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Full Length Research Paper

Isolation and Identification of Fungi Associated With Tigernut Milk Drink *(Kunun Aya)* In Dutse, Jigawa State

Nura, M., Abubakar, A., Auyo, M, I., Sunday, E., and Kutama, A. S.

Department of Biological Sciences, Federal University, Dutse, Nigeria

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The study was carried out to isolate and identify the types of fungi associated with Tigernut milk Drink "*Kunun Aya*". The samples of kununaya were collected from five different areas within Dutse town. These include; Yalwawa, Fagoji, Gida dubu, Yan tifa, and Takur site. The experiment was carried out on fresh and spoilt samples, where the fresh samples were used for the first experiment, and the same samples were kept for three days to spoil and used for the second experiment. The serial dilution method was adopted for the experiments. The species of fungi isolated and identified were; *Aspergillus flavus, Aspergillus niger, Penicillium* species and *Saccharomyces cerevisae*. The results showed that, the population of fungi were higher in spoilt samples than in the fresh ones.

Keywords: fungi, tigernut, Kunun Aya, Dutse

INTRODUCTION

Jigawa state is one of the 36 states in Nigeria, located in the north eastern part of Nigeria and it was founded in 27th of August, 1996. The capital of the state is Dutse. Jigawa state share border with Kano, Katsina, Bauchi, Yobe and Republic of Niger. Dutse is the capital city of Jigawa state. Dutse is situated at 11.76 north latitude, 9.34 East longitude and 460 meters elevation above the sea level. It is home to federal university Dutse which opened in November 2011. The estimated population of Dutse is 17,697 (2007). The research was carried out in Dutse town where some areas were randomly selected within Dutse metropolis. The areas are; Yalwawa, Fagoji, Gida dubu, Yan tifa and Takur site. The research was conducted on Tigernut milk drink (Tigernut milk drink) which is one of the popular local drink of the town.

Tigernut milk drink is a traditional non-alcoholic beverage with a spicy nutty taste. It is widely consume for its nutritional and medicinal benefits to its consumers and for its non-alcoholic properties (Abegas, 2007). It has immense social, economic, nutritional and medicinal benefits. Tigernut milk drink like other locally made drinks is widely consumed in Nigeria. In most Nigerians cities the sales and consumption of this locally made beverage is high. Due to the non-alcoholic nature of this drink, it is widely accepted and consumed as a substitute for alcoholic drinks. The drink is usually sold at the motor parks, school premises and market places and even served during social gatherings (Abegas, 2007)'

In recent years, several developments in society have contributed to changes in the global beverage market. Consumers are increasingly aware of the impact of diet on their health and well-being. Beverages are not only consumed to provide refreshment and hydration, but also

*Corresponding Author's Email: kutamasak@yahoo.com

to increase well-being and to help in preventing nutrition-related disorders (Tenge and Geiger, 2001).

A range of microbes can be associated with drink manufacture, though only a few are able to cause spoilage. Microbiological spoilage leads to deterioration of the sensory quality and typically appears as off-flavors, odours and visual changes in the product (Stratford, 2006).

Tigernut milk drink is rich in nutrients (Abaejah *et al.,* 2006). It depends the internal mechanisms and prevents both constipation and diarrhea. Tiger nut has never been found to produce allergy (Belewa and Abodunrin, 2008). It is also used as a flavoring agent for ice cream and biscuits (Cantalejo, 1997).

Despite high nutritive value of this drink, its production in Nigeria has been hampered due to the deteriorating effects of some microorganisms on the drink (Abegas *et al.,* 2006). Osuntogun and Aboaba (2004) reported that Tigernut milk drink is prone to microbial deterioration due to either unhygienic methods of preparation or by use of contaminated raw materials and utensils (Osuntogun and Aboaba, 2004).

The aim of this research is the isolation and identification of common fungi associated with Tigernut milk drink.

Study Area

The study area was Dutse Metropolis, where the samples of Tigernut milk drink were obtained from randomly selected different areas within the town. The selected areas are: Yalwawa, Gida Dubu, Fagoji, Garu and Takur site in Dutse metropolis.

Sample Collection

Samples of freshly prepared Tigernut milk drink were obtained from randomly selected areas which include: Yalwawa, Gida Dubu, Fagoji, Garu and Takur site of Dutse town in Dutse local government Jigawa state. The samples were collected in 50cl plastics bottles. The bottles were labeled and taken to Biological science laboratory for analysis.

Preparation of Media

Two solid media potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) were used for the isolation. The media were used in accordance with the manufacturers' instructions. A pure culture of each isolate was obtained by sub-culture, using the SDA plates.

