Effects of Drying Methods on the Content, Structural Isomers, and Composition of Astaxanthin in Antarctic Krill

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ABSTRACT: Antarctic krill (Euphausia superba) is one of the important bioresources in Antarctic waters, containing many bioactives (e.g., astaxanthin), which have a highly potential value for commercial exploitation. In this study, the effects of processing methods on the content, structural isomers, and composition of astaxanthins (free astaxanthin and astaxanthin esters) were studied. Three drying methods, comprising freeze-drying, microwave drying, and hot-air drying, were used. Free astaxanthin (Ast), astaxanthin monoesters (AM), and astaxanthin diesters (AD) in boiled krill (control) and dried krill were extracted and analyzed using high-resolution mass spectrometry with ultraviolet detection. After the three processes, total astaxanthin loss ranged from 8.6 to 64.9%, and the AM and AD contents ranged from 78.3 to 16.6 and 168.7 to 90.5 μg/g, respectively. Compared to other kinds of astaxanthin esters, astaxanthin esters, which linked to eicosapentaenoic acid and docosahexaenoic acid, as well as the Ast, were more easily degraded, and AM was more susceptible to degradation than AD. All-E-astaxanthin easily transformed to the 13Z-astaxanthin than to the 9Z-astaxanthin during the drying process, but the proportions of optical isomers changed due to drying by no more than 5%. The results suggested that freeze-drying, low-power microwave drying (≤1 kW), and low-temperature hot-air drying (≤60 °C) are optimal drying methods for ensuring the quality of krill products.

1. INTRODUCTION

With tremendous biomass, Antarctic krill (Euphausia superba) constitutes the largest biological population of a single species in Antarctic waters.1 Many unsaturated fatty acids, proteins, and bioactive compounds, such as astaxanthin and chitin, can be extracted from krill.2 Some studies have reported that dietary supplements containing krill can regulate intestinal microbiota and relieve hyperlipidaemia to improve atherosclerosis.3,4 In another study, the presence of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and astaxanthin in krill oil improved the cognitive ability and alleviated depression in rats.5 A valuable health supplement, krill oil, has gained popularity worldwide.9

As the main type of natural xanthophyll in krill, astaxanthin has strong antiangiogenic, anti-inflammatory, and antioxidant activities.2–9 Astaxanthin could become a potential remedy for the treatment of diabetic nephropathy.10 Animal experiments have shown that astaxanthin can prevent liver damage caused by ageing.11 inhibit neuronal apoptosis, and enhance cognitive function.12 Astaxanthin exists in three forms in nature: free astaxanthin (Ast), astaxanthin monoesters (AM), and astaxanthin diesters (AD) (Figure 1). Krill contains approximately 30–40 mg/kg of astaxanthin, with 25–35% of AM, 55–64% of AD, and <5% of Ast.13 The fatty acids C14:0, C16:0, C16:1, C18:1, C20:0, C20:5, and C22:6 are present in AM and AD.14 Herein, astaxanthin isomers with different chiralities at C3,3′ are referred to as R/S (optical) isomers and consist of two enantiomers (3S,3′R/3R,3′S) and a meso form (3S,3′R).15 Maoka et al. ascertained 3R,3′R to be the most abundant isomer (62–71%) in krill.16 Isomers with different configurations of double bonds are referred to as cis/trans isomers, and all-trans (all-E)-astaxanthin, 13-citrine (13Z)-astaxanthin,
and 9-cistrine (9Z)-astaxanthin are the most common geometric isomers. Krill processing involves boiling, drying, grinding, and refining. In most cases, the contents of astaxanthin vary markedly during these processes due to a large number of double bonds in its structure.17 The drying method used to process krill plays an important role in determining the content of astaxanthin. Thus, it is critical to clarify how xanthophyll carotenoid such as astaxanthin degrades during different drying methods. Little is known about how the manufacturing process affects astaxanthin. It was reported that thermal treatment (deactivation of lipases and proteases at 92–98 °C for 10 min) resulted in an approximately 17% decrease in the amount of astaxanthin in the product.18 The study about the stability of astaxanthin from the lactic acid fermented shrimp byproduct had been reported.19 A previous study of the isomerization of Ast dissolves in organic solvents reported that higher temperatures could promote isomerization.20 Because astaxanthin isomers vary in their chemical and biological properties and display different antioxidant capacities,21,22 understanding how the drying process affects the formation of isomers is important for generating an effective health supplement. Numerous studies have assessed the effects of thermal processing and drying on the contents of astaxanthin in Pacific white shrimp (Penaeus vannamei) and microalgae (Haematococcus pluvialis),23–25 but few studies have focused on the effects of drying method on the structure and composition of astaxanthins in krill.

