Journal of Bioscience and Biotechnology Discovery
Volume 6(5), pages 46-52, December 2021
Article Number: 670CD8AE1
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2021.158
https://integrityresjournals.org/journal/JBBD
Full Length Research

Comparative study on the growth rate and physico-chemical properties of yeast (*Saccharomyces cerevisiae*) using *Zea mays l. (*corn) and *Triticum asetivum l. (*wheat) flour substrates

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Received 9th September, 2021; Accepted 25th November, 2021

ABSTRACT: Significance of yeasts in food technology as well as in human nutrition, as alternative sources of protein to cover the demands in a world of low agricultural production and rapidly increasing population, makes the production of food grade yeasts important. The comparative study on the growth rate and physicochemical properties of yeast was carried out using corn and wheat flour substrates. 50 g of wheat and corn flour each was weighed into a calibrated culture plastic rubber. 120 ml of distilled water was added with 5 ml of lemon juice. The sample was stirred and kept under room temperature at 26°C. The growth of the yeast were observed every three days for two weeks and recorded in millimetre. Yeast (*Saccharomyces cerevisiae*) culture grown using corn flour gave a mean growth of 12.18±0.38 for the first week and 15.24±1.21 for the second week. On the other hand, the growth of yeast produced from wheat flour gave 8.29±0.35 and 10.46±1.23 for first and second week respectively. The result also showed that yeast produced from corn flour grew rapidly than the wheat flour. The acid value of yeast produced from corn flour was 17.95 mgKOH/g, saponification value of 9.5 mgKOH/g, ester value of 164.37 mgKOH/g, iodine value of 87.63 gI_2/100g, peroxide value of 16.0 mEq/kg, free fatty-acid of 9.02%, refractive index of 1.50, specific gravity of 0.902, density of 5 g/cm^3 and pH of 5, while yeast produced from wheat flour had an acid value of 14.3 mgKOH/g, saponification value of 5.3 mgKOH/g, ester value of 152.1 mgKOH/g, iodine value of 66.0 gI_2/100g, peroxide value of 10.1 mEq/kg, free fatty-acid of 5.2%, refractive index of 0.6, specific gravity of 0.561, density of 3.2 g/cm^3 and pH of 5. The result showed that the physico-chemical properties of yeast produced from corn flour were higher than that produced from wheat flour with p value of 0.001.

Keywords: Corn, *Saccharomyces cerevisiae*, wheat.

INTRODUCTION

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. The first yeast originated hundreds of millions of years ago, and at least 1,500 species are currently recognized. They are estimated to constitute 1% of all described fungal species (Kurtzman and Piskur, 2006; Hoffman et al., 2015). They constitute approximately 1% of all known fungal species. Yeasts (*Saccharomyces cerevisiae*) are single-celled organisms which developed from multi-celled precursors, with some species having the ability to cultivate multi-celled properties by developing threads of linked potential cells known as pseudo hyphae or false hyphae (Shittu et al., 2008). Yeasts normally measure 3 to 4 µm in diameter, but some can grow up to 40 µm in size,

depending on species and environs. Nearly all yeasts procreate asexually by mitosis, and several of them do so by the unequal division process known as budding. Yeasts, with their unicellular growth habit, strikingly differ from molds, which grow hyphae. Fungal species that are single-celled or can grow hyphae (depending on temperature or other conditions) are called dimorphic fungi, "dimorphic" means "having two forms" (Shittu et al., 2008).

The production of alcoholic beverages from fermentable carbon sources by yeast is the oldest and most economically important of all biotechnologies. Yeast plays a vital role in the production of all alcoholic beverages and the selection of suitable yeast strains is essential not only to maximise alcohol yield, but also to maintain beverage sensory quality (Walker and Stewart, 2016). The yeast species S. cerevisiae convert carbohydrate to carbon dioxide and alcohols by fermentation. For thousands of years, this carbon dioxide has been found useful in baking and the alcohol in alcoholic beverages. It is also a centrally vital prototype organism in modern cell biology research, and is one of the most comprehensively researched eukaryotic microorganisms. Scholars have used *S. cerevisiae* to collect data about the biology of the eukaryotic cell and eventually human biology (Novák et al., 2010).

