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RESEARCH PAPER

# Evaluation of physical and chemical properties and total phenolic content in baker's yeast obtained from grape juice

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# تقييم الخواص الفيزيائية والكيميائية والمحتوى الفينولي الكلي في خميرة الخبز المأخوذة من عصير العنب

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**Abstract.** Baker`s yeast is mainly produced from molasses in various parts of the world, and other sources, including grape juice. In this study, the grape juice was chosen. This study aimed to produce a biomass from dry baker`s yeast. Its physical and chemical properties was evaluated. The biomass from baker's yeast *S. cerevisiae* was equal to 41.50±0.01 g/L. The following fermentation conditions, i.e. temperature (30.1°C), pH (4.75), sugar concentration (158.36 g/L), ratio of carbon to nitrogen (11.9), and initial concentration of yeasts (2.5 g/L) were used. The fermentation was carried out for a period 12 h. Grape juice was subjected to four different heat treatments as follows: pasteurized grape juice at (65, 70, and 75°C) for 10 min, and sterilized grape juice in the autoclave at 121°C for 20 min. The effect of each treatment was determined on inhibition of the enzyme polyphenol oxidase present in grape juice. The total phenolic content was determined in the yeast. Heat treatments gave the best phenolic content in the resulting yeast. The heat treatments of the juice succeeded in reducing the activity of the enzyme polyphenol oxidase and autoclave heat treatment gave the best phenolic content in the yeast.

**Keywords:** : Baker`s yeast, grape juice, fermentation, phenolic content.

**الملخص:** يتم إنتاج الخميرة المستخدمة في المخابز بشكل أساسي من دبس السكر في أجزاء مختلفة من العالم ، ومصادر أخرى ، بما في ذلك عصير العنب. في هذه الدراسة تم اختيار عصير العنب. وعليه كان الهدف من هذه الدراسة هو إنتاج كتلة حيوية من خميرة المخابز الجافة. حيث تم تقييم الخواص الفيزيائية والكيميائية للخميرة. فقد كانت الكتلة الحيوية من خميرة الخباز *S. cerevisiae* تساوي ٤١,٥٠ ± ٠,٠١ جم / لتر. وقد تم استخدام ظروف التخمير التالية ، درجة الحرارة (٣٠,١ درجة مئوية) ، و درجة الحموضة (٤,٧٥) ، وتركيز السكر (١٥٨,٣٦ جم / لتر) ، ونسبة الكربون إلى النيتروجين (١١,٩) ، والتركيز الأولي للخمائر (٢,٥ جم / لتر). تم إجراء التخمير لمدة ١٢ ساعة. تعرض عصير العنب لأربع معاملات حرارية مختلفة على النحو التالي: عصير العنب المبستر عند (٦٥ ، ٧٠ ، ٧٥ درجة مئوية) لمدة ١٠ دقائق ، وعصير العنب المعقم في جهاز التعقيم عند ١٢١ درجة مئوية لمدة ٢٠ دقيقة. تم تحديد تأثير كل معاملة على تثبيط إنزيم بوليفينول أوكسيديز الموجود في عصير العنب و تحديد محتوى الفينول الكلي في الخميرة. أعطت المعالجات الحرارية أفضل محتوى فينولي في الخميرة الناتجة. نجحت المعالجات الحرارية للعصير في تقليل نشاط إنزيم بوليفينول أوكسيديز وأعطت المعالجة الحرارية بجهاز التعقيم أفضل محتوى فينولي في الخميرة.

**الكلمات المفتاحية:** خميرة المخابز ، عصير العنب ، التخمير ، محتوى الفينول.

## Introduction

Fermentation is the process of using microorganisms to produce valuable products, such as antibiotics, industrial enzymes, food, and chemicals. Microorganisms which multiply predominantly by budding are collectively called "yeasts" (Saranraj et al., 2017). Phaff (1990) gave the definition for yeasts as unicellular eukaryotes, which at some stage in their life cycle, divide by budding. The known organisms is the strain of *Saccharomyces cerevisiae* used in the brewing and baking industries. If the term "yeast" is not further specified, *Saccharomyces cerevisiae* is the commonly referred organism. This strain of yeast has been extensively studied and applied widely both in the laboratory and in industry. Baker's yeast (*Saccharomyces cerevisiae*) is one of the oldest products of industrial fermentation.

