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Evaluation the effect of Di-potassium hydrogen phosphate (K2HPO4) on the accumulation of some secondary metabolites in Spirulina Cyst

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ABSTRACT

Marine microalgae constitute a natural source of a variety of drugs for pharmaceutical, food and cosmetic applications which encompass carotenoids, among others. In this study the inducible effect of K2HPO4 on the accumulation of some apo carotenoids includes, a and b types chlorophyll, beta carotene, astaxanthin, lycopene and pheophytin were conducted on five treatment and three replication under CRD design. cellular extracts were extracted with DMSO solvent. analysis of the obtained data through spectrophotometric absorbtion for which studied metabolites have done by GeneStat 12.1 statistical software and the grouping of means on 5% probability level (P<0.05) performed via duncan's test. The result showed significant difference between treatments. the metabolites content in cellular extracts on stationary phase of algae calculated in highest level of 4/5 and 3/588 (mg/g) for chlorophil a and beta carotene respectively in fifth treatment level. Also astaxanthin and lycopene was 1/505 and 2/84 (mg/g) respectively in second treatment. There was none significant differences between the chlorophyll b and pheophytin pigments compare to control.

Key words: Spirulina, apocarotenoids, DMSO, K2HPO4

INTRODUCTION

Apo Carotenoids are bio-active compounds derived from carotenoids. These compounds metabolically are derived from the failure of the oxidative of C40 [7,8] (Figure 1).Given that there are a significant amount of carotenoids in microalgae, needed to find a species with high carotenoid is necessary. Ahmed *et al*. exprimented apo carotenoid in cell extracts of 12 species of saltwater microalgae in shoreline and subtropical regions showed that 8 carotenoids, Ntra xanthine, lutein, epoxide, Lutein, Zeaxanthin, alpha and beta carotene were as the major carotenoid in different species [1]. the large number of existing species of microalgae constitutes a unique reservoir of biodiversity, which supports potential commercial exploitation of many novel products besides vitamins, pigments and polyunsaturated fatty acids [4,6].

In this study aimed about the evaluation of potassium di-hydrogen phosphate (K2HPO4) effects on the accumulation of some secondary metabolites in Spirulina cysts at the stationary phase.