Preparation of Sample

Two experiments were conducted, one for fresh, and the other for spoilt Tigernut milk drink sample. The fresh Tigernut milk drink samples were used for first experiment, the same samples were left for seven days to spoil.

Fivefold serial dilutions of each sample were prepared. Serial dilution was carried out, where 1ml of each sample was transferred into a test tube containing 9.0 ml of sterile distilled water and the test tube was shaken and labeled as 10^{-1}, from this tube 1.0ml was also transferred into another tube containing 9.0ml of the sterile distilled water and labeled as 10^{-2}. The procedure was repeated up to 10^{-5} using sterile syringes. The test tube 10^{-2} was used.

Inoculation

1.0ml from the dilution factors of each 10^{-2} test tube was transferred into sterile petri-dishes, containing prepared potato dextrose agar (PDA). The diluted samples were used to inoculate the prepared media using pour plate method. The agar plates were allowed to solidify and placed in an inverted position for 5 days at room temperature.

Isolation of Fungi

The samples of Tigernut milk drink were placed on potato dextrose agar (PDA) and incubated at room temperature for 5 days. After incubation, colonies of different shape and colors were observed on the plates (cheesbrough 2000). Pure culture of each colony type on each plate was obtained. This was done by sub-culturing each of the different colonies onto the SDA plates and incubated at room temperature again for 5 days Jiha (1995).

Identification

The identification of fungi was based on macroscopic and microscopic examination.

Macroscopic examination was based on color and nature of the hyphae.

In microscopic examination, the technique of James and Natalie (2001) was adopted for identification of unknown isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide, where a small portion of the mycelium was spread very well on the slide with the aid of the needle.

A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with ×10 and ×40 objective lenses. The species encountered was identified in accordance with Cheesbrough (2002).

STATISTICAL ANALYSIS

The data were analyzed by using non parametric method of analysis. The method used was Kruskel-walles method (H test). The following table show the number of fungi in all the samples and their ranking.

Table 1: Identification table

S/N	Fungi species	Macroscopy	Microscopy
1	*Aspergillus flavus*	The upper surface of the colony was yellow- green with edge, granular surface and green coloration on the reverse side.	The conidiophores was thick walled, hyaline and slightly roughened, erect, long aseptate with a vesicle with short conidial chains.
2	*Aspergillus niger*	The colonies were widely spread black with smooth white edges and spongy surface densely packed and brown on the reverse side.	The conidiophore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head.
3	*Penicellium* species	Greenish or grey green colored colonies with greenish black color inside	Mycelium consist of highly branched network of septate hyphae. Many branched conidiophores sprout on the mycelium bearing conidiophore
4	*Saccharomyces cerevisiae*	The colonies were creamy or white in color.	Cells are large, globose and also budding.

Table 2: Name and Number of fungal isolate obtained in fresh samples

S/N	Species /Areas	Yalwawa	Gida dubu	Fagoji	Yan tifa	Takur site
1	*Aspergillus flavus*	5	5	6	6	6
2	*Aspergillus niger*	9	6	7	8	8
3	*Penicellium* species	6	8	6	7	7
4	*S. cerevisae*	3	5	5	6	5
Total		23	24	24	27	26

RESULTS

Total of four fungal species *Aspergillus niger, Aspergillus flavus, Penicillium* species and *Saccharomyces cerevisiae* were isolated from five samples of Tigernut milk drink. They were identified by studying their macroscopic and microscopic characters (Table1) and compared with identification keys by Samson *et al*. (2004).

NUMBER OF COLONY

The following tables show the fungal species and the number of their colony of both fresh and spoilt samples of Tigernut milk drink found in Gida Dubu, Yalwawa, Fagoji, Takur Site and Yan Tifa.

After the finding the variance of each species of both fresh and spoilt samples, and the data do not met normality assumption (constant variance). Non parametric method Kruskel-walles H-test were used.

By using Kruskel-walles H-test formular, -11.3 and -9.9 were calculated for both fresh and spoilt samples respectively. For K-1=4 degree of freedom at 0.05 significant level. From X^2 table $X^2_{0.95}$ = 9.49 was obtained. Since -11.3 and -9.9 are less than 9.49, null hypotheses (H_0) was accepted and concluded that there was no significant difference on these fungi found in Tigernut milk drink samples of the areas.