It is still one of the most important fermentation products based on the volume of sales and its use for bread making, a stable food for large section of world population. The Baker's yeast *Saccharomyces cerevisiae* has been associated with human beings for more than 6000 years due to its use in food production, baking, wine and beer (Saranraj et al., 2017).

*Saccharomyces cerevisiae* was the first eukaryotic organism to be sequenced in 1996 (Goffeau et al., 1996), and is clearly the most ideal eukaryotic microorganism for biological studies. The impact of Baker's yeasts on the production, quality and safety of foods and beverages is intimately linked to their ecology and biological activities. Recent advances in understanding the taxonomy, ecology, physiology, biochemistry and molecular biology of Baker's yeasts have stimulated increased interest in foods and beverages. This has led to a deeper understanding of their roles in the fermentation of established products, such as bread, beer and wine. As the food industry develops new products and processes, yeasts present new challenges for their control and exploitation.

Food safety and the linkage between diet and health are the issues of major concern to the modern con-

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sumer and Baker's yeasts are emerging in this context (Saranraj et al., 2017). On the positive side, there is increasing interest in using Baker's yeasts as novel probiotic and biocontrol agents, and for the nutrient fortification of foods (Gelinas, 2006; Prem Kumar et al., 2015a). Baker's yeast, *Saccharomyces cerevisiae*, is still one of the most important biotechnological products because of its several industrial applications.

Baker's yeast as a commercial product has several formulations that can be grouped into two main types: compressed yeast, called fresh yeast, and dried yeast (Beudeker et al., 1990). Compressed yeast is the traditional formulation of baker's yeast and is ready for immediate use. Dried yeast is available in two forms: active dry yeast (ADY) and instant dry yeast (IDY). Active dry yeast (ADY) is normally sold in airtight packages, vacuum seal or filled with an inert gas such as nitrogen. It is not a problem to maintain quality, but it should be rehydrated before use. Unlike ADY, instant dry yeast (IDY) does not have the cell damage during rehydration. IDY is the most expensive among the three type of baker's yeast. Baker's yeast is marketed in two ways, either as compressed cakes or as a dry powder, however there is also a saleable intermediate of the process known as 'Cream yeast' (Gill et al., 2013).

Yeast are a unicellular fungi or plant-like microorganism that exists in or on all living matter i.e. water, soil, plants, and air. They are microbial eukaryote, associated with ascomycetes and are rich in protein and vitamin B (Dunn et al., 2015). As a living organism, yeast primarily requires sugars, water and warmth to stay alive. In addition, albumen or nitrogenous material is also necessary for yeast to thrive. There are hundreds of different species of yeast identified in nature, but the genus and species most commonly used for baking is *Saccharomyces cerevisiae*.

The scientific name *Saccharomyces cerevisiae*, means a mold which ferments the sugar in cereal (i.e. saccharo-mucus cerevisiae) to produce alcohol and carbon dioxide. Yeasts are usually spherical, oval or cylindrical in shape and a single cell of *S. cerevisiae* is around 8 μm in diameter. Each cell has a double-layered wall, which is permeable to certain substances and food material is taken into the cell and metabolites (Slonimski et al., 2013).

Cell division or cell reproduction generally takes place by budding. In the budding process, a new cell forms as a small outgrowth of the old cell, the bud gradually enlarges and then separates. Although, most yeast reproduce only as single cells, under some conditions some yeasts can form filaments (Madigan et al., 2003; Sivasakthivelan et al., 2014).