In this study, the influence of drying methods (freeze-drying, microwave drying, and hot-air drying) on the content and structural isomers of astaxanthin in Antarctic krill was studied. Additionally the composition and stability of astaxanthin esters were investigated.

### 2. RESULTS AND DISCUSSION

#### 2.1. Composition of Astaxanthin Esters in the Absence of Drying

Astaxanthin esters in boiled krill that

**Table 1. Identities and Relative Contents of Astaxanthin Esters in Antarctic Krill Determined Using HPLC-HRMS**

| compound  | formula       | m/z | retention time (Rt) (min) | relative content (μg/g) |
|-----------|---------------|-----|--------------------------|-------------------------|
| Astaxanthin | C₄₀H₅₂O₄ | [M + H-FA]⁺ | 597.39384 | 6.06 | 7.18 ± 0.03 |
| Asta-C12:0 | C₂₀H₂₀O₅ | 579.38458 | 779.56090 | 11.06 | 2.33 ± 0.15 |
| Asta-C14:0 | C₂₀H₂₀O₅ | 579.38458 | 807.59220 | 16.52 | 5.05 ± 0.04 |
| Asta-C16:0 | C₂₀H₂₀O₅ | 579.38458 | 835.62350 | 15.67 | 17.9 ± 0.07 |
| Asta-C16:1 | C₂₀H₂₀O₅ | 579.38458 | 833.60785 | 16.62 | 1.13 ± 0.22 |
| Asta-C18:0 | C₂₀H₂₀O₅ | 579.38458 | 861.63915 | 14.08 | 15.6 ± 0.13 |
| Asta-C18:1 | C₂₀H₂₀O₅ | 579.38458 | 857.60785 | 11.08 | 14.4 ± 0.41 |
| Asta-C18:2 | C₂₀H₂₀O₅ | 579.38458 | 855.59220 | 10.37 | 12.4 ± 0.37 |
| Asta-C20:0 | C₂₀H₂₀O₅ | 579.38458 | 881.6087 | 11.26 | 6.26 ± 0.05 |
| Asta-C22:6 | C₂₀H₂₀O₅ | 579.38458 | 907.62195 | 11.69 | 2.81 ± 0.33 |
| Asta-C12:0/C14:0 | C₂₀H₁₆O₃ | 761.54669 | 789.58070 | 19.02 | 8.49 ± 0.26 |
| Asta-C12:0/C16:1 | C₂₀H₁₆O₃ | 761.54669 | 815.59900 | 20.31 | 2.02 ± 0.19 |
| Asta-C12:0/C20:5 | C₂₀H₁₆O₃ | 761.54669 | 863.59756 | 20.29 | 2.48 ± 0.13 |
| Asta-C14:0/C14:0 | C₂₀H₁₆O₃ | 789.57745 | 789.57745 | 20.71 | 20.8 ± 0.09 |
| Asta-C14:0/C16:0 | C₂₀H₁₆O₃ | 789.57745 | 817.61027 | 23.01 | 22.8 ± 0.43 |
| Asta-C14:0/C18:0 | C₂₀H₁₆O₃ | 789.57745 | 837.57275 | 20.63 | 3.85 ± 0.23 |
| Asta-C14:0/C20:5 | C₂₀H₁₆O₃ | 789.57745 | 863.59756 | 19.07 | 4.91 ± 0.36 |
| Asta-C14:0/C22:6 | C₂₀H₁₆O₃ | 789.57745 | 889.61072 | 1117.82187 | 19.53 | 0.18 ± 0.15 |
| Asta-C16:0/C16:0 | C₂₂H₂₀O₃ | 817.61027 | 817.61027 | 25.42 | 15.6 ± 0.09 |
| Asta-C16:0/C18:1 | C₂₂H₂₀O₃ | 817.61027 | 843.63397 | 25.29 | 33.6 ± 0.10 |
| Asta-C16:0/C20:5 | C₂₂H₂₀O₃ | 817.61027 | 863.59756 | 24.61 | 14.5 ± 0.14 |
| Asta-C16:1/C16:1 | C₂₂H₂₀O₃ | 815.59381 | 815.59381 | 22.86 | 2.46 ± 0.24 |
| Asta-C16:1/C18:1 | C₂₂H₂₀O₃ | 815.59381 | 843.63397 | 22.62 | 5.37 ± 0.27 |
| Asta-C16:1/C18:4 | C₂₂H₂₀O₃ | 843.63397 | 837.57275 | 25.11 | 13.2 ± 0.13 |
| Asta-C18:1/C18:4 | C₂₂H₂₀O₃ | 843.63397 | 1119.83752 | 20.18 | 3.74 ± 0.09 |
| Asta-C18:1/C20:5 | C₂₂H₂₀O₃ | 843.63397 | 863.59756 | 1145.8320 | 19.78 | 4.86 ± 0.27 |
| Asta-C20:5/C20:5 | C₂₂H₂₀O₃ | 863.59756 | 863.59756 | 1165.82190 | 17.83 | 1.97 ± 0.38 |
| Asta-C20:5/C22:6 | C₂₂H₂₀O₃ | 863.59756 | 889.61072 | 1191.83752 | 18.08 | 1.97 ± 0.19 |

