EXTRACTION AND CHROMATOGRAPHIC IDENTIFICATION OF CAROTENOIDS OF RED PEPPER

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ABSTRACT

The aim of this investigation was to extract the pigments from red pepper and to identify its components. Dried red pepper extracts showed higher absorption intensity than those of the fresh red pepper. The HPLC-chromatogram shows that the red pepper pigments consist of about 20 fractions, which have been identified according to their absorption spectrums. The major component of red pepper pigments in the fresh sample was the red color of capxanthin (52.31 %). The other components in the extract were zeaxanthin, capsorubin, violaxanthin, cryptoxanthin and lutein. Drying of red pepper samples facilitated the extraction of more pigment components such as anthraxanthin, capsorubin esters as well as α and β- carotenes. The optical density of the red pepper extracts increased with increasing the extraction time and temperatures in solid: solvent ratio of 1:2, compared with the 1:4 ratio. The calculated rate constant κ (min^{-1}) values for the pigment extraction were increased with increasing temperature in both untreated and steam-blanched dried red pepper, which ranging 0.013 to 0.023 min^{-1} in the untreated extracts (1:2). Also, the activation energy values (of the extraction process) ranged between 23.1 to 58.9 KJ/mol for untreated and steam-blanched dried samples.

The color parameters of the ethanolic extracts, (a) and chroma values were increased while (b) and hue values were decreased with the progress of extraction time and with increasing extraction temperature. The equilibrium moisture contents of red pepper powder were higher in the untreated red pepper compared with the steam-blanched samples, which varied between 0.094 to 0.455 g H_2O/g DM

in the a_w range of 0.11 to 0.85, respectively. Halsey equation found to be the best isotherms equation to fit the experimental data for a wide range of water activity (0.11-0.85).

INTRODUCTION

Pepper, specifically Capsicum annuum, is a general name for plants coming from Capsicum species of Solanaceae family, whose products are used as vegetables and spices (Doymaz and Pala, 2002). Paprika is one of the most commonly used natural colorant in the food industry. The selection of pepper cultivar with a high level in carotenoids and suitable for industrial processing is another aspect of research on the improvement of paprika quality (Reeves, 1987). Red pepper is used mainly in ground form. It's market value depends largely on the red color. Although color does not necessarily reflect nutritional, flavor or functional properties, it relates to consumer preference based on the appearance of the product. Therefore, color evaluation of red pepper powder is especially significant (Chen *et al* 1999).

Ramesh *et al* (2001) found that cutting and steam blanching the red pepper samples for 3 min., resulted in the increased retention of carotenoids and tocopherol, justifying the fact that, blanched products are more stable for carotenoid losses due to low oxygen atmosphere during steam blanching and inhibition of oxidative enzymes. Aguilera *et al* (1987) stated that the character of red pepper extract color depends on the solvent used. Thus, ethanol has the advantage of extracting much of the non-pungent matter from chilies, being commonly available at reasonably low costs and leaving a safe residue.

Dehydration is an important preservation process which reduces water activity through the decrease of water content, avoiding potential deterioration and contamination during long storage periods (Vega *et al* 2007) Since the activity of water in a sample of paprika depends on the manufacturing procedure used, it is clear that the drying system, including its processing steps, will have an influence on pigment stability. Pigment degradation in paprika coincides with

the destruction of vitamins C and E continuously with degradation of the yellow and red carotenoids, due to their oxidation in air (Ramesh *et al* 2001).

Carotenoids are very stable when they are present in intact plant tissue, but when processed, carotenoids are isolated and are vulnerable to the effects of heat, light and oxygen tension (Klieber & Bagnato, 1999). HPLC procedure is the most widely used technique for pepper carotenoid analysis due to its speed, the absence of alteration in the sample, and the possibility of multiple on-line detection (Biacs *et al* 1989 and Almela *et al* 1991). Choi and Ha (1994) found that Capxanthin, among dried red pepper, was the most abundant carotenoid (97.8 mg %) followed by β-carotene, cryptoxanthin, violaxanthin, cryptocapsin and capsorubin.

The purpose of this investigation was the extraction of red pepper pigments and identification of individual carotenoid content by developed HPLC methods. Also, to study the total extractable color and its identification by spectrophotometry, as well as, to determine the effects of steam-blanching, drying process and extraction ratios by the ethanol on L, a, b color parameters of red pepper powder.

MATERIALS AND METHODS

2.1. Materials

Red pepper samples (Capsicum annuum L.) were purchased from local market, Cairo, Egypt, during 2005. Red pepper fruits were cut perpendicular to strips (about 1 cm wide) seeds and stems were separated from the pods, then the samples were divided into two portions; the first untreated (control), while the second portion was steam-blanched with saturated steam at approximately 100 °C for 5 min. under atmospheric conditions.

Blanched samples were immediately cooled to prevent further thermal stress. Drying of untreated and treated red peppers was performed in an air oven at 70 °C to a final moisture content of about 10% and the final product was ground in a laboratory-size mill.

Extraction procedure was carried out on fifty gram samples in a 500 ml agitated glass beaker immersed in a temperature controlled bath with shaker. Sampling was done through a capillary tube connected to a syringe. Ethanol (95%) was used as solvent in a solid: solvent ratios; 1:2 and 1:4. Temperatures of extraction were 30, 40

and 50 °C. Sampling was performed at 1,3,5,7,10,15,30,60,90,120,150 and 180 minutes.

