Dough Rising Ability of *Tamarindus Indica, Citrus Limon* and *Hibiscus Sabdariffa* Yeast Isolates

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ABSTRACT

Evaluation of dough rising ability of Tamarindus indica, Citrus limon and Hibiscus sabdariffa yeast isolates was carried out using Culturation ,morphological identification, fermentation and lyophilization standard procedures to determine dough rising ability of the three plants wild yeasts in fermentation of carbohydrate (maize). Results have shown that, the three plants wild yeasts correlate positively with the standard yeast (Saccharomyces cerevisea) in maize dough rising ($\alpha = 0.05 > p$ – value 0.02, 0.027 and 0.01). Commercialization of wild plant yeasts in order to earn and conserve foreign exchange for the country is recommended.

INTRODUCTION

Fermentation produces lactic acid and lactate, carbon dioxide, and water. In yeast and most plant cells fermentation produces ethyl alcohol, carbon dioxide, and water. Alcoholic fermentation is a process that was known to antiquity. Before 2000 B.C the Egyptian apparently knew that crushed fruits stored in a warm place would produce a substance with a pleasant intoxicating power [1]. Alcoholic fermentation occurs when yeast cells convert carbohydrate sources to ethanol and carbon dioxide. Fermentation reactions are common in muscle cells, yeast, some bacteria, and plants [2]. Yeasts are eukaryotic microorganisms classified in the kingdom fungi, with 1500 species currently estimated to be only 1% of all fungal species. Most reproduce asexually by mitosis, and many do so by an asymmetric division process called budding. Yeasts are unicellular, although some species with yeast forms may become multicellular through the formation of a string connected budding cells (pseudohyphae) as seen in most moulds. By fermentation the yeast species *Saccharomyces cerevisiae* converts carbohydrates to carbon dioxide and alcohol. For thousands of years the carbon dioxide

has been used in baking and the alcohol in alcoholic beverages. Yeast microbes are probably one of the earliest demonstrated organisms. People have used yeast for fermentation and baking throughout history [3]. Yeast, most commonly *S. cerevisiae* is used in baking as a leavening agent, where it converts the fermentable sugars present in dough into the gas carbon dioxide. This causes the dough to expand or rise as gas forms pockets or bubbles. When the dough is baked, the yeast dies and the air pockets "set", given the baked product a soft and spongy texture. The use of potatoes, water from potato boiling, eggs, or sugar in bread dough accelerates the growth of yeast. Most yeast used in baking is of the same species common in alcoholic fermentation. Additionally, *Saccharomyces exiguus* (*S. minor*), a wild yeast found on plants, fruit, and grains occasionally used for baking sugar and vinegar provide the best conditions for yeast to ferment [4]. Some probiotic supplements use the yeast *Saccaromyces boulardii* to maintain and restore the natural flora in the gastrointestinal tract. *S. boulardi*i has been shown to reduce the symptoms of acute diarrhoea in children, prevent reinfection of *Clostridi um difficile*, reduce bowel movements in diarrhea predominant patients, and reduce the incidence of antibiotic, travelers, and HIV/AIDS associated diarrheas [5].

Tamarindus indica belongs to the subfamily *Caesalpiniondeceae* of family *Fabaceae*. It is a large tropical tree with a short massive trunk, small yellow flowers and flat reddish brown pods. The tree can grow up to 90ft (27.4m) tall but is usually less than 50ft (15.2m) [1].

Citrus limon (aka lemon) belongs to the flowering plants family *Rutaceae*. *Citrus limon* is believed to have originated in the part of Southeast Asia. *Citrus limon* has been cultivated in an ever widening areas since ancient times. The plant is large shrub or small tree, reaching 5 – 15 tall, with spiny shoots and alternately arranged evergreen leaves with an entire margin. The flowers are solitary or in small corymbs, each flower 2 – 4cm diameter, with five (rarely four) white petals and numerous stamens, they are often very strongly scented. The fruit is a hesperidium, a specialized berry, globose to elongated, 4 – 30cm long and 4 – 20cm diameter, with a leathery rind surrounding segments or "Liths" filled with pulp vesicles [5]. *Hibiscus sabdariffa* has more than 300 species which are distributed in tropical and subtropical regions around the world. Roselle belongs to *Malvaceae* family. It is an erect mostly branched, annual shrub. Stems are reddish in color and up to 3.5m tall. Leaves are dark green to red, alternate, glabrous long – petiolate, palmately divided into 3 – 7 lobes with serrate margins. Flowers are red to yellow with a dark center containing short peduncles. The flowers have both male and female organs. Seedpods are enclosed in their red, fleshy calyces which are commonly used for making food and tea [6]. The current study sets to determine dough rising ability of ; *Tamarindus Indica*, *Citrus limon* and *Hibiscus sabdariffa* using their wild yeast isolates in fermentation of carbohydrate (maize).

