

The carotenoid characteristics of the important wild shrimp *Trachysalambria curvirostris* (Stimpson, 1860) in China*

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Abstract *Trachysalambria curvirostris* is an economically important shrimp in the coastal waters of the Western Indo-Pacific. However, there is no information about its carotenoid composition and distribution. The carotenoid profiles including concentration, composition, molecular forms (free or esterified) and configuration (geometrical and optical isomers) of raw and dried *T. curvirostris* (Stimpson, 1860) were qualitatively and quantitatively assessed. In the raw shrimp, astaxanthin and β -carotene were the two dominant carotenoids, which contributed 75.18%–88.86% and 2.86%–16.83% of the total carotenoid content. Traces of echinenone, canthaxanthin and astacene were also detected. The carotenoid content in the waste (80.28 mg/kg dry weight) was nearly two-fold higher than that in the meat (49.69 mg/kg dry weight). Of the total astaxanthin in these shrimp, 85.18%–90.59% was esterified. All-*trans* astaxanthin was the predominant geometrical isomer followed by 13-*cis*, 9-*cis*, di-*cis* and 15-*cis* isomers. A mix of three stereoisomers was found in all parts and the percentages of *meso* (3*S*, 3'*R*) and (3*S*, 3'*S*) forms were significantly higher than the (3*R*, 3'*R*) isomer. Compared to the carotenoid content of the raw shrimp, only 26.17 mg/kg remained in the dried shrimp. Nearly half of the carotenoids were lost during the drying process. Substantial hydrolysis and isomerization also occurred.

Keyword: *Trachysalambria curvirostris*; carotenoids; astaxanthin; dried shrimp

1 INTRODUCTION

The cocktail shrimp, *Trachysalambria curvirostris* (Stimpson, 1860) is a commercially important shrimp species that is widely distributed in the coastal waters of the Western Indo-Pacific (a range from Korea, Japan, China, Australia, Sri Lanka, India and the East Africa) (Cha et al., 2004). This shrimp is one of the most important target species of the shrimp fishery, which is responsible for almost one fifth of the total shrimp catches in China from 2010 to 2016, with annual harvests of more than 310 000 tonnes (Bureau of Fisheries of the Ministry of Agriculture of the People's Republic of China, 2017) and contributing over 50% of the total shrimp catch in Korea (Cha et al., 2004).

As a small shrimp, *T. curvirostris* is mainly used for producing shelled fresh shrimp and further

processing to dried shrimp. The products made from *T. curvirostris* are popular among consumers because of their color, texture and superior flavor compared to similar products from other shrimps. Color, being the most important sensory attribute for consumers to evaluate product quality, has a direct effect on the price and marketability of shrimp products (Latscha, 1989). For example, in the Chinese market, the price of well-colored *T. curvirostris* dried shrimp is \$10–24/kg USD higher than poorly colored products. However, there is still no information available on the pigment composition or distribution in this shrimp.

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In addition, as most dried shrimp are traditionally prepared by sun drying or hot air drying, loss of color and other physical and sensory properties often occur during the drying process (Namsanguan et al., 2004; Niamnuy et al., 2008), but information about the pigment profile in dried shrimp is still limited.

This study is focused on the carotenoid profiles, including concentration, composition, molecular forms (free or esterified) and configuration (geometrical and optical isomers) in the raw and dried shrimp *T. curvirostris*, so as to better understand and utilize this bioresource.

2 MATERIAL AND METHOD

2.1 Animals and sample preparation

Three lots (60 shrimps per lot) of wild adult *T. curvirostris* of both sexes (female: male=1:1) were caught off the west coast of China in February, 2017. The shrimp samples were placed in a styrofoam box with ice and transported to the Key Laboratory of Experimental Marine Biology of the Institute of Oceanology of the Chinese Academy of Sciences. All the shrimps were healthy and in their intermolt period. The gonads of the females were immature. The mean body length and weight of *T. curvirostris* were 12.26 ± 0.17 cm, 13.32 ± 0.63 g, respectively. Each lot shrimps were equally divided into two portions. One portion was fresh-processed to obtain the raw meat and waste (including head, shell and tail) samples and the remaining portion was used for dried meat preparation.

