ANTIOXIDANT ACTIVITY OF YELLOW PEPPER CAROTENOIDS

AZOUZ, A.[1], K. H. TOLBA[2] AND E.M. RIZK[2]

1. Oils and Fats Res. Dept., Food Tech. Res. Inst., ARC, Giza, Eygpt
2. Horticultural Crops Res. Dept. Food Tech. Res. Inst., ARC, Giza, Eygpt

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Abstract

As the demand for natural antioxidant increases with the increasing of consumer health awareness towards the hazard effect of synthetic antioxidants, there is a considerable interest in exploring new sources of natural compounds like carotenoids. Carotenoids were extracted from yellow pepper and identified by High Performance Liquid Chromatography (HPLC). Results revealed that, violaxanthin was the predominant carotenoids identified in yellow pepper extract followed by cryptoxanthin and lutein. 100, 200, 300 and 400 ppm of extracted carotenoids were tested for their efficiency in retarding the oxidative rancidity by Rancimat method and their capability to stand for the destructive effects of heating process at 180°±10°C for 5 hr/day for consecutively 5 days compared with the most common used synthetic antioxidant; BHT. Physicochemical characteristics of heated oils were monitored throughout heating process and results showed the high efficiency of the extracted carotenoids as antioxidant.

INTRODUCTION

It is widely believed that increasing fruit and vegetable consumption reduces risk factors for cancer, cardiovascular disease and a number of other diet–related chronic diseases. These foods contain relatively high levels of beneficial phytochemicals, including antioxidants that inhibit in vitro low– density lipoprotein (LDL) cholesterol oxidation and many account, in part, for their protective effect against certain human degenerative diseases that are associated with oxygen free radical damage. It is generally assumed that the vitamin and provitamins antioxidants (ascorbic acid, tocopherols and carotenoids) contained in them accounts for their beneficial effects. A large number of vegetable and fruit have been explored for their antioxidant potential (Leong and Shui 2002).

Carotenoids are the widest distributed group of pigments. They are responsible for many of the brilliant red, orange, and yellow colors of fruits, vegetables and flowers. Nutritional interest was initially on the provitamin A- carotenoids, particularly in vegetables. Expanded interest in plant carotenoids indicated their anti-carcinogenic, anti-ulcer or anti-aging properties. Carotenoids appear to be involved in protection against both singlet oxygen and triplet oxygen (as radical chain–breaking antioxidants). Carotenoids are most effective biological quenchers of O_2 . Singlet oxygen is known to be capable of damaging DNA (Rizk et al., 2006). Many diseases, such as cancer and strokes, involve oxidative processes mediated by free radicals. Carotenoids, by their antioxidant effect, can show benefits in such diseases. There exists evidence of the effectiveness of β-carotene in the treatment of certain kinds of cancer, for example, smoking–related cervical intraepithelial neoplasia and cervical and stomach cancer. β–carotene have shown its effects on the immune response in rats, and by this means tumor growth is inhibited. The antimutagenicity of carotenoids in Mexican green peppers was studied. The antimutagenicity inhibition by nitroarenes was higher than 90%. Pepper carotenoids were more efficient antimutagens than pure β-carotene, suggesting that other carotenoids (e.g. lutein, zeaxanthin) in the pepper extracts showed a synergistic effect with β-carotene. Also, it was mentioned that the antimutagen activity might be from blocking the entrance of toxic compounds into the cell or by their antioxidant activity. Antioxidants are often added to lipid–containing products to delay or slow the rate of oxidation and to increase the shelf–life of foods by 15-200%. Synthetic antioxidants, are widely used in the food industry because they are effective and less expensive than natural antioxidants. However, the safety of synthetic antioxidants has been questioned in recent years as they cause pathological enzyme and lipid alterations and have carcinogenic effects. Furthermore, evidence is accumulating that natural antioxidants in foods may have clear benefits because they have anticarcinogenic effects and inhibit biologically harmful oxidation reactions in the body (Samah and Mohamed 2002).

