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Shrimp Waste Oil: A Natural Supplement of Resistance Carotenoids Against Oxidation

[1]Parisa Sadighara, [1]Naiema Vakili Saatloo, [2]Nersi Jafary Omid and [3]Atefeh Araghi
[1]Department of Food Safety, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Food Safety, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

ABSTRACT
Background: Marine oils have high levels of polyunsaturated fatty acids. These kinds of fatty acids have the strongest evidence for health benefits. Moreover, crustaceans such as shrimp have natural carotenoids. In this study, shrimp waste oil obtained by solvent extraction. **Materials and methods:** The total carotenoid of oil was assayed. Then, the oil samples were incubated with different dilution of synthetic carotenoids (125, 250 and 500 ppm). The negative control was considered without any of synthetic carotenoids. The samples were exposed to oxidant agent. Inhibition of lipid peroxidation was evaluated by thiobarbituric acid reactive substances assay. **Results:** There was no significant difference ($p \leq 0.05$) between groups even with high group (500 ppm). The levels of lipid oxidation in oil without any antioxidant and with 500 ppm of β carotene were 9.07±1.9 and 8.6±8.1, respectively. Our results indicated shrimp waste oil is a good source of natural carotenoids against oxidation. **Conclusion:** Therefore, this kind of oil is associated to contribute of a source of carotenoids and omega-3 in manufacturing food and supplement.

Key words: Marine oil, natural carotenoids, medical food

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INTRODUCTION
The wastes of shrimp have a complex mixture of biomaterials. The waste is constituted of about 50% of shrimp total body weight which is also environmental contaminants. Therefore, utilization of these wastes can prevent environmental contamination. It is a good natural source of carotenoids (Sachindra *et al*., 2006).

Carotenoids have also been documented for health promoting function. These components are known to provide a range of biological effects (provitamin, antioxidant, coloring etc.). The role of carotenoids as a source of pigment and in immune defense system has been established. Astaxanthin, the main carotenoid found in shrimp, has an antioxidant activity which was reported to be higher than that of α-carotene, β carotene and lutein and is also higher than tocopherol against certain reactive oxygen species (Chew *et al*., 2011). Astaxanthin was also shown to have 500 fold stronger free radical antioxidant activity than vitamin E. Moreover, it have a key role in protection against UV-light photooxidation, inflammation, cancer, *Helicobacter pylori* infection, aging and age-related macular degeneration (Sarada *et al*., 2006). Furthermore, shrimp contains the Omega-3. The body can't produce these fatty acids. They must be consumed in different sources. Several studies in experimental animal and epidemiologic studies have shown that Omega-3 has antiartherosclerotic potential (Von Schacky *et al*., 1999). Therefore, Shrimp rich in Omega-3 is also rich in carotenoids. It is expected shrimp oil has biological function.

The edible oils are susceptible to oxidation during processing and storage. The oxidation of oils is influenced by many factors such as their composition (Halvorsen and Blomhoff, 2011). Marine oils are susceptible to oxidation, because of their polyunsaturated fatty acids (PUFA). The objective of this survey was to determine resistance of shrimp waste oil to oxidation. For this purpose, the resistance of shrimp waste oil to oxidation once without any antioxidant and once with different concentration of antioxidant was examined.

MATERIALS AND METHODS
Test materials: Shrimp wastes of *Penaeus semisulcatus*, comprising of head and carapace were collected from the processing plants. Then, the wastes were air-dried in the shade and turned into powder.

Corresponding Author: Parisa Sadighara, Department of Food Safety, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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Extraction of shrimp waste oil: Shrimp waste oil obtained by standard methods. Soxhlet extraction is a standard technique which has been used for many decades (Wang and Weller, 2006). The sample (5 g) was placed in test tube and dissolved in the solvent. Since hexane is the most popular and is also a good solvent for oil extraction it was used for the experiment. At the end of the extraction, solvent was evaporated.

Determination of total carotenoids of shrimp waste oil: Total carotenoids were determined by β carotene standard curve and by spectrophotometric method at 470 nm. The total carotenoids content of samples was calculated on the basis of the standard curve of β carotene (Thaipong *et al*., 2005).