Yeasts flourish in habitats where sugars are present, such as fruits, flowers and bark of trees. However, commercial yeasts of today are quite different from wild strains due to genetic manipulation, allowing them to grow in previously unsuitable conditions (Liti et al., 2009; Prem Kumar et al., 2015b).

Yeasts are of great economic importance. Yeasts, especially different strains of *Saccharomyces cerevisiae* have long been used for the production of alcoholic beverages, solvents and other chemicals. In the modern bakery, yeasts are used for manufacturing of different kinds of bread and confectionaries. It is responsible for leavening the dough and imparting a delicious flavor to the product (Warringeret al., 2011).

Molasses is the most used raw material in the production of Baker's yeast, and it may be sourced from sugar beet, or sugar cane, and it contains about 50-55% of fermentable sugars, and some vitamins and minerals. These are important in cell proliferation containing fermentable sugars, such as date and grape juices (Gelinas et al., 2000).

Yeasts have a positive image with consumers, as they are considered a safe source of ingredients and additives for food processing (Boze et al., 1992; Bekatorou et al., 2006; Tsunatu et al., 2017). Preparations of baker's and brewer's yeasts have been available for many years as dietary, nutrient supplements because of their high contents of B vitamins, proteins, peptides, amino acids and trace minerals. Yeasts are often considered as an alternative source of protein for human consumption (Buzzini et al., 2005; Chaucheyras-Durand et al., 2008; Pienaar et al., 2012).

Many products are now derived from yeasts and, according to Abbas (2006), about 15–20% of the global industrial production of yeasts is used for this purpose. The production of antioxidants, aromas, flavors, colors and vitamins could be done by yeasts. Interest in food phenolics has increased, because of their antioxidant and free radical scavenging abilities (Lugasi and Hovari, 2003), metal chelators and enzyme modulators (Dulger et al., 2002). Many phenolics can exhibit antioxidant activity as their extensive, conjugated electron systems allow ready donation of electrons, or hydrogen atoms, from the hydroxyl moieties to free radicals. However, the antioxidant efficacy, in terms of reaction stoichiometry and reaction kinetics may vary considerably (Lugasi et al., 2003). This is dependent on structural features, such as the number and positions of the hydroxyl moieties on the ring systems, and the extent by which the unpaired electron in the oxidized phenolic intermediate can delocalise throughout the molecule. Thus, most phenolics, especially flavonoids are very effective scavengers of hydroxyl and peroxyl radicals. Phenolics are chelators of metals and inhibit the Fenton and Haber-Weiss reactions abilities (Lugasi et al., 2003; Dulger et al., 2002), which are important sources of active oxygen radicals. Phenolic compounds inhibited the development of cancerous tumours, reduce a risk for cardiovascular disease, and have showed antibacterial, anti-inflammatory, antispasmodic and anti-diarrheic properties (Abdoul-latif et al., 2012).

Fermentation is a good technology with great potential for application on the production or extraction of antioxidant active compounds from natural sources. New bioactive compounds could be found during fermentation. Moreover, modification of fermentation process could be tailored to increase the bio accessibility of bio-

active compounds. Production of bioactive compounds yet remains a quite unexplored potential, which could be accomplished by utilizing new fermentation process. Therefore, in the future, it can be anticipated that fermentation could be used to design food with health effects. Some fermentation processes are available on the applications of production of antioxidant activity compounds (Shahat, 2017). However, the underlying mechanisms affecting anti-oxidative activity during fermentation are varied, and the production of antioxidant active compounds during fermentation (Hur et al., 2014).

Some of the most compelling evidence of a protective effect of diets against cancer, in recent years, is the evidence on the intake of fruits and vegetables (Block et al., 1992; Fokou et al., 2017). EPIC (European Prospective Investigation into Cancer and Nutrition) is an important study that indicates that these retrospectively obtained results, at least respecting to cancer, might have been somewhat overestimated, however, still a significant reduction of consumption of fruits and vegetables on colorectal cancer (Bouayed0 et al., 2010).

