Analysis of Polar Components in Salt by GC-MS

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Abstract Edible salt is the most common and extensive seasoning agent in people's daily life, and its edible safety is directly related to human health. The polar components of edible salt were analyzed by gas chromatography-mass spectrometry (GC-MS). The results showed that there were more than 20 polar organic compounds in salt, mainly C14-C22 long-chain fatty acids. The highest content of erucic acid (50.610%) was detected. Meanwhile, phthalate esters (PAEs) and elemental sulfur were also detected.

1. Introduction
In modern analysis technology, gas chromatography-mass spectrometry (GC-MS) is a kind of high-efficiency analysis technology. The technology uses the separation ability of gas chromatography to realize separation of components in mixture, and uses mass spectrometry to identify the separated components, which can be used for qualitative and quantitative analysis. Because of its good separation, high sensitivity and low detection limit, GC-MS is suitable for trace and trace analysis, and is one of the reliable technical means for food safety inspection. The technology has been widely used in food safety detection, analysis of environmental pollutants, drug composition identification, doping detection and so on [1, 2]. Compared with other foods, salt is a necessity of human life and is irreplaceable. At present, for the edible safety of salt, more emphasis is placed on the analysis of inorganic elements, while the study of organic compounds in salt is less [3]. In the study, the polar organic compounds in salt were studied by GC-MS, which can provide a scientific reference for food safety supervision of edible salt.

2. Samples and methods
2.1. Samples Treatment
Salt samples (120.0000 g) purchased from supermarkets was weighed accurately and put into a 250 mL beaker, and then added appropriate amount of refined chloroform into the beaker containing the sample (subject to the sample just passing). Then the samples were extracted with CHCl₃ by ultrasonic for 3 times and each time for 20 minutes, and the three extracts were mixed. Finally, the organic substances extracted were weighted after CHCl₃ was completely volatilized.

The organic substances extracted were esterified with BF₃ in methanol to convert carboxylic acids to their corresponding methyl esters more amenable to GC analysis [4]. The same volume of refined
Dichloromethane and refined methanol mixture (volume ratio 1:1) were added to the above extracted samples to dissolve the organic matter, and 5 mL BF₃/MeOH reagent added, followed by shaking gently. Finally, the sample was sealed and kept in a water bath at 60 °C for 10 h.

The sample was transferred into a 120 mL separation funnel after methyl esterification. Then 60 mL of ultra pure water and 20 mL of refined ether were added into the separation funnel. The sample was shaken fully and then left to stand. After the liquid level was stratified, the organic phase was retained, and then 40 mL ultra pure water was added again. The sample was still shaken fully and allowed to stand. After the liquid level was stratified, the organic phase in the separation funnel was transferred to a weighing bottle and weighed after ether volatilization.

2.2. Instruments and sample conditions

After the ether is completely evaporated, 1-2 drops of refined dichloromethane solvent were slowly added along the wall of the bottle contained the above extracted samples, and then gently shaken. After the solvent volatilized to a small amount, 1.0 ul of solution was injected and analyzed using an Agilent 6890N/MSD5973N gas chromatography mass spectrometer(GC-MS) equipped with a J&W HP-5 column (30 m × 0.32 mm i.d. × 0.25 μm film thickness). The carrier gas was helium (99.999%) at a column flow of 1.2 mL/min. The initial oven temperature was 80°C for 2 min, and then was raised with 4 °C/min to 295 °C, and held for 20 min. MS conditions were as follows: electron ionization (EI) at 70 eV; an ion source temperature of 230 °C; quadrupole rod temperature of 150 °C, interface temperature of 280 °C and spectrum library with NIST05L (U.S.A.)[4].

3. Results and discussion

The GC / MS total ion current chromatogram of polar fraction (methyl ester products) from the salt is shown in Figure 1. Fatty acids appeared at 14.504 min and erucic acid appeared at 23.630 min, with the highest peak. A total of 29 polar components were observed in the salt sample; these components were predominantly composed of fatty acids, phthalate esters (PAEs) and sulfur rings (Table 1). The relative abundance of the three type was as follows: fatty acids (94.764%) > PAEs (3.566%) > sulfurs (1.67%), respectively (Figure 2). The fatty acids were divided into saturated fatty acids (SFAs) and unsaturated fatty acids (USFAs). The content of unsaturated fatty acids was higher than that of saturated fatty acid in the sample.

The saturated fatty acid components detected in the sample consisted mainly from C₁₄ to C₂₄, and the even carbon content was higher than that of odd carbon, which was accordance to the fatty acids in biological. In biological systems, the content of even carbon was generally higher than that of an odd carbon number in saturated fatty acid series compounds (carbon number in 12 to 24, typical) [5].

