



## Determination of Optimum Conditions for Ergosterol Production by *Saccharomyces cerevisiae*

Esraa S. Ethafa & Alaa J. A. Al-Manhel\*

Department of Food Science, College of Agriculture, University of Basrah, Iraq

\*Corresponding author email : [alaa.abd@uobasrah.edu.iq](mailto:alaa.abd@uobasrah.edu.iq), (ESE) [esraasabah909@gmail.com](mailto:esraasabah909@gmail.com)

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**Abstract:** Ergosterol is an important provitamin in the present-day of industrial biotechnology. Seven yeast strains were obtained from the market of Basrah city and subjected to screening for their ergosterol production ability using liquid-state fermentation, the cultural conditions, and nutritional requirements for optimal production of ergosterol by *Saccharomyces cerevisiae* under laboratory conditions were determined. Y.6 is the best isolate of the yeast that produced ergosterol. It was identified as *S. cerevisiae*, with a similarity rate of 97% by using the Vitek2 device, this *S. cerevisiae* (Y.6) was further subjected to optimization conditions. The results showed that the best medium for production was yeast extract peptone dextrose broth. The effect of two cheaper carbon sources, molasses and date juice were investigated. Maximum ergosterol (0.55% ) was produced using a medium containing date juice, with a replacement ratio of 75%, an incubation time of 72 hours, pH 5, at a temperature of 30 °C, and an inoculation volume of 4 ml. The percentage of ergosterol was (0.47, 0.55, 0.74, 0.66, 0.68 and 0.78 %), respectively.

**Keywords:** Ergosterol production, Optimum conditions, *Saccharomyces cerevisiae*, Screening.

### Introduction

Vitamin D<sub>2</sub> (Ergocalciferol) is a fat-soluble vitamin, which has an important role in the absorption of calcium, phosphate, the prevention of osteoporosis and rickets. This vitamin is found in some seafoods and animal products such as fish, milk, cheese, and butter, while plant foods do not contain D<sub>2</sub>, so vegetarians have symptoms of this vitamin deficiency (Martineau *et al.*, 2019; Amrein *et al.*, 2020).

Vitamin D represents a group of 11 sterols. The most important of which is vitamin D<sub>2</sub> or calciferol which consists of ergosterol (produced

in a semi-biological way). It is created by exposing animal sterols derived from cholesterol to ultraviolet rays, as well as vitamin D<sub>3</sub>. Provitamin D<sub>2</sub> is another name for ergosterol (ergosta-5,7,22-trien-3-ol). It is one of the most common microbial sterols, a white crystalline organic substance belonging to the family of steroids (Wu & Ahn, 2014; Amrein *et al.*, 2020).

Ergosterol (5,7-dieneoxysterol) is a sterol found in fungal cell membranes and it is responsible for regulating membrane permeability and elasticity (Rodrigues, 2018).

Ergosterol is naturally present in mushrooms, bread yeast, and cod liver oil (Kadakil & Tepe, 2019). Commercially, ergosterol is produced from yeast (Tan *et al.*, 2003).

It is an immunoreactive lipid as well as a naturally occurring hormone that promotes development and reproduction (Rodrigues, 2018; Cirigliano *et al.*, 2019). It is found in most microorganisms, but its distribution is irregular, as bacteria do not contain sterols or contain very low amounts (0.01-0.001%). Ergosterol is present in cell membranes of fungi, such as *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Cladosporium*, ranged from 0.1-0.8 %. However, *Saccharomyces* and *Candida* species are a major source of ergosterol (Tan *et al.*, 2003), with high productivity ranging between 0.04-4.6% of the dry weight of the cell, the main yeast species used is *Saccharomyces cerevisiae* (Shang *et al.*, 2006; Nahlik *et al.*, 2017; Blaga *et al.*, 2018). Several studies have shown that there are large numbers of people suffering from vitamin D deficiency, reaching nearly a billion people worldwide, more than 50% in Asia and the Middle East (Bokhari & Albaik, 2019). Vitamin D is of a great importance in reducing diseases such as heart disease, atherosclerosis, and treating osteoporosis, by improving the body's immunity, as well as helping to control the absorption and transfer of some minerals such as calcium and phosphorus. It also lessens the prevalence of aged individuals, particularly Alzheimer's disease, and serves as a preventive treatment for various types of cancer (Hirsch, 2011; Ledesma-Amaro *et al.*, 2013).

Vitamin D sources, which include sunlight and some foods such as cod liver oil, salmon, and tuna, require consumption of 400-2000 international units (10-50 mcg.day<sup>-1</sup>), depending

on a variety of parameters such as age, body weight, disease type (Pludowski *et al.*, 2018). Therefore, recent studies have emphasized the necessity and importance of fortifying food products, like yogurt, cheese, juice, bread, and others with this vitamin, to realize these benefits (Moulas & Vaiou, 2018; Zahedirad *et al.*, 2019; Maurya *et al.*, 2020). The aim of the present study was to determine the optimum conditions for ergosterol production by *S. cerevisiae*.

## Materials & Methods

### Collection of samples

Dry bakery yeast strains (*S. cerevisiae*) were obtained from the local markets of Basrah city, which bears the trademarks; Saf-Instant, American (Y.1), European (Y.2), Natu, Chinese (Y.3), Yuva, Indian (Y.4), Beşler, Turkish (Y.5), Domo, Lebanese (Y.6), Angel, Egyptian (Y.7). These yeasts were activated in yeast extract peptone dextrose (YEPD) medium (Himedia, India) (Blaga *et al.*, 2018; Al-sahlany *et al.*, 2020).

### Inoculum preparation

The inoculum was prepared by transferring cells of activated yeast into a 250 ml conical flask containing 50 ml of YEPD media, and incubating at 28±2 °C for 20 hours in a shaking incubator (Sartorius, Germany) at 180 rpm.min<sup>-1</sup> (Blaga *et al.*, 2018; Al-Jumaiee *et al.*, 2019).

### Screening of yeasts for ergosterol production

Yeast extract peptone dextrose was used as a production medium, 50 ml of ergosterol production medium was prepared in 250 ml conical flasks, each flask was inoculated with 5 ml of yeast cells (1 x 10<sup>6</sup> CFU.ml<sup>-1</sup>) and incubate for 30 hours at 28°C at a speed of 200 rpm (He *et al.*, 2000; He *et al.*, 2003). The cells were centrifuged (Tafesa, Germany) at 2700 rpm for

5 minutes, washed with 10 ml of distilled water, centrifuged again, and their wet weight recorded. Then 3 ml of 15% KOH alcoholic solution was added and stirred for 1 minute before being put in an 85°C water bath for 1 hour. The solution was left to cool at room temperature (approximately 30 minutes), then the sterol was extracted by adding 1 ml of distilled water and 3 ml of N-heptane, blended on vortex (Fisher Scientific, USA) for 3 minutes, and left for 30 minutes without agitation. The layer of N-heptane was transferred to another test tube, frozen for 24 hours until the analysis was done. Then 1 ml of sterol was taken and diluted five times with ethanol (Holloway, 2011).