Polyphenols can further act by inhibiting cell proliferation, which is deregulated in cancer. This inhibition has been demonstrated in vitro in many tumor cell lines. Although the anti-proliferative effects of polyphenols in general and in particular of flavonoids and iso-flavonoids in cell cultures seems well established, there are relatively few data regarding the *in vivo* anti proliferative activity, and virtually nothing is known about the clinical relevance of this bioactivity (Birt et al., 2001). This anti-proliferative effect suggests that polyphenols may have an effect via regulating the cell cycle or inducing apoptosis in tumor cells. In fact, many studies have shown the effect of polyphenols on the cell cycle of tumor cells in cultures in in vitro assays.

Therefore, in this presented study, grape juice was selected as the sole source of carbon for producing dry biomass from yeast due to its richness in phenolic compounds. Then the phenolic content of the yeast obtained from grape juice was evaluated. This study distinguished from previous studies using an organic medium, while previous studies used commercial media to obtain dry yeast with good phenolic content (Shaha, 2017).

It is known that grapes contain a good amount of the enzyme, polyphenol oxidase, which causes oxidation of phenolic compounds and reduces their quantity. It also causes enzymatic browning, which may negatively effect of the final product quality. In this study, the effect of heat treatment on the activity of the enzyme polyphenol oxidase was evaluated at different temperatures with the aim of choosing the best heat treatment in reducing the enzyme activity and thus maintaining a good phenolic content in the resulting yeast.

# Materials and Methods

## Commercial materials

All materials used in these experiments are collected from HiMeda Company, Mumbai, India. Glucose and vitamin solutions were sterilized by filtration and added to the autoclaved medium.

## Origin and Reactivation of the Yeast *S. cerevisiae*

Dried powder yeast form of S. cerevisiae (ATCC20408/S288c) was used in this study. It was produced by the Biomatric-The Biostability Company. The yeast was reactivated on agar plates containing YPGA medium composed of yeast extract 10 g/L, peptone 10 g/L, glucose 20 g/L, agar 20 g/L with a pH 6, incubated at 30°C for 24 h.

## Preparation of Grape Juice

Baladi grapes (Figure 1), which is one of the white grape varieties, originating in Spain were used. It is known as Cayetana grape. Baladi grapes are among the varieties available in Syria. Its yield is up to 20%. It is a domestic variety characterized by the size of its large mass and has a single conical shape. The grains are spherical in shape, large size, yellowish-white in color, and the peel is thin and light pink in color. The pulp is flaky, has a good taste, has a characteristic flavor, it is a late-ripening variety. It is a popular and luxurious table variety, suitable for remote transportation and long winter storage.

Baladi grape samples were collected from the Sheikh Badr area in the countryside of Tartous city in Syria. The grape berries were removed from their clusters, cleaned and washed with warm water. The juice was extracted by breaking and pressing in doubly folded cloth.

**Figure 1.** The baladi grape

## Thermal Treatment for Grape Juice

The heating effect on the grape juice was pasteurized at (65, 70 and 75°C) for 10 min, while fourth treatments was sterilized grape juice in the autoclave at 121 °C for 20 minutes. Then the effect of each heat treatment on inhibiting the activity of the enzyme polyphenol oxidase in grape juice was determined by estimating the phenolic content in the yeast.

## Preparation of Culture Medium Based on Grape Juice and Inoculums

The method cited by Kocher and Uppal (Kocher et al., 2013) was used with minor modifications. The obtained grape juice from the above preparation was supplemented by mineral salts: magnesium sulfate 0.44 g, urea 12.70 g, and ammonium sulfate 5.30 g. Finally, the medium was distributed in an Erlenmeyer of 250 mL with a ratio of 100 mL per flask and sterilized at 120 °C for 20 min. The pre-culture was obtained by inoculating two colonies of the yeast *S. cerevisiae* in 250 mL shake flasks containing 100 mL of grape juice, mentioned above. The pre-culture was incubated at 30 °C for 3 h, and used further as inoculums for the yeast biomass production.