MATERIALS AND METHODS

Sample Collection and Handling

The plant materials namely; *Tamarindus Indica, Citrus limon* and *Hibiscus sabdariffa* were collected in clean polythene bags in May, 2012, from Kafur local government, Katsina State, Nigeria. The collected samples were transported to the Department of Biological Science, Nigerian Defence Academy, Kaduna, Nigeria for authentication and confirmation of taxonomic identity. Press of plant materials were prepared and voucher specimens were deposited *(T. Indica:201002; C.limon* :201001and *H.sabdariffa:201003)* in the Department herbarium.

Morphological Identification of Wild Yeasts Associated with T. indica, C. limon and H. sabdariffa : Plant Materials

The plant materials, namely: *T. indica* (fruit pulp), *C. limon* (fruit) and *H. sabdariffa* (calyces) were sprinkled with sterile distilled water in sterile petri-dishes and allowed to decomposed for 7 days. The decomposed plant materials were separately mixed with sterile distilled water and poured in sterile measuring cylinders (10ml).

Media Preparation:

Potatoes dextrose agar (PDA) and nutrient agar (NA)

Potatoes dextrose agar (PDA) was prepared according to manufacturer's instruction. Therefore, fifteen grammes (15g) of PDA was weighed using electronic weighing balance (Sartorius 1401, Germany) and put into 250ml distilled water in conical flasks (500ml). The preparation was properly homogenized and autoclaved at 121^0C for 15 minutes. The sterile medium was allowed to cool at 55^0C, subsequently poured in sterile labeled Petri-dishes (60x15mm) and bijou bottles for slants. The poured plates and bijou bottles were allowed to gel. Similar procedure was employed to prepare 7 grammes of nutrient agar.

Growth of microbial wild cultures

The previously prepared inocula were radially inoculated unto the separate labeled PDA and nutrient agar plates using sterile wire loops. The inoculated plates were inculcated at 30^oC for 24hours. The PDA plate cultures were culturally and microscopically examined for yeasts. Subsequently, the yeast cells were sub cultured on slants and incubated at 30^0C for 24hours. The slant yeast cultures were stored in refrigerator prior to morphological identification. These were kept in refrigerator prior to molecular characterization of microbial wild cultures.

Morphological identification of the wild yeast isolates

Smears of the yeast cultures were prepared for morphological identification using microscopy. Therefore, small drop of lacto phenol (LP) was placed on a clean microscopic slide. Thereafter small portion of the yeast colony was removed and placed into the drop of lacto phenol to suspend the cells. A clean cover slip was placed and the preparation was viewed under the compound microscope using x 10 and x 40 objective

lenses. Digital eyepiece (model 5821 oplenic optronics, kina) was used to capture yeasts images with the help of minisee software.

Evaluation of wild yeast isolates in fermentation of carbohydrate (maize) through dough rising

The wild yeast isolates were propagated and harvested. The harvested yeast cells (yeast slurry) as well as standard commercial yeast *Saccharomyces cerevisea* (slurry) were used for the fermentation. Separate dough was prepared with each yeast slurry by weighing 10 grammes of maize powder and separately mixed with the yeast slurry in 100ml measuring cylinder with 10ml sterile distilled water. The preparations were incubated at room temperature and monitored for dough rising.

Optimization, storage and packaging of particulate yeasts / Optimization of particulate yeasts production

Potatoes dextrose agar (PDA) was prepared according to manufacturer's instruction and autoclaved at 121^0C for 15 minutes. The sterile medium was allowed to cool at 55^0C and subsequently poured into sterile plates. The medium was allowed to gel and the PDA plates were labeled accordingly. Each of the yeast colonies from the stored *T. indica*, *C. limon* and *H. sabdariffa* yeast cultures were inoculated separately unto the labeled PDA plates using radial streaking method with the help of sterile wire loop. The inoculated PDA plates were inverted and incubated at 30^0C for 24 hours. The cultures were sub cultured unto freshly prepared sterile PDA plates and incubated at 30^0C for 24 hours. The yeast cells were harvested in clean plastic bottles (120ml) using sterile distilled water.

