Optimization of Phenolic Compound Production By Local 
*Aspergillus Niger* B1b Isolate

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Abstract. The objective of this study was to optimize the factors for the production of Phenolic Compound from *Aspergillus Niger* B1b was obtained from laboratories of the College of Agricultural Engineering Sciences, was isolated from soil and genetic diagnosis. Phenolic compounds were produced from locally isolate under different conditions and extract from filtrate of it by ethyl acetate and identified using high performance liquid chromatography instrument (HPLC). Experiments proved that the optimum conditions for phenolic compounds production of *Aspergillus Niger* isolate were, fructose and yeast extract as sources of carbon and nitrogen with concentrations 51 µg/ml, 72 µg/ml respectively. Optimum Temperatures 30°C, aeration was 200 rpm, inoculum size 10 spore/ml (80, 102.4, 104.3) µg/ml, respectively. while incubation period was 9 days and the pH with concentrations (125, 131.1) µg/ml, respectively.

1. Introduction

In food processing, storage, and transportation, oxidation of lipid causes the nutritive value and quality of food to deteriorate. It has been reported that certain diseases are related to toxic substances produced by lipid oxidation [1]. Antioxidants are used to protect food quality by preventing oxidation of its lipids. The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene, may be a safety concern [2]. A natural antioxidant, is an effective antioxidant for lipid-containing foods but has limited use [3]. Therefore, both the development and utilization of more effective antioxidants of natural origin are desired. These products vary from whole cells to extracellular secondary metabolites of various microorganisms [4]. Fungi are remarkable organisms that readily produce a wide range of natural products as secondary metabolites and some are considered to be beneficial due to their medical, industrial and agricultural importance. These secondary metabolites are antibiotics, phenolic compounds, steroids, terpenes and polyketides which may possess different bioactivities including antioxidant activity [5]. Antioxidants have the capability to neutralize reactive oxygen species (ROS), which include superoxide anion (O²⁻), alkoxy radical (RO), nitric oxide (NO), hydrogen peroxide (H₂O₂), peroxyl radical (ROO⁻) and hypochloride.
(HOCl) [6]. Such ROS are responsible for many degenerative or pathological processes and antioxidants thus can protect the human body from such situations like aging, cancer, coronary heart disease, Alzheimer’s disease, neurodegenerative disorders, atherosclerosis, cataracts, and inflammation [7]. Earlier, synthetic antioxidants were commonly used for this purpose but because of their carcinogenic nature, there is a need to find out natural and more effective economic antioxidants [8].

2. Materials and Methods

Aspergillus niger B1b was isolated from soil and was molecularly identified in the laboratories of the Faculty of Agricultural Engineering Sciences, University of Baghdad, Department of Food Science.[10]

2.1. Isolating and purifying: were determined as mentioned by [10]

2.2. Screening of isolates for antioxidant production as mentioned by [10]

2.3. Determination of total phenolic content [10]:

2.4. Czapek Doxs Broth: The media was prepared according to [9]: 3 gm sucrose, 0.2gm NaNO₃, 0.1gm K₂HPO₄ , 0.05g MgSO₄, and 0.05gmKCl were dissolved in 100 ml distilled water. The media was autoclaved and used for screening the antioxidants producing fungi. The same media was used for screening the antioxidants producing molds.

2.5. Medium optimization using one factor at a time classical method:

Studied nutritional and environmental conditions to determine the best ones in the production of antioxidants through the determination of the amount of total phenolic compounds the Total phenolic compounds produced as shown below:

2.5.1. Optimum carbonate source:

To find out the best carbon source, sucrose in the Czapek dox’s medium was replaced with same concentration of one of the sugars (glucose, maltose, lactose, Fructose, Mannitol, sucrose) separately, 50ml Volume of 250ml ph amended to 5.

2.5.2. Optimum nitrogen sources:

to find out the best nitrogen source, NaNO₃ in Czapek dox’s medium was substituted with one or the other inorganic nitrogen source (Ammonium sulphate, Ammonium nitrate) or nitrogen rich organic supplement (yeast extract, peptone, Beef extract).

