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CAROTENOID CONTENT, SENSORY PROPERTIES AND MICROBIOLOGICAL QUALITY OF STORED YELLOW CASSAVA *FUFU*

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ABSTRACT

The carotenoid content, sensory properties and microbiological assessment of stored cassava fufu from two cultivars of yellow cassava (TMS 01/1368 and TMS 01/1412) being multiplied for distribution in South-East and South-South Nigeria were investigated using standard techniques. There is scanty information on microbiological safety, carotenoid content, and sensory evaluation of reconstituted fufu from the newly released yellow cassava roots. That is the background against this study is carried out. In terms of carotenoid content, TMS 01/1368 had the highest carotenoid value of 7.76 µg/g and 7.48µg/g respectively, for both fresh root and fermented mash. While TMS 01/1412 had 6.26 µg/g carotenoid for fresh root and 6.69µg/g carotenoid for fermented mash. The sensory properties of the yellow cassava fufu samples revealed that they had no much difference in all the parameters and were acceptable. Results indicated acceptability of the yellow cassava cultivars and their use for fufu preparation. The pH decreased from an average of 4.66 on day one to 3.67 on the eight day for TMS 01/1368 while the cultivar TMS 01/1412 decreased from an average of 4.65 on day one to 3.54 on the eight day. The titratable acidity of the both samples did not show significant increase. The % moisture content increased from day one to day eight for the both cultivars. The values were (47.23% - 50.98%) for TMS 01/1368 and (52.04 - 60.12) for TMS 01/1412. The microbial counts increased from the initial average of 1.66 x 10^5 cfu/g on day one to an average count of 11.6 x 10^5 cfu/g on eight day for TMS 01/1368 while TMS 01/1412 increased from 2.33 x 10^5 cfu/g on day one to an average count of 12.0 x10^5 cfu/g on the eight day. The spoilage organisms associated with the products during storage include Staphylococcus areaus, Bacillus cereus, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer and Candida albicaus.

Key Words, Cassava, *Fufu*, Safety, pH, TTA, Moisture content, Micro-organisms DOI: http://dx.doi.org/10.4314/jafs.v14i1.7

INTRODUCTION

Cassava (*Manihot esculenta*) is an important crop widely cultivated in Sub-sharan Africa. Cassava is to Africa peasant farmers what rice is to Asia farmers or wheat and potatoes are to the European farmers (El- sharkawy, 2003). Although cassava is grown virtually in all parts of the sub- continent, production is specific in the humid tropics (Okereke *et a*l., 2001).

Cassava plays a major role in efforts to alleviate Nigeria's food crisis because of its efficient production of food energy, all year round availability, tolerance to extreme stress conditions and suitability to various farming and food system (Awah and Tumanteh, 2001). Yellow cassava is a new, yellow- fleshed breed of one of the most popular root corps in the tropics. Yellow Cassava is similar to ordinary varieties of cassava (*Manihot esculntum*) but it has a yellow flesh inside the root which is generally white in ordinary varieties (Egesi, 2011). The yellow cassava varieties are grown in Nigeria for their high concentration of β-carotene which is a precursor to vitamin A. Since cassava is a food staple, yellow cassava shows great potential to alleviate vitamin A deficiency in Africa. Yellow cassava could be processed into different forms for human delicacies and animal feeds. Cassava chips has a wide application for dough and paste, for composite flour making and starch as source of fermentable sugar required in the production of alcoholic beverages (Amuth and Gunasekaeam, 2001). Processed cassava flour has been reported to be good weaning food, feed ingredient and bakery substitute (Lekule and Sarwath, 1992). In Nigeria, the majority of cassava produced, (90%) is used for human food (IITA, 2010). Yellow cassava can be processed into *fufu,* abacha, garri and other cassava products. Cassava *fufu* is one of the food products of cassava root which is produced by fermentation. The preparation of *fufu* is a traditionally based process to produce *fufu*, garri, *akpu*, cassava flour, edible starch, and tapioca. Ezedinma and Oti (2001), stated that the traditional methods of cassava processing into *fufu* are often contaminated with undesirable extraneous matters that make them unhygienic thereby affecting demand and consumption. The processing is carried out manually with simple inexpensive tools and equipment within the reach of the small scale farmers. The use of traditional method for production of *fufu* from yellow root cassava varieties have been recommended for maximum utilization of nutrient in the newly released yellow cassava varieties (Omadamiro *et al*., 2012). Fresh yellow root cassava is processed by peeling the cassava, cutting into small sizes, steeping in water for 48 hours, grating into mash, re - steeping for 48 hours, - wet sieving- sediment – dewatering (bagging and pressing) - yellow *fufu* mash (Omodamiro *et al.*, 2012). Achi and Akomas (2006) reported the isolation of *Lactobacillus* spp, *Bacillus* spp, yeast and coliform during fermentation of cassava using different processing methods. Fermentation enhances cassava roots and preparation to a dimensional food, *fufu,* which is transported from one part of the country to another on a very high demand because of its nutritional value; a good storage means is highly important. Yellow cassava has potentials for supporting the growth of micro-organisms because of its nutrients. Cooking of *fufu* alone without proper handling does not confer preservation of retted yellow cassava *fufu*. Micro-organisms gain entry into food during storage and handling and due to their activities alter the smell, taste, colour or chemical composition of the food significantly and render it inedible (William and Shaw, 1992). However, there is scanty information on microbiological safety, carotenoid content, and sensory evaluation of reconstituted *fufu* from the newly released yellow cassava roots. The study adds value to wider utilization of the yellow cassava *fufu,* careful target for health consumer and guide for safety measures. The knowledge on microbiological quality of food eaten in places is valuable in identifying and solving nutritional and health problems of the population.

