Extraction and Stability of Daucus Carota Extract

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Abstract

Rapid solvent extraction technique was employed to effectively extract carotenoid, the pigment of Daucus carota. The extraction was done using hexane, acetone and methanol mixture in the ratio (50:25:25 v/v/v). The stability of the extracted pigments was studied under different processing and storage conditions. The carrot samples were pretreated by blanching, by 0.2% ammonium sulphite and combination of blanching and 0.2% ammonium-sulphite treatment. All pigment samples were stored at 25^oC in the presence of light, and in the dark cupboard; at 4^oC and 40^oC. Results revealed that the untreated samples had the least concentration of carotenoid (5.8782mmol/L); the blanched carrot sample had the highest concentration(6.3013mmol/L), while the sample pretreated with 0.2% ammonium sulphite and that with the combined blanching and 0.2% ammonium sulphite treatment were found to be 6.0321 and 5.929mmol/L respectively. The results revealed that the blanched carrot had the highest percentage pigment retention (65.11%) after 6-day storage at 4^oC.

Key words: Carrot; Carotenoid; Antioxidant; Blanching; Storage Stability

1. Introduction

Carrots are by far the richest source of carotenoids-just one cup provides 16,679 IUs of beta-carotene and 3,432 Res (retinol equivalents), or roughly 686.3%, the RDA for vitamin A (Wikipedia.org, 2008). High carotenoid intake has been linked with 20% decrease in post-menopausal breast cancer and up to 50% (Canfield et al, 1993) decrease in the incidence of cancers of bladders, cervix, prostate, colon, larynx and esophagus. Extensive human studies suggest that a diet including as little as one carrot per day could conceivably cut the rate of lung cancer in half. From studies, heavy long term cigarette smoker was given synthetic beta-carotene, and it did not appear to prevent them from developing lung cancer. Synthetic beta-carotene is not only biochemically identical to the real stuff found in carrots, but scientist now think that carrots protective effects are the result of a team effort among several substances abundant in carrots, including alpha-carotene. Beta-carotene is an excellent source of antioxidant compounds, and the richest vegetable source of pro-vitamin A carotene. Carrots antioxidant compounds help protect against cardiovascular disease and cancer and also promote good vision, especially night vision (Anonymous, 2008). Vegetables and fruits is a category of foods where great variety of carotenoids is found (Goodwin, 1976).

Carotenoids are organic pigments that are naturally occurring in chromoplasts of plants and some other photosynthetic organisms like algae's, some type of fungus, and some bacteria. There are over 600 known carotenoids (Baloch et al, 1997), and divided into two classes, xanthophyll and carotenes. They absorb blue light. Carotenoids serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photo damage. In humans, carotenoids such as beta-carotene are a precursor to vitamin A, a pigment essential for good vision, and carotenoids can also act as antioxidants (Brithon, 1995). People consuming diets rich in carotenoids from natural foods, such as fruits and vegetables, are healthier and have lower mortality from a number of chronic illness. However, a recent meta-analysis of 68 reliable antioxidant supplementation experiments involving a total of 232,606 individuals concluded that consuming additional beta-carotene from supplements is unlikely to be beneficial and may actually be harmful, although this conclusion may be due to the inclusion of studies involving smokers (Anonymous, 2004).

Antioxidants in the simplest sense are substances capable of slowing the rate of oxidation in autoxidation materials. The choice of an antioxidant for a given purpose is governed by the requirements of the system and the characteristics of the antioxidants available. Desirable features of antioxidants include effectiveness at low concentrations, being nontoxic, convenience, safety and low cost. They are particularly important in the protection of foods, rubber products, petroleum products, lubricants, plastics, cosmetics, and some pharmaceuticals. Antioxidants used in foods must be limited to substances generally recognized s safe. The presence of an antioxidant in a food must be declared on the product label listing the antioxidant and the purpose of its use. Not all countries allow antioxidants in food, and up to date regulatory information must be obtained for each country. The selection of antioxidants and the level required for optimum effectiveness is based on the substrate, it is the method of preparation, packaging, and distribution. This paper is aimed at determining the antioxidant property of Daucus Carota extract (carrot) as well as studying the stability of the extracted pigment under different storage conditions.

