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EFFECT OF LIGHT AND HEAT ON STABILITY OF CRUDE CAROTENOID EXTRACT FROM NATURAL SOURCES

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ABSTRACT: Carotenoids are accessory coloring pigments found occurring naturally in the plants and some photosynthetic organisms. Recently there has been an increased focus on the health benefits and food applications of these natural pigments. The stability and bioavailability of carotenoids vary significantly across a range of processing paradigms. The present study was planned to investigate stability of carotenoids (β-carotene, lutein and lycopene) subjected to different ranges of temperature and light. It concluded that most stable extract under light is marigold followed by tomato and least stable is carrot. Marigold extract (lutein) is most heat stable followed by tomato extract (lycopene) then carrot extract (β- carotene).

INTRODUCTION: There are over 600 carotenoids, classified into two classes, xanthophylls (which contain oxygen) and carotenes (hydrocarbons with no oxygen). Carotenoids constitute hydrocarbons consisting of eight isoprenoid units joined in a manner that the arrangement of isoprenoid units is reversed at the center of the molecule so that the two central methyl groups are in a 1, 6-positional relationship and the remaining non-terminal methyl groups are in a 1, 5-positional relationship.

The majority of carotenoids are derived from a 40-carbon polyene chain backbone of the molecule. This chain may be terminated by cyclic end-groups (rings) and may be complemented with oxygen-containing functional groups. β- carotene, in carrots, is a familiar carotene, while lutein, the major yellow pigment of marigold petals, is a xanthophyll.

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The structure of a carotenoid ultimately determines what potential biological activity of the pigment. The characteristic pattern of alternating single and double bonds in the polyene backbone of molecules allows them to absorb excess energy from other molecules, while the nature of the specific end groups on carotenoids may influence their polarity.

The former may account for the antioxidant properties of biological carotenoids, while the latter may explain the differences in the ways that individual carotenoids interact with biological membranes[1]. In human beings, carotenoids exhibit several important functions. Their provitamin A activity contributes the major one. Deficiency of vitamin A is attributed to premature death among children in developing nations. In humans, Beta-carotene acts as a major precursor for vitamin A synthesis [1, 2, 3].

Dietary source of beta- carotene is fruits and vegetables, such as carrots, spinach, peaches, apricots, and sweet potatoes [4]. Other provitamin A carotenoids include alpha-carotene (found in carrots, pumpkin, and red and yellow peppers) and cryptoxanthin (from oranges, tangerines, peaches,

nectarines, and papayas). Carotenoids possess potential role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen. Lycopene, that gives tomatoes their red color, is particularly effective in quenching the destructive potential of singlet oxygen [5].

Lutein and zeaxanthin, xanthophylls found in corn and in leafy green vegetables such as kale and spinach, are believed to function as protective antioxidants in the macular region of the human retina [6, 7, 8]. Astaxanthin found in salmon, shrimp, and other sea-food, is another naturally occurring xanthophyll with potent antioxidant properties [9].

In developed countries, 80–90% of the carotenoid intake comes from fruit and vegetable consumption. Among 700 naturally occurring carotenoids identified thus far, as many as 50 are present in the human diet and can be absorbed and metabolized by the human body [10].

How-ever only six (β-carotene, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin), representing more than 95% of total blood carotenoids, are present in the blood of people from different countries and have been associated with health benefits.

MATERIALS AND METHODS: Pigments were isolated from plant material (β-Carotene from carrots, Lutein from marigold petals, Lycopene from tomatoes, purchased from the local market. All experiments and experimental procedures, beginning with extraction, were performed under dim light as much as possible and inside vessels and equipment covered with aluminum foil or black cloth, preventing possible pigment photo-oxidation.