Materials and methods

Sources of Experimental Samples

Fresh roots of two yellow cassava varieties namely: UMUCASS 36 (TMS 01/1368) and UMUCASS 37 (TMS 01/1412) were obtained from Cassava Programme of National Root Crops Research Institute, Umudike. The cassava roots were harvested at the 11th month after planting.

Preparation of *fufu* Samples

Thirty kilograms of root tubers of each of the two yellow cassava varieties (TMS 01/1368) and (TMS 01/1412) were used for the production of *fufu.* Roots of each variety were washed and peeled, cut into small sizes of about 7-8 cm, washed and steeped in 25 litres of clean tap water in two basins of same volume and diameter (25 litre and 54cm) respectively and properly labelled and allowed to ferment for 48 hours for 3 days at room temperature, after which the roots were grated and the resulting mash re-steeped for 24- 48 hours, wet sieved to obtain the sediment wet yellow cassava mash. (Omodimiro *et al.,* 2011).

Having obtained yellow cassava wet mash, 200g of the wet mash was put into a clean stainless pot and 250 ml of water was added to form mash slurry and was constantly stirred with a wooden rod on fire for about 30 minutes until the ready-to-eat *fufu* dough was formed. It was allowed to cool for 45 minutes. After cooling the sample was collected with sterile containers and covered immediately and taken to the laboratory for total carotenoid content and some were parked equally on serving plates for sensory evaluation. Ten cooked *fufu* samples 50g of each variety were wrapped in transparent cellophane of low density polyethene, normally used to wrap and hawk fufu. They were wrapped and kept on dry, clean trays stored for 8 days at ambient temperature ($28\pm2^{o}C$) and were examined every day and samples taken for pH, Total titratable acidity (TTA), moisture content and microbial safety of the wrapped samples for the duration of storage. Replicate samples were collected from each batch for the analysis.

Total Carotenoids Content Analysis

The Harvest Plus procedure for carotene analysis was used to analyze the total carotenoids content of the fresh cassava roots, and reconstituted *fufu.* The Harvest plus method of Rodrigueze-Amaya and Kimura (2004) for carotenoid analysis procedure is described as follows: Five (5) grams of each fresh root sample was grinded with the aid of hyflosupercel in 50ml of cold acetone and filtered with suction through a Buchner funnel with filter paper; the filtrate was extracted with 40ml of petroleum ether using separating funnel. Saturated sodium chloride solution was used to prevent emulsion formation.

The lower phase (water) was discarded while the upper phase was collected into a 50ml volumetric flask, making the solution pass through a small funnel containing anhydrous sodium sulfate to remove residual water. Then, the separating funnel was washed with

petroleum ether and the standard flask made up to 50ml mark. The absorbance at 450nm wavelength was taken using spectrophotometer (model 6406 uv- visible, jenway) and the total carotenoid content was calculated as follows;

$$\text{Total Carotenoid } (\mu g/g) = \frac{A \times \text{volume (ml)} \times 10^4}{A1\%1cm \text{ X sample weight g}}$$

Where A = absorbance, Volume = total volume of extract (50ml), A1% 1 cm = absorption coefficient of β-carotene in P.E. (2592)