DISCUSSION

All the five samples of Tigernut milk drink were found to be contaminated with the same number of fungal species. The presence of the fungal species in these samples indicate

Table 3: Name and Number of fungal isolate obtained in spoilt samples

S/N	Species /Areas	Yalwawa	Gida dubu	Fagoji	Yan tifa	Takur site
1	*Aspergillus flavus*	17	17	16	19	17
2	*Aspergillus niger*	15	19	20	24	23
3	*Penicellium* species	12	20	19	23	20
4	*S. cerevisae*	11	14	15	10	13
Total		55	70	70	76	73

Table 4: Number of fungi in fresh samples and their ranks

Yalwawa	R	Fagoji	R	Gida dubu	R	Yan tifa	R	Takur site	R
5	4	5	4	6	10	6	10	6	10
9	20	6	10	7	15	8	18	8	18
6	10	8	18	6	10	7	15	7	15
3	1	5	4	5	4	6	10	5	4
Total	35		36		39		53		47

(R= rank)

Table 5: Number of fungi in spoilt samples and their rank

Yalwawa	R	Fagoji	R	Gida dubu	R	Yan tifa	R	Takur site	R
17	10	16	8	17	10	19	13	17	10
15	6.5	20	16	19	13	24	19	23	18.5
12	3	19	13	20	16	23	18.5	20	16
11	2	15	6.5	14	5	10	1	13	4
Total	21.5		43.5		44		51.5		48.5

(R=rank)

poor hygienic method of preparation of Tigernut milk drink, unhygienic working environment or using unsterilized materials. Fungal isolates were identified by their cultural and morphological characteristics (Table 1). The species of fungi found are *Aspergillus flavus, Aspergillus niger*, *Saccharomyces cerevisae,* and *Penicillium* spp. The study also indicate that spoilt Tigernut milk drink contain higher

percentage of fungi than fresh ones due to the increase in the population of the fungal species on storage.

In both fresh and spoilt samples, Sample collected from Yan tifa had the higher fungal population, and *Aspergillus niger* with the higher number of occurrence of 8 and 24 in both fresh and spoilt samples respectively. This indicate that, Yan tifa sample was prone to microbial contamination than the rest. And this also indicate the poor hygienic condition of the area. The occurrence of these fungi may cause diverse effects on human health as they have the potential of producing mycotoxins. The concentrations of these substances may increase during storage of Tigernut milk drink due to the increase in the population of the fungal species, hence, daily intake of such Tigernut milk drink containing mycotoxins could result in bioaccumulation in the body which could be hazardous to human health. *Aspergillus flavus* and *Aspergillus niger* are known to produce aflatoxins, ochratoxins which are carcinogenic and are capable of causing kidney and liver disorders, invasive and noninvasive aspergillosis, allergic and sinusitis (Bryce, 1999; Bennett and Klich, 2003 and Sumson *et al* (2004)

Aspergiilus flavus strain produces two must common aflatoxins (B1 and B2) (Amaike *et al.,* 2011). *A. flavus* has a minimum growth temperature of 12c and a maximum growth temperature of 48c, the optimum growth temperature is 37c (Agrios and George 2005). The four major Aflatoxins produced by A. flavus are B1, B2, G1 and G2. The production of the major toxins are as a result of particular strains of A. *flavus.* Aflatoxin B1 is the most toxic compounds including aflatrem, gliotoxin, kojic acid, aspertoxin and aspergillus acid (Hedayati, *et al.,* 2007). *A flavus* aflatoxins can lead to acute hepatitis, immunosuppression, hepatocellular carcinoma and neutropenia in humans. It is highly possible for *A. flavus* to invade arteries of the lung or brain and cause infarction (Crawford *et al.,* 2005). *Aspergillus flavus* is the second leading cause of Aspergillosis (Crawford *et al.,* 2005).

Aspergillus niger's strains have been reported to produce potent mycotoxins called Ochratoxins (Abarca *et al.,* 1994). Recent evidence suggests some true A. niger strains do produce Ochratoxin A. it also produces the Isoflavoneorobol (Samson *et al.,* 2011). A niger is less likely to cause human disease than some other Aspergillus species. Human may become ill but this is due to a serious lung disease Aspergillosis that can occur (Handwert and Brian, 2005). *Aspergillus niger* is one of the most common causes of Otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and in severe cases, damage to the ear canal and tympanic membranes (Handwert and Brian, 2005).

Some species of *Saccharomyces cerevisae* are opportunistic pathogens that can cause infection in people with compromised immune systems (Cogliati, 2013). S cerevisae is not normally considered to be a pathogen. In healthy people, disease resulting from *S. cerevisae* colonizing in a particular area are very rare (Main, 1988). 1 percent of all vaginal yeast infections occur due to *S. cerevisae* in the vagina, but symptoms associated with it are identical to the symptom caused due to another organism more commonly associated with yeast infections *Candida albican* (McCullough, 1988).

Penicelliumglabrum produce citromycetin which is known to cause allergy, asthma and some respiratory problems (Cooley *et al.,* 2004, frisvad *et al.,* 1998).

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