

## Determination of Fermentation Potentials of *Tamarindus indica* Yeast Isolates

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**Abstract:** Determination of fermentation potentials of *Tamarindus indica* yeast isolates was carried out using isolation, morphological identification and physiological (fermentation) procedures. Ty1; Ty2 and Ty3; Ty4 isolates are morphologically similar as small cylinder to ovoid and small spherical yeast cells respectively. The fermentation process indicates gradual changes in pH (5.5-1.96) and brix (20-7.40%). Long lag phase (132 h) was observed in the natural fermentation. The alcohol production potentials of the four yeast isolates have shown variations: Ty3NF (D3:6.48%)>Ty2SF (D6:5.47%)>Ty3SF (D6:5.08%)>Ty4SF (D6:4.34%). Thus *T. indica* could be a good source of wild yeast and biofuel.

**Keywords:** Fermentation potentials, lag phase, tamarindus indi, yeast Isolates

### INTRODUCTION

Fermentation refers to a process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simple compounds without the use of oxygen. Fermentation results in the production of energy in the form of two ATP molecules and produces less energy than the aerobic process of cellular respiration. The other end products of fermentation differ depending on the organism. In many bacteria, fungi, protists and animal cells (notably muscle cells in the body), fermentation produced lactic acid and lactate, carbon dioxide and water. In yeast and most plant cells, fermentation produces ethyl alcohol, carbon dioxide and water (Hoet *et al.*, 1998).

Yeast is unicellular organisms that reproduce asexually by budding. In general, yeasts are larger than most bacteria. Yeast can have beneficial and non-beneficial effects in foods. Yeast plays an important role in the food industry as they produce enzymes that favor desirable chemical reactions such as the leavening of bread and the production of alcohol and invert sugar. Fermented foods from Africa include: Vinegar, Pickled fruit and vegetables, fermented fruit and vegetable seeds. The common yeast found in fermenting fruit and vegetables include: *Saccharomyces cerevisiae*, *S. pombe*, *Debaromyces zygocacharamyces rouxii*, *Candida* species such as *Geotrichum candidum*.

*Tamarindus indica* belongs to *ceasalpiniaceae* (*Fabaceae*) family. Tamarind is a large beautiful evergreen tropical tree that can grow up to 100' tall although 80' is more common. The trunk has cracks, down and across, the bark is brown-gray. It has a dense and spreading crown of bi-pinnate foliage and numerous yellow, with red and purple filaments,

flowers and the brown woody curved pod contains the seeds; black, shiny squares surrounded by pulps. The fruit pulp is rich in tartaric and citric acids, high amount of vitamin C and sugar. The compressed fruit pulp is used in syrup, juice concentrates and as spices in exotic food specialties like drinks, chutney, curries, pickles and meat sauces (Boundy Mill, 2012).

The present study sets to investigate fermentation potentials of *T. indica* yeast isolates.

### MATERIALS AND METHODS

**Sample collection and handling:** The *Tamarindus indica* fruits were collected in clean polythene bag from Kafur Local Government, Katsina State, Nigeria, in May 2013., The *T. indica* samples were transported to the Department of Biological Science, Nigerian Defence Academy, Kaduna, Nigeria where authentication and confirmation of taxonomic identity of the plant was done using voucher specimens. Plant press of the specimens was deposited in the Departmental herbarium.

**Isolation and identification of *T. indica* yeast:** *T. indica* fruit pulps were sprinkled with sterile distilled water in sterile Petri-dishes and allowed to decompose for 7 days. The decomposed fruit pulps were mixed with sterile distilled water and poured into sterile measuring cylinder (10mL). This served as inoculum for the yeast isolation.

**Medium preparation:** Potatoes Dextrose Agar (PDA) was prepared according to manufacturer's instruction. Therefore, 15g of the PDA was weighed using electronic weighing balance (Sartorius 1401, Germany)

and mixed with 250mL distilled water in a conical flask (500mL). The preparation was properly homogenized and autoclaved at 121°C for 15min. The sterile medium was allowed to cool at 55°C and subsequently poured into sterile labeled Petri-dishes and bijou bottles. These were allowed to gel.

**Yeast cultivation:** The previously prepared inoculum was radially inoculated into the PDA medium using sterile wire loop. The preparation was incubated at 37°C for 24h.

Smears from the culture were prepared and stained with lactophenol. The preparations were microscopically examined using x10 and 40 objective lenses. The Yeast cells were subcultured on the PDA plates and incubated at 37°C for 24 h. The slant cultures were stored in refrigerator.

**Tamarind yeast fermentation:** The fermentation potentials of the Tamarind yeast isolates were evaluated using natural and standardized fermentations of Tamarind juice extract.

