



EFFECT OF CAROTENOIDS EXTRACTED FROM SHRIMP SHELL ON LIPID OXIDATION IN SESAME OIL

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Abstract

Shrimp shell composition had determined on dry base for moisture , ash , lipid and protein content were 72, 11, 1, 16% for wet samples and 14.5, 25, 2.06 and 31% for dry samples respectively.

Different methods were employed for carotenoids extraction from dried shrimps shell . The first method (T1) used different combinations of organic solvents (Ethanol : petroleum ether , 1:1 V/V) by soxhlet device at 50°C. The second (T2) and Third (T3) methods used separately acid and basic infusion for 48 hours with 0.1N , HCL and 0.1N NaOH respectively. The fourth method (T4) based on sunflower oil extraction . The fifth treatment (T5) included cold extraction with ethanol (100%). The sixth method used solvent combination of Hexane : Acetone (1:1 V:V) . The seventh method used combination of Hexane : Acetone (2:1 V/V). The T6 was adopted as the highest carotenoid yield was obtained and valued 0.08 µg/g with 80% extraction yield as compared to the other of or mentioned methods.

Deproteinization to carotenoids were conducted on shrimp samples before extraction and valued 76.5% for wet samples and 58.5% for dry samples. Crud extract had added in different levels of 0.01, 0,2, 0.04. 0.5 and 1% in sesame oil compared to control and BHT 0.02% and samples stored to 30 days at 65°C. Peroxide Value and Thiobarbituric acid were determined for sesame oil samples after every two days storage. The obtain results revealed that the 1% crud extract in sesame oil have the strongest effect as natural antioxidant against oil oxidation to 28 days compared with other group.

Key word : Carotenoids , Shrimp shell , anti oxidant, Sesame oil.

Introduction

Shrimp waste is considered as an important sources of carotenoids the natural antioxidants which are also responsible for the color of many important fish and shellfish. Most important sea foods, such as shrimp, lobster, crab, crayfish, trout, salmon redfish, red snapper and tuna, have orange-red integument and also existed on flesh containing pigments (Sindhu and Sherife, 2011).

Oxidation of lipids and proteins during processing and storage of food products decreases consumer acceptability of foods by causing undesirable changes in flavor, texture, appearance and nutritional quality, as well as producing toxic compounds. Consumption of these potentially toxic products can give rise to several diseases. Oxidation of foods can be minimized by removing pro oxidants, such as free fatty acids, metals and oxidized compounds, by protecting foods from light and air, and by adding antioxidants. Carotenoids may serve a number of

physiological functions (Kamath *et al.*, 2008) . First, carotenoids may function as antioxidants, which protect against the damaging oxidizing effects of free radicals (Egan *et al.*,1981, Mukai, 2005). Second, the carotenoids may also improve resistance to pathogens by increasing the production of antibodies or the proliferation of immune system (Goodwin, 1984).

Processing of crustaceans such as shrimps generates large quantities of solid wastes accounting for approximately 35–45% of whole shrimp weight (Sachindra *et al.* 2005; 2006). These waste spoils rapidly, thus causing environmental problems. Furthermore, as shrimp waste being a rich source of protein, chitin, carotenoid and enzymes, considerable interest has been shown recently to recover these valuable components as marketable products. The shrimp shell waste is a rich source of astaxanthin and it is the major carotenoid present in crustacean waste, and occurs as carotenoprotein complexes, where carotenoids are bound

to proteins (Ghidalia 1985; Shahidi *et al.* 1998).

This investigation was carried out to utilize shrimp shell wastes as to examine the best methods of extraction of Carotenoids from shrimp shells waste by different solvents and Vegetable oil and to assess the antioxidant capacity of crude carotenoids extracted in sesame oil stored at elevated temp. for different periods.

Materials and methods

Preparation of shrimp's Shells waste

Shrimp's shells waste materials were purchased from local markets in Baghdad city, belongs to *Penaeus semisulcatus*, *Penaeus japonicus* and *Exopalaemon styliferus*, transported to the laboratory under iced conditions. The shrimps shell were scraped free of meat, washed under running water and air dried in the shade over night at room temperature. Then were grinded into powder, dried yield were placed in to plastic container and stored at (-20°C) until use. Samples were homogenized in a laboratory mixer prior to extraction of carotenoids and estimation of different components such as Moisture, protein, lipids and ash were conducted for the shell.

