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Studies on carotenoid in *Artemia parthenogenitica*

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Abstract

Carotenoids extracted from halophilic bacteria, Artemia, Artemia cysts and UV-treated Artemia were both qualitatively and quantitatively analyzed. The carotenoid content was calculated by using spectrophotometer. The secondary study was done with UV treatment at 5, 10, 15, and 20 min interval for 5 consecutive days on Artemia. A control was maintained. In mortality studies, the percentage mortality of Artemia gradually increased and was the maximum on the final day due to long exposure (20 min). On qualitative estimation of carotenoid by centrifugation, there were changes seen on the total carotenoid content. On qualitative examination of carotenoid by TLC, there was a gradual decrease from Artemia control of Artemia UV treated (5, 10, 15 and 20 min). There were no changes qualitatively.

Keywords: Carotenoids, *Artemia*, *Artemia* cysts, UV

Introduction

Carotenoids are the accessory light-harvesting pigments (4). Besides their role in pigmentation effects and vitamin A activity, carotenoids may protect tissues from oxidative damage through. The first colourless carotenoid phytoene synthesized from geranyl geranyl pyrophosphate (GGPP) have been isolated from photosynthetic bacteria *Rhodobacter* sp, non-photosynthetic bacteria *Erwinia* sp and *Thermus thermophilus*, cyanobacterium *Synechococcus* sp strain pcc 7942, fungus *Neurospora crassa* and higher plants (16). Over 200 carotenoid pigments including a great number of xanthophylls, have been isolated from marine animals (14). Marine animals are able to metabolize the supplemented carotenoids into suitable forms and store them.

Most current research is focused on a proposed role for carotenoids as lipids antioxidants, which are able to protect against oxidation and other destructive process mediated by free radicals (11). Blue green algae have chlorophyll a as the major light harvesting pigments along with carotenoids and phycobilins as accessory pigments (15).

Carotenoids have proved to be particularly valuable chemo systematic markers in micro algae and this has found practical application in oceanography (1) scavenging of singlet O_2. At higher O_2 pressures carotenoids act as pro oxidants (5). Carotenoids may be an important biological antioxidant and are thought to play a role in controlling oxidatively induced diseases such as cancer and atherosclerosis (7). The antioxidant property of carotenoids depends on environmental conditions and the nature of oxidation catalyst. There

are exciting prospects for the biotechnological production of carotenoids and the use of carotenoids as health promoting or disease preventing substances (18).

The brine shrimp *Artemia* has great significance as an important and standard live feed to over 85% of the marine aquaculture species of diverse groups (8). *Artemia* is regarded as a lifeline for the aquaculture industry, as no satisfactory substitute as live feed for larval fishes and crustaceans has yet been found (19). The wide size range of *Artemia* and their physical forms such as decapsulated cysts, newly hatched nauplii, meta nauplii, juveniles and adult stages as well as processed forms like frozen, freeze dried, dried and flakes (10 & 17) are very suitable for commercial aquaculture. In the present study carotenoids were quantitatively and qualitatively analyzed from *Artemia*, *Artemia* cyst and the UV-treated *Artemia*. The percentage mortality of UV-treated *Artemia* was also studied.

Material and Methods

An *Artemia* population inhabiting the condenser pond was selected. Along with this the *Artemia* cysts were also collected and stored in a beaker. Later on five beakers were cleaned properly and used for growing *Artemia*. Equal numbers of *Artemia* (75 No) were grown in each beaker. They were fed properly with micro algae. Antibiotics were added to control any bacterial and fungal contaminations. The *Artemia* grown separately in the five beakers were taken for UV treatment. UV-β rays were subjected to the *Artemia* in the beakers. After regular time intervals (5, 10, 15 and 20 min.) the corresponding beakers were taken out from the UV chamber. This was continuously done for 5 days at 24 h interval. After 5 days of UV treatment, the *Artemia* were checked for mortality. The cumulative mortality index (CMI) was calculated based on (3). The data obtained in the present study were subjected to relevant statistical analysis following the procedure given in (20). The carotenoids were extracted from the *Artemia* cyst; *Artemia* (whole animal) and UV treated *Artemia* (whole animal) by following the methodology of (13).

Results

Artemia was identified based on the morphological and biological characteristics and the species was confirmed as *Artemia parthenogenitica* (9). The mortality study conducted on the effect of UV irradiation in *Artemia* has been illustrated in Table 1. The result indicated that there was increase in the mortality of *Artemia* on long UV exposure (20 min). The % mortality was found to be 98.66 % on the final day at 20 min UV exposure. There was no mortality in the control. The mortality has occurred gradually (i.e.) as the days increased, % mortality also increased.

Table 1. Percentage mortality of *Artemia parthenogenitica* exposed to UV stress at different time intervals.

