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Short communication

Chromaticity Stability of Beta-Carotene from Pumpkin (Cucurbita Moschata)

Rashidi Othman[1]*, Ainaa Eliah Abu Bakar[1], Noraini Mahmad[1], Norshazila Shahidan[2]

[1] *International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, KAED, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia.*

[2] *School of Food Industry, Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin, Besut Campus, 22200, Besut, Terengganu, Malaysia.*

**Corresponding author E-mail: rashidi@iium.edu.my*

Abstract

This study focused on the chromaticity of Beta-carotene extracted from pumpkin *(Cucurbita moschata)* by using extracts of *ethyl acetate*. Beta-carotene was then being dissolved in the PLA coating solution and was then being tested with different treatments which were UV-A exposed and different temperature within a period of time. For UV-A, beta-carotene was exposed until 8 hours, while for the different temperature (-25°C, RT, and 40°C), it was exposed until 6 hours. For the CIElab result, the colour changes due to the beta-carotene being exposed under a UV-A light and showed the result was sensitive to UV-A light exposure. For a different temperature, it can be concluded that, it was a slightly different within the temperatures selected due to the Beta-carotene was stable with the temperatures, eventhough it was exposed for a longer time. As a conclusion, it can be said that, beta-carotene was sensitive and not stable with the light especially rather than the temperature.

Keywords: *Beta-carotene, Chromaticity, and Stability.*

1. Introduction

Colours play some important roles as to attract the consumers to buy the products. With today's sophisticated formulations and transparent packaging, it is important to improve and maintain the product's key visual and sensorial appearance, due to consumers were preferred colour to a specific item [1]. By applying the colour to the products, it will help the consumers to differentiate the products easily by making different colours on the different products. It can help in imparting the colour to the variety of materials which can described as a substrate by enhancing the highly colorant on the products [2]. By doing that, the consumers are eager to know the real products inside the colourful packaging or the colour of the products itself. By choosing high quality colours to the products, it is one of the basis factor for successfully communicating from the brands of the product to the consumers. Colours as a means of behaviour-centred communication [3].

Carotenoids form one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables. Carotenoids are plant pigments responsible for red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colours of some birds, insects, fish, and crustaceans. Carotenoids are a class of naturally occurring pigments which play a vital role towards the photosynthesis of plants, photosynthetic bacteria and algae. It can also be found in some of the non-photosynthetic bacteria, yeasts and crustaceans [4]. The colorant pigment from the animals can be produced in a small scale due to the animals cannot synthesize carotenoids. In addition, the familiar examples of carotenoid coloration come from the oranges of carrots, citrus fruits, the pinks from flamingos and salmon, and the reds of peppers and tomatoes. Carotenoids can be divided into two parts, which is hydrocarbon carotenes (contain carbon and oxygen) and oxygenated xanthophylls (contain carbon, oxygen, and hydrocarbon).

Beta carotene, alpha carotene, lycopene, lutein and cryptoxanthin are represented in the diet and human body, which is close to 90% of the carotenoids. Over 600 carotenoids have been found in nature, 40 are present in a typical human diet itself and only 14 from them considered as metabolites that have been identified in blood and tissues [5].

Beta-carotene is an orange coloured compound which comes from the carotenoid family and mostly being found in the fruits and vegetables, for example pumpkins, tomatoes, mangoes, carrots and spinach [6]. The name carotene is derived from the word "carota", which comes from the carrot due to the chemical was first being discovered via crystallization of carrot roots in 1831. The free radical scavenging nature of beta-carotene is involving in the trapping of singlet oxygen, which is provide the overall reducing environment in the hepatic tissues, and can help in anti-cancer potential for the long term exposure of beta-carotene [7]. Beta-carotene is the most abundant form of vitamin A in fruits and vegetables. By consuming beta-carotene, it will help in maintaining human health and well-being due to the enzymatically converted to retinol (Vitamin A) in the human intestine by the beta-carotene 15, 15'monoxygenase and directly contribute to the most common and safe source of vitamin A. Besides its pro vitamin A function, beta-carotene can perform and have ability to protect human health from disease such as can exhibit antioxidant activity), lower metabolic syndrome in middle-aged adults can enhance immune system performance and can reduce the risk of cardiovascular diseases. More recently, carotenoids can against serious disorders such as cancer [8]. Beta-carotene is the most widely studied carotenoid and is one of the major carotenoids in our diet and in human blood and tissues. Beta-carotene is an effective source of vitamin A in both conventional food and dietary supplements.

