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EVALUATION OF SOME LOCAL EGYPTIAN PLANTS AS A SOURCE OF CHLOROPHYLL PIGMENTS*

By

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تقييم لبعض النباتات المصرية المحلية كمصدر لصبغ اليخضور

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تم تحضير صبغ اليخضور (الكلورفيل) كمصدر للون الأخضر الطبيعي من أربع مصادر نباتية محلية هي : السبانخ والسلق والبرسيم والبقدونس. حيث تم إعداد الخامة النباتية بعدة طرق مختلفة إما بالتزهية أو بدونها (طازجة) أو المجففة في تيار من الهواء داخل الأفران الكهربائية أو أفران الطاقة الشمسية أو الهواء الجوي وذلك لكل من العينات التي تم لها عملية التزهية أو بدونها. وقد تم تقييم هذه الطرق كمياً وكيفياً باستخدام كروماتوجرافيا الطبقة الرقيقة وكروماتوجرافيا السائل عالي الأداء. وطيف الأشعة فوق البنفسجية حيث أوضحت عمليات التقييم أن السبانخ تحتوي على نسبة عالية من صبغ اليخضور يلي ذلك البرسيم ثم البقدونس والسلق ويفضل تجفيف العينات النباتية بأفران الطاقة الشمسية أو الأفران الكهربائية. كما شملت الدراسة تحضير صبغ اليخضور أ & ب ومشتقاتهم في درجة نقاوة عالية بهدف استخدامها كمواد قياسية استخدمت في مراحل التقييم الكمي والكيفي.

Key Words: Chlorophyll pigments, *Spinacia oleracae, Beta vulgaris*, *Medicago sativa, Petroselinum sativum,* Qualitative and quantitative evaluation.

ABSTRACT

Four plant materials *viz. Spinacia oleracae* Linn. (spinach), *Beta vulgaris* Limm (chard), *Medicago sativa* Linn. (alfalfa) and *Petroselinum sativum* Hoff. (parsely) were studied as local sources for the preparation of chlorophyll pigments as natural green colour additives. Processing of the plant materials were carried out under different conditions *viz.* blanching, non-blanching followed by drying in air or electrical oven or in solar dehydrating oven. The qualitative and quantitative evaluation of the different plant materials revealed the following: spinach contains the highest chlorophyll content followed by alfalfa then parsely and finally chard. The use of electrical oven and solar dehydrating oven were shown to be more efficient for drying of plant materials. The study includes preparation of chlorophylls a and b and their derivatives in highly purified form to be used as standards for the evaluation of both of the plant materials and the different methods applied.

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INTRODUCTION

Chlorophyll is the green colouring matter found entirely in the plant kingdom. It usually occurs as mixture of green pigments necessary for the photosynthesis process in higher plants. Chlorophyll and its derivatives have found many important industrial uses. Since chlorophyll is physiologically harmless, it is of great value as a natural colouring additive for edible fats and oils, chewing gum, candy, confectionery, egg white, gelatine, pharmaceuticals and many other food products. Moreover, the chemical relationship between chlorophyll and the red pigment of the blood has suggested its application in medicine.

Plant materials used as sources for chlorophyll pigments may be grass meal, spinach, alfalfa, stinging nettle or corn which may be either fresh or more usual, comminuted and dried [1].

This work deals with the evaluation of selected plant materials as local sources for the production of chlorophyll pigments as colour additives as well as the methods and techniques applied in their processing, extraction and purification.

EXPERIMENTAL

Plant Materials

Four plant materials *viz. Spinacia oleracae* Linn. (spinach), *Beta vulgars* Linn. (chard), *Medicago sativa* Linn. (alfalfa) and *Petroselinum sativum* Hoff. (parsely) were studied as local sources for the preparation of chlorophyll preparation of chlorophyll pigments.

APPARATUS AND TECHNIQUES

TLC: Cellulose layer (aluminum sheet, Merck) developed with pet ether (60-80°C) / acetone / *n*-propanol (90:10:0.5) [2] (Table 1).

HPLC Apparatus and Conditions

Apparatus: HPLC Waters Associate Analytical Liquid Chromatograph Model ALC/GPC 205 U equipped with:

- UV detector model 450 variable wave length Waters Associate.

- Solvent programmer model 660 Waters Associate.

- Processors 745 data module Waters Associate.

Conditions: Column µ-Bondapak C-18 (4 mm x 30 cm) Waters Associate.