Preliminary detection of ergosterol was carried out, by scanning at a wavelength of 230-330 nm using a UV-visible spectrophotometer (Optima, Japan). The appearance of four peaks in the curve indicated the presence of ergosterol, but if the curve was in a flat straight line, it indicated the absence of ergosterol in the sample. Then, the content of ergosterol in yeast isolates was estimated according to Holloway (2011) using the following equations:

$$\% \text{ Ergosterol} + \% 24(28) \text{ DHE} = \left[ \left( \frac{A_{281.5}}{A_{290}} \right) \times F \right] / \text{Sample weight}$$

$$\% 24(28) \text{ DHE} = \left[ \left( \frac{A_{230}}{A_{518}} \right) \times F \right] / \text{Sample weight}$$

$$\% \text{ Ergosterol} = (\% \text{ Ergosterol} + \% 24(28) \text{ DHE}) - \% 24(28) \text{ DHE}$$

Whereas

F: the dilution factor in ethanol

DHE: Dehydroergosterol

290 and 518: E values (percentages per centimeter) estimated for crystallization of ergosterol and 24(28) DHE.

### Estimation of yeast biomass

The method described by He *et al.* (2000) was followed in estimating the obtained biomass, Yeast cells were collected after the end of the fermentation period by a refrigerated centrifuge (Tafesa, Germany) for 10 min at 4000 rpm and washed once with distilled water. The cells were dried in an air oven at 80°C for 6-8 hours (until the weight was constant). Yeast biomass was estimated with a sensitive balance (Denver, Germany).

### Identification of yeast by Vitek 2 .technique

Yeast was identified according to the instructions of Biomerieux (Anonymous, 2010) by Vitek 2 (VK2C8300, U.S.A.), at Al-Bayan Al-Ahally specialist laboratories in Basra city. Before testing, a suspension of the isolate was inoculated onto YEPD. Inoculum suspension for the VITEK 2 was prepared in sterile normal saline at turbidity equal to a 2.7 McFarland standard, as estimated using a DensiChek instrument (bioMérieux). The test card was filled automatically with prepared culture suspension, sealed, and incubated by VITEK 2 instrument, optical density readings were taken automatically every 15 min. The final profile result was compared with the database, and the identification of the organism was achieved.

### Selection of culture media for ergosterol production

Three types of commercial media (sabouraud dextrose broth (HiMedia, India) (Shobayashi *et al.*, 2005), YEPD (He *et al.*, 2003), Moreover, soybean meal 8 g, glucose 8 g, KH<sub>2</sub>PO<sub>4</sub> 0.6 g, urea, 0.1 g, NaNO<sub>3</sub> 0.2 g, and MgSO<sub>4</sub> 0.1 %

(Damini *et al.*, 2013) were initially tested for ergosterol production.

### **Optimization studies for ergosterol production**

Optimization experiments were achieved by using 50 ml of YEPD in 250 ml Erlenmeyer flasks, inoculated with 5 ml of *S. cerevisiae*. The flasks were incubated in a shaker incubator (200 rpm) at 28°C. After the incubation period (30 h), Ergosterol production and biomass were measured for each experiment.

### **Optimum carbon sources for ergosterol production**

The carbon source has replaced glucose in the production media with low-cost culture media at different replacement ratios: 25, 50, 75, and 100%, including cane molasses (the total sugar content of which is 65%), and date juice (the total sugar content of which is 45%).

### **Preparation of date palm juice:**

Date flesh Zahdi variety (purchased from Basrah markets) was crushed and cut into small pieces, distilled water was added to 1 Kg of date in a ratio (1:1), and the mixture was heated in a water bath at 80°C for 3h. Then, filtered through a cloth and autoclaved at 121°C for 10 minutes (Gabsi *et al.*, 2013; Khassaf *et al.*, 2019).

### **Preparation of cane molasses**

Sugarane molasses (obtained from sugar cane Factory, Maysan) was prepared according to Rasmey *et al.* (2018), also, was characterized through total sugar contents using the Lane-Eynon technique.

### **Optimum pH for ergosterol production**

Optimal pH for production of ergosterol was estimated by preparing the production medium (50 ml) with different pH values (3, 3.5, 4, 4.5,

5, 5.5, 6, 6.5, and 7) using 1N of HCl or NaOH solutions, inoculated with 5 ml of yeast cells and incubated for 30 hours at 28°C in a shaker incubator (200 rpm). Optimum pH value was utilized in the later experiment.

### **Optimum temperature for ergosterol production**

In order to estimate the effect of incubation temperature in ergosterol production, YEPD medium was inoculated with 5 ml of cells and incubated (30 hours) in a shaker incubator (200 rpm) at different temperatures including 20, 25, 28, 30, and 35°C. The optimal temperature was subsequently utilized based on the ergosterol production.

### **Optimum incubation period for ergosterol production**

The effect of the incubation period in ergosterol production was determined, YEPD medium (50 ml) was inoculated and incubated at different periods including 24, 48, 72, 96, and 120 hours, in a shaker incubator (200 rpm). The optimal incubation period was subsequently utilized based on the ergosterol production.

### **Optimum inoculum volume for the production of ergosterol**

Yeast extract peptone dextrose medium (50 ml) was inoculated at different inoculum (1, 2, 3, 4, and 5) ml, respectively, and incubated in a shaker incubator (200 rpm). Optimum inoculum volume was subsequently utilized based on the ergosterol production.

### **The optimum agitation speed for the production of ergosterol**

To determine the effect of incubation temperature in ergosterol production, yeast extract peptone dextrose medium was incubated

at different agitation speeds, including 0, 50, 100, 150, and 200 rpm. The optimum pH, temperature, incubation period and inoculum volume were utilized based on the ergosterol production.