Pumpkin is considering as a fruit botanically, even though it is commonly known by the consumers as vegetable terms. The pumpkin is included in the category of functional vegetables because of the highly rich in phenolics, flavonoids, vitamins (including beta-carotene, vitamin A alpha-tocopherol, and vitamin C), carbohydrates, and amino acids. Seeds and flesh of pumpkin are commonly used for medicinal purposes and culinary. Pumpkin has their own multiple uses as fruits, vegetables, therapeutic and medicinal properties. It is also can consider as the traditional Chinese medicine. It is important to know and use less known species in order to fulfil all the needs for the future generations. In addition, pumpkin is one of the species which are considered as the healthy fruit and can meet all the conditions needed, especially in terms of nutritional value. Carotenoids are the most responsible natural plant pigments, which contribute to the orange colour of pumpkin. Three species of pumpkin that are reported to consist of beta-carotene, which are (Cucurbita pepo, C. maxima, and C. moschata), which each of it have a value of beta-carotene (0.06-7.4mg/100g), alpha-carotene (0-7.5mg/100g) and lutein (0-17mg/100g). Pumpkin is considering as an extensively used as vegetable processed food and stock feed in different part of the world. In some studies, done by Azizah *et al.* (2009) reported that pumpkins consist of β-carotene and lycopene. According to Zaharah *et al.* (2006), the area for pumpkins plantation in Malaysia was around 138 ha, where pumpkin abundantly planted in Kelantan (79.6 ha), Terengganu (59.6 ha) and Johor (93.5 ha). Although very little information is available about the production statistics of pumpkin, the average yield of fruit is reported to be 25000 kg/ha. The major pumpkin producing states in Malaysia are Terengganu, Kelantan, Perak and Kedah (MARDI, 2007).

2. Materials and Methods

2.1. Chromaticity stability test

For some natural product extracts, the colour for stability test was investigated from the previous studies where it took CIELAB values as an index [9]. Spectrophotometric analysis was also adapted to similar research [10]. The sample readings were averaged, and colour of each sample was expressed in terms of CIE values for lightness (L*), red-green (a*), yellow-blue (b*), chroma (C*ab) and hue (h ab). a* which takes positive values depicts a shift towards red whereas, negative a-value depicts a shift towards green. b* which takes positive value depicts a shift towards yellow whereas, negative b-value depicts a shift towards blue Carotenoids form one of the most important classes of plant pigments and play a crucial role in defining the quality.

2.2. UV light exposure

The sample with different concentration (1 mg/mL, 2 mg/mL, and 3 mg/mL) were dissolved in Polylactic Acid (PLA) and were tested under UV light after 8 hours exposed. Then, the coating will be read with the CIE lab.

2.3. Temperature

For temperature stability test, the sample were conducted with different points which were (-25 ºC, RT, and 40 ºC). with the same concentration of samples were used (1 mg/mL, 2 mg/mL, and 3 mg/mL). The coating was tested in the dryer. The CIE lab reading were taken within 6 hours of exposing through the different temperature.

2.4. Coating on Poly Lactic Alcohol (PLA)

4 g of sodium hydroxide (NaOH) powder was measured and dissolved in 100ml of ultra-pure water (18Ω) with the ratio of 1:10. The solvent then was heated up on the hotplate at 70-80ºC and stirred by using the magnetic stirrer. Then, the sample was mixed with the solvent with different concentration, (1mg/mL), (2mg/mL), and (3mg/mL). The sample and the solvent were poured in the mold and was kept in overnight.

3.5. Statistical Analysis

The results obtained in this study are represented as mean values of three individual triplicates. One-way analysis of variance was performed with a statistics software programme, XLSTAT version 2014. The significant differences between the mean values were determined by using the Dunnett’s (two sided) multiple range test at a significance level of $p<0.0001$.

