ISOLATION AND IDENTIFICATION OF YEAST ASSOCIATED WITH MELON SEED FERMENTATION

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Abstract

The aim of this study is to isolate and identify yeasts from different stages of melon seed fermentation produced using two melon seed varieties (Feterita and Wad akar). 20 samples were obtained from different stages of egusi production (grain flour, malt, fermented dough at zero time, fermented dough after 12 h fermentation with and without starter, fermented dough after 24 h fermentation with and without starter and the end product). The yeast counts of egusi prepared from white egusi (FSH) were 3.3×10^3 , 6.1×10^4 , 3.6×10^4 and 8.3×10^5 C.F.U/ml, while yeast counts of egusi prepard from brown egusi (WSH) were 3.5×10^3 , 4.3×10^5 , 6.3×10^5 and 1.6×10^6 C.F.U./ml at time intervals of 3, 9, 12 and 24 hours, respectively. Only two genera of yeast were detected, these were Saccharomyces, with a frequency ranged between 40 and 100% in white melon seed and 80% in brown melon seed and Candida, with a frequency of 60%.

Key words; *yeast, melon seed, fermentation, egusi, saccharomyces.*

INTRODUCTION

Spoilage is always a concern to anyone who purchases melon seeds but an increase in the rate of melon seed consumption in Nigeria means that understanding spoilage is of increasing importance to consumers as many of the microorganisms responsible for the spoilage can present health problems to humans or animals should the spoiled melon seed be ingested.

Most handlers of melon seed in developing countries are ignorant of basic food safety measures. Consequently, melon seed are commonly exposed to various contaminates at different stages of handling. The high incidence of food and water borne disease in Nigeria according to Ministry of Health is above 7million annually (WHO, 2015). The major problem of melon seed is the incidence of microbial contamination. Pest infestation in stored grains can be the source of contaminating melon seed. Everyother sources of contamination such as air pollution duing stepping and rodents attacks. There are urgent need to carry out a microbial study on melon seed as to ascertain its quality and safe for consumption.

Melon plant is a runner, with a hairy green stem and green leaves that are shaped like duck legs. It matures in 3-4 months. Depending on the variety, white or yellow flowers are conspicuous on the plant towards pod-bearing stage. Melon plant is a legume which bears light green netted pods almost up o the size of broad-leaved pumpkin pods. The pods are the fruits which bear the seeds (Van der Vossen *et al*., 2004). Melon seed is a native of tropical countries, and Nigeria is one of largest producers in the world (Njoku *et al* 1994). There are two major types in Nigeria – the Bava (*Adenopsis guinensis*) and Egusi (Citrulllus lanatus). Both have white seed protected by a brown crown (Van der

Vossen *et al* 2004). Melon seeds produce important vegetable oil used for cooking, cosmetics, in the pharmaceutical industries and as important staple oil in Southern Africa (Van der Vossen *et al*., 2004).

This research work is aimed at reducing the microbial loss and spoilage caused by microorganisms which is highly significant. The result from this work will help to identify the microbial compositions of melon seed and this will assist in formulation of antibiotics effective against the microbes to drastically reduce their attack on the fruits.

MATERIALS AND METHODS

The materials used in this work include Nutrient agar, MacConkey agar, Peptone water, Petri dish, autoclave, inoculating wire loop, forceps, Bunsen burner, Conical flask, Antibiotic discs, Weighing balance, Test tube rack, plastic pipette, wire loop, Microscope, Incubator, beakers, glass slide, sterile cotton wool, test tube rack, universal container.

COLLECTION OF SAMPLES

Five representative spoilt melon seeds were purchased from sellers in open markets in Oko. The samples were taken to the laboratory in sterile containers for analysis within 24 hours.

SAMPLE PREPARATION

The traditional method of 'egusi' preparation was adopted. Sun-dried melon seeds (Citrullus lanatus) were shelled and washed. The seeds were boiled in excess volume of water for 2hrs in a pressure cooker, and then the excess water was allowed to evaporate to dry, leaving moist boiled seeds that were aseptically mashed and wrapped in flamed warm banana leaves to ferment for 5 days at ambient room temperature (28±2°C) according to the documented traditional method of [4], which include de-hulling of the melon seeds, washing and boiling, mashing of the seeds, packaging in banana leaves, and fermentation at 28°±2°C to produce 'egusi'. The 'egusi' condiment was made in seven replicates to facilitate the 24-hourly microbiological evaluations.