Extraction of Pigments: The colorant was extracted from sample (carrot, marigold petals and tomatoes) through cold saponification by using AOAC method (1984). For this a sample was freshly grounded and extracted with 100 ml of extractant (Hexane: Acetone: Ethanol: Toluene, 10:7:6:7 v/v/v/v), for carring out cold saponification, the resulting slurry was treated with 40% methanolic KOH (7.5ml) after standing in dark for 16 hrs. Hexane (100ml) was then added to the flask and swirled gently for one minute after diluting to volume with 10% Na_2SO_4 and shaking vigorously for 1 min. The flask was allowed to stand in dark for 1 hr. until two layers were formed. The upper layer was collected and vacuum dried to obtain pigment extracts. The concentrated pigment extracts were kept in amber vessel.

RESULTS AND DISCUSSION: The observations regarding different light treatments of different extracts are presented in following **tables 1-3**.

TABLE 1: EFFECT OF DIFFERENT LIGHT TREATMENTS ON TOMATO EXTRACT

Hours	Control	UV	Sunlight	Tube-light	Darkness
24	100	90.2	91.9	94.6	96.9
48	100	85.5	87.3	90.2	95.47
72	100	77.5	83.9	86.1	94.56
96	100	72.6	76.5	83.9	91.8
120	100	68.1	72.1	80.1	88.6
144	100	64.1	66.5	77.5	88.1
168	100	58.7	60.3	72.6	86.1
192	100	54.5	56.3	70.3	84.3
216	100	49.6	51.9	64.1	81.4

TABLE 2: EFFECT OF DIFFERENT LIGHT TREATMENTS ON CARROT EXTRACT

Hours	Control	UV	Sunlight	Tube-light	Darkness
24	100	91.2	88	90.3	96.5
48	100	85.6	81.1	84.7	89.6
72	100	81	72.2	74.5	84.8
96	100	71.9	62.5	69.8	70.1
120	100	64.7	58.8	66.7	67.3
144	100	59.1	53.8	62.1	62.4
168	100	49.3	46.7	55.5	58.4
192	100	44	39.1	51.7	54.3
216	100	33.3	36.1	44.9	48.1

TABLE 3: EFFECT OF DIFFERENT LIGHT TREATMENTS ON MARIGOLD EXTRACT

Hours	Control	UV	Sunlight	Tube-light	Darkness
24	100	94.5	94	97.6	98.3
48	100	91.1	91	95.9	95.2
72	100	89.3	87.5	94.4	95.5
96	100	84.6	82.9	92.4	93.5
120	100	81.8	79	90.3	92.5
144	100	79.9	73.4	88.4	91.2
168	100	76.1	69.5	86.9	89.8
192	100	72.5	66	85.7	88.4
216	100	69.2	62.1	84.5	87.5

Prominent effect of light can be seen on the different color extracts. The % retention of tomato extracts (lycopene) of control was 100. For UV treated sample it was 90.2, for sunlight it was 91.9, for tube light it was 94.6 while maximum was for darkness at 96.9. After 216 hr the values of % retention were like: 100, 49.6, 51.9, 64.1and 81.4 for control, UV, sunlight, tube-light and darkness respectively.

The maximum loss was in the UV treated extracts as % retention declined from 90.2 at 24 hours to 49.6 after 216 hours, followed by the decline in% retention of sun light treated extract declined from 91.9 to 51.9, however the decrease in % retention under tube light treated extract was 94.6. to 64.1.

For carrot extract % retention was 100, 91.2, 88, 90.3 and 96.5 for control, UV, sunlight, tube-light, and darkness respectively after 24 hours. It declined continuously and reached 33.3, 36.1, 44.9 and 48.1 after 216 hours for UV, sunlight tube-light and darkness respectively. The destruction of coloring pigments as reported in terms of decline in % retention decreased in following order ; UV > sunlight > darkness > tube-light.

The% retention for marigold extracts for UV treatment decreased from 94.5after 24 hours to 69.2 after 216 hours. For sunlight treatment the decrease reported was 94.0 to 69.2, for tube-light 97.6 to 84.5 and for darkness the decline observed was 98.3 to 87.5.

From above data it can be concluded that most stable extract under light is marigold followed by tomato and least stable is carrot.