2.5.3. Optimal incubation temperature:

Flasks in the incubator were incubated at different temperatures(20,25,30,35-40-45) taking into consideration optimal conditions obtained from previous experiments.

2.5.4. Optimal aeration:

The flasks in the shaking incubator were incubated at different speeds, including 50,100, 150, 200, and 250 rpm each, taking into consideration the optimum conditions obtained from previous experiments.

2.5.5. The optimal inoculum volume:

The volume of the inoculum was studied using different sizes of 10³, 10⁴, 10⁵, 10⁶, 10⁷, 10⁸ and 10⁹ spore/ml each, taking into consideration the optimum conditions obtained in previous experiments.
2.5.6. Optimal incubation period
Flasks in the incubator were incubated at different periods (3, 6, 9, 12, 15, 18, 21) days taking into consideration optimal conditions obtained from previous experiments.

2.5.7. Optimal pH
The production medium was prepared with different hydrogen numbers (2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, 11.5 and 12.5) to modify the acidic function with a consideration of the optimal conditions obtained in previous experiments.

2.5.8. Statistical Analysis
The statistical analysis system-SAS (2012) was used in data analysis in order to study the effect of different treatments on the studied characteristics according to a complete random design (CRD), the significant differences between means where compared through the use of least significant difference test (LSD) with a probability level of (0.05).

3. Results and Discussion

3.1. Optimal conditions for the Phenolic Compound production
Studied some of the factors affecting the production of antioxidant compounds from the fungi *Aspergillus niger* B1b which included the environmental factors and medium constitution affecting, in order to determine the best conditions in which access to higher production of these methods.

3.2. The optimal carbon sources:
As shown in Fig. 1 The impact of different carbon sources, fungi production highest of Phenolic Compound when the use of fructose as a source of carbon with concentrations 51 µg/ml, distract (maltose, sucrose, glucose, galactose, Mannitol ) Separately, with concentrations(46, 45, 43, 27, 13) µg/ml. The results of the statistical analysis showed that were significant differences between fructose and other carbon sources, at a significant level of 0.05. Fructose proved to be the most promising to produce bioactive compounds(Phenolic Compound) . That explains why the difference in the concentration of phenolic compounds produced using different carbon sources that *Aspergillus* sp. It has the capacity to take advantage of some of the sources of carbon in growth and biomass, but do not have the capacity to be used in the production of secondary metabolism [11].

![Figure 1. The effect of different sources of carbon in the phenolic compounds production from *Aspergillus niger* B1b](image-url)
3.3. The optimal nitrogen sources:
Nitrogen is an element of the core elements of the role in building the cell growth and biomass, since it could not obtain the appropriate critical mass, and thus the production of article required without nitrogen Source in growth media. Nitrogen enters in the amino acid composition of nitrogenous bases and some vitamin [12].

As shown in Fig.2 The impact of different nitrogen sources, the highest production of Phenolic Compound when the use of yeast extract as a source of carbon with concentrations 72 µg/ml, distract (peptone, beef extract, ammonium sulphate, ammonium nitrate, sodium nitrate), with concentrations (67, 65, 44, 37, 30) µg/ml Separately. this may be due to the fact that yeast extract is not only a source of nitrogen, but offers a microorganisms requirements of micro elements including vitamins that fungi need it for growth and production [13]. The results of the statistical analysis showed that there were significant differences between yeast extract and other nitrogen sources, at a significant level of 0.05.

![Figure 2. The effect of different sources of nitrogen in the phenolic compounds production from Aspergillus niger B1b](image)

[14] refer to The yeast extract gave a strong influence in the production of antioxidants of the fungi Aspergillus candidus, culture media, have a major effect on the microbial growth and production. The parameters are concerned there is usually a problem between getting maximal growth and maximal bioactivity because conditions that allow good cell growth could be unfavorable to metabolite production. This shows that the growth medium can also have impact on secondary metabolites [11].

