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# β-Carotene—properties and production method from The Yeasts

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#### Abstract

Carotenoids are natural stains produced by different groups of living organisms, such as plants, animals, and micro-organisms. They take many colors, such as yellow, orange, red, and purple. Microorganisms have received a lot of attention from researchers to obtain carotenoids from their natural sources, as they are characterized by the easy of extraction and purification, low costs, and production is not related to the seasonal changes experienced by the process of extracting carotene from plants, in addition to that carotene The output has no negative effects. Carotenoids are of great importance and have wide applications in many areas because of their functions and properties that made them interesting, as they play a role in protecting cells from the harmful effects of free radicals and reducing the risk of injury Some types of cancerous diseases, cardiovascular diseases, and the prevention of Alzhemeir's disease due to its antioxidant properties and carotenoids are also included in the food industry as they are used as food colorants that contribute to attracting consumers to goods and goods and are added as food supplements to animal feed and recently entered the pharmaceutical industries.

## Introduction

Carotenoids are a group of natural dyes produced by a group a wide variety of plants, algae, and microorganisms such as bacteria, molds, and yeasts (Perez-Fons *et al.*, 2011). They are largely unsaturated organic compounds. They share a general molecular formula (C40H56).

They belong chemically to polyenes and are considered types of turbines. They follow the Tetraterpenoids section because their chemical composition is composed of Isoprenoid units. The colors of carotenoids yellow, red, and pink are returned to easily oxidized and have multiple double bonds (Rodriguez-Amaya, 2001). In 1960 AD the number of known natural carotenoids was about 80 compounds and reached 500 compounds in 1980 AD and recently its number has become more than 700 species, among the most important types of carotenoids produced by yeasts:

# **1.** β-carotene

It is used in food coloring and as a complementary food supplement as a precursor of Vitamin A at a concentration of between 2-50 ppm, as it is added to juices, drinks and some products such as butter, ghee and cheese (Britton *et al.*, 1995).

## 2. Torulene

Characterized by its attractive colors, it has 13 double bonds and is Its use as an antioxidant is due tohaving this number of double bonds which made them more efficiency of  $\beta$ -carotene which contains in its composition fewer double bonds (Sakaki *et al.*, 2002).

## 3. Astaxanthin

Red stain causes the appearance of attractive colors in marine invertebrates, fish and birds. As in carotene stain, it is a colored substance in salmon, trout and shrimp reddish pink (Jackson *et al.*, 2008).

# 4. Canthaxanthin

It is an important keto-carotene stain, mainly used in the food and cosmetic industries (Hannibal *et al.*, 2000).

Carotenoids are divided into two parts according to the presence of the oxygen element in their composition:

## 1. Carotenes:

Chromo lipids include oxygen-free compounds, as they are unsaturated hydrocarbons mainly soluble in non-polar solvents. It consists of eight isoprene units, these units are arranged in such a way that their arrangement is reflected in the center of the molecule. Most

carotenoids contain 40 carbon atoms and some of them contain alcoholic or aldehyde groups. Carotenoids also consist of condensation of isoprene molecules with loss of hydrogen as a result of this condensation.

# 2. Xanthophylls

The compounds contain oxygen in the form of active groups, as they are similar to carotenoids in the composition of their carbon structure, and they are considered a keto derivative of carotenoids, and each compound of xanthophyls is often a compound of corresponding carotenoids (Britton *et al.*, 2008).

## **Carotene Stain**

Carotene stain in red, yellow, pink, orange and red coral colors represent a group of natural antioxidant stains that are abundant in plants such as citrus, carrots, mangoes and apricots (Sun, 2018). It also produces various types of microorganisms such as bacteria *Corynebacterium michiganense*, *Micrococcu sroseus*, *Brevibacterium* spp., *Bradyrhizobium* spp., *Gordonia jacobaea*, and *Dietzia natronolimnaea* (Nasri-Nasravadi and Razavi, 2010) and And many algae like *Dunaliella*, *Dictyococcus*, and *Haematococcus*) Some types of filamentous fungi and a few fungi that belong to the class of Ascomycetes fungi as well as yeasts for example *Cryptococcus*, *haffia*, *Rhodosporidium*, *Rhodotorula*, and *Sporobolomyces* (Marova *et al.*, 2012). Most of which are due to Basidiomycetes (Libkind *et al.*, 2005).