Storage and packaging of the particulate yeasts

The propagated harvested yeast cells were lyophilized using sublimation in a vacuum freeze dryer (LGJ-12, Beijing Songjuanhun-Xing Develop co. ltd). Therefore, the harvested yeast cells namely: Ty1, Ty2, Ty3, Ty4; Cly1, Cly2, Cly3; Hsy1, Hsy2 were separately put in sterile 10ml beakers and pre frozen in refrigerator and subsequently loaded on stainless steel tray(240mm in diameter) on the pre freezing shelf . The pre freezing shelf was subsequently put into cold trap of the lyophilizer and covered with insulation lid .The samples were removed from the cold trap and put on the drying shelf. The sealed ring was checked and the organic glass barrels covered. The drain valve was tied in clockwise; the vacuum pump and vacuum gauge were opened. The vacuum degree was allowed to decline (less than 20 Pa). The drain (inlet) valve was opened and the vacuum yeast samples were packaged in sterile Medi scan plastic bottles.

RESULTS AND DISCUSSION

The result for the local plants wild yeast isolates on the fermentation of carbohydrate source (maize) through dough raising are presented in the table 1. It was observed

that, the dough raising ability of the yeast isolates is directly proportional to time. The dough raising ability of the yeast isolates was found promising and effective after 120 minutes (2 hours). The correlations are highly positive correlated which shows that there is positive relationship between the standard and wild yeasts in maize dough raising ability. It also shows significance relationship between the wild and standard yeast since the $\alpha = 0.05 >$ p- value of the both (0.02, 0.027 and 0.01) respectively.

Table 1: Maize dough rising ability of local plants wild yeast isolates

Time (min)	Yeast isolates/dough rising ability									
	TY 1	TY 2	TY 3	TY 4	HSY 1	HSY 2	CLY 1	CLY 2	CLY 3	C^+
10	12	12	14	15	13	12	12	14	13	15
30	14	13	14	17	13	14	13	15	14	18
60	17	14	15	17	14	14	15	16	15	20
90	18	15	15	17	14	14	16	17	17	22
120	19	17	15	17	14	14	16	17	17	23
DRAY/100ml	80	71	73	83	68	68	72	79	76	98

Legend:
TY = Tamarindus *indica* yeast isolate
HSY = *Hibicus sabdariffa* yeast isolate
CLY = Citrus limon yeast isolate
C^+ = Position control standard Baker's yeast (*Saccharomyces cerevisae)*
DRAY /100ml: Dough rising ability of yeast per 100 millitres

The use of yeast in industrial processes cannot be over emphasized. Yeasts are found very useful in bread making alcohol and antibiotic production. Yeasts are found naturally from the surrounding. The result of yeast isolates which shows different morphology of the yeast support the occurrence of variety of wild yeast in plant materials. Fruits, vegetable, drinks and other agricultural products are very important

microhabitats for different yeast species [7]. The presence of the different yeast isolate in the study plants further supports the use of plants as sources yeast in fermentation process. [8] reported the occurrence o f *Saccharomyces cerevsiae* in *Cocos nucifera, Dimocarpus longan* spp, *Annona muricata, Bambusa vulgaris, Salacca zalacca,* and *Mangifera indica.* Yeast of plants origin are found very useful industrially in a wide range of fermentation processes: medicinally as a source of B-complex vitamins and as a stage in the production of various antibiotics and steroid hormones and as feed and foodstuffs. Yeasts are very common in the environment, and are often isolated from sugar-rich material. Examples include naturally occurring yeasts on the skins of fruits and berries (such as grapes, apples), and exudates from plants (such as plant saps or cacti) [9]. The morphological yeast isolates being from plants commonly found in the savanna, could easily be propagated and utilized for ethanol fermentation. This will go a long way in providing cheap natural source of yeast for local fermentations. Yeast organisms cause bread dough to rise by consuming sugars in the dough and produce ethanol and carbon dioxide as waste products. The carbon dioxide forms bubbles in the dough and cause it to expand. Nearly all the ethanol evaporates from the dough when the bread is baked [10]. The result of dough rising ability of maize by *T. Indica, C. Limon* and *H. Sabdariffa* yeast isolates has shown positive correlation ($\alpha = 0.05$) p – value, 0.02, 0.027, 0.01) respectively between the standard yeast *Saccharomyces cerevisae* and the yeast 2 isolates of the three plants. This informed the use of the plant yeast isolates in dough rising, typically in bread making. [11] reported on the use of yeast of plant origin to rise dough.

CONCLUSION

The current study has shown that *T. Indica, Citrus limon and Hibiscus sabdariffa* plant parts are good sources of wild yeasts with fermentation potentials. Wild plant yeasts from the three plants could be use as alternative in dough rising during processing of carbohydrate foods.

RECOMMENDATION

Wild yeasts from *Tamarindus indica, Hibiscus sabdariffa* and *Citrus limon* are recommended for dough rising in the fermentation of carbohydrate food source.

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