3.4. Temperature
The temperature of the incubation is an important factor affecting both the growth and production of phenolic compounds the fungi strains, As shown in Fig.3 effect of different temperature of incubation (20, 25, 30, 35, 40, 45) C°. The capability of the production of total phenolic compounds by Aspergillus niger B1b, For 5 days incubation. It was observed an increase in the concentration of total phenolic compounds produced with high temperature reached the highest value 30 C° The concentration of total phenolic compounds 80 µg/ml, After they were 55 and 57 µg/ml in 20 and 25 C° temperature respectively. With the note marked decline by focusing the temperature (35, 40, 45) C° having reached 75, 67 and 15 (µg/ml) respectively. The results of the statistical analysis showed that were significant differences
between 30°C and other incubation temperatures, at a significant level of 0.05. The concentration of total phenolic compounds differed depending on the temperatures incubation which affected the viability of microorganism on growth was the temperature of the incubation of the 30°C is the best in the mold growth that has resulted in increasing the production of phenolic compounds in the filtrate while the concentration of phenolic compounds at temperatures (20, 25, 30, 35, 40 and 45) °C as a result of the decrease of fungi growth and this is consistent with the [15] the extracted methanol for fermented soybean by fungi *Aspergillus awamori* gave the highest effective antioxidant at a temperature of 30°C was accompanied by an increase in total phenolic compounds and fungi growth, while the less effective at temperature 25 and 35°C.

The best temperature of incubation fungi growth depends on the type of genetic characteristics of strain [16] therefore the incubation temperature 30°C is best for growth and production of the highest concentration of phenolic compounds and then the highest of the effectiveness of the antioxidant compounds the mediation of the fungi *Aspergillus niger B1b*.

![Figure 3. The effect of the different temperature in the total phenolic compounds production from *Aspergillus niger B1b*](image)

**3.5. The effect of aeration rates**

As shown in Fig. 4 The impact of aeration rates for production of total phenolic compounds the from *Aspergillus niger B1b*

![Figure 4. The effect of aeration rates in the total phenolic Compounds production from *Aspergillus niger B1b*](image)
They found that the best production of phenolic compounds was obtained when the aeration rate of 200/minute with a concentrated 102.4 \( \mu \text{g/ml} \). The results of the statistical analysis showed that were significant differences between ventilation rate 200rpm/ min and other ventilation rates, at a significant level of 0.05. Note from the figure increased the concentration of total Phenolic compounds with high rate of air producing up to 200 rpm, the more speed increase dissolved oxygen in the center, and thus grow the production of phenolic compounds, as the speed of the rotation of the utmost importance in mixing the components of the Center and provide nutrients and oxygen to the cells of the developing countries to be able to [16].

3.6. The inoculation size:
The size of the inoculation is one of the important factors in any biological production process, in order to ensure the highest production as well as to shorten the time required to reach the stage of final production. The inoculation is the preparation of the proper size as a basis for the production of phenolic compounds the mediation of the brewing process, as production growth depend on the inoculation in terms of quantity and quality differ. inoculation must develop among the rich media for the purpose of ensuring access to a physiological condition is good, of course, the inoculation to attend the several stages and then transferred to the brewers productivity in sterile conditions [17]. As shown in Fig.5 the impact of the use of inoculation in different sizes, the production of total phenolic compounds from \textit{Aspergillus niger} B1b It was to get the highest production of phenolic compounds when the volume of inoculation \( 10^7 \) Spore/ml with concentrations \( (104.3) \mu \text{g/ml} \).

