Current Trends in Biotechnology and Pharmacy
Vol. 11 (2) 113-120 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)


# Comparative Microbiological and Physico-Chemical Properties of Commercially available Baker's Yeast and Fruit Juice Isolate (FJ1)

**Hiranmay Malik, Priya Katyal* and Param Pal Sahota**
Department of Microbiology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana-141004, Punjab, India.
*For Correspondence - drpkatyal@pau.edu

**Abstract**

Seven commercially available Baker's yeasts formulations were procured from local market and stored under the conditions mentioned by the manufacturer. Microbiological analysis of the product was done using five different selective media viz. Rose Bengal chloramphenicol agar for moulds, Baired Staph agar for *Staphylococcus aureus*, McKonkey agar for faecal coliforms, *B. cereus* selective agar for *Bacillus cereus* and *Salmonella-Shigella* agar for *Salmonella*. The result revealed that all the commercial products differ w.r.t. to the presence of contaminants. The level of contaminants in some of the samples was above the safe limit. These contaminants may cause a substantial loss economically in the bakery industry and may also cause health problems due to the production of cytotoxic substances. Further these products were used for isolation of different yeast strains present in them using normal microbiological procedures and the isolates were compared w.r.t morphological (texture, colour, surface, elevation and margin), physiological (growth, pseudohyphae formation, budding and the presence of pellicle) and biochemical (sugar fermentation ability in both non-sugar dough as well as in high- sugar dough, invertase activity, carbon utilization as well as assimilation, nitrogen assimilation, carbohydrate and trehalose content) characteristics with a laboratory isolate (FJ1). Freeze tolerance of all these yeast isolate was studied upto 90 days.

**Key words**: Baker's yeast, microbial contaminants, invertase activity, trehalose, fermentation

## Introduction

Commercial yeasts have many practical uses in baking, distilling, wine and brewing industries and are also being used for the production of enzymes, amino acids, vitamins, and substances for therapeutic purposes such as hormones, antibiotics and vaccines. However, the most widely recognized role of yeasts is their leavening and fermenting ability in bread and other fermented products (1). As industrialization increased the manufacture of fermented products, the demand of yeast grew exponentially (2). Baker's yeast (*Saccharomyces cerevisiae*) is the common name for the strains of yeast generally used as a leavening agent in baking. It is still one of the most important fermentation products based on the volume of sales and its use for bread-making which is a staple food for a large section of world's population (3). In addition to producing high-quality yeast for baking, it is an important objective for yeast manufacturers to increase product shelf life. New products with better and sophisticated properties make the search for new and improved industrial strains a promising talk. Despite the rich yeast flora of fruit juices, there is little or no information on the dough leavening ability of these yeast isolates (4). Keeping it in mind an attempt is hereby made to isolate yeast from fruit juices and a potent isolate named as

Vol. 11 (2) 113-120 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

FJ1 was compared with the commercial yeast strains.

The shelf life of commercially manufactured yeast is often taken for granted because of the intrinsic factors such as lowering the pH during manufacturing lowers the chances of contaminants. However, the microbial spoilage ecology of commercially manufactured yeast subsequent to initial contamination is often influenced by a number of factors. Commercially manufactured yeast is nutrient-rich and is comprised of water (70%), proteins (15%), carbohydrates (10.5%), minerals (3%) and fats (1.5%), and is thus actually highly susceptible to contamination and growth of a variety of spoilage microorganisms (5). To meet customer demands, commercial yeast manufacturers must consistently produce a high-quality product with guaranteed storage life. Although there has been research executed on the bacterial populations associated with commercially manufactured compressed yeast blocks (6), limited work has been carried out on the bacterial populations associated with commercially manufactured cream and dry yeast products (7). In fact, the bacterial populations responsible for the spoilage of these three different commercially manufactured yeast products are largely unknown (8).

Therefore this study was conducted to highlight the major pathogenic micro-organisms present in the different baker's yeast formulations including FJ1 and to compare these yeast preparations w.r.t morphological, physico-chemical and cultural characteristics. Furthermore, their sugar fermentation ability on both non-sugar dough and high- sugar dough, growth on maltose and sucrose respectively, invertase activity, carbohydrate and trehalose content were also compared.