2.2. HPLC-analysis

HPLC-analysis of the red pepper extracts was carried out with an Agilent reverse phase HPLC- system consisting of degasser, pump system, autosampler, thermostated column and a photo diode array detector (DAD). The column used was a WATERS spherisob 5 μm ODS_2 columns of 4.6x250 mm dimensions. A gradient solvent consisting of two solutions (Acetonitrile/H_2o/Triethylamin "A" and ethylacetate "B") were used. Flow rate was adjusted to 1 ml/min and 41 min. run time was applied. Identification and peak area quantification of the color components were carried out on-line according to their retention time absorption spectrum in the range of λ= 350-750 nm and to their retention time and compared with pigment standards. Color extract samples were filtered through 0.2 μm PTFE membrane filter and injection volume was 15 μl.

2.3. Color measurements

Extractable color was determined photometrically according to AOAC (2000) measuring absorbance at 460 nm in a model Spekol 11 Carl zeiss JENA spectrometer.

Color parameters of the red pepper extracts were determined according to the tristimulus color system described by Francis (1983) using spectrophotometer (MOM, 100D, Hungary). Color coordinates X, Y & Z were converted to corresponding Hunter L, a & b color coordinates according to formula given by manufacturer. L value indicates lightness or darkness in a scale from 100 to 0, the a value represents the coordinates of greenness (-) and redness (+), while the b value signifies change from blueness (-) to yellowness (+). Hue angle (θ) is defined as a color wheel, with red-purple at angle of 0° and 360°, yellow at 90°, bluish-green at 180° and blue at 270°. Hue angles (θ) were calculated as follows

$$\theta = \tan^{-1} (b/a) \qquad\qquad (1)$$

On the other hand, the color intensity or chroma (C) represents color saturation or purity, calculated as follows

$$C = (a^2+b^2)^{1/2} \qquad\qquad (2)$$

2.4. Sorption isotherm experiments

Equilibrium moisture experiments were carried out by applying the gravimetric method as described by Wolf *et al* (1984). Each experiment was performed in triplicate and the average values were used in the analysis. Sorption isotherm curves were plotted by taking 0.11 to 0.85 aw on x-axis and the corresponding equilibrium moisture content of the untreated and steam blanched red pepper powders on y-axis measured at room temperature of 30 °C.

Five sorption isotherm models were applied to fit the experimental sorption data and the parameters of the models were estimated by regression analysis using the linear form of the isotherm models as reported by Kim *et al* (1990); Kim *et al* (1991) and Kaymak-Ertekin & Sultanoglu (2001) as follows:

Halsey equation
$$\ln M = (1/B) \ln A - (1/B) \ln [\ln (1/a_w)] \qquad (3)$$

Chung and Pfost equation
$$\ln a_w = -A \exp (-B.M) \qquad (4)$$

Henderson equation
$$1 - a_w = \exp (-A.M^B) \qquad (5)$$

Iglesias and Chirife equation
$$\ln [M + (M^2 + M_{0.5})^{1/2}] = A + B. a_w \qquad (6)$$

BET equation
$$a_w/ [M . (1-a_w)] = (1/M_0 . C) + [(C-1)/ (M_0 . C)]. a_w \qquad (7)$$

Where M is equilibrium moisture content, a_w is water activity, A&B are constants while $M_{0.5}$ is the moisture content at water activity of 0.5; M_0 is a monolayer moisture content and C is energy constant.

RESULTS AND DISCUSSION

3.1. Absorption spectrum and identification of red pepper pigment extracts:

The spectrum of the extracted color solution was measured in the visible region between 380 - 700 nm. The absorption spectrum of all extracts was found to flatten at wave length higher than 600 nm,

which show that the absorption intensity of the color component of red pepper lay in the region of 400 -600 nm. Dried red pepper samples showed higher absorption intensity than those of the fresh red pepper extracts which could be referred to the higher amount of color components extracted from the dried samples as a result of the cell wall damage caused by the blanching and drying processes. Positive effect of drying on the amount of the extractable color components could be confirmed by the results obtained by Daood *et al* (1996). They found that drying of red pepper at relatively low temperature was advantageous for color retention, extracted amount of β-carotene and hygroscopic properties of dried products.

Table (1) and Fig. (1) show the HPLC-chromatogram of the pigment components extracted from red pepper. The elution of carotenoids and chlorophyll components with the applied HPLC-system starts with the peak of Neoxanthin at 10.23 minutes.

The HPLC-chromatogram shows that the red pepper pigments consist of about 20 fractions, which have been identified according to their absorption spectrums. The major component of red pepper pigments in the fresh sample was the red color of free and esterified capxanthin, which makes about 52.31% of the total area under the chromatograms of the fresh sample. This result agrees with the capxanthin percentage of red pepper (26-56%) and 33.4% reported by Almela *et al* (1991) as well as by Choi and Ha (1994), respectively. The other components in the extract of fresh red pepper sample were the yellow zeaxanthin (7.56%), the free red capsorubin (6.77%), the pink-colored violaxanthin (4.13%), the orange red cryptoxanthin (2.72%), the lutein (1.99%) and the yellow cryptoxanthin (1.5%). β-carotene was not detected in the fresh red pepper extracts, may be because of its resistance to diffusion from inside the intact cell wall materials to the surrounding ethanol solvent. However, chlorophyll A and B and their derivatives were present in the pigment extracts of the fresh red pepper samples (13.48%).

As seen in Table (1), pigment profile of the dried red pepper samples showed a reduction in the extracted free capxanthin and increase in its extracted ester forms. Besides, the drying process has facilitated the extraction of other pigment components such as Anthraxanthin, capsorubin esters as well as α- and β- carotenes. This phenomenon indicates that the drying process caused damage to the cell walls and thereby increasing the total amount of the extracted

pigments. However, the reduction in free capxanthin could be referred to the oxidative and enzymatic reactions taking place during the period of drying. According to Gross (1991), free carotenoid pigments are less stable for oxidation than their carotenoid esters and the oxidation products of capxanthin did not accumulate but were rapidly oxidized to low-molecular compounds. On other side, blanching of fresh red pepper prior to drying led to a substantial increase in the amount and fractions of the extracted pigments.

