# PROPERTIES OF CHLOROPHYLL PIGMENTS EXTRACTED FROM SPINACH (*Spinacia oleracea* ) AND CHARD (*Beta vulgaris* ) AS A FOOD COLORANTS

*Journal*

*J. Biol. Chem. Environ. Sci., 2008, Vol. 3(1): 201-221*
*www.acepsag.org*

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## ABSTRACT

Chlorophyll pigments from spinach (*Spinacia oleracea* ) and chard (*Beta vulgaris* ), were extracted, determined, identified and adsorped to solid matrixes (dextrin, flour, lactose, skim milk and starch ). The effects of pH values and temperatures on the chlorophyll pigments and thermal stability were evaluated. Also, the chlorophylls in spinach pigments were used for coloring hard candy, noodle and glazing jelly as healthy food products. The concentration of chlorophylls in spinach and chard were 123.67 and 69.80 mg/ 100g, respectively.

Chlorophylls (a) and (b) were presented in both of spinach and chard by 92.15 and 31.52 mg/100g, respectively for the first source, and 52.97 and 16.83mg/100g respectively for the second source. Starch was the most effective adsorpant for spinach pigment followed by dextrin, while flour was the best carrier for adsorption chard pigment followed by lactose.

Neutral pH gave the highest retention rate of spinach chlorophylls, while the highest retention rate of chard chlorophylls was observed at pH 9.0 .The tested chlorophylls exhibited good stability to heat.

Addition of 0.260, 0.077 and 0.026 % spinach pigment to hard candy, noodle and glazing jelly gave the highest scores for color and overall acceptability.

**Key words:** Chlorophyll, spinach, chard, extraction, identification, adsorption, stabilization, hard candy, noodle, glazing jelly.

## INTRODUCTION

It is now well accepted that quality of food and drink is one of life's great pleasures. Color plays an important role in our enjoyment of foodstuffs; it is appreciated for its aesthetic role and as a basis for the assessment of quality (Bridle and Timberlake, 1997).

An increasing trend in food industries is mainly oriented towards the replacing of synthetic colorants with natural colorants. Chlorophylls are an important class of biological compounds that are widely distributed in green plants. The major chlorophylls in foods include chlorophyll (a&b). Chlorophyll (a) was reported to be present at a concentration two to three times higher than chlorophyll (b) (Schwartz and Lorenzo, 1990).

In food science, chlorophyll (a) and chlorophyll (b) and their copper complexes are of interested as food dyes (E140, E141) (Torsten and Thomas, 2004).

All of the green vegetables in the world`s healthiest foods- asparagus, bell peppers, broccoli, Brussels sprouts, green cabbage, celery, collard greens, green beans, green peas, kale, leaks, green olives, parsley, romaine lettuce, sea vegetables, spinach, swiss chard, and turnip greens are concentrated sources of chlorophyll.While all green plants contain chlorophyll (a) and most vegetables that we eat contain both chlorophyll (a) and chlorophyll (b), some vegetables contain particularly high amounts of total chlorophyll. Best studied of all the vegetables is spinach (Spinacia oleracea) with this vegetable containing about 300- 600 milligrams per ounce. Chlorophyll is one of the primary pigments in olives, but olives contain only 30-300 micrograms per ounce (about 1/1000 th as much as spinach) (Breinholt et al., 1995).

Chlorophylls are the most abundant plant pigments in nature. Recent studies have shown that chlorophyll derivatives can exhibit health- promoting activities, a part from their use as food and pharmaceutical colorants. Chlorophylls, the most abundant pigments in green plants are gaining increasing importance in the human diet not only as food colorants, but also as healthy food ingredients.

Epidemiological studies have shown correlation between the consumption of chlorophylls and the decreased risk of colon cancer. In recent years there has been a growing interest in natural and semi-synthetic chlorophyll derivatives, not only as food colorants, but as food supplements. A part from their role as colorants, disclosing reports attribute to these substances manifold potential health benefits, introducing the possibility that they may be protective agents against the development of several chronic diseases (Tais et al., 2007).

Research on the health benefits of chlorophyll has focused on the area of cancer (including treatment and prevention). This research got underway when damage to genes (or more precisely, to the genes`DNA) by carcinogenic substances called aflatoxins (or more precisely aflatoxin B1 or AFB1) was found to be prevented by chlorophyllin (Breinholt et al., 1995).

Several reports have demonstrated that plant pigments play important roles in health. Chlorophyll molecules are extracted and used as natural colorants and antioxidants to restore the natural level of these molecules in food products or to prepare fortified products (Benoit, 2002). Chlorophyll (a) exhibited considerable antioxidant activity only at high concentrations of about 1m Mol/L, but due to its high level found in plants, it may play a role in protecting against lipid oxidation. The potential antioxidant activity of some chlorophyll derivatives is likely to have important implications regarding health benefits by diet management and it deserves further studies (Ursula et al., 2005).