“Asta, astaxanthin; FA, fatty acid; [M + H-FA]⁺: the mass-to-charge ratio of astaxanthin ester fragment ions, which were lost by the formation of any of the fatty acid molecules.”

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did not undergo drying were separated using a C30 column. The molecular species of astaxanthin were identified by high-performance liquid chromatography (HPLC) coupled with high-resolution mass spectrometry (HRMS), and the detailed data are shown in Table 1. A total of 28 astaxanthin derivatives were identified in this experiment: Ast, nine AM, and 18 AD. The retention time of Ast was 6.02 min, whereas those of AMs and ADs were 10−16 and 17−26 min, respectively.

Due to the lack of some astaxanthin ester standards, we used the standards of Asta-C16:0 and Asta-C16:0/C16:0 for quantitative analysis, which relied on accurate masses and characteristic ions. The qualitative definition of AM is mainly based on the exact molecular mass of the compound and the characteristic ions of astaxanthin. The qualitative definition of AD is also based on characteristic ion fragments of [M + H-FA]+. For AD, the same chemical formula may occur in combination with different fatty acids. Therefore, the type of AD cannot be determined from the precise molecular weight and characteristic ions of astaxanthin. However, the characteristic ions of [M + H-FA]+ can be used to reliably distinguish astaxanthin esters. As shown in the spectrum of Asta-C16:0/C16:0 in Figure 2, the molecular ion peak at 1073.85732, the characteristic ions of astaxanthin, and [M + H-FA]+ 817.60419 are clearly visible.

The peak area of Asta-C16:0 was tenfold greater than that of Asta-C16:0/C16:0, which showed that the response values of AM and AD were different. Therefore, analysis of the contents of astaxanthin esters was carried out using a quantitative method according to the molar content of the two standards.
and the corresponding peak area. In the control sample, diesterified astaxanthin was the major component, with a relative percentage of 66.9%, and monoesterified astaxanthin accounted for a large percentage (30.4%). The most abundant astaxanthins in krill corresponded to Asta-C16:0/C18:1 (13.2%), followed by Asta-C14:0/C16:0 (9.0%), and Asta-C14:0/C14:0 (8.2%), with Asta-C16:0 (7.0%), Asta-C16:0 (7.1%), and Asta-C16:0/C16:0 (6.2%) being the most representative astaxanthin esters (Table 1). Notably, among AD, seven species contained EPA or DHA, and Asta-C20:5/Asta-C22:6 was present.

The number, relative proportion, and composition of the astaxanthin molecular species obtained in the present work differ slightly from those reported in other studies.14 In this study, detected astaxanthins comprised Ast (2.6%), AM (30.4%), and AD (66.9%), consistent with Yamaguchi et al.13 We and Takaichi et al. both detected high concentrations of Asta-C18:4, which Grynbaum et al. and Xie et al. did not observe. The types of algae consumed by Antarctic krill vary geographically and temporally. In the winter, ice algae, which contain Asta-C18:4, can provide krill adequate food. Consumption of these algae might explain the presence of Asta-C18:4 in krill. Another reason for our finding of Asta-C18:4 in krill might be the higher mass resolution and mass accuracy instrument in this study than in previous studies, allowing more astaxanthin esters to be detected. In addition, in the present study, Asta-C18:0 and Asta-C20:1 were not detected, which are inconsistent with the results of Grynbaum et al.14 This difference might be due to seasonal differences between studies or the destruction of several astaxanthins in the present study during the short boil time.

