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Production of Safe Green Natural Colorant from Carrot Leaves (*Daucus carota* L)

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THE AMOUNT of agricultural wastes seems to be high and could be used to obtain some important products. Carrot wastes represent about 36% of the total crop. These wastes, could be used to produce compounds of beneficial and economic values such as natural green coloring matters to be used in manufacturing food products. The results obtained ascertained the possibility of producting natural green colour from carrot (leaves) to be used in food industries.

Carrot leaves chlorophyll content degraded to about 50% of the initial content after 1 week storage under refrigerated conditions at 5°C in polyethylene bags. The loss reached to 92% at the end of storage (six weeks) under the same conditions. Gradual increase of pheophytin content occurred during the first 3 weeks of storage and gradually decreased until the end of storage. The best treatment for extraction of green colour from the carrot leaves was by hot water at 95±3°C for 3 minutes containing 0.5% MgO and 1% $CaCo_3$. Natural green colour was obtained as powder using the freeze-drying method. Addition of natural green colour (powder) to some foods increased their ash content. An increase in mineral salts such as Ca, Mg, Fe, Na, Cu and Zn occurred, in addition to improving taste, appearance and overall acceptability of such enriched foods with this natural green colour.

The amount of vegetable wastes seems to be high and could be used to obtain some important products such as chlorophyll from carrot leaves. The percent of wastes reached about 36.16 ± 13.52% of the whole plant (Mahmoud, 1992). An increasing demand exists for natural colorant in foods, among which is the chlorophyll as a green colorant.

The average acreage cultivated with carrots in Egypt is about 11100 Feddans (4662 Hectars) yielding about 128200 Tons (Ministry of Agriculture, 2000). Carrots can be processed into jams, syrups, juices and dry sheets or compote (Mona, 1990). After manufacturing the remainder of carrot leaves (by- product) is about 35% from the total product (Mahmoud, 1992). Carrot leaves (by-product) reached approximately 44900 tons. These wastes contained 87.44, 12.56, 20.35, 3.10, 10.86, 56.30 and 5.56% moisture content, dry matter, ash, crude protein, ether extract, crude fiber, total carbohydrates and total sugars respectively,

Higazy (1984), Gazal (1989) and Mahmoud (1992). These vegetable wastes seem to be high and could be used to obtain valuable products such as safe green colorant for foods.

Chlorophyll as natural colorant derived from green plants ranges from 30 to 200 mg per gram on dry weight basis and an endogeneous protein content of 150-500 mg/kg (DM basis). It could be used as a powder, or as a paste with a moisture content of 10-30% (Bortlik and Gauthier, 2000). If natural additives are not available the amount of artificial additives should be below safe levels, since some synthetic colorant in many foods, have been found to be toxic to experimental animals. Natural plant products began to receive much attention as sources of safe antioxidants (Frankel *et al.*, 1996).

Some plant leaves such as young green barley leaves contain strong antioxidants (Osawa *et al.*, 1992). These natural antioxidants are agents protecting from free radicals damages including atherosclerosis (Iwakami, 1965), liver diseases (Suematsu and Abe, 1982), diabetes (Saito *et al.*, 1979), aging (Sagai and Ichinose, 1980), and mutagenic and carcinogenic (Marnett *et al.*, 1985 ; Feinman, 1988 ; Esterbaure *et al.*, 1991 and Chaudhary *et al.*, 1994).

The present investigation was carried out to study the possibility of green colour production from carrot leaves for using in food industries. The effect of storage under refrigerated conditions on chlorophyll content, ash minerals and organoleptic properties was investigated.

Material and Methods

Materials

Carrot leaves (by-product) used in this study were obtained from the local market of Giza, Egypt. Fresh mature whole carrot plants were selected. The green leaves were removed from the plants and used for the study.

Methods

1- Extraction methods of green matter from carrot leaves included the following

a) Removing the parts (leaf stems) no green colour from carrot leaves.

b) Washing the leaves using distilled water.

c) Cutting the carrot leaves into small spieces (2-3 mm.).

d) Weighing samples of 100g chopped leaves in clear muslin cloth.

e) Plunging wrapped fresh leaves in hot water to prevent enzymatic oxidation or hydrolysis according to the method described by Harborne (1973). Hot water containing $CaCo_3$ and/or MgO (Negi and Roy, 2000).