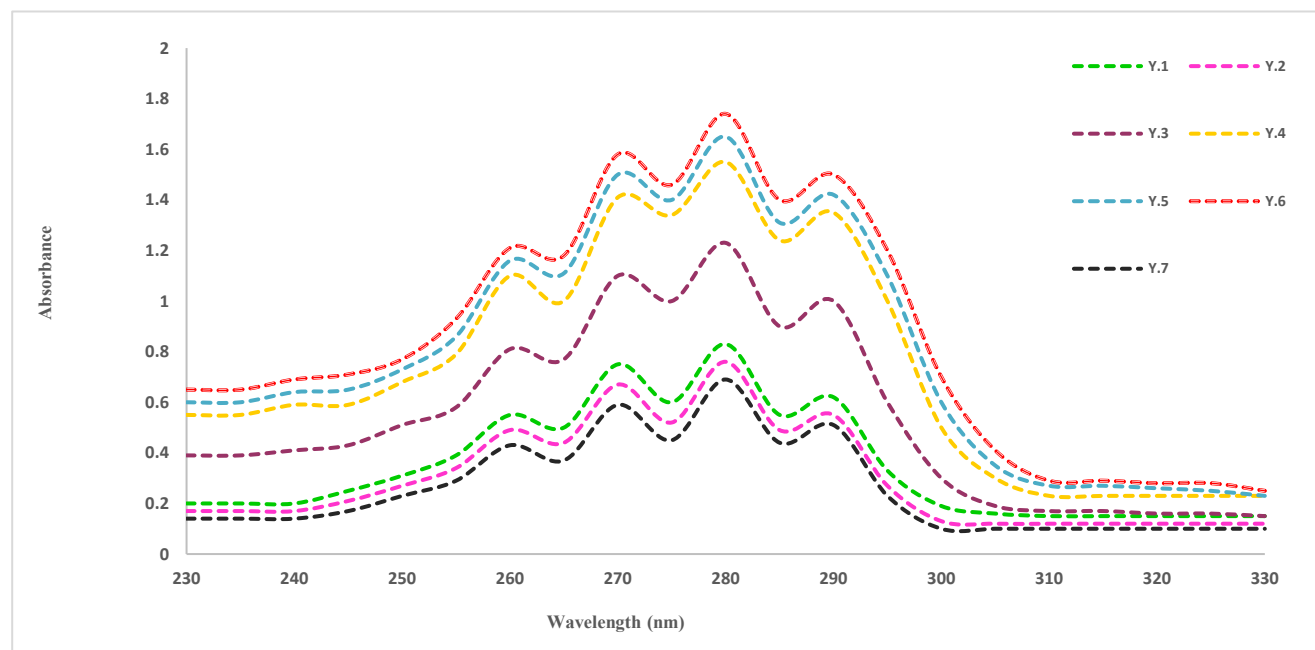
### Statistical Analysis

One-way ANOVA was used to assess statistically significant differences. The experiments cited above were analyzed by using SPSS Statistics V23.0 (Statistical Package for Social Sciences, San Antonio, TX, USA) resulting in the production of ergosterol from *S. cerevisiae*. All statistical conclusions were based on a ( $p < 0.05$ ) level of confidence

### Results & Discussion

#### Screening the isolates to choose the best isolate in the production of ergosterol:

A screening process was carried out for the yeasts, by conducting a scan at a wavelength of 230-330 nm using a UV-visible spectrophotometer for the initial detection of the presence of ergosterol. It was noticed from fig. (1) that 4 peaks appeared in the curve indicating the presence of ergosterol. Furthermore, the height of the absorbance peaks was evident, which was dependent or corresponded to ergosterol (sterol) concentration. This result is consistent with the data of many studies (Arthington-Skaggs *et al.*, 1999; Holloway, 2011).



Y.1 :Saf-Instant, Y.2: European, Y.3: Natu, Y.4: Yuva, Y.5: Beşler , Y.6: Domo, Y.7: Angel

Fig. (1): UV absorption spectra of the ergosterol from *S. cerevisiae*.

As for the amount of ergosterol produced, it was estimated according to the method of Arthington-Skaggs *et al.* (1999). The results in fig. (2) showed that the highest amount of

ergosterol produced was from the yeast type Y.6 (Domo, Lebanese), which had productivity of 0.47%, the lowest amount of ergosterol produced by yeast type Y.7 (Angel, Egyptian),

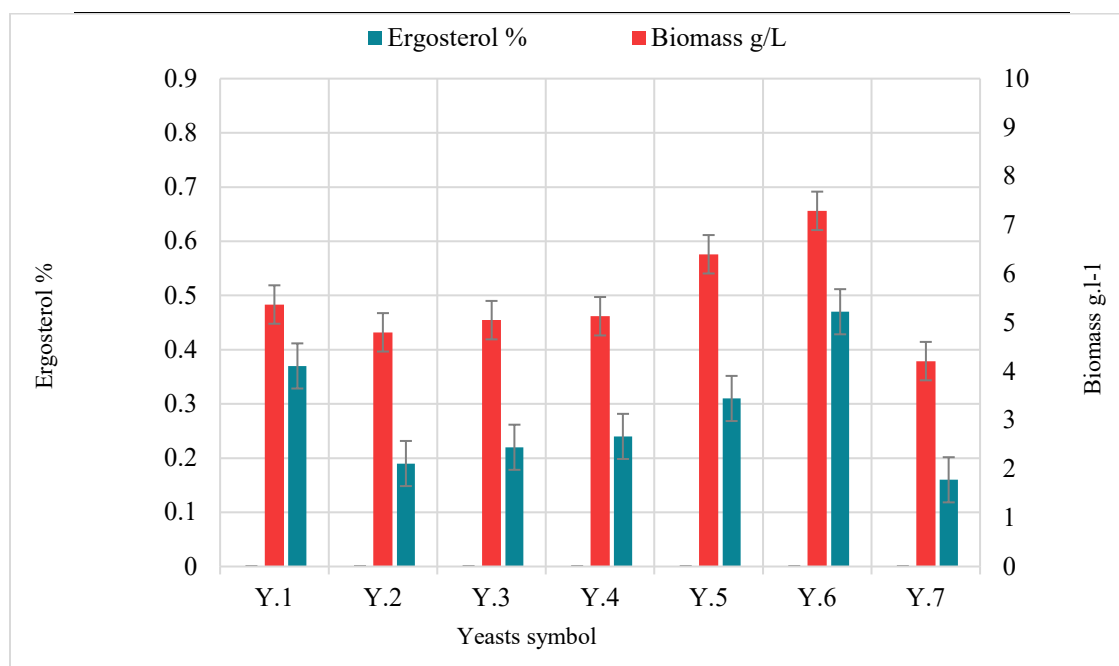
which was 0.16%. This result was less than what was mentioned by Gutarowska *et al.* (2015). The amount of ergosterol produced ranged between 1.4-6.4%. The ergosterol yield produced by fermentation of the *S. cerevisiae* is dependent on the final amount of ergosterol content in the cells and yeast biomass (Nahlik *et al.*, 2017).

The results show there was a significant difference at  $p \leq 0.05$  between the type of yeasts. The yeast with the highest production of ergosterol (Lebanese origin) was selected for diagnosis using the Vitek2 system as shown in table (1). The results which included 64 tests, showed that it belongs to the yeast *S. cerevisiae*, with a concordance rate of 97%.

Table (1): Biochemical tests using the Vitek 2 system for *S. cerevisiae*.

Test NO.	Test	Results
3	L-Lysine-Arylamidase	-
4	L-Malate assimilation	-
5	Leucine-Arylamidase	+
7	N-Acetyl-glucosamine assimilation	-
10	D-Gluconate assimilation	-
12	Glycerol assimilation	-
13	Tyrosine Arylamidase	-
14	Beta-N-Acetyl-Glucosaminidase	-
15	Arbutin assimilation	-
18	Arginine GP	-
19	Erythritol assimilation D-galactose assimilation	+
20	.Gentiobiose assimilation	-
21	D-Glucose assimilation	+
23	Lactose assimilation	-
24	Methyl-A-D-glucopyranoside assimilation	+
26	Amygdalin assimilation	-
27	Gamma- glutamyl- Transferase	-
28	D- Maltose assimilation	+
29	D- Raffinose assimilation	+
30	Pnp-N-acetyl-Bd-galactosaminidase 1	-
32	D-Mannose assimilation	+
33	D-Cellobiose assimilation	-
34	D-Melezitose assimilation	+
38	L-Sorbose assimilation	-
39	L-Rhamnose assimilation	-
40	Xylitol assimilation	-
42	D-Sorbitol assimilation	-
44	D- Melibiose assimilation	+
45	Urease	-
46	Alpha- Glucosidase	+
47	D-Turanose assimilation	+
48	D-Trehalose assimilation	+