The raw meat and waste were weighed immediately to determine the yield of the different body parts (% of total body weight) and then were stored separately at -80°C for later analysis.

Dried meat was prepared according to the method reported by Namsanguan et al. (2004) by boiling raw shrimp in NaCl solution and then drying in a hot air dryer at 50°C until the average moisture reached 28%–30% before shucking the shell. The determination of moisture content was conducted according to AOAC procedures (AOAC, 1995).

The raw meat was well chopped and then homogenized before subsampling. Both the waste of raw shrimp and the dried meat samples were vacuum freeze dried and macerated before subsampling.

2.2 Measurement of total carotenoids

The carotenoids in shrimp samples were extracted with acetone (AR grade) according to the modified

method Johnston et al. (2000). The total carotenoid content was determined by using an ultraviolet-visible spectrophotometer (U-2900, HITACHI Co. Ltd., Japan) at 478 nm. The carotenoid content, expressed as mg carotenoid/kg dry weight, was calculated according to the following formula:

$$\text{Carotenoid concentration (mg/kg)} = (A_{478 \text{ nm}} \times D) / (0.22 \times W),$$

where A is the absorbance, D is the dilution multiple of the extract and 0.22 is absorbance at 478 nm of a 1-mg/kg astaxanthin standard and W is the dry weight of the sample, in grams.

2.3 Identification of major carotenoids by HPLC

The separation of carotenoids was performed on an Agilent 1200 HPLC system (Agilent Technologies Inc., CA, USA) with a Luna 3 μ Silica column (150 mm \times 4.6 mm, Phenomenex, USA) following a modified method described by Capelli and Cysewski (2007). An isocratic elution program with two solvents, 83:17 (v/v) hexane (HPLC grade, Merck & Co. Inc., Kenilworth, NJ, USA)/acetone (HPLC grade, Merck & Co. Inc., Kenilworth, NJ, USA), using a flow rate of 1.0 mL/min and an injection volume of 20 μ L at 25°C . Carotenoids were identified at 478 nm based on their retention times in comparison with commercially available standards. Other carotenoids without standards were identified by comparing their retention times with published data (Schiedt K and Liaaen-Jensen, 1995; Capelli and Cysewski, 2007).

To determine the composition of free and esterified astaxanthin, the amount of free astaxanthin was measured both before and after hydrolysis of the carotenoid extract. Hydrolysis of astaxanthin esters was achieved by using the method of Wade et al. (2005).

Further separation of optical isomers of astaxanthin was performed according a modified method of Wang et al. (2008). Baseline separation of three stereoisomers of astaxanthin was achieved using a Chiralpack IC column (25 cm \times 4.6 cm, Daicel Chiral Technologies [China] Co. Ltd., China) with methyl tert-butyl ether and acetonitrile (35:65, v/v) at a flow rate of 1.0 mL/min and measured at 470 nm at room temperature. Peaks were identified by comparing the retention times of sample peaks using (3*S*, 3'*S*) astaxanthin, racemic astaxanthin (1:2:1 mixture of the [3*S*, 3'*S*], [3*R*, 3'*S*] and [3*R*, 3'*R*] isomers) standards, purchased from CaroteNature (Lupsingen, Switzerland).