### 2.2. Effect of Drying Method on Total Astaxanthin Content

To eliminate the effects of moisture on the measurement results, we reduced the moisture content of all samples as much as possible. In this experiment, the total astaxanthin content in boiled krill (control samples) was 125 μg/g (dry weight). As shown in Figure 3, the astaxanthin amount in the samples varied with the processing method. Compared with the drying temperatures and powers, the selected drying methods, such as freeze-drying, hot-air drying, and microwave drying, had a stronger influence on astaxanthin. Among the three drying methods, freeze-drying had the least effect on astaxanthin recovery. After freeze-drying, the content of astaxanthin decreased to 113 μg/g (dry weight). Under hot-air drying, the amount of astaxanthin gradually decreased as drying temperature increased, with astaxanthin content decreasing from 116 to 103 μg/g (dry weight) as drying temperature increased from 40 to 60 °C. There was a significant decrease in astaxanthin content as the drying temperature exceeded 60 °C, and more than 60% of astaxanthin was lost when the temperature reached 100 °C, demonstrating that the drying conditions had a strong influence on astaxanthin content. Among the three drying methods, freeze-drying had the least effect on astaxanthin recovery. After freeze-drying, the content of astaxanthin decreased to 113 μg/g (dry weight). Under hot-air drying, the amount of astaxanthin gradually decreased as drying temperature increased, with astaxanthin content decreasing from 116 to 103 μg/g (dry weight) as drying temperature increased from 40 to 60 °C. There was a significant decrease in astaxanthin content as the drying temperature exceeded 60 °C, and more than 60% of astaxanthin was lost when the temperature reached 100 °C, demonstrating that the drying conditions had a strong influence on astaxanthin content. A similar pattern was observed under microwave drying, with astaxanthin content decreasing with increasing microwave power. The percent degradation of astaxanthin at different powers increased from 10.6% (at a power of 1 kW) to 58.0% (at a power of 5 kW).

The different drying methods had different effects on the astaxanthin in Antarctic krill. Freeze-drying is a common method used in the dry heating of labile compounds. We found that the samples were freeze-dried for 12 h at −50 °C lost 8% of their astaxanthin content. Miao et al. reported that H. pluvialis lost only 5% of its astaxanthin during storage at −18 °C for 76 weeks.30 The thick cell walls of H. pluvialis isolate astaxanthin from oxygen, thus protecting it from damage due to external conditions. Accordingly, the loss of astaxanthin in freeze-dried krill in this study was higher than that of H. pluvialis in Yang’s experiment. A small amount of astaxanthin loss at low temperatures might also be attributed to astaxanthin esters account for 95% of the total content in H. pluvialis and krill.

In addition to freeze-drying, low-temperature (≤60 °C) drying and low-power (1 kW) drying were suitable for obtaining high astaxanthin contents. However, at higher temperatures or power, the loss of astaxanthin increased substantially. Sun et al. reported that hot-air drying at 60 °C combined with freeze-drying yielded krill powder of better quality, but astaxanthin content decreased rapidly above 60 °C.22 The results of the current study suggest that 60 °C is the optimum temperature for hot-air drying. Astaxanthin, β-carotene, and α-carotene belong to the carotenoid family and show similar responses in thermal treatment experiments due to their similar chemical structures. The total loss of α-carotene and β-carotene caused by microwaving at 3 kW was lower than that under hot-air drying. Thus, compared to conventional heating, microwave heating at 300 W was found to be less harmful to anthocyanins and other antioxidants.33 In the present study, microwave drying (>1 kW) had milder effects than did hot-air drying (≥60 °C), with retaining more astaxanthin under microwave drying.

### 2.3. Effect of Drying Method on the Composition of Astaxanthins

In this study, derivatives of astaxanthin detected in the extracts of dried krill were present in the boiled krill, and the drying process did not produce new astaxanthin esters. The evolution of the relative contents of 17 types of derivatives of astaxanthin during drying (Figure 4) suggests that the degradation of astaxanthin esters during hot-air and microwave drying was more extensive than that of during boiling. During the boiling process, the content of each astaxanthin ester diminished by 3 to 90%. The loss rate was the highest for Ast, and the esterified forms were more stable than the free form, as the proportion of astaxanthin esters to total astaxanthin increased after drying. The stability of astaxanthin linked to unsaturated fatty acids was lower than that of other astaxanthin esters except in the case of Asta-C18:3 and Asta-C18:4.

