THE ROLE OF CAROTENOIDS AS NATURAL FAT ANTIOXIDANT

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Abstract

Carotene extracted from fresh carrots, and dried carrot powder were used to study their effects as natural antioxidant at levels of 0.05, 0.01 and 0.2% (w/w). Carotene and carots powder were added to refined corn oil and the oxidizability were determined in dark (oven at 63±1oC) and light. The addition of carotene, in all cases, improved the stability of refined corn oil, and the stability was proportionally increased as the carotene concentration increased. Carrot powder, obtained from carrot dried in indirect sunlight, increased the stability of refined corn oil (exposed to light) from 4 days to 11 days also, the oil had better colour compered to the other treatments.

INTRODUCTION

Carotenoids play a great part in the colour of several vegetable oils. Carotenoids are widely distributed in nature. They are found in plants, insects, and birds producing a wide array of brilliant yellow to red colors. The color of yellow corn, tomatoes, apricots and carrots, just to name a few, is due to carotenoids. The name "Carotene" was given to the yellow pigment of the carrot (Daucus Carota) from which it was first isolated in (1831).

Carotenoids have advantages and disadvantages. Among the advantages, they are natural color materials wich are stable in the pH range of most food products; unaffected by reducing substances like ascorbic acid; non-corrosive; excellent supply of provitamin A, content of B-carotene and B-apo-δ carotenal; and highly desirable yellow-orange-red colors. Some of their disadvantages, the limited color range and sensitivity to oxidative degradation, Alexander Emodi (1978). Plant extracts containing carotenes and various carotenoids are frequently used as natural colouring materials but they also posses antioxidant activity, especially in the presence of light. They act by quenching singlet oxygen produced during photosynthesis and their capability of inhibiting free radical-induced in lipid peroxidation. Terao (1993).

The presence of carotenoids in edible oils may also help in preventing the formation of singlet oxygen by blocking light transmation through the oil, owing to their multiple conjugated double-bond system. (Jan Ponkorny, 1991). Krinsky (1989) reported that carotenoid pigments, including hydrocarbons such as beta-carotene or xanthophylls are very widely distributed in nature, where they play an important role in protecting cells organisms from the harmful effects of light, air and sensitizer pigments.

In addition to this protection, is the fact that carotenoids can also act as antioxidant in conditions other than photosensitization. Sies *et al* (1992) discussed the metabolism and antioxidant properties of vitamin E, beta-carotene and other carotenoids. Palozza *et al.* (1994) showed that the antioxidant effect of betacarotene depend on the oxygen pressure, it is antioxidant at low oxygen pressure and proxidant at high oxygen pressures; carotenoids are generally belived to be an effective physical quaencher of oxygen and enhance the oxidative stability of oil, Carlsson *et al* (1976).

However, Matsushita and Terao (1980) reported that the prooxidant activity of carotenoids in stabilizing edible oils had not yet clearly established.

Haila and Heinonen (1995) studied the effects of B-carotene and α-tocopherol on the photo oxidative stability of purified rape seed oil. They noticed that in the presence of both B-carotene and α-tocopherol, oxidation was inhibited more effectively than by α-tocopherol alone. It had been concluded that there was a potential antioxidant benefit in combining B-carotene and α-tocopherol in food systems.

Meanwhile Kiritsakis and Dugan (1985) reported that the presence of 4 or 6 ppm of B-carotene decreased the peroxide formation in the early stages of light oxidation of olive oil. They concluded that carotenes either acted as singlet oxygen quenchers or were oxidized, thus sparing the oil until the carotenes were destroyed by oxidation. Carlsson *et al* (1976) also reported that B-carotene is an efficient singlet oxygen quencher, that can provide protection at a level of 0.01% by weight. They concluded that the inclusion of nontoxic, effective singlet oxygen quencher in foods containing unsaturated oils could substantially improve their shelf life. This work was carried out to evaluate the effect of low levels of carotenoids in improving the oxidative stability of refined corn oil in dark and light.

MATERIALS AND METHODS

Materials

Fresh carrots and refined corn oil were obtained from the local market.

