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RESEARCH ARTICLE

COMPARATIVE STUDY OF COMMERCIALLY AVAILABLE BAKER'S YEASTS TO EVALUATE THEIR GAS PRODUCTION ABILITY

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ABSTRACT

Yeast (*Saccharomyces cerevisiae*) is a biological leavening agent used in wheat breads, pizza dough, pita bread, crackers and many other bakery products. The organism converts the fermentable sugars present in the dough into Carbon dioxide gas and ethanol.CO_2 gas acts as a major leavening agent during bread dough processing while small amount of leavening is also contributed by alcohol production during fermentation. An enormous number of strains of *S.cerevisiae* exist, many of which have already been selected for baking. The potential characteristics of a particular baker's yeast are determined by the strain of yeast that is selected. The actual characteristics of baker's yeast, from a particular strain are determined by its composition. Baker's yeasts belong to the different strains of *S. cerevisiae* and are available in different forms such as Active Dry yeast, Instant yeast, Cream yeast, compressed yeast and rapid rise yeast etc. After comparing the leavening activity of 6 different commercially available brands of baker's yeasts i.e. DCL (E.U.),Saf-instant (France), Efkay (U.A.E), Saf-levure (France), Rossmoor and English. The results showed that the brand name as DCL (E.U.) had the highest leavening activity while the brand name Saf-instant (France) had the least leavening activity.

INTRODUCTION

Yeasts can be considered as man's oldest industrial microorganism (**Oldest Beer from Shipwreck Yields Dead Yeast, 2013**). Scientists hypothesized that a mixture of flour meal and water was left for a longer period than usual on a warm day and the yeasts present in the flour caused it to ferment before baking. The bread thus formed had a low weight and more tasty than the old flat breads. Growth vats for the culturing of *S.cerevisiae* and centrifugation methods (**Sasidharan *et al*., 2012**) was found to be which led to the production of modern yeast. The slurry yeast that was made at small baker's and grocery shops became cream yeast, which is a suspension of live yeast cells in the growth medium and subsequently compressed yeast, the fresh cake yeast became the standard leavened for bread making (**Bellido *et al*., 2009**). Different companies then produced instant yeast which has been used for different purposes and also commercially more well-known both as the fresh yeast (**Kollár *et al*., 1991**) and dry yeast (**Bangham, 2014**). Baker's yeast or *S.cerevisiae* (**Mukhtar *et al*., 2010**) is a single celled (**Zinser *et al*., 1991**), eukaryotic fungi (**Bangham, 2014**) and is the name for the type of yeast used to leave the bread.

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The latin translation of *S.cerevisiae* (**Ruiz and Ariño, 2007**) is "Sweet fungi of Beer". *S.cerevisiae* is an oval shaped fungi (**Kolodziej *et al*., 1996**) and is devoid of flagella (**Phillips, 2008**). Baker's yeast vary in size ranging from 1-5µm in width and from 5-30µm in length (**Tibayrenc *et al*., 2010**). A single yeast cell contains a protoplasm (http://www.classofoods.com/page1_3.html, 2013) containing fats, proteins, organic ions, vacuoles, nucleus and is surrounded by a plasma membrane (**Osumi, 1998**). A yeast cell has 6000 different genes (**Forsburg, 2001**) and 16 different chromosomes (**Wickner *et al*., 1983**).

Baker's yeast is available in a vast variety of forms, the main difference is the moisture contents. Though each type is advantageous over other, the choice of which form is to utilize depends entirely on the requirements of the recipe. Dry yeast forms are more preferential due to longer shelf life (**Malik and Hoffmann, 1993**).The different forms of yeast can be inter-convertible if the liquid content and temperature of the yeast is manipulated. The different forms of baker's yeast include wild yeast and starter cultures, Active dry yeast **(http://journalofafrenchfoodie.com/category/bread/, 2013),** instant yeast (**http://www.brewuk.co.uk/store/liquid-yeast, 2013**), fresh yeast (**http://www.breadworld.com/rr_vs_ ady.aspx, 2013**), bread machine yeast (**Bushuk and Hulse, 1974**), liquid yeast

(**Elkassabany and Hoseney, 1980**), nutritional yeast (**Watanabe *et al.*, 2011**), rapid rise yeast (**Ishihara *et al.*, 1992**), cream yeast, autolysed yeast, hydrolyzed yeast etc.

