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Effect of extraction conditions on the yield and antioxidant activity of crude extracts from shrimp peel

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Abstract

This study was designed to examine the effect of 6 white shrimp (*Litopenaeus vannamai*) peel preparation methods on the extractions yield and astaxanthin content comparing with extracts using the conventional method. These methods were use of fresh shrimp peel, fresh and grinded shrimp peel, dried and grinded shrimp peel, boiled shrimp peel, boiled and grinded shrimp peel, and boiled-dried and grinded shrimp peel. The results showed that boiled-dried and grinded shrimp peel gave the highest extractions yield and astaxanthin content in extracts which were 9.87 mg extract/g shrimp peel and 38.76 μg/g extract ($p<0.05$) respectively. In addition, the effect of ethanol extraction by using maceration extraction and soxhlet extraction on DPPH radical scavenging assay on the extracts was investigated. It was found that the extract of fresh and grinded shrimp peel obtained by soxhlet extraction gave the highest inhibition efficiency (IC_{50} value = 0.59 mg/mL). This study may be useful to assist efficient astaxanthin extraction for food product applications.

Keywords: astaxanthin, shrimp peel, antioxidant activity, DPPH

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1. Introduction

Shrimps are aquatic animals which are the highest value fishery product exported from Thailand. Nowadays, the white shrimp (*Litopenaeus vannamai*) production industry has been expanding in order to keep up with demand for its products. These products include fresh refrigerated shrimp, frozen shrimp and canned shrimp. The industry generates wastes from its production processes which include shrimp heads, peels, and tails. These constitute about 40-50% of the total shrimp weight [1], Generally, the industry sells the wastes for animal feed at a low price. Some studies, however have found that shrimp wastes contain many value components such as chitin, mineral, astaxanthin, and carotenoids, etc. [2, 3]. Astaxanthin extraction from shrimp residues is another way to value from these wastes.

Astaxanthin is a carotenoid pigment which is red in color and found frequently in nature. It gives shrimp, mantis shrimp, and crabs their color [4]. The molecular structure of astaxanthin consists of a long line of hydrocarbon which has an intramolecular double bond. The structure has a non-polar property which can be oil-soluble and a polar property which make it a little water soluble. The 2 tips of the structure are rings of oxygen atoms. Astaxanthin can be formed differently or with chemical reaction with protein or lipoproteins were called carotenoproteins or carotenolipoproteins. These lead to colors which appears green or blue instead of red or orange [5]. Furthermore, astaxanthin has antioxidant effect. The absorption of oxygen free radicals causes degradation of carotenoids molecule instead of other molecules or tissues [6]. Another study showed that astaxanthin is 10 time more powerful as an antioxidant than β-carotene, lutein, zeaxanthin, and canthaxanthin [7]. Astaxanthin is an anti-inflammatory. It can reduce the risk diabetes, inhibit growth of some cancer cells and reduce the risk of coronary heart disease [8].

Astaxanthin extraction from white shrimp wastes is an interesting area for research. Currently, most astaxanthin available in the market is synthetic, while the demand for natural products is increasing because concerns about safety of the synthetically produced product [9]. The aim of this study was to examine the extraction and antioxidant activity of extracts from white shrimp peel prepared using different methods.

2. Materials and Methods

2.1 Preparation of shrimp peel

The fresh shrimp peels used in the experiment were white shrimp peels from Thai Union Group Public Company Limited, Samut Sakhon, Thailand. During transportation to Nakhon Pathom Rajabhat University, the samples were preserved by adding ice at the ratio of 1:1 by weight. The peel was cleaned and prepared as

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described by Mezzomo et al. [10]. The peel was processed using 6 methods. These were;

1. No processing, fresh shrimp peel (less than 70% moisture content (m.c.))

2. Fresh and grinded shrimp peel obtained by grinding using MX-900 MW Panasonic blender for 15 s (less than 70% m.c.),

3. Dried and grinded shrimp peel obtained by drying at 60 °C for 5 h and grinding in the grinder for 15 s (less than 10% m.c.),

4. Boiled shrimp peel obtained from boiling at 100 °C for 10 min (less than 70% m.c.),

5. Boiled and grinded shrimp peel obtained by boiling 100 °C and grinded in the grinder for 15 s (less than 70% m.c.),

6. Boiled-dried and grinded shrimp peel obtained by boiling at 100 °C for 10 min, drying at 60 °C for 5 h and grinding in the grinder for 15 s (less than 10% m.c.).