Proximate Composition of shrimp shell

Moisture, ash, total protein and total lipids were quantified according to the method of AOAC (1984). Moisture was determined by drying 5g sample at 105°C for 24 hrs in on air Oven until reached constant weight. Lipid was determined by extracting given quantity of sample with petroleum ether in Soxhlet Apparatus for 8 hrs. The crude protein estimation was conducted on a dried sample by kjeldahl procedure.

A conversion factor of 6.25 was used to determine protein content. Ash content was determined by furnace at 550°C for 8 hrs. All analyses were completed in triplicate.

Deproteinization of Shrimp shell Waste

Alkali deproteinization of shell waste was carried out according to the method of Sindhu and Sherefi (2011) with NaOH (1N) (1 : 20) W:V for 2 hours at room temperature. The deproteinization residue was dried and total nitrogen in the supernatant was determined by Microkjeldahl's method. Protein content was calculated. Percentage of deproteinization was calculated as follows:

$$X \times 100 \frac{\text{Protein in Supernatant}}{\text{Protein in waste}} = \text{Deproteinization\%}$$

Extraction of Crude Carotenoids from shrimp Shell Waste

Six different methods were employed for Carotenoids

extraction from dried and wet shrimp shell waste to evaluate the best method to obtain better yield of crude carotenoids. (T1) was carried out by hot extraction with Soxhlet device by using a mixture of Petroleum ether : Ethanol (1:1) V:V for 8 h. (T2) and (T3) was carried out according to method by Jeddi *et al.* (2013) by acid and alkaline treatment, 0.5g samples were solved in hydrochloric acid or sodium hydroxide (1N) for 48h. The solution was centrifuged and the supernatant was used for assay. (T4) included extraction by using sunflower oil (w/V). Cold extraction of carotenoids from shrimps shell waste was investigated according to the methods Sachindra, (2006) by using different organic solvents, as (20g) of wet and dry shrimp shell was homogenized extracted in (T5) with ethanol 100%. (T6) included extraction by Hexane : Acetone (1:1) V:V, with aid of magnetic stirrer for 4h. this was repeated three times and the waste became colorless. After extraction, Crude carotenoids were collected in separator funnel for primary. The lower phase discarded and The upper epiphyses was centrifuged on 3000rpm and then the crude extracted evaporated to dryness under a stream of nitrogen at 45°C (Harborne, 1973). (T7) was carried out as the procedure in T6 except using another mixture solvent being Hexane : Acetone (2:1). The T6 gave the highest carotenoids yield which was 0.08µg/g with 80% extraction compared to other methods, So this procedure was adopted in this investigation.

Crude Carotenoids as antioxidants in sesame oil

Sesame Oil was purchased from a local market in Baghdad and was free of commercial antioxidant. Different levels of carotenoids extract 0.01, 0.02, 0.04, 0.05, 1% were added to treatments 2- 6 respectively, 0.02% BHT was added to the seventh treatment (T7), the first treatment (T1) was control and free of antioxidant. All samples were stored at 65°C for 30 days to assess peroxide value and TBA value.

Peroxide value Determination

The peroxide value of the sesame Oil was determined according to standard method of AOAC (1980). as a sample of 5g of Oil was dissolved with 30ml of (60% Glacial acetic acid + 40% Chloroform), 0.5% Potassium Iodine (Saturated Solution) was added to Oil for 2 minutes with stirring, using 0.01N Sodium thiosulfate solution as the titrant. Starch solution was used as indicator (1g dissolved in 100 ml D.W). The peroxide value was calculated by using the following formula :-

$$P.V \text{ (milliequivalents /kg oil)} = 1000 \times N \times S / g$$

Thiobarbituric acid Value (TBA) Determination

The test carried out according to A.O.C.S (2001).

The absorption was carried out by Spectrophotometer type PC 1650 UV American Com. on 530 nm . Blank sample was also done.

The TBA value was calculated by using the following formula :

$$\text{Malonaldehyde (mg/kg)} = \text{Abs} \times 7.8$$

$$\text{TBA} = \text{Abs} \times 7.8(\text{malonaldehyde/kg}).$$

Statistical analysis

Used SAS – Statistical Analysis System (2012) .