S. cerevisiae requires growth factors at very low concentrations in order to perform specific catalytic or structural roles and these include vitamins, purines and pyrimidines, nucleotides and nucleosides, amino acids, fatty acids and sterols. Complex media, such as malt wort or wine must, should be able to provide these accessory growth factors for alcohol fermentations, but commercially available yeast “foods” may also be employed to supplement media. These are based on mixtures of yeast extract, ammonium phosphate and minerals (for example, magnesium and zinc) and may be employed in alcohol fermentations to ensure consistent yeast activity. Sources of sugars for beverage fermentations can either be directly extracted from sugar-rich plants (for example, from sugarcane in the case of molasses or fruits in the case of wine must) or from starch-rich plants (Walker and Stewart, 2016).

Food and beverage processing using microorganisms is the most suitable technology for the development of innovative fermented food products. Solid state fermentation is used for processing of vinegar, soy sauce, tea, and cheese (Ghosh, 2015). Wine, beer, distilled beverages, and yogurt are developed by submerged fermentation. Both methods of fermentation are influenced by numerous factors, including temperature, pH, nature, and composition of the medium, dissolved O_2, dissolved CO_2, operational system, and feeding with precursors, among others. Variation in these factors may affect the rate of fermentation, the product spectrum and yield, the sensory properties of the product (appearances, taste, smell, texture), physic-chemical properties, nutritional quality, and the production of metabolites that promote human health attracting consumers attention towards fermented products, namely beverages (Vilella, 2019). High water activity is required for *S. cerevisiae* cells which typically possess a minimum a_w of around 0.65. Water is absolutely essential for fermentation, and high sugar-containing media can impose osmotic stress (reduced water availability) on cells to adversely affect cell physiology (Walker and Stewart, 2016).

Yeasts, as well as other microorganisms, contain a variety of lipids, whose content and composition can be effected by growth conditions and/or genetically, which makes them suitable for the production of highly specific lipids (Blagovic et al., 2001). In the recent times, yeasts have been used to generate electricity in microbial fuel cells, and produce ethanol for the biofuel industry subject to the substrate used for growing the yeast (Madhani and Fink, 1998). *S. cerevisiae* is utilized in winemaking, where it converts the sugars present in grape juice (glucose and fructose) known as must, into ethanol. A pure yeast culture is therefore usually added to the must to repress the wild yeasts and dominate the fermentation. This ensures a reliable and predictable fermentation (Landry et al., 2006). Nearly all yeasts added to wine are strains of *S. cerevisiae*, yet not all strains of the species are appropriate. Diverse *S. cerevisiae* yeast strains vary in physical and fermentative features; hence the actual strain of yeast selected can have a direct effect on the finished wine. Significantly, research has shown that *S. cerevisiae* added to the development of new wine produce a distinctive good flavour profiles to promote complexity in wines (Landry et al., 2006). Conservation and comercialization of yeast cultures in fresh liquid or pressed forms are not economically advantageous. Thus, dehydrated yeasts present numerous advantages, such as lower cost, convenient for transport and storage, and ease of handling (Luna-Solano, 2003). All these benefits, as well as other health benefits of *S. cerevisiae* led to this study to investigate the physical and chemical properties of yeast grown on corn and wheat flour substrates as well compare the growth rate of yeast on the two substrates (Landry et al., 2006). A huge part of the earth’s population is undernourished, due to poverty and inadequate distribution of food. Consequently, the invention of microbial biomass for food consumption is a main concern for the industry and the scientific community.This study aimed is to compare the growth rate and physicochemical properties of yeast (*S. cerevisiae*) using corn and wheat flour substrates.

MATERIALS AND METHODS

Experimental site

This research work was carried out at Maeve Academic Research Laboratory, Awka, Anambra state, Nigeria.

Plate1. Corn grains.

Plate 2. Wheat grains.

Source of experimental materials

Grains of corn (*Zea mays* L.) (Plate 1) and wheat (*Triticum aestivum*) (Plate 2) were procured from Eke-Awka market while yeast (*Saccharomyces cerevisiae*) was sourced locally in Awka from the baker's store.