2.2 Extraction of astaxanthin using conventional method from shrimp peel

Astaxanthin extraction was performed according to the procedure described by Mezzomo et al. [10]. In accordance with the procedure; 5 g of prepared shrimp peel was added into 200 mL mixed solvent containing petroleum ether, acetone and distilled water at a ratio of 15:45:10 v/v/v. The mixture was kept in dark at 5°C for 24 h. Then, the liquid was separated by filtering through Whatman no.4 filter paper. A vacuum evaporator at 60°C and pressure of 178 mbar was used to remove the solvent. After that, the crude extract was stored in a dropping bottle at -18 to -20°C [9].

2.3 Astaxanthin content analysis

Astaxanthin content was measured according to the modified method described by Tolasa et al. [11]. An astaxanthin standard solution was prepared by weighing 0.003 g astaxanthin (Astaxanthin 97 % (HPLC) from Haematococcus pluvialis; Sigma Aldrich, St. Louis, MO, USA) and 0.1 g butylated hydroxytoluene (BHT). The volume was adjusted with 10 mL dichloromethane, diluting with petroleum ether mixed with acetone and distilled water in a ratio of 15:75:10 v/v/v, to 1, 2, 3, 4, 5, 6 and 7 µg/mL. The absorbance was measured at 472 nm using a UV-Vis spectrophotometer, UV-1800 (Shimadzu Corporation, Tokyo, Japan). The astaxanthin content analysis was performed in triplicate, compared with astaxanthin standard curve and the results were expressed in the unit of equivalent µg/g extract (µg/g extract).

2.4 Ethanol extraction of astaxanthin

The extraction experiments were divided into the following sets; 1) Control - shrimp peel sample was blended with 96% ethanol at the ratio of shrimp peel per ethanol as 1:2 (weighing percentage) using a blender (MX-900 MW, Panasonic) for 5 min. The shrimp peel was filtered out using vacuum filter, 2) Maceration extraction - shrimp peel samples were prepared by using fresh and grinded shrimp peel and boiled-dried and grinded shrimp peel. 500 mL 96% ethanol was added to 105 g shrimp peel samples and it was kept at room temperature for 5 days. (The sample was wrapped with Aluminum foil to protect light, and filtered out using a vacuum filter), 3) Soxhlet extraction - shrimp peel samples were prepared by using 5 g samples of fresh and grinded shrimp peel and boiled-dried and grinded shrimp peel. Extraction was performed using Soxhlet apparatus (Soxhlet extraction, Extraction System B-811, BUCHI). 150 mL of a 96% ethanol solution was used and the apparatus was run at 180°C for 2 h (20 rounds of extracts fat removal). The extracts from all 3 methods were analyzed to examine astaxanthin content. They were evaporated using a vacuum filter at 60°C, 178 mbar pressures, weighing the extracts to analyze extraction yield, and kept in screw cap bottles at -20°C until being analyzed in the next step of the method [9].

2.5 DPPH radical scavenging assay

An antioxidant effect examination was carried out with DPPH radical scavenging assay. It was compared with standard ascorbic acid. The DPPH solution was prepared by weighing DPPH 0.0039 g and adding 100 mL methanol in order to give 100 µM concentration. Then, 3 mL of astaxanthin solution was put in the test tube, DPPH 100 µM solution was then added to the 1 mL capacity of the test tube. The test tube was shaken and kept in dark for 30 min. The absorbance of the sample at 517 nm was measured using a UV-visible spectrophotometer. The light absorption was recorded of (Asample), DPPH 100 µM solution light absorption and methanol at 517 nm and (Acontrol). Methanol was used to estimate auto zero on the machine. From this result the DPPH antioxidant efficiency was calculated from the equation below. These have the extract con-centration that can decrease DPPH antioxidant content to 50% (IC50) [9].

$$\% \text{ DPPH radical scavenging} = [(A_{control} - (A_{sample} - A_{sample\ blank})/A_{control}] \times 100$$

As $A_{control}$ = absorbance of DPPH solution and methanol

A_{sample} = absorbance of DPPH solution and astaxanthin extracts solution

$A_{sample\ blank}$ = absorbance of astaxanthin extracts Solution

Table 1 Extractions yield, astaxanthin content and DPPH radical scavenging activity from 6 different preparation methods using conventional extraction method