- Solvent: methanol / acetonitrile 25:75 9v/v) (isocratic).

- Flow rate: 1.5 ml/min.

- Detector wave length at 445 nm.

- Chart speed: 0.5 cm/min.

The different samples were subjected to HPLC analysis according to Braumann *et al.* [6] and the retention times (R_t) of the separated peaks were correlated with those of authentic chlorophyll pigments (Table 2).

UV-Spectrophotometry

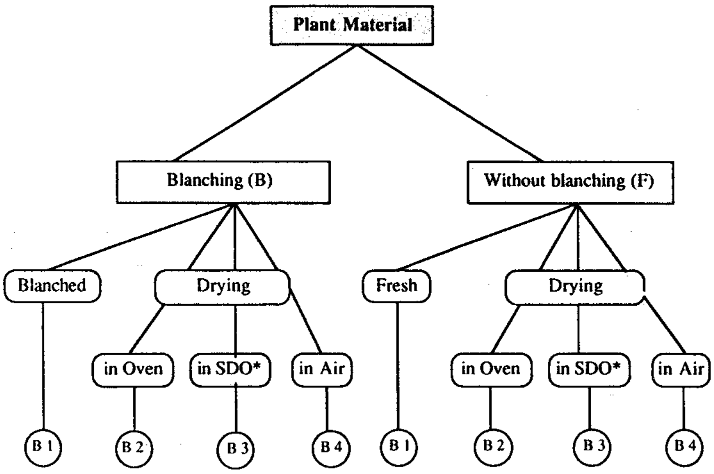
Pye-Unicam double beam spectrophotometer SP8-100.

The percentage of chlorophyll retention was calculated according to Vernon (1960). The absorbencies were measured at 645, 655, 662, 666 and 700 nm. (Table 3) and the percentage of chlorophyll retention was calculated according to the equation given by Vernon [7].

- EHRET drying Oven, model TK/L 3340.
- Solar dehydrating oven designated by Solar Energy Department of National Research Center.

Processing of Plant Materials

Processing of the plant materials was carried out under different conditions *viz.* blanching (B) or non-blanching (F) followed by drying in air or electrical oven or in solar dehydrating oven (Scheme 1).



Processing of Plant Materials

F1 = fresh sample.

F2 = fresh samples dried in electric oven.

F3 = fresh sample dried in solar dehydration oven.

F4 = fresh sample dried in air

B1 = blanched sample.

B2 = blanched sample dried in oven.

B3 = blanched sample dried in solar dehydration oven.

B4 = blanched sample dried in air.

SOD = Solar Dehydration oven.

Blanching Process

Fresh plant materials were blanched in aqueous alkaline solution for stabilization of their green pigments by using the following method [3].

Table 1
R_f values and colours of chlorophyll pigments in differently treated plant materials

Spot No.	Component	Rf	Colours in		Spinach							Alfalfa							Parsely							Chard						
			dl	uv	F1	F2	F3	F4	B1	B2	B3	F1	F2	F3	F4	B1	B2	B3	F1	F2	F3	F4	B1	B2	B3	F1	F2	F3	F4	B1	B2	B3
1	pheophytin a	087	Gy	R Br	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
2	pheophytin b	077	–	R Br	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	chlorophyll a	062	Bl Gr	dV	+++	++	++	+	+++	++	++	+++	++	++	+	+++	++	++	+++	++	++	+	+++	++	++	+++	++	++	+	+++	++	++
4	unkn chl deg	0.51	Bl Gr	P	-	-	-	+	-	++	++	-	-	-	+	-	++	++	-	-	-	+	-	++	++	-	-	-	+	-	++	++
5	chlorophyll b	045	YG	dV	+++	++	++	+	++	++	++	+++	++	++	+	+++	++	++	+++	++	++	+	++	++	++	+++	++	++	+	++	++	++
6	unkn chl deg	040	Gr	fP	-	-	-	+	-	++	++	-	-	-	+	-	++	++	-	-	-	+	-	++	++	-	-	-	+	-	++	++
7	unkn chl deg	023	fGr	fP	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	+	+	+
8	unkn chl deg	012	fGr	fP	-	-	-	-	–	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+

Absorbent: Cellulose (Aluminum sheet, Merck).