It is essential to inactivate enzymes (such as chlorophyllase) which can easily degrade chlorophylls during the extraction with sodium, calcium or magnesium carbonate (Bahceci et al., 2005). To prevent the conversion of chlorophylls to pheophytins anhydrous $Na_2Co_3$ (3.5g) was added to adjust the pH to 8-9, (Bellomo and Fallico, 2007). Chlorophylls are the most common green pigments found in plants. As an integrated part of vegetable foodstuffs, chlorophylls have been a nature component of the human diet throughout history (Seljasen et al., 1998).

In vivo, these pigment play a key role in photosynthesis (Schoefs , 2002). They have a complex four ring structure, and the centers of the molecule are coordinated with an $Mg^{2+}$ ion. Along hydrophobic hydrocarbon tail (phytyl) is attached to the ring structure. Chlorophyll

(a) has a methyl group bound to ring 11, while chlorophyll (b) has an aldehyde group in this position (Catalina et al., 2008)

Chlorophylls are susceptible to many degradation reactions caused by weak acid, oxygen, light, temperature changes and / or enzymes. Replacement of the central $Mg^{2+}$ ion in chloro pigments with hydrogen leads to pheophytins, which are related to the color change from bright green to olive brown (Mangos and Berger, 1997).

Degree of greenness is important in determining the final quality of thermally processed vegetables, which acquire their green color due to chlorophyll. The color change from bright green to olive brown during processing is attributed to conversion of chlorophylls to pheophytins due to the loss of central magnesium ion. Several investigators have studied the rate and extent of chlorophyll conversion and which follows first-order reaction rate kinetics (Shalini, et al., 2008).

The aims of this study are to evaluate the chlorophyll levels in spinach and chard grown in Egypt and determine the stability of investigated pigment. Using specific carriers for the extracted colorant and utilizing the chlorophylls as natural coloring in some food products.

## MATERIALS AND METHODS

**Materials:**

Samples of Spinach (*spinacia oleracea*) and chard (*Beta vulgarris*) were purchased from local market in Cairo, Egypt, during the winter months (December- March). Chemicals and solvents were reagent grade.

**Methods:**

**Extraction and determination of green colorants (chlorophylls):**

Chlorophylls were extracted from fresh spinach and chard according to the Official method **A.O.A.C. (1990)** as follows: 10g of plant tissues were weighed and placed into blender cup that contains 0.1g $CaCo_3$ to prevent pheophytin formation during extraction. Acetone 85% was added to extract the pigment and the mixture was filtered with suction. Extraction and filtration were repeated till the tissue become colorless. The filtrates were transferred to volumetric flask and made up to volume with 85% acetone. 50 ml of the final extract were pipette into separating funnel containing 50 ml ether. $H_2O$ were added carefully until pigments have entered ether layer,

$H_2O$ layer was then discarded. The ether solution was washed until all acetone is removed, ether solution was then transferred to 100 ml volumetric flask and made up to mark by ether and mixed.

**Determination of chlorophylls:**

Measurements of chlorophylls (a) and (b) were carried out by direct determination of the absorbance at different wavelengths, using a standard spectrophotometer Jenway 6105 (UV.VIS.). Assuming an 80% acetone extract, the absorbance was found to be measured at 663 and 645 nm in 1cm cells (A.O.A.C.1990).

The concentration can then be calculated from the following formulae:

Total chlorophyll (mg/1) = $20.2\ A_{645} + 8.02\ A_{663}$

Chlorophyll a (mg/1) $= 12.7\ A_{663} - 2.69\ A_{645}$

Chlorophyll b (mg /l) $= 22.9\ A_{645} - 4.68\ A_{663}$

These can be converted to chlorophyll content on a fresh weight basis as follows:

$$\text{Chlorophyll a (mg/g)} = \frac{\mathbf{12.3\ A_{663} - 0.86\ A_{645}}}{\mathbf{\alpha.\ 1000.\ W}} .V$$

$$\text{Chlorophyll b (mg/g)} = \frac{\mathbf{19.3\ A_{645} - 3.6\ A_{663}}}{\mathbf{\alpha.\ 1000.\ W}} .V$$

Where: $A_{645}$ and $A_{663}$ = the absorbencies at 645 and 663 nm.

V = the volume in ml.

$\alpha$ = the length of light path in the cell (usually 1cm).

W = the fresh weight in grams.

**Concentration and adsorption of chlorophylls in solid supports**

The collected extracts of chlorophylls were concentrated by removal of solvent in a rotary vacuum evaporator at 40 °C. The concentrated pigments were adsorbed to solid matrixes (lactose, dextrin, flour starch, and skim milk) in different ratios (1:1, 1:2, 1:3, and 1:4 carrier/pigment) and the mixtures were dried in oven at 40 °C for 24 hr.