This study was conducted to shed the light on yellow pepper as one of the most important sources of carotenoids; and to identify their efficiency as antioxidant. Also, to evaluate their capability to retard thermal oxidation during heating sunflower seed oil.

MATERIALS AND METHODS

Materials:

Yellow pepper (*Capisum annum*), yield of 2005 season, was obtained from the Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Butylated hydroxy toluene (BHT) was provided from Tokyo KaseiKogyo Co. (Tokyo, Japan)

Sunflower oil: Refined sunflower oil without adding antioxidant was obtained from Safola Saime, Egypt.

Methods

Extraction and concentration of carotenoids

Carotenoids from sweet yellow pepper were extracted using the method of Marimion (1984). Finely grounded dried pods were extracted for 4 hrs using petroleum ether (b.p. 40-60). After filtration, the extract was diluted with 600 ml ether, 100 ml

30% methanolic KOH was added and the mixture was stirred for 8 hr. The ether layer was collected, washed, and concentrated. The concentrated carotenoids were dried in oven at 40°C for 24 hr.

Identification of carotenoids

Carotenoids from yellow pepper were identified by Kanuer HPLC pump 64 according to the method reported by Ohmacht, (1979) using C18 column (3.9×150 mm) with a mobile phase of methanol and ethyl acetate in a liner gradient for 20 min. The detection wave length was set at 475 nm and the flow rate was 1.8 ml/min.

Oil heating process:

Sunflower oil was heated without any addition (control sample), and with adding 200 ppm BHT. Sunflower oil samples were mixed with different concentrations of 100, 200, 300 and 400 ppm of carotenoids extracted from yellow pepper. Oil samples were heated continuously on a gas cooker at 180°C for 5 hrs/day for 5 consecutive days. At certain periods of heating, aliquot samples were taken and stored at 5°C for subsequent determinations.

Determination of the physical and chemical characteristics of heated sunflower oil

Refractive index at 25°C, smoke point, colour at 35 yellow, acid, peroxide, iodine values, and oxidized fatty acids were determined according to A. O. A. C. (2000). Changes in viscosity of oils during heating were determined by using a Brookfield Viscometer RVDV-1+C/P connected with water bath Brookfield TC500. Viscosity was measured at 25°±0.01°C according to the method described by Howard (1991). Determination of the thiobarbituric acid value (TBA) was carried out according to Sidwell et al. (1954).

Determination of induction period with Rancimat method

Rancimat 679 (Metrom Ltd., CH 9100 Herisau, Switzerland) was used for the determination of oxidative stability of oils. The induction time was automatically determined, i.e., the time from the start of the experiment to the intersection point (Mendez et al. 1996)

RESULTS AND DISCUSSION

Carotenoids compounds were extracted from yellow pepper, identified by HPLC technique and calculated results are shown in Table (1). Extracted carotenoids were separated based on their functional groups into eight fractions namely violaxanthin, lutein, zeaxanthin, cryptoxanthin, α-carotene and β-carotene with calculated concentrations of 33.07, 13.37, 10.96, 14.10, 10.90, 6.73%, respectively and 10.87% unidentified compounds. These results are confirmed by Matus et al. (1991). The violaxanthin was the most abundant carotenoid in dried yellow pepper extract followed by cryptoxanthin, and lutein.

Rizk et al. (2006) extracted and identified the carotenoids compounds from red pepper and reported that capsanthin was the predominant carotenoid followed by capsorubin and β-carotene.

Antioxidative efficiency of extracted caratenoids:

Different concentrations of carotenoids compounds extracted from yellow pepper; 100, 200, 300 and 400 ppm, were examined for their antioxidative efficiency in retarding the accelerated oxidative rancidity induced by Rancimat technique compared with synthetic antioxidant; 200 ppm BHT, and control sample. Results in Fig (1) demonstrated that, addition of BHT increased the oxidative stability of sunflower oil by 1.6 fold of its original value. Adding different levels of extracted carotenoids had variable efficiency as antioxidants. While 100 ppm had slightly lower efficiency than BHT (1.3 fold of control), 200, 300 and 400 ppm had almost the double (2.5) triple (3.13) and forth times induction period than the oxidative stability of control sample, respectively. Results showed that 300 and 400 ppm of extracted carotenoids proved their high efficiency as antioxidants.