As seen in Table (1), the calculated total area under the HPLC-chromatogram of the blanched-dried samples was 3.6-fold and 2.8-fold those of fresh and non-blanched dried samples, respectively. Such improvement in the amount of the extracted pigment could be referred to the combined effect of blanching and drying on disintegration of the cell wall, which in turn, facilitates the extraction process and protect the free capxanthin from enzymatic oxidation. Also, the blanching and drying processes have facilitated the extraction of the esterified forms of capxanthin, cryptocapsin, cryptoxanthin, capsorubin as well as α- and β- carotenes. However, the amount of chlorophyll breakdown components was increased as a result of heat treatments during blanching and drying. From these results, it could be concluded that blanching and drying of fresh red pepper would enhance the amount of essential red pigment fractions such as capxanthin, capsorubin, cryptoxanthin, cryptocapsin and β-carotene from red pepper samples.

The obtained results agree with those reported by Camara and Monéger (1978) as well as Biacs *et al* (1989) and Gross (1991) concerning the presence of capsorubin mainly in its polyester form in the extracts of red pepper powders. Furthermore, they stated that capxanthin and capsorubin and their esterified form were the major color components in ripe red pepper. The presence of some chlorophylls and their derivatives in the extracts of the red pepper samples in the present investigation agree with the statement of Matus *et al* (1991) and Gross (1991). They found that the ratio of chlorophylls to total carotenoids decreases with progress in the maturity stage of the red pepper fruit.

The presence of lutein and cryptocapsin in only small amounts in the extracts of red pepper agrees also with the finding of Camara and Monéger (1978).

The area ratio between β-carotene and capxanthin in extracts of the dried red pepper powders obtained in the present work agree, to a great extent, with those reported by Gross (1991).

Table (1) Identification of the major carotenoid pigments of red pepper extracts.

| No.	Rt. (min.)	Pigment component	Fresh Samples		Untreated dried samples		Steam-blanched dried samples	
			Area	%	Area	%	Area	%
1	10.23	Neoxanthin	30.74	1.06	-	-	91.69	0.88
2	10.6-11.28	Capsorubin	196.55	6.77	79.77	2.15	144.85	1.39
3	13.22	Violaxanthin	119.97	4.13	45.26	1.22	82.69	0.79
4	13.80	Capxanthin	959.18	33.03	421.29	11.34	830.69	7.98
5	15.54	Anthraxanthin	-	-	21.00	0.57	61.92	0.59
6	16.60	Lutein	57.96	1.99	78.67	2.12	106.47	1.02
7	17.40	Zeaxanthin	219.61	7.56	82.43	2.22	167.47	1.61
8	21.6	Capxanthin ester I	17.89	0.62	33.09	0.89	155.72	1.49
9	23.8	Capxanthin ester II	78.07	2.69	35.97	0.97	201.45	1.94
10	24.8	Chlorophyll B	42.94	1.48	242.64	6.53	209.59	2.01
11	25.9	Chlorophyll A	105.1	3.62	467.13	12.57	271.8	2.61
12	26.8	Cryptocapsin	79.15	2.72	69.61	1.87	247.90	2.38
13	27.6-30.9	Chlorophyll degradation derivatives	243.43	8.38	302.1	8.13	1606.52	15.43
14	32.3	Cryptoxanthin	43.56	1.50	54.02	1.45	827.7	7.95
15	34.0	β-Carotene	-	-	229.63	6.18	1264.6	12.15
16	35.31	Capsorubin ester I	-	-	271.08	7.29	959.04	9.21
17	36.01	α-Carotene	-	-	126.45	3.40	266.12	2.56
18	36.52	Capsorubin ester II	-	-	310.74	8.36	615.95	5.92
19	37.84	Capxanthin ester III	-	-	186.17	5.01	422.27	4.06
20	39.64	Capxanthin ester IV	463.71	15.97	370.14	9.96	331.67	3.19
		Unknown pigments	246.49	8.48	289.44	7.77	1546.23	14.84
		Total area	2904.35		3716.63		10412.34	
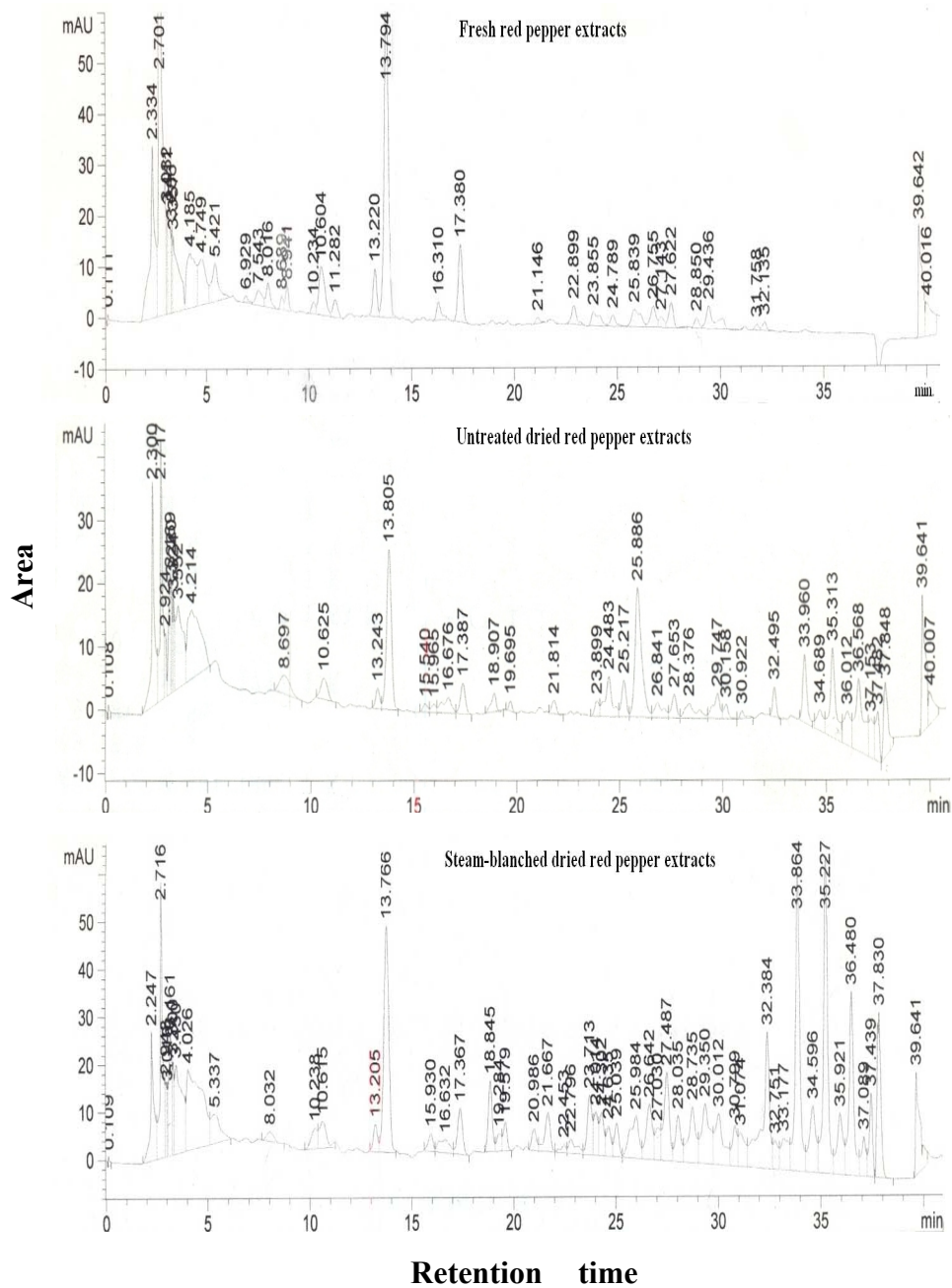
Fig. 1. HPLC_Chromatogram of red pepper extracts