3. Results and Discussion

3.1. Chromaticity of UV-A stability test

Table 1 showed the CIElab reading on different concentration of beta-carotene (1mg/mL, 2mg/mL, 3mg/mL) that has being exposed under the UV-A light. The reading for L* showed above 100 for every beta-carotene concentration. For the 1mg/ml concentration, a* value showed inconsistent when beta-carotene been exposed to 2 and 4 hours. The number showed less than -10 when it reached to 6 hours. For the 2mg/mL and 3mg/mL concentrations, the a* value showed below than -10 and decreased from the 0 hour to the 6 hour. The degradation of color showed a small degree of changes as compared to the 1mg/ml concentration. The color changes start right after the samples being exposed for 2 hours under the UV-A light. The final color for 6 hours of exposed was slightly yellowish as compared to 0 hour.

Table 1: CIELab reading on different beta-carotene concentration with exposure to UV-A light.

Time of exposure	L*	a*	b*	C	h
1 mg/mL beta-carotene					
Hour 0	104.65^{c}	-8.65^{c}	2.55^{c}	9.28^{c}	161.73^{c}
Hour 2	396.42^{b}	-158.89^{b}	43.95^{b}	165.53^{b}	164.59^{b}
Hour 4	410.80^{a}	-159.01^{a}	58.88^{a}	169.65^{a}	159.71^{a}
Hour 6	409.82^{a}	-154.00^{a}	60.26^{a}	165.61^{a}	158.54^{a}
Hour 8	103.66^{c}	-5.40^{c}	6.29^{c}	8.60^{c}	125.93^{c}
2 mg/mL beta-carotene					
Hour 0	105.25^{a}	-7.38^{b}	3.08^{b}	8.00^{a}	157.28^{a}
Hour 2	101.40^{b}	-3.09^{a}	6.68^{ab}	7.37^{a}	116.85^{b}
Hour 4	101.32^{b}	-3.80^{a}	8.70^{a}	9.49^{a}	113.59^{b}
Hour 6	101.33^{b}	-3.79^{a}	8.76^{a}	9.54^{a}	113.37^{b}
Hour 8	101.33^{b}	-3.77^{a}	8.70^{a}	9.48^{a}	113.40^{b}
3 mg/mL beta-carotene					
Hour 0	105.21^{a}	-7.18^{b}	3.00^{b}	7.78^{b}	157.27^{a}
Hour 2	101.33^{b}	-3.75^{a}	8.70^{a}	9.48^{a}	113.33^{b}
Hour 4	101.23^{b}	-3.64^{a}	9.52^{a}	10.21^{a}	111.24^{b}
Hour 6	101.33^{b}	-3.76^{a}	8.70^{a}	9.48^{a}	113.35^{b}
Hour 8	101.33^{b}	-3.75^{a}	8.70^{a}	9.48^{a}	113.33^{b}

Data expressed as the mean (n=3) of triplicates with the significant differences at 0.01% level ($P<0.0001$) utilising One-Way Anova. ^{a,b,c,d,e} shows significant difference in same column.

The value of b* for the (1, 2, 3 mg/mL) of concentrations showed a positive result, and the value was below 10 for 2 and 3 mg/ml. It can be concluded from this experiment, the carotenoids play their role in undergo degradation of color for example in bleaching, when it has been exposed to prolonged UV-irradiation. This is caused by the photon energy input in the UV itself which was extremely dependent from the free radicals-madiated. From this experiment, the molecular of beta-carotene affected the bleaching level of color.

3.2. Chromaticity of stability test on different temperature

Table 2 showed the CIElab reading on different of beta-carotene (1mg/mL, 2mg/mL, 3mg/mL) at different temperature which were (-25℃, RT, and 40℃).The value for L* of 1mg/ml on -25 ºC were more than 200 and slowly showed decreasing in a* and b* value. The value for 0 hour and 6 hours were slightly different. For the 2mg/ml, the value of L* showed increased until the 4 hours and went down when the time reached 6 hours. The a* value showed the negative result for the 0 hour, and showed a positive result on the following hours. For the 3mg/ml, the a* value showed a negative results and the L* value were not more than 30. For the 1mg/ml at RT condition, the L* values were decreasing in a slightly number and the a* value were in negative results, and the b* value declined towards the 6 hours of exposed. For 2mg/ml, the L* were increasingly until 6 hours and the a* value showed decreasingly. For the 40ºC condition, the value of a* were in negative results and the values of b* were in positive results. Beta-carotene promoted a potential health beneficial to human especially in terms of natural colorants that can help in changing the used of synthetic colorants.