Sensory evaluation

Twenty-member sensory panel was used for the sensory evaluation using the method described by (Iwe, 2002). The panelists were asked to indicate their preference for colour, mouldability, odour, hand-feel, and general acceptability using a 9-point Hedonic scale, where 9 indicates like extremely, 5 indicates neither like nor dislike and 1indicates for dislike extremely.

pH

Ten grams of cooked *fufu* sample was aseptically removed and homogenized with 100mI of sterile distilled water. The water was decanted and its pH determined in duplicates. The pH meter was calibrated using buffer of pH 4.0 and 7.0. The pH was determined using a Kent pH meter model 7020 equipment with glass electrode.

Total Titratable acidity (TTA)

Total titrating acidity of cooked *fufu* samples was determined by titrating 25 ml of the decanted water used for pH determination. TTA is calculated as % Lactic acid.

$$\% \text{ Lactic acid} = \frac{\text{ml of 0.1 N NaOH x O.9}}{\text{Weight of samples}}$$

Moisture content

The moisture content of the samples was determined by the standard AOAC (2010) method. A 5.0 g of the *fufu* samples was weighed into a weighed moisture can. The can and its content were dried in the oven at 105°C for 3 h in the first instance. They were cooled in desiccator and reweighed. The weight was recorded while the samples were retained in the oven for further drying. The drying, cooling and weighing was continued repeatedly until a constant weight was obtained. The moisture content was determined by difference and expressed as percentage of the weight of sample analysed. All analyses were carried out in duplicate.

Moisture content MC % = $\frac{W_2 - W_1}{\text{Weight of sample}}$ X 1 100

Where

w_1= weight of moisture can with sample before drying.

W_2= weight of can with dried sample.

Microbiological Analysis

One gram from each of the *fufu* wraps was separately homogenized in 9 ml of sterile peptone water. The dilution was serially made until 10^{-5} dilution was obtained. Isolation and identification was done according to the method of Ogbulie *et al.,* (2005). For bacterial isolation, nutrient agar, macConkey agar were used, while sabouraud dextrose agar was used for fungi isolation. Total viable counts of bacteria were determined by enumerating the colony forming units (cfu/g) by pour plating 1 ml of 10^{-5} diluent incubated at 37^0C for 48h. Total fungi counts were determined by pour plating also and incubated at 37^0C for 3 days. The experiments were carried out in triplicates. Pure cultures of bacterial and fungal isolates were obtained respectively. Discrete colonies were aseptically transferred by streaking using sterile wire loops onto sterile nutrient agar, macConkey agar slants for bacteria and sabouraud dextrose agar slants for fungi to obtain pure cultures.

Characterization and Identification of Isolates

Bacteria isolates were characterized and identified by initially examining colonies morphology on their cultural properties followed by physiological and biochemical tests (Motility, citrate, coagulase, indole, starch fermentation, Gram stain, spore stain, catalase and oxidase). The fungal isolates were characterized by their cultural properties stained with cotton-blue lactophenol solution and observed under low power objective lens (Chessbrough, 2002; Ogbulie *et al*., 2005).

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel (windows 8.0) to show the responses of pH, TTA and moisture content with increasing period of storage of yellow cassava *fufu.* Data were analysed using mean± standard deviations of triplicate experiments and results were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The total carotenoid content of the yellow cassava fresh mash (grated mash) and the fermented mash (product) of the yellow cassava varieties is shown in Table 1. The result ranged from 6.57- 7.75 µg/g for fresh mash samples while the fermented mash ranged from 6.32- 7.48 µg/g. The TMS 01/1368 had the highest carotenoid content for both fresh and

fermented mash. The carotenoid values obtained were in line with the carotenoid values obtained by (Omodamiro *et al.*, 2012). The carotenoid values obtained in this study were better when compared with yellow roots cassava *fufu* flours that give 1.20 – 2.00 µg/g (Omodamiro *et al.*, 2012). Reduction in carotenoid contents during processing of yellow cassava roots into some food forms has been reported (Bai *et al.*, 2010).