Under freeze-drying, the greatest losses were more than 20%, as shown by Ast and AM connected to EPA and DHA. In contrast, Asta-C14:0/C14:0 and Asta-C14:0/C16:0 were the most stable and remained almost undecomposed. The loss of total astaxanthin was 8.5%, whereas the loss of most AM- and AD-containing unsaturated fatty acids was even greater.

During hot-air drying, the detected content of astaxanthins decreased with increasing drying temperature (Figure 4). The percentage loss of Ast was higher than that of other astaxanthins and approached 100% at a drying temperature of 100 °C. In a range of 40 to 60 °C, the loss of astaxanthins was moderate. The astaxanthins were greatly damaged at temperatures above 60 °C. Under hot-air drying, Asta-C18:3 and Asta-C18:4 were more stable than the other AM. When the drying temperature reached 100 °C, most AM was degraded by approximately 90%, such as Asta-C16:0, Asta-C20:5, and Asta-C22:6, but Asta-C18:3 and Asta-C18:4 were degraded by only approximately 40%. For AD, the percent loss of Asta-C16:0/C20:5 was lower than that of Asta-C18:1/
C18:1 at temperatures below 60 °C, but the pattern was reversed at higher temperatures, as was observed for Asta-C14:0/C20:5, Asta-C18:1/C20:5, and Asta-C20:5/C22:6. This behavior was attributed to the highly unsaturated nature of fatty acids linked to astaxanthin, which makes them more susceptible to degradation than less unsaturated lipids at higher temperatures.

The degradation of astaxanthins by microwave increased with increasing power at an approximately constant rate. The maximum loss of Ast is about 83%, the loss of Asta-C20:5 is about 77%, and the losses of other astaxanthins are lower than this value. However, under hot-air drying conditions, the maximum loss of some astaxanthins exceeded 90%. Compared with the trend of astaxanthin loss with increasing temperature under hot-air drying, that under microwave drying was less variable.

In this study, the loss of Ast during the drying processes was much higher than that of AM or AD (Figure 4). Similarly, compared with Ast in *H. pluvialis*, the stability of astaxanthin esters was higher. This phenomenon may be due to the substitution of the hydroxyl groups at both ends of astaxanthin with fatty acids, increasing ester stability. For astaxanthin esters, high-efficiency microwave drying was less harmful to astaxanthin esters than high-temperature hot-air drying, which may be related to the drying time. Research on the storage of dehydrated pumpkin revealed that the degradation of carotenoids was related to storage time, with the loss of carotenoids increasing with storage time. In the present study, the microwave drying time was shorter than the hot-air drying time such that some astaxanthin esters might have been destroyed in the long-term, high-temperature aerobic environment.

The content of astaxanthin in krill varied with the drying method. In general, freeze-drying was the method least destructive to astaxanthins, followed by microwave drying and then hot-air drying. As shown in Figure 4, the esterified forms of astaxanthin had higher stability, and most of the AD was more stable than AM during drying. Similarly, in a study of the effect of heat treatment on astaxanthin esters in *P. vannamei*, Yang et al. found that AD was more stable than AM. This phenomenon may be due to the fact that both ends of AD are linked to fatty acids and have stable chemical properties. In the present study, we found that compared to other AM, four esters, Asta-C16:0, Asta-C18:1, Asta-C20:5, and Asta-C22:6, were less thermally stable. In addition, Yang et al. reported higher loss rates of Asta-C20:5 and Asta-C22:6 than that of Asta-C18:0 or Asta-C18:2, and the loss rates of other type of AM were not reported.

### 2.4. Effect of Drying Method on Optical Isomers.

Astaxanthin has three all-E-astaxanthin optical isomers, which have different compositions. The boiled krill contained 12% 3S,5′S, 9% 3S,3′R, and 79% 3R,3′R (Figure 3). Freeze-drying was the mildest of the drying methods tested, and it had no effect on the optical isomer composition of astaxanthin (p > 0.05). Hot-air drying temperatures of 40–60 °C did not significantly affect the stereostructure of astaxanthin; however, when the temperature increased from 60 to 100 °C, the proportion of 3S,3′R increased by 4.8% and that of 3R,3′R decreased by 3.4% (p < 0.01). Microwave drying had a similar effect. When the power increased from 1 to 5 kW, the proportions of 3R,3′R and 3S,3′S decreased slightly and the proportion of 3S,3′R increased by 2.3% (p < 0.05). Thus, the composition of the optical isomers of astaxanthin was not affected by freeze-drying but was significantly affected by both hot-air and microwave drying.