The carotenoid oxidation played an important role in maintaining the colour changes and bioactivity of the pigments. The level of air circulation in the sample was one of the major decreasing for the colour changed because of the interaction from the oxygen as well as the beta-carotene. From the result, it showed that beta-carotene with PLA tested lead towards the colour changed due to the exposure with different temperature. Due to that, the higher the temperature tested, the more the colour changed. Besides, the concentration of Beta-carotene used also affected the exposure to the temperature. Based on the result, the colour changed drastically from the 2 and 3 mg/ml of concentration as compared to the 1mg/ml of concentration. From that, the longer the Beta-carotene being exposed with the highest concentration to the high temperature, the more colour changed. From [11] & [12], they found that, the formation of cis-isomers and oxidation products that determined by the HPLC due to the two hours of heating of pure beta-carotene at 180ºC.

4. Conclusion

For the stability test of beta-carotene, the samples were being set on different time of exposure, which were on UV-A light and with different temperature in Polylactic acid (PLA) polymer formed. The test that has been carried out and were tested with the CIElab color space as to measure the color changes from beta-carotene treatment in a specific time exposed. For UV-A treatment, beta-carotene showed a color changes from yellowish color to a slightly yellow color, from the initial of the exposure until the ends. For the better future, it can be help by exploring on the beneficial compound that can help in making the pure beta-carotene become stable on the environment effect**,** due to the beta-carotene itself cannot undergoes their own stability because of the carotenoids are easily oxidised by oxygen, light, and heat.

Table 2: CIELab reading on different beta-carotene concentration with exposure on different temperature.