The treatments were as follow

Sample No. 1: Carrot leaves were diping for 3 min in hot water 95 ± 3°C containing MgO at the concentration of 0.10%.

Sample No. 2: Carrot leaves were diping for 3 min in hot water 95 ± 3°C containing MgO at the concentration of 0.25%.

Sample No. 3: Carrot leaves were diping for 3 min in hot water 95± 3°C containing MgO at the concentration of 0.50%.

Sample No. 4: Carrot leaves were diping for 3 min in hot water 95 ± 3°C containing MgO at the concentration of 0.50% and $CaCo_3$ at the concentration of 1%.

Sample No. 5: Carrot leaves were diping for 3 min in hot water 95 ± 3°C containing $CaCo_3$ at the concentration of 1%.

Sample No. 6: Cavrot leaves were diping for 3 min in hot water 95 ± 3°C (without any additives).

Sample No. 7: Carrot leaves without any treatment or additives (control).

F) Extraction of leaves juice by pressing.

g) Cooling and freezing.

h) Selection of the best pre-treatment for drying using freeze-drying to obtain green matter powder.

2- Method of sweet preparation

500 g sugar were added to 500 ml boiling water with mixing, slowly poured 125 g starch were soluble in 150 ml cold water, continuously mixing until to ripening stage, green colour powder were added at concentration 1% during mixing, cooling, cutting and packing.

3- Method of ice-cream preparation

Pour 200 ml cold milk in a deep narrow bowl, add 85g ice-cream powder, whip with electric mixer at high speed for 3-4 minuets, add green colour powder at concentration 0.5% during whipping, packing and freezing.

4- Methods of analysis

a) Extraction of chlorophylls and pheophytins were accomplished according to the method described by James *et al.*, (1996) and some modifications.

A sample of carrot leaves washed in a strainer with double distilled water to remove the dressing. The sample was then patted dry with paper towels, two 5g (two 5 ml leaves juices). Each sample was blended with enough acetone 80% (V/V). the solutions were then filtered through a Whatman No. 4 filter. The filter cake was then washed with 50ml of 85% (V/V) acetone, which was sufficient to remove any remaining pigments from the filter cake and complete to 100ml for each sample. Sample 1 was measured at 660 and 642.5 nm. Sample 2 was acidified with 1 drop of concentrated HCL and allowed to stand in the dark for 2 hours before being measured at 666.5 and 653 nm. Total chlorophyll, chlorophyll A, chlorophyll B, pheophytin A and pheophytin B were calculated by the following equations:

- Total chlorophyll, mg/litre = (7.12 X OD at 660 nm) + (16.8 X OD at 642.5 nm).

- Chlorophyll A, mg/litre = (9.93 X OD at 660 nm) - (0.777 X OD at 642.5 nm).

The rate of these increments was 31% for pheophytin a and 70% for pheophytin b.Gradual decrease occurred after the fourth week. The rate of this decrease was 40 and 13% for pheophytin a and pheophytin b compared to zero time evaluation. Rapid conversion of chlorophyll to pheophytin suggests rapid acidic removal of Mg or an enzymatic reaction that is possibly by the enzyme chlorophyllase. These results were in accordance with Yamauchi, *et al.*, (1995); Heaton *et al.*, (1996) ; James, *et al.*, (1996) and Leunda *et al.*, (2000). Effects of heat treatment and additives on total chlorophyll and chlorophytll pigments during carrot leaves extraction were also evaluated.

For extraction of green matter from plant parts, some treatments such as heat treatment including blanching in hot water containing some additives such as MgO or $CaCo_3$ were used.Negi and Roy (2000) reported that the blanching in hot water at 95 ± 3°C for 3 minutes in the presence of MgO [5g/l] prevented pheophytin formation.