Sample preparation	Extraction yield (mg/g shrimp peel)	Astaxanthin content (µg/g extract)	DPPH radical scavenging (%)
Fresh	$2.64^{d*} \pm 1.16$	$13.99^{b} \pm 0.73$	$80.56^{b} \pm 0.97$
Fresh/grinded	$6.33^{bc} \pm 1.26$	$14.31^{b} \pm 0.76$	$84.56^{a} \pm 1.18$
Dried/grinded	$7.75^{ab} \pm 1.62$	$33.90^{a} \pm 1.22$	$61.91^{c} \pm 0.31$
Boiled	$4.60^{cd} \pm 1.13$	$19.07^{b} \pm 1.88$	$50.49^{d} \pm 0.71$
Boiled/grinded	$3.88^{cd} \pm 0.50$	$15.13^{b} \pm 2.93$	$52.67^{d} \pm 0.38$
Boiled-dried/grinded	$9.87^{a} \pm 2.74$	$38.76^{a} \pm 1.75$	$43.46^{e} \pm 0.98$

* Mean with different letters in the same column are significant difference ($p<0.05$)

2.6 Statistical analysis

Statistical evaluation used Complete Randomized Design; CRD and analyzed the results with ANOVA (Analysis of variance). Duncan's multiple range test (DMRT) at 95% confident level was used to compared significant difference in the results. Each experiment was repeated 3 times with the results being entered into the statistical package.

3. Results and discussion

3.1 Effect of preparation method on extraction yield and astaxanthin content

Experiments were carried out with 6 methods of shrimp peel preparation. These were fresh shrimp peel (control), fresh and grinded shrimp peel, dried and grinded shrimp peel, boiled shrimp peel, boiled and grinded shrimp peel, and boiled-dried and grinded shrimp peel. Experiments were carried out to find extraction yield and astaxanthin content in shrimp peel extracts using conventional method. The finding found that extraction yield and astaxanthin content in shrimp peel extracts were significantly different ($p<0.05$). The boiled-dried and grinded shrimp peel had the highest extraction yield and astaxanthin content in shrimp peel extracts, followed by the dried and grinded shrimp peel (Table 1). Mezzomo et al. [10] found that boiled-dried and grinded had the highest extraction yield and the concentration of astaxanthin compared with shrimp peel which is not boiled-dried and grinded which is the same result that was found in this research. In it's natural state astaxanthin is a complex compound with a protein called caroteno-protein complex which matches with a protein or glycoprotein at a hydrophobic position. Heating boiled shrimp peel therefore broke this caroteno-protein bonds causing the release of astaxanthin. Drying had the effect of reducing moisture content in the shrimp peel. Grinding shrimp peel had the effect of reducing the size of particles in the sample and increasing the overall surface area of the samples. This increased the area the solvent was able to act upon thereby increasing the astaxanthin extraction with the solvent (petroleum ether, acetone and distilled water). The particle reduction is useful in reducing the solvent pathway to reach the solute inside the solid matrix of the shrimp peel sample. It therefore improves the diffusion mechanism [10].

This research studied primary information by experimenting on the efficiency of antioxidant using DPPH radical scavenging assay (concentrated to 1 mg/mL extract sample). It found that fresh shrimp peel and fresh and grinded shrimp peel samples had higher antioxidant efficiency than samples produced using different methods. The amount of extract yielded from fresh and grinded shrimp peel was higher than the fresh shrimp peel sample. Samples that had been boiled-dried and grinded had a greater yield of extracts than other experimental sets. Fresh and grinded shrimp peel, and boiled-dried and grinded shrimp peel were therefore selected for further study.

3.2 Effect of extraction method on extract yield and astaxanthin content in shrimp peel extracts

The effect of shrimp peel samples preparation to extracts yield and astaxanthin content in shrimp peel extracts were investigated. Conventional methods were used to study the effect of extraction with maceration extraction and soxhlet extraction. A 95% ethanol solution was used as a solvent due to its food safety credentials. The study of shrimp peel extraction produced extracts of 8.32, 4.54 and 4.35 mg extract/g shrimp peel for the samples that were spun with ethanol (controlled set), fresh and grinded shrimp peel and boiled-dried and grinded shrimp peel using maceration extraction respectively. Fresh and grinded shrimp peel and boiled-dried and grinded shrimp peel samples were processed using soxhlet extraction giving extract yields of 7.31 and 10.65 mg extract/g shrimp peel, respectively (Table 2). Mezzomo et al. [10] found that astaxanthin extraction of pink shrimp using maceration extraction had extracts yield content less than soxhlet extraction. They felt that the soxhlet extraction probably outperformed the maceration extraction due to the high temperature, the solvent recycle and the solvent interaction. In addition,

Table 2 Extracts yield content and astaxanthin content in shrimp peel extracts of grinding fresh shrimp peel with ethanol (controlled set) using maceration extraction (ME) and soxhlet extraction (SE)

