K_2CO_3 in different containers and control for nine Months at room temperature.

Container	Jan	Feb	March	April	May	June	July	August	Sept.
Ceramic	5.50	5.50	5.50	5.95	6.20	6.50	6.50	6.50	6.60
Metal	5.50	5.50	5.20	5.30	5.23	5.50	6.30	6.40	6.50
Plastic	5.50	5.50	5.60	5.60	6.20	6.60	6.50	6.60	6.60
Glass	5.50	5.50	5.50	5.80	6.200	6.40	6.50	6.50	6.50
Control	5.50	6.00	6.05	8.50	12.10	12.50	13.50	13.50	13.70

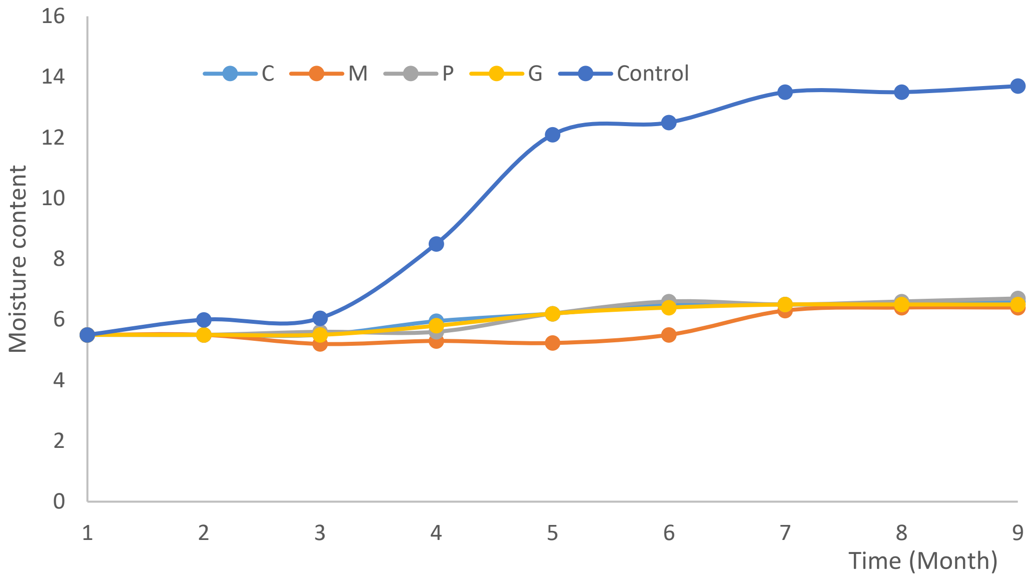

Fig 1: Plot of moisture content values against time across containers at 0% of K_2CO_3 and room temperature

Table 2: Average Temperature and Relative Humidity of Storage Environment during Storage

Months	Morning		Afternoon		Evening		Average	
	Temp. (^{0}C)	RH (%)	Temp. (^{0}C)	RH (%)	Temp.(^{0}C)	RH (%)	Temp. (^{0}C)	RH (%)
1	28.5	35	29.3	29	29.1	25	28.96	29.67
2	29.2	26	29.7	26	29.4	24	29.43	25.33
3	31.3	26	32.3	44	33.7	49	32.43	39.67
4	30.0	62	32.7	65	33.0	64	31.90	63.67
5	28.5	65	29.0	64	28.7	65	28.73	64.67
6	28.0	68	28.5	66	28.5	66	28.33	66.67
7	28.0	72	28.5	68	28.5	70	28.33	70.00
8	28.0	70	29.5	68	29.0	69	28.83	69.00
9	27.5	74	28.5	68	28.0	72	28.00	71.33