Methods :

Isolation of carotenoids:

Fresh carrots were used for carotene isolation. The fresh carrots were washed and cut to a small pieces, The sample was divided into three portions and treated as follows:-

1- The first portion

Carotenoids were extracted according to Kew and Berry (1970) method from the first portion as follows:

Extraction: The prepared sample was well mixed with freshly distilled haxane (1:2 w/w) in a blender for three mins. The mixture was then filtered using Buchner funnel. The residue was rinsed with fresh hexane for several times. These rinsings were added to the original extract. The hexane extract was concentrated in a rotary evaporator under vaccum at 50^{O}C.

Saponification: Saponification was carried out to remove the fixed oils and the chlorophylls extracted with carotenoids. The concentrated extract was washed twice with 200 ml portions of methanolic potassium hydroxide solution (100g. potassium hydroxide, 750 ml of methanol and 250 ml water) in a separatory funnel. The upper layer was washed for several times with 10% dihydrogen potassium phosphate solution until the pH reached 7.5. The aqueous layer was discarded. The hexane extract was dried over anhydrous sodium sulphate and concentrated in a rotary evaporator .

Steam distillation : After removal of hexane, the d-limonine was removed from the remaining concentrate of crude pigment by steam distillation which was carried out at 50°C and under 10 mm HG. by passing atmopheric steam into the crude pigment extract. After colling to room temperature, the mixture was transferred to a separatory funnel and a double volume of fresh distilled hexane was added. The aqueous layer was discarded and the hexane layer was dried over anhydrous sodium sulphate. The hexane extract was concentrated using a rotary evaporator untile the complete removal of hexane. The crude pigment mixture was then weighed.

II- The second portion : Was dried in indirect sunlight under atmospheric condition for 4 days then ground in a blender to make the powder form.

II- The third portion : Was dried in an oven under vaccum at 50°C for three days, then ground in a blender to make the powder form.

Antioxidant properties of carotenoids : (crude extract and powder form)

1. To study the antioxidant effect of the carotene (extract and powder) three concentrations were added to freshly prepared corn oil (initail peroxide was zero) these concentrations were 0.05. 0.1 and 0.2% w/w from carotene. The peroxide values were followed periodically every 48 hrs at 63°C ± 1, oven test method as suggested by Thompson (1960).

2. The three concentrations from cartoene (extract and powder) were also added to refined corn oil, samples were kept under a flourescent lamp. To estimate the oxidative deterioration, the peroxide value of the oxidized oils was determined every 48 hours.

Light storage conditions for the oxidation study:

The light storage box consisted of two rectangular chambers, the first chamber was (60 cm x 30 cm x 50 cm) for sample storage and the wooden box (70 cm x 50 cm x 60 cm) for light sources. Samples were placed on the wire netting which was 10 cm above the bottom of the chamber. The light source was two sylvania flourescent lamps 60 watt each, white flourecent lamps were placed on the bottom of the wooden box and the temperature was 30oC. as reported by Lee and David (1988)

RESULTS AND DISCUSSION

1- Effect of addition of different concentration of carrotenoids as antioxidant on the stability of refined corn oil:

Carotene extracted from carrots at 0.05, 0.1 and 0.2% w/w were added to corn oil and the stability of all samples was determined by measuring the changes in peroxide value during the incubation at 63oC. The obtained results are graphically illustrated in fig (1) and the induction periods are tabulated in table 1. From table 1and fig (1), it can be noticed that antioxidant effect of carotene extract from carrots was found to increase as the added concentration was increased. The addition of the carotene to corn oil increased the induction period from 4 days for the control (refined oil) to 5, 6-7 and 7 days of corn oil containing 0.05. 0.1 and 0.2% w/w of natural extract of pigment respectively.

Table 1. Effect of the addition of carotene extracted by solvent from carrots on the stability of corn oil.

	Induction Period at 63°C in days		
Control	0.05%	0.1%	0.2%
4	5	6-7	7

2- Effect of addition of carrot powder obtained from carrots dried in indirect sunglight on the stability of refined corn oil :

Low concentrations (0.05, 0.1 and 0.2% w/w) of the carrot powder were added to the refined corn oil, stability was measured at 63oC ± 1. The obtained data are presented in table 2 and graphically in fig 2. It is evident from these results that the addition of (0.05, 0.1 and 0.2% w/w) of carrote powder to corn oil increased its stability from 4 days to 7,8 and 9 of the added natural corrat powder respectively.