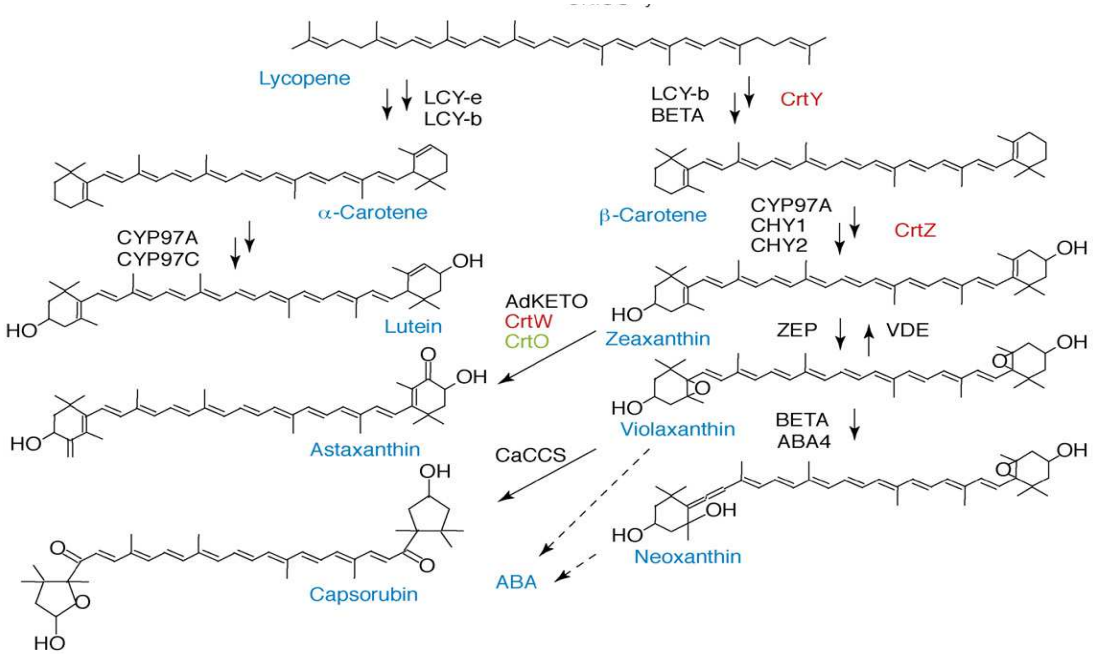

Figure 1: The biosynthesis pathway of carotenoids. Components with blue color, enzymes (vegetable enzymes) with black color, red for the bacterial enzyme, the green color for chetolase enzyme (CtrO) of cyanobacteria and green algae are shown

MATERIALS AND METHODS

In this study was used the modified giullards solution with vitamin B-complex as a microalgae growth medium. The salinity of sea water which used for culture medium, was 12-13 grams per liter so that disinfected by chlorine 70% solution. Then five levels of K2HPO4 were applied as a desired treatments in suspension culture under complately randomized design (CRD) (table 1).

Table1: Applied treatments of K2HPO4 in the suspension culture of Spirulina

تیمار	T0	T1	T2	T3	T4
K2H2PO4 mg/l	0	5	20	35	50

Metabolites extracted using DMSO (dimethyl sulfoxide) solvent under 55 ° C temperature condition. After filtering extract from any solid materials, the ratio of absorption read in suitble range wavelength for Apo carotenoids includes chlorophyll a and b, b-carotene, astaxanthin, lycopene, and pheophytin.

RESULTS AND DISCUSSION

Carotenoid production appears to be one of the most successful case studies of blue biotechnology. The rising market demand for pigments from natural sources has promoted large-scale cultivation of microalgae for synthesis of such compounds, so significant decreases in production costs are expected in coming years. In tis research, DMSO were used as an extraction solvent in this project. extraction usually resorts to hexane and has advantages over alkaline treatment because all lutein and zeaxanthin are converted to their free forms, while carboxylic acids and chlorophylls remain in the aqueous phase, this method has been optimized for Spirulina almeriensis [2,5]. statistical analysis were perfoemed by GenStat 12.1, and Duncan's test were used at 5% ($P < 0.05$) of probability levels.

in the stationary phase type chlorophyll a, in control treatment was calculated 4.5mg and beta-carotene with 3/588mg in fifth treatment, astaxanthin and lycopene, with 1/505 and 2/84 mg per grame respectively measured in second teatment. No significant were detected in chlorophyll b and pheophythin between treatments (Figure 2,3 and 4).

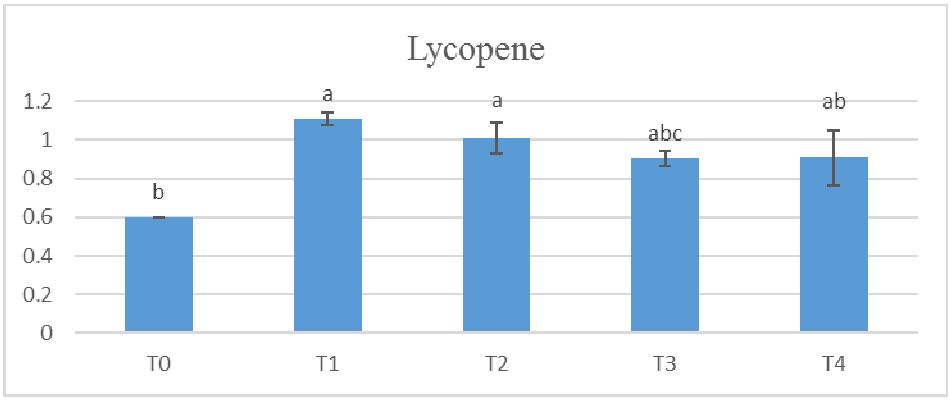

Figure 2: Lycopene absorption average at 476 nm wavelength (horizontal axis: T0-T4 are the treatments)