Aims and Objectives

The basic aim of this study is to determine the gas production abilities of the different brands of yeast that are available locally. In addition to the evaluation of gas producing capacities, the CFU/g of the yeast sachets and a comparative analysis was also made on the basis of the yeast cells morphology.

MATERIALS AND METHODS

Samples

Six different brands of commercially available baker's yeast were collected from the local markets. These brands include DCL yeast, Rossmoor, English yeast, Saf-levure, Saf-Instant and Efkay yeast. These packets of yeast weighed 11g except Saf-levure [38] which was bought in 125 g jar and Efkay yeast as 15g sachet. All the packets were kept at the room temperature prior to opening and during the experiment the packets were refrigerated.

Ingredients for the dough making

Five kilograms of wheat flour of the Ashrafi brand aata was used to monitor the leavening capacities of different brands of yeast Granulated non-powdered sugar from the local market, National un-iodized salt and distilled water was bought from the local stores. Thermometer was used to measure the room temperature before starting the fermentation process. The wheat flour bag was refrigerated during the procedure. Vernier calipers which was not having zero error was used to measure the diameter of the gas measuring cylinder. Magnetic hot plate was used to heat the water for the Active dry yeast sample. Steel spatula and small glass plates were used to transfer the yeast sample and sugar, salt to the flour. The measurement of the weight of the different components was measured using a calibrated weighing machine.

Measurement of each component of dough

Hundred grams of flour, 1g of yeast of different brands,1g of sugar,1g of salt were measured using the electric weighing machine. The amount of distilled water i.e. 60mL was kept constant for all the subsequent dough making procedures.

Kneading of the dough

Flour was mixed with sugar, yeast and salt in a glass bowl (200mL) and mixed with distilled water (60mL) at regular intervals during the kneading process. The mixing time/kneading time was kept constant for all the procedures.

Setting up the fermentation assembly

The technique employed by **Ali and Hasnain (2011)** was used.

Determination of the morphological and colonial characters of each of the yeast brands

Slides were made of different brands of yeast and then gram stained.

Determination of CFU count of each of the yeast sample

Preparation of the selective media for the growth of yeast

PDA (potato dextrose agar) was prepared using the Oxoid® PDA formulation i.e. 39g makes 1 liter. The medium was dissolved in distilled water and then autoclaved at 121°C. The medium was then allowed to cool down for a while and then approximately 20ml of the medium was poured aseptically in the disposable plates. The plates were then covered and left overnight for pre-incubation. Next day the plates that did not show any microbial growth i.e. free of contamination were selected and refrigerated.

Preparation of diluent

Diluent used in this research is referred to as the Maximum recovery diluent (MRD). For one litre of MRD i.e. 1000mL,1g of Bacteriological peptone (Oxoid®), 850g NaCl (Analytical grade of Oxoid company) was dissolved in 1000mL of distilled water. The MRD was then autoclaved at 121°C for 120min. The MRD was then dispensed in sterile test tubes aseptically using a sucker and 10mL pipette and then kept in a jar wrapped and refrigerated for further use.