**Identification of chlorophylls**

Chlorophylls pigments which extracted from spinach and chard were identified by thin- layer chromatography (TLC) plates according to the method of Bacon (1965) as follows: 12 grams of cellulose powder M N 300 and 68 ml of redistilled water were mixed with a fast electric stirrer for 4min. The slurry was applid with an applicator as a 250u layer to grease free glass plate (25-20cm) and allowed to partially dry at room temperature for 25 min. The plates were then placed in a ventilated oven at 105 °C, horizontally for 10 min. and vertically for 25 min., after that the plates were placed in a dissicator. After the plate had cooled enough, 0.01 ml of the acetone extract of spinach and chard pigments was applied as a spot or streak in dim light and the chromatogram was developed in the dark at room temperature with petroleum ether (b.p. 60/80 °C): acetone: n-propanol (90:10:0.40 v/v/v).This mixture was usually added to the tank, which was lined with chromatography, about 20 min. before inserting the plate. Development was over a distance of 15 cm and took less than 30 min. after that the plate was removed from the developing tank and allowed to dry. The $R_f$ value was calculated.

**Stabilization of chlorophylls:**

**Effect of pH**

Changes in chlorophylls (spinach and chard) caused by pH were measured according to the method described by Elbe and Huang , (1974) as follows: mixing 1 ml of pigment solution and 4 ml of Mcllvaines 0.1 M buffer of various pH ranging from 2.0 – 9.0 The buffered solutions were introduced into 10 ml vials which were flushed repeatedly with nitrogen gas to limit oxygen. Vials were maintained at 4 °C in the dark, and absorbance readings were made initially and after 7 days using spectrophotometer at 663 and 645 nm

**Effect of temperature**

The method described by Saguy , (1979) with some modifications was applied to study the effect of temperature on chlorophylls, 1ml of chlorophylls solution and 9 ml of optimum buffer into unsealed vials were placed in a thermostatically controlled water bath at different temperatures ranging from 50- 100 °C for 30 min. The samples were further cooled down immediately in an ice water bath

and absorbance measurements were read by spectrophotometer at 663 and 645 nm.

**Thermal stability of chlorophylls**

Holding chlorophylls pigment solution at 70 to 100 °C was extended for 180 min in water bath and removed each 30 min then cooled immediately in an ice bath followed by measuring absorption of the solution by spectrophotometer at 663 and 645 nm.

**Technological methods:**

**1- Hard candy processing**

Hard candy was manufactured in the laboratory using the traditional procedure as described by Staniec, (1994). The formulation of control sample is shown in Table (1). Water, sucrose, corn syrup, and citric acid were mixed together and heated to reach 157.5C with continues stirring and then the mixture was cooled to reach 110 C. Color and flavor were added to the formula and then formulation and cooled to reach room temperature and then packaging was carried out for the control treatment. Synthetic green color was added by ratio 20.5 mg/100g of mixture. Different ratios of the investigated green pigment of spinach were added by 0.13, 0.26, and 0.39 gm pigment /100gm of mixture.

**Table (1): Formulation of control hard candy.**

| Ingredients | Weight (%) |
|---|---|
| Sucrose | 48.48 |
| Corn syrup | 25.90 |
| Water | 25.26 |
| Flavoring oil | 0.21 |
| Citric acid | 0.15 |

**2- Manufacturing of noodles:**

Noodle samples were prepared from flour (72% extraction) in the laboratory using the method described by Collins and Pangloli, (1997) using the past Matic 1000 Simac Machine corporation; Millano, ltaly. Each sample was manufactured using 150 g wheat flour and blended with different ratios of the investigated green natural

pigments 0.026, 0.051, and 0.077 gm /100 gm of mixture. Enough water was added for each mixture and the mixing times ranged from 4-6 min to get plastic homogenous dough. The produced dough was kneaded and extruded in a continuous extrusion press equipped with special die shape. Noodle was cut to appropriate lengths, hardened for 15 min in air and dried at 40 °C for 24hr.The product was packed in polyethylene bags for further analysis.

**3- Glazing jelly Processing:**

Glazing jelly was prepared in the laboratory according to the method described by Rizk et al, (2002) using ingredients given in Table (2) as follows: sucrose and caragenan mixture was boiled first in water, then calcium chloride (dissolved in a small volume of water) was added to the mixture. Sorbic acid (dissolved in 2 ml isopropyl alcohol) and potassium sorbate (dissolved in water) were added to the mixture. Glucose was added lately with continuous stirring. After the complete dissolving of ingredients, heating was stopped and color was added. Formulation and cooling of jelly samples were done in the refrigerator for 5hr. For the control treatment, synthetic green color was used by ratio 20.5 mg/ 100g of mixture. Different ratios of the adsorbed green pigments were added by 0.13, 0.26, and 0.39gm/100gm of mixture.

**Table (2): Formulation of glazing jelly sample.**

| Ingredients | Weight (%) |
|---|---|
| Sucrose | 35.85 |
| Water | 54.35 |
| Glucose | 9.06 |
| Caragenan | 0.38 |
| Sorbic acid | 0.08 |
| Potassium sorbate | 0.13 |
| Calcium chloride | 0.15 |

**Organoleptic evaluation.**

The organoleptic properties of the processed green cooked noodle, hard candy and glazing jelly were assessed by taste panelists of the staff members of the Food Science Department, Faculty of Agriculture, Ain Shams University.