The values presented is an average of triplicate determinations. The level of significance was estimated at ($p < 0.05$) .

Result and Discussion

Composition of Shrimp Shell

Table 1 shows the proximate composition of fresh and dried Shell waste that valued 72, 11, 1, 16% for fresh samples and 14, 25, 2.06, 31% for Moisture, Ash, Lipid and Protein content respectively. The results are in agreement with Sindhu and Sherife (2011) investigation on the composition of *Arteteus alcocki* , values obtained were 14.57% moisture , 29.40% Ash, (dry base), 29.71% protein (dry base), 2.06% Lipid (dry base) for dry samples and 70.7, 10.3, 1.03 and 5.40% for wet samples to moisture, Ash, lipid, protein respectively. The values obtained results is also in agreement with Ravichandran *et al.* (2009) who found the values of 12.3, 26.6, 9.8 and

Table 1: Proximate composition of fresh and dried shrimp Shell.

Component %	Shrimp Shell	
	Fresh	Dried
Moisture	72	14.5
Ash	11	25
Lipid	1	2.06
Protein	16	31

Data for triplicate determinations.

Table 2: Deproteinisation yield of Shrimp Shell Waste by alkali solution

Deproteinisation %	
Wet Samples	Dry Samples
76.5	58.5

Data for triplicate determinations.

32.5% for moisture, Ash, Lipid, Protein (on dry base) respectively and reported the lipid content in shrimp shell is dependant and related to the seasonal variation, species, physiological status, diet.

Deproteinization of Shrimp Shell Waste

Table 2 showed the yield values for dry and fresh shrimp subjected to alkali deproteinization and were 58.5

and 76.5% respectively.

Sindhu and Sheref (2011) had reported that deprotenization by alkali or enzyme increases the extraction yield of carotenoids from Shell waste. The values obtained in the present study also correlates with that reported by Holando and Netto (2006) who found that deprotenization yield of shrimp *Arteteus alcocki* was 77.68% for wet samples and 51.53% for dry samples . The deprotenization is important to allow different extraction of carotenoids from shrimp shell wastes.

Carotenoids Extraction from Shrimp Shell Waste

Table 3 shows the Maximum carotenoid yield was 0.82mg/g and obtained with T1 that employed extraction with ethanol : petroleum ether (1:1,V:V) at 50°C for 8 hours. The carotenoids yield for T6 was adopted in the present work being a cold treatment for shorter time. The different extraction methods examined in this investigation suggested that higher carotenoids yield could be obtained with organic solvents mixture comprised of an equal parts of polar and non polar solvent that might ensure suitable polarity to extract carotenoids from shrimp shell. It was also observed that higher yield was obtained with dried samples as compared to wet samples for all examined extraction methods.

Cianici *et al.* (2002) ad reported that extraction yield varies significantly among extraction solvents, wet and dry samples and deproteinization and non deproteinisation of shrimp sell samples. The carotenoids yield obtained in present work was higher than that reported by sachindra *et al.*, (2005) that valued 43.9 mg/g with a mixture of Isopropanol : Hexane (1:1, V:V) compared to 40.60 mg/g with Acetone. The previous reports suggested that Hexane : Acetone was the best solvents mixture to ensure efficient extraction of carotenoids from shrimp wastes (AOAC, 1984).

Peroxide Value of Sesame Oil During Storage

Fig. 1. Shows peroxide value of sesame oil contained to different levels of added crude carotenoids during storage at 65°C.

It is appeared that added carotenoids at level 1% was effective in suppressing lipid oxidation in sesame oil for 28 days as compared to (control sample) that developed peroxide value to 67.5 meq /kg oil after 6 days of storage that developed rancid flavor and thus became unacceptable for human consumption.

On the other hand incorporation of BHT 0.02% had lower effect on the lipid oxidation activity during storage and rancidity developed after 8 days of storage with a value of 12.25 meq/kg oil.