Heat effect: Results pertaining to heating of different extracts under different time and temperature are present in following **tables 4-6**.

TABLE 4: EFFECT OF HEATING TOMATO EXTRACT AT DIFFERENT TEMPERATURE

Time	Control	40°C	50°C	60°C	70°C	80°C
5 min	100	96.1	93.7	89.9	83.3	81.1
10 min	100	95.3	90.1	77.4	72.5	70.0
15 min	100	86.3	77.4	71.4	67.4	61.5
20 min	100	68.1	60.6	53.0	50.3	45.6

TABLE 5: EFFECT OF HEATING CARROT EXTRACT AT DIFFERENT TEMPERATURE

Time	Control	40°C	50°C	60°C	70°C	80°C
5 min	100	95.3	92.6	72.6	61.7	55.8
10 min	100	88.0	70.8	58.8	53.4	49.6
15 min	100	71.9	63.1	51.7	48.4	46.7
20 min	100	59.7	58.8	44.3	39.3	36.0

TABLE 6: EFFECT OF HEATING MARIGOLD EXTRACT AT DIFFERENT TEMPERATURE

Time	Control	40°C	50°C	60°C	70°C	80°C
5 min	100	97.6	96.7	95.8	94.5	94
10 min	100	95.9	94.7	92.4	91.2	89.8
15 min	100	92.7	91.1	90	88.3	86.3
20 min	100	88.4	87.5	86.9	84.3	82.9

Tomato extract (lycopene) when heated for 5 min at different temperature, viz., 40, 50, 60, 70, 80°C, % retention value decreased from 100 to 96.1 at 40ºC, 93.7 at 50ºC, 89.9 at 60ºC, 83.6 at 70°C and 81.1 at 80°C. After 10 minutes of heating at same temperatures the values of % retention were 95.3 at 40ºC, 90.1 at 50°C, 77.4 at 60°C, 72.5 at 70°C and 70 at 80°C. There was further decrease in % retention after heating for 15 min and the reading after 20 minutes of heating same were 68.1 at 40ºC, 60.6 at 50º, 53 at 60ºC,50.3 at 70ºC and 45.6 at 80ºC respectively.

The extract of carrot (β-carotene) showed decrease in % retention when heated for 5 to20 minutes (at the intervals of 5, 10, 15 and 20 minutes) from 95.3 to 59.7 at 40ºC, 92.6 to 58.8 at 50ºC, 72.6 to 44.3 at 60ºC, 61.7 to 39.3 at 70ºC and 55.8 to 36 at 80°C.

Similarly, for marigold extract (lutein) % retention when heated at 40ºC, (at the intervals of 5, 10, 15 and 20 minutes) decreased from 97.6 to 88.4, at 50^{o}C the decline was from 96.7 to 87.5, at 60^{o}C decline was from 95.5 to 86.9, at 70^{0}C decline was from 94.5 to 84.3and at 80^{o}C it was from 94.0 to 82.9.

Marigold extract (lutein) is most heat stable followed by tomato extract (lycopene) then carrot extracts (β-carotene).

Most investigators have reported the stability of solvent extracted pigments in hours whilst others have reported in 1 to 2 days. Craft (1992) Spectro-phometrically monitored 32% to 97% retention of β-carotene depending on the extraction solvent type over a 10 day period. These findings were consistent with those reported by Pesek and Warthesen (1988), Kopas-Line and Warthesen (1995) as a general trend. According to Pesek and Warthesen (1987), the carotenoids in a carrot juice system retained only 25 % of the original color after 4 days of light exposure at 4 ºC.

CONCLUSION: The major cause of carotenoid destruction during processing and storage is oxidation. Whatever processing method may be chosen retention of carotenoids decreases with longer processing times, higher processing temperature. High temperature and short time is good alternative. Exclusion of oxygen, protection from light and low temperature keep carotenoids protected. The stability of carotenoids differ in different foods, thus optimum conditions for pigment retention differ from one food to another.

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