Chapter 9
Analysis of Soluble Organic Polar Fractions from Sea Salt by GC–MS

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Abstract In order to investigate the organic compounds from sea salt, soluble organic polar fractions as a result of chloroform extraction followed by being esterified with BF_3 in methanol were determined using gas chromatography-mass spectrometry (GC–MS). There were greater than 20 soluble organic polar fractions presented in sea salt, and the major components were C_{15}–C_{28} fatty acid compounds series, plasticizers and sulfur. The total amount of fatty acids accounted for more than 50%, and C_{16} was the main peak. The relative abundance of *n*-hexadecanoic acid was the highest (41.19%) among the soluble organic polar fractions from the analyzed sample. At the same time, a certain amount of phthalate acid esters (PAEs) such as dibutyl phthalate (DBP), diisobutyl phthalate (DIBP) and bis(2-ethylhexyl) phathalate (DEHP) and sulfur (S6, S7 and S8) were detected, and the contents of the two component types were relatively close. Among the three PAEs, the relative abundance of DIBP (17.678%) was the highest. Further, their effects on marine organisms and ecosystem should be concerned based on the potential risks of PAEs.

Keywords Salt · Polar components · GC–MS · Fatty acid · Plasticizer

9.1 Introduction

Salt is one of the necessities of people's production and life, and it is widely used in food, leather, ceramics, glass, soap and dye industries. Inorganic elements from salt such as sodium chloride, iodine and heavy metal elements were mainly focused on (Huang et al. 2020), while the studies for organic compounds from salt were few. Fang et al. determined volatile organic acids from edible salt samples, and the results showed that butyric acid and isovaleric acid were presented in some salt samples

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(Fang et al. 2019). The determination and analysis of the organic compounds from salt is helpful for people to fully understand the composition of salt. The analysis and identification soluble organic polar fractions of salt from inland natural lake water were performed by GC–MS in our previous studies, and soluble polar fractions extracted by chloroform from the samples could be divided into three types (fatty acids, PAEs, and sulfur ring) (Chang et al. 2020a). The effect of PAEs on organisms have gradually recognized and concerned by people. In the study, the organic polar (non-hydrocarbon) fractions from sea salt were analyzed by GC–MS, which could help us to further understand the composition characteristics of organic compounds from salt.

9.2 Samples and Methods

Sea salt samples were purchased from supermarkets. The detailed pretreatment process and analysis of the sample were given in the literature (Chang et al. 2020a). Briefly, samples of sea salt (120.0000 g) were weighed accurately and placed in a beaker. An appropriate amount of refined chloroform just submerged the sample were added into the sample to extract organic compounds. The extracts were subsequently mixed after 3 separate ultrasonic treatments (30 min each), and the organic compounds extracted three times were combined and put it into a 500 mL beaker. Then, methyl esterification of the extracts in a water bath was carried out at 60 °C for 10 h after chloroform in the extracts volatilized completely (Chang et al. 2020a; Pancost et al. 2000; Duan et al. 2014). Finally, the composition of polar fractions was performed using an Agilent GC6890N/MSD5973N system. Agilent J&W HP-5 column (30 m × 0.32 mm i.d. × 0.25 μm film) was used as capillary column, and high purity helium (He) (99.999%) were applied in the GC–MS detection system as the carrier gas. The sample injection was 1 μL, and the flow rate of carrier gas was 1.0 mL/min with 40 cm/sec of linear velocity. The organic substances from the sea salt samples were identified using NIST05 L (U.S.A.), and the peak area was normalized to calculate the content of each component.