**Statistical analysis:**

The obtained data were subjected to analysis of variance (ANOVA) and followed by LSD to carry out the multiple comparisons as described by Statistical Analysis System (SAS, 1996).

## RESULTS AND DISCUSSION

**Determination and identification of chlorophylls**

The chlorophyll contents in spinach and chard pigments were 123.67 and 69.80 mg/ 100g fresh weight, respectively. Chlorophylls extracted from spinach and chard was separated based on their functional groups into two fractions by thin layer chromatography (TLC) as shown in Fig. (1). The calculated $R_f$ values for spinach and chard chlorophyll fractions were 0.37 and 0.60 and they were identified as chlorophyll (b) yellow green and chlorophyll (a) blue green, respectively. The concentration of chlorophyll (a) and (b) in spinach pigment were 92.15 and 31.52 mg/100g, respectively, while the concentration of chlorophylls (a) and (b) in chard pigment were 52.97 and 16.83 mg/100g, respectively. These results are in agreement with those reported by Schwartez et al., (1981), Schwartez and Lorenzo, (1990) and Ayerra et al., (1998). They also mentioned that chlorophylls (a) and (b) were the most abundant pigments detected in spinach, with chlorophyll (a) levels being higher than those of chlorophylls (b).

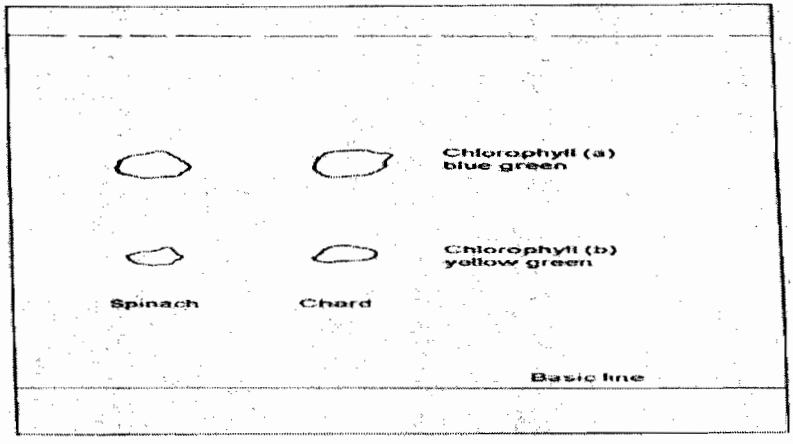


**Fig. (1): TLC chromatogram of Chlorophyll pigments extracted from spinach and chard.**

**Adsorption of chlorophylls**

Adsorption of chlorophylls extracted from spinach on different carriers (dextrin, flour, lactose, skim milk and starch) at different ratios (1:1, 2:1, 3:1 and 4:1 pigment: carrier w/ w) are presented in Table (3). It was noticed that starch had the highest concentration of pigment in the ratios 1:1, 2:1, 3:1 and 4:1 with concentrations of 29.34, 55.50, 98.19 and 163.62 mg/100g, respectively. On the other hand, no significant difference (P> 0.05) was observed between starch and dextrin in (1:1) ratio. The concentrations were 89.27 and 139.34 mg/100g. for dextrin at ratio 3:1 and 4:1 respectively.

**Table (3): Color concentration (mg / 100g carrier) of chlorophylls extracted from spinach and suspended on different carriers at different ratios (w/w).**

| Pigment : Carrier (w/w) | Applied carriers | | | | | L.S.D |
|---|---|---|---|---|---|---|
| | Dextrin | Flour | Lactose | Skim milk | Starch | |
| 1:1 | $29.22^{Da}$ | $15.00^{Dc}$ | $25.33^{Db}$ | $28.64^{Da}$ | $29.34^{Da}$ | 1.4448 |
| 2:1 | $39.71^{Ce}$ | $31.15^{Cf}$ | $46.37^{Cb}$ | $41.82^{Cd}$ | $55.50^{Ca}$ | 0.9579 |
| 3:1 | $89.27^{Bb}$ | $58.44^{Bf}$ | $65.91^{Be}$ | $73.31^{Bd}$ | $98.19^{Ba}$ | 1.4465 |
| 4:1 | $139.34^{Ab}$ | $126.71^{Ac}$ | $121.82^{Ae}$ | $123.62^{Ad}$ | $163.62^{Aa}$ | 1.3708 |
| L.S.D | 2.0766 | 2.4002 | 1.2612 | 2.1735 | 1.7414 | |

Means within a column showing the same capital letters are not significantly different (P> 0.05).

Means within a row showing the same small letters are not significantly different (P> 0.05).

Results in Table (4) show the concentrations of chard chlorophyll on different carriers at different ratios. It was found that, the highest concentration at (1: 1) ratio was for skim milk (56.14 mg/ 100g) followed by flour (53.43 mg/ 100g). The same trend was noticed in (2:1) ratio where, the skim milk had the highest concentration (103.04 mg/100g) followed by flour (86.58 mg/100g). Flour had the highest concentration (170.06 mg/100g) at (4:1) ratio followed by lactose (154.40 mg/100g). Generally, it can be concluded

that, flour was the best carrier for chlorophyll extracted from chard followed by lactose.