3.2. Factors affecting the extraction of red pepper pigments

The real extraction process involved transfer of multiple molecular components, which is difficult to quantify and model. The response variable monitored was the overall color of the extract, which is a commercial index of quality and is commonly determined spectrometrically at 460 nm (Aguilera *et al.* 1987). Once a solvent (ethanol) has been chosen, main extraction variables are solid to solvent ratio and steam-blanching pretreatment to affect changes in the internal structure of the solid.

Fig. (2) illustrates the relation between optical density and extraction time of untreated and steam-blanched dried red pepper in dilution ratio of 1:2 & 1:4. From this figure, it could be noticed that, the optical density increased with increasing the extraction time and temperatures in solid: solvent ratio of 1:2 compared with dilution of 1:4. The steam-blanching pretreatment has increased the mobility of color particles and hence, the rate of mass transfer was increased due to the decrease of the resistance for mass transfer around the cell wall. The dilution ratio (1:2) accelerated the rate of color extraction as a result of increment the differences in concentration between the colors concentrates inside the cell and in the surrounding solvent. It is clear from Fig. (2) that the O.D – τ curve follows an exponential function which can be mathematical expressed according to (Toledo, 1991) as follows:

$$Y = Y_\circ . \exp (\kappa . x) \qquad\qquad (8)$$

Where Y is an obtained optical density (O.D), $Y_\circ$ is a theoretical value of the O.D at zero time, X is the extraction time in min. and κ is the slope of the reaction (extraction) velocity or rate constant in min^{-1}.

Table (2) shows the rate constants resulted from the relationship between O.D and extraction time (τ) of dried red pepper extracts. From linear portion of the O.D/τ curve, the calculated rate constant (κ) increased with increasing of the temperature in both untreated and steam-blanched dried red pepper extracts.

Fig. (3) represents the Arrhenius plot to calculate the activation energy (Ea) of dried red pepper extracts according to the following equation (Aguilera *et al* 1987).

$$\kappa = \kappa_\circ . \exp(- Ea/RT) \qquad\qquad (9)$$

Where κ is a rate constant per min., $\kappa_\circ$ is a rate constant at zero time, Ea is an activation energy, R is a general gas constant and T is an absolute temperature.