49	Nitrate assimilation	-
51	Saccharose/Sucrose assimilation	-
52	D-Galacturonate assimilation	-
53	Esculin hydrolysis	-
54	L-Glutamate assimilation	-
55	D-Xylose assimilation	-
56	DI-Lactate assimilation	+
58	L-Arabinose assimilation	+
59	Citrate (Sodium) assimilation	-
60	Glucuronate assimilation	-
61	L-Proline assimilation	-
62	Keto-D-gluconate assimilation-2	-
63	Tyrosine Arylamidase	-
64	Acetate assimilation	-



Y.1 :Saf-Instant, Y.2: European, Y.3: Natu, Y.4: Yuva, Y.5: Beşler , Y.6: Domo, Y.7: Angel

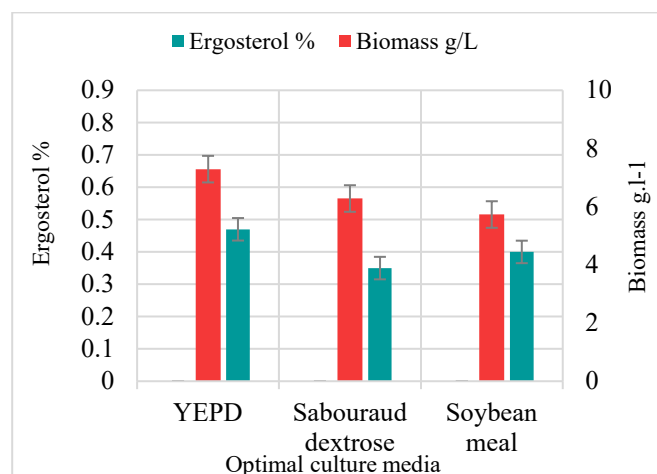
Fig. (2): Various types used for screening of yeast for ergosterol production. L.S.D ( $p < 0.05$ ) = 0.504

### The optimum media for the production of ergosterol

Different culture media were selected to study their efficiency to enhance ergosterol production. The results showed that the best media for production is YEPD, which produced the highest yield of ergosterol, was reached 0.47%, with a biomass weight of 7.29 g.L<sup>-1</sup> (Fig. 3), while the production of each of the

Sabouraud dextrose and Soybean meal media of ergosterol reached (0.35 and 0.41%), respectively. The ratio and type of carbon source and nitrogen source in the production media directly affects the amount of ergosterol and biomass production. The results were in agreement with several studies that used Yeast extract Peptone Dextrose (YEPD) media in the production of ergosterol (He *et al.*, 2000). Also,

the obtained value was in agreement with Ma *et al.* (2018), when the same components of the media were used in the production of ergosterol, while Damini *et al.* (2013) showed that the best media was soybean meal to produce the highest amount of ergosterol.



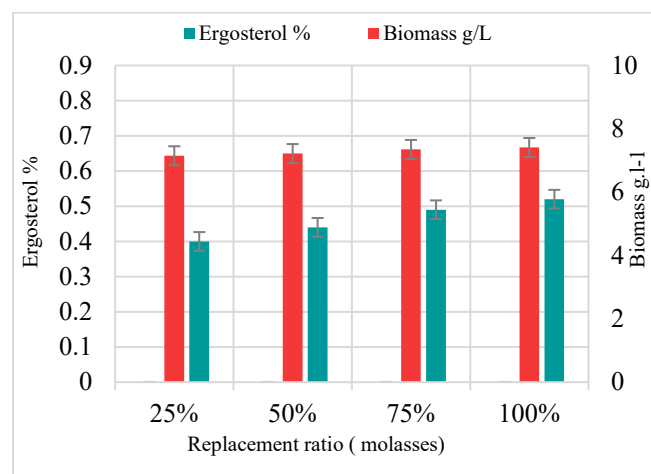
**Fig. (3): The best media for the production of ergosterol. L.S.D ( $p < 0.05$ ) = 0.216.**

The difference in productivity may be due to the amount of glucose present in the media, an increase in glucose by 16% leads to an increase in the amount of ergosterol produced. The results showed there was a significant difference ( $p < 0.05$ ) between the best medium for producing ergosterol. The studies conducted on the biomass and the content of ergosterol showed that 10% of glucose gives the highest yield of ergosterol; however, high glucose concentration may restrain cell growth (He *et al.*, 2000).

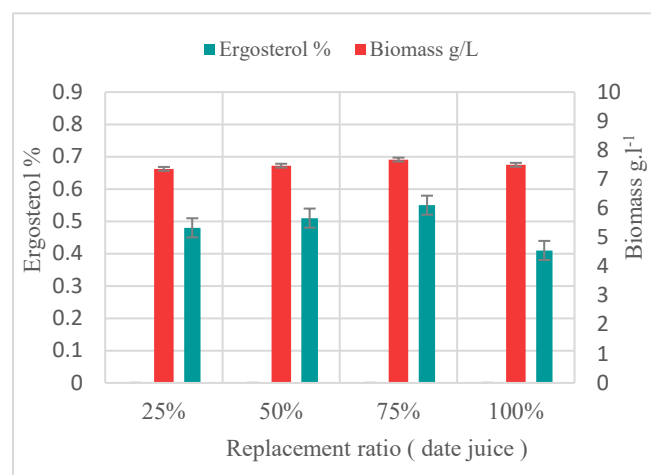
### The effect of carbon source

The results illustrated in figs. (4 and 5) show the effect of different carbon sources on the productivity of ergosterol from *S. cerevisiae*. Based on these results, ergosterol was reached 0.55% using date juice as the carbon source with a replacement ratio of 75%, and high compared with the molasses. At the 100% replacement rate, the productivity decreased, and this is may

due to the date juice containing some compounds that affect yeast growth and, consequently, ergosterol production, including acetic, formic, and propionic acids (Al-Jasass *et al.*, 2010). Therefore, date juice were chosen as the carbon media to carry out the remaining fermentation processes. The results agreed with some studies that used local alternatives for the carbon source, He *et al.* (2007) who found that the maximum ergosterol production was 52.6 mg.g<sup>-1</sup> when using a molasses medium.