2.0 MATERIALS AND METHODS

2.1 Sample Preparation

The sample of Daucus Carota (carrot) were first rinsed in water to remove any dirt and unwanted material and then allowed to drain. The carrot was skinned and sliced into smaller sizes, then crushed by means of a homogenizer (Warring Blender) for two minutes with little water added to increase the surface area for efficient treatment. About 100g of the sample was weighed using an electronic weighing balance and placed in four separate beakers labeled A, B, C and D. Sample A is the untreated is the untreated carrot, Sample B was subjected to steam blanching for two minutes and allowed to cool. A 0.2% ammonium sulphite solution was prepared by weighing 20g ammonium sulphite and made to 100ml distilled water. The solution was added to sample C. To sample D, the 0.2M ammonium sulphite solution was also added and then subjected to blanching for 2 minutes to serve as a combined pretreatment process.

2.2 Extraction of Carotenoid Pigment

For each of the four samples, a solution of hexane, acetone and methanol was prepared in the ratio (50:25:25 v/v/v), and then added to each sample, followed by 20mL of distilled water to separate the solution into two layers. This was shaken thoroughly and allowed to settle for 15 minutes. Two layers were formed, an upper non-polar layer and a bottom polar layer, the bottom layer was the beta-carotene which is the carotenoid pigment (lipid soluble extract) and water. The mixture was filtered in order to obtain the residue (pigments) and the water soluble pigment extract.

2.3 Determination of Antioxidant Property and Stability

In order to determine the antioxidant property from the water-soluble extract, each resulting solution was poured into four test tubes; and 1mL of 0.1 M cone. H_2SO_4 was added. To each of the test tube 2mL of ethanol was also added and heated in a Bunsen burner for 3 minutes and allowed to cool. The four tubes were then subjected to boiling water for 10minutes, in a water bath and allowed to cool. The upper layer was then transferred into separate test tubes and their absorbance was read at 540nm using UV-Spectrophotometer.

Pigment extract were analyzed for stability test by using a brand UV-spectrophotometer (perkin-elmer 701), for the stability test, the effect of light, dark, temperature, blanching 0.2% ammonium sulphite treatment, both blanching and 0.2% ammonium sulphite treatment were studied. For each condition, pigment extracts were stored in a small bottle. Dark conditions were provided by wrapping a glass bottle with several layers of aluminum foil, while the light effect was provided by exposing samples to fluorescent room light during the day. The untreated samples (sample A) were stored at 25^{o}C light and 25^{o}C dark conditions, 4^{o}C, and 40^{o}C for 5 days for the temperature effects. Similarly, the pretreated samples (B, C and D) were also stored at the same temperature conditions as above and their absorbance were read at 450nm and then converted to % retention for accurate comparison among the treated samples.

3.0 Results and Discussion

The analysis of the results obtained from the four samples are presented in Table1-5

Table 1: Antioxidant property of the extracted pigments

Sample	Concentration(mmol/L)
A	5.8782
B	6.3013
C	6.03205
D	5.929

Table 1 shows that sample A (untreated carrot) has the least concentration of antioxidants (5.8782mmol/L) and sample B (blanched carrot) had the highest antioxidant concentration of 6.3013mmol/L. This could be attributed to the fact that blanched carrot liberates natural

antioxidant and phenolic compounds. Whereas, sample C (ammonium sulphite treated carrot) and sample D (combined blanching and ammonium sulphite carrot) had a concentration of 6.03205mmol/L and 5.929mmol/L, respectively.

Table 2: Percentage Retention of carrot Pigment in untreated sample at different Temperatures and Storage Conditions.