**Preparation of *T. indica* juice extract:** Thirty percent ( $\frac{30}{100}$ ) of *T. indica* juice extract was prepared. Therefore, 600g of *T. indica* was weighed using electric weighing balance.

The weighed sample was put into an aluminium pot with 2 L of distilled water. The preparation was boiled using burnsen burner. It was allowed to cool and sieved using muslin cloth. Specific Gravity (S.G), sugar brix and pH of the extract was measured using hydrometer, sugar refractor meter and Hanna microprocessor pH meter (model 3305), respectively.

**Propagation of tamarind yeast isolates:** PDA was prepared according to manufacturer's instruction and autoclaved at 121°C for 15 min. The sterile medium was allowed to cool at 55°C and subsequently poured into sterile plates. The medium was allowed to gel and the plates were labeled accordingly. The yeast colonies from the stored *Tamarind* yeast cultures were separately inoculated into the PDA plates using radial streaking with the help of sterile wire loop. The inoculated PDA plates were inverted and incubated at 37°C for 24 h.

**Preparation of 0.5 McFarland turbidity standard:** A 1%  $\frac{1}{100}$  solution of sulphuric acid ( $H_2SO_4$ ) was prepared by adding 1ml of concentrated  $H_2SO_4$  to 99mL of water in a volumetric flask. The preparation was properly mixed. A 1%  $\frac{1}{100}$  solution of barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride ( $BaCl_2 \cdot 2H_2O$ ) in 50mL of distilled water in a volumetric flask. A 0.6mL of the barium chloride solution was added into 99.4mL of the  $H_2SO_4$  solution and mixed properly. A small volume of the turbid

standard solution was put into a screw cap bottle. The standard was kept at room temperature (20-28°C) prior to inocula standardization (Cheesbrough, 2000).

**Standardization of the yeast inocula:** Sterile distilled water was used to wash the propagated yeast cells into sterile conical flasks (250ml). The yeast suspensions were adjusted to 0.5 McFarland turbidity standards by adding sterile distilled water and measuring the absorbance at 540nm using WPA spectrophotometer.

**Preparation of tamarind yeast starter cultures:** The standardized *Tamarind* yeast inocula were separately mixed with 50mL *Tamarind* extract in sterile conical flask (100 mL). The preparations were aseptically plugged with cotton wool and wrapped with aluminium foil. These were incubated at 37°C for 24 h. These were incubated at 37°C for 24 h. These served as starter cultures.

**Natural and standardized fermentation of Tamarind juice extract:** The previously prepared starter cultures of the *Tamarind* yeast isolates: Ty1, TY2, TY3 and TY4 were used for both natural and standardized fermentations.

The prepared *Tamarind* juice extract was used in its natural form (pH = 2.62, brix = 13% and S.G = 1.05). However, for standardized fermentation, the *Tamarind* juice extract was reconstituted through pH and brix adjustment. The pH was adjusted to pH = 5.5 using 150mL 5m NaOH solution. The sugar brix was adjusted from 13 to 20% using 200g sucrose. Subsequently, using eight sterile plastic containers (2.5 L) (pitted with sterile syringe straw withdrawing setup), 150mL of *Tamarind* juice extract was put into each container and labelled with the yeast types. The preparations were pasteurized at 71°C for 15 sec (High temperature short time, HTST) (Willey *et al.*, 2008). These were allowed to cool and subsequently the starter cultures were added. They were incubated at room temperature (25±2°C) (natural fermentation) and 30°C (standardized fermentation), for twelve days. Sugar brix and pH were measured on daily basis using sugar refractor meter and Hanna microprocessor pH meter (model 3305), respectively. After the twelve days fermentation, the process was arrested by pasteurizing the ferments at 71°C for 15 min using water bath. The pasteurised ferments were refrigerated prior to alcohol estimation.

**Estimation of alcohol in the tamarind ferments:** The pasteurized ferments were separately centrifuged in Hechich 7200 universal centrifuge (England) at 3000 rpm for 10 minutes. The supernatants were separately put into conical flasks (100ml). The samples were neutralized with 5m NaOH solution. One hundred mL of each neutralized sample was measured using measuring cylinder and put into volumetric flask.

Subsequently, the samples were transferred into distillation flasks and 50mL of water was added to each sample. The preparations were distilled back into the same volumetric flasks at the boiling temperature of alcohol (78-79°C) (Oke *et al.*, 2003). The distillates (95mL) were collected and each made to 100mL with water. Using pipette, 50 mL of each preparation was pipetted and put into specific gravity bottle. These were weighed with analytical weighing balance (B. Bran LA 164, England). The constant weights of the samples were recorded and their specific gravity calculated. The specific gravity data were used to estimate the percentage alcohol from the Hehner's alcohol table.