Time of exposure	L*	a*	b*	C	h
1 mg/mL beta-carotene (Temperature : -25ºC)					
Hour 0	237.43^{a}	-112.05^{b}	181.59^{a}	213.38^{a}	121.69^{a}
Hour 1	236.13^{ab}	-108.74^{ab}	171.72^{ab}	203.25^{ab}	122.35^{ab}
Hour 2	236.08^{ab}	-108.80^{ab}	173.29^{ab}	204.62^{ab}	122.12^{b}
Hour 4	236.04^{ab}	-108.38^{a}	171.60^{ab}	202.96^{ab}	122.28^{ab}
Hour 6	235.82^{ab}	-108.25^{a}	170.23^{b}	201.73^{b}	122.45^{ab}
2 mg/mL beta-carotene (Temperature : -25ºC)					
Hour 0	109.94^{c}	-6.35^{bc}	-34.58^{cd}	35.34^{bcde}	259.83^{ab}
Hour 1	254.70^{a}	1.42^{bc}	14.71^{ab}	14.77^{cde}	84.48^{d}
Hour 2	250.21^{a}	6.96^{b}	8.26^{abc}	10.81^{e}	49.61^{d}
Hour 4	253.47^{a}	2.86^{bc}	12.61^{abc}	13.25^{d}	74.86^{d}
Hour 6	198.55^{b}	48.26^{a}	-43.48^{d}	67.83^{ab}	323.10^{a}
3 mg/mL beta-carotene (Temperature : -25ºC)					
Hour 0	23.10^{a}	-16.77^{a}	39.68^{ab}	43.08^{ab}	121.69^{a}
Hour 1	23.18^{a}	-15.92^{a}	39.81^{ab}	42.90^{ab}	111.79^{a}
Hour 2	27.55^{a}	-4.00^{a}	29.36^{ab}	30.81^{b}	112.94^{a}
Hour 4	26.87^{a}	-6.93^{a}	46.12^{ab}	49.55^{ab}	111.53^{a}
Hour 6	28.16^{a}	-18.80^{a}	48.33^{ab}	51.86 ^{ab}	111.27^{a}
Time of exposure	L*	a*	b*	C	h
1 mg/mL beta-carotene (Temperature :RT)					
Hour 0	235.23^{ab}	-108.37^{a}	170.16^{b}	201.74^{b}	122.4^{abc}
Hour 1	233.40^{ab}	-107.88^{a}	168.11^{b}	199.75^{b}	122.70^{ab}
Hour 2	233.42^{ab}	-107.60^{a}	167.06^{b}	198.72^{b}	122.78^{ab}
Hour 4	231.57^{b}	-106.82^{a}	163.90^{b}	195.63^{b}	123.10^{a}
Hour 6	234.32^{ab}	-107.99^{a}	168.61^{b}	200.23^{b}	122.64^{ab}
2 mg/mL beta-carotene (Temperature :RT)					
Hour 0	226.68^{ab}	54.11^{a}	-10.42^{bcd}	55.94^{abc}	231.74^{abc}
Hour 1	201.46^{b}	61.90^{a}	-51.44^{d}	85.06^{a}	328.92^{a}
Hour 2	249.93^{a}	-25.47^{c}	10.41^{abc}	36.09^{bcde}	166.30^{bcd}
Hour 4	252.76^{a}	-24.47^{c}	17.20^{ab}	37.09^{bcde}	155.25^{bcd}
Hour 6	254.69^{a}	1.22b^{c}	15.78^{ab}	15.83^{cde}	85.67^{d}
3 mg/mL beta-carotene (Temperature :RT)					
Hour 0	27.59^{a}	-18.55^{a}	47.35^{ab}	50.86^{ab}	111.39^{a}
Hour 1	26.84^{a}	-7.20^{a}	46.08^{ab}	49.61^{ab}	111.81^{a}
Hour 2	24.99^{a}	-17.39^{a}	42.91^{ab}	46.30^{ab}	112.12^{a}
Hour 4	23.12^{a}	-16.60^{a}	39.70^{ab}	43.03^{ab}	112.68^{a}
Hour 6	29.08^{a}	-20.51^{a}	37.35^{ab}	43.06^{ab}	118.80^{a}
Time of exposure	L*	a*	b*	C	h
1 mg/mL beta-carotene (Temperature: 40ºC)					
Hour 0	235.23^{ab}	-108.37^{a}	170.16^{b}	201.74^{b}	122.49^{abc}
Hour 1	233.40^{ab}	-107.87^{a}	168.44^{b}	200.02^{b}	122.64^{ab}
Hour 2	233.40^{ab}	-107.87^{a}	168.44^{b}	200.02^{b}	122.64^{ab}
Hour 4	234.32^{b}	-107.99^{a}	168.61^{b}	200.23^{b}	122.64^{ab}
Hour 6	234.32^{ab}	-107.98^{a}	168.61^{b}	200.23^{b}	122.64^{ab}
2 mg/mL beta-carotene (Temperature: 40ºC)					
Hour 0	29.93^{d}	-6.89^{bc}	51.37^{a}	51.85^{abcde}	97.64^{cd}
Hour 1	30.41^{d}	-3.59^{bc}	52.20^{a}	52.65^{abcde}	94.07^{d}
Hour 2	23.10^{d}	-16.77^{bc}	39.68^{a}	43.08^{bcde}	112.91^{cd}
Hour 4	27.60^{d}	-10.71^{bc}	47.38^{a}	48.89^{abcde}	103.33^{cd}
Hour 6	24.56^{d}	-2.50^{bc}	42.19^{a}	42.26^{bcde}	93.39^{cd}
3 mg/mL beta-carotene (Temperature: 40ºC)					
Hour 0	23.12^{a}	-16.59^{a}	39.70^{ab}	43.03^{ab}	112.68^{ab}
Hour 1	33.20^{a}	-20.98^{a}	56.92^{a}	60.66^{a}	110.24^{a}
Hour 2	26.51^{a}	-14.70^{a}	32.45^{ab}	35.72^{ab}	115.84^{ab}
Hour 4	26.49^{a}	-14.81^{a}	31.18^{ab}	34.55^{ab}	116.26^{ab}
Hour 6	23.10^{a}	-16.77^{a}	39.68^{ab}	43.08^{ab}	112.91^{ab}

Data expressed as the mean (n=3) of triplicates with the significant differences at 0.01% level (P<0.0001) utilising One-Way Anova. ^{a,b,c,d,e} *shows significant difference in same column.*

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