**Fig. (4): The best carbon source using molasses and in different proportions of the culture media. L.S.D ( $p < 0.05$ ) = 0.288.**



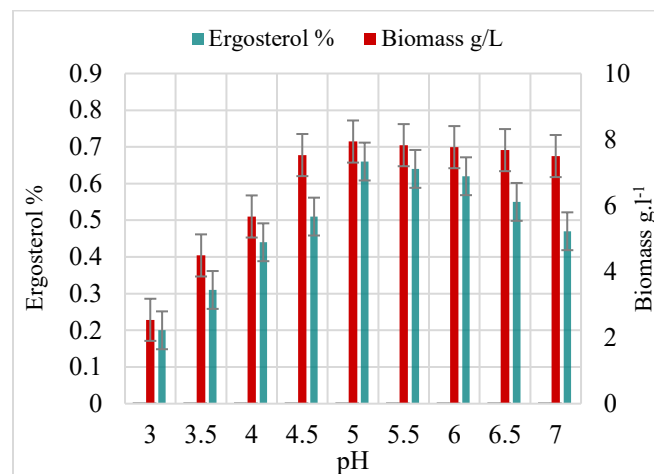
**Fig. (5): The best carbon source using date juice with different substitution ratios for the culture media. L.S.D ( $p < 0.05$ ) = 0.288.**



### Effect of pH

The effect of pH on ergosterol production was studied using different pH ranging from 3-7, as shown in fig. (6), the productivity of ergosterol increased with the increase of the initial pH value of the media until it reached its maximum at 5 with a productivity of 0.66%, then it decreased again in the neutral pH, to reach the productivity of 0.47 % at pH 7. The pH of the growing medium is important for ergosterol production because it determines the availability of the nutrients. The pH of a yeast growth medium plays a significant role by inducing enzymes secretion, morphological changes in microbes, and affecting the microbe's stability in the growth medium, generally, yeast prefers slightly acid conditions, hence, the pH influences ergosterol productivity because of its effect on the solubility of media components and its effect on the ionization process of the medium in addition to its availability for yeast growth. It is unnecessary the congruity between the optimal pH for ergosterol production and the optimal pH for microorganism growth (Grothe *et al.*, 1999). He *et al.* (2000) utilized pH 6 to produce ergosterol from bread yeast, and the results were consistent with Shang *et al.* (2006), who found that the maximum value of ergosterol was at 5.5.

The difference between our present study's results with the results of previous studies can be explained by the difference in carbon sources. He *et al.* (2000) demonstrated that the optimal pH for ergosterol production is depends on the type of carbon sources, such as glucose or sucrose.

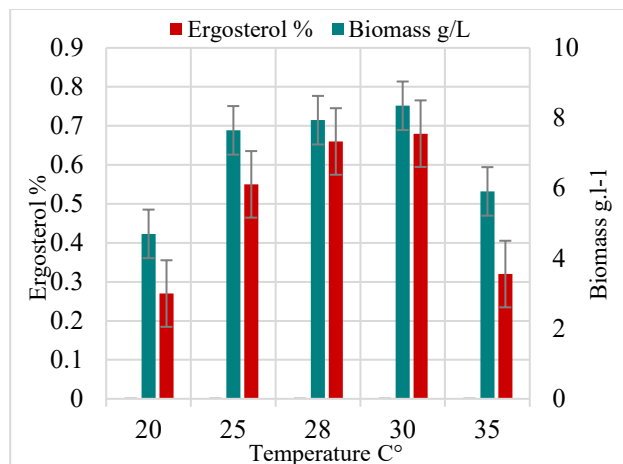


**Fig. (6): The optimum pH to produce the largest amount of ergosterol. L.S.D (  $p < 0.05$  ) = 0.648.**

### Temperature

Temperatures ranging from (20, 25, 28, 30, and 35) °C were utilized to determine the optimal production of ergosterol. According to fig. (7), the temperature of 30°C produced the best productivity of (0.68 %) with a biomass weight of 8.35 g.L<sup>-1</sup>. The temperature of 30°C may have a stronger influence on the solubility of oxygen and the kinetic energy of the molecules than other temperatures, increasing metabolic products and therefore enhancing ergosterol production. Temperature influences the activity of many microorganisms, such as growth and other critical activities. It was a key component in anabolism and catabolism activities, particularly in the fermentation industries (Anderson & Smith, 1976). The results demonstrate that there was a significant difference ( $p < 0.05$ ) in the optimal temperature to produce ergosterol. Several research has been conducted to determine the influence of incubation temperature on biomass development and ergosterol synthesis. Endo *et al.* (2009) found that the optimal temperature for ergosterol synthesis was 30°C. While Li *et al.* (2009)

studied a wide variety of temperatures ranging from 10 to 30°C, the optimal temperature for ergosterol synthesis was 25°C.



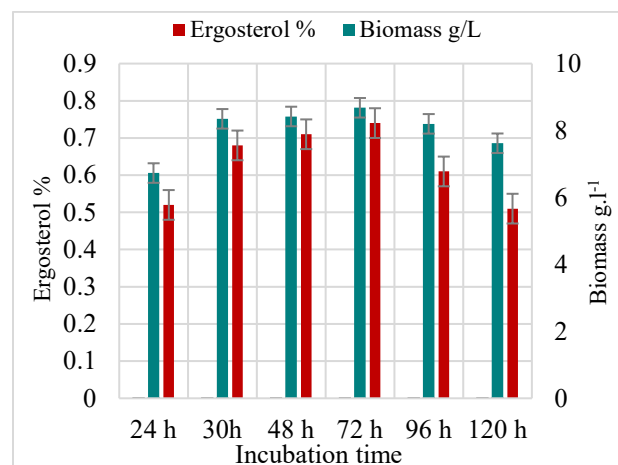
**Fig. (7): The optimum temperature to produce the largest amount of ergosterol.**  
L.S.D (  $p < 0.05$  ) = 0.364.

The results were also in agreement with the findings of Shang *et al.* (2006) who found that the highest value of ergosterol produced from *S. cerevisiae* was at 30°C, and agreed with Shobayashi *et al.* (2005) when they used a temperature of 30°C to produce ergosterol from the yeast *S. cerevisiae*.

### Incubation period

The incubation period is an important parameter for ergosterol production. Different incubation periods were used to choose the best incubation period to produce the highest amount of ergosterol, ranging between (24, 48, 72, 96, and 120 hours). The result of the optimum in fig. (8) that the highest productivity was at the 72-hours of incubation period, which amounted to 0.74 % with a biomass weight of 8.68 g.L<sup>-1</sup>. The results showed there was a significant difference ( $p < 0.05$ ) in the incubation period. The reason for the decrease in ergosterol after the 72-hours of incubation may be due to the beginning of the depletion of nutrients from the media, as well as

increasing the by-products of cells, which led to inhibition of their growth or death, and then a decrease in production. The results agreed with that stated by Veen *et al.* (2003) who found these the optimum incubation period was 3 days and the temperature was 28°C. Also, Abd-Elsalam *et al.* (2017) indicated that the ergosterol production was increased gradually over 72 hours.