Solvent : Petroleum ether (60-80°C)/acetone/n-propanol (90:10:0.45)

F1 = fresh sample, F2 = fresh sample dried in oven, F3 = fresh sample dried in solar dehydrating oven, F4 = fresh sample dried in air, B1 = blanched sample,

B2 = blanched sample dried in oven, B3 = blanched sample dried in solar dehydrating oven, unkn = unknown, chl = chlorophyll, deg = degradation, pheo = pheophytin,

d1 = day light, uv = ultraviolet light, fp = faint pink, dv = dull violet, Gy = gray, Gr = green, Bl = blue, Y = yellow, R Br = reddish brown

Table 2
Retention times obtained from HPLC chromatgrams

Peak	F1		F2		F3		F4		B1		B2		B3		
No.	R_t	Rel %	R_t	Rel %	R_t	Rel %	R_t	Rel %	R_t	Rel %	R_t	Rel %	R_t	Rel %	Components
1											1.23	6.8			-
2											1.53	1.4	1.54	30.4	-
3			2.26	37.8	2.26	35.6	2.26	37.0	2.26	25.5	2.26	31.7	2.26	16.2	-
4			2.82	1.90	2.82	0.3	2.82	1.6	2.81	1.6	2.81	1.2			-
5			3.11	1.0	3.09	0.1	3.09	1.3	3.05	1.3	3.09	2.0	3.12	3.2	-
6													3.61	3.2	-
7	3.90	19.0	3.91	14.1	3.90	12.6	3.90	11.7	3.90	15.7	3.90	2.9	3.90	4.2	Chlorophyll b
8											4.4	19.0	4.5	8.3	-
9	4.65	81.0	4.65	39.4	4.65	49.4	4.65	44.1	4.65	45.7	4.72	11.5	4.62	30.2	Chlorophyll a
10											8.53	5.5			-
11													9.52	0.3	-

The plant material was first washed with water and cutting off the roots and large branches. About 100 gm of plant materials (representative samples) were blanched by dipping in muslin bag into 2% sodium carbonate at 80°C for 5 minutes, then removed, cooled under drained water for 10 minutes and squeezed to remove excess water. The blanched plant material was packed into nylon bags and flattened to form a sheet layer (0.5 cm thickness).

The fresh plant materials either blanched or non-blanched, were dried under different conditions.

Drying Methods

The following methods were used for drying both fresh non-blanched and blanched plant materials.

1. Air drying: drying in air was carried out at room temperature, in shade and wall ventilated place.
2. Oven drying: drying was set at 45°C, in a controlled electric oven equipped with an air circulating system.
3. Solar dehydration oven drying: drying in hot air circulating system.

Table 3

Spectrophotometric determination of chlorophyll in differently treated plant materials

Plant Materials		Chlorophyll A	Chlorophyll B	Total Chlorophyll
Spinach	F1	1.08	0.21	1.29
	F2	0.36	0.08	0.44
	F3	0.33	0.09	0.42
	F4	0.12	0.03	0.15
	B1	0.87	0.18	0.95
	B2	0.40	0.08	0.48
	B3	0.39	0.09	0.48
Alfalfa	F1	0.59	0.48	1.07
	F2	0.29	0.09	0.38
	F3	0.34	0.08	0.42
	F4	0.10	0.06	0.16
	B1	0.54	0.38	0.92
	B2	0.37	0.11	0.48
	B3	0.30	0.11	0.41
Parsely	F1	0.58	0.22	0.80
	F2	0.31	0.13	0.44
	F3	0.12	0.10	0.32
	F4	0.05	0.09	0.14
	B1	0.39	0.18	0.57
	B2	0.17	0.08	0.25
	B3	0.20	0.08	0.28
Chard	F1	0.56	0.28	0.84
	F2	0.32	0.11	0.43
	F3	0.36	0.15	0.41
	F4	0.07	0.12	0.19
	B1	0.38	0.19	0.57
	B2	0.28	0.13	0.41
	B3	0.24	0.12	0.36

Evaluation of the Different Plant Materials and the Applied Processing Techniques

The qualitative and quantitative evaluation of the different plant materials as well as different processing techniques were carried out using TLC, HPLC and UV/VIS spectrophotometry.

This study includes preparation of chlorophylls a and b and their derivatives in a highly purified form to be used as standards for the evaluation of both plant materials and the different processing methods applied. Chlorophyll pigments a and b were prepared by using the two-phase extraction method [4], which effectively separated chlorophyll a and b in a pure form. Further more purification and separation of both of chlorophyll a and b were accomplished by reverse-phase TLC on kiselguhr impregnated with paraffin oil using methanol : acetone : *iso* proponal : water : benzene (60 : 20 : 20 : 10 : 2) [5].