Table 1 Yield, moisture and total carotenoid content in raw meat, waste and dried meat of *T. curvirostris*

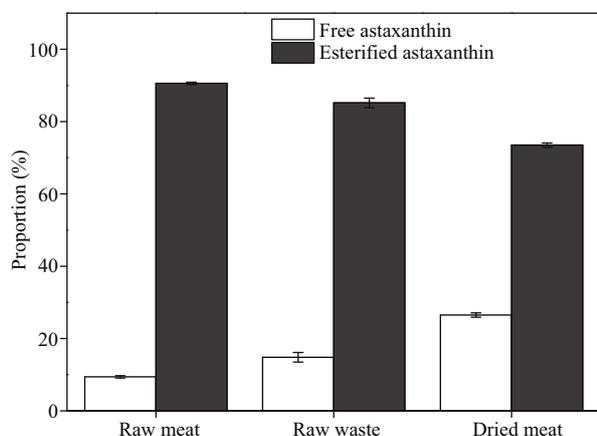
Item	Raw meat	Raw waste	Dried meat
Yield (% of raw shrimp biomass)	42.30±1.24 ^a	57.69±1.24 ^b	15.30±0.34 ^c
Moisture (%)	74.40±0.04 ^a	60.50±0.15 ^b	29.23±0.01 ^c
Total carotenoid content (mg/kg Dwt)	49.69±2.85 ^a	80.28±1.54 ^b	26.17±1.25 ^c

Dwt: dry weight tissue. Data are presented as mean±SD ($n=3$). Different superscripts within a row indicate a significant difference ($P<0.05$).

Table 2 Composition (% of total carotenoids) of major carotenoids found in raw meat, waste and dried meat of *T. curvirostris* (by HPLC following de-esterification)

Carotenoid	Raw meat	Raw waste	Dried meat
Astaxanthin	88.86±1.12 ^a	75.18±1.84 ^b	84.56±1.43 ^c
β -carotene	2.86±0.27 ^a	16.83±2.10 ^b	0.99±0.46 ^c
Echinenone	0.96±0.06 ^a	1.51±0.27 ^b	1.49±0.49 ^c
Canthaxanthin	0 ^a	0.41±0.11 ^b	0 ^a
Astacene	0 ^a	0.42±0.09 ^b	1.75±0.31 ^c
Others	7.32±1.47 ^a	5.64±0.55 ^b	11.20±1.21 ^c

Data are presented as mean±SD ($n=3$). Different superscripts within a row indicate a significant difference ($P<0.05$).

**Fig.1 The proportion (% of total astaxanthin) of free and esterified astaxanthin in the raw meat, waste and dried meat of *T. curvirostris* (by HPLC)**

Data are presented as mean±SD ($n=3$).

2.4 Statistical analysis

To verify differences in carotenoid values among different body parts, one factor analysis of variance (ANOVA) was employed using SPSS 19.0 statistical software (SPSS Inc, Chicago, IL, USA). For all tests, $P<0.05$ was accepted as the level of statistical significance.

3 RESULT

3.1 Carotenoid concentration and composition in raw and dried shrimp

The raw shrimp biomass yielded 42.3% raw meat and 57.7% raw waste. The moisture content of these was 74.4% and 60.5%, respectively. Raw shrimp biomass yielded 15.3% dry meat, with a moisture content of 29.3% (Table 1). The total carotenoids in raw meat, waste, and dried meat were 49.69±2.85, 80.28±1.54 and 26.17±1.25 mg/kg dry weight tissue, respectively (Table 1), which are statistically significant differences ($P<0.001$).

The composition of carotenoids in raw meat, waste and dried meat are given in Table 2. In the raw shrimp, astaxanthin and β -carotene were the two major carotenoids constituting 88.86% (meat)–75.18% (waste) and 2.86% (meat)–16.83% (waste) of the total carotenoids, respectively. Echinenone, canthaxanthin (waste only) and astacene (waste only) along with other unidentified carotenoids were also detected, although their levels were low. Compared to the raw materials, astaxanthin and β -carotene in the dried meat decreased significantly ($P<0.05$). Astacene, undetected in the raw meat, appeared in the dried meat.

3.2 Molecular forms of astaxanthin in raw and dried shrimp

Astaxanthin in *T. curvirostris* exists in free and esterified forms. In all samples, the levels of esterified astaxanthin were always higher than the free form (Fig.1). The highest degree of esterification was found in the raw meat (90.59%), whereas the lowest was in the dried meat (73.49%).