**Stabilization of chlorophylls**

**Effect of pH**

Results in Table (5) show that retention % of chlorophylls extracted from spinach and chard as a function of pH values (7 days/ 4 °C). It was found that, the lowest retention of chlorophylls extracted from spinach(1.16%) at pH 2.0.On the other hand the highest degradation rate of chlorophylls of spinach was (98.84%) at pH 2.0. Retention rate of chlorophylls significantly increased from (1.16%) at pH 2.0 to (68.26%) at pH 3.0. From the mentioned results, it could be noticed that, the retention rates of chlorophylls were significantly decreased from (68.26%) at pH 3.0 to (57.31%) at pH 5.0, and significantly increased from (57.31%) at pH 5.0 to (90.87%) at pH 7.0.The highest values of the chlorophyll retention of spinach were (90.87) at pH 7.0 and (89.08) at pH 9.0. Retention of chlorophylls extracted from chard was (0.46%) at pH 2.0 The highest retention of chlorophylls of chard was 88.37% at pH 9.0 Results also showed that, retention rates of chlorophylls extracted from spinach as a function of pH values were more than chlorophylls extracted from chard.

**Table (4): Color concentration (mg / 100g carrier) of chlorophylls extracted from chard and suspended on different carriers at different ratios (w/w).**

| Pigment : Carrier (w/w) | Applied carriers | | | | | L.S.D |
|---|---|---|---|---|---|---|
| | Dextrin | Flour | Lactose | Skim milk | Starch | |
| 1:1 | 49.99 $^{Dc}$ | 53.43 $^{Db}$ | 31.04 $^{De}$ | 56.14 $^{Da}$ | 34.95 $^{Dd}$ | 1.1474 |
| 2:1 | 80.02 $^{Cc}$ | 86.58 $^{Cb}$ | 64.03 $^{Ce}$ | 103.04 $^{Ca}$ | 75.65 $^{Cd}$ | 0.8892 |
| 3:1 | 95.03 $^{Bd}$ | 101.10 $^{Bc}$ | 138.94 $^{Ba}$ | 108.53 $^{Bb}$ | 88.16 $^{Be}$ | 1.3759 |
| 4:1 | 146.36 $^{Ac}$ | 170.06 $^{Aa}$ | 154.40 $^{Ab}$ | 142.54 $^{Ad}$ | 119.49 $^{Ae}$ | 1.6082 |
| L.S.D | 1.7271 | 1.387 | 4.6933 | 2.1129 | 2.1835 | |

Means within a column showing the same capital letters are not significantly different (P>0.05).

Means within a row showing the same small letters are not significantly different (P> 0.05).

**Table (5): Retention % of chlorophylls extracted from spinach and chard as a function of pH values (7 days / 4 °C).**

| pH values | chlorophylls of spinach | | chlorophylls of chard | |
|---|---|---|---|---|
| | Retention % | Degradation % | Retention % | Degradation % |
| 2 | 1.16h | 98.84a | 0.46h | 99.54a |
| 3 | 68.26e | 31.74d | 9.82g | 90.18c |
| 4 | 62.98f | 37.02c | 3.57h | 96.43b |
| 5 | 57.31g | 42.69b | 51.93c | 48.07f |
| 6 | 78.84d | 21.16e | 62.90b | 37.10g |
| 7 | 90.87a | 9.13h | 43.20e | 56.80d |
| 8 | 82.42c | 17.58f | 47.74d | 52.26e |
| 9 | 89.08b | 10.92g | 88.37a | 11.63h |
| L.S.D | 1.1359 | 1.3121 | 0.8495 | 0.6762 |

Means within a column showing the same letters are not significantly different (P> 0.05).

## Effect of temperature

Retention % of chlorophylls extracted from spinach in solution of pH 7.0 as a function of temperature is presented in Table (6). Data showed that no significant differences (P > 0.05) were observed between the retention rates at (50- 60 °C)/30 min. The lowest retention rate of chlorophylls of spinach was (87.17%) at 100 °C /30 min. and the highest retention rate was 99.32% at 50 °C /30min.

**Table (6): Retention % of chlorophylls extracted from spinach in solution of pH 7.0 as a function of temperature.**

| Temperature (°c /30 min) | mg/100 | Retention % | Degradation % |
|---|---|---|---|
| 50 | $6.35^{a}$ | $99.32^{a}$ | $0.68^{e}$ |
| 60 | $6.27^{a}$ | $98.04^{ab}$ | $1.96^{d}$ |
| 70 | $6.22^{ab}$ | $97.31^{b}$ | $2.69^{d}$ |
| 80 | $5.89^{bc}$ | $92.08^{c}$ | $7.92^{c}$ |
| 90 | $5.73^{c}$ | $89.65^{d}$ | $10.35^{b}$ |
| 100 | $5.57^{c}$ | $87.17^{e}$ | $12.83^{a}$ |
| **LSD** | **0.363** | **1.7531** | **0.7315** |

Means within a column showing the same letters are not significantly different (P> 0.05).