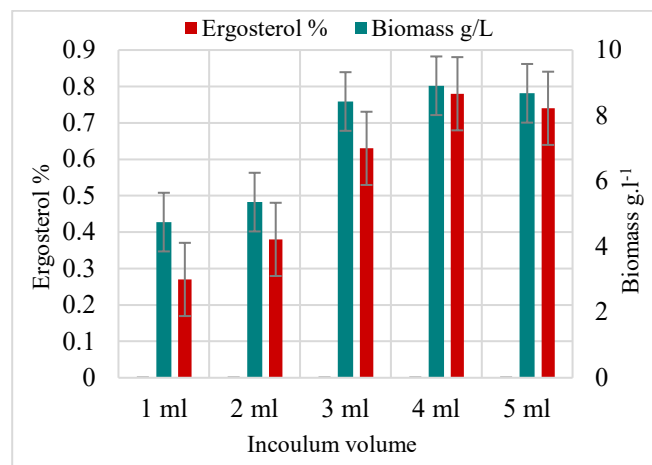


**Fig. (8): The best incubation period to produce the maximum amount of ergosterol.**  
L.S.D (  $p < 0.05$  ) = 0.432

### Inoculum volume

To determine the influence of inoculum volume on ergosterol synthesis, the production media was affected with various quantities of inoculum. The results in fig. (9) indicated that the maximum ergosterol production was at a 4 ml (8 %) inoculum volume, with a 0.78 % yield and biomass of 8.91 g.L<sup>-1</sup>. There was a significant variation in inoculum volume ( $p < 0.05$ ) according to the data. The reason for this is that the low volume of the inoculum may affected on the fermentation process, resulting in a low amount of ergosterol produced, whereas increasing the volume of the inoculum results in microorganism competition for media components and nutrients, early depletion, as well as oxygen consumption as a result of growth and cell agglomeration, and thus a decrease in ergosterol productivity (van Kuijk *et*

*al.*, 2016). The results were similar to those observed by He *et al.* (2000), who found that the optimal inoculum volume for synthesizing ergosterol from yeast was 10%. Damini *et al.* (2013) also employed a 10% inoculum to produce ergosterol from *S. cerevisiae*. While Abd-Elsalam *et al.* (2017) noted that utilizing a 3 ml inoculum volume resulted in the highest synthesis of ergosterol from *Saccharomyces boulardii*.



**Fig. (9): The optimal inoculum volume to produce the largest amount of ergosterol.**

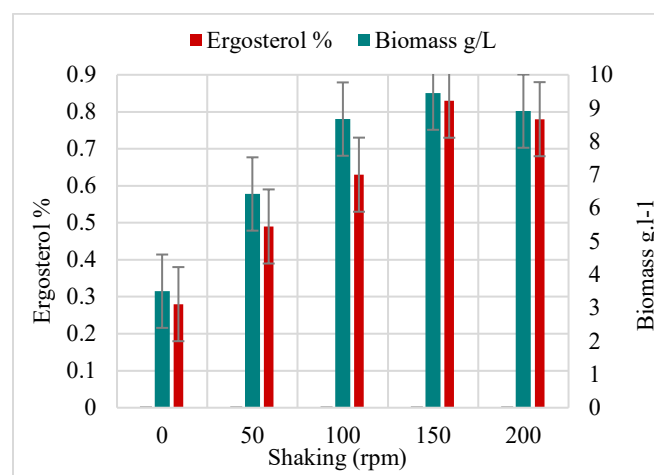
L.S.D ( $p < 0.05$ ) = 0.364

### Incubator shaking speed

The influence of the incubator's shaking speed on the production of ergosterol from the local isolate *S. cerevisiae* was shown in fig. (10). The maximum ergosterol synthesis occurred at a shaking speed of 150 rpm, with a productivity of 0.83 % and a biomass weight of 9.45 g.L<sup>-1</sup>. The rate of shaking helps to avoid cell agglomeration, increases the solubility of oxygen in the production media, and mixes its components. By influencing the metabolic pathways of ergosterol-producing yeast, oxygen has a variety of impacts on ergosterol production during aerobic fermentation (Potumarthi *et al.*, 2007). Furthermore, ergosterol production was

based on dissolved oxygen content (Blaga *et al.*, 2018).

The results revealed that shaking speed had a significant difference ( $p < 0.05$ ). Many studies have stressed the importance and need of utilizing agitation during fermentation to increase ergosterol synthesis as a result of increased oxygen supply (Shang *et al.*, 2006; He *et al.*, 2007; Damini *et al.*, 2013; Abd-Elsalam *et al.*, 2017). The acquired value corresponded with the value obtained by Endo *et al.* (2009), but it differed from the result obtained by He *et al.* (2000), who found that the production of ergosterol at the speed of shaking was 200 rpm.



**Fig. (10): Optimum shaking speed to produce the largest amount of ergosterol.**

L.S.D ( $p < 0.05$ ) = 0.364

The difference in productivity is due to the speed of shaking in yeast growth and its effectiveness in producing ergosterol, which prevents cell agglomeration and it improves the oxygen penetration in the medium and equal distribution of nutrients, whereas low aeration (static incubation) inhibits growth and stimulates ergosterol synthesis (He *et al.*, 2000).

### Conclusions

Ergosterol is the primary sterol in the cell membranes of yeast and it is also present in

membranes in the cell wall of filamentous fungi. The possibility of using the yeasts that are available in the local markets of Basrah city for the production of ergosterol was studied. The *S. cerevisiae* was used in the production of ergosterol and biomass, A series of laboratory experiments were carried out to determine the optimum conditions required in improving ergosterol production. The use of an alternative growth medium as a carbon source was studied to minimize the cost of ergosterol production. High yield of ergosterol 0.55% for date juice and the biomass was 7.68 g.L<sup>-1</sup> at 75% replacement ratio, On the other hand, maximum ergosterol production was observed under optimal cultivation conditions at 72 h of incubation period, 30°C and pH 5. Also, the productivity using the shaking incubator was superior to the incubation under static conditions. The findings in this study suggest that the yeast *S. cerevisiae* has the potential to be utilized as a local isolate for ergosterol production.

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## Conflicts of Interest:

The authors declare no conflicts of interest.

## ORCID

A.J.A. Al-Manhel: <https://orcid.org/0000-0002-6625-4287>

E. S. Ethafa: <https://orcid.org/0000-0002-1227-8224>

## Contributions of Authors

**E.S.E. A.:** Investigation and methodology, writing-original draft preparation.

**A.J.A.A.:** Writing-original draft preparation, designing and planning the experiments.

## References

- Abd-Elsalam, I. S., Adham, N. Z., Shetiaia, Y. M., & Hassan, H. (2017). Fermentation production of ergosterol using *Saccharomyces boulardii* ATCC 236. *The Egyptian Journal of Experimental Biology (Botany)*, 13(2), 361-366.  
<https://dx.doi.org/10.5455/egyjebb.20170913073156>
- Al-Jasass, F. M., Al-Eid, S. M., & Ali, S. H. H. (2010). A comparative study on date syrup (dips) as substrate for the production of baker's yeast (*Saccharomyces cerevisiae*). *Journal of Food, Agriculture & Environment*, 8(2), 314 - 316.  
<https://doi.org/10.17660/ActaHortic.2010.882.76>
- Al-Jumaiee, S. A. J., Al-Hussainy, K. S. J., & Al-Manhel, A. J. A. (2019). Effect of different level of  $\beta$ -glucan extracted from baker's yeast *Saccharomyces cerevisiae* and barley bran in the physicochemical properties of fish patties at cooling storage periods. *Basrah Journal of Agricultural Sciences*, 32(1), 79-87.  
<https://doi.org/10.37077/25200860.2019.131>
- Al-sahlany, S. T. G., Altemimi, A. B., Al-Manhel, A. J. A., Niamah, A. K., Lakhssassi, N., & Ibrahim, S. A. (2020). Purification of bioactive peptide with antimicrobial properties produced by *Saccharomyces cerevisiae*. *Foods*, 9(3), 324.  
<https://doi.org/10.3390/foods9030324>
- Amrein, K., Scherkl, M., Hoffmann, M., Neuwersch-Sommeregger, N., Kostenberger, M., Berisha, A. T., Martucci, G., Pilz, S., & Malle, O. (2020). Vitamin D deficiency 2.0: an update on the current status worldwide. *European Journal of Clinical Nutrition*, 74, 1498-1513.  
<https://doi.org/10.1038/s41430-020-0558-y>
- Anderson, J. G., & Smith, J. E. (1976). *Effects of temperature on filamentous fungi*. Pp, 191-218. In: Skinners, F.A., & Hugo, W.B. (Eds.). *Inhibition and inactivation of vegetative microbes*. Academic Press, New York, 378pp.
- Anonymous. (2010). Vitek2 Systems product Information. Durham, North Carolina 27704-0969, 2-22.
- Arthington-Skaggs, B. A. (1999). Quantitation of ergosterol content: novel method for determination of fluconazole susceptibility of *Candida albicans*.