Preparation of yeast dilution

The yeast packet was opened and marked date of opening on the packet. One gram of yeast was weighed and then dispensed in 100 mL sterile MRD containing bottle (as 1g of yeast cannot be dispensed in 9 mL of MRD containing test tube. It will soak the water and will clog). The bottle is thoroughly shaken and then 1mL of the diluted sample is dispensed in 9mL containing MRD. This makes the 1:1000 dilutions. Now aseptically transfer 1ml from the 1:1000 diluted test tubes to 9mL MRD. This will give 1:10000.Continue making the dilution in the similar manner up to 1:1000000 dilution. At each dilution step, the diluted tube was shaken by grasping the tube between the palms of both hands and rotating quickly to create a vortex. This will aid in the distribution of the sample and break all the clumps of the yeast culture.

Determining the CFU of each brand

Hundred microlitre of 10^{-6} dilution was dispensed aseptically in 6 plates using Jester (100 microlitre tip) and it is spread evenly onto the surface of the solidified agar plates with the help of sterile cotton swab. The plates were then wrapped and marked with the date of incubation and the brand name as well. The plates were incubated at two different temperatures and for two different durations. 3 plates of 10^{-6} dilution were incubated at 30°C for 3 days time and three plates of 10^{-6} dilution were incubated at ambient temperature for a week (7 days). As the number of colonies that appear in 3 days time for DCL and Saf-Instant was far beyond the countable range so higher dilutions were prepared.

For DCL 10^{-8} dilution was made and for Saf-Instant 10^{-20} dilution was made. The remaining procedure of incubations was same for the plates dealing with 10^{-8} and 10^{-20}. After the appearance of the countable range of colonies, CFU was calculated for each of the yeast brands. The CFU/g was calculated from the data and the observations were recorded in a tabulated format.

Graphical representation and statistical analysis

The CFU/g of each of the yeast brands was plotted against the cost (in PKR) of each of the yeast brands. Another graph was made in which the CFU was plotted against the temperature of each of the yeast brands.

Colonial characters

The colonial characteristics were recorded for each of the yeast brands.

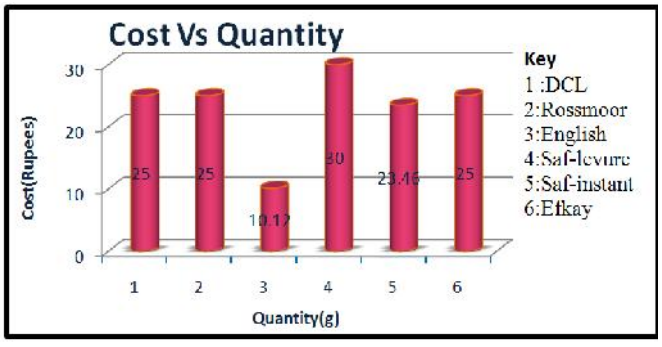
Isolation, purification and maintenance of pure cultures

Each of the yeast culture on the 10^{-6} marked PDA plate was streaked on PDA plate using a sterile wire loop. The method was carried out aseptically. After having the isolated colonies a single colony was inoculated on the PDA slant in the MacCartney bottle. The agar slants were prepared by first making the PDA medium using the previously mentioned method. Nine milliliter of the melted, autoclaved PDA was dispensed in the McCartney tubes. The McCartney tubes were left at room temperature for overnight for pre-incubation. The tubes containing no visible growth were selected and then the culture was inoculated on their slanted surface. The tubes were incubated at 37°C for a day for the appearance of the colonies of the yeast.