## Materials and Methods

### Isolation and maintenance of yeast

The yeast strains used in this study were isolated from seven different commercial baker's yeast formulations and a laboratory isolate FJ1 was obtained from fermenting orange juice by standard microbiological procedures and were maintained by periodic sub-culturing on Glucose Yeast Extract (GYE) agar.

***Microbiological analysis of Commercial Baker's yeast :*** The comparative qualitative microbiological analysis was done for the presence of Molds, *Bacillus, Staphylococcus*, Faecal coliforms and *Salmonella* in all the yeast preparations by the methods given by (3). Different selective media were used for checking the presence and absence of various pathogenic micro-organisms.

***Comparative cultural characteristics of different yeasts :*** Growth in liquid media was examined to know the cellular morphology of different yeast isolates using Malt extract (ME) broth by the method (9). Similarly, Malt extract agar was used for the observation of morphological/ cultural properties in solid media. Cultural characteristics include colony texture, color, surface, elevation, and margin.

### Physiological and biochemical characteristics of different yeasts

***Fermentation and assimilation of carbon compounds :*** The fermentation basal medium of (10) was used and fermentation of different carbon compounds (galactose, xylose, fructose, arabinose, sucrose, maltose, mannitol, dextrose, cellobiose, starch and lactose) by different commercial yeasts and the FJ1 isolate was studied. Similarly, carbon assimilation was checked using the basal medium of (9) supplemented with different carbon sources (starch, citric acid, sucrose, arabinose, melibiose, rhamnose, lactose, glucose, raffinose, maltose and mannitol). Comparative growth was observed as a measure of Optical Density at 540 nm after 24 hrs of incubation.

### Assimilation of N compounds

The assimilation of $NaNO_2$ and $KNO_3$ was studied by auxanographic method of (9).

### Pseudohyphae formation

Ability to form pseudohyphae was checked by Dalmau plate method (10) using rice agar. Results were noted microscopically wherein the

coverslip was examined under a microscope for the presence/absence of pseudohyphae.

**Sugar fermentation ability in non-dough and high-sugar dough** : The ability of all the strains to ferment maltose and sucrose as non- sugar and high-sugar dough were tested with Bromo Cresol Purple or BCP method, whereby, growth was reported as the intensity of yellow color developed against the color of the control substrate (purple) and were expressed as plus/ minus (yellow/purple).

**Total carbohydrate and trehalose determination :** Total carbohydrate and trehalose contents of yeast were determined by the Anthrone reagent method (11). Standard curve for trehalose and carbohydrate estimation was made by using trehalose and glucose as standard solutions upto 100 µg/ ml.

**Invertase activity** : Invertase activity was measured spectrophotometrically at 525 nm by the methods of (12). Results obtained in terms of Optical Density were defined in terms of glucose released, where one unit of invertase is defined as µg of glucose released at 30°C per minute per mg of yeast (dry basis) under the experimental conditions.

**Freeze tolerance** : Freeze tolerance ability of different yeast strains was compared by the method of (13) wherein methylene blue solution (0.01% in distilled water) was used to identify the non-viable cells in the haemocytometer chamber.

**Results and Discussion**

Comparison of the microbiological quality of commercial yeast strains and laboratory isolate FJ1 is presented in Table 1. Various contaminating micro-organisms including *Bacillus cereus, Staphylococcus and Molds* were prevalent in most of the commercial preparations. *Salmonella* and *E.coli* was not found in any of the commercial yeasts preparations. Faecal coliforms were present only in Prestige and Kothari preparations. The presence of *Staphylo coccus* in almost all the commercial yeasts and in FJ1 possibly signifies the aerial mode of contamination. Earlier also, a study conducted by (1) aimed at verifying the quality of different commercial yeasts used in breadmaking showed that the liquid baker's yeast was characterized by the lowest microbial contamination and by the highest leavening activity. Thus dry and compressed forms were contaminated by different microorganisms, and the extent of contamination depended on the type of baker's yeast formulation. They reported that, one of the major contaminating bacteria found prevalent in commercial yeast preparations was *Bacillus* which is the main cause of ropiness in the bread. In the present study, *Staphylococcus* was found to be the most prevalent bacterium followed by *Bacillus*.