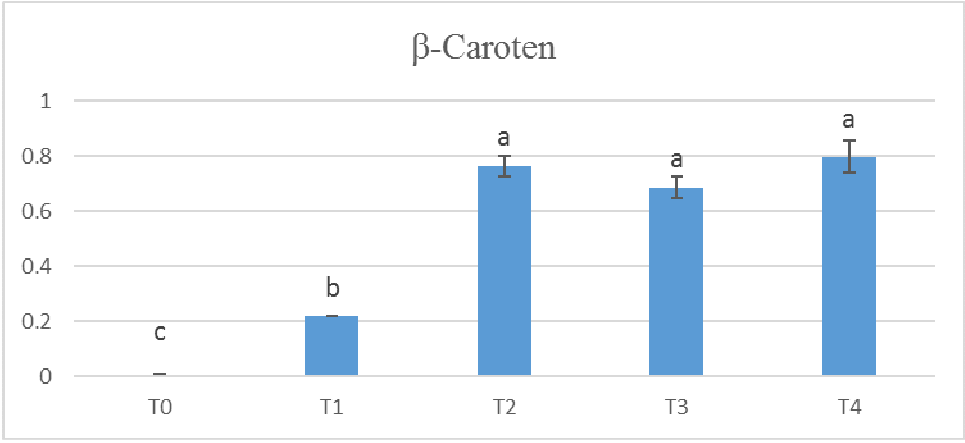

Figure 3: Beta-carotene absorbtion ratio at 460 nm wavelength

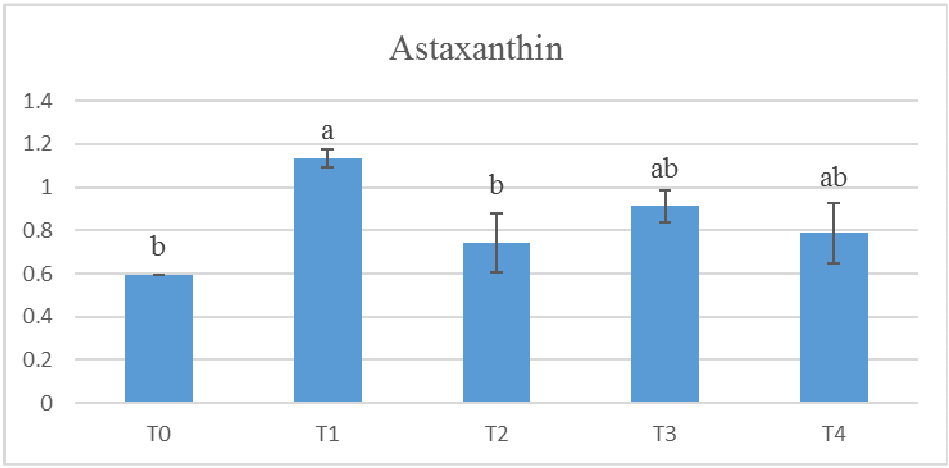

Figure 4: Average uptake of astaxanthin in per unit cell extract that was extracted with the same volume of acetone at 489 nm wavelength (horizontal axis: T0-T4 are the treatments)

As it clare the amount of beta-carotene for the treatment of 50 mg (T4) are in maximum and in the control treatment are in least amount (Figure 3). This result claim a suitable effect of Dipotasium hedrogen phosphate on iduce the accumulation of beta-carotene in algal cyst.

Recent studies showed that the Dunaliella Salina with a mean of 4.5 milligrams per gram of material is rich source of beta-carotene, while the level of Taiwanese species of Dunaliella Salina 6.55 mg/ g have been reported [6].

In other experiment, entitled carotene extracted from Spirulina platensis were discussed about the using ultrasound waves for beta-carotene extracted from Spirulina platensis and effect of various parameters such as the extraction

solvent, the biomass proportion to the amount of solvent, temperature, and electrical noise effect of different treatments on the extraction of astaxanthin were studied. The results showed that the optimum conditions for extraction of carotene from an economic point of Spirulina is 12 times increase in the amount of beta-carotene [3]. Some techniques, such as radiation-induced biosynthesis of astaxanthin has been studied in Haematococcus Plvyalys [3].

Advances in knowledge of the underlying physiology, biochemistry and molecular genetics of carotenoid-producing microalgae are now urged—which would have a major impact upon development and optimization of this (and alternative) microalga-based technologies.

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