It was recently observed that the production of carotene from plants became limited due to the high costs of production compared to the percentage of returns, which led to the tendency of most research and studies to obtain carotene from microorganisms that became alternative sources for plants in view of the low cost of production and avoiding environmental pollution with waste from agricultural and industrial waste (Mata-Gómez *et al.*, 2014), In addition, the carotene from these microorganisms avoids the problems caused by the seasonal and geographical changes facing its production of plants (Frengova and Beshkova, 2009). Yeasts are more suitable in carotene production than other microorganisms especially algae, since they are single-celled organisms, as well as their rapid growth rate (Ausich, 1997).

## Types of Carotene and its Chemical Composition

There are three types of carotene: alpha-carotene, beta-carotene, and kama-carotene. Figure (1) shows the chemical composition of the three types of carotene.



y- Carotene

Figure 1: The syntactic formula for the isoprene unit included in the synthesis of the various carotenoids and the three types of carotenoids (Erich and Annu, 2006).

# Beta-carotene Biosynthesis in yeasts



Figure 2: Beta-carotene biosynthesis steps (Hayman et al., 1974).

## Yeasts sources of β-carotene

Many yeasts produce  $\beta$ -carotene and the following table shows the most important yeasts produced for  $\beta$ -carotene:

Name of Yeats	Source
Candida utilis	Shimada et al., 1998
Phaffia rhodozyma	Stachowiak and Czarnecki, 2007
Rhodosporidium diobovatum	Nasirian et al., 2018
Rhodotorula acheniorum	Nasrabadi amd Razavi 2011
R. glutinis	Sinisa et al., 2013
R. gracilis	Somashekar and Joseph 2000
R. graminis	Maldonade et al., 2008
R. minuta	Malla Obaida, 2017
R. rubra	Chanchay et al., 2012
R. mucilaginosa	Malla Obaida et al., 2018
Saccharomyce cerevisiae	Verwaal et al., 2007
Sporidiobolus pararoseus	Borba et al., 2018
Sporobolomyces pararoseus T.	Fuchman, 1980
Sporo. roseus	Sinisa <i>et al.</i> , 2013
Sporo. ruberrimus	Hadi et al., 2007
Sporo. shibatanus	Marova <i>et al.</i> , 2012

#### Table 1: Yeasts produced of β-carotene

## β-Carotene - its Importance and Uses

β-Carotene is one of the most important and most common types of natural carotenoids made by plants and microorganisms, with about 70% of total carotene (Eldahshan and Singab, 2013). In recent years, interest in it has increased greatly, due to the abundance of evidence indicating its benefits and importance to human health (Bogacz-Radomska and Harasym, 2018). It is a source of vitamin A in the body as it is converted in the human intestine into vitamin A necessary to maintain vision as it is an anti-oxidant that protects the body from free radicals, and works to strengthen the immune system in the body and contribute to stimulating cell growth and differentiation (Terlecki *et al*, 2014) so It is given to those who suffer from a deficiency in the body in doses ranging from 0.4 - 20 mg daily (Coma, 1991). In a study by Giuseppe *et al*. (2007) it has been shown to help protect lymphocytes from the risk of free radicals, particularly the hydrogen peroxide (H2O2) and nitrogen oxide (NO2) that causes cell destruction and membranes.

Numerous studies have shown that it is a very effective antioxidant inhibitor against some plants oils such as corn oil, soybean oil, and sunflower oil and it is believed that it contributes to reducing the incidence of cancer that starts with the formation of Free Radicals as it reduces the incidence of lung cancer among smokers. Studies indicate its role in reducing the incidence of breast cancer, prostate, bladder, stomach, and colon (Perera and Yen, 2007) It has a preventive role against many degenerative diseases such as cardiovascular disease, atherosclerosis, allergies, asthma, rashes, kidney tumors, headache, and increased activity in children (Elvira-Torales et al., 2019) as well as its systemic functions in sustaining epithelial tissues, maintaining growth, and increasing reproductive efficiency (Niizu, 2003) Preventing cataracts and age-related macular degeneration (Saini and Keum, 2004) and protecting the skin from sunburn-induced burns as well as from damage caused by oxidation and UV exposure (Britton et al., 2008), as well as being used as a coloring material for food and beverages and giving them attractive color, flavor and taste as being a safe and harmless substance to human health rather than food dyes not authorized for food use (Machado et al., 2019). It is also used in the coloring of cosmetics (Das et al., 2007), and it is one of the additives for animals feed (Tang et al., 2019).