## Fermentation Process

The fermentation was carried out within a biological fermenter with a capacity of 6 liters with an engineering design and initial volume of the fermentation medium (i.e. grape juice) was 3 liters. The initial conditions for the fermentation process were: temperature (30.11 °C), pH (4.75), sugar concentration (158.36 g/L), ratio of carbon to nitrogen (11.9), initial concentration of yeasts (2.5 g/L), stirrer speed (630 r.p.m), air flow (20 min/L), and period fermentation was 12 h. The temperature of the fermentation medium was set at the required degree using the cooling and heating coils in the biological fermenter. The pH was also adjusted by pumping appropriate quantities of 10 % (w/v) NaOH and 10% (v/v) $H_2SO_4$ as needed into the fermentation medium.

## Biomass Concentration

The measurement of biomass was followed by estimation of cell dry weight, expressed in g/L. One mL of yeast culture was centrifuged at 5000 rpm for 5 min. The supernatant obtained was washed twice with water and dried by incubation at 105 °C until at a constant weight (Jiménez-Islas et al., 2014).

## Total Phenolic Content (TPC) in the Yeast Biomass

Total phenolic content of yeast extracts obtained from grape juice medium was estimated using the Folin-Ciocalteu reagent method (Kahkonen et al., 1999; Ainsworth and Gillespie, 2007). One ml of each sample extracts were mixed with 250 µL of 10% (v/v) Folin-Ciocalteu reagent, followed with the addition of 500 µL saturated sodium carbonate (10%, w/v aqueous solution) after 2 min of incubation at room temperature. The mixture was placed in the dark for 1 hour. Absorbance was then measured at λ750 nm. The concentration of total phenols was calculated based on a calibration curve using gallic acid. The phenol content was expressed as gallic acid equivalent (GAE), which reflects the phenol content, as the amount of gallic acid units in liter of extract (mg GAE $L^{-1}$). Three replicates were used for total phenolic content.

## Physicochemical Characteristics of the Obtained Yeast

Proximate composition: total protein, nitrogen, moisture, dry matter and ashes were determined in accordance with the AOAC procedures (1975; 1990). Total carbohydrates were determined through the colorimetric method from Dubois et al. (1958). Total lipids were extracted through the procedure of Blight et al. (1959) and determined gravimetrically. Fibers were quantified through the method from Asp et al. (1983). Density, pH and energy of dry yeast were determination with the COFALEC (2012): General characteristics of dry baker's yeast.

Test for dispersibility in water: Weigh 5 g of dry baker's yeast or 20 g of fresh baker's yeast into a 400 ml beaker and add 50 ml of distilled water at 40 °C. Leave the product undisturbed for 5 min and thereafter, stirred for 2 min. Take into a one liter graduated cylinder, 900 ml of distilled water at 40 °C in the case of dry baker's yeast and at 30 °C for fresh baker's yeast. Pour the slurry from the beaker into the water in the graduated cylinder. Wash the beaker with 50 ml of distilled water, poured it into the cylinder and left it undisturbed for 5 min. Checked for any deposits at the bottom of the cylinder. If no deposits appeared at the bottom of the cylinder, the material shall be considered to have passed the test (Rad et al., 2017).

## Dough Raising Capacity

Fresh baker's yeast (4 g) or 1.0 g of dry baker's yeast with 100 g of wheat flour were mixed. Sucrose (1.0 to 1.5 g) was added to a suitable quantity of water (about 55 ml). These were mixed by Knead well Press into a glass beaker until formed a dough. The level of the dough by means of a scale, from the bottom of the beaker was noted. It was kept covered for one h at 27 °C. At the end of this period, level was recorded again. The product shall be deemed to have satisfied the test if the level was at least 80 percent of the original for dry baker's yeast and 110 percent for fresh baker's yeast (Rad et al., 2017).