O'Brien *et al* determined the effects of various storage temperatures on the shelf life and bacterial populations associated with commercially manufactured cream, compressed and dry yeast. Results showed that Cream and compressed yeast samples became bacteriologically and visually spoiled over time when stored at elevated temperatures (10, 25 and 37°C). This also revealed the populations of Enterococcaceae including *Enterococci,* predominant in the finished dry yeast product, while *Lactobacillus* sp. is the dominant bacterial population associated with cream and compressed yeast products.

Various morphological/cultural properties presented in Table 2 showed marked difference in the shape, color, surface, elevation and margin whereas no such difference was present in the texture of the yeast isolates. The shape varied from oval to coccus. Color varied from cream to white. Platinum yeast showed striated surface, rest was smooth. Elevation showed marked difference wherein Allinson, Kothari and Falora showed raised elevation, FJ1 showed pulvinate and Kipps, Red star and Platinum showed convex elevation. Margin varied from entire to smooth. Among Physiological properties Kothari, Kipps and FJ1 isolate showed a significant presence of pseudo hyphae (Table 2). Pellicle formation was not seen in any of the yeast preparation instead bottom sediments were seen. Budding was

Vol. 11 (2) 113-120 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

**Table 1.** Comparative microbiological analysis of various commercial yeasts and lab isolate

| SAMPLE | *B. Cereus* | *Staphylococcus* | *Moulds* | *Salmonella* | *E.coli* | *Faecal Coliforms* |
|---|---|---|---|---|---|---|
| Prestige | + | + | + | - | - | + |
| Kipps | + | + | + | - | - | - |
| Kothari | + | + | + | - | - | + |
| Platinum | - | + | + | - | - | - |
| Red Star | - | + | + | - | - | - |
| Falora | + | + | + | - | - | - |
| Allinson | - | - | + | - | - | - |
| FJ1 | - | + | - | - | - | - |

**Table 2.** Morphological and physiological properties of different commercial yeasts and lab isolate

| Sample | Morphological/cultural properties | | | | | Physiological Properties | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Shape | Texture | Color | Surface | Elevation | Margin | Pellicle | Budding | Pseudo-hyphae |
| Prestige | Oval | Butyrous | White | Smooth | Convex | Entire | - | + | - |
| Kipps | Oval | Butyrous | White | Smooth | Convex | Entire | - | + | + |
| Kothari | Oval | Butyrous | White | Smooth | Raised | Entire | - | - | + |
| Platinum | Coccus | Butyrous | White | Striated | Convex | Smooth | - | - | - |
| Red Star | Coccus | Butyrous | Cream | Smooth | Convex | Smooth | - | + | - |
| Falora | Coccus | Butyrous | White | Smooth | Raised | Smooth | - | - | - |
| Allinson | Oval | Butyrous | Cream | Smooth | Raised | Smooth | - | - | - |
| FJ1 | Oval | Butyrous | White | Smooth | Pulvinate | Entire | - | + | + |

**Table 3.** Comparative assimilation of inorganic nitrogen source

| Sample | $KNO_3$ (Nitrate) | $NaNO_2$ (Nitrite) |
|---|---|---|
| Prestige | - | - |
| Kipps | - | - |
| Kothari | - | - |
| Platinum | - | - |
| Red Star | - | - |
| Falora | + | + |
| Allinson | + | - |
| FJ1 | + | - |

observed in Prestige, Kipps, Red Star and FJ1 isolate.

Assimilation of inorganic source of nitrogen was checked in nitrate and nitrite as shown (Table 3). The bleak ability of assimilation was shown by the yeast preparations. FJ1, Falora and Allinson were found to assimilate nitrate and Falora alone was found to assimilate Nitrite. Ability to ferment dough was seen in both the non-sugar and high-sugar dough. All the yeasts were able to ferment Maltose (non-sugar). The data indicated that only Kothari, Kipps and Platinum were able to ferment Sucrose in the high-sugar dough.