Reagents and equipment

The reagents and equipment used include aqueous sodium hydroxide, sodium hydroxide, alcohol phenolphthalein, diethyl ether, petroleum ether, powered potassium iodide, glacial acetic acid, chloroform, sodium thiosulphate and alcoholic potassium hydroxide, 250 ml calibrated plastic containers, distilled water, sieve and lemon orange.

Sample preparation

Plant samples (corn and wheat grains) were blended to fine powder using an electric blender and kept in sterile bags till further use. Using the method of Aransiola et al. (2012), 50 g of wheat and corn flour each was weighed into a calibrated culture plastic rubber and 2 g of yeast was dissolved in 5 ml of distilled water and added to each of the substrates. 120 ml of distilled water was then added with 5 ml of lemon juice. The sample was stirred using a sterile stirrer and covered with a muslin cloth and kept under room temperature. The growth of the yeast was observed every three days for two weeks and recorded in millimetre accordingly (Plates 3 and 4).

Physico-chemical properties of *S. cerevisiae* on corn and wheat flour substrates

Acid value

The method used was according to Ogbuanu et al. (2015). 1 g of the sample was dissolved in 25 ml of solvent with light heat to dissolve. 2 drops of phenolphthalein was added and titrated with 0.1N Potassium hydroxide to faint pink colour that persists for 20 to 30 sec. The volume (ml) of standard KOH used was recorded and used for the calculation.

$$\text{Acid value (mgKOH/g)} = \frac{\text{V xN xeq.wt}}{W}$$

Where: V = ml of KOH, N = Normality of KOH, eq.wt = eq.wt of KOH and W = Weight of the sample.

Saponification value

1 g of the sample was weighed into a beaker and dissolved with 3 ml of distilled water. This was then transferred into a 250 ml conical flask. 25 ml of 0.5 Normality (N) alcoholic KOH was added. The conical flask with its content was attached to a reflux condenser and heated on a boiling water bath for 30 min. After which it was allowed to cool at room temperature. This was then titrated with 0.5N HCl using 2 drops of phenolphthalein indicator. A blank was done without the sample. The volume of the standard HCl used was recorded for both the blank and the sample and used for the calculation (Ogbuanu et al., 2015).

$$\text{Saponification value (mgKOH/g)} = \frac{(\text{Tb} - \text{Ts}) \text{ x N x eq.wt}}{\text{W}}$$

Where: Tb = titre value for the blank, Ts = titre value for the sample, V = ml of KOH, N = Normality of KOH, eq.wt = eq.wt of KOH and W = Weight of the sample.

Plate 3. Yeast (*Saccharomyces cerevisiae*) culture on corn and wheat flour substrates.

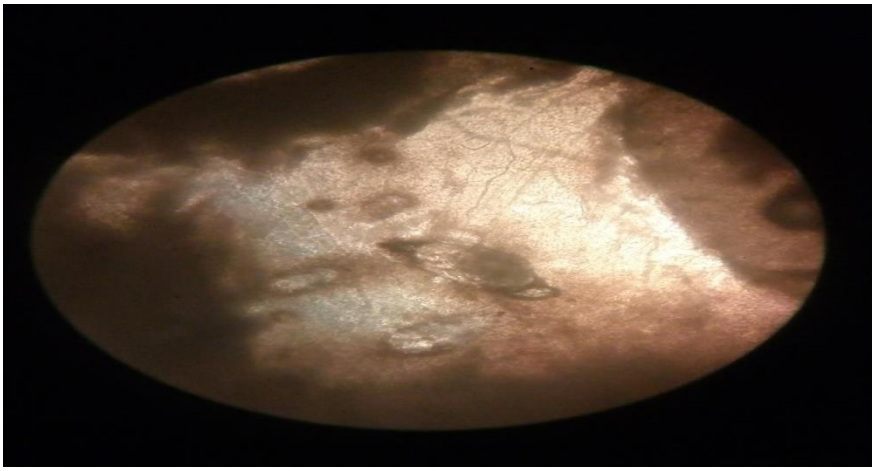

Plate 4. Microscopic view of yeast (Magnification 40x).