Assay of shrimp waste oil against oxidation: Shrimp waste oil was exposed to $CuSO_4$. $CuSO_4$ induced lipid peroxidation. Then, the different concentrations of synthentetic carotenoids (125, 250 and 500 ppm) were added to samples. The oxidative stability was assayed by measuring levels of malondialdehyde (MDA) according to the previous method (Sicinska *et al*., 2006). Briefly, the samples mixed with 20% trichloroacetic acid then they were centrifuged. Thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was measured at 532 nm. Lipid peroxidation was expressed in nmol mL^{-1}. The values were expressed in nmoles MDA mg^{-1} protein, using a molar extinction coefficient of $1.56\times105\ M^{-1}\ cm^{-1}$.

Statistical analysis: The data were tested by analysis of ANOVA in SPSS software. The evaluation was made by comparing group. The difference more than 95% ($p\leq0.05$) was considered significant.

RESULTS AND DISCUSSION

The concentration of total carotenoid pigment in the extracts was calculated using the standard curve obtained by commercial β carotene. The level of carotenoid pigment in Shrimp waste oil was 88 ppm.

The results of lipid peroxidation are given in Table 1. The values of level of lipid peroxidation were not significant difference between groups ($p\geq0.05$). The level of lipid oxidation was not significantly different between in without any antioxidant and with different concentration of β carotene (125, 250 and 500 ppm). The level of lipid peroxidation in without any antioxidant was similar with different concentration of antioxidant. Therefore, this oil has strong antioxidant to help protect it from oxidative damages. Moreover, there was no difference between the three treatment groups (125, 250 and 500 ppm), which confirms oil has enough antioxidant for resistance to oxidation.

Table 1: Level of lipid oxidation in samples after the exposure to $CuSO_4$

Component	Level of lipid peroxidation (nmol mL^{-1})
Shrimp waste oil	9.07±1.9
Shrimp waste oil+125 ppm β carotene	8.78±8.2
Shrimp waste oil+250 ppm β carotene	8.72±8.2
Shrimp waste oil+500 ppm β carotene	8.60±8.1

The level of lipid oxidation was not significantly different in 125, 250 and 500 ppm concentration of antioxidant compared to sample without antioxidant

There is an interest in using natural antioxidants in oils. The synthetic antioxidants such as butylated hydroxytoluene (BHT) are suspected as promoters of carcinogenesis (Al-Reza *et al*., 2010). Carotenoids are soluble in the apolar solvents including edible fats and oils. Therefore, they can easily be used in the oil industry. The shrimp waste oil contains its own carotenoids. These carotenoids have significantly been shown to exhibit a high antioxidant activity. Shrimp waste is one of the important natural sources of carotenoid. The major carotenoid is astaxanthin and its esters (Sachindra *et al*., 2006). Astaxanthin in shrimp waste oil seems to prevent lipid peroxidation and to maintain appropriate levels of MDA. It showed powerful antioxidants and anti inflammatory activity. In previous study, astaxanthin had protective effects in the CCl_4-treated rat liver by inhibiting lipid peroxidation and stimulating the cellular antioxidant system. In other survey, the antioxidant activity of astaxanthin against oxidative stress induced by paraquat in cell cultures was observed (Lawlor and O'Brien, 1995). Several studies demonstrated that astaxanthin crosses the Brain-Blood Barrier (BBB) to reach brain regions. It has neuroprotective properties against oxidative modification in lipids and proteins in brains (Mattei *et al*., 2011). Astaxanthin can delay oxidative rancidity in edible oils (Antone *et al*., 2012). The phenolic antioxidant in shrimp waste could play a role in providing hydrogen or electrons to free radicals (Seymour *et al*., 1996).

Furthermore, a rich source of omega-3 fatty acids is identified in marine oil. The oils of marine products is generally accepted that have high level of polyunsaturated fatty acids which are considered to have effects on health, particularly on cardiovascular system. Heart disease is significant problem that cause annually considerable deaths. The cardiovascular disease is strongly related to dietary. Omega-3 in shrimp waste oil is very sensitive to oxidation. But, in this study shrimp waste oil without any synthentetic carotenoids had a high antioxidant activity (Table 1). Therefore, shrimp waste oil has natural carotenoids which resistance to oxidation and a good source of omega-3 fatty acids. It has an opportunity for production of medical foods.

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CONCLUSION

The results of this study showed ability of shrimp waste oil against oxidation and a good source of natural antioxidants. Astaxanthin/Omega-3 supplement has associated to human health. Therefore, it could be considered by food and supplement industries for special medical purposes.

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