The composition of optical isomers differs among different organisms. For example, 3S,3′S accounts for a large proportion of the isomers in *H. pluvialis*, whereas 3R,3′R is more abundant than 3S,3′S in Antarctic krill. In the current study, hot-air drying decreased the 3S,3′S ratio and increased the 3S,3′R ratio. Liu et al. conducted in vitro and in vivo experiments and found that the antioxidation and antiaging activities of different stereoisomers of astaxanthin decreased in the order 3S,3′S > 3R,3′R > synthetic astaxanthin (3S,3′S/3S,3′R/3R,3′R = 1:2:1). Additionally, 3S,3′S and 3R,3′R both showed strong...
inhibition in an erythrocyte hemolysis test, whereas synthetic astaxanthin did not show the same response.38

These three configurations of astaxanthin have been shown to promote lymphocyte proliferation in mice, with 3S,3′S having the strongest effects.39 In the present study, under hot-air and microwave drying, the 3S,3′R R ratio gradually increased with increasing temperature and power, which may have influenced the activity of astaxanthin. Furthermore, freeze-drying was the only method that did not affect the optical isomers, although subsequent studies are needed to confirm this finding.

2.5. Effect of Drying Method on Geometrical Isomers.

The industrial process used to produce krill powder involves preboiling the krill at a high temperature, which produces 9Z- and 13Z-astaxanthin.22 In this study, the effect of drying method on the stability of astaxanthin was evaluated by comparing the isomerized astaxanthin before and after drying. The contents of all-13-E, 9Z-, and 13Z-astaxanthin, their percentage changes, and their relative proportions are summarized in Table 2.

During freeze-drying, the contents of all-13-E, 13Z-, and 9Z-geometrical isomers of astaxanthin decreased by an average of 10, 3.60, and 6.63%, respectively. However, high temperature and microwave power strongly affected the structure of astaxanthin. The loss of all-13-E-astaxanthin was no more than 23.19% at drying temperatures of 40–60 °C and reached 15.32% at 1 kW. However, the contents of all-13-E-astaxanthin decreased 65 and 54% when the temperature reached 100 °C and microwave power reached 5 kW. At drying temperatures of 40–60 °C, the percentage of 13Z-astaxanthin increased from 13 to 15%, whereas that of 9Z-astaxanthin did not change. As the temperature increased from 60 to 100 °C, the percentage of all-13-E- decreased from 73 to 62%, whereas that of 13Z-astaxanthin increased by 3% and that of 9Z-astaxanthin increased by 8%. As microwave power increased from 1 to 5 kW, the content of all-13-E-astaxanthin decreased from 15 to 53%. However, at 1 kW, the percentage of 13Z-astaxanthin increased to 4.66%, and that of 9Z-astaxanthin decreased to 2.34%, indicating that the all-13-E-astaxanthin was readily transformed to 13Z-astaxanthin.Under microwave drying, the proportions of all-13-E- and 13Z-astaxanthin changed greatly, while the percentage of 9Z-astaxanthin increased by only 3%. The results revealed that all drying processes led to the loss of E/Z isomers and that higher temperature or microwave power exacerbated this process. Freeze-drying caused slight all-13-E-astaxanthin, whereas hot-air (≥60 °C) and microwave drying (>1 kW) strongly influenced the isomerization of astaxanthin. The production of the 13Z-astaxanthin was greater than that of the 9Z-astaxanthin under the drying process.