Samples preparation	Extraction method**	Extraction Yield (mg/g shrimp peel)	Astaxanthin content (µg/g extract)
Fresh/grinded /Ethanol	-	$8.32^{b*} \pm 0.14$	$8.86^{b} \pm 0.21$
Fresh/grinded	ME	$4.54^{c} \pm 1.68$	$13.43^{b} \pm 0.76$
	SE	$7.31^{b} \pm 1.79$	$373.70^{a} \pm 118.51$
Boiled-dried/grinded	ME	$4.35^{c} \pm 0.14$	$50.84^{b} \pm 0.91$
	SE	$10.65^{a} \pm 0.72$	$438.06^{a} \pm 205.76$

* Mean with different letters in the same column are significant difference ($p<0.05$)

the use of solvent at its boiling temperature decreased its viscosity, and surface tension, allowing the solvent to more easily reaches the soluble substances inside the solid matrix of the shrimp peel in the sample.

The study of astaxanthin content of shrimp peel extracts found that soxhlet extraction method had higher astaxanthin content than the maceration extraction method. It was found that spinning fresh shrimp peel with ethanol increased yield and astaxanthin content from fresh shrimp extracts. Yields obtained through soxhlet extraction were higher than maceration extraction and the extraction by spinning with ethanol. It is thought that this is due to the fact that soxhlet extraction is carried out with continuous heat until the ethanol evaporated. In addition, the ethanol condensed in the samples when the extract was close to the siphon level. The extract therefore would remain at the bottom of the flask until the process is. In this process the solvent was able to act on the sample several times. Moreover, the heat decreased viscosity, and surface tension of inhibitors. The overall effect of this was to increase the efficiency of astaxanthin extraction from the shrimp peel [12].

3.3 Antioxidant activity of astaxanthin using DPPH radical scavenging assay

The effect of antioxidant using DPPH radical scavenging assay worked by the antioxidant giving hydrogen or electron atoms to DPPH free radicals. This caused a change in DPPH radical structure [5]. The absorbance at 517 nm of a shrimp peel extract sample was measured to find the concentration of the extract that could decreased the DPPH free radicals content to 50% (IC_{50}). If IC_{50} is low, then there is high antioxidant activity. It was found that fresh and grinded shrimp peel extract that was extracted using maceration extraction and soxhlet extraction had IC_{50} as 0.61 and 0.59 respectively. The efficiency that was found higher than fresh shrimp peel extract spun with ethanol (controlled set) and boiled-dried and grinded shrimp peel using both maceration extraction and soxhlet extraction (Table 3). These had IC_{50} of 0.83, 0.97 and 1.18 mg/mL respectively. The efficiency of elimination DPPH of shrimp peel extract spun with ethanol, however, had higher efficiency than boiled-dried and grinded shrimp peel using maceration extraction and soxhlet extraction. This showed that the method of shrimp peel sample preparation has an effected on the antioxidant efficiency of shrimp peel extract. This is due to the heat of boiling and grinding shrimp peel before extraction. The high temperature effects the stability of astaxanthin in which it changes from one isomer to another. This change from *trans*-form to *cis*-form causes the antioxidant activity of astaxanthin to be decreased [13].

The analysis of the antioxidant properties of shrimp peel extract spun with ethanol, fresh and grinded shrimp peel and boiled-dried and grinded shrimp peel using maceration extraction and soxhlet extraction by DPPH found that shrimp peel extract acts as an antioxidant. Astaxanthin has a structure that consists of polyene hydrocarbon and 2 tips. Here closed tips are surrounded with oxygen atom and hydroxyl groups (OH) to present hydrogen radicals and free radicals. The result in changing from free radical to a stable molecule is that the polyene structure offered an electron to the free radical [14].

4. Conclusion

This study showed that primary shrimp peel sample preparation and extraction methods affected yield of extracts obtained. Astaxanthin content in shrimp peel extracts, and efficiency of antioxidant using DPPH radical scavenging assay was also affected. The appropriate shrimp peel sample preparation for astaxanthin extraction was found to be boiled-dried and grinded shrimp peel. This is because yield of the extract and astaxanthin content in shrimp peel extract were higher than other experimental sets. However, fresh and grinded shrimp peel had highest efficiency in antioxidant when it was analyzed using DPPH radical scavenging assay. In addition to this result, astaxanthin extraction from fresh and grinded shrimp peel and boiled-dried and grinded shrimp peel using soxhlet extraction presented highest astaxanthin content in shrimp peel extract. The efficiency examination of