Table 2. Effect of the addition of carotene Powder obtained from carrote dried in indirect sun light on the stability of corn oil at 63°C.

	Induction Period at 63°C in days		
Control	0.05%	0.1%	0.2%
4	5	8	9

3- Effect of addition of carrot powder obtained from carrots dried in oven under vaccum at 50oC on the stability of corn oil:

To study the antioxidant effect of carotene obtained from carrots as powder through drying in an oven under vaccum at 50°C, three concentrations were added to refined prepared corn oil (initial peroxide value was zero), these concentrations were 0.05, 0.1 and 0.2% w/w. The peroxide values, of the corn oil treatments kept in oven at 63°C, was followed every 48 hr for 21 days. The obtained results are shown in table 3 and are graphically presented in fig 3. From the results in table 3 and fig 3 it can be observed that carotene acted as antioxidant at all concentrations since the induction period increased from 4 days to 6,6-7, and 7-8 days,respectively.

Therefore, it is beleived that, in many cases, carotenoids play a role in the prevention of lipid peroxidation as reported by Abdus Sattar *et al* (1976). They said that carotene was considered to serve as a filter for light of low wave length and studying the antioxidative properties of some carotenoids and vitamin A had shown that both B-carotene and vitamin A were inhibitors of spontaneous fat and oil oxidation. These findings were confiermed by Warner and Frankel (1987), Witas and Wedzinskaa (1988) and Jan-Pokorny (1991).

Table 3. Effect of the addition of carotene Powder obtained from carrots dried in oven under vaccum at 50°C on the stability of corn oil at 63°C.

	Induction Period at 63°C in days		
Control 4	0.05% 6	0.1% 6-7	0.2% 7-8

Effect of addition of carotene on the stability of corn oil when exposed to flourescent light :

Carotene extracted from carrots was added separately to corn oil at 0.05, 0.1 and 0.2% w/w and then exposed to flourescent light at room temperature. Oil stability was determined and the obtained results are shown in table 4 and graphically illustrated in fig 4. It is clear that the addition of caroten at all concentrations increased the stability since the induction period was increased from 4 days to 6, 7 and 8 days respectively.

Table 4. Effect of the addition of carotene extracted by solvent on the stability of corn oil as exposed to fluorescent light.

	Induction Period of oil under florescent light		
Control 4	0.05% 6	0.1% 7	0.2% 8

Table 5 and fig 5 show that the addition of carotene powder, which was obtained from air dried carrot in indirect sunlight, generally increased the oils induction peroid from 4 days to 8,9 and 11 days respectively.

Table 5. Effect of the addition of Powder carrotes dried in indirect sunlight on the stability of corn oil as exposed to fluorescent light.

	Induction Period of oil under florescent light		
Control 4	0.05% 8	0.1% 9	0.2% 11

Table 6 and fig 6 show the effect of addition of carotene powder which was obtained from carrots dried in an oven under vaccum at 50°C on the stability of refined corn oil. It can be noticed that there is a gradual increase in the stability, the induction peroid increased from 4 days to 7,8 and 9 days respectively.

Table 6. Effect of the addition of Powder carrotes dried in indirect sunlight on the stability of corn oil as exposed to fluorescent light.

	Induction Period of oil under florescent light		
Control 4	0.05% 7	0.1% 8	0.2% 9

This agree with Abdus Sattar *et al* (1976) and Jan Pokorny (1991), who reported that plant extracts containing carotenes and various carotenoids are frequently used as natural colouring materials, but they also possess antioxidant activity, especially in the presence of light. They act by quenching singlet oxygen produced during photosynthesis, and the presence of carotenoids in edible oils may also help protect against the formation of singlet oxygen by blocking light transmission through the oil.

Conclusion :

It was concluded from the above mentioned results that carrot powder can be used as natural antioxidant. In addition, the carrot powder (dried carrot in indirect sunlight) increased the stability more than the carrot powder obtained from carrot dried in oven under vaccum.

The stability of oil samples in white flourescent light is more than that at oven test (dark). This may be due to the low temperature at flourescent light (30oC).