3.3 Geometrical and optical isomers in the raw and dried shrimp

With respect to astaxanthin geometrical isomers (Fig.2), all-*trans* was the predominant form followed by 13-*cis*, 9-*cis*, di-*cis* and 15-*cis*. All five geometrical isomers were detected in raw shrimp but 15-*cis* was not detected in dried meat. The highest ratio (70.38%) and the lowest ratio (64.64%) of all-*trans* astaxanthin were obtained in the waste and the dried meat, respectively. In raw shrimp, the 13-*cis* isomer was the predominant *cis*-astaxanthin constituting 14.45%–15.65% of the total astaxanthin. The ratio of 9-*cis*, di-*cis* and 15-*cis* astaxanthin varied around 6.21%–12.18%, 4.40%–6.71% and 0.82%–2.25%,

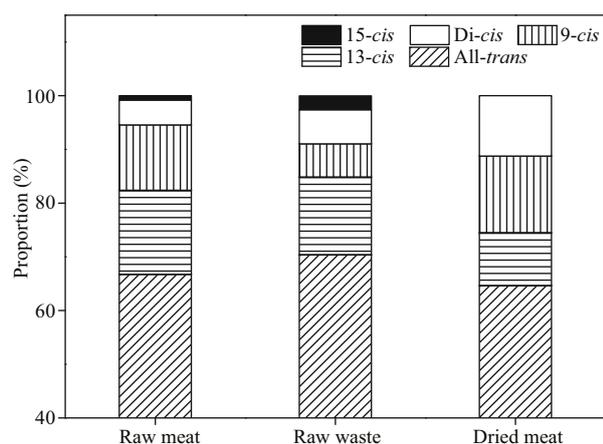


Fig.2 Composition (% of total astaxanthin) of astaxanthin geometrical isomers in raw meat, waste and dried meat of *T. curvirostris*

All values are averages of three independent repetitions.

respectively. In the dried meat, 9-*cis* astaxanthin was the highest *cis* isomer (14.30%) followed by the di-*cis* isomer (11.24%) and 13-*cis* isomer (9.83%).

Results presented in Table 3 show that three stereoisomers, i.e. two enantiomers (3*S*, 3'*S*) and (3*R*, 3'*R*) and a *meso* form (3*S*, 3'*R*), were present in *T. curvirostris*. However, the levels of specific configurational isomers in this shrimp were significantly different ($P < 0.001$) in different parts. The (3*R*, 3'*R*) isomer, ranging from 4.21% to 16.24% of the total astaxanthin was always the lowest stereoisomer, and the *meso* (3*S*, 3'*R*) isomer was always the highest stereoisomer, no matter which part of the shrimp. However, proportions of the (3*S*, 3'*S*), (3*S*, 3'*R*) and (3*R*, 3'*R*) isomers varied greatly in the raw meat (2:3:1), waste (3:3:1) and dried meat (5:7:2).

4 DISCUSSION

4.1 Carotenoid profiles in raw shrimp

The carotenoid content of shrimps is species-specific and varies in different body parts. For example, the total carotenoid content in the meat and waste were 17.4 and 66.65 mg/kg in *P. monodon*, 10.4 and 43.02 mg/kg in *Penaeus indicus*, 11.1 and 61.23 mg/kg in *Metapenaeus dobsonii*, 16.0 and 143.57 mg/kg in *Parapenaeopsis stylifera* (Sachindra et al., 2005), 15.9 and 76.66 mg/kg in *Solonocera indica* and 21.4 and 168.91 mg/kg in *Aristeus alcocki* (Manjabhat et al., 2006), respectively. This study indicated that the total carotenoid content in the meat of *T. curvirostris* is much higher than in the shrimps mentioned above and the level in the waste was

Table 3 Composition (% of all-*trans* astaxanthin) of major astaxanthin stereoisomers in the raw meat, waste and dried meat of *T. curvirostris* (by HPLC following de-esterification)