Iodine values

2 g of the sample was dissolved in 100 ml of petroleum ether to get 2% fat Solution. 10 ml of the sample was mixed with 25 ml of HCl solution and stoppered. This was kept in the dark for 1 hour after shaking thoroughly. A blank was also prepared in which the sample was replaced with 10 ml of chloroform. After 1 hour, the necks of the bottles were rinsed with about 50 ml of distilled water. 10 ml of 10% KI solution was added and these were titrated with 0.1N Sodium thiosulphate to a pale straw color, then 1 ml of 1% starch solution was added and titration, with thorough shaking, continued until the colour disappears. The volume of thiosulphate used was recorded and used for the calculation (Ogbuanu et al., 2015).

Iodine value (gI_2/100g) = (Bl - Tl) x 6.35

Where: Bl = titre value for the blank and Tl = titre value for the sample

Peroxide value determination

According to Lubrizol standard test procedure (2006), 5 g

of the sample was allowed to dissolve in a solvent mixture (Glacial acetic acid and chloroform [2:1] v/v) for 30 seconds and was shaken vigorously for another 30 seconds. 0.5 ml of 5% potassium iodide was added and allowed to shake for 1 minute. This was titrated with 0.1N of the $Na_2S_2O_3$ using starch indicator, a blank was similarly titrated.

$$Peroxide\ value = \frac{(S-B)xN\ x\ 1000}{W}$$

Where: S = Sample titre, B = Blank Titre, N = Normality Thiosulphate and W = Weight of sample

Determination of ester value

According to Crowe and White (2001), 2 grams of the sample was refluxed for 1 hour with 25 ml of aqueous sodium hydroxide in a water bath. The condenser was washed down with 5 mL of distilled water and the content allowed to cool down to room temperature. The excess alkali was titrated with 0.5 M HCl using phenolphthalein as indicator. A blank titration was repeated without the oil sample.

Ester value = Saponification value – Acid value.

Specific gravity

The specific gravity was determined using the specific gravity bottle. The bottle was dried and weighted. The weight of the empty bottle was recorded. It was then filled with distilled water and dried. It was removed, wiped dry and weighted. The bottle was emptied, dried and then refilled with the melted fat sample. It was then cleaned and wiped completely dry and weighted. The specific gravity was calculated using the formula below (Crowe and White, 2001).

$$\text{Specific gravity} = \frac{W1-W2}{W3-W}$$

Where: W_1 = Weight of bottle + oil, W_2 = Weight of oil, W_3 = Weight of ottle +water and W = Weight of bottle

Refractive index

This was carried out using a narrow glass tube. The tube was filled with sample and a silver-colored metal bulb was added. The real depth of the metal was measured using a meter rule. Then the apparent depth was determined as seen by the eye (Crowe and White, 2001). The refractive index was calculated as follows:

$$\text{Refractive index} = \frac{Real\ Depth}{Apparent\ depth}$$

Flash point

The flashpoint was carried out according to method of Crowe and White (2001). Flash point is the lowest temperature of the test specimen, at which the application of an ignition source causes the vapor of the test specimen to ignite momentarily and the flame to propagate across the surface of the liquid under the specified conditions of test. 3 ml of the sample was placed in crucible and placed in a muffle furnace with a thermostatic temperature recorder. This was then heated to the point of ignition. The temperature was recorded at this moment.

Boiling point

The boiling point was carried out according to method of Crowe and White (2001). Boiling point is the lowest temperature of the test specimen, at which the liquid just begin to agitate. 3 ml of the sample was placed in a crucible and placed in a muffle furnace with a thermostatic temperature recorder. This was heated until sample began to agitate. The temperature at this moment was recorded as the boiling point.

Statistical analysis

Data was analysed using two way Analysis of Variance via Sigma plus and means separated using LSD at $p \leq 0.05$.

RESULTS

Mean growth of *Saccharomyces cerevisiae* on corn and wheat flour substrates

Yeast (*S. cerevisiae*) culture from corn flour had a mean growth of 12.18±0.38 for the first week and 15.24±1.21 for the second week. On the other hand, the growth of yeast produced from wheat flour gave 8.29±0.35 and 10.46±1.23 for first and second week respectively (Table 1). From the result, it showed that yeast produced from corn flour grew rapidly than from wheat flour.