The activation energy values ranged between 23.1 to 58.9 KJ/mol for untreated and steam-blanching dried samples. Aguilera *et al.* (1987) found that the Ea value of red pepper extract was 6.1 kcal/mol (25.5 KJ/mol).
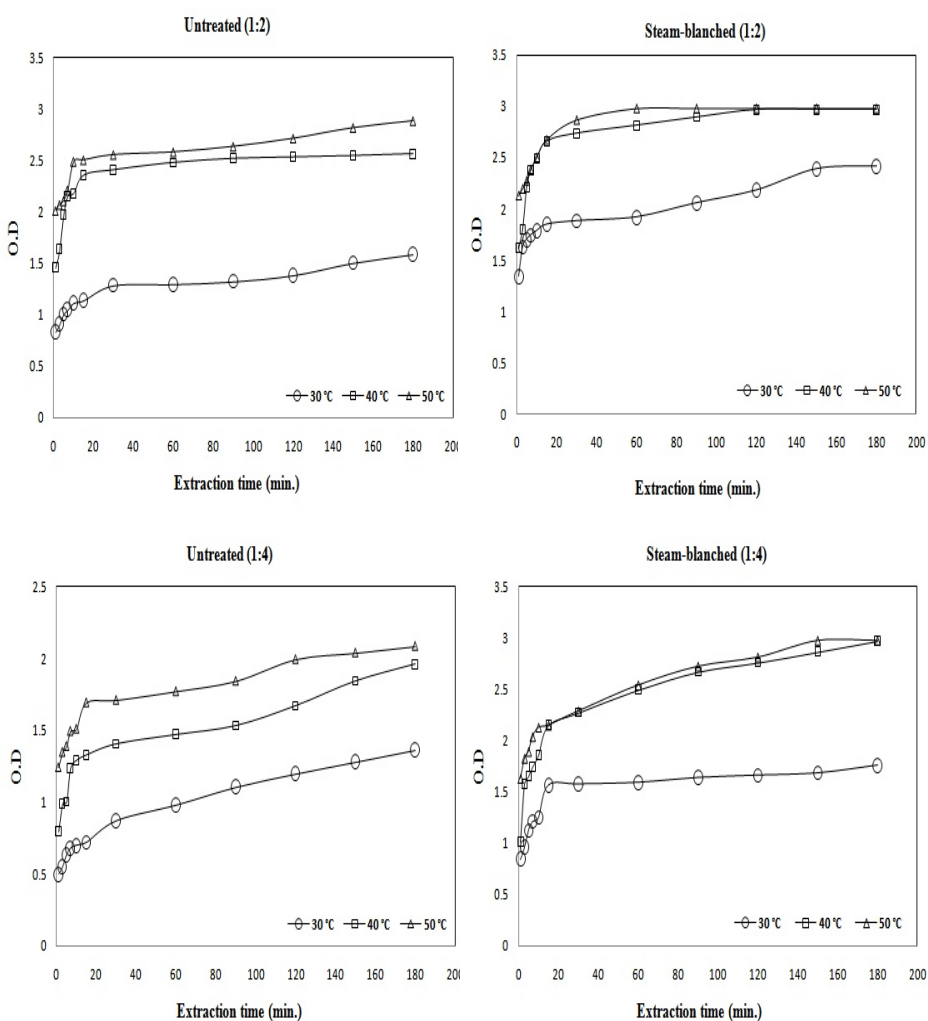

Fig (2) Extraction curves of untreated and steam-blanched red pepper with ethanol (solid: Solvent; 1:2 and 1:4)

Table (2) Rate constants (κ) per min. of dried red pepper extract calculated from a linear portion of the O.D/τ curves at different temperatures.

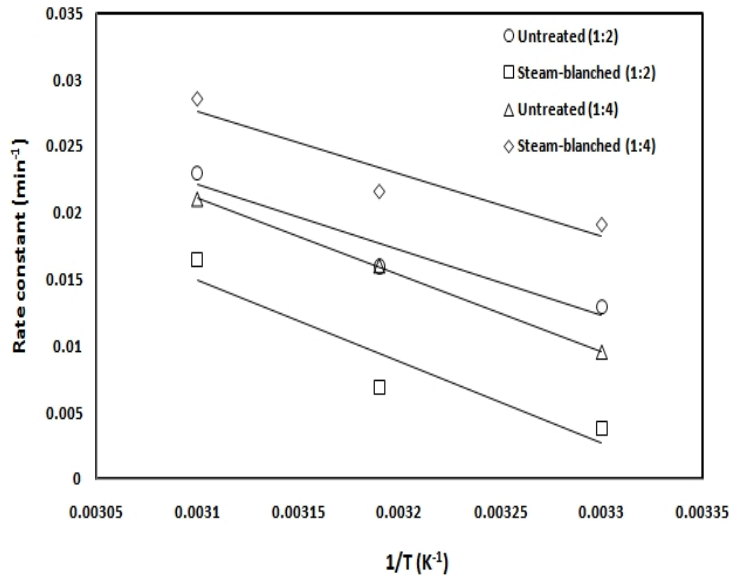| Treatment	30 °C		40 °C		50 °C	
	K	r	κ	r	κ	r
Untreated red pepper extracts (1:2)	0.013	0.91	0.016	0.82	0.023	0.97
Steam-blanched red pepper extracts (1:2)	3.84×10^{-3}	0.66	6.87×10^{-3}	0.74	0.016	0.99
Untreated red pepper extracts (1:4)	9.53×10^{-3}	0.91	0.016	0.81	0.021	0.98
Steam-blanched red pepper extracts (1:4)	0.019	0.84	0.022	0.79	0.028	0.97

Fig (3) Arrhenius plot of rate constants for extraction of untreated and steam-blanched red pepper samples.

In general, total extraction yield and extraction efficiency of pigments increased with increasing temperature. This finding agrees with Perva-Uzunalić *et al* (2004) as well as Jarèn-Galán *et al* (1999). The effect of temperature is positive due to increase in the mobility of color particles to move outside from the cell wall (κ values) and diffuse in the surrounding media.

3.3. Color parameters of red pepper extracts

Table (3) illustrates the color parameters (a values, b values, chroma (C) and hue angle "θ") of untreated and steam-blanched red pepper extracts with dilution ratios of 1:2 and 1:4.

Table (3) Color parameters of untreated and steam-blanched dried red pepper extracts diluted with ethanol in ratio of (1:2) & (1:4).