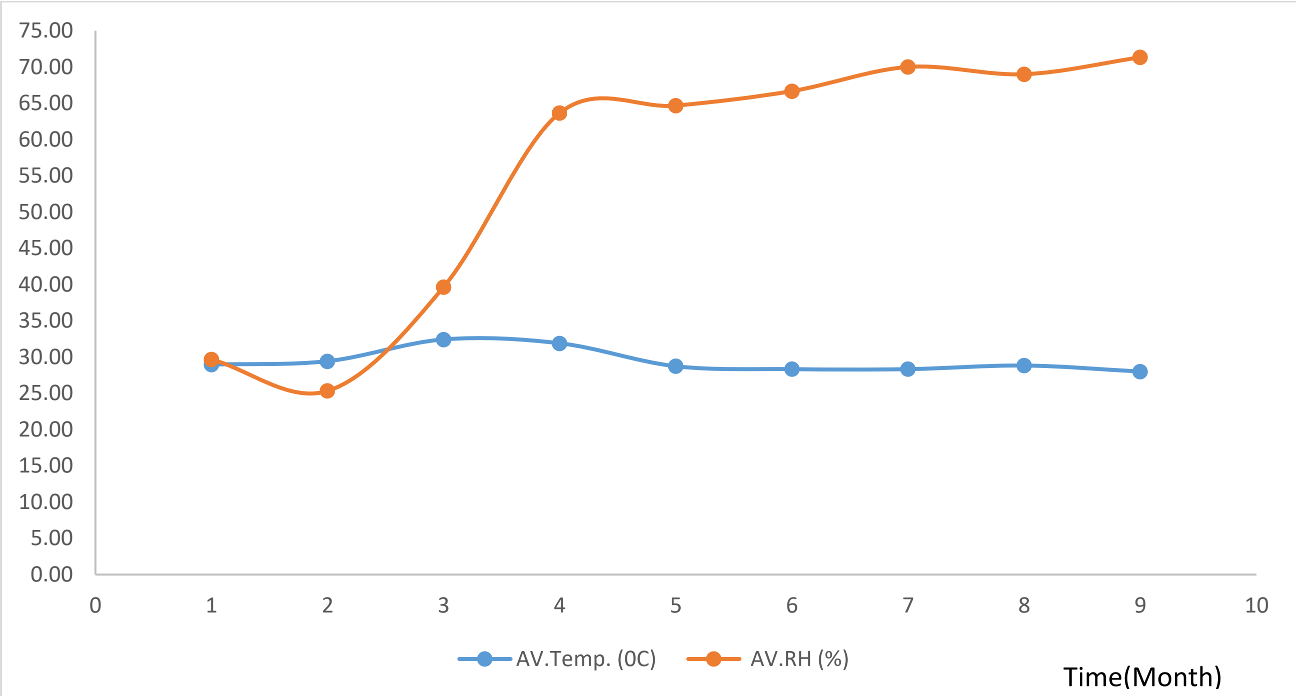

Fig2:Plot of Average Temperature and Relative Humidity of Storage Environment during Storage against time

Table 3: Microbial Load Count of BombaxCostatum Samples using two media (Nutrient Agar (NA) and Maconcay Agar (MA)

Medium	Dilution Factor	Before Storage (logcfug-1)	Load after Storage (logcfug-1)				
			Control	Plastic	Glass	Ceramic	Metal
Nutrient Agar (Microbial Load)	10.1	3.95	4.50	3.96	3.97	3.97	3.90
	10.2	4.78	5.43	4.85	4.83	4.83	4.86
	10.3	5.60	6.18	5.74	5.70	5.70	5.73
	10.4	6.30	7.12	6.31	6.49	6.49	6.32
	10.5	7.03	7.88	7.28	7.27	7.27	7.04
Average		5.53	6.22	5.63	5.57	5.65	5.57
MaconcayAgar (Microbial load)	10.1	3.51	4.15	3.55	3.57	3.59	3.6
	10.2	4.14	5.11	4.5	4.31	4.3	4.40
	10.3	4.83	6.07	4.9	4.95	5	5.12
	10.4	5.34	7.04	5.61	5.70	5.98	5.96
	10.5	5.95	7.95	6.76	6.48	6.98	6.60
Average		4.74	6.06	5.06	5.00	5.13	5.14