9.3 Results and Discussion

The soluble organic polar fractions profile from the sea salt sample was obtained by GC analysis (Fig. 9.1), and the results of their compounds identification were shown in Table 9.1. The fatty acid compounds with the carbon number distribution range between 12 and 22 were detected in the samples, and their total relative abundance was more than 50%; n-C_{16} fatty acid represented the main peak in sea salts with retention times 17.265 min. The other components consisted of phthalate acid esters and sulfur, and the abundances of the two types were less than 20%, respectively. Among the soluble organic polar components detected, *n*-hexadecanoic acid

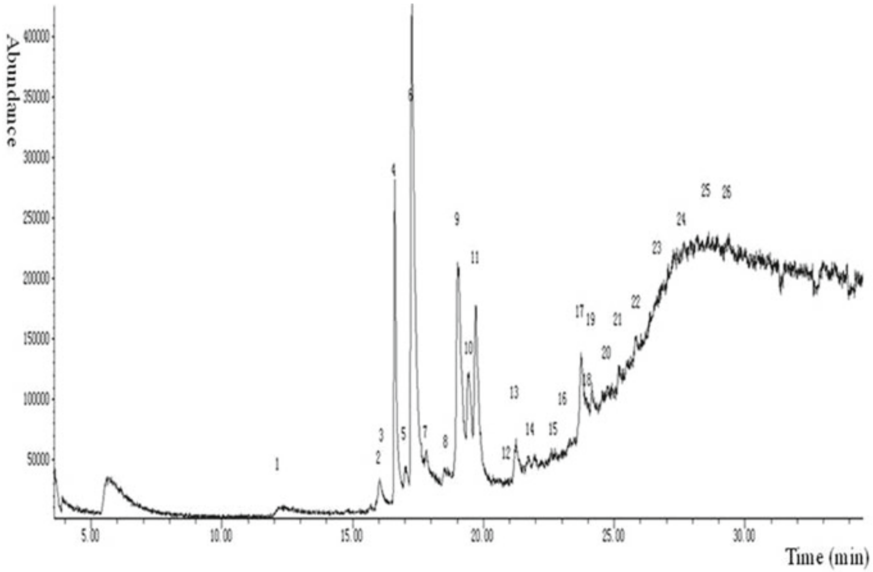

Fig. 9.1 GC profile of soluble organic polar components (methyl esters) from sea salt obtained by chloroform extraction

(41.19%) was the most abundant fatty acid, accounting for almost half of the soluble organic components; followed by *n*-octadecatrienoic acid accounted for one tenth of the total organic matter, and the relative abundances of the other fatty acids in the samples were minor, which were all less than 6%. Among the observed the fatty acids from the sea salt sample, the total abundances of saturated fatty acids was almost five times greater than that of unsaturated fatty acids. The ratio of total amount of odd carbon *n*-fatty acids to that of even carbon *n*-fatty acids was defined as carbon preference index (CPI_{total}) of *n*-fatty acids (Chang et al. 2020b), and the CPI_{total} index could be used to analyze the characteristics of *n*-fatty acids from various samples. The CPI_{total} in the sea salt samples was 0.005, which suggested that the total amount of the former was lower than that of the latter obviously. Furthermore, the content characteristic of the saturated fatty acids that were showed in the study were in accordance with the previously observed characteristic of even carbon content being higher than odd carbon content (Petrovic and Arsic 2016).

The unsaturated fatty acid composition in the sea salt sample were 9-hexadecenoic acid ($C_{16}H_{30}O_2$) (0.122%), 9-Octadecenoic acid ($C_{18}H_{34}O_2$) (5.798%), and erucic acid (4.658%).

Sulfur (S6, S7 and S8) was founded in the sea salt, and the relative abundance of them was as follows: S8 (19.39%) > S6 (0.006%) > S7 (0.003%), respectively. In addition to the above substances observed, phthalate acid esters (PAEs) were detected from the sea salt, and the relative abundance of PAEs was close to that of sulfur rings (Fig. 9.2).