![Figure 5. The effect of inoculation size in the total phenolic Compounds production from \textit{Aspergillus niger} B1b](image)

Note during the figure increased the concentration of phenolic compounds produced with an increase in the size of the vaccine to the size of the optimal inoculation \( 10^7 \) spore/ml, noting from figure low concentration of phenolic compounds produced when sizes inoculation \( (10^3, 10^4, 10^5 \text{ and } 10^6) \) spore/ml. The results of the statistical analysis showed that were significant differences between size of inoculum \( 10^7 \) spore/ml and other sizes of inoculum , at a significant level of 0.05. The reason for the lack of proportionality between the size of the inoculation to the size of the media fermentation and thus the inoculation will need a long time to configure the required numbers for the production of biomass of cells and thus the production of secondary metabolism of phenolic compounds, the more time needed to increase the number of inoculation will lead to
the consumption of most of the materials necessary for growth Without access to the preparation of the required production cells, and found it to stay away from this size of the inoculation up happens in a severe drop in the concentration of phenolic compounds produced, partly because of the decline in the incidence of severe competition between cells for oxygen and nutrients and factors of growth in the fermentation media and consumption of most of the material in the media, Which may lead to rapid change in the conditions of the fermentation media and become inappropriate for the production process. The size of the productive process determines the amount of the inoculation to be added, as determined by the quantity of inoculation added to the type of organism, the user, the concentration of phenolic compounds produced varies depending on the size of the inoculation (17).

3.7. incubation period:
As shown in Fig.6 effect incubation period in the production of phenolic compounds the from Aspergillus niger B1b. these included the duration (3, 6, 9, 12, 15, 18 and 21) days and in temperature 30°C, they found that the best production of total phenolic compounds was obtained in the 9 day with concentrations 125µg/ml. The results of the statistical analysis showed that were significant differences between the period of incubation 9 days and other time periods, at a significant level of 0.05. Note from the figure that there is a difference in the concentration of antioxidant compounds the isolation for the duration of the incubation the concentrations started increasing with the duration of incubation and culminated in the 9 day, then dropped the concentrations with the progress of the incubation, the increase in the period of the incubation during the fermentation process, resulting in a decrease in the concentration of Phenolic Compounds and then decline in the effectiveness of the antioxidant compounds that is due to analyze them or may be due to the composition of some inhibitory antioxidant compounds, they lead to a decrease in the concentration of phenolic compounds as a result of the lack of fungi growth, which affects the overall content of phenolic compounds in the extracted This is consistent with what the [18] He indicated that the concentration of the total phenolic compounds was low in the first days of fermentation he reasoned that, natural phenolic compounds are linked to the proportion of a few generally free phenols (dissolved), the drop in the concentration of Phenolic compounds with the progress of the incubation causes Polymerization of phenolic compounds, as well as the stress-producing molds.

![Figure 6. The effect incubation period in the production of Phenolic Compounds from Aspergillus niger B1b](image)
3.8. pH
As shown in Fig. 7 the impact of pH on growth and production of phenolic compounds, they found that the best No. pH is 8.5. The concentration of phenolic compounds produced 131.1µg/ml. The figure shows the increase in the concentration of Phenolic compounds with high pH to optimum pH 8.5 these values also witnessed a decrease whenever away pH the value up or down, and the results of the statistical analysis showed that were significant differences between the pH 8 and other pH, at a significant level of 0.05. The pH of the fermentation mediums is one of the important factors affecting the work of the enzymatic systems of the various microorganism including fungi, and affect the readiness of materials needed by the mold growth also affect the situation ionic article basis, [11] find The best pH to produce phenolic compounds from the *Aspergillus* spp. was 7, the concentration of total phenolic compounds extracted 16.74mg/ml.

![Figure 7. The effect of Ph the production of phenolic compounds from *Aspergillus niger* B1b](image)

4. Conclusions
The highest phenolic compound amount (45.3µg/ml) was obtained from *Aspergillus* B1b. Increased productivity of *Aspergillus niger* isolation from total phenolic compounds when using fructose as a source of carbon, and yeast extract as sources of nitrogen. Optimum Temperatures, ph and incubation period were 30°C, 8.5 and 9 days, while the aeration was 200 rpm and inoculum size 7×10 spore/ml.

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