Yeast survivability was determined as CFUs per gram of dry matter. A microbial test can be used to measure the viability and survivability of yeast cells. Twenty-five grams of yeast was mixed with 175 mL of water. The viability of the suspensions was checked by plate counting. Yeast cell suspensions were counted on yeast extract glucose-chloramphenicol (YGC) agar (YGC Agar, Merck) after 5 days of incubation at 25 °C. Logarithmic dilutions were carried out in saline, and diluted suspensions were

cultured on YGC agar and incubated at 25 °C for 5 days (Rad et al., 2017).

### Statistical analysis

Experiments were performed in three replicates and all results were expressed as mean ± standard deviation (SD) using Windows software, version 7.0 (Origin Lab, 2010).

## Results and Discussion

Biomass from baker's yeast *S. cerevisiae* in grape juice medium was determined as a sole carbon source, and it was equal to 41.5±0.01 g/L at the fermentation conditions of temperature (30.11°C), pH (4.75), sugar concentration (158.36 g/L), ratio of carbon to nitrogen (11.9), initial concentration of yeasts (2.5 g/L) and a period of fermentation (12 h). Similarly, Nancib et al. (1997) obtained biomass from baker's yeast *S. cerevisiae* was 40 g/L, when date fruit byproducts were used. Khan et al. (2017) used six different strains of *S. cerevisiae* in fermentation medium containing date extract (with 60% sugars), 2 g/L ammonium sulfate and 50 mg/L biotin. Their results showed that the theoretical yields were about 42.8%. In addition, Al Obaidi et al (1986) studied two substrates (i.e., date syrup and molasses) for the propagation of baker's yeast strain *S. cerevisiae* on a pilot plant scale. The results showed that higher productivity of baker's yeast was observed when date extract was used. In fact, the optimal biomass production (6.3 g/L) was depicted at 24 h using *Saccharomyces cerevisiae* DIV13Z087C0VS on a medium containing sweet cheese as a sole carbon source (Boudjema et al., 2015). On the other hand, the production of baker's yeast from apple pomace gave a yield of 0.48 g/g (Bhushan et al., 2006). Therefore, it was concluded from these studies that the medium containing the grape juice as a sole carbon source is an excellent fermentation medium for baker's yeast production. The total phenolic content was estimated by Folin-Ciocalteu method using gallic acid as the standard reference (Figure 2).

Total phenolic content in the yeast extract were 1275.39±0.01, 1623.3±0.01, 1739.17±0.01, and 2087±0.01 mg/L at thermal treatment of grape juice 65, 70, 75, and in autoclave at 121°C, respectively. It was noted that the heat treatment of the juice succeeded in reducing the activity of the enzyme polyphenol oxidase by about 50% at temperature 65°C, 70% at temperature 70°C, 75% at temperature 75 °C, and 90% at temperature 121°C, respectively. The best heat treatment was observed in the case of autoclave, as it helped to maintain the best phenolic content in the resulting yeast. Shahat (2017) were determined for four commercial mediums after sterilized in the autoclave at 121 °C for 20 min. The phenolic contents were 1387±0.01, 1990±0.01, 1129±0.01 and 982±0.01 mg/L for the yeast-peptone-dextrose medium, corn meal, oat meal and sugar cane medium, respectively. The best result was with corn meal, and was close to the result of this current study at 121 °C for 20 min. Consequently, the yeast produced from grape juice can play an important part in the human health and nutrition due