These results are in accordance with those reported by Rizk et al. (2006) who reported that, the number of double bonds, carbonyl groups and cyclopentane rings in the carotenoids structure enhanced their antioxidant activity and suggested that carotenoids could be used as highly efficient antioxidants.

Effect of adding carotenoids extracted from yellow pepper on physicochemical properties of heated sunflower oil:

Oil heated at high temperatures is subjected to a series of degradation reactions, thermal oxidation and formation of a variety of decomposition compounds. This decomposition results in poor performance of oil and off-flavor formation and may influence the nutritional quality and the safety of food. Antioxidants are used to prevent or delay the thermal and oxidative deterioration during heating process and to extend their shelf-life and improve their stability. Different concentrations of carotenoids compound extracted from yellow pepper, (100, 200, 300 and 400 ppm) were added to sunflower oil. Treated oils were heated at 180°±10°C for consecutively 5 days and their quality characteristics were monitored through determining the changes in physicochemical properties that occurred during heating process. Changes occurring during heating oil without any additives (control) and with adding 200 ppm BHT were simultaneously determined with the data previously reported by Rizk et al. (2006) in the first part of this study.

Refractive Index

Due to the thermal oxidation during heating and the formation of decomposed compounds, refractive index (RI) of oil increased. Results in Fig (2) showed that, addition of 200 ppm BHT had moderate effect on decreasing the increment in RI values during heating process for 5 days. While 100 ppm had similar effect as BHT during the first 3 days of heating and lost its effect in retarding the thermal oxidation during the last 2days. The addition of 200, 300 and 400 ppm extracted carotenoids had better effect on RI values than BHT during the 5 days of heating and this effect was more pronounced with the increment of extract concentrations.

The increment of RI during thermal treatment was also reported by Samah and Fyka (2004), Samah and Azouz (2005).

Colour

Data in Fig (3) showed that, the addition of different concentrations of extracted carotenoids increased the colour of sunflower oil at zero time and lowered the effect of thermal oxidation on colour development. 400 ppm of extracted carotenoids had the highest effect in retarding the changes that occurred in colour during heating process followed by 300, 200 ppm of extracted carotenoids. BHT and 100 ppm carotenoids had the lowest efficiency as antioxidant. Darkening of oil colour during thermal treatment was previously reported by Baixauli et al. (2002).

Smoke Point

Effect of heating process on smoke point of oil treated or untreated with different levels of carotenoids extracted from yellow pepper were determined compared with the effect of adding 200 ppm of BHT as a synthetic antioxidant and results are shown in Fig (4). Data revealed that smoke point values decreased during heating process, BHT and 300 ppm carotenoids slowed down this decrement. Adding 100, 200 ppm carotenoids had lower antioxidative efficiency, while adding 400 ppm had the best effect in retarding the formation of thermal degradated products which leave the oil as smoke. For safety reasons, the temperature of frying oil should be kept below its smoking point (Farhang 2005).

Viscosity

Viscosity of oils heated for 5 consecutive days at $180^{\circ}\pm10^{\circ}C$ with or without adding natural (100, 200, 300 and 400 ppm caretenoids) and synthetic (200 ppm BHT) antioxidants were determined and results are given in Fig (5). Results demonstrated that, viscosity (cp) was increased during heating process for all tested treatments as a result of the formation of high molecular weight compounds. Addition of antioxidants hindered the formation of these compounds resulting in lower viscosity values. 100 ppm extracted carotenoids had the lowest antioxidant activity while 300 and 400 ppm extracted carotenoids had the highest antioxidative efficiency with a slight difference being more pronounced with the higher concentration. 200 ppm of natural and synthetic antioxidants had the same efficiency in retarding the formation of high molecular weight compounds. Increased oil viscosity during thermal treatment was also reported by Fyka and Samah (2003).