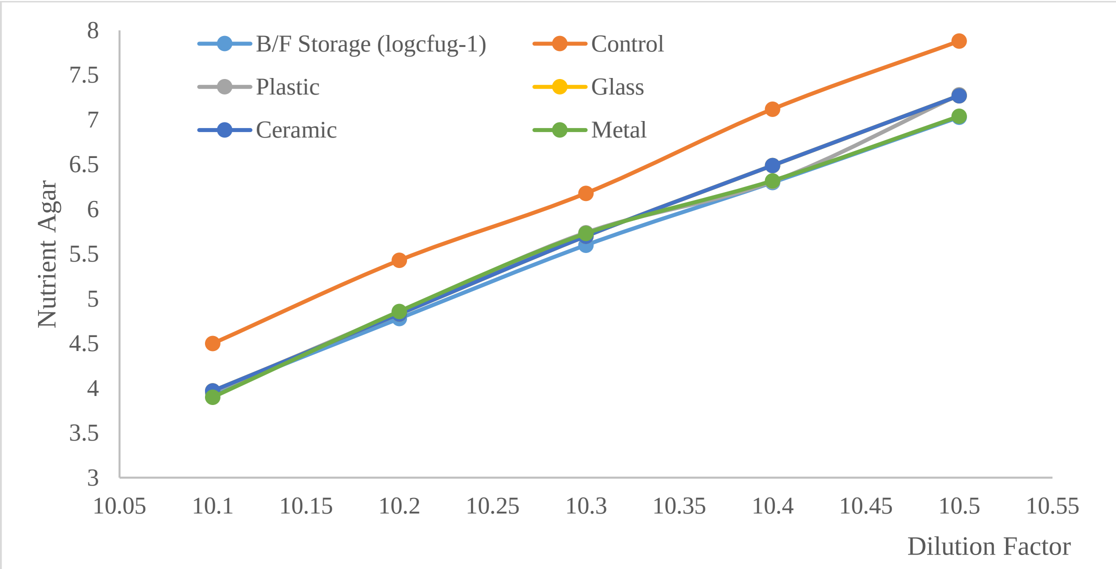

Figure 3: Microbial Load Count of Bombax Costatum Samples Nutrient Agar (NA) medium