Extraction time (min.)	30 °C				40 °C				50 °C			
	a	b	C	hue	a	b	C	hue	a	b	C	hue
Untreated sample extracts (1:2)												
1	3.83	4.51	5.92	49°43′	4.36	4.39	6.19	45°17′	5.03	4.27	6.60	40°21′
5	4.28	4.42	6.15	45°50′	4.76	4.29	6.41	41°59′	5.69	4.15	7.04	36°07′
10	4.89	4.28	6.50	41°20′	5.20	4.15	6.65	38°39′	6.88	3.99	7.95	30°06′
30	5.47	4.04	6.80	36°30′	6.28	3.92	7.40	31°47′	7.71	3.75	8.57	26°06′
60	5.59	3.68	6.69	33°25′	6.74	3.56	7.62	27°55′	7.95	3.37	8.63	22°46′
90	5.73	3.48	6.70	31°22′	7.04	3.33	7.79	25°10′	8.22	3.13	8.80	20°48′
180	6.18	2.97	6.86	25°38′	8.12	2.82	8.60	19°17′	9.25	2.58	9.60	15°38′
Steam-blanched sample extracts (1:2)												
1	3.88	4.40	5.87	48°29′	5.14	4.30	6.70	40°01′	5.26	4.26	6.77	39°00′
5	4.36	4.32	6.14	44°42′	5.38	4.19	6.82	37°57′	6.58	4.12	7.76	32°12′
10	4.99	4.18	6.51	40°01′	5.61	4.02	6.90	35°45′	7.80	3.95	8.74	27°01′
30	5.61	3.92	6.84	34°59′	6.80	3.85	7.81	29°40′	8.04	3.71	8.85	24°42′
60	5.79	3.55	6.79	31°22′	6.93	3.50	7.76	27°01′	8.18	3.32	8.83	22°17′
90	5.88	3.37	6.78	29°40′	7.60	3.29	8.28	23°16′	8.64	3.05	9.16	19°17′
180	7.27	2.84	7.81	21°18′	9.25	2.70	9.64	16°10′	10.02	2.45	10.32	13°29′
Untreated sample extracts (1:4)												
1	2.91	6.07	6.73	64°25′	3.21	5.99	6.80	61°51′	3.77	5.92	7.02	57°30′
5	3.26	6.02	6.85	61°36′	3.62	5.92	6.94	58°37′	4.38	5.75	7.23	52°38′
10	3.69	5.93	6.98	58°09′	4.07	5.82	7.10	55°02′	5.09	5.52	7.51	47°12′
30	4.26	5.74	7.15	53°28′	4.73	5.60	7.33	49°43′	5.63	5.23	7.68	42°55′
60	4.37	5.46	6.99	51°20′	4.99	5.32	7.29	46°56′	5.88	4.81	7.60	39°21′
90	4.62	5.31	7.04	48°59′	5.23	5.13	7.33	44°25′	6.07	4.64	7.64	37°14′
180	4.89	4.88	6.91	45°00′	5.90	4.63	7.50	37°57′	6.96	4.09	8.07	30°32′
Steam-blanched sample extracts (1:4)												
1	3.01	5.97	6.69	63°12′	3.34	5.90	6.78	60°32′	3.96	5.84	7.06	55°46′
5	3.38	5.92	6.82	60°15′	3.74	5.84	6.93	57°20′	4.51	5.69	7.26	51°33′
10	3.83	5.83	6.98	56°39′	4.27	5.75	7.16	53°28′	5.17	5.47	7.53	46°40′
30	4.41	5.67	7.18	52°13′	4.88	5.56	7.40	48°44′	5.81	5.16	7.77	41°40′
60	4.55	5.39	7.05	49°43′	5.12	5.25	7.33	45°50′	5.94	4.75	7.61	38°39′
90	4.80	5.22	7.09	47°27′	5.37	5.09	7.40	43°31′	6.19	4.57	7.69	36°30′
180	5.14	4.79	7.03	42°55′	6.09	4.52	7.58	36°30′	7.07	3.99	8.12	29°14′

As seen in Table (3), the "a" and chroma "C" values increased while "b" values and hue angles decreased with the progress of extraction time from 1 to 180 min. and with increasing extraction temperature from 30 to 50 °C.

In dilution of (1:2) the "a" values were higher compared with (1:4) dilution. Hue angle decreased towards the pure red axis with increasing of extraction time and temperatures as could be seen in Table (3). Dried red pepper extracts typically have hue angles between 13 to 49, which is the range from red to orange. This result agrees with the results of Ergünes and Tarhan (2006) and Chen *et al* (1999).

3.4. Equilibrium moisture contents and sorption isotherms of pepper powders

Figure (4) shows the moisture sorption isotherms of dried red pepper at room temperature   (30 °C). As seen, the isotherm curves have the characteristic sigmoidal shape. The equilibrium moisture content at each water activity, which varied between 0.094 to 0.455 g H_2o/g DM in the a_w range of 0.11 to 0.85. From Fig. (4), it could be seen, that the equilibrium moisture content of untreated samples was higher than those of the steam-blanched. Several equations have been proposed to describe the moisture sorption data of dried red pepper (Table 4). It could be seen, that Halsey equation gives the best fit to the experimental data for a wide range of water activity, where the correlation coefficient (r^2) was in the range of 0.985, compared with lower r^2 values for other used equations. The BET (Brunauer-Emmett-Teller) equation was employed to calculate the monolayer moisture content of the dried red pepper sample as mentioned by (Kaymak-Ertekin and Sultanoğlu 2001).

