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YEAST CAROTENOIDS IMPORTANCE IN FOOD AND FEED INDUSTRIES AND ITS HEALTH BENEFITS– A REVIEW

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ABSTRACT

Yeast carotenoids represent a group of valuable molecules for the pharmaceutical, chemical, food and feed industries, not only because they can act as vitamin A precursors, but also for their coloring, antioxidant and possible tumor-inhibiting activity. The synthesis of different natural commercially important carotenoids (carotene, torulene, torularhodin and astaxanthin) by several yeast species belonging to the genera *Rhodotorula, Sporobolomyces* and *Phaffia* were considered as potential pigment sources. In this review, the different yeast carotenoids and their health benefits are discussed.

Key words: Carotenoids, Colourants, *Phaffia*, *Rhodotorula*, Yeast

Humans acquire carotenoids through diet, from vegetables and fruits as well as from animal products. Carotenoids represent a group of valuable molecules for the pharmaceutical, chemical, food and feed industries, not only because they are vitamin A precursors, but also for their coloring, antioxidant and possible tumor-inhibiting activity. Despite the availability of a variety of natural and synthetic carotenoids, there is renewed interest in microbial sources. The growing scientific evidence has showed that carotenoid pigments obtained from microbial sources have potential benefits in human and animal health and has led to increased commercial interest in search of alternative natural sources. Now a days biological sources of carotenoids received major focus because of the stringent rules and regulations applied to chemically synthesized/purified pigments. Compared with the extraction from vegetables or chemical synthesis the microbial production of carotenoids is of paramount interest, mainly because of overcoming the problems of seasonal and geographic variability in the production and marketing of the colorants of plant origin and because of the economic advantages of microbial processes using natural low-cost substrates as carbohydrate sources (Frengova and Beshkova, 2009).

Commercial production of natural carotenoids from microorganisms is a new approach more eco-friendly than synthetic manufacture by chemical procedures. Despite the availability of a variety of natural and synthetic carotenoids, there is currently renewed interest in microbial sources (Bhosale 2004). Carotenoid pigments are common to a broad range of microorganisms. The chemical structure of carotenoids (the presence of double bonds) determines specific properties of these compounds. Being strong antioxidants, carotenoids perform the function of quenching free radicals in many microorganisms. In some bacteria, carotenoids are also involved in photosynthesis.

Pigmented yeasts are an interesting subject from the biotechnological point of view. As a rule, these yeasts have a broad spectrum of utilization of different carbon sources and grow well at high temperatures, forming a large biomass. Except for the anamorphous stage of the archiascomycete yeasts of the genera *Taphrina* and *Protomyces*, all known pigmented yeasts are related to three classes of basidiomycetes. Several yeast species belonging to the genera *Rhodotorula* and *Phaffia*, has consider these microorganisms as potential pigment sources. Yeasts are more convenient than algae or molds for large-scale production in fermenters, due to their unicellular nature and high growth rate (Frengova and Beshkova, 2009).

At present, a large quantities of pigments is actually satisfied through synthetic way. However, in food and cosmetic industries, the application of chemically synthetic carotenoids is restricted because of their toxicity. Therefore, commercial production of the carotenoids using carotenoids-produced microorganisms has gained more importance owing to its highly efficient and easy manipulation.

Carotenoid production by yeasts: Carotenogenic yeasts are considered to be ubiquitous due to its world-wide distribution in terrestrial, freshwater and marine habitats, and their ability to colonize a large variety of substrates. Carotenoid production by yeasts namely, *Phaffiarhodozyma*, *Rhodotorula*,*Rhodosporidium*, *Sporidiobolus*, *Sporobolomyces*, *Cystofilobasidium*, *Kockovaella*, *Phaffia* are known as potential natural alternative sources of carotenoids. They offer versatility in commercial carotenoid production in industrial-scale fermentation because of their unicellular nature, relatively high growth rate and their amenability to easily applied processes.

Phaffia rhodozyma: The red yeast *Xanthophyllomyces dendrorhous*, which was formerly known as *Phaffia rhodozyma* was isolated by Herman Phaff in the 1960s, during his pioneering studies of yeast ecology. Initially, the yeast was isolated from limited geographical regions, but isolates were subsequently obtained from Russia, Chile, Finland, and the United States. The biological diversity of the yeast is more extensive than originally envisioned by Phaff and his collaborators, and at least two species appear to exist, including the anamorph *Phaffia rhodozyma* and the teleomorph *Xanthophyllomyces dendrorhous*. The yeast has attracted considerable biotechnological interest because of its ability to synthesize the economically important carotenoid astaxanthin (3,3'-dihydroxy-beta, beta-carotene-4,4'-dione) as its major pigment. This property has stimulated research on the biology of the yeast as well as development of the yeast as an industrial microorganism for astaxanthin production by fermentation.