Retention % of chlorophyll extracted from chard in solution of pH 9.0 as a function of temperature is given in Table (7). Results showed the same findings for retention % of chlorophylls extracted from spinach. No significant differences (P> 0.05) were obtained between the retention rates at (50- 80 °C)/30 min. The lowest degradation rate was (2.46%) at 50 °C and the highest degradation rate was (7.80%) at 100 °C /30 min. For the pervious results, it can be concluded that, the chlorophylls extracted from chard are stable for different temperatures.

**Table (7): Retention % of chlorophylls extracted from chard in solution of pH 9.0 as a function of temperature.**

| Temperature (°c /30 min) | mg/100 | Retention % | Degradation % |
|---|---|---|---|
| 50 | $48.77^{a}$ | $97.54^{a}$ | $2.46^{e}$ |
| 60 | $48.73^{a}$ | $97.46^{a}$ | $2.54^{de}$ |
| 70 | $48.52^{a}$ | $97.04^{a}$ | $2.96^{d}$ |
| 80 | $48.12^{a}$ | $96.25^{ab}$ | $3.75^{c}$ |
| 90 | $46.99^{a}$ | $93.98^{bc}$ | $6.02^{b}$ |
| 100 | $46.10^{a}$ | $92.20^{c}$ | $7.80^{a}$ |
| **LSD** | **2.7183** | **2.4063** | **0.457** |

Means within a column showing the same letters are not significantly different (P> 0.05).

**Thermal stability**

Table (8) showed thermal stability of chlorophylls extracted from spinach at different temperatures in solution of pH 7.0. The degradation rates of chlorophylls were greatly influenced by temperature and holding time. The degradation of pigment was increased significantly by increasing of temperature (70-100 °C) and holding time (30- 180 min). These results are in agreement with those reported by Shalini et al., (2008). While the retention rates of chlorophylls were decreased significantly with increasing temperatures (70-100°C) and holding time. A certain stabilizing effect of chlorophylls was observed between 30 and 60 min. The higher stability of chlorophyll was noticed when using 70 and 80 °C for (30-150 min.), and the lower stability of chlorophyll was related to 100 °C for 150-180 min.

**Table (8): Thermal stability° of chlorophylls extracted from spinach at different temperatures in solution of pH 7.0.**

| pH values | chlorophylls of spinach | | chlorophylls of chard | |
|---|---|---|---|---|
| | Retention % | Degradation % | Retention % | Degradation % |
| 2 | 1.16$^{h}$ | 98.84$^{a}$ | 0.46$^{h}$ | 99.54$^{a}$ |
| 3 | 68.26$^{c}$ | 31.74$^{d}$ | 9.82$^{f}$ | 90.18$^{c}$ |
| 4 | 62.98$^{d}$ | 37.02$^{c}$ | 3.57$^{g}$ | 96.43$^{b}$ |
| 5 | 57.31$^{e}$ | 42.69$^{b}$ | 51.93$^{c}$ | 48.07$^{f}$ |
| 6 | 78.84$^{d}$ | 21.16$^{e}$ | 62.90$^{b}$ | 37.10$^{e}$ |
| 7 | 90.87$^{a}$ | 9.13$^{h}$ | 43.20$^{e}$ | 56.80$^{d}$ |
| 8 | 82.42$^{c}$ | 17.58$^{f}$ | 47.74$^{d}$ | 52.26$^{e}$ |
| 9 | 89.08$^{b}$ | 10.92$^{g}$ | 88.37$^{a}$ | 11.63$^{h}$ |
| L.S.D | 1.1359 | 1.3121 | 0.8495 | 0.6762 |

° Expressed as the retention% of the original pigment color.

Means within a column showing the same capital letters are not significantly different (P> 0.05).

Means within a row showing the same small letters are not significantly different (P> 0.05).

Thermal stability at different temperatures of chlorophylls extracted from chard in solution of pH 9.0 is shown in Table (9). The chlorophyll retention data showed trends similar to the chlorophylls extracted from spinach. The stability of chlorophylls extracted from chard was greater than stability of chlorophylls extracted from spinach at 100 °C for holding time (30-180 min).

**Table (9): Thermal stability° of chlorophylls extracted from chard at different temperatures in solution of pH 9.0.**

| Temperature °c | Tolerance period (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | LSD |
| 70 | $95.83^{Aa}$ | $95.18^{Aa}$ | $93.33^{Ab}$ | $91.62^{Ac}$ | $90.13^{Ad}$ | $89.84^{Ad}$ | 0.7877 |
| 80 | $93.34^{Ba}$ | $91.42^{Bb}$ | $89.40^{Bc}$ | $86.46^{Bd}$ | $84.23^{Be}$ | $83.46^{Be}$ | 1.5169 |
| 90 | $92.07^{Ca}$ | $91.31^{Ba}$ | $88.97^{Bb}$ | $85.31^{BCc}$ | $83.79^{BCd}$ | $81.68^{Ce}$ | 1.3933 |
| 100 | $92.00^{Ca}$ | $89.26^{Cb}$ | $86.12^{Cc}$ | $84.31^{Cd}$ | $83.02^{Ce}$ | $79.87^{Df}$ | 1.2375 |
| L.S.D | 1.1816 | 1.6092 | 1.4389 | 1.6043 | 1.1004 | 0.9501 | |

° Expressed as the retention% of the original pigment color.
Means within a column showing the same capital letters are not significantly different (P> 0.05).
Means within a row showing the same small letters are not significantly different (P> 0.05).