RESULTS

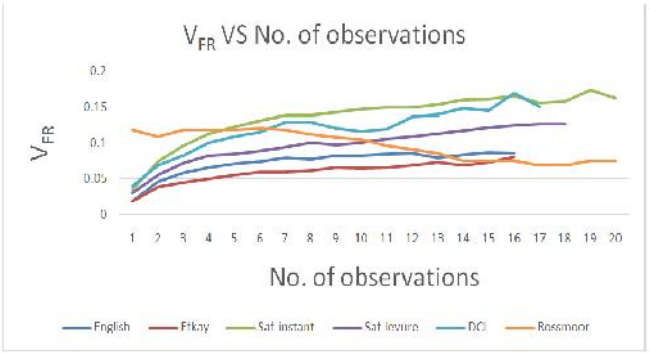
Yeast available in local markets

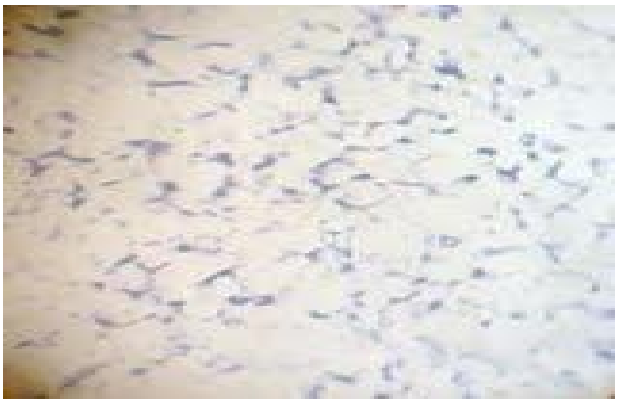
S#	Brand name	Quantity per pack(g)	Cost (Rupees)	Store/Super Market	Manufacturer Country Site
1.	DCL	11	25	Home Needs	E.U.
2.	ROSSMOOR	11	25	Home Needs	Pakistan
3.	ENGLISH	11	25	One-Ten	Pakistan
4.	SAF-LEVURE	125	115	Imtiaz Super Market	France
5.	SAF-INSTANT	11	30	Imtiaz Super Market	France
6.	EFKAY	15	32	K.U. Super Store	U.A.E



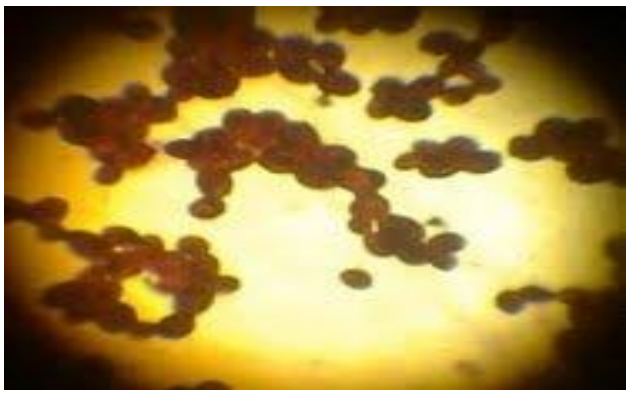
Graph showing a relationship between Cost and Quantity of yeast brands