DAYS	PERCENTAGE MORTALITY				
	CONTROL	5 min	10 min	15 min	20 min
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	73.33
3	0.00	0.00	22.66	52.00	90.66
4	0.00	30.66	49.33	89.33	98.66
5	0.00	54.66	73.33	89.33	98.66

The control *Artemia* showed no mortality. As the time of UV exposure increased the mortality rate also increased. (Table 2)

Table 2. Two - way ANOVA due to UV effect and exposure duration

SOURCE	SS	DF	MS	F	P
Total variance	36912.32	24	-	-	-
Variance due to UV effect	13805.44	4	3451.36	7.13	<0.05
Variance due to exposure duration	15361.78	4	3840.45	7.93	<0.05
Error Variance	7745.10	16	484.07	-	-

The CMI values calculated for the *A.parthenogenitica* are reported in Table 3. The CMI of control *Artemia* was the least (0). The reduction of stress (37.34%) was high in 10 min UV exposed *Artemia.* It has been decreased in 15 min UV treated *Artemia* and was found to be 27.41%. Again there was an increase in reduction of stress (33.36%) in 20 min UV treated *Artemia* from that of 15 min UV treated *Artemia.*

Table 3. CMI Values of Reduction of Stress in *Artemia parthenogenitica*

UV IRRADIATION (min)	CMI	REDUCTION OF STRESS (%)
CONTROL	0	0.00
5	297	100
10	474	37.34
15	653	27.41
20	980	33.36

The amount of total carotenoid contents estimated from the *Artemia* cysts, *Artemia* (whole animal), *Artemia* control and UV treated *Artemia* (5, 10, 15 and 20 min) are illustrated in Table 5. The total carotenoid extracted from 1g dry weight of *Artemia* cyst was 0.6168 mg. (Table 4) The carotenoid content present in the *Artemia* (whole animal) and *Artemia* control was quantified as 0.2304 and 0.2259 mg/g respectively. The total carotenoid estimated from 1g each of 5, 10, 15 and 20min UV treated *Artemia* was 0.2232, 0.1908, 0.1377, 0.0963 mg respectively. There was a gradual decrease in carotenoid content as the time of UV exposure increased.

Table 4. Carotenoids extracted from *Artemia* whole animal, *Artemia* cysts and *Artemia* UV treated (Qualitative)

EXPERIMENTAL ORGANISMS	Rf VALUE	CAROTENOIDS*
Artemia control	0.800	+
Artemia whole animal	0.813	+
Artemia cysts	0.433 0.566	+ Astaxanthin diester
Artemia 5min UV	0.780	2- isoprentenyl-3,4-didehydro rhodopsin
Artemia 10min UV	0.833	+
Artemia 15min UV	0.785	2- isoprentenyl-3,4-didehydro rhodopsin
Artemia 20min UV	0.807	+

* Reference: D'Abramo; + Unidentified

Table 5. Total Carotenoids (mg/g) estimated from different supernatants of *Artemia* (quantitative)

SUPERNATANTS	*ARTEMIA* (WHOLE)	*ARTEMIA* CONTROL	UV EXPOSURE TO *ARTEMIA*			
			5 min	10 min	5 min	20 min
FIRST	0.0946	0.0846	0.0822	0.0752	0.0566	0.0482
SECOND	0.1236	0.1148	0.0844	0.0628	0.0552	0.0492
THIRD	0.0624	0.0426	0.0384	0.0294	0.0204	0.0084
MIXTURE	0.2304	0.2259	0.2232	0.1908	0.1377	0.0963

UV exposure has played a vital role in the quantitative changes of carotenoid. But there was no change qualitatively. It is found that as the time of UV exposure increased the content of carotenoid decreased. When long exposure was given (20 min) for consecutive 5 days there was 98.66% mortality and the total carotenoid has also been decreased to 0.0963 mg/g. The non-UV treated *Artemia* showed larger amount of carotenoid (0.2304 mg/g). In general, it was found that, there was a gradual decrease in the total carotenoid content. The total carotenoid present in the *Artemia* cyst was (0.6168 mg/g). Regarding qualitative changes in total carotenoid, there were no changes found. All the carotenoids were found to be present.

The separation of carotenoids through TLC in different samples viz., *Artemia* cyst, *Artemia* (whole animals), *Artemia* control, *Artemia* (UV treated) are given in the Table 4. In *Artemia* cysts, the carotenoid level was much decreased, whereas in *Artemia* (whole animal), *Artemia* control and *Artemia* UV treated, the carotenoid contents gradually decreased. In *Artemia* cyst, the presence of Astaxantin diester was found by the Rf value 0.566. The Rf value 0.780 and 0.785 revealed the presence of 2-iso prentenyl – 3, 4 – didehydro rhodopsin in the 5 and 10 min UV treated *Artemia*. The two-way ANOVA shows that there was a significant difference ($P<0.05$) between UV exposure and time intervals.

Discussion

Before the demonstration of different carotenoids in *Artemia,* nothing suggested that this organism differed from related Crustacea as far as the (non-specific) fate of its carotenoids was concerned (6). Like other Crustacea, *Artemia* mobilizes carotenoids in the gonads and the eggs. Since the *Artemia* feed on micro algae such as *Chlorella, Dunaliella, Chlorococcum* etc., there is chance for the production or synthesis of carotenoid metabolites in *Artemia* from the micro algae (2).

It is found that the UV-β rays play a vital role in the decrease of the carotenoids. There might be changes in the thiamine-dimer base pairs due to irradiation. If such changes were found to occur then there might be changes in base pair and mutation should have occurred. If mutation has occurred, then this might be the reason for the reduced synthesis of carotenoids.

Despite the enormous economic potential of carotenoids, their generally accepted application in foods, feeds and the current strong interest in " Natural " pigments, few known microorganisms lend themselves to a commercial exploitation. Only β – carotene is produced, on a limited scale and at high cost from one microbial source. The industrial fermentation of zeaxanthin from *Flavobacterium* sps is said to be in a final stage and that of astaxantin from *Phaffia rhodozyma* might be so in a not too distant future. No industrial scale fermentation processes are currently in use for lutein because alternative, cheaper plant sources exist (12).

Whether the genetic engineering of carotenoid biosynthetic pathways will remain restricted to academic studies or will eventually result in commercial products that are competitive with their synthetic equivalents is hard to predict.

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