Abidi and Warner (2001) reported elevated levels of high molecular weight polymeric compounds which correlated with the extent of frying oil degradation and these served as indicators for frying oil stability.

Acid Value

During heating process the thermal decomposition of oils released free fatty acids. Data in Fig (6) revealed that, there is a dramatic increase in acid values after the first day of heating at 180°±10°C followed by a gradual increase in all tested treatments with prolonged heating time. Addition of natural and synthetic antioxidants had variable effect on slowing–down the formation of thermal decomposed products; free fatty acids, while 100 ppm caretenoids had almost no effect as antioxidant, 200 ppm BHT and the carotenoids had the same effect. Meanwhile, 400 ppm carotenoids had the highest antioxidative efficiency followed by 300 ppm carotenoids.

Effect of thermal treatment on free fatty acids formation was also determined by Samah and Azouz (2005).

Peroxide Value

The evolution of peroxide compounds formation during heating process as an indication for the degree of initial oxidation of oils were determined for oils with or without adding antioxidants. A sharp increase in the formation of peroxide compounds during the first two days of heating sunflower oil at 180°±10°C with adding BHT, 100 ppm extracted carotenoids or without any additives (control) was noticed reaching almost 10, 11 and 13 folds of its initial peroxide values, respectively. While the addition of 200, 300, 400 ppm extracted carotenoids hindered the formation of peroxides during the first two days of heating. This effect was more pronounced with increasing the concentration of antioxidant added. Results in Fig (7) also revealed that, there was a gradual increase in peroxide values with almost a same rate during the following 3 days regardless the kind of treatment.

The increase in peroxide compounds formation during thermal treatments of oils was also confirmed by Samah and Azouz (2005).

Iodine Value

Iodine value as an indication for the degree of unsaturation was determined in the heated samples. During thermal treatment, the unstable unsaturated fatty acids are affected by high temperatures forming more stable ones accompanied by a decrease of the iodine values, results are shown in Fig (8). Addition of antioxidants had variable effects in lowering the destructive effect of thermal treatment. 100 ppm added carotenoids had no effect as antioxidant, while antioxidative efficiency of other treatments could be ordered as follows; BHT had the lowest effect followed by 200, 300 and 400 ppm of extracted carotenoids which had the highest efficiency.

Thiobarbituric acid value (TBA)

Thiobarbituric acid value (TBA) was determined in all oil samples and results are shown in Fig (9). TBA value is used to measure the thermal degradated products formed during thermal treatments of oil. TBA values increased sharply after the first two days of heating almost reaching 10 and 3 folds of its initial value after the first and second day of heating process, respectively followed by a slight increase during the rest of heating days. Oil without any additives and with 100 ppm extracted carotenoids followed the same trend, while adding synthetic antioxidant inhibited the formation of thermal degredated products during heating time. This trend was also observed by the addition of 200, 300 and 400 ppm extracted caroteniods depending on the concentration added. These levels of carotenoids exhibited more antioxidative efficiency than synthetic antioxidant (BHT). The increase in TBA during thermal treatment of oil was also reported by Rizk et al. (2006).

Oxidized Fatty Acids

Percentage of formed oxidized fatty acids as a result of thermal degradation effect were determined during heating process and results are shown in Fig (10). Results illustrated that, 200 ppm carotenoids had a good antioxidative efficiency as BHT. While increasing the concentration of added carotenoids revealed more antioxidative efficiency in lowering the formation of oxidized fatty acids and retarding the oxidative rancidity of oils exposed to high temperatures.

Fyka and Samah (2003) also observed the increase of % oxidized fatty acids during frying process.