Red yeasts can use diverse sources of carbon, including low cost raw materials, such as molasses, whey and agricultural industrial by products, such as crude glycerol. The red yeast is able to grow under a wide range of initial pH conditions from 2.5 to 9.5 and over a wide range of temperatures from 5 to 26°C.

It is the only known red yeast that produces astaxanthin. In fact, astaxanthin contributes to 80-90% of its total carotenoids. Carotenoids are produced during late log phase or stationary phase by the mevalonate isoprenoid pathway. The astaxanthin yields of *P. rhodozyma* on both synthetic media and agricultural substrates are highly variable ranging from 174 Cg/g on Eucalyptus hydrolysates to 7200Cg/g on hydrolyzed corn syrup, with intermittent production on various substrates. The variability in yield is due to the inherent variability in the *P. rhodozyma* strains used and/or the carbon source in the media (Frengova and Beshkova, 2009). The red-pigmented fermenting yeast *Phaffia rhodozyma* contains astaxanthin as the principal carotenoid pigment. Echinenone, 3-hydroxyechinenone and phoenicoxanthin were also isolated and identified; isocryptoxanthin and canthaxanthin were absent. Evidence is presented for a new carotenoid, 3-hydroxy-32 42 -didehydro-â,ø-caroten-4-one. A possible biosynthetic scheme for the formation of astaxanthin in *P. rhodozyma* was also suggested (Arthur *et al.*, 2003)

Rhodotorula sp: Yeasts in the genus *Rhodotorula* synthesize carotenoid pigments. Most of the research is focused on the species *Rhodotorula glutinis*, however some papers deal with other species such as *R. gracilis*, *R. rubra* and *R. graminis*. The main compounds produced by these red yeasts are torulene and torularhodin, with minute quantity of â-carotene (Bhosale and Gadre, 2001) . Feed supplement with a *Rhodotorula* cell mass has been found to be safe and nontoxic in animals. Its use in the nutrition of laying hens has also been documented (Abd El-Rhman *et al.*, 2012). As â-carotene content in wild strains of *R. glutinis* is quite low, efforts have been made to increase it through strain improvement, mutation, medium optimization and manipulation of culture conditions (temperature, pH, aeration, C:N ratio). These studies resulted mainly in an increased yield of torulene and torularhodin, which are of minor interest. Some succeeded in increasing the target carotenoid and mutants derived from the wild type were able to produce 76-fold more â-carotene than the parent strain, *i.e.* 70 mg/L

Various species of Rhodotorula were isolated from plant leaves, flowers, fruits, slime fluxes (or exudates) of deciduous trees, soil, refinery waste water, air and yoghurt (Aksu and Eren, 2007). The production of carotenoids by genus *Rhodotorula* is affected by species, medium constituents and environmental conditions. The amount of carotenoids produced by this genus can be arranged as low (less than 100 ìg·g^{-1}), medium (101 to 500 ìg·g^{-1}) and high (more than 500 ìg·g^{-1}) as reported by many authors (Saenge *et al.*, 2011)

Sporobolomyces: In *Sporobolomyces* species, the main produced pigment is beta-carotene. Production of torulenes was not clearly confirmed, nevertheless, some oxidized carotenoid derivatives are produced in *Sporobolomyces* strains. In most of red yeasts oxidative and osmotic stress was reported to induce carotenoid production probably as a part of stress response. (Marova *et al.*, 2012). The genus *Sporobolomyces* contains about 20 species. The most common one is *Sporobolomyces roseus* and *Sporobolomyces salmonicolor, Sporobolomyces* colonies grow rapidly and mature in about 5 days. *Sporobolomyces* produces yeast-like cells, pseudohyphae, true hyphae, and ballistoconidia. The yeast-like cells are the most common type of conidia and are oval to elongate in shape (Nanjudasamy and Praveen, 2010).The optimal growth temperature is 25-30 °C. The colonies are smooth, often wrinkled, and glistening to dull.

S. roseus produces other carotenoids such as torulene and torularhodin. Total carotenoid content of *S. roseus* on synthetic medium has ranged from 82.3 Cg/g to 237 Cg/g (Maldonade *et al.*, 2008).