Generally it could be recommended that carrot powder (from carrot dried in indirect sunlight) protect edible oil from light oxidation, and as a result it increase the shelf life of oils during storage.

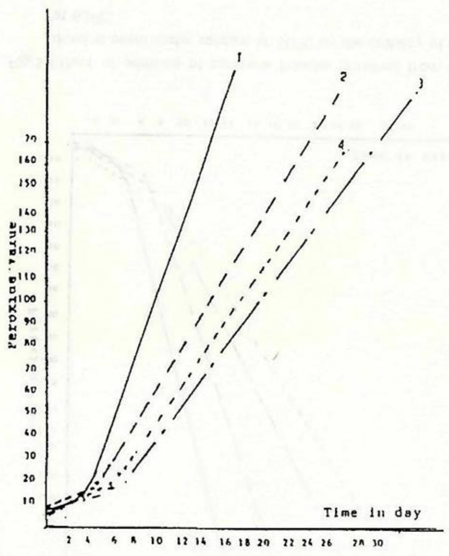

Fig.1 Effect of addition of carotene extracted by solvent from carrots on the stability of corn oil.

1. Control.
2. Control + 0.05%
3. Control + 0.1%
4. Control + 0.2%

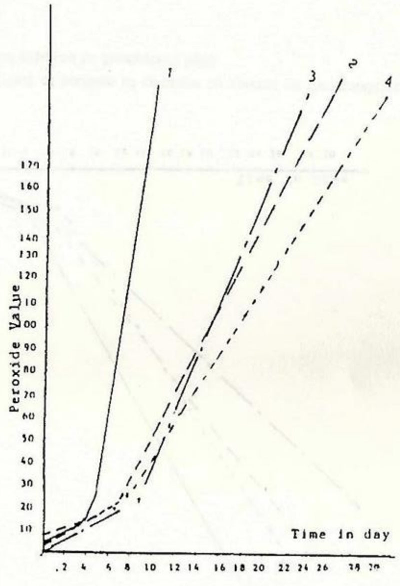

Fig.2 Effect of addition of carotene Powder, obtained from carrote dried in indirect sun light, on the stability of corn oil at 63°C.

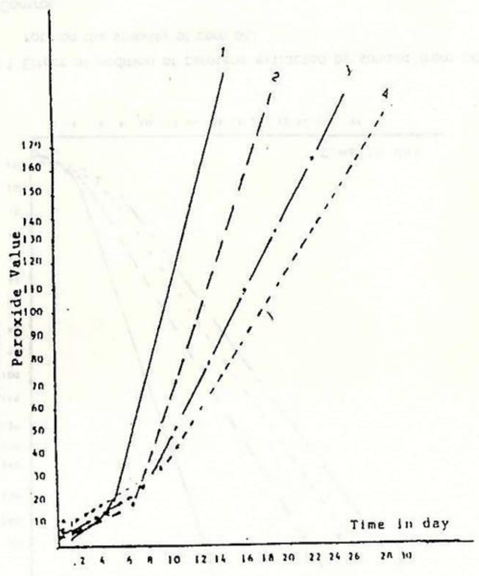

Fig.3 Effect of addition of carotene Powder obtained from carrote dried in ovem under vaccum at 50°C on the stability of corn oil at 63°C.

1. Control.
2. Control + 0.05%
3. Control + 0.1%
4. Control + 0.2%

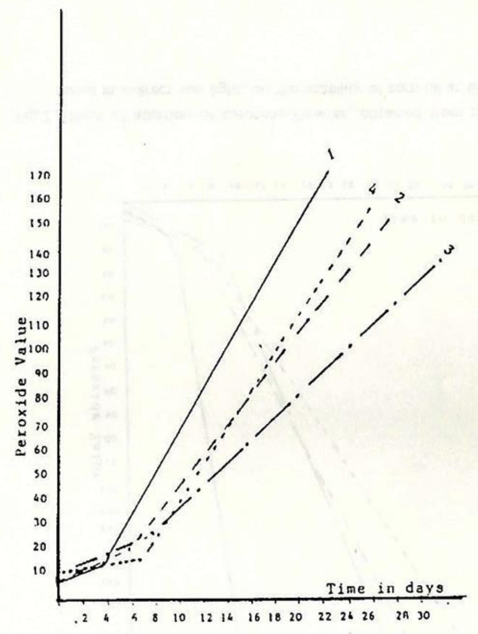

Fig.4 Effect of addition of carotene by solvent on the stability of corn oil exposed to fluotescent light.