Stereoisomer	Raw meat	Raw waste	Dried meat
3 <i>S</i> , 3' <i>S</i>	33.81±1.34 ^a	42.17±0.07 ^b	36.76±1.68 ^c
3 <i>S</i> , 3' <i>R</i>	49.95±0.86 ^a	43.59±0.22 ^b	48.71±0.82 ^c
3 <i>R</i> , 3' <i>R</i>	16.24±0.48 ^a	14.21±0.25 ^b	14.52±1.07 ^c
Proportion	2:3:1	3:3:1	5:7:2

Data are presented as mean±SD ($n=3$). Different superscripts within a row indicate a significant difference ($P < 0.05$).

comparable. In addition to the species-specific difference, the carotenoid content in wild shrimps may also be influenced by sex, life stage, pathogens, different habitat, seasonal changes in food composition, etc. (Yanar and Yanar, 2004). Thus, a more detailed investigation of the biology of this wild shrimp is needed.

Like other shrimps (Mandeville et al., 1991; Sánchez-Camargo et al., 2011), the highest carotenoid content of this shrimp was observed in the waste, which comprised half of the total body weight. Combined with the statistical data (Bureau of Fisheries of the Ministry of Agriculture of the People's Republic of China, 2017), the production of *T. curvirostris* waste is around 180 000 tonnes per year in China and most of the waste is discarded as worthless trash. Further, calculation of the carotenoid content shows that nearly 9 tonnes high-value carotenoids (mostly astaxanthin) are discarded every year. Utilization of this waste would provide an excellent source of pigment and nutrients for animal feeds, increasing the profitability of the industry, in addition to contributing to the reduction of clandestine disposal in the environment.

Esterified astaxanthin is the dominant form of astaxanthin found in *T. curvirostris*. Similar distributions of astaxanthin and its esters have been reported by Sachindra et al. (2005) and Manjabhat et al. (2006) in various Indian shrimps. Previous studies reported that the percentage of esterified astaxanthin was 53.9%–68.1% in *P. indicus*, 63.8%–72.4% in *P. monodon*, 67.1%–76.8% in *M. dobsonii*, 46.2%–73.6% in *P. stylifera* (Sachindra et al., 2005), 65.4%–79.7% in *S. indica*, and 69.95%–82.4% in *A. alcocki* (Manjabhat et al., 2006). Compared to *T. curvirostris*, the amounts of astaxanthin esters reported in these shrimps was significantly lower. This may explain why the quality of products made from *T. curvirostris*

is superior to those for other shrimps. For human consumption, foods or nutraceuticals with a higher percentage of astaxanthin esters can provide a significantly longer shelf life (Lorenz and Cysewski, 2000; Etoh et al., 2012).

In nature, the predominant geometric isomer of astaxanthin is the all-*trans* astaxanthin, the kinetically and thermodynamically most stable form (Bjerkeng et al., 1997). *Cis* isomers at the 9, 13 and 15 position are also observed (Rodriguez-Saiz et al., 2010). In *Haematococcus pluvialis*, the ratio of all-*trans* to *cis* isomers is nearly 3:1 and the main *cis* isomers were 9-*cis* and 13-*cis* astaxanthin (Yuan and Chen, 2000). The level of 13-*cis* astaxanthin detected in the raw *P. vannamei* was 2.25% (Yang et al., 2015). *Cis* isomers deposited in coho salmon (*Oncorhynchus kisutch*) comprise 3%–8% of total astaxanthins (Arai et al., 1987). In this study, all-*trans*, 9-*cis*, 13-*cis*, 15-*cis* and di-*cis* astaxanthin were detected, and constituted 30%–35% of the astaxanthin, which is a higher level than that reported for other species. Several studies have reported that *cis* astaxanthin isomers are selectively absorbed into human plasma (Østerlie et al., 2000) and have higher antioxidant potential in vitro and in vivo (Yang et al., 2017). Combining these studies with the results obtained in the current work suggests that *T. curvirostris* could be used as a natural source of *cis*-astaxanthin-enriched functional food.