Quantitative evaluation

Comparative quantitative study of the chlorophyll pigment contents of the four plant materials were carried out by using both of spectrophotometric analysis and high-performance liquid chramatographic analysis.

By using the external standard method, the highly purified chlorophylls a and b previously prepared were used for the calculation of their response factors (RF), hence different volumes of 5 µl to 25 µl of the stock standard solution containing known concentrations of chlorophyll a and b in ether were injected. The concentrations were calculated according to Watanabe *et al.* [8] and the corresponding response factors are given by data module attached to the HPLC instrument. The mean average values of response factors corresponding response factor are given by data module attached to the HPLC instrument. The mean average values of response factors corresponding for both chlorophylls a and b were found to be 44.03 and 50.21 respectively.

The concentration of chlorophylls (Table 4) in the different samples of plant materials were carried out and calculated according to the following equation [9]:

$$\text{Chl} - \text{a or b conc (ug)} = \frac{\text{Peak Area}}{\text{Response Factor x 10}}$$

$$\text{wt (\%)} = \frac{\text{Conc } \mu\text{g x total volume (ml)}}{\text{volume injected x dry wt of the original samples}} \text{x } 10^{-4}$$

RESULTS AND DISCUSSION

Qualitative investigation of the blanched plant materials (Bl) by TLC showed no degradation products. However, HPLC showed slight degradation as indicated by the presence of two minor peaks.

The drying of the plant materials either in an electric oven or solar dehydrating oven is more efficient than air drying. Moreover, the green colour of the dried samples subjected to blanching process was shown to be more intense and distinct

Table 4

HPLC determination of chlorophyll in differently treated plant materials

Plant Materials		Chlorophyll A	Chlorophyll B	Total Chlorophyll
Spinach	F1	1.00	0.45	1.54
	F2	0.26	0.17	0.43
	F3	0.21	0.16	0.37
	F4	0.17	0.10	0.27
	B1	0.84	0.32	1.06
	B2	0.30	0.10	0.40
	B3	0.32	0.13	0.45
Alfalfa	F1	0.76	0.27	1.03
	F2	0.45	0.15	0.60
	F3	0.50	0.18	0.68
	F4	0.10	0.12	0.22
	B1	0.50	0.26	0.76
	B2	0.36	0.17	0.53
	B3	0.35	0.14	0.49
Parsely	F1	0.68	0.31	0.99
	F2	0.31	0.11	0.42
	F3	0.50	0.18	0.68
	F4	0.10	0.12	0.22
	B1	0.49	0.22	0.71
	B2	0.28	0.15	0.43
	B3	0.28	0.11	0.93
Chard	F1	0.62	0.21	0.83
	F2	0.27	0.11	0.38
	F3	0.22	0.18	0.40
	F4	0.12	0.05	0.17
	B1	0.48	0.11	0.59
	B2	0.28	0.18	0.46
	B3	0.25	0.13	0.38

than of non-blanched plant materials . The non-blanched plant materials dried in oven (F2) and solar dehydrating oven (F3) showed by HPLC two minor degradation products in addition to the major peaks corresponding to chlorophylls a and b. Moreover, the air dried samples (F4) showed slight degradation on cellulose TLC as well as HPLC. On the other hand, blanched plant materials followed by drying either in an electrical oven (B2) or solar dehydrating oven (B3) showed relatively higher degradation as indicated by TLC and HPLC than other samples.

The air drying of the blanched plant materials (B4) was excluded, as it takes long time for complete drying (several days which facilitates the growth of microorganisms (fungi and/or bacteria) on such humid plant materials.

Comparative quantitative study of the chlorophyll pigments content was carried out by spectrophotometric and HPLC analyses. The results obtained revealed that spinach contains the highest chlorophyll content among the other plant materials, followed by alfalfa, then parsely and finally chard. Moreover, the fresh samples (F1) contain the highest chlorophyll content than other samples. The non-blanched samples dried either in oven (F2) or solar dehydrating oven (F3) showed comparable values and contain higher chlorophyll content than air drying (F4) while the blanched samples (B1) showed contents nearly to fresh samples (F1). On the other hand, the blanched samples dried either in oven (B2) or solar dehydrating oven (B3) have chlorophyll content comparable to that of fresh samples dried under the same conditions.

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