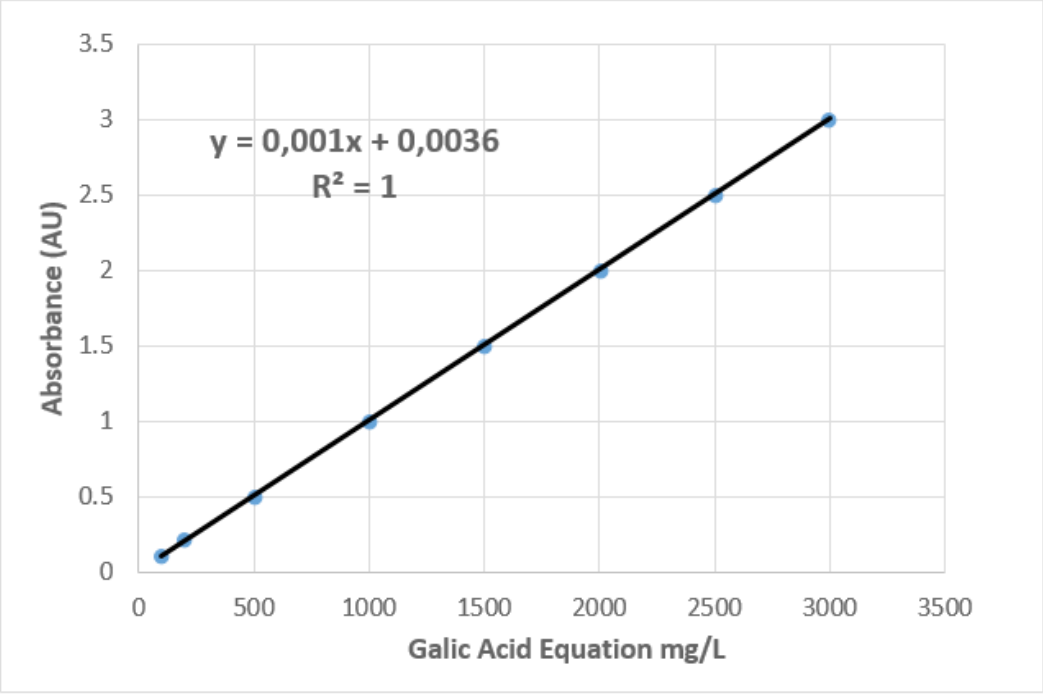


**Figure 2.** Standard calibration urve for galic acid at a wavelength of 765 nm

**Table 1.** The Physico-chemical characteristics of the obtained yeast.

| parameter | Requirement value for dry yeast | Result value for obtained yeast |
|---|---|---|
| Moisture (%) | < 8% | 5±0.013% |
| Dry matter (%) | (92-96)% | 95±0.021% |
| Total protein (%, dry matter) | 46%±10% | 42.5±0.014 % |
| Nitrogen (% / dry matter) | 7.5%±1.5% | 7 ±0.0321% |
| Total carbohydrate (%, dry matter) | 20%±9% | 18±0.014% |
| Total fat (%, dry matter) | 6%±2% | 7.5±0.0342 % |
| Fibers (%, dry matter) | 28%±5% | 23±0.015 % |
| Ashes (%, dry matter) | 6%±2% | 4±0.0212 % |
| Density (g/cc) | (0.75-0.95) | 0.89±0.012 |
| pH value | 6±2 | 6±0.01 |
| Energy value (kcal/100 g dry matter) | 373 – 310 kcal/100g dry matter | 324.975±0.01 kcal/100g dry matter |

to its biological effectiveness and its antioxidant and anticancer activities. The results of physico-chemical characteristics of yeast are shown in Table 1 with the corresponding values according to the COFALEC (2012).

Dry yeast cells was dispersed in water as required and no yeast cell was deposited. The level of dough became 80 percent of the original as required , and yeast cells have satisfied the test. Colony counting of the samples showed that the number of cfu/mg yeasts was $15\times10^{10}$ cfu per milligram in the dry matter of yeast. According to the COFALEC (2012), Coliform count was below 1000 cfu/g, thus the yeast produced from grape juice was acceptable.

## Conclusion

This study showed that the heat treatments of the juice reduced the activity of the enzyme polyphenol oxidase and autoclave heat treatment gave the best phenolic content in the yeast. It is clear that the use of grape juice can be possible as the sole source of carbon in order to produce bread yeast. It demonstrated a good yield of dry biomass from bread yeast and these could be used by food industries, and it contained high level of phenolic content. It can also make a nutritional supplement and could beneficial to human health.

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