YEAST ENUMERATION

From suitable dilutions of sample 0.1ml was aseptically transferred onto solidified potato dextrose agar containing 0.1g chloramphenicol per one liter of medium to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod. Plates were then incubated at 28ºC for 48 hours as described by Harrigan [8]. Suspected colonies of yeast were re-streaked on sterile PDA plates and incubated at 28ºC for 48 hours. This procedure was repeated three times to obtain pure isolates of yeast to be ready for identification tests.

IDENTIFICATION OF YEASTS

Pure isolates of yeasts were identified according to Lodder [9]; Barrnett *et al.* [2010] as follows: The microscopic examination test was accomplished for yeasts from growing cultures which were inoculated into 10ml sterile liquid culture medium composed of 20g glucose, 5g yeast extract, 10g peptone and 1000ml distilled water, and the culture was examined microscopically after incubation at 28ºC for 72 hrs. The shapes of the yeasts cells and the form of budding were observed and registered. For sporulation test, smear was prepared according to Harrigan and McCance [2011] and stained by 5% malachite green then observed under the

microscope. The ascospore formation (Sporulation) test was carried out according to Barrnett *et al*. [2022], the following media were used:

1-Yeast extract, malt extract and glucose peptone agar
2- Acetate agar which was prepared by dissolving 9.8g potassium acetate, 1.0g glucose, 1.2g sodium chloride, 0.7g magnesium sulphate, and 2.5g yeast extract and 20g agar in a liter of distilled water.
3- Potato Dextrose Agar (PDA) (Barrnett *et al*., 1983)

Each of the above media was prepared into bottles as slopes after being autoclaved at 121ºC and 1.06kg\cm² for 15 minutes. Each isolate was then inoculated in each slope and incubated at 28ºC for one to four weeks, and then they were examined microscopically for ascospore formation. For the test for growth at 37ºC and 40ºC, YMA media contained (3g yeast extract, 3g malt extract, 10g glucose, 5g peptone and 20g agar in 1000ml distilled water) (McClay *et al*., 1959) was inoculated with fresh culture of yeast and incubated aerobically at 37ºC and 40ºC.

For the test for growth on high D-Glucose concentration, 56 grams of D-glucose were dissolved each in 1% yeast extract solution. Then 3% agar was added and was dissolved by boiling, distributed into test tubes and autoclaved for 15minutes at 121ºC and then sloped and inoculated by streaking and at 25ºC for 4 weeks .The growth in slopes was then examined.

The fermentation of glucose was carried out by filling **t**est tubes with 10-15 ml of yeast extract broth containing 2% D-glucose with inverted Durham tubes, then autoclaved at 121ºC for 20 minutes. Each test tube was inoculated with a fresh yeast suspension then incubated at 25ºC for 3 weeks, and examined frequently for bubbles of gas.

For assimilation of carbohydrates, small test tubes, each containing 10ml sterile medium composed of 0.5% peptone water with 4% test sugar were used. The tubes were inoculated with the selected yeast cultures in duplicate. A Vaseline- paraffin (Vaspar) layer, 2cm deep, was added on the top surface of one tube of the medium. The culture was incubated at 25ºC for four to five days. Fermentation was detected by the lifting of the Vaspar layer.

Urea broth medium (Difco) was used for urea hydrolysis test, in which, urea broth was dispensed into tubes aseptically, in aliquots of 5 ml then autoclaved at 121ºC for 20 minutes. A loop full of cells from one or two day old culture was suspended in the broth and incubated at 37ºC. The tubes were examined every half hour for a change of color to red which indicates urease activity.

The Cycloheximide resistance test was similar to the glucose fermentation test with the addition of cycloheximide to the medium (0.1%) and examining for resistance of yeast to cycloheximide.[10]

RESULTS

The count of yeasts of the fermented melon seed samples is shown in Table 1. The yeast counts of egusi prepared from white egusi (FSH) were 3.3×10^3, 6.1×10^4, 3.6×10^4 and 8.3×10^5 C.F.U/ml, while yeast counts of egusi prepared from brown egusi (WSH) were 3.5×10^3, 4.3×10^5, 6.3×10^5 and 1.6×10^6 C.F.U./ml at time intervals of 3, 9, 12 and 24 hours, respectively.