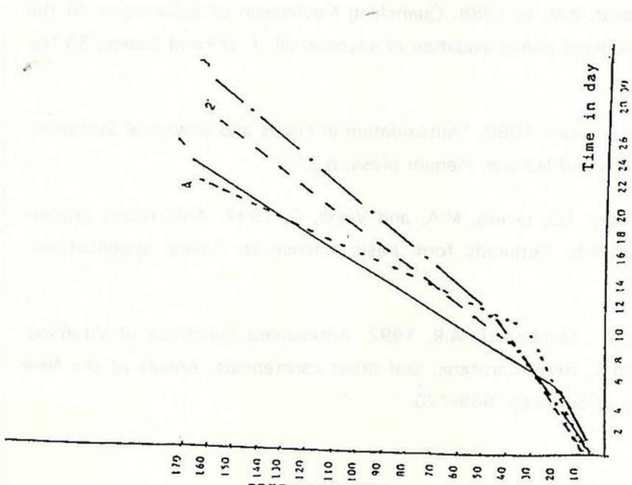

Fig.5 Effect of addition of Powder carrote dried in indirect sunlight on the stability of corn oil exposed to fluorescent light.

1. Control.
2. Control + 0.05%
3. Control + 0.1%
4. Control + 0.2%

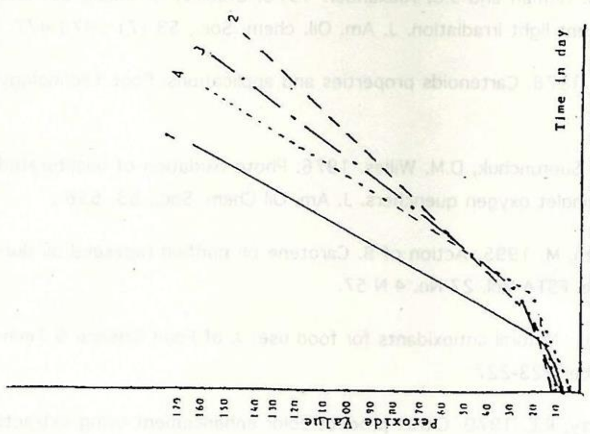

Fig.6 Effect of addition of Powder carote dried in oven under vaccum at 63°C on the stability of corn oil exposed to fluorescent light.

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دور الكاروتينات كمضادات أكسدة طبيعيه فى الظلام وفى ضوء الفلورسنت

عواطف إبراهيم إسماعيل

معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية - جيزة - مصر.

تم استخلاص الكاروتين من ثمار الجزر الطازجة ، كما تم تجفيف الجزر بطريقتين :-

١ - فى الجو العادى فى ضوء الشمس الغير مباشر.

٢ - فى الفرن تحت تفريغ على درجة ٥٠ م للحصول على الكاروتين فى صورة بودرة.

تم دراسة تأثير الكاروتينات كمضاد للأكسدة فى كل من الحالات السابقة بثلاثة تركيزات مختلفة وهى ٠,٠٥٪ ، ٠,١٪ ، ٠,٢٪ وزن /وزن وتم إضافتها الى زيت الذرة المكرر. ثم تم تقدير الثبات :-

١ - بوضع العينات فى الفرن على درجة ٦٣ م.

٢ - تحت ضوء الفلورسنت الأبيض ووجد أن إضافة الجزر (المجفف فى الجو العادى فى ضوء الشمس الغير مباشر). أفضل طريقة لزيادة ثبات الزيت عند تعريض العينات لضوء الفلورسنت حيث زادت فترة التحضين من ٤ يوم فى العينة الأساسية الى ١١ يوم بعد الإضافة حيث أن هذه العملية تعتبر إقتصادية فيمكن إستخدامها بدلاً من طريقة الإستخلاص بالكيماويات.