CONCLUSION

Carotenoids extracted from yellow pepper proved their high efficiency as natural antioxidants in retarding the thermal oxidation of sunflower oil during the measurement of oil's oxidative stability by Rancimat at 100°C. Also, different concentrations of extracted carotenoids have similar and even higher activity than the synthetic antioxidant; (BHT) in keeping oil's quality characteristics during thermal treatment.

Table 1. Identification of carotenoids extracted from yellow pepper

CAROTENOIDS	*CONCENTRATION %*
Violaxanthin	33.07
Unidentified	6.35
Unidentified	4.52
Lutein	13.37
Zeaxanthin	10.96
Cryptoxanthin	14.10
α-Carotene	10.90
β-Carotene	6.73

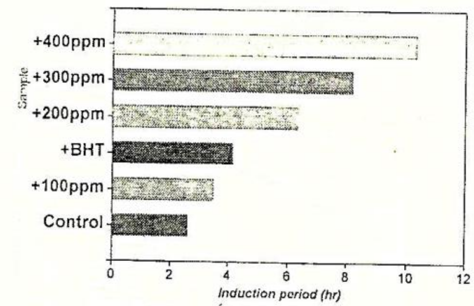

Fig (1) Effect of adding different concentrations of carotenoids and BHT on oxidative stability of sunflower oil

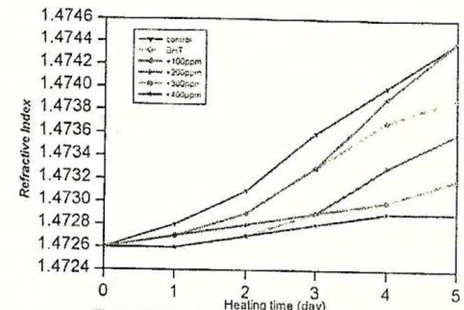

Fig (2) Effect of adding different concentrations of yellow pepper carotenoids and BHT on refractive index during heating process

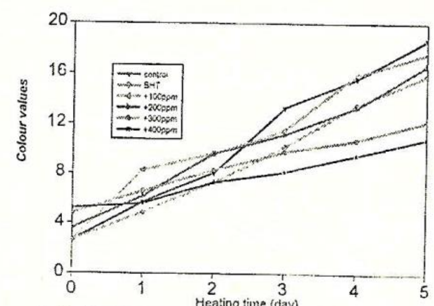

Fig (3) Effect of adding different concentrations of yellow pepper carotenoids and BHT on colour values during heating process

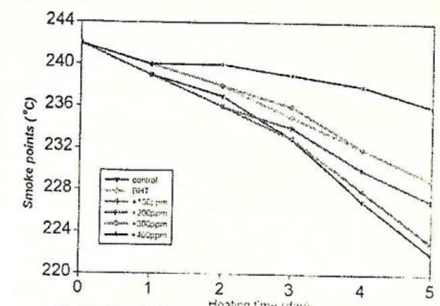

Fig (4) Effect of adding different concentrations of yellow pepper carotenoids and BHT on smoke point °C during heating process

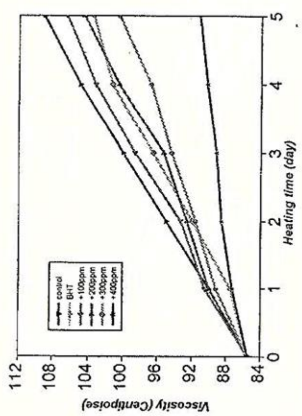

Fig (5) Effect of adding different concentrations of yellow pepper carotenoids and BHT on viscosity (Centipoise) during heating process

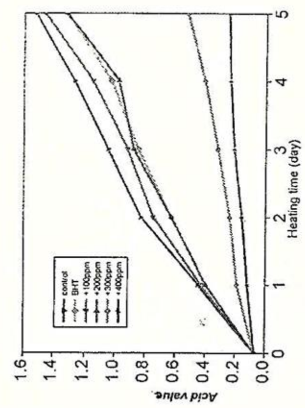

Fig (6) Effect of adding different concentrations of yellow pepper carotenoids and BHT on acid value during heating process