There are many different forms of processing to which food may be subjected before consumption. These two varieties have between 6.32 – 7.75 µg/g of pro- vitamin A and these have not reached the Harvest – plus benchmark of 15µg/g which is adequate to meet daily requirement for humans, as described by (Njoku *et al.*, 2013) It is important to note that β – carotene is a precursor of vitamin A, where it cleaved by dioxygenase using molecular oxygen to form retinal in the intestine (the retinal so formed is hydrolysed to retinol which is absorbed by the intestinal mucosal cells and hence vitamin A deficiency could lead to night blindness. Vitamin A is also involved in cell differentiation, synthesis of glycoproteins, reproduction and overall growth and development (Woolfe, 1992). A sustainable way of mitigating vitamin A deficiency is by breeding food staples such as cassava to produce vitamin A by itself, a process known as biofortification (Graham and Rosser, 2002).

Table 2 shows the sensory evaluation of the freshly prepared yellow cassava cooked *fufu* . There were no significant (> 0.05) differences in the colour, odour and overall acceptability of the cooked *fufu* of the two varieties. The slight odour during storage at day 6 could be attributed to the spoilage organisms identified in the study. This did not affect the acceptance of the yellow *fufu* and may serve as a probiotic food for mankind. However, there was significant difference in the means of the mouldability and Hand feel. The result revealed that the cooked *fufu* products had no much difference in colour, odour, and overall acceptability and were acceptable.

Figure 1 presents the results of the trend of the pH during the storage of the yellow cassava *fufu* at ambient temperature. The initial pH values were 4.66 and 4.56 for TMS 01/1368 and TMS o1/1412 respectively.

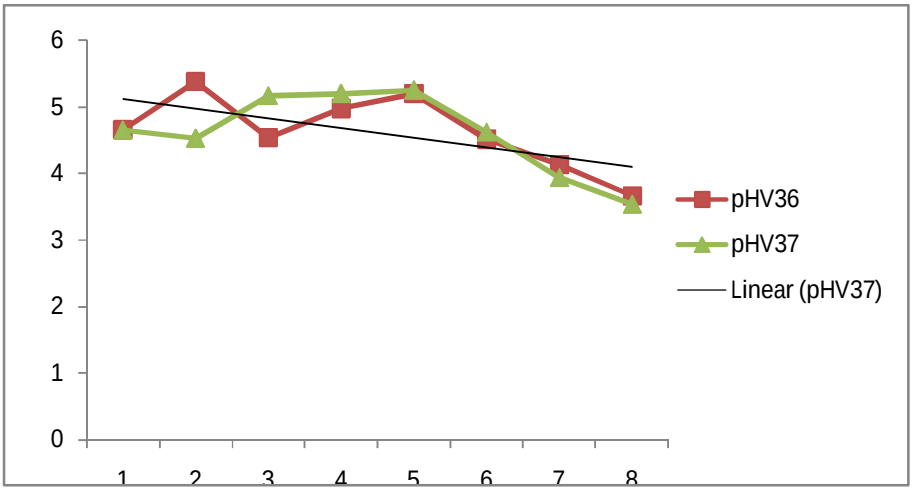

Fig. 1: Trend analysis of PH during the period of storage of the yellow cassava *fufu*

The values decreased to 3.67 and 3.54 respectively at the 8 day of storage at ambient temperature. The pH trend of the food samples decreased following the storage period, a situation which favoured the incidence of more fungal colonies during storage at ambient temperature.

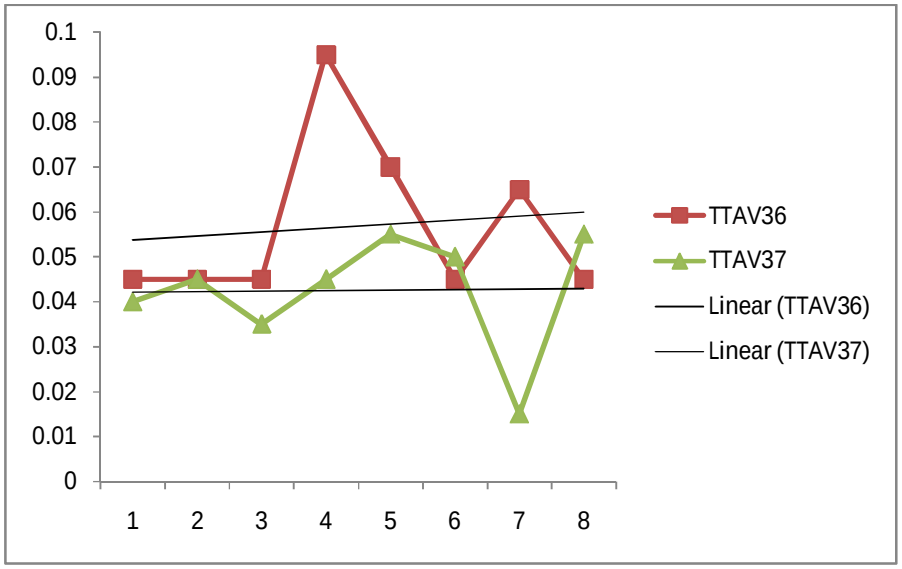

Fig. 2: Trend analysis of the titratable acidity (TTA) during the period of storage of yellow cassava *fufu*.