Time(days)	%Retention			
	25°C light*	25°C dark+	4°C refig**	40°C Oven*+
0	100	100	100	100
1	68.7	67.61	78.74	70.88
2	65.43	66.53	54.53	39.26
3	55.8	55.99	38.17	0.16
4	0.0033	0.22	21.8	0.15
5	0.00	0.33	16.36	0.13
6	0.001	0.17	12.00	0.11

*Sample stored under light
+Sample stored in a dark cupboard
**Sample stored under refrigeration
*+Sample stored under oven

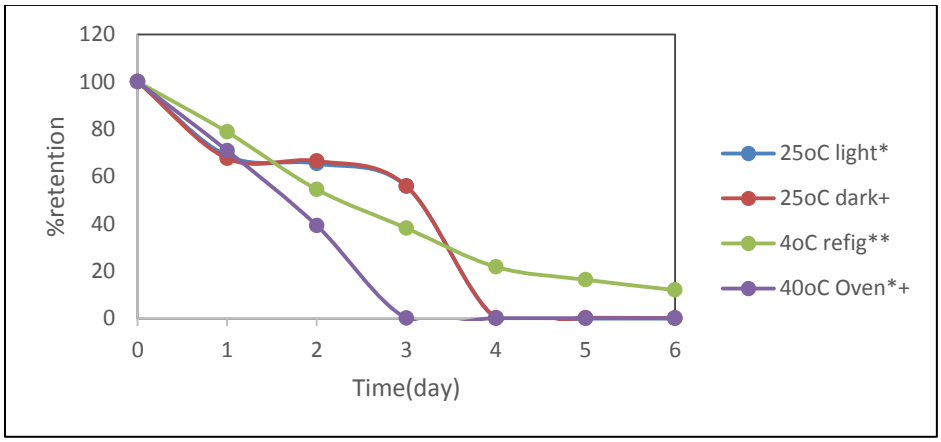

Figure 1: Percentage Retention of carrot Pigment in Untreated Sample at Different Temperatures and Storage Conditions

For the untreated carrot samples, results showed that the percent retention of pigment after 6 days' storage period was 12.0% for samples stored at 4°C. All color was lost after 3 days for all samples stored at 25°C. After 2 days there was a significant difference between the samples treated with ammonium sulphite solution stored at 40°C (Fig 1). It can also be observed from the result that storing the carrot sample at 4°C significantly improved pigment retention and

indicating that as the time increases from 1 to 6 days, the pigment decreases (degraded) simultaneously.

Table 3: Percentage Retention of Carrot Pigment In Blanched Sample at Different Temperatures and Storage Conditions

Time(days)	%Retention			
	25°C light*	25°C dark+	4°C refig**	40°C Oven*+
0	100	100	100	100
1	83.42	82.4	88.5	81.38
2	75.28	72.23	83.42	66.12
3	65.11	61.04	79.35	54.93
4	55.95	54.93	74.26	0.41
5	0.2	0.2	67.14	0.31
6	0.1	0.1	65.11	0.2

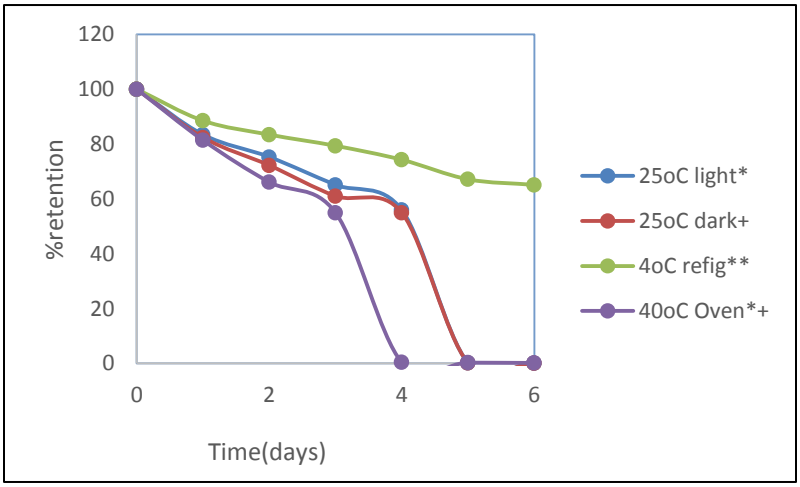

Figure 2: Percentage Retention of Blanched Carrot Pigment (sample B) at Different Storage Temperatures

For the blanched carrot samples, result showed that the percent retention of pigment after 6days storage period at 4°C was 65.1% and 25°C light and 25°C dark samples lost all color after 4days, while 40°C samples lost all color after 3 days (Fig.2). The data showed that storage under light and in dark conditions had no significant effect on pigment retention. It can also be deduced from result above that, blanching the carrot sample had a significant effect on carrot pigment for all temperatures indicating the possible involvement of carotenoid destroying enzymes in untreated sample.