**Table 4.** Dough fermentation ability in non-sugar and high-sugar dough

| Sample | Low Sugar (Maltose) | High Sugar (Sucrose) |
|---|---|---|
| Prestige | + | - |
| Kipps | + | + |
| Kothari | + | ++ |
| Platinum | + | + |
| Red Star | + | - |
| Falora | + | - |
| Allinson | + | - |
| FJ1 | + | - |

**Table 5.** Comparative ability of commercial yeasts and lab isolate to ferment different carbon sources

| SampleSugar | Prestige | Kipps | Kothari | Platinum | Red Star | Falora | Allinson | FJ1 |
|---|---|---|---|---|---|---|---|---|
| Galactose | ++ | ++ | + | ++ | ++ | - | + | + |
| Xylose | ++ | ++ | + | ++ | ++ | + | + | + |
| Fructose | ++ | ++ | + | ++ | ++ | - | + | ++ |
| Arabinose | ++ | ++ | + | ++ | + | - | - | ++ |
| Sucrose | ++ | ++ | + | ++ | ++ | + | + | ++ |
| Maltose | ++ | ++ | + | ++ | ++ | - | + | ++ |
| Mannitol | ++ | ++ | + | ++ | ++ | + | + | + |
| Dextrose | ++ | ++ | + | + | ++ | + | + | + |
| Cellobiose | ++ | ++ | + | + | ++ | + | + | + |
| Lactose | ++ | ++ | + | ++ | ++ | - | + | + |

* All values are mean of three replicates
* Incubation temperature- 25±2°C
* Incubation period- 72 hrs.

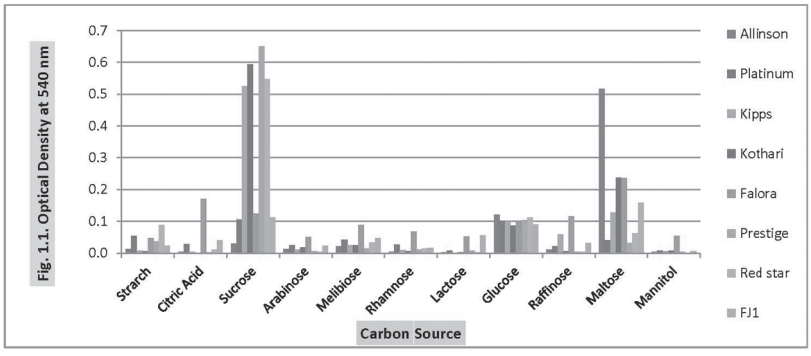


**Fig. 1.** Carbon assimilation by commercial yeasts and FJ1 isolate

Vol. 11 (2) 113-120 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

The carbon assimilation was analysed in terms of increase in optical density at 540 nm after 72 hr of incubation at 28°C. Sucrose was the most preferred carbon source with Prestige yeast showing maximum assimilation followed by maltose and glucose. Allinson showed maximum assimilation for maltose and glucose while others did not show any significant difference in their assimilation values. Other sources of carbon showed bleak assimilation.

Relatively higher carbohydrate content was observed in Allinson and FJ1 isolate. It ranged between 28-34% among the different yeast isolate studied. No significant difference in the carbohydrate content of different yeast strains was observed. The carbohydrate content of FJ1 was at par with the other commercial isolates. The results were in agreement with the study done by (14) which suggested a carbohydrate content ranging between 30-32% with commercial baker's yeast strains for high sugar bread dough containing relatively high carbohydrate content in the range 34-39%.

Trehalose has been reported by many investigators to have certain roles in heat and desiccation resistance and cryoresistance in frozen bread dough method (15, 16). Resistance to dehydration of *S. cerevisiae* containing high trehalose content was also increased if a high level of intracellular trehalose accumulated in stationary-phase cells or cells incubated in the absence of nitrogen source (17). Trehalose content (Table 9) was found to be maximum in FJ1 isolates (5.00%), thereby possibly depicting its cryotolerance property followed by Falora (3.38%), Platinum (3.27%), Kipps (2.79%), Allinson (2.73%), Kothari (2.06%), Prestige (1.81%) and Red Star (1.51%). A significant difference was observed in the trehalose content of various commercial yeasts and lab isolate. Among all commercial yeast strains, Falora contained the highest trehalose content (3.381 %).