The result of titratable acidity of yellow cassava *fufu* during storage for 8 days at ambient temperature is shown in figure 2. This indicates the acidity (estimated as % lactic acid) of each food sample. The result revealed that the TTA of both samples did not respond to increase in storage period as their values were very low.

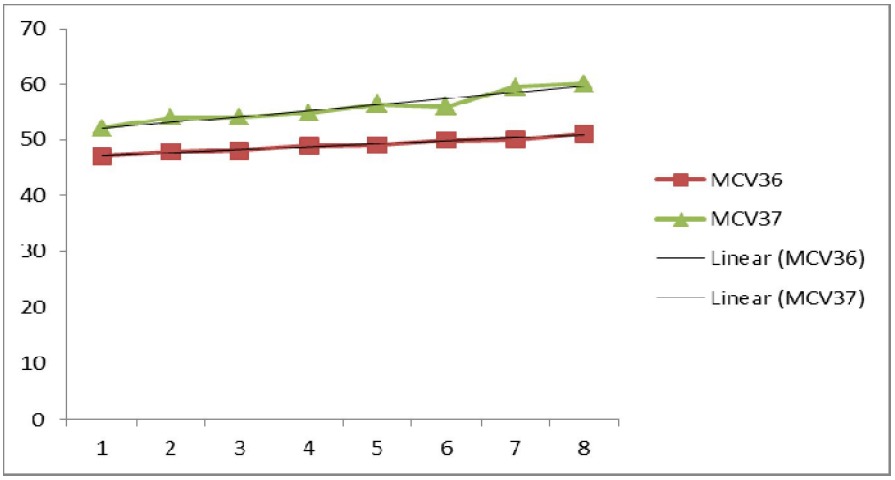

Fig. 3: Trend analysis of the moisture content during the period of storage of yellow cassava *fufu*

The result of the moisture content during storage for 8 days is presented in Figure 3. The mean values of the *fufu* samples ranged from 47.23 – 50.98 for TMS 01/ 1368 and 52.04 – 60.12 for TMS 01/1412. This indicates that TMS 01/1412 had the highest mean value of the percentage moisture content. The moisture content increased significantly ($p< 0.05$) as the storage period increased irrespective of the poly ethylene film during the 8 days storage at ambient temperature. The increase in the percentage moisture content of stored *fufu* can be attributed to the degrading activity of the different micro-flora during storage(Egbebi *et al.*,2011). Although, Polyethylene films generally have good barrier against moisture (Ukpabi *et al*., 1998), but low density polyethylene have higher water vapour permeability.

Table 3 shows the microbial counts of the cooked yellow cassava *fufu* stored at ambient temperature (28 ± 2^oC) for at least 8 days. The microbial counts of the cooked *fufu* ranged from 1.66×10^4 cfu/g to 11.6×10^4 cfu/g for TMS 01/1368 while TMS 01/1412 ranged from 2.33×10^4 cfu/g to 12.0×10^4 cfu/g. The result showed that the microbial count increased gradually with increase in storage time for both cassava varieties. The result also revealed significant difference ($p<0.05$) of the microbial count from day 5 to the 8^{th} storage day for both varieties. The result also indicate that wrapping the cooked *fufu* with the cellophane film after preparation can extend its storage for up to 4 days before it can be reconstituted. The microbial load of the cooked *fufu* during the storage period increased with increase in moisture content. This is because moisture is extremely vital in bringing about deterioration of food. Furthermore, product deterioration during storage could be as a result of combined effect of moisture and microbial proliferation in the product (Bothast *et al*., 1991). The pH, total titrabable acidity, moisture content and storage temperature of the yellow cassava *fufu* samples favoured the growth of microorganisms. Some micro-organisms isolated from the yellow *fufu* samples are shown in Table 4. The organisms include *Staphylococcus aureus, Lactobacillus plantarum, Candida albicans, Aspergillus niger. Aspergillus flavus and Rhizopus stolonifer*. This is similar to the findings of Obadina *et al*.,(2007), who isolated some fungi from *fufu* flour stored at ambient condition. The presences of these organisms is an indication of post- processing contamination which is of public health significance. Although, the high temperature used in the preparation of the cassava *fufu* is enough to eliminate most of the micro- organisms but post processing contamination may occur which affected the quality of the products. The food samples may be contaminated during mixing, and moulding . This observation corroborate with previous report by Anifantaki *et al*.,(2002). The increase in microbial number of these organisms as the days of storage increases indicates proliferation with deterioration of nutritional quality which are utilized by spoilage organisms (Bueno *et al*., 2004). However, the presences of *Staphylococcu aureus* in the fufu samples is due to contamination from the skin, mouth or nose of the food handler which can be introduced directed into foods during processing. The *Aspregillus* spp and *Rhizopus stolonifer* in the food may lead to food poisoning, since many of these fungi are toxin producing organisms.