Table 9.1 Soluble organic polar components (methyl esters) identification from sea salt detected by GC–MS

Peak number	Chemical formula	Molecular mass	Compound name	Percentage (%)
1	S_6	192	S_6	0.006
2	S_7	224	S_7	0.003
3	$C_{16}H_{32}O_2$	256	Pentadecanoic acid, methyl ester	0.025
4	$C_{16}H_{22}O_4$	278	Diisobutyl phthalate (DIBP)	17.678
5	$C_{17}H_{32}O_2$	268	9-Hexadecenoic acid, methyl ester	0.122
6	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester	41.19
7	$C_{16}H_{22}O_4$	278	Dibutyl phthalate (DBP)	0.03
8	$C_{18}H_{36}O_2$	284	Heptadecanoic acid, methyl ester	0.024
9	S_8	256	Cyclic octaatomic sulfur	19.39
10	$C_{19}H_{36}O_2$	296	9-Octadecenoic acid, methyl ester	5.798
11	$C_{19}H_{38}O_2$	298	Octadecanoic acid, methyl ester	10.087
12	$C_{20}H_{40}O_2$	312	Nonadecanoic acid, methyl ester	0.007
13	$C_{21}H_{42}O_4$	358	8, 10-Dimethexy-octadecanoic acid, methyl ester	0.137
14	$C_{21}H_{42}O_2$	326	Eicosanoic acid, methyl ester	0.024
15	$C_{22}H_{44}O_2$	340	Heneicosanoic acid, methyl ester	0.046
16	$C_{23}H_{46}O_4$	386	10, 12-Dimethexy-octadecanoic acid, methyl ester	0.105
17	$C_{23}H_{44}O_2$	352	Erucic acid, methyl ester	4.658
18	$C_{23}H_{46}O_2$	354	Docosenoic acid, methyl ester	0.018
19	$C_{24}H_{38}O_4$	390	Bis(2-ethylhexyl) phathalate (DEHP)	0.052
20	$C_{24}H_{48}O_2$	368	Tricosanoic acid, methyl ester	0.04
21	$C_{25}H_{50}O_4$	414	12, 14-Dimethexy-octadecanoic acid, methyl ester	0.058
22	$C_{25}H_{50}O_2$	382	Tetracosanoic acid, methyl ester	0.034
23	$C_{26}H_{52}O_2$	396	Pentacosanoic acid, methyl ester	0.071

(continued)

Table 9.1 (continued)

Peak number	Chemical formula	Molecular mass	Compound name	Percentage (%)
24	$C_{27}H_{54}O_2$	410	Hexacosanoic acid, methyl ester	0.26
25	$C_{28}H_{56}O_2$	424	Heptacosanoic acid, methyl ester	0.056
26	$C_{29}H_{58}O_2$	438	Octacosanoic acid, methyl ester	0.081

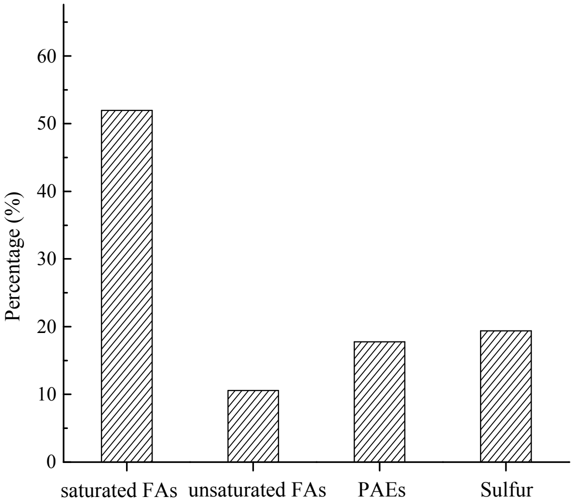

Fig. 9.2 Relative content of different types from soluble organic polar fractions in sea salt