## Organoleptic evaluation of hard candy products:

Color, flavor, texture, clarity, mouth feel, and overall acceptability of hard candy containing 20.5 mg/100g synthetic green color and hard candy containing 0.13, 0.26 and 0.39% chlorophyll pigments extracted from spinach were evaluated. Sensory evaluation was statistically analyzed and shown in Table (10). No significant difference ( P> 0.05 ) were observed between hard candy contained synthetic colored and 0.26% natural pigment for color, texture, clarity, mouth feel and overall acceptability. The hard candy containing 0.26% pigment had superior organoleptic properties compared with other natural colored hard candy. The inferior color (11.50) and

overall acceptability (75) were recorded in hard candy prepared with 0.13% chlorophylls pigment extracted from spinach.

**Table (10): Effect of adding chlorophyll pigments extracted from spinach as a natural food colorant on selected sensory parameters of hard candy products.**

| Treatments | Tested parameters | | | | | Overall Acceptability (20) | Total score (100) |
|---|---|---|---|---|---|---|---|
| | Color (20) | Flavor (15) | Texture (15) | Clarity (15) | Mouth feel (15) | | |
| 1 | 19.6$^{a}$ | 13.7$^{ab}$ | 14.6$^{a}$ | 14.9$^{a}$ | 14.3$^{a}$ | 18.9$^{a}$ | 95.7$^{a}$ |
| 2 | 13.9$^{b}$ | 13.5$^{ab}$ | 14.5$^{a}$ | 12.9$^{b}$ | 14.1$^{a}$ | 14.2$^{b}$ | 82.4$^{b}$ |
| 3 | 18.2$^{a}$ | 14.3$^{a}$ | 14.6$^{a}$ | 14.4$^{a}$ | 14.4$^{a}$ | 18.8$^{a}$ | 94.3$^{a}$ |
| 4 | 11.5$^{c}$ | 13.0$^{b}$ | 14.2$^{a}$ | 11.4$^{c}$ | 12.8$^{b}$ | 12.1$^{c}$ | 76.0$^{c}$ |
| L.S.D | 1.4027 | 0.8905 | 0.5835 | 0.7406 | 0.8417 | 1.2187 | 3.984 |

Treatment 1:- hard candy + synthetic green color as a control (20.5mg/100g).
Treatment 2:- hard candy + 0.39 % pigment.
Treatment 3:- hard candy + 0.26 % pigment.
Treatment 4:- hard candy + 0.13 % pigment.
Means within a column showing the same letters are not significantly different (P> 0.05).

**Organoleptic evaluation of noodles products:**

Table (11) shows the sensory evaluation scores for the green noodles prepared with different levels of chlorophyll pigments extracted from spinach and green noodle purchased from local market as a control. Means comparison for color, taste, flavor, aroma, overall acceptability and total score used to evaluate the cooked noodle. A significant difference (P< 0.05) was recorded between the control sample and all treatments.

Scores of green noodle purchased from local market were lower than scores of all treatments. However, treatments 3 and 4 (0.051 and 0.077%) pigments improved the overall acceptability scores to 19.1 and 19.5, respectively. It could be concluded that noodle prepared from flour (72% extraction) and containing 0.051 and 0.077% chlorophyll pigments extracted from spinach had recorded superior acceptability than other treatments.

**Table (11): Effect of adding chlorophyll pigments extracted from spinach as a natural food colorant on selected sensory parameters of noodle products.**

| Treatments | Tested parameters | | | | Overall Acceptability (20) | Total score (100) |
|---|---|---|---|---|---|---|
| | Color (20) | Taste (20) | Flavor (20) | Aroma (20) | | |
| 1 | 12.4[c] | 17.6[b] | 17.3[b] | 18.4[a] | 13.4[c] | 77.0[c] |
| 2 | 16.0[b] | 18.1[ab] | 18.3[ab] | 18.4[a] | 17.1[b] | 86.6[b] |
| 3 | 18.7[a] | 18.5[ab] | 19.2[a] | 19.1[a] | 19.1[a] | 92.1[ab] |
| 4 | 19.4[a] | 18.8[a] | 19.0[a] | 19.1[a] | 19.5[a] | 96.1[a] |
| L.S.D | 1.488 | 1.1463 | 1.2809 | 1.0319 | 1.118 | 5.6969 |

Treatment 1:- green color noodle sample from local market (control).
Treatment 2:- noodle + 0.026 % pigment.
Treatment 3:- noodle + 0.051 % pigment.
Treatment 4:- noodle + 0.077 % pigment.
Means within a column showing the same letters are not significantly different (P> 0.05).