Astaxanthin in krill usually consists of all-13-E-astaxanthin, but when exposed to light, heat, organic solvents, acids, and bases, the structure is easily isomerized to form a mixture of Z and E isomers.21 Both 13Z- and 9Z-astaxanthin were generated during the drying process, and the isomerism of astaxanthin increased as the drying temperature and microwave power increased. Yang et al. found thermal processing apparently promoted the isomerization of astaxanthin.23 As shown in Figure 3, the proportion of all-13-E-astaxanthin gradually decreased under drying, indicating that some astaxanthin was isomerized during the drying process, and the production of 13Z- was greater than that of 9Z-astaxanthin. In addition, we found that low drying temperatures (≤60 °C) could reduce all-13-E-astaxanthin. It was reported previously that AM linked to palmitic acid is more readily transformed to 13Z-astaxanthin than Ast.40 Thus, in the present study, the higher content of 13Z-astaxanthin than that of 9Z-astaxanthin in dried krill was possibly due to the abundance of AM. Zhao et al. reported that Ast was preferentially transformed to 13Z-astaxanthin rather than 9Z-astaxanthin under microwave treatment.41 We obtained similar results (Table 2). Furthermore, we found that microwave power below 1 kW reduced the isomerization phenomenon and helped preserve all-13-E-astaxanthin krill dry products. In rat microsomes, the antioxidant capacities of these isomers decreased in the order 9Z > 13Z > all-E-astaxanthin.21 Further studies are needed to determine the effect of astaxanthin structural changes on biological activity.

In the study, HPLC was used to measure the total content of astaxanthin in the lipid extracts of krill samples. The drying mode (freeze-drying, hot-air drying, or microwave drying) and conditions (temperature or microwave power) affected total astaxanthin content and isomer composition. The astaxanthin esters were identified by liquid chromatography coupled with mass spectrometry, and the main astaxanthin esters in the dried krill samples were quantitatively analyzed. Krill (not dried) contained a total of 28 types of astaxanthin derivatives. Ast had the lowest stability regardless of the drying process. The stability of most AD was higher than that of AM, and the astaxanthin esters linked to EPA and DHA were more easily destabilized. As drying temperature or microwave power increased, astaxanthin content gradually decreased. The drying

| Table 2. Content and Changes of Astaxanthin Isomers in Antarctic Krill |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|
| drying process             | condition     | 13Z-astaxanthin | all-E-astaxanthin | 9Z-astaxanthin |
|                            | Content (μg/g) | Change (%)      | Content (μg/g)      | Change (%)      | Content (μg/g)      | Change (%)      |
| boiling                   | 15.65 ± 0.06  | 90.71 ± 0.08    | 15.39 ± 0.06       | 13/75/12        |
| freeze-drying            | 15.09 ± 0.03  | 15.07 ± 0.05    | 14.37 ± 0.05       | 14/74/12        |
| hot-air drying            | 15.02 ± 0.09  | 8.82 ± 0.11     | 13.81 ± 0.11       | 13/75/12        |
| microwave drying          | 100 °C        | 72.22 ± 0.18    | 31.62 ± 0.21       | 14/74/12        |
|                            | 2 kW          | 16.38 ± 0.15    | 76.81 ± 0.10       | 13/75/12        |
|                            | 3 kW          | 14.23 ± 0.06    | 54.65 ± 0.15       | 13/75/12        |
|                            | 4 kW          | 14.25 ± 0.18    | 50.81 ± 0.21       | 13/75/12        |
|                            | 5 kW          | 13.41 ± 0.28    | 42.71 ± 0.18       | 13/75/12        |
conditions of 60 °C and 1 kW were optimal as they minimized astaxanthin degradation and ensured maximum drying efficiency. High-power microwave drying (≥1 kW) and high-temperature drying (≥60 °C) increased the isomerization of astaxanthin. These results show that freeze-drying, low-power microwave drying (1 kW), and low-temperature hot-air drying (≤60 °C) are conducive to the production of Antarctic krill powder with high astaxanthin content.

3. CONCLUSIONS
The effects of different drying methods on astaxanthin in Antarctic krill were studied. HPLC was used to measure the total content of astaxanthin in the lipid extracts of krill samples. The drying mode (freeze-drying, hot-air drying, or microwave drying) and conditions (temperature or microwave power) affected total astaxanthin content and isomer composition. The astaxanthin esters were identified by liquid chromatography coupled with mass spectrometry, and the main astaxanthin esters were identified and stored at −80 °C prior to treatment.

4. MATERIALS AND METHODS

4.1. Materials. Antarctic krill were acquired from the Marine Fisheries Group Co., Ltd. (Dalian, P.R. China) and were stored at −80 °C. The all-E-astaxanthin standard with a purity of 95.8 ± 0.5% was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The standard products for 13Z-astaxanthin, 9Z-astaxanthin, Asta-C16:0, and Asta-C16:0/C16:0 were purchased from Swiss Carotenature (Münzingen, Switzerland). Methanol, tert-butyl methyl ether, isopropanol, and acetonitrile were of HPLC grade (Merck, Darmstadt, Germany). Acetone was of HPLC grade (Duksan, Korea). Anhydrous magnesium sulfate and sodium hydroxide were all of high purity (GuoYao, Shanghai, China).