The monolayer value ($M_\circ$) indicates the amount of water strongly adsorbed on specific sites, and it is generally considered to be an optimum at which the food is very stable. The $M_\circ$ values of untreated and steam-blanched samples were 0.079 and 0.069 g H_2o/g solids respectively (Table 4). Kim *et al* (1991) found that the $M_\circ$ values for red pepper varied between 0.06 and 0.1 g H_2o/g solids, depending on storage temperature. The same finding were found by Labuza *et al* (1985), they stated that the value of $M_\circ$ was always less than 0.1 kg/kg (dry basis), which was the maximum value reported for food materials.
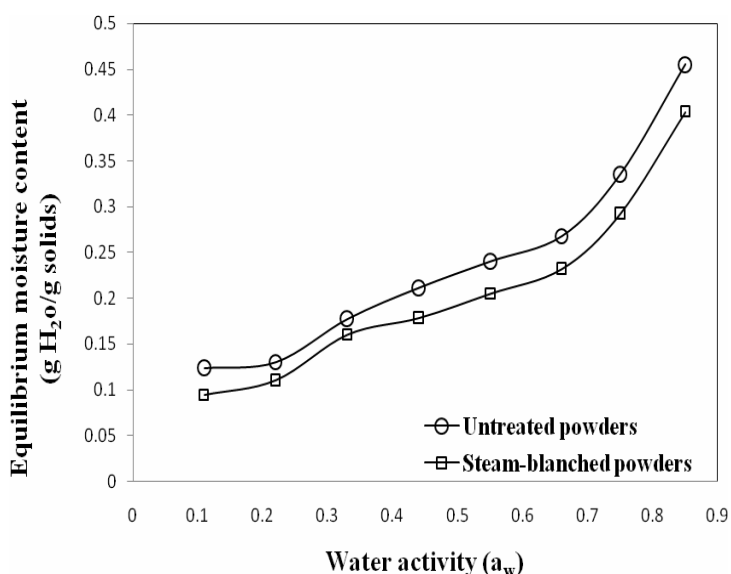
Fig (4) Moisture sorption of dried red pepper powders at room temperature (30 °C).

Table (4) Estimated sorption model constants for dried red pepper powders.

Model		Untreated powders	Steam-blanched powders
Halsey	**b**	-0.5096	-0.5450
	r²	0.9845	0.9819
Chung & Pfost	**b**	-7.6740	-8.4539
	r²	0.9547	0.9563
Henderson	**b**	2.0076	1.8957
	r²	0.9341	0.9554
Iglesias & Chirife	**b**	0.7421	0.7432
	r²	0.9255	0.9249
BET	**M$_o$**	0.0785	0.0690
	C	-8.0958	-8.8361
	r²	0.9256	0.9306

Conclusion

The HPLC-chromatogram shows that the red pepper pigments consist of about 20 fractions. The major component in the fresh sample was the free and esterified capxanthin (52.31 % of the total area) followed by zeaxanthin, capsorubin, violaxanthin, cryptoxanthin and lutein. Both the steam-blanching and drying process of the fresh pepper has facilitated the extraction of other pigment components, due to the decrease of the resistance for mass transfer around the cell wall. The rate constant (κ) values increased with increasing of the extraction temperature and activation energy (Ea) values ranged between 23.1 to 58.9 KJ/mol for all dried samples. Color parameters, a and chroma values increased with the progress of extraction time and with increasing extraction temperature, while b and hue angle values decreased. The equilibrium moisture content of untreated samples was higher than those of the steam-blanched ones and the pepper powder could be safely stored at relative humidities lower than 45% at room temperature without a remarkable increase in the absorbed moisture.

REFERENCES

Aguilera, J.M.; G.A. Escobar; J.M. Delvalle and R.S. Martin (1987). Ethanol extraction of red peppers: kinetic studies and microstructure. International J. Food Sci. and Tech., 22:225-232.

Almela, L.; J.M. López-Roca; M.E. Candela and M.D. Alcázar (1991). Carotenoid composition of new cultivars of red pepper for paprika. J. Agric. Food Chem., 39(9):1606-1609.

AOAC (2000). Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists International. Gaithersburg, Maryland, USA.

Biacs, P.A.; H.G. Daood; A. Pavisa and F. Hajdu (1989). Studies on the carotenoid pigments of paprika. J. Agric. Food Chem., 37(2): 350-353.

Camara, B. and R. Monéger (1978). Free and esterified carotenoids in green and red fruits of *Capsicum annuum*. Phytochemistry, 17: 91-93.

Chen, Q.; H.K. Koh and J.B. Park (1999). Color evaluation of red pepper powder. Transactions of the ASAE, 42(3):749-752.

Choi, O. and B. Ha (1994). Changes in carotenoid pigments of oleoresin red pepper during cooking. J. Korean Soc. Food Nutr., 23(2): 225-231.

Daood, H.G.; M. Vinkler; F. Markus; E.A. Hebshi and P.A. Biacs (1996). Antioxidant vitamin content of spice red pepper (paprika) as affected by technological and varietal factors. Food chemistry, 55(4): 365-372.

Doymaz, I. and M. Pala (2002). Hot-air drying characteristics of red pepper. J. Food Eng., 55:331-335.

Ergünes, G. and S. Tarhan (2006). Color retention of red peppers by chemical pretreatments during greenhouse and open sun drying. J. Food Eng., 76: 446-452.

Francis, F.J. (1983). Colorimetry of foods. In Peleg, M. and E.B. Bagly. Physical Properties of foods. pp 105-123. The AVI publishing company Inc. Westport, Connecticut, USA.

Gross, J. (1991). Pigments in vegetables "Chlorophylls and Carotenoids" AVI Van Nostrand Reinhold, New York. pp. 198-208.