Table 4: Percentage Retention of Pigment in ammonium sulphite treated carrot sample at different Temperatures and storage conditions

Time(days)	%Retention

	25°C light*	25°C dark+	4°C refig**	40°C Oven*+
0	100	100	100	100
1	86.4	87.89	91.92	82.04
2	66.1	76.09	84.38	67.91
3	57.4	66.42	82.15	56.32
4	51.01	56.54	76.63	0.42
5	0.2	0.21	69.08	0.32
6	0.1	0.1	57.4	0.1

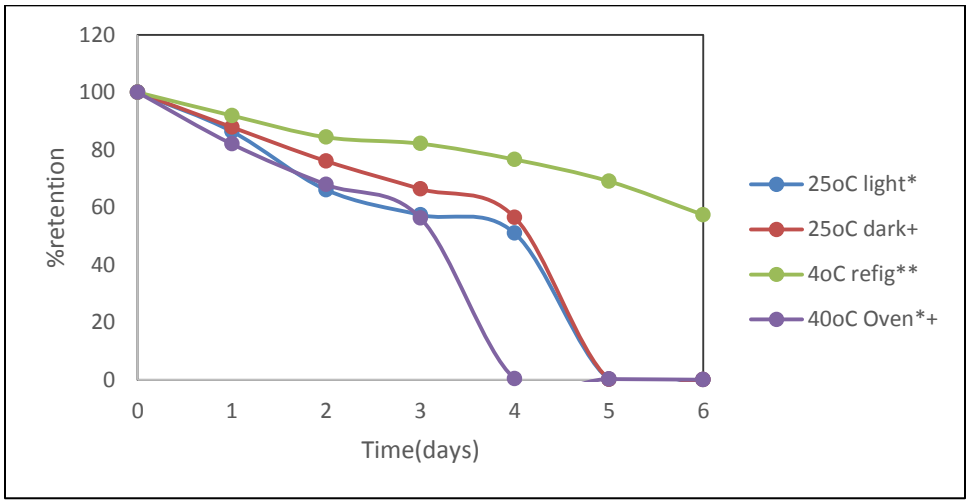

Figure 3: Percentage retention of pigment in Ammonium sulphite treated carrot sample at different Temperatures and Storage Conditions

For the ammonium treated sample, the percentage retention of pigment after 6days storage at 4°C was 57·4%. All color was lost after 4 days for 25°C light and 25°C dark samples, while 40°C sample lost all color after 3 days (Fig.3)

Table 5: Percentage Retention of Pigment in Ammonium Sulphite treated Blanched Carrot Samples at Different Temperatures and Storage Conditions.

Time(days)	%Retention			
	25°C light*	25°C dark+	4°C refig**	40°C Oven*+
0	100	100	100	100
1	77.2	73.51	83.68	68.11
2	68.65	68.11	62.7	42.16
3	60.76	55.57	42.16	0.43
4	0.4	0.43	29.2	0.42
5	0.32	0.21	23.78	0.32
6	0.22	0.23	19.5	0.21