V_{FR} vs No. of observations of the average values

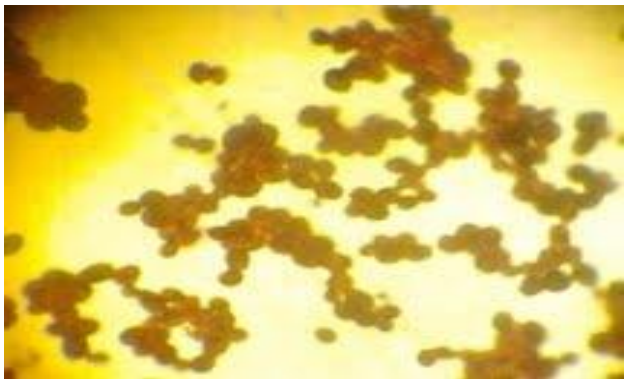




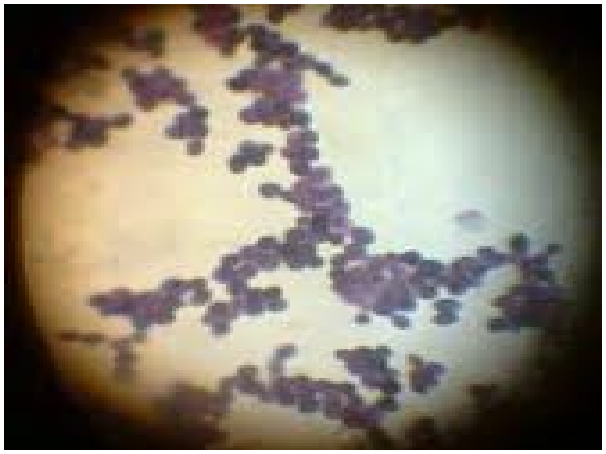
English



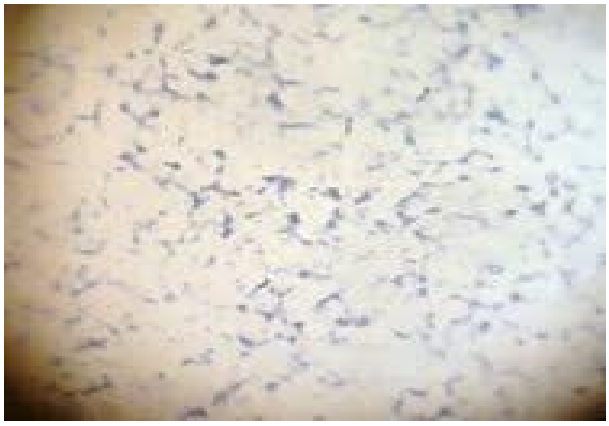
DCL



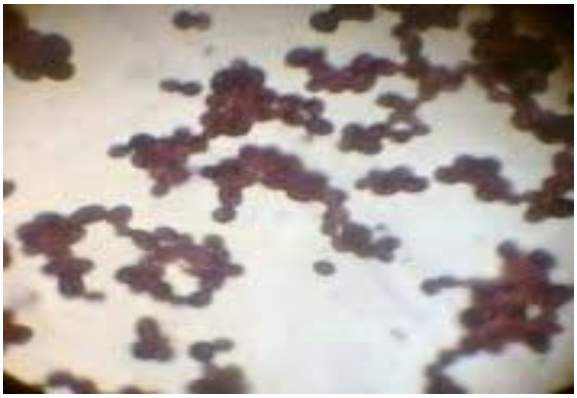
Efkay



Rossmoor



Saf-levure



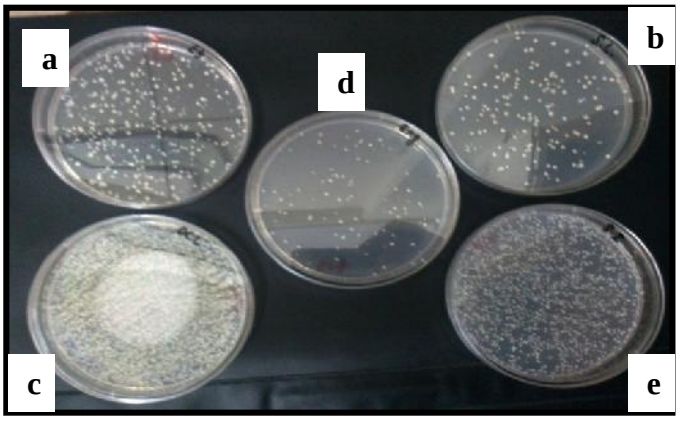
Saf-instant

Figure 1. Gram reaction of the different brands of yeast

Morphological characters of different brands of yeast

Names of yeast brands	CFU at Temperature(°C) 15°C	CFU at Temperature(°C) 30°C
DCL	432[c]	441[c]
English	117.66[a]	83.66[a]
Saf-instant	217.66[ab]	813[d]
Saf-levure	148.66[ab]	118[a]
Rossmoor	46[a]	46[a]
Efkay	398.66[c]	306.33[bc]

[a]Values are the mean of three different replications. Different alphabets within each row are significantly different at p≤0.05.