Jaren-Galán, M.; U. Nienaber and S.J. Schwartz (1999). Paprika (Capsicum annuum) oleoresin extraction with supercritical carbon dioxide. J. Agric. Food Chem., 47:3558-3564.

Kaymak-Ertekin, F. and M. Sultanoğlu (2001). Moisture sorption isotherm characteristics of peppers. J. Food Eng., 47:225-231.

Kim, H.K.; K.S. Jo; M.H. Park; Y.S. Chang and Z.I. Shin (1990). Comparison of sorption characteristics of red pepper powders with their seeds mixing ratio. Korean J. Food Sci. & Tech., 22:817-820.

Kim, H.K.; Y. Song and K.L. Yam (1991). Water sorption characteristics of dried red pepper. International J. Food Sci. and Tech., 29:339-345.

Klieber, A. and A. Bagnato (1999). Color stability of paprika and chilli powder. Food Australia, 51(12):592-596.

Labuza, T.P.; A. Kaanane and J.Y. Chen (1985). Effects of temperature on the moisture sorption isotherms and water activity shift of two dehydrated foods. J. Food Science, 50: 385-391.

Matus, Z.; J. Deli and J. Szabolcs (1991). Carotenoid composition of yellow pepper during ripening: isolation of β-cryptoxanthin 5,6-epoxide. J. Agric. Food Chem., 39(11): 1907-1914.

Perva-Uzunalić, A.; M. Skerget; B. Weinreich and Z. Knez (2004). Extraction of chilli pepper with supercritical Co_2: Effect of pressure and temperature on capsaicinoid and color extraction efficiency. Food Chemistry, 87: 51-58.

Ramesh, M.N.; W. Wolf; D. Tevini and G. Jung (2001). Influence of processing parameters on the drying of spice paprika. J. Food Eng., 49:63-72.

Reeves, M.R. (1987). Re-evaluation of Capsicum color data. J. Food Chem., 52:1047-1049.

Toledo, R.T. (1991). Fundamentals of food process engineering. 2nd Ed., p. 490., Van Nostrand Reinhold, New York, USA.

Vega, A.; P. Fito; A. Andres and R. Lemus (2007). Mathematical modeling of hot air drying kinetics of red bell pepper. J. Food Eng., 79:1460-1466.

Wolf, W.; W.E.L. Spiess and G. Jung (1984). The water vapor sorption isotherms of microcrystalline cellulose and purified potato starch. J. Food Eng., 3:51-73.

إستخلاص الكاروتينات من الفلفل الأحمر والتعرف على مكوناتها بالتحليل الكروماتوجرافى

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إستهدف البحث إستخلاص اللون من ثمار الفلفل الأحمر والتعرف على مركبات اللون الموجودة فى الفلفل الأحمر الطازج والمجفف والمعامل مبدئياً بالسلق بالبخار وخاصة الكاروتينويد بواسطة كروماتوجرافيا السائل عالية الأداء (HPLC) وقد إحتوى مستخلص صبغة الفلفل الأحمر على حوالى 20 مركب تم تصنيفهم طبقاً لوقت البقاء (R_t) وطول موجة الطيف الممتص. وإتضح أن المكون الأكبر من الكاروتينويد هو فى العينة الطازجة هو الكابزانثين (52.31%) وكانت بقية المكونات التى تليه من حيث المساحة هى الزيازانثين، الكابسوروبين، الفيولازانثين، الكربتوزانثين والليوتين بينما فى العينة المجففة ظهرت بعض المركبات الأخرى نتيجة لكل من عملية السلق والتجفيف للثمار الطازجة حيث أظهر كروماتوجرام الـHPLC مركبات مثل إنثرازانثين، إسترات الكابسوروبين والألفا والبيتا-كاروتين.

وبدراسة نمط الإمتصاص الضوئي عند طول موجى 460 نانوميتر لمستخلصات الفلفل الأحمر وجد أن شدة الإمتصاص تزداد بإزديـاد زمن الإستخلاص ودرجات الحرارة المستخدمة فى الإستخلاص وخاصة عند التخفيف بنسبة 1:2 (عينة فلفل: إيثانول) مقارنة بنسبة 1:4. وتم حساب ثابت معدل إستخلاص اللون (دقيقة$^{-1}$) ووجد أنه يزداد بزيادة درجة الحرارة من 30 حتى 50 مْ للعينات المجففة. كذلك تم حساب طاقة التنشيط لعملية الإستخلاص ووجد أنها تتراوح من 23.1 حتى 58.9 كيلو جول/ مول للعينات غير المعاملة والمسلوقة بالبخار.

تم تقدير قيم اللون السطحى (a ، b والكروما و hue) للمستخلصات اللونية ووجد أن كلاً من قيم a والكروما تزداد مع تقدم وقت عملية الإستخلاص وزيادة درجة الحرارة بينما قيم b ، زاوية hue تنخفض. وقد أظهرت نتائج تقدير عملية الإمتزاز الرطوبى للمساحيق المجففة للفلفل الأحمر إرتفاع المحتوى الرطوبى الإتزانى للعينات غير المعاملة مقارنة بالعينات المسلوقة بالبخار حيث كانت فى مدى من 0.094 – 0.455 جم ماء/جم مادة جافة للعينات فى مدى نشاط مائى من 0.11 – 0.85 وتم إستخدام العديد من المعادلات المستخدمة لتوصيف عملية الإمتزاز الرطوبى الحرارى وإتضح أن أفضل المعادلات المستخدمة هى معادلة هلسى حيث أعطت أفضل معامل إرتباط بين قيم المحتوى الرطوبى الإتزانى والنشاط المائى (0.985).