Since baker's yeast is highly sensitive to high osmotic pressure created by sugar and/or salt in bread dough, yeast with low invertase helps to prevent them adverse effect of high osmotic pressure (18). Thus, lower invertase activity of these new strains was considered beneficial for leavening ability in low- and high sugar bread doughs. There was a marked difference in the invertase activity among the different isolates. It varied from 205.04 unit/mg in Allinson to 30.32 unit/mg in Falora. FJ1 isolate showed relatively higher (165.14 unit/mg) invertase activity than Red Star, Kothari, Kipps, Prestige and Falora.

**Table 6.** Comparative carbohydrate, trehalose and invertase activity of different commercial yeasts and lab isolate.

| Commercial yeasts/ FJ1 | Carbohydrate content % (g/100g DW) | Trehalose Content % (g/100g DW) | Invertase Activity (unit/mg) |
|---|---|---|---|
| Prestige | 29.66 | 1.81 | 75.45 |
| Kipps | 31.78 | 2.79 | 130.97 |
| Kothari | 29.67 | 2.06 | 44.13 |
| Platinum | 28.76 | 3.27 | 186.49 |
| Red Star | 30.99 | 1.51 | 62.64 |
| Falora | 32.22 | 3.38 | 30.32 |
| Allinson | 33.62 | 2.73 | 205.0 |
| FJ1 | 33.89 | 5.00 | 165.14 |
| CD (5%) | 2.99 | 0.29 | 12.30 |

Vol. 11 (2) 113-120 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

**Table 7.** Freeze tolerant ability of different commercial yeasts and lab isolate.

| Commercial yeasts/FJ1 | 10 days | 20 days | 30 days | 40 days | 50 days | 60 days | 90 days |
|---|---|---|---|---|---|---|---|
| Allinson | 100 | 100 | 98.0 | 97.0 | 93.0 | 90.0 | 72.0 |
| Prestige | 100 | 96.0 | 94.0 | 90.0 | 88.0 | 83.0 | 64.0 |
| Kipps | 100 | 100 | 95.0 | 90.0 | 84.5 | 70.5 | 58.0 |
| Kothari | 100 | 97.0 | 92.0 | 88.5 | 81.0 | 76.5 | 62.5 |
| Platinum | 100 | 97.0 | 93.0 | 90.0 | 82.0 | 73.5 | 59.5 |
| Falora | 100 | 98.0 | 94.0 | 90.5 | 83.0 | 73.0 | 54.0 |
| Red Star | 100 | 100 | 97.0 | 87.5 | 81.2 | 72.5 | 61.0 |
| FJ1 | 100 | 100 | 97.0 | 93.0 | 90.0 | 87.0 | 71.0 |

*All values are mean of three replicates **CD (5%)** = 5.05 (after 90 days of incubation)

Freeze tolerance ability of the yeast depicts its ability to survive under refrigeration conditions. The freeze tolerance ability of the isolates was noted for 90 days which showed asignificant difference in Kipps and Falora while no significant difference was observed in all other yeasts. FJ1 isolate in comparison to Allinson yeast showed lesser viability after 90 days.

**Conclusion**

Yeast is the foremost constituent in the baking industry. Prevalence of contaminating microorganisms was underestimated because of the inherent low pH of the substrate (molasses) being used for its multiplication. But this study concluded the presence of various food-borne pathogens in the commercial yeast preparations. Contaminants like *B. cereus*, *Staphylococcus* and Molds have been found to be prevalent. Faecal coliforms were present only in two commercial brands. Assimilation of inorganic nitrogen in the form of nitrate was observed in FJ1, Falora and Allinson yeast, whereas nitrite was assimilated by Falora yeast. All the isolates were able to ferment lactose (non-sugar dough) whereas only Kothari, kipps and platinum were able to ferment sucrose (high-sugar dough). Sucrose was the most preferred carbon source (with Prestige yeast showing maximum assimilation) followed by maltose and glucose. Allinson yeast showed maximum assimilation of maltose which is the main sugar available for fermentation in flour. Allinson and FJ1 have significantly higher carbohydrate content (33.62 and 33.89%), while for all other 6 brands it was at par. Trehalose content was highest for FJ 1 (5.0 g/100 g DW) followed by Falora, Platinum and Allinson, whereas invertase activity was highest in Allinson.