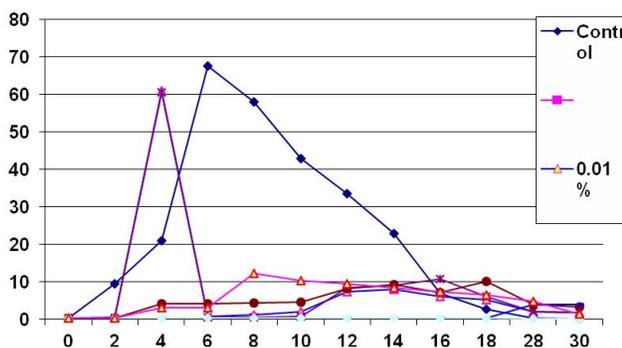
Table 3: Extraction of carotenoids from shrimp wastes by different methods

Treatments	Extraction solvents	Type of extraction	Yields of crud carotenoids		% extract	
			Dry sample	Wet sample	Dry sample	Wet Sample
T1	Ethanol : petroleum ether 1:1	Soxhlet device	0.82	0.60	82	60
T2	HCLIN	Acid treatment	0.16	0.15	16	15
T3	NaOH	Alkaline treatment	0.42	0.23	42	23
T4	Sunflower oil 1:1	Oil treatment	0.45	0.34	45	34
T5	100% ethanol	Cold extraction	0.55	0.42	55	42
T6	Hexene:acetone 1:1	Cold extraction	0.80	0.71	80	71
T7	Hexene:acetone 1:2	Cold extraction	0.60	0.42	60	42

The obtained results suggested that carotenoids from shrimp shell have strong effect on reducing oxidative rancidity especially after 28 days storage at 65°C.

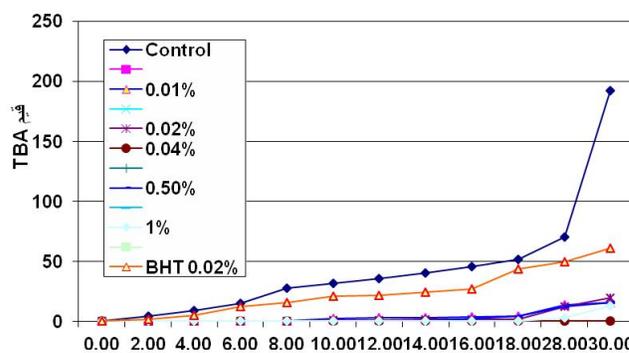
Use of natural antioxidant in food has received special attention because of worldwide trend to avoid the use of synthetic food additives. The obtained results appeared to be in well agreement with other investigation as Raposa (2012) had reported that Crude Carotenoids from shrimp shell which content astaxanthine was stable for 9 weeks at different temperature in different vegetable oil. This activity for carotenoids due to presence of many bioactive compounds such as astaxanthin, leutein, β -Carotein and α -Carotein (Awad, 2016).

Carotenoids are valued as powerful antioxidants and the astaxanthine is novel carotenoids nutraceutical occurring in many crustaceans and red yeast, Crude extracted and fractions rich in astaxanthine showed strong antioxidant activity as indicated by radical scavenging and the ability of carotenoids to quench singlet oxygen has been linked to the conjugated double bond system. (Foot *et al.*, 1970). The strong antioxidant activities exhibited by the carotenoids extract of shrimp shell might be due to the combined action of astaxanthin and β -carotein, lutein, violoaxanthin and salsylic acid present.

**Fig. 1:** Peroxid value for sesame oil contained added crude carotenoids from shrimp shells as stored at 65°C

TBA value to sesam oil during storage

The obtained result in fig. 2 showed that TBA value in sesame oil increased from an initial value of 0.42 to 192.1 mg (malonaldehyd/kg oil) after 30 days of storage at 65°C. Samples with 0.5% and 1% added crude carotenoids extract were effective to control lipid oxidation up to 14 and 18 days respectively during storage at 65°C compared to sample with 0.02% BHT which increase the TBA from 0.41 to 61.0 (malonaldehyd/kg oil) up to 30 days. These results were in agreement with Fabio, (2009) who founded an inverse relationship between the added carotenoid astaxanthine and resulted malonaldehyde in stored oil.

**Fig 2:** TBA value for sesame oil contained added crude carotenoids from shrimp shell as stored at 65°C

Conclusion

The obtained overall results of the current study suggested the efficiency of using some natural antioxidant extracted from shrimp shell sources in extent shelf-life of sesame oil. One interesting observation on this study was that carotenoids from shrimp shell can be used up to 1% in sesame oil to extent the shelf-life during storage for 28 days at 65°C.

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