Table 3 DPPH scavenging effect of shrimp peel extract using maceration extraction (ME) and soxhlet extraction (SE)

Samples preparation	Extraction method	IC_{50} (mg/mL)*
Fresh/grinded /Ethanol	-	$0.83^{c} \pm 0.01$
Fresh/grinded	ME	$0.61^{d} \pm 0.02$
	SE	$0.59^{d} \pm 0.01$
Boiled-dried/grinded	ME	$0.97^{b} \pm 0.03$
	SE	$1.18^{a} \pm 0.11$
Ascorbic acid	-	$0.04^{e} \pm 0.00$

*Results are means of three different experiments.

shrimp peel extract in antioxidant of the DPPH radical scavenging assay was carried out. It showed that extraction from fresh and grinded shrimp peel using soxhlet extraction and maceration extraction were the best in terms of obtaining samples with the highest concentration of antioxidants. Overall fresh and grinded sample preparation extracted with soxhlet extraction was identified as being the appropriate method for application in the food processing industry. This is due to the astaxanthin content in the extract and its high activity as an antioxidant. Currently, astaxanthin is used primarily as a red color. For this reason the extraction that gives more astaxanthin extract but less antioxidant properties may be more relevant

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References

[1] Simpson BK, Haard NF. The use of proteolytic enzymes to extract carotenoproteins from shrimp wastes. **Journal of Applied Biocchemistry**. 1985; **7**: 212-222.

[2] Cira LA, Huerta S, Hall GM, Shirai K. Pilot scale lactic acid fermentation of shrimp wastes for chitin recovery. **Process Biochemistry**. 2009; **37**: 1359-1366.

[3] Handayani AD, Sutrisno A, Indraswati N, Ismadji S. Extraction of astaxanthin from giant tiger (*Panaeus monodon*) shrimp waste using palm oil: Studies of extraction kinetics and thermodynamic. **Bioresource Technology**. 2008; **99**: 4414-4419.

[4] Sachindra NM, Bhaskar N, Siddegowda GS, Sathisha AD, Suresh PV. Recovery of carotenoids from ensilaged shrimp waste. **Bioresource Technology**; 2005; **98**: 1642-1646.

[5] Sangsuriyawong A, Limpawattana M, Siriwan D, Kaewnern P, Klaypradit. W. Effect of phospholipid concentration and mixing methods on properties of astaxanthin extract-loaded liposomes. **Journal of Agriculture**. 2016; **32**: 321-333.

[6] Klaypradit W, Limpawattana M. Astaxanthin: more values than solely as a colorant. **Journal of Food Technology: Siam University**. 2010; **5**: 7-12.

[7] Naguib YM. Antioxidant activities of astaxanthin and related carotenoids. **Journal of Agriculture and Food Chemistry**. 2000; **48**: 1150-1154.

[8] Jyonouchi H, Sun S, Lijima K, Gross MD. Antitumor activity of astaxanthin and its mode of action. **Nutrition and Cancer**. 2000; **36**: 59-65.

[9] Taksima T, Limpawattanab M, Klaypradit W. Astaxanthin encapsulated in beads using ultrasonic atomizer and application in yogurt as evaluated by consumer sensory profile. **LWT – Food Science and Technology**. 2015; **62**: 431 – 437.

[10] Mezzomo N, Maestri B, Satos RL, Maraschin M, Ferreira SRS. Pink shrimp (*P. brasiliensis* and *P. paulensis*) residue: Influence of extraction method on carotenoid concentration. **Talanta**. 2011; **85**: 1383-1391.

[11] Tolasa S, Cakli S, Ostermeyer U. Determination of astaxanthin and canthaxanthin in salmonid. **European Food Research and Technology**. 2005; **220**: 787-791.

[12] Mezzomo N, Mileo BR, Friedrich MT, Martínez J, Ferreira SRS. Supercritical fluid extraction of peach (*Prunus persica*) almond oil: Process yield and extract composition. **Bioresource. Technology.** 2010; **14**: 5622-5632.

[13] Dutta D, Chaudhuri UR, Chakraborty R. Structure, health benefits, antioxidant property and processing and storage of carotenoids. **African Journal of Biotechnology**. 2005; **4**: 1510-1520.

[14] Liu X, Osawa T. *Cis* astaxanthin and especially 9-*cis* astaxanthin exhibits a higher antioxidant activity in vitro compared to the all-*trans* isomer. **Biochemical and Biophysical Research Communications**. 2007; **357**: 187-193.