Fatty acids (FAs) as the main components of lipids within the body play a key role in cellular structures and functions, such as the regulation of apoptosis, the main energy pool in adipose tissue and the activity of hypothalamic neurons (Rudolph et al. 2001; Michael and Watt 2020). In our study about soluble organic polar substances from *Hydrilla verticillata* (an aquatic plant) which is widely found in freshwater, the carbon number distribution range from C_{12} to C_{24} was observed in the plant. In the plant, *n*-hexadecanoic acid with relative abundance of 47.06% representing the main fatty acid peak was observed and the total relative abundance of saturated FA was almost twice that of unsaturated FA (Chang et al. 2020b). Moussa and Almaghrabi (Moussa and Almaghrabi 2016) reported that hexadecanoic acid with relative abundance 48.13% was the highest abundant fatty acid among the saturated fatty acid compositions from *Peganum harmala* plant. Hexadecanoic acid was detected also as the main component in some essential oils involving in those from *Oryzasativa* L. (74.57%) (Sayaka and Mitsuo 2006), *Ardisia brevicaulis* Diels leaves (43.32%) (Pu et al. 2009) and from *Allium roseum* var. *randiflorum* subvar. *typicum* Regel leaves (75.9%) (Rouis-Soussi et al. 2014). C_{15}–C_{28} fatty acid compounds constituted the main polar component of sea salt samples, in which the highest abundant of *n*-hexadecanoic acid was analyzed, which was almost half of the total amount of the soluble organic components.

In previous research on the soluble organic polar substances in salt from inland natural lake water, the fatty acids with the carbon number distribution range from C_{14} to C_{24} were observed, and among them erucic acid was the highest relative abundance (50.610%), accounting for half of the total amount of soluble organic polar substances, the followed by *n*-hexadecanic acid with relative abundance of 13.637% and then 9-octadecenoic acid (8.747%) (Chang et al. 2020a).

In addition, these results showed that the distribution characteristic of fatty acids in salt from different areas could be closely associated with the biological characteristics of water source of the salt.

Although the content of sulfur in the sea salt sample is significantly higher than that in salt from natural lake (Chang et al. 2020a), the existence forms of sulfur element, that is S6, S7 and S8 in the two salts is the same. Phthalate acid esters, as one group of endocrine dispersing chemicals have been found in water, soil, atmosphere, plants and animals based on their wide application in production, and the environmental impact and the harm to organisms caused by phthalate acid esters also attracted the attention of scholars (Cai et al. 2017; Kong et al. 2019; Paluselli et al. 2018; Takeuchi et al. 2018; Vered et al. 2019; Gao et al. 2018; Li et al. 2020). Vered et al. reported that high contents of PAEs (DBP and DEHP) were found in *Herdmania momus* and *Microcosmus exasperatus* which were obtained from the Israel Mediterranean and Red Sea coasts (Vered et al. 2019). The effect of phthalate acid esters on organisms has also attracted the attention of many researchers. Lu et al. reported that three PAEs exposures, including DBP, mono-(2-ethylhexyl) phthalate, and DEHP, could result in developmental abnormalities in zebrafish larvae and induced cytotoxicity in HEK293T cells (Lu et al. 2021). Phthalate acid esters involving in DIBP, DBP and DEHP were detected in the sea salt, which were also founded in edible salt sampls from inland natural lake water (Chang et al. 2020a). The phenomenon indicates that PAEs are common in water, which should be taken seriously. In addition, the proportion of plasticizer content in the sea salt is relatively high, accounting for more than one tenth of the total soluble organic polar substances, which also suggested that the plasticizer content in sea water should not be ignored. In view of the universality of plasticizers, their biological effects should be further studied. It should be paid more attention to the study of the types and concentrations of plasticizers in seawater, their migration characteristics from water to soil or organisms, their effects on marine organisms and marine ecology and their enrichment and degradation characteristics in organisms.

9.4 Conclusions

The analysis results by using GC–MS showed there were greater than 20 soluble organic substances existed in sea salt. These compounds were mainly devided into three types: fatty acids, PAEs, and sulfur. The characteristics of soluble organic polar fractions in salt and the carbon number distribution range of fatty acids have close association with the water quality characteristics of their sources. The total amount of the even carbon *n*-fatty acids was higher obviously than that of odd carbon *n*-fatty acids from the sea salt. The content order of the above three components was fatty acids > sulphur ~ PAEs. The relative abundance of phthalate acid esters (17.76%) in soluble organic polar components extracted by chloroform from sea salt was not low, which should be paid more attention.

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