**Organoleptic evaluation of glazing jelly products:**

Panel scores indicated significant differences between means of color, clarity, grainess and overall acceptability for green glazing jelly containing different ratios of chlorophyll pigments extracted from spinach (0.13, 0.26 and 0.39%) with glazing jelly sample prepared with 20.5 mg/100g synthetic green color as a control.

Data of Table (12) showed that, the synthetic colored sample gave the highest scores of sensory properties. On the other hand, no significant differences (P> 0.05) were observed between natural colored samples for color, taste, texture, grainess, bleeding and overall acceptability. It could be observed also that, glazing jelly containing 0.13% chlorophyll pigment recorded highest score of taste, clarity, texture and grainess. From the aforementioned results, using 0.13% of chlorophylls pigment extracted from spinach to prepare glazing jelly product gave superior acceptability than other ratios of tested samples.

**Table (12): Effect of adding chlorophyll pigments extracted from spinach as a natural food colorant on selected sensory parameters of glazing jelly products.**

| Treatments | Tested parameters | | | | | | Overall Acceptability (20) | Total score (100) |
|---|---|---|---|---|---|---|---|---|
| | Color (20) | Taste (10) | Clarity (20) | Texture (10) | Grainess (10) | Bleeding (10) | | |
| 1 | 19.8$^{a}$ | 9.8$^{a}$ | 19.8$^{a}$ | 9.8$^{a}$ | 10.0$^{a}$ | 10.0$^{a}$ | 19.5$^{a}$ | 98.9$^{a}$ |
| 2 | 18.3$^{b}$ | 9.5$^{a}$ | 16.9$^{c}$ | 9.7$^{a}$ | 9.3$^{b}$ | 10.0$^{a}$ | 17.0$^{b}$ | 90.7$^{c}$ |
| 3 | 17.9$^{b}$ | 9.5$^{a}$ | 17.2$^{c}$ | 9.7$^{a}$ | 9.3$^{b}$ | 10.0$^{a}$ | 17.5$^{b}$ | 91.1$^{bc}$ |
| 4 | 18.1$^{b}$ | 9.6$^{a}$ | 18.9$^{b}$ | 9.8$^{a}$ | 9.7$^{ab}$ | 10.0$^{a}$ | 17.2$^{b}$ | 93.3$^{b}$ |
| L.S.D | 0.997 | 0.5014 | 0.6233 | 0.4112 | 0.6467 | 0.0 | 1.117 | 2.3418 |

Treatment 1:- jelly + synthetic green color as a control (20.5mg/ 100g of mixture).
Treatment 2:- jelly + 0.39% pigment.
Treatment 3:- jelly +0.26% pigment.
Treatment 4:- jelly +0.13% pigment.
Means within a column showing the same letters are not significantly different (P> 0.05)

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# خصائص الصبغات الكلوروفيلية المستخلصة من السبانخ (*Spinacia oleracea*) والسلق (*Beta vulgaris*) كملونات غذائية

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يهدف البحث الى إستخلاص صبغة الكلوروفيل من السبانخ والسلق والتعرف على مكوناتها بالتحليل الكروماتوجرافى ودراسة تركيز الصبغة وخصائص تحميلها على بعض المواد.ودراسة تأثير كل من pH ودرجة الحرارة على ثبات الصبغة وإستخدام صبغة الكلوروفيل المستخلصة من السبانخ كملونات غذائية طبيعية فى المكرونة والحلوى الصلبة وجيلى التجليس .

وأظهرت النتائج المتحصل عليها أن تركيز صبغات الكلوروفيل المستخلصة من السبانخ والسلق هو.123.67 ، 69.80 ملليجم / 100 جم علي التوالي واثبت التحليل الكروماتوجرافي وجود مركبي كلوروفيل (ا) وكلوروفيل (ب) في الصبغة المستخلصة من السبانخ بتركيز92.15 و 31.52 ملليجم/ 100جم علي التوالي كذلك وجد كلا من كلوروفيل (ا) وكلوروفيل (ب) في الصبغه المستخلصة من السلق وكان تركيزهما52.67 و16.83 ملليجم/100جم علي التوالي .

أظهرت النتائج أن النشا كان أفضل مادة تحميل للصبغة المستخلصة من السبانخ بينما الدقيق كان أفضل مادة تحميل للصبغة المستخلصة من السلق . بصفة عامة يمكن القول بان رقم ال pH المتعادل افضل رقم اس ايدروجيني لثبات كلوروفيل السبانخ بينما كان pH 9 هو الافضل للحفاظ علي كلوروفيل السلق .أيضا وجد أن كلوروفيل المستخلص من السبانخ و السلق ذو ثبات حراري جيد .

اوضحت الدراسة ان إضافة 0.26 و 0.077 و 0.026 % من الصبغة المستخلصة من السبانخ الى الحلوى الصلبة و المكرونة و جيلى التجليس على الترتيب سجلت اعلي قيم للون و القبول العام مقارنة بالكنترول .