Table 1. Changes in yeast count (C.F.U./ml) during *Robe* Production from different sites

Sample	3	9	12	24
White egusi	3.3×10^3	6.1×10^4	3.6×10^4	8.3×10^5
Brown egusi	3.5×10^3	4.3×10^5	6.3×10^5	1.6×10^6

The data in Tables 2 and 3 show the results of morphological and biochemical identification tests of east isolates from different stages of egusi production by using two melon variety. The results indicated that, only two genera of yeast were detected in the various stages of fermentation. These were *Saccharomyces* and *Candida.*

Table 2. Morphological characteristics and biochemical tests for identification of yeast isolates from different stages of egusi production by using tow sorghum variety (Feterita & Wad Akar)

Tests Sample. No	Colony Shape	Cell Shape	Vegetative growth	Pseudohypha	Growth at Sporulation	37ºC	Growth in 42ºC	glucose 50%	60%
AI	Smooth-White to Creamy	Spherical	Budding	-ve	-ve	+ve	+ve	+ve	-ve
A2	″	Spherical	Budding	-ve	-ve	+ve	+ve	+ve	-ve
A3	"	Spherical	Budding	-ve	-ve	+ve	+ve	+ve	-ve
A4	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
A5	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
B1	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
B2	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve

B3	White-butyrous	Elongated	Budding	-ve	+ve	+ve	-ve	+ve	-ve
B4	"	Elongated	Budding	-ve	+ve	+ve	-ve	+ve	-ve
B5	"	Elongated	Budding	-ve	+ve	+ve	-ve	+ve	-ve
C1	Smooth-White to Creamy	Spherical	Budding	-ve	-ve	+ve	+ve	+ve	-ve
C2	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
C3	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
C4	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
C5	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
D1	"	Ovoid	Budding	-ve	-ve	+ve	+ve	+ve	-ve
D2	White-butyrous	Elongated	Budding	-ve	+ve	+ve	-ve	+ve	-ve

Table 3. Morphological characteristics and biochemical tests for identification of yeast isolates from different stages of egusi production by using raw sorghum variety (Feterita & Wad Akar)

Tests Sample. No	Fermentation of glucose	Urea Hydrolysis	Cycloheximide resistance	Assimilation of carbon compounds						Genus
				Glu.	Suc.	Lac.	Mel.	Raff.	Gla.	
A1	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces
A2	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces
A3	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces
A4	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces

A5	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces
B1	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces
B2	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	Saccharomyces
B3	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	Candida
B4	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	Candida
B5	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	Candida

DISCUSSION

The microbiological analysis revealed high counts of yeasts in the tested *egusi* samples which indicated improper hygiene measures during its preparation. These counts were higher than those reported by Sulieman *et. al. 2018* who found that yeast and mould were devoid in some samples of *Egusi* while few numbers were detected in other samples ($1x10^2$ CFU/ml). Morphological characteristics tests (Table 2) indicate that 70% of the colonies were Smooth-White to creamy and the rest were white-butyrous. 55% of colonies were ovoid while 25% were spherical and 20% were elongated. All isolates reproduced by budding, did not form pseudohyphae and only 25% were spore-former. All yeast isolates were able to grow at 37ºC and 80% were able to grow at 42ºC. All yeast isolates were able to grow at 50% glucose level.

The biochemical tests results (Table 3) indicate that all yeast isolates fermented glucose did not hydrolyzed urea and did not resist Cycloheximide. However, all isolates assimilated glucose, but did not assimilate either lactose or melibiose. On the other hand, only 4 isolates (20%) assimilated raffinose and 16 isolated (80%) assimilated glactose.

CONCLUSION

Two genera of yeast were detected in the various stages of *Egusi* fermentation. These were *Saccharomyces*, its frequency ranged between 40 and 100% in variety *white melon* and 80% in *brown melon* and *Candida*, its frequency was 60% in *White melon* and 20% in brown melon. In conclusion, it is preferably to produce *egusi* from Brown melon than *white melon* variety.

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