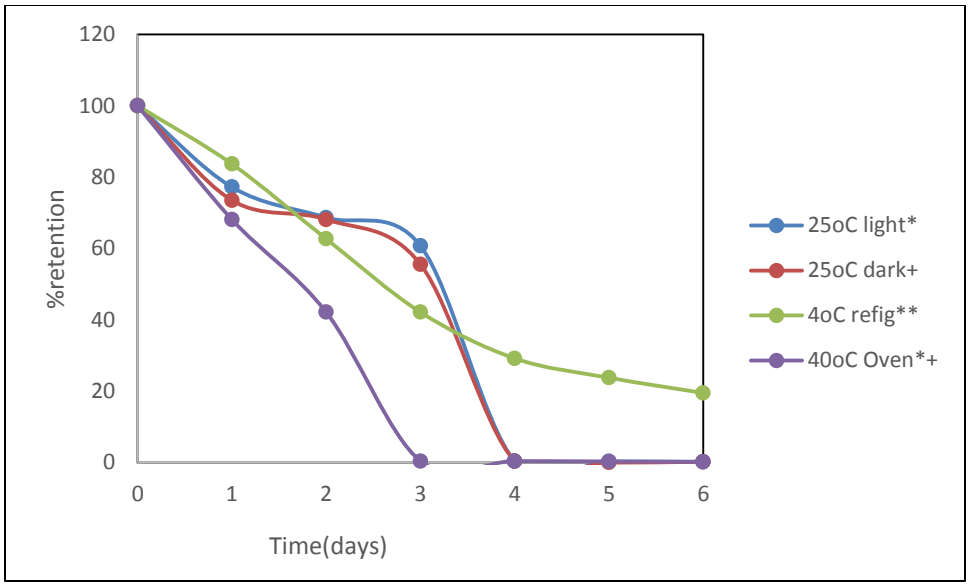

Figure 4: Percentage Retention of Pigment in Ammonium Sulphite treated Blanched Carrot Sample at Different Temperatures and Storage Conditions

For both blanched and ammonium sulphite treated carrot samples, result showed that the percent after 6days storage at 40^{o}C was 19.5%. All color was lost after 3days for 25^{o}C light and 25^{o}C dark conditions while 40^{o}C sample lost all color after 2days (Fig.4). The combined blanching and Ammonium Sulphite treatment on pigment retention was performed to determine if it has any synergistic effect, but the data showed very similar result with untreated carrot.

4.0 Summary and Conclusion

The determination of antioxidant property of carrots and stability of extract has been carried out. The investigation has revealed that carrot extract (Sample A) had the least concentration of carotenoid which was found to be 5.8782mmol/L, Sample B had the highest concentration of 6.3013mmol/L, while samples C and D had a concentration of 6.03205mmol/L and 5.929mmol/L, respectively. The high antioxidant property of sample B can be as a result of the fact that blanched carrot liberates natural antioxidant and phenolic compounds.

The stability of the extracted pigment under different processing and storage conditions were studied, i.e. untreated (sample A), blanched (sample B), 0.2% Ammonium Sulphite treated (sample C), and the combined blanching and 0.2% ammonium sulphite treatment (sample D). All pigments were stored at 25^{o}C under light, 25^{o}C in the dark cupboard, 4^{o}C and 40^{o}C. Sample B had the highest percentage retention of carrot pigment after 6days of storage at 4^{o}C (65.1%). Sample A had the least percentage retention of 12.0%, while samples C and D had 57.4 and 19.5 percentage retention, respectively, under the same storage conditions.

The work showed that if appropriate processing and storage conditions could be met, then carrot pigment could retain their natural color. It is obvious that the storage of pigments in a food system is possible even for the natural commercial scale production. The significance of this

research lies in the demonstration of the production of carotenoid pigments which can be used as natural food colorants.

REFERENCES

Baloch, A.K., Buckle, K.A and Edwards, R.A. (1997): "Effect of Processing Variables on the Quantity of Dehydrated Carrots" Journal of Food Technology. Vol. 12, pg. 14, 18-21.

Brithon, G. (1995): "Carotenoid" European Journal of Biochemistry, vol.4, pg. 25, 118- 119.

Canfield, L., Krinsky, N., and Olson, J. W. (1993): "Carotenoids in Human Health," Annuals of New York Academic of Sciences, pg. 691.Http:1" carrots," available online at en.wikipedia.org/wiki/carrot#cite, by anonymous (2008), accessed on 16/11/2008. Http:2" properties and reactivity of carotenoid," available online at www.nothernnontarioflora.ca/fruits_term_types.cfm,by anonymous (2004), accessed on 25/11/2008.

Goodwin, T.W. (1976) "Chemistry and Biochemistry of plant pigments" Academic press, New York. Vol. 2