Astaxanthin contains two chiral carbons and therefore may be present in various stereoisomeric forms (Britton, 1995). In general, astaxanthin from natural sources tends to occur predominantly as either the (3*S*, 3'*S*) or (3*R*, 3'*R*) form, while synthetic astaxanthin contains a mixture of the three isomers in approximately a 1:2:1 ratio (Turujman et al., 1997). Three stereoisomers were identified in *T. curvirostris* and the ratio of these isomers was similar to those found in previous studies on other shrimps in the Penaeidae, where the percentage of (3*S*, 3'*S*) astaxanthin ranged from 32% to 45%, *meso* astaxanthin 40%–50% and (3*R*, 3'*R*) astaxanthin 15%–30% (Matsuno et al., 1984; Latscha, 1989). However, this differs from the distribution in *E. superba* in the Euphausiidae, which contains over 99% of (3*R*, 3'*R*) astaxanthin and in *Macrobrachium nipponense*, *Palaemon paucidens* and *Paratya compressa compressa* in the Palaemonidae, which have over 99% of (3*S*, 3'*S*) astaxanthin (Matsuno et al., 1984). Little is yet known as to why shrimps in the Penaeidae have such high percentages of *meso* astaxanthin, or where this *meso* isomer originates.

4.2 Comparison the carotenoid profiles of raw and dried shrimp meat

The dried meat of *T. curvirostris* obtained after hot air drying, had a substantial loss of total carotenoids, including β -carotene and astaxanthin. Astacene, an oxidation product of astaxanthin and not found in raw meat, was detected in the dried meat, which indicates that the shrimp drying process not only caused some degradation of the original carotenoids but also produced by-products. The percentage of free astaxanthin was increased nearly 3-fold compared to that in raw shrimp. The dehydration process also led to isomerization of astaxanthin. The all-*trans* astaxanthin showed a slight decline after drying. The 9-*cis* astaxanthin increased and the 13-*cis* and 15-*cis* isomers decreased. The most remarkable change was the 2-fold increase of di-*cis* astaxanthin in the dried shrimp as compared to the raw shrimp. The optical patterns of astaxanthin in dried shrimp were also changed compared to the raw meat. The proportion of (3*S*, 3'*S*) isomer increased while those of *meso* and (3*R*, 3'*R*) isomers decreased. The severe degradation, hydrolysis and isomerization of carotenoids during the drying of shrimp may be due to the traditional hot air drying conditions. From the carotenoid protection perspective, new drying techniques should be able to minimize the hydrolysis of astaxanthin esters and isomerization of astaxanthin, thereby preserving the maximum amount of carotenoids in the dried shrimps.

5 CONCLUSION

Trachysalambria curvirostris contains a high concentration of carotenoids, with the predominant carotenoid being esterified astaxanthin. Five geometrical isomers, i.e. all-*trans*, 9-*cis*, 13-*cis*, 15-*cis* and di-*cis* astaxanthin, and three optical isomers, i.e. (3*S*, 3'*S*), (3*S*, 3'*R*) and (3*R*, 3'*R*) astaxanthin were observed. The study also revealed that the levels of carotenoids, degree of esterification of astaxanthin and ratio of different geometrical and optical isomers in the raw meat, waste and dried meat of *T. curvirostris* were significantly different. The high content of carotenoids in shrimp waste coupled with the huge amount of unused shrimp biomass available, suggests this waste could be exploited for recovery of carotenoids (mainly astaxanthin esters) for use as pigment additives in feeds. Severe degradation and isomerization of carotenoids occurred during the drying process suggesting that the method of dehydrating shrimp should be carefully controlled,

and improved, in the manufacturing of shrimp products.

6 DATA AVAILABILITY STATEMENT

All data generated and/or analyzed during this study are included in this published article

7 ACKNOWLEDGEMENT

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