## RESULTS

**Morphology of *T. indica* Yeast isolates:** The morphology of the *T. indica* yeast isolates is presented in Fig. 1 to 4. Ty1; Ty2 and Ty3; Ty4 isolates appeared morphologically similar as small cylindrical to ovoid and small spherical yeast cells, respectively.

The result of pH and brix measurement of standardized and natural fermentations of *Tamarindusindica* juice extract using four *T. indica* yeast isolates is presented in Table 1. It indicates gradual changes in the pH and brix in both the standardized and natural fermentations. Short lag phases (48 h) were observed in the standardized

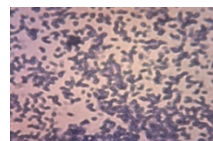


Fig. 1: Ty1, Tamarindus indica yeast 1 isolate cells (x40)

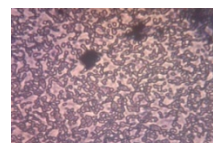


Fig. 2: Ty2, Tamarindus indica yeast 2 isolate cells (x40)

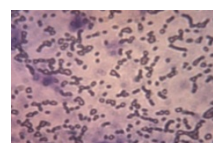


Fig. 3: Ty3, Tamarindus indica yeast 3 isolate cells (x40)

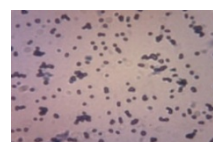


Fig. 4: Ty4, Tamarindus indica yeast 4 isolate cells (x40)

Table 1: pH and Brix Measurements of Standardized and natural fermentations of *Tamarindus indica* juice extract using four *T. indica* yeast Isolates

Days	TY1SF		TY2SF		TY3SF		TY4SF	
	pH	Brix(%)	pH	Brix(%)	pH	Brix(%)	pH	Brix(%)
1	5.50	20.0	5.50	20.0	5.50	20	5.50	20.0
2	5.36	19.0	5.34	19.0	5.37	19	5.21	19.0
3	5.01	17.0	5.03	17.0	4.92	16	4.85	16.0
4	4.76	15.0	4.82	15.0	4.72	14	4.57	13.2
5	4.82	12.8	4.85	13.0	4.82	12	4.69	11.4
6	4.64	11.0	4.62	11.0	4.62	11	4.48	10.2
7	4.60	11.0	4.58	10.6	4.54	11	4.54	10.0
8	4.62	10.8	4.59	10.6	4.57	11	4.55	10.0
9	4.62	10.8	4.59	10.6	4.57	11	4.55	10.0
10	4.62	10.8	4.59	10.6	4.57	11	4.55	10.0
11	4.62	10.8	4.64	10.5	4.61	11	4.62	10.0
12	4.62	10.8	4.64	10.5	4.61	11	4.62	10.0
Days	TY1NF		TY2NF		TY3NF		TY4NF	
	pH	Brix(%)	pH	Brix(%)	pH	Brix(%)	pH	Brix(%)
1	2.62	13	2.62	13	2.62	13	2.62	13.0
2	2.63	13	2.63	13	2.63	13	2.63	13.0
3	2.65	13	2.65	13	2.65	13	2.65	13.0
4	2.76	13	2.75	13	2.72	13	2.74	13.0
5	2.64	12	2.64	12	2.64	12	2.64	12.0
6	2.47	12	2.48	12	2.47	12	2.47	12.0
7	2.43	12	2.42	12	2.46	12	1.96	11.5
8	2.46	12	2.51	12	2.52	12	2.46	10.2
9	2.48	10	2.54	10	2.53	10	2.48	9.60
10	2.49	8.0	2.55	8.0	2.54	8.0	2.50	8.60
11	2.53	7.5	2.72	7.5	2.72	7.5	2.71	8.00
12	2.48	7.5	2.66	7.5	2.68	7.5	2.68	7.20

Table 2: Specific gravity measurements and % alcohol of *Tamarindus indica* Yeast isolates fermentations

Tamarindus yeast isolate/Fermentation type	Day 3		Day 6		Day 9	
	S.G	Alcohol	S.G	Alcohol	S.G	Alcohol
Ty1NF	0.9977	1.57	0.9951	3.49	0.9980	1.34
Ty2NF	0.9969	2.20	0.9982	1.19	0.9968	2.27
Ty3NF	0.9911	6.48	0.9948	3.69	0.9966	2.43
Ty4NF	0.9963	2.02	0.9948	3.69	0.9972	1.96
Ty1SF	0.9961	2.79	0.9942	4.12	0.9942	4.12
Ty2SF	0.9979	1.42	0.9924	5.47	0.9924	5.47
Ty3SF	0.9943	4.05	0.9929	5.08	0.9929	5.08
Ty4SF	0.9963	2.02	0.9939	4.34	0.9939	4.34

Ty: *Tamarindus* yeast isolate; Nf: Natural fermentation; Sf: Standardizing fermentation; 1, 2, 3, 4: yeast starter cultures; SG: Specific gravity

fermentations. However, long lag phases (132 h) were observed in the natural fermentations. The pH fluctuations were noticed to decelerate the activity level of the fermenting *Tamarind* yeast isolates.