***Cryptococcus* sp:** *Cryptococcus neoformans* is the only yeast known that consistently produces a melanin or melanin-like pigment from many p-diphenols. Pigment production from diphenols by other members of the genus is very rare. However, pigment production by some *Cryptococcus* species can occur when hydroquinone is present in the substrate. *Cryptococcus* species may also produce small amounts of pigment from diphenols after prolonged incubation (10days or more).

The strains of *Cryptococcus neoformans* produced brown cell-associated intracellular pigments while others produced pink extracellular pigments from l- or dl-tryptophan. Additionally, various other *Cryptococcus* species produced brown or pink pigments when cultured on tryptophan

Health benefits of yeast carotenoids: The biological role of the carotenoids as vitamin A precursors in humans and animals and owing to their antioxidant properties and suspected activity in preventing some forms of cancer as well, carotenoid pigments represent a group of most valuable molecules for industrial applications. The pharmaceutical, chemical, feed and food industries have shown increased interest in the use of carotenoids, mainly as provitamin A, but also as natural food and feed colorants

Aquaculture industry: The red yeast *P. rhodozyma* is currently used for the production of astaxanthin, an important carotenoid pigment that can be exploited in aquaculture to give an appealing pink color to the fish of farmed salmonid fish. Astaxanthin is vital in aquaculture feed: it improves the egg quality and fry survival, protects against oxidation of lipids in salmon which contain high levels of polyunsaturated fatty acids, has pro-vitamin A activity, improves fish liver histology and improves shrimp and prawn survival rates.

The most expensive ingredient in salmonid feeds is astaxanthin, and though the actual revenues are privately held, it has been estimated that the market for astaxanthin in US $100 milion per year. *P. rhodozyma* could be a source of astaxanthin for salmonids raised in aquaculture. The recommended dosages of carotenoids are 1-50 mg/day to enhance immune response (Hayek, 2000), 40-70 mg astaxanthin / kg of feed or 30- 120mg/kg of total carotenoids in aquaculture.

Animal health and Feed industry: Usually animal feeds are poor in carotenoids and are added as feed supplements. Animals are incapable of producing carotenoids but are able to assimilate the ingested carotenoids. Carotenoids are beneficial to animals as they i) act as antioxidants and precursors of vitamin-A, ii) improve cell communication and enhance immune response in ruminants and dogs iii) reduce incidence of mastitis in dairy cows, iv) assimilate into milk as vitamin-A, thereby improving the keeping quality of milk (Noziere *et al.*, 2006), improve reproductive efficiency.

Additionally, the innate defense mechanisms of fish were modulated by dietary carotenoids from *P. rhodozyma* and *Dunaliella salina*. Several studies have been done using astaxanthin esters in mammals to prove its effectiveness in the treatment of muscle diseases, for example, equine exertional rhabdomyolysis or to increase the production of breeding and production mammals (porcine, bovine, and ovine).

The administration of astaxanthin to layer hen diet increases fertility, improves the overall health status of these animals, and decreases chicken mortality. Egg production and the yellow coloration of yolk is also increased, while salmonella infections reduced dramatically probably due to a stronger membrane formation. It also provides greater pigmentation to chicken and meat, a desirable attribute to some consumers.

When disrupted cells *P. rhodozyma*, without cell walls, are added to the diets of animals, astaxanthin is readily absorbed from the gut and effectively colors the flesh of penreared salmonids, and also helps to impart a desirable golden color to the egg yolk and flesh of poultry. The yeast also contains a high level of unsatured fat, protein and vitamins that contribute to good growth of animals. These attributes enhance the potential utility of *P. rhodozyma* as a source of astaxanthin in animal diets.

Nutraceutical and Antioxidant potential: Currently there is a wide variety of astaxanthin products sold in health food stores in the form of nutritional supplements. Most of these products are manufactured from algae or yeast extracts. Due to their high antioxidant properties these supplements have been attributed with potential properties against many diseases. Thus, research on the actual benefits of Astaxanthin as a dietary supplement is very recent and basically limited to *in vitro* assays or pre-clinical trials.

Yeast *R. glutinis* (containing carotenoid pigments torulene, torularhodin and carotene in proportion 58:33:2) showed significant effect on the prevention of liver tumor development. However, *R. glutinis* effects were relatively more significant in groups where *R. glutinis* was administered suggesting that *R. glutinis* is quite effective in the prevention of liver tumor development.

Rapta *et al.*, (2005) found that Zn^{2+} ions induced changes in yeasts (*R. glutinis* and *R. rubra*) leading to more efficient scavenging and antioxidant capacities compared with Ni^{2+} ions, and antioxidants (carotenoids) present in yeast cell walls showed higher ability to scavenge free radicals than those from inside the cells. Later, the in vivo antioxidant and protective effects of astaxanthin isolated from *X. dendrorhous* against ethanol-induced gastric mucosal injury were established in animal models, especially rats.