#### β-Carotene extract From Yeasts

Carotenoids accumulate in certain parts of the cell, but its presence in the plasma membrane only or in the cell wall is not yet known. Moreover, the distribution of carotenoids and their derivatives in the cell parts has not been studied (Mata-Gómez et al., 2014). Many techniques were used to extract carotenoids from yeasts, but they encountered many difficulties and restrictions due to the characteristics of the cell wall, which constitutes an obstacle to the extraction processes, and this calls for improvement and development in the techniques used. Several types of technologies, including mechanical, chemical, and enzymatic methods were used to tear the cell walls and carotenoids were obtained from yeasts Phaffia rhodozgma and at high concentrations of 190.35µg/ g when applying the biomass freeze and enzymatic degradation technique, although many patents relating to the production of carotenoids were registered From microorganisms around the world. Among the most important techniques used to break down cell walls in yeasts and obtain carotene are soaking and freezing diatomaceous soils, treatment with Dimethyl Sulfoxide (DMSO), enzymatic degradation, ultrasonic waves

(Michelon *et al.*, 2012), using glass beads (Aksu and Eren, 2007), Dry Freeze (Park *et al.*, 2007), Soaking and Freezing with Liquid Nitrogen (Valduga *et al.*, 2009), using a mixture of DMSO solvents, acetone, and petroleum ether (Taskin *et al.*, 2011) or using acetone, and hydrochloric acid (Gu *et al.*, 1997).

### β-Carotene estimation using High Performance Liquid technology

## **Chromatograph (HPLC)**

Several methods have been used to analyze the mixture of  $\beta$ -Carotene compounds, including a highly sensitive and specific chromatographic technique in stabilizing the results including paper chromatography (Deb and Madhugiri, 2012), thin layer (Khaled *et al.*, 2013) and gas-liquid chromatography (Buzzini *et al.*, 2007). ) And HPLC (Malla Obaida *et al.*, 2016) and high-performance liquid chromatography - mass stratification (Sacchetti *et al.*, 2005).

Several studies concerned with the appointment of beta-carotene using chromatographic technique were performed. Beta-carotene tincture was diagnosed in yeasts R. rubra, Ph. rhodozyma, Rhodotorula sp., Sporobolomycas, Salmonicolor, and R. glutinis using HPLC (Voaides and Dima, 2012). The  $\beta$ -Carotene content was 43.9 µg/g in yeast R. glutinis from the total content of carotenoids while the other ingredients Torulene reached 23  $\mu$ g/g and Torularhodin 182 $\mu$ g/g (Yehia *et al.*, 2013). In Sporolomy ruberrimus Hllo, the separated orange stains were identified by means of highly efficient liquid chromatography (HPLC), as the extracted carotene stain contained (5) components:  $\beta$ - carotene, Toraularhodin, Apocarotenoid ester, Canthaxanthin, and Astaxanthin in quantities (250, 750, 200, 250, and 100)  $\mu$ g/l, respectively (Hadi *et al.*, 2006). Maldonade *et al.* (2007) were able to diagnose  $\beta$  – carotene and Torulene stains in a mixture of carotenoids obtained from yeasts R. mucilaginosa and R. glutinis based on HPLC technology. Kim et al. (2010) were able to separate the  $\beta$ - carotene stain from the orange carotenoids stains from yeast Rhodosporidium sp. Buzzini et al. (2007) found that carotenoids extracted from yeasts Sporobolomy, Sporidioblus, Rhodotorula, and Rhodosporidium, whose quantities ranged between 16.4-184 µg/ gm contain (4) constituents of torularhohodin, torulene,  $\alpha$  -carotene, and  $\beta$ -carotene.

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