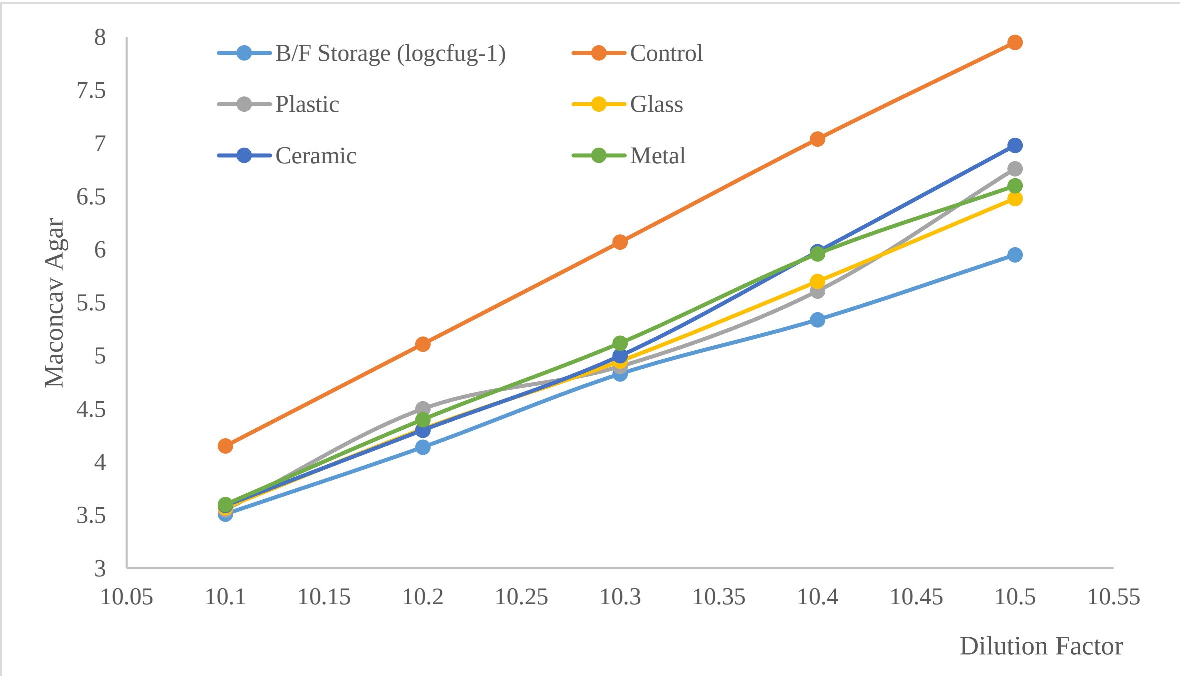

Figure 4: Microbial Load Count of BombaxCostatum Samples using Maconcay Agar (MA) medium

Table 4: Sensitivity of Microbial Load Count of BombaxCostatum Samples after Storage

Medium	Dilution Factor	Before Storage (%)	Control (%)	Plastic (%)	Glass (%)	Ceramic (%)	Metal (%)
NA (Microbial)	10.1	0.00	13.92	-0.25	0.51	0.51	-1.27
	10.2	0.00	13.60	1.46	1.05	1.05	1.67
	10.3	0.00	10.36	2.21	1.79	1.79	2.32
	10.4	0.00	13.02	0.16	3.02	3.02	0.32
	10.5	0.00	12.09	-0.71	3.41	3.41	0.14
MA (Microbial)	10.1	0.00	18.23	1.14	1.71	2.28	2.56
	10.2	0.00	23.43	1.45	4.11	3.86	6.28
	10.3	0.00	25.67	1.45	2.48	3.52	6.00
	10.4	0.00	31.84	5.06	6.74	11.99	11.61
	10.5	0.00	33.61	4.20	8.91	12.31	12.61

Discussion

Moisture content

Table 1 and figures 1 contain average Moisture Content of powdered *Bombax Costatum* calyx samples treated with different levels of K_2CO_3 and stored hermetically in four different containers and control, for Nine Months. The moisture content for ceramic, Metal, Plastic, Glass and control containers as determined during the storage increased from 5.5 to 6.60%wb, 5.5 to 6.40%wb, 5.5 to 6.7%wb, 5.5 to 6.50%wb and 5.50 to 13.70%wb respectively. The result showed that all containers provided effective sealing and barrier from external moisture, as the level of moisture increase after nine months of storage was still within the acceptable moisture level for storage of flours. Except the control which showed substantial increase in moisture due to deliberate exposure to the room atmosphere. According to the result, metal provided more effective sealing, followed by glass, ceramic and lastly plastic. The result also showed that moisture content of samples decrease with increase in percentage of K_2CO_3 and increase with time.This result is in agreement with the result obtained by Gernah and Gbakaan(2003). The low percentage of moisture of *Bombax Costatum* calyx used for this study is in agreement with Mkaanem (2018) for leafy and calyx materials, especially for the purpose of storage. The supposition that moisture affects some functional properties of *Bombax Costatum* is justified by values of viscosity, bulk density, water absorption capacity and swelling capacity of samples exposed to the room environment, which absorbed more moisture than the sealed containers that were adequately protected from moisture invasion, as shown by the moisture content of the control sample. The relative humidity and temperature of stored environment were monitored. The result in figures 1 and 2 showed that moisture content increases with increase in relative humidity.