The result of specific gravity measurements and percentage alcohol of four *Tamarind* yeast isolates for 9 days natural and standardized fermentations are shown in Table 2. Variations in specific gravity and percentage alcohol were noticed from day 3 today 9, indicating inverse relationship. The natural fermentation process evidence more promising result as the standardized fermentation halted at the 6<sup>th</sup> day. The alcohol production potential for the four yeast isolates shows variations: Ty3NF (D3:6.48%)>Ty2SF (D6:5.47%)>Ty 3SF (D6:5.08%)>Ty4SF (D6:4.34%).

## DISCUSSION

The *Tamarind* yeast isolates have shown morphological similarities. Yeast is very common in the environment and is often isolated from sugar rich material. Typically, naturally occurring yeast are found on the skins of fruit and berries (such as grapes, apples or peaches) and exudates from plants (such as plant saps and lacti) (Suhel *et al.*, 2005). The useful physiological properties of yeast have led to their use in the biotechnology. Many kinds of yeast are used for making foods: bakers yeast in bread production, brewer's yeast in beer fermentation, yeast in wine fermentation and for Xylitol production (Rao *et al.*, 2004).

The pH and brix measurements which indicate fluctuations further confirm the potentials of the *Tamarind* yeast isolates in fermentation process. Most nutrients needed by the yeast growth are contained in the *Tamarind*, due to the effective sugar notification observed in the fermentations. Good source of nitrogen is therefore obtainable in *Tamarind* for the yeast growth, since in absence of ammonium sulphate the yeast were physiologically active in their performance as all have shown evidence of fermentation: odour, bubbles and reduced pH values.

The performance of *Tamarind* yeast 3 and 4 which appeared more promising further justifies the use of *Tamarind* in local fermentations. This suggests the use of *Tamarind* as potential candidate for alcoholic

beverage production. Metabolism of carbohydrates by certain species of yeast under anaerobic or low oxygen condition could be very useful in exploiting the *Tamarindusindica* fruit pulp for production of beverages. Carbohydrate containing plant materials are fermentable by yeast to produce dilute solution of ethanol. Spirit could be produced by distilling the dilute solutions of ethanol (Boundy Mill, 2012).

Alcoholic fermentation (ethanol fermentation) ensures the conversion of sugars such as glucose, fructose and sucrose into cellular energy, producing ethanol and carbon dioxide as metabolic waste products. Because yeast performs this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process. Alcoholic fermentation occurs in the production of alcoholic beverages and ethanol fuel and in the rising of bread dough (James, 2007). The present study which shows appreciable performance of *Tamarind* yeast 3, especially in natural fermentation and that of *tamarind* yeast 2 especially in standardized fermentation informed the use of *tamarind* as source of yeast that could be very useful for industrial fermentation to produce alcohol as well as in leavening of bread. Yeast plays an important role in the food industry as they produce enzymes that favor desirable chemical reactions such as the leavening of bread and the production of alcohol (Mountney and Gould).

The performance of the *Tamarindus* yeast 3 especially in day 3 to produce alcohol informed the frequent use of *Tamarindusindica* plant parts in different fermentation process in the production of local drinks to enhance flavour. The *Tamarindusindica* yeast 3 isolate may probably contain glucoamylase which easily locates the complex sugars into simple sugars. *Saccharomyces* yeasts have been genetically engineered to ferment Xylose (one of the major fermentable sugars present in cellulosic biomasses (Suhel *et al.*, 2005). It further suggest that, ethanol can be efficiently produced from more inexpensive feedstock such as agricultural residues, paper wastes and wood chips (Bratet *et al.*, 2009).

*Tamarind* yeast could probably be a good source of nutritional yeast when used as dietary supplement, thus serving as a good substitute of protein and vitamins, especially the B. complex vitamins as well as other

cofactors required for growth (Centina-Sauri and Sierra, 1994).

### CONCLUSION

*Tamarindus indica* fruit pulp could be a good candidate for yeast extract and bioethanol production.

### RECOMMENDATION

Further research should be carried out to harness the potentialities of *Tamarindus* yeast extract as food additive or flavor and as source of free glutamic acid.

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