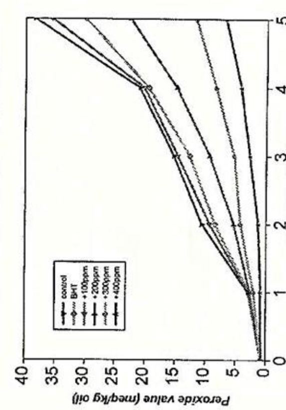

Fig (7) Effect of adding different concentrations of yellow pepper carotenoids and BHT on peroxide value (meq/kg oil) during heating process

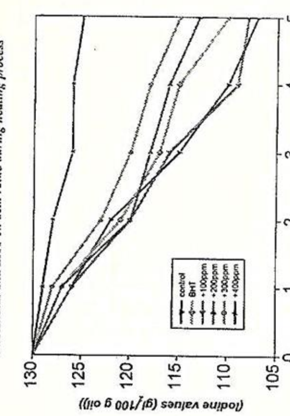

Fig (8) Effect of different concentrations of yellow pepper carotenoids and BHT on iodine values (I_2 g /100g oil) during heating process

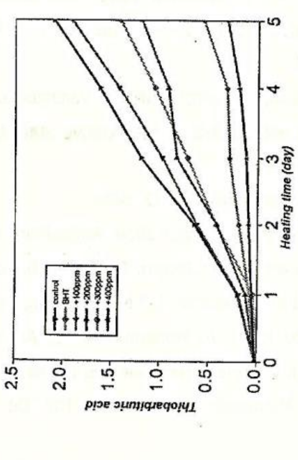

Fig (9) Effect of adding different concentrations of yellow pepper carotenoids and BHT on thiobarbituric acid during heating process

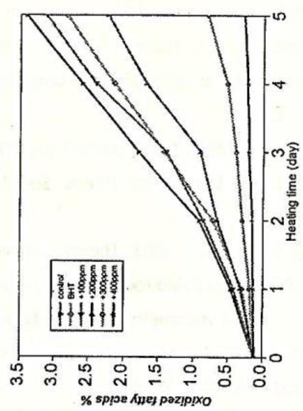

Fig (10) Effect of adding different concentrations of yellow pepper carotenoids and BHT on oxidized fatty acids % during heating process

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النشاط المضاد للأكسده للكاروتينيدات فى الفلفل الاصفر

علاء عزوز [١] ، قرنى حنفي طلبه [٢] ، عفت مهدى رزق [٢]

١. قسم بحوث الزيوت والدهون

٢. قسم بحوث الحاصلات البستانية – معهد بحوث تكنولوجيا الأغذية– مركز البحوث الزراعية

يزداد البحث عن المصادر الطبيعية لمضادات الاكسدة بزيادة الوعى التغذوى والصحى للمستهلك كمحاولة لتقليل التاثير الضار الناتج عن استخدام المضادات الاصطناعية. تم استخلاص الكاروتينيدات من ثمار الفلفل الأصفر والتعرف عليها بواسطه جهاز. HPLC وكان مركب violaxathin هو الكاروتين السائد فى المستخلص يتبعه cryptoxanthin ثم الــ lutein.

تم استخدام جهاز الرانسيمات في اختبار كفاءه تركيزات مختلفه من الكاروتينيدات المستخلصه من ثمار الفلفل الاصفر (١٠٠، ٢٠٠، ٣٠٠، ٤٠٠ جزء فى المليون من المستخلص) فى منع التزنخ الأكسيدى للزيت ، كذلك قدرته على حمايه الزيت خلال عمليه التسخين المستمره ٥ ساعات يوميا لمده ٥ أيام متواصله على ١٨٠ ±١٠°م بالمقارنه بــ BHT. واثبتت نتائج تقدير التغييرات الطبيعية والكيميائية للزيت أثناء التسخين، مدي الكفاءه العاليه للكاروتينيدات كمضادات اكسدة.