4.2. Antarctic Krill Sample Preparation. Initially, 7 kg of frozen krill were thawed at room temperature (18 °C). The thawed samples were then boiled in 4 L of water for 3–5 min and then cooled to room temperature. The boiled krill samples were centrifuged, dehydrated, uniformly mixed, and then divided into 13 portions. Five portions were subjected to microwave drying, six portions were subjected to hot-air drying, one portion was subjected to freeze-drying, and the last portion was directly placed in a freezer at −80 °C without any treatment. Microwave drying consisted of five powers (1, 2, 3, 4, and 5 kW), and the speed of the conveyor was adjusted to make the final moisture content of the sample consistent between treatments. To prepare the hot-air-dried samples, approximately 500 g of krill was uniformly tiled on glass Petri dishes and placed in a preheated oven. Drying was carried out at 40, 50, 60, 70, 80, and 100 °C. The moisture content of the samples was measured every 30 min until it became constant. For freeze-drying, a portion of the krill was plated on a Petri dish and dried in a vacuum freeze-dryer at a temperature of −55 °C for 12 h. All samples were stored at −80 °C prior to treatment.

4.3. Extraction of Astaxanthin. All samples were individually comminuted using acetone as a solvent to extract astaxanthin, following a previously reported method with slight modification. Each undried sample was mixed with anhydrous magnesium sulfate and acetone at a ratio of 1:2:5 (w/w/v), and each dried sample was directly combined with five volumes of acetone. The mixture was extracted by ultrasonication using the ultrasonic cleaner (Kunshan, China) at 40 kHz for 15 min. The ultrasonic extraction dispersed the particles uniformly in the extract, thus significantly improving the extraction efficiency. The mixture was centrifuged at 4 °C for 10 min, and the above extraction steps were repeated; finally, the supernatants were combined. To ensure that the astaxanthin was not destroyed, the temperature was controlled at 0–4 °C. Each supernatant was mixed and placed in a brown glass bottle, sealed with nitrogen, and stored in a freezer at −20 °C.

4.4. Analysis of Astaxanthin Composition. Astaxanthin was identified by HPLC quadrupole/high-resolution MS (HRMS) (LC-Q Exactive Orbitrap MS). A mixture of acetone and krill extract was diluted at 1:10 (v/v) and analyzed using a 0.25 μm filtration membrane. A C18-reversed phase column (250 × 2.1 mm, 1.9 μm) was used under the following conditions: eluent A: 90% isopropyl alcohol acetonitrile solution with 10 mM ammonium formate and 0.1% formic acid; mobile phase B: 60% acetonitrile aqueous solution with 10 mM ammonium formate and 0.1% formic acid. The elution velocity, column temperature, and injection volume were 0.3 mL/min, 45 °C, and 2 μL, respectively. The linear gradient elution condition was as follows: B increased from 40 to 65% in 0 to 3 min; B increased from 65 to 75% in 3–8 min; B increased from 75 to 80% in 8–12 min; B increased from 80 to 85% in 12–35 min; B remained at 85% for 35–40 min; B decreased from 85 to 40% at 41 min. Then, the system was maintained for 10 min to stabilize the column.

The analysis was carried out using the HPLC-HRMS (Thermo Scientific, USA) technology, and the source temperature was set at 320 °C in the positive ion mode. The resolution of the full-scan mode was set as 70000 full width at half-maximum (FWHM). The C-trap maximum capacity (automatic gain control (AGC) target) was 3 × 106. The C-trap maximum injection time was 100 ms. The resolution of MS/MS was set as 17500 FWHM. The maximum capacity of the C-trap (AGC target) was 1 × 105. The maximum injection time of the C-trap was 50 ms. The collision energy values were 10 and 18 eV.

4.5. Determination of Total Astaxanthin Content. The astaxanthins were transformed into Ast after hydrolysis for quantification. The method described by Wade et al. was used to study the decomposition of astaxanthin esters in Antarctic krill. Chromatographic conditions in the method described by Sun et al. were used. The analysis was carried out on an HPLC system (Agilent, USA) with a UV detector using methanol and methyl tert-butyl ether at a flow rate of 1.0 mL/min, an injection volume of 20 mL, and a column
statistically significant at p < 0.05.

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Notes
The authors declare no competing financial interest.

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