Table (2) shows data explaining effect of heat treatment and some additives on chlorophyll and chlorophyll pigments during carrot leaves extraction. From these data, it could be noticed that the treatment No. 4 using hot water containing 0.5% MgO and 1% $CaCo_3$ at 95 ± 3°C for 3 minutes was the best pre-treatment before freeze-drying to produce natural green colour as a powder, which could be used in food products.

TABLE 2. Effect of heat treatment and additives on chlorophyll and chlorophyll pigments during carrot leaves extraction (mg/L).

Components / Treatments	Total chlorophyll	Chlorophyll a	Chlorophyll b	Pheophytin a	Pheophytin b
1- Hot water containing 0.1 % MgO	4.11	2.17	1.93	1.05	0.85
2- Hot water containing 0.25 % MgO	5.04	2.77	2.25	1.12	1.02
3- Hot water containing 0.5 % MgO	5.21	2.90	2.30	1.01	0.98
4- Hot water containing 0.5 % MgO + 1% $CaCo_3$	6.56	3.84	2.70	0.78	0.72
5- Hot water containing 1 % $CaCo_3$	5.00	2.85	2.14	0.76	0.73
6-Hot water without any additives	3.07	1.58	1.57	2.05	1.85
7-without any additives or any treatment	5.75	3.63	2.10	1.35	1.30

This powder was added into two types of foods (sweet and ice – cream), followed by the organoleptic evaluation, to explain the effect of these additives on flavour, colour (appearance), and overall acceptability. As well as ash and

minerals contents of such foods was studied because the natural green colour powder containing of high level of ash.

TABLE 3. Change in ash content after addition of natural green colour.

Treatments	Ash content %
1- Sweet without any additives	0.8%
2- Sweet with natural green colour	2.0%
3- Ice- cream without any additives.	1.0%
4- Ice- cream with natural green colour.	1.6%
5- Natural green colour powder.	35.6%

Table (3) shows change of ash content after natural green colour addition. From these data, it could be noticed that the ratio of ash content in treatment No. 5, (natural green colour powder) was 35.6%. This ratio resulted in high percent of ash content of treatments containing powder. This would explain the increase of ash content in treatment No. 2 (containing 1% powder) and treatment No. 4 (containing 0.5% powder).

Mineral salts are important in human nutrition and necessary for vital activities of human body. Different vegetables are considered to be main sources of mineral salts. Table (4) shows changes of mineral content after the addition of natural green colour powder.

TABLE 4. Change in mineral content after the addition of natural green colour powder (mg/100g).

Treatments / Components	1	2	3	4	5
Ca	0.688	7.831	0.823	8.468	91.170
Mg	0.870	2.930	1.220	1.690	53.000
Fe	0.730	2.847	0.347	1.487	3.719
Na	20.310	60.990	31.780	38.490	712.900
Cu (ppm.)	0.093	0.100	0.063	0.087	0.126
Zn	0.272	0.322	0.147	0.252	0.668

- Treatment No. 1 : Sweet without any additives.
- Treatment No. 2 : Sweet with natural green colour powder.
- Treatment No. 3 : Ice- cream without any additives.
- Treatment No. 4 : Ice- cream with natural green colour powder.
- Treatment No. 5 : Natural green colour powder.

From the data in Table (4) minerals were, 91.17mg, 53.00 mg, 3.719mg, 712.9mg, 0.126 ppm and 0.668mg/100g for Ca, Mg, Fe, Na, Cu and Zn respectively in

treatment No. 5 which shows the highest values compared to the other treatments especially the control.

Addition of powder to the sweet (treatment No. 2) caused an increase in mineral salts ratios. These ratios were 1038, 237, 290, 200, 7.5 and 18% for Ca, Mg, Fe, Na, Cu and Zn, respectively compared to the control which gave relatively lower values (treatment No. 1).

Addition of the powder to ice – cream (treatment No. 4) caused an increase in mineral salts ratio. These ratios were 929, 39, 329, 21, 38 and 71% for Ca, Mg, Fe, Na, Cu and Zn respectively compared to the control which gave relatively lower values (treatment No. 3).

5) Organoleptic evaluation

Data shown in Table (5) explain the effect of natural green colour powder addition on the organoleptic characteristics of sweet and ice-cream, concerning taste, appearance and overal acceptability using statistical analysis.