- Journal of Clinical Microbiology*, 37(10), 3332-3337.  
<https://doi.org/10.1128/jcm.37.10.3332-3337.1999>
- Blaga, A. C., Ciobanu, C., Cascaval, D., & Galactionb, A. I. (2018). Enhancement of ergosterol production by *Saccharomyces cerevisiae* in batch and fed-batch fermentation processes using n-dodecane as oxygen-vector. *Biochemical Engineering Journal*, 131, 70-76.  
<https://doi.org/10.1016/j.bej.2017.12.010>
- Bokhari, F. F., & Albaik, M. (2019). *Vitamin D and Its Deficiency in Saudi Arabia*. IntechOpen  
<http://dx.doi.org/10.5772/intechopen.88745>
- Cirigliano, A., Macone, A., Bianchi, M. M., Oliaro-Bosso, S., Balliano, G., Negri, R., & Rinaldi T. (2019). Ergosterol reduction impairs mitochondrial DNA maintenance in *S. cerevisiae*. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, 1864, 290-303.  
<https://doi.org/10.1016/j.bbalip.2018.12.002>
- Damini, D., Jayasmita, D., Sukriti, P., Selvarajan, E., Suganthi, V., & Mohanasrinivasan, V. (2013). Fermentative production of ergosterol using *Saccharomyces cerevisiae*. *Journal of Applied Sciences Research*, 9(2), 1214-1221.
- Endo, A., Nakamura, T., & Shima, J. (2009). Involvement of ergosterol in tolerance to vanillin, a potential inhibitor of bioethanol fermentation, in *Saccharomyces cerevisiae*. *FEMS Microbiology Letters*, 299(1), 95-99.  
<https://doi.org/10.1111/j.1574-6968.2009.01733.x>
- Gabsi, K., Trigui, M., Barrington, S., Helal, A. N., & Taherian, A. R. (2013). Evaluation of rheological properties of date syrup. *Journal of Food Engineering*, 117, 165-172.  
<http://dx.doi.org/10.1016/j.jfoodeng.2013.02.017>
- Grothe, E.; Moo-Young, M., & Chisti, Y. (1999). Fermentation optimization for the production of poly ( $\beta$ -hydroxybutyric acid) microbial thermoplastic. *Enzyme and Microbial Technology*, 25(1-2), 132-141.  
[https://doi.org/10.1016/S0141-0229\(99\)00023-X](https://doi.org/10.1016/S0141-0229(99)00023-X)
- Gutarowska, B., Skóra, J., & Pielech-Przybylska, K. (2015). Evaluation of ergosterol content in the air of various environments. *Aerobiologia*, 31(1), 33-44.  
<https://doi.org/10.1007/s10453-014-9344-4>
- He, X., Huai, W., Tie, C., Liu, Y., & Zhang, B. (2000). Breeding of high ergosterol-producing yeast strains. *Journal of Industrial Microbiology and Biotechnology*, 25(1), 39-44.  
<https://doi.org/10.1038/sj.jim.7000004>
- He, X. P., Zhang, B. R., & Tan, H. R. (2003). Overexpression of a sterol C-24(28) reductase increases ergosterol production in *Saccharomyces cerevisiae*. *Biotechnology Letters*, 25, 773-778.  
<https://doi.org/10.1023/a:1023572403185>
- He, X., Guo, X., Liu, N., & Zhang, B. (2007). Ergosterol production from molasses by genetically modified *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, 75, 55-60.  
<https://doi.org/10.1007/s00253-006-0807-6>
- Hirsch, A. L. (2011). *Industrial Aspects of Vitamin D*. 73-93. In: Feldman, D., Pike, W., & Adams, J. S. (Eds.). *Vitamin D*. 3rd Edition, Academic Press, 2144pp.  
<https://doi.org/10.1016/B978-0-12-381978-9.10006-X>
- Holloway, M. J. (2011). *Biochemical and pharmacological characterization of cytochrome b5 reductase as a potential novel therapeutic target in Candida albicans*. Ph. D. Thesis, College of Medicine, University of South Florida, 129pp.
- Kadakal, C., & Tepe, T. K. (2019). Is Ergosterol a new microbiological quality parameter in foods or not? *Food Reviews International*, 35(2), 155-156.  
<https://doi.org/10.1080/87559129.2018.1482495>
- Khassaf, W., H., Niamah, A.K., & Al-Manhel, A. J. (2019). Study of the optimal conditions of levan production from a local isolate of *Bacillus subtilis* subsp. *subtilis* w36. *Basrah Journal of Agricultural Sciences*, 32(2), 213-222.  
<https://doi.org/10.37077/25200860.2019.211>
- Ledesma-Amaro, R., Jimenez, M. A., & Revuelta, J. L. (2013). *Microbial production of vitamins*. In: *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*. 246, Pp, 571-594. In McNeil, B., Archer, D., Giavasis, L., & Harvey, L. (Eds.). *Woodhead Publishing Series in Food Science, Technology and Nutrition*, Oxford, 610pp.
- Li, Y., Wadsö, L., & Larsson, L. (2009). Impact of temperature on growth and metabolic efficiency of *Penicillium roqueforti*—correlations between produced heat, ergosterol content and biomass. *Journal of*