FJ1 isolate was selected on the basis of its highest trehalose content whereas among the commercial strains Allinson was selected as reference strain on the basis of its preferred carbohydrate assimilation, high carbohydrate content, moderate trehalose content and high freeze tolerance. Both these strains are presently being tested for their dough rising ability and fermentation efficiency for use in bread making in our laboratory.

**References**

(1) Reale, A., Di Renzo, T., Succi, M., Tremonte, P., Coppola R. and Sorrentino, E. (2013). Microbiological and fermentative properties of baker's yeast starter used in bread making. J. Food Sci. 78:1224–231.

(2) Gomez-Pastor, R., Perez-Torrado, R., Garre, E. and Matallana, E. (2011). Recent advances in yeast biomass

production. INTECH Open Access Publisher.

(3) Nasr, N. F., Zaky, A. S.and Daw, Z. Y. (2010). Microbiological quality of active dry and compressed baker's yeast sold in Egypt. J Pure and Applied Microbiol. 4: 455-62.

(4) Boboye, B. and Dayo-Owoyemi, I. (2009). Comparative evaluation of the sensory properties of doughs fermented with yeasts isolated from orange. J. Biotech.8(3): 389-92.

(5) Reed, G. and Nagodawithana, T. W. (1991). Baker's yeast production. In: Yeast Technol. Pp 261-314. Springer, Netherlands.

(6) Viljoen, B. C. and Lues, J. F. R. (1993). The microbial populations associated with post-fermented dough and compressed baker's yeast. Food Microbiol. 10:379–86.

(7) O'Brien, S. S., Lindsay, D. and Von Holy, A. (2004). The presence of Enterococcus, coliforms and E. coli in a commercial yeast manufacturing process. Int. J. Food Microbiol. 94: 23-31.

(8) O'Brien, S. S., Lindsay,D. and Von Holy, A. (2007). Microbiological shelf-life studies on commercially manufactured yeast. J. Food Quality. 31:627–44.

(9) Lodder, J. and Kreger-van Rij, N. J. W. (1952). The yeasts, a taxonomic study. North Holland Publishers, Amsterdam, London.

(10) Wickerham, L. J. (1951). Taxonomy of yeasts. Techn Bull 1029, U.S. Dept. Agr. Washington D C.

(11) Yemm, E. W. and Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57(3): 508.

(12) Oda, Y. and Ouchi, K. (1990). Effects of invertase activity on the leavening ability of yeast in sweet dough. Food Microbiol. 7: 241-48.

(13) Trevors, J. T., Merrick, R. L., Russell, I., Stewart, G. G. (1983). A comparison of methods for assessing yeast viability. Biotechnol. Lett. 5(2): 131-34

(14) Wongkhalaung, C. and Boonyaratana-kornkit. (2007). Characterization of new Baker's yeast strains and their leavening ability in bread dough. Kasetsart J. Nat. Sci. 41: 751-763.

(15) Hino, A., Mihara, K., Nakashima, K. and Takan, H. (1990). Trehalose levels and survival ratio of freeze-tolerant versus freeze-sensitive yeasts.Appl. Environ. Microbiol. 56(5):1386-391.

(16) Meric, L., Lambert-Guilois, S., Neyreneuf, O. and Richard-Molard, D. (1995). Cryoresistance of Baker's yeast *Saccharomyces cerevisiae* in frozen dough: Contribution of cellular trehalose. Cereal Chem. 72 (6):609-15.

(17) Gadd, G. M., Chalmers, K. and Reed, R. H. (1987). The role of trehalose in dehydration resistance of *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. 48: 249-54.

(18) Oura, E., Suomalainen, H. and Viskari, R. (1982). Breadmaking.Econ. Microbiol.7: 86-141.