Photoprotectant and Cosmetic use: The synthesis of photoprotective compounds is a common response of several organisms when exposed to high irradiation; however, there are few reports of photoprotection afforded by carotenoids in microorganisms in general and yeasts in particular. Moreover, there is still controversy regarding the photoprotection effciency of carotenoids in the range of UV-B wavelengths, the photoprotective role of carotenoids in yeasts by contrasting the responses to UV-B of pigmented and naturally occurring albino strains of *Sporobolomyces ruberrimus* and *Cystofilobasidium capitatum* in different conditions. Fungi, their fundamental role is to provide protection against reactive oxygen species (ROS), specifically O_2. Furthermore they provide indirect protection against both UV-A (315–400 nm) and visible light (photosynthetically active radiation, PAR 400–700nm) by means of ROS quenching. Several yeast species are known to accumulate carotenoid pigments as secondary metabolites. In these microorganisms, carotenoid synthesis is associated with growth. Maximum carotenoid accumulation is observed in stationary phase in relation to cell ageing, and is probably a general mechanism of defence against oxidative stress. The synthesis of torularhodin, torulene, and â-carotene is common in several genera of *Rhodotorula*, *Sporobolomyces*, and *Cystofilobasidium*. In these microorganisms, carotenoids may contribute to preserve the viability of ageing cells by quenching oxygen radicals, possibly compensating for their lack of antioxidant enzymes. At present the wrinkles and sagging of the human skin are considered to be caused by reduction in skin elasticity, influenced by aging, dryness, oxidation, sunlight (UV rays) etc., carotenoids actions on skin, such as inhibition of pigmentation, inhibition of melanin-generation and of light-induced aging have been reported. In this study a cream preparation combined with astaxanthin from yeast, was studied for cosmetic effect for on the skin by performing a patch test and a skin repeated application test on human beings to assess the safety of the skin and on skin moisture retention capacity (Tomoka *et al.*, 2010)

Antihypertensive effects: Hussein *et al.*, (2005) reported that the administration of astaxanthin extracted from *P. rhodozyma* induced a significant reduction in blood pressure and delayed the incidence of stroke in stroke-prone spontaneously hypertensive rats, suggesting that astaxanthin has antihypertensive effect. In a study using aortic rings of spontaneously hypertensive rats, astaxanthin induced a significant reduction of the contractile responses of the aorta to which may contribute to the antihypertensive effect of astaxanthin. In a histopathological study, astaxanthin decreased coronary artery wall thickness compared with the control, indicating the possibility that astaxanthin ameliorates hypertension-induced vascular remodeling. Astaxanthin has anti-infl ammatory, antidiabetic, antihypertensive, and antioxidative activities.

Food industry

Yeast carotenoids are one of the leading food colorants in the world. Carotenoids has been applied to a range of food and beverage products to improve their appearance to customers, including items such as margarine, cheese, fruit juices, baked goods, dairy products, canned goods, confectionary and health condiments. Carotene is used in various pet foods as both a colorant and a precursor to Vitamin A. carotenoids can be applied to an array of animal foods designed for pets, including dogs, cats, fish, and birds. The antioxidant and precursory vitamin A properties increase the appeal and application of â-carotene in pet foods

Conclusion: Carotenoids are playing an important role in human health in the developed world as colorants, feed supplements, and nutraceuticals in the food, medical, and cosmetic industries.

The nutritional value of carotenoids, such as á and â-carotene, â-cryptoxanthin and astaxanthin has been known for many years, and their antioxidant properties and their effciency in the prevention of certain human diseases have also been claimed. Consenquently, interest in these compounds from a nutritional aspect has increased substantially and a multimillion dollar market has been established in the last 20 years. However, it is also a well-established fact that chemical synthesis is fulfilling most of this demand. The high commercial demand for many carotenoids have long been met by chemical synthetic technology. However, some of the by-products resulting from such chemical processes may have undesirable side eVects on consumption. For this reason, the production of carotenoids from microbial sources has been the focus of extensive research. Furthermore, the microbial production of carotenoids could also be of interest because of the problems of seasonal and geographic variability in the production and marketing of colorants of plant origin. In the current literature, yeasts *Rhodotorula* (producing _-carotene, torulene and torularhodin as the major carotenoids pigments) *Phaffia* (producing mainly astaxanthin), *Sporobolomyces* and *Cryptococcus* are described as a potential source of carotenoids with medical or industrial interest.

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