Bacteria and Microbial loads

Tables 3-4 and figures 3-4 show the microbial and bacteria counts of *Bombax costatum* sampled from different storage containers in two media, that is Nutrient Agar and Macconcay

Agar. The total viable count (Tvc) of the initial sample (before storage) ranged from 3.51 logcf/g^{-1} to 7.03 logcfu/g^{-1}. After nine months storage of the sample, there was an increase in the microbial count which ranged from 4.15logcfu/g^{-1}-7.95logcfu/$g^{-1}$for control, while samples stored in plastics, glass, ceramics and metal had microbial counts that ranged from 3.55logcfu/g^{-1}- 7.12logcfu/g^{-1}, 3.57logcfu/g^{-1} – 6.98logcfu/g^{-1}, 3.59logcfu/g^{-1} – 7.27logcfu/g^{-1} and 3.60logcfu/g^{-1} to 7.04logcfu/g^{-1} respectively. The overall mean TVC (Total Viable Count) obtain from this study ranged from 4.75logcfu/g^{-1} to 6.06logcfu/$g^{-.}$ The Total Viable Counts (TVC) was high on the control sample after storage with TVC ranging from 4.15logcfu/g^{-1} – 7.95logcfu/g^{-1}. This may be due to exposure of the sample, as it was not covered to avoid air or other particles to come in contact with it. Gilbert et al (2000), states that the presence of microbial load in foods may be due to harvesting methods and also the postharvest handling of such food. Again the control sample after storage has high number of bacteria colonies compared to those in containers, which also is an indication that it was more exposed to this microbes than the later. According to EC (2002), the Hazard Analysis and Critical control point total quality management (HACCP-TQM) technical guidelines lay down the microbial quality for raw foods where the food containing TVC less than 4.4 -6.69, 6.70-7.69 and greater than 7.69logcfu/g^{-1} is rated good, average, poor (spoiled food) respectively. The mean TVC in this study across dilutions and containers showed that Plastic ranged from 3.55logcfu/g^{-1}- 7.12logcfu/g^{-1}, glass ranged from 3.57logcfu/g^{-1} – 6.98logcfu/$g^{-1,}$ metal ranged from 3.60logcfu/g^{-1} to 7.04logcfu/g^{-1}, and ceramic ranged from3.59logcfu/g^{-1} – 7.27logcfu/g^{-1},the control had TVC ranged from 4.15logcfu/g^{-1}-7.95logcfu/g^{-1}.The average TVC across all dilutions and containers ranged from 4.75logcfu/g^{-1} to 6.06logcfu/g^{-1}. In line with HACCP-TQM technical guidelines, both the sample before storage and samples in containers after storage belong to the category of good food with glass presenting the best protection, followed by metal, ceramic, plastic and lastly control. This confirms that metal,glass and ceramic are actually good storage systems for storage of *Bombax costatum*, as it has equally preserved viscosity with minimum loss and provided good barrier for moisture permeability and protection of food proximate constituents, especially at 0% of K2CO3. The Presence of these microorganisms reflect that samples or storage containers have been exposed to contaminants and favourable condition for multiplication of the microorganisms (Aycicelc et al (2004). Gilbert et al (2000) and Cheesbrough,(2006) also stated that plate count of microorganisms found in food is one of the microbiological indicator for food quality and most foods are regarded as harmful when they have large population of microorganisms, even if the organisms are not known to be pathogenic. Although the result obtained from this study is rated to be good according to HACCP –TQM, proper handling of food during storage should be ensured to avoid contamination to reduce risk of this microorganism getting into human body. The sensitivity percentage of samples was computed to range between -0.25% to 12.61% for sealed containers and 13.92% to 33.61% for control as shown in table 4.

Conclusion

The hermitic storage of *Bombax costatum* using ceramic, metal, glass and plastic containers presented effective performance. Both moisture content analysis and microbial counts performed on *Bombax costatum* before and after storage showed that, all containers provided effective protection to stored samples and the nutritional quality of the food was equally retained across all containers. The safety of the stored *Bombax costatum* was established to be good for samples stored in metal and glass, and average for samples stored in plastic and ceramic containers, by comparing the total viable counts of microbes for each container with the HACCP-TQM scale.

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