Conclusion

The cassava *fufu s*amples harbour microbial contaminates. The contaminants which affected the quality of the food may have come from cross contamination by processors, utensils, packaging materials and storage. The changes in pH, moisture content and titrable acidity(TTA)were caused by the microorganisms and made the cassava *fufu* unsafe for consumption after the fourth day keeping. However, the quality of the food must be improved by processing the food in hygienic conditions, as to minimize the risk of contamination. Breeders are already working hard to release new yellow cassava varieties with higher carotenoid content for good health.

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APPENDIX

Table 1: Total carotenoid content (µg/g) of the yellow cassava fresh root (grated mash) and the fermented mash (Product)

Samples	Fresh root	Fermented mash
TMS 01/1412	7.76	7.48
TMS 01/1368	6.26	6.69

Table 2: Sensory Evaluation of fresh Yellow Cassava *fufu*

Sample	Colour	Moldibility	Odour	Hand feel	Overall Acceptability
TMS 01/ 1412	7.00±0.9	7.65±1.0	5.90±1.1	6.80±1.0	7.75±0.8
TMS 01/ 1368	7.55±0.9	5.75±2.0	5.85±1.3	5.95±1.6	7.45±1.7

Values are means of triplicate ± standard deviations.

Table 3: Microbial Count of cooked Yellow cassava *fufu* stored at Ambient Temperature

Number of Days	**Varieties**	cfu/g ±SD
1	TMS 01/1368	1.66 ± 0.57
	TMS O1/1412	2.33 ± 0.57
2	TMS 01/1368	2.00 ± 0.00
	TMS 01/1412	2.66 ± 0.57
3	TMS 01/1368	2.66 ± 0.57
	TMS 01/1412	3.66 ± 1.15
4	TMS O/1368	3.33 ± 0.57
	TMS 01/1412	3.00 ± 0.00
5	TMS 01/1368	4.00± 0.01
	TMS 01/1412	6.66 ± 2.08
6	TMS 01/1368	9.00 ± 0.01
	TMS 01/1412	10.0 ± 0.01
7	TMS 01/1368	7.33 ± 3.78
	TMS 01/1412	11.3 ± 2.08
8	TMS 01/1368	11.6 ± 1.52
	TMS 01/1412	12.0 ± 1.73

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Cultural and morphological Characteristic	**Gram reaction**	**Motility**	**Catalase**	**Coagulase**	**Oxidase**	**Indole**	**Citrate**	**Glucose**	**Sucrose**	**Mannitol**	**Lactose**	**Maltose**	**Inositol**	**Identified isolates**
Creamy, regular, smooth raised colonies 1-2µm	+ve coci	_	+	+	_	_	_	A	+	A	_	A	+	*Staphylococcus aureus*
Gray, round, wavey, edge Flat and irregular rods 2-5µm	+ve rods	_	+	_	+	_	+	A	A	A	A	A	_	*Bacillus cereus*

FOR FUNGI

Cultural	**Staining**	**Identified isolates**
Identification		
Black ,irregular, roughened and powdery	+VE With hyphae	*Aspergillus niger*

Fluffy white with black spores. Fluffy yellow - green	+ve with hyphae	*Aspergillus flavus*
Gray white with brown -black patches. Hyphae rapidly cover the media	+ve with hyphae	*Rhizopus stolonifer*
Whitish irregular slightly round.	+ve cocci pseudo hyphae	*Candida albicaus.*

Table 4: Morphological and biochemical characteristics of bacterial and fungal isolates

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