- applied microbiology*, 106(5), 1494-1501.  
<https://doi.org/10.1111/j.1365-2672.2008.04110.x>
- Ma, B. X., Ke, X., Tang, X. L., Zheng, R. C., & Zheng, Y. G. (2018). Rate-limiting steps in the *Saccharomyces cerevisiae* ergosterol pathway: towards improved ergosta-5, 7-dien-3 $\beta$ -ol accumulation by metabolic engineering. *World Journal of Microbiology and Biotechnology*, 34(4), 1-12.  
<https://doi.org/10.1007/s11274-018-2440-9>
- Martineau, A. R., Thummel, K. E., Wang, Z., Jolliffe, D. A., Boucher, B. J., Griffin, S. J., & Hitman, G. A. (2019). Differential effects of oral boluses of vitamin D2 vs vitamin D3 on vitamin D metabolism: A randomized controlled trial. *The Journal of Clinical Endocrinology & Metabolism*, 104(12), 5831-5839.  
<https://doi.org/10.1210/jc.2019-00207>
- Maurya, V. K., Bashir, K., & Aggarwal, M. (2020). Vitamin D microencapsulation and fortification: Trends and technologies. *Journal of Steroid Biochemistry and Molecular Biology*, 196, 105489.  
<https://doi.org/10.1016/j.jsbmb.2019.105489>
- Moulas, A., & Vaiou, M. (2018). Vitamin D fortification of foods and prospective health outcomes. *Journal of Biotechnology*, 285, 91-101.  
<https://doi.org/10.1016/j.jbiotec.2018.08.010>
- Nahlik, J., Hrnčířík, P., Mares, J., Rychtera, M., & Kent, C. A. (2017). Towards the design of an optimal strategy for the production of ergosterol from *Saccharomyces cerevisiae* yeasts. *Biotechnology Progress*, 33(3), 838-848.  
<https://doi.org/10.1002/btpr.2436>
- Pludowski, P., Holick, M. F., Grant, W. B., Konstantynowicz, J., Mascarenhas, M. R., Haq, A., Povoroznyuk, V., Balatska, N., Barbosa, A. P., Karonova, T., Rudenka, E., Misiorowski, W., Zakharova, I., Rudenka, A., Lukaszewicz, J., Marcinowska-Suchowierska, E., Laszcz, N., Abramowicz, P., Bhattoa, H. P., & Wimalawansa, S. J. (2018). Vitamin D supplementation guidelines. *The Journal of Steroid Biochemistry and Molecular Biology*, 175, 125-135.  
<https://doi.org/10.1016/j.jsbmb.2017.01.021>
- Potumarthi, R., Ch., S., & Jetty, A. (2007). Alkaline protease production by submerged fermentation in stirred tank reactor using *Bacillus licheniformis* NCIM-2042: effect of aeration and agitation regimes. *Biochemical Engineering Journal*, 34(2), 185-192.  
<https://doi.org/10.1016/j.bej.2006.12.003>
- Rasmeey, A. H. M., Hassan, H. H., Abdulwahid, O. A., & Aboseidah, A. A. (2018). Enhancing bioethanol production from sugarcane molasses by *Saccharomyces cerevisiae* Y17. *Egyptian Journal of Botany*, 58(3), 547-561.  
<https://dx.doi.org/10.21608/ejbo.2018.1820.1126>
- Rodrigues, M. L. (2018). The multifunctional fungal ergosterol. *MBio*, 9(5), e01755-18.  
<https://doi.org/10.1128/mbio.01755-18>
- Shang, F., Wen, S., Wang, X., & Tan, T. (2006). Effect of nitrogen limitation on the ergosterol production by fed-batch culture of *Saccharomyces cerevisiae*. *Journal of Biotechnology*, 122(3), 285-292.  
<http://dx.doi.org/10.1016/j.jbiotec.2005.11.020>
- Shobayashi, M., Mitsueda, S. I., Ago, M., & Fujii, T. (2005). Effects of culture condition on ergosterol biosynthesis by *Saccharomyces cerevisiae*. *Bioscience, Biotechnology, and Biochemistry*, 69(12), 2381-2388.  
<https://doi.org/10.1271/bbb.69.2381>
- Tan, T., Zhang, M., & Gao, H. (2003). Ergosterol production by fed-batch fermentation of *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology*, 33(4), 366-370.  
[https://doi.org/10.1016/S0141-0229\(03\)00132-7](https://doi.org/10.1016/S0141-0229(03)00132-7)
- van Kuijk, S. J. A., Sonnenberg, A. S. M., Baars, J. J. P., Hendriks, W. H., & Cone, J. W. (2016). The effect of particle size and amount of inoculum on fungal treatment of wheat straw and wood chips. *Journal of Animal Science and Biotechnology*, 7, 32-39.  
<https://doi.org/10.1186/s40104-016-0098-4>
- Veen, M., Stahl, U., & Lang, C. (2003). Combined overexpression of genes of the ergosterol biosynthetic pathway leads to accumulation of sterols in *Saccharomyces cerevisiae*. *FEMS Yeast Research*, 4(1), 87-95.  
[https://doi.org/10.1016/s1567-1356\(03\)00126-0](https://doi.org/10.1016/s1567-1356(03)00126-0)
- Wu, W. J., & Ahn, B. Y. (2014). Statistical optimization of ultraviolet irradiate conditions for vitamin D2 synthesis in oyster mushrooms (*Pleurotus ostreatus*) using response surface methodology. *PLoS One*, 9(4),

e95359.

<https://doi.org/10.1371/journal.pone.0095359>

Zahedirad, M., Asadzadeh, S., Nikooyeh, B., Neyestani, T. R., Khorshidian, N., Yousefi, M., & Mortazavian, A. M. (2019). Fortification aspects of vitamin D in

dairy products: A review study. *International Dairy Journal*, 94, 53-64.

<https://doi.org/10.1016/j.idairyj.2019.01.013>

## تحديد الظروف المثلى لإنتاج الأركوستيروول بواسطة *Saccharomyces cerevisiae*

اسراء صباح عذافة وعلاء جبار عبد المنهل

قسم علوم الاغذية، كلية الزراعة، جامعة البصرة، البصرة، العراق

**المستخلص:** يعد الأركوستيروول الفيتامين المهم في مجال التكنولوجيا الحيوية الصناعية في الوقت الحاضر، يتم تحديد الظروف المزرعية والمتطلبات التغذوية للإنتاج الأمثل للأركوستيروول بواسطة *Saccharomyces cerevisiae* تحت ظروف المختبر. تم إجراء عملية غربلة لاختيار أفضل عزلة من الخمائر، وكانت العزلة التي تحمل الرمز Y.6 (لبنانية المنشأ، علامة Domo) أفضل عزلة أنتجت إركوستيروول. تم تشخيصها باستخدام جهاز Vitek وظهرت نسبة تطابق 97% مع *S. cerevisiae*، تمت دراسة الظروف المثلى للإنتاج بطريقة التخمر السائل. أظهرت النتائج أن أفضل وسط إنتاج هو YEPD، ووجد أن عصير التمر هو أفضل مصدر كربوني محلي بديل، مع نسبة استبدال 75% من الوسط القياسي، ووقت حضانة 72 ساعة، ودرجة الحموضة = 5، عند درجة حرارة 30 درجة مئوية وحجم اللقاح 4 مل. كانت نسبة الأركوستيروول (0.47، 0.55، 0.74، 0.66، 0.68 و 0.78) على التوالي. يحتاج تحسين الأركوستيروول المنتج من خميرة *S. cerevisiae* إلى المزيد من العمل في كل من الصناعات الغذائية والصيدلانية.

الكلمات المفتاحية: إنتاج الأركوستيروول، ظروف مثلى، *Saccharomyces cerevisiae*، غربلة.