TABLE 5. Effect of addition of natural green colour powder on the organoleptic characteristics of sweet and ice-cream.

Samples Parameters	1-Sweet without any additives	2-Sweet with natural green colour powder	3- Ice-cream without any additives	4- Ice-cream with natural green colour powder
Taste	a 8.5 ± 0.29	a 8.5 ± 0.29	a 8.8 ± 0.29	a 8.8 ± 0.29
Appearance	a 7.6 ± 0.29	ab 8.3 ± 0.44	a 8.6 ± 0.29	a 8.6 ± 0.29
Overal Acceptability	a 7.8 ± 0.25	ab 8.3 ± 0.14	a 8.1 ± 0.29	ab 8.6 ± 0.29

a-b Means within a row with the same superscript are significant different ($P<0.05$).

From these data it could be observed that the sweet containing natural green colour powder (sample No. 2) doesn't effect on taste and significant increase of appearance and overal acceptability compared to the control. Ice-cream containing natural green colour powder doesn't effect on taste and appearance and slightly improved on overal acceptability compared to the control.

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إنتاج لون أخضر طبيعى آمن من أوراق الجزر واستخدامه فى بعض المنتجات الغذائية

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مع زيادة الإنتاج الزراعى من محاصيل الخضر فإنه بالتالى تحدث زيادة فى كمية المخلفات الناتجة من تلك المحاصيل. ومحصول الجزر ينتج عنه مخلفات "أوراق الجزر" تمثل ٣٦% تقريباً من الكمية الإجمالية للمحصول وليس لها فائدة اقتصادية. والهدف الرئيسى لهذه الدراسة هو إمكانية إنتاج لون أخضر طبيعى من أوراق الجزر واستخدامه فى الصناعات الغذائية نظراً لما للألوان الصناعية من أضرار.

ومن أهم النتائج التى تم الحصول عليها:-

١- إمكانية إنتاج لون أخضر طبيعى من أوراق الجزر ثابت عند استخدامه فى تلوين نوعين من الأغذية احدهما يستخدم فيه المعاملة الحرارية (بعض أنواع الحلوى) والأخر يستخدم فيه التبريد مثل الآيس كريم (المثلجات اللبنية).

٢- محتوى أوراق الجزر من الكلورفيل حدث لها فقد بنسبة ٥٠% بعد الأسبوع الأول من التخزين فى البولىإثيلين تحت ظروف التبريد (٥°م) وفى نهاية فترة التخزين تم فقد ٩٢% منه بعد ٦ أسابيع تحت نفس الظروف.

٣- فى بداية فترة التخزين تحت نفس الظروف حدثت زيادة تدريجية فى محتوى الأوراق من الفيوفيتين فى الثلاث أسابيع الأولى ثم حدث نقص تدريجى فى محتوى الأوراق من الفيوفيتين فى الثلاث أسابيع الأخيرة من فترة التخزين.

٤- للحصول على المستخلص الأخضر من أوراق الجزر تم معاملتها حرارياً على ٩٥± ٣°م لمدة ٣ دقائق مع إضافة أكسيد الماغنسيوم أو كربونات الكالسيوم بنسب مختلفة. وكانت أحسن المعاملات التى أضيف إليها ٠,٥ % أكسيد ماغنسيوم + ١% كربونات كالسيوم من ماء المعاملة وهى المعاملة رقم ٤ والتى تم تجفيفها بالتجميد والحصول على اللون الأخضر منها والذى أضيف إلى بعض أنواع الأغذية.

٥- إضافة اللون الأخضر الطبيعى إلى بعض الأغذية أدى إلى زيادة النسبة المئوية للرماد فى هذه الأغذية.

٦- إضافة اللون الأخضر الطبيعى إلى كل من الحلويات والآيس كريم أدى إلى زيادة ملحوظة فى تركيز المعادن مثل الكالسيوم والماغنسيوم والحديد والصوديوم والنحاس والزنك.

٧- إضافة اللون الأخضر الطبيعى إلى بعض الأغذية أدى إلى التحسن فى الطعم والمظهر والقابلية العامة لهذه الأغذية.