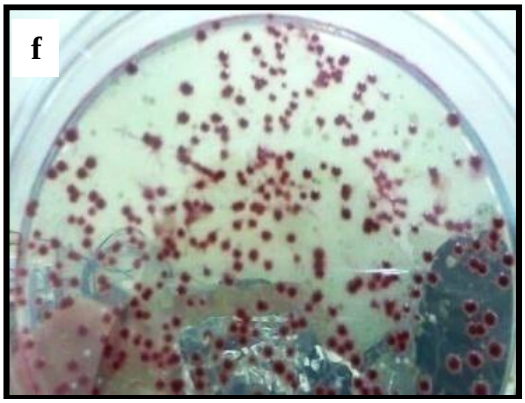


Figure 2. Colonies of different brands of yeast. a: Efkay, b: Saf-levure, c: DCL, e: Saf-instant, d: English f:English yeast

The McCartney tubes were then checked for the pure cultures performing gram staining (mentioned earlier) of the yeast cultures. The tubes were then refrigerated if they were devoid of any sort of contamination. Special care was taken in case of the isolation of English yeast as there was mixed culture of red and cream colored yeast colonies. After streaking 3 times on PDA plates, pure colonies were obtained. These yeast cultures were checked regularly in order to do the sub-culturing if there was drying of the medium.

DISCUSSION AND CONCLUSION

In this research the different brands of commercially available yeast sachets were tested for their comparative gas production abilities. The dough's that were prepared using the Instant yeast showed positive sign of fermentation by producing leavening of the dough. The dough that did not require a large amount of time to show leavening. This research was carried out at an ambient temperature. The purpose of this is to analyze the gas production in the dough's, which usually home bakers experience while baking. The season when this experimentation was done was hot temperature weather i.e. during the month of July and August. Therefore, the leavening was done at the optimum temperature. The temperature noted during the days of experimentation was in the range of 30-33°C. This temperature also favored the growth of *S.cerevisiae*. In case of English yeast, the leavening activity was affected by the bacterial contamination. The yeast sachets when morphologically analyzed showed a huge amount of

gram positive and gram negative micrococcus. The yeast cells were only few in counts. When the further purification of these yeast cultures were performed, the originally red colored colonies of the yeast with the green peripheries(similar as that of pseudomonas pigmentation) lost this red pigmentation and became light pink in color with no pigmentation of the peripheries. It was also found out that the yeast sachet which contained the highest number of yeast cells also had the highest leavening activities. But in the case of Saf-instant it was observed that although the numbers of viable yeast cells were more but the gas production ability was bit lower possibly due to the strain of *S.cerevisiae* used in the packets. More or less all the yeast brands had some sort of bacterial contamination in the yeast sachets. In case of Saf-levure small gram positive short rods were observed.

In a number of literatures it is stated that Lactobacilli are added to the yeast sachets in order to preserve the yeast cells and to give them a longer shelf life. It was observed that when 100µL of the yeast cell suspension is inoculated on the surface of PDA and incubated in a box which is sealed completely with the help of a tape, uncountable colonies were developed. The colonies that were developed were too many and too tiny. The plates when opened also had a large amount of gas produced because the media plates were bumped and specific aroma of fermentation could also be sensed. This showed that when the yeast cells are provided enough amounts of sugars and anaerobic condition they multiply and replicate to a large amount in a lesser time period. In case of incubation temperature, a higher temperature than ambient temperature favoured the growth of yeast cells. A comparison between the incubation temperature at ambient temperature and at 30°C tested. For ambient temperature, the incubation duration was seven days and for 30°C it was two to three days. Although the incubation duration was more in case of ambient temperature, still the colonies were more in the case of incubation at 30°C. In the case of Rossmoor, the countable yeast colonies appeared within the period of 2 days. If a general ranking is given to the yeast brands depending on the V_{FR} values. The volumetric flow rate followed the order DCL = Saf Instant > Saf-Levure > Rossmoor > English > Efkay.

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