Characterization of *S. cerevisiae* produced from and corn wheat flour

Table 2 showed that the acid value of yeast produced from corn flour was 17.95 mgKOH/g, saponification value of 9.5 mgKOH/g, ester value of 164.37 mgKOH/g, Iodine

Table 1. Mean growth of *Saccharomyces cerevisiae* on corn and wheat flour substrates at 2 weeks.

Substrate	Week 1 (mm)	Week 2 (mm)
Corn wheat	12.18±0.38	15.24±1.21
Wheat four	8.29±0.35	10.46±1.23

Results are in mean±standard error.

Table 2. Characterization of *Saccharomyces cerevisiae* produced from wheat and corn flour.

Parameters	Corn flour (%)	Wheat flour (%)
Acid value (mgKOH/g	17.95	14.3
Saponification Value (mgKOH/g)	9.5	5.3
Ester value (mgKOH/g)	164.37	152.1
Iodine value (gI_2/100g)	87.63	66.0
Peroxide value (mEq/kg)	16.00	10.1
FFA (as oleic %)	9.02	5.2
Refractive index	1.50	0.6
Specific gravity	0.902	0.561
Density (g/cm^3)	5	3.2
pH	5	5

Table 3. Statistical analysis on the growth rate of *Saccharomyces cerevisiae* on corn and wheat flour substrates.

Source of variation	DF	SS	MS	F	P
Substrate	2	150.448	75.224	2.993	0.107*
Parameter	9	46052.455	5116.939	203.562	<0.001*
Residual	8	201.096	25.137		
Total	19	46611.362	2453.230		

**p<0.05.

value of 87.63 gI_2/100g, peroxide value of 16.0 mEq/kg, FFA of 9.02%, refractive index of 1.50, specific gravity of 0.902, density of 5 g/cm^3 and pH of 5. On the other hand, yeast produced from wheat flour had acid value of 14.3 mgKOH/g, saponification value of 5.3 mgKOH/g, ester value of 152.1 mgKOH/g, iodine value of 66.0 gI_2/100g, peroxide value of 10.1 mEq/kg, FFA is 5.2%, refractive index 0.6 specific gravity of 0.561, density of 3.2 g/cm^3 and pH of 5. The result showed that the physical and chemical properties of yeast produced from corn flour were higher than that produced from wheat flour.

Statistical analysis on the growth rate of *S. cerevisiae* on corn and wheat flour substrates

The growth rate of yeast on the test substrates gave a p value of 0.107, showing that there is a significant difference between corn flour substrate and wheat flour. Also, there was a significant difference (p value of 0.001) in the physicochemical properties of yeast on corn flour and wheat flour (Table 3).

DISCUSSION

Growth substrates for *Saccharomyces cerevisiae* used in this study were maize and wheat. This agrees with Walker and Stewart, (2016) that sources of sugars for beverage fermentation (yeast) can either be directly extracted from sugar-rich plant or from starch-rich plant. In this study, the growth of yeast from corn flour substrate was higher in the first 2 weeks than in the wheat substrates. This suggests that growth factors necessary for the growth of yeast were more favourable in the maize substrate than in the wheat, one of the factors could be as a result of high water retaining capacity in maize than in wheat as stated by Walker and Stewart (2016) that high water activity is required for *S. cerevisiae* cells and water is absolutely essential for fermentation.

Data presented from this study showed that yeast produced from corn flour and wheat flour are physico-chemically different. According to Vilella (2019), variation growth factors in yeast production may affect physiochemical properties towards fermented products. The distinct variations in the fats and oil contents showed that yeast with higher fat can be gotten from corn flour than wheat flour. This agrees with Blagovic et al. (2001), that yeast contains a significant amount of lipids. This can serve as a guide for nutritionist to make appropriate recommendation in food dieting. This implies that corn flour produced good quality of yeast that can be used in baking, brewing and for other nutritional products.

Evidence from this study showed that yeast produced from corn flour has more nutritional value than the one produced from wheat flour. Therefore, yeast produced from corn flour substrate have more physical and chemical properties than those produced from wheat flour.

Conclusion and Recommendation

Based on the results presented in this study, it is preferable to use corn flour to produce the largest live mass of baker's yeast and to consume it fresh or in the production of bread so that the consumer can benefit from its high nutritional value. It is also recommendthat further studies on other yeast growth substrates such as cassava flour being carried out to confirm their efficacy in yeast production.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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