![Figure 1. Total ion current chromatogram of polar fraction in sample from salt.](image)

![Figure 2. Relative abundance of different type of components from salt.](image)
Table 1. Polar fraction identification (methyl esters) in salt.

| Peak number | Molecular formula | Molecular weight | Compounds                                      | Content (%) |
|-------------|-------------------|------------------|------------------------------------------------|-------------|
| 1           | C₁₀H₁₀O₄          | 194              | Dimethyl phthalate                              | 0.033       |
| 2           | S₆                | 192              | Sulful S₆                                      | 0.509       |
| 3           | C₁₂H₁₆O₂          | 242              | Tetradecanoic acid, methyl ester                | 0.601       |
| 4           | C₁₅H₃₀O₂          | 256              | 12-methyl- tetradecanoic acid, methyl ester     | 0.285       |
| 5           | S₇                | 224              | Sulful S₇                                      | 0.070       |
| 6           | C₁₆H₃₂O₂          | 256              | Pentadecanoic acid, methyl ester                | 0.482       |
| 7           | C₁₆H₃₂O₄          | 278              | Diisobutyl phthalate (DIBP)                     | 0.150       |
| 8           | C₁₃H₃₄O₂          | 270              | Pentadecanoic acid, 14-methyl-, methyl ester    | 0.272       |
| 9           | C₁₃H₃₂O₄          | 268              | 9- Hexadecenoic acid, methyl ester              | 2.332       |
| 10          | C₁₃H₃₄O₂          | 270              | Hexadecanoic acid, methyl ester                 | 13.637      |
| 11          | C₁₆H₃₂O₄          | 278              | Diibutyl phthalate (DBP)                        | 1.204       |
| 12          | C₁₈H₃₆O₂          | 284              | Heptadecanoic acid, methyl ester                | 0.494       |
| 13          | C₁₇H₃₂O₄          | 292              | Methyl 2-ethylhexyl phthalate                   | 1.763       |
| 14          | S₈                | 256              | Cyclic octaatomic sulfur                        | 1.091       |
| 15          | C₂₁H₄₂O₂          | 326              | Phytyanic acid, methyl ester                    | 1.814       |
| 16          | C₁₀H₃₆O₂          | 296              | 9-Octadecenoic acid, methyl ester               | 8.747       |
| 17          | C₁₁H₃₆O₂          | 296              | 16-Octadecenoic acid, methyl ester              | 0.195       |
| 18          | C₁₁H₃₈O₂          | 298              | Octadecanoic acid, methyl ester                 | 6.662       |
| 19          | C₁₂H₄₀O₂          | 312              | Nonadecanoic acid, methyl ester                 | 0.074       |
| 20          | C₂₁H₄₂O₄          | 358              | 8,10-Dimethyloctadecanoic acid, methyl ester    | 1.215       |
| 21          | C₂₁H₄₀O₂          | 324              | Methyl trans-11-eicosenoate                     | 0.557       |
| 22          | C₂₁H₄₀O₂          | 324              | Methyl cis-11-eicosenoate                       | 0.168       |
| 23          | C₂₂H₄₂O₂          | 326              | Eicosanoic acid, methyl ester                   | 0.619       |
| 24          | C₂₂H₄₄O₂          | 340              | Heneicosanoic acid, methyl ester                | 0.113       |
| 25          | C₂₂H₄₄O₂          | 352              | Erucic acid, methyl ester                       | 50.610      |
| 26          | C₂₃H₄₆O₂          | 396              | Tricosanoic acid, 3,5-dimethyl-, methyl ester    | 0.395       |
| 27          | C₂₃H₄₆O₂          | 354              | Docosenoic acid, methyl ester                   | 0.648       |
| 28          | C₂₃H₄₈O₄          | 390              | Bis(2-ethylhexyl) phthalate (DEHP)              | 0.416       |
| 29          | C₂₃H₅₀O₄          | 414              | 12,14-Dimethoxy-octadecanoic acid, methyl ester | 4.864       |

In nature, fatty acids come from different sources such as fruits, vegetable oils, seeds, nuts, animal fats, and fish oils. Fatty acids are the primary component of lipids and play an important role in biological systems, including as primary constituents of cell membranes, an energy source, and regulating the activity of enzymes and inflammatory processes[6-8], and can exist as free forms and bound forms, such as cholesterol and phospholipids [1, 9]. Among the various fatty acids, the content of erucic acid was the highest, 50.610%; second, n-hexadecanic acid (13.637%) and 9-octadecenoic acid (8.747%); then octadecanoic acid (6.662%). The rest of the fatty acids were less than 3%. Eric acid is an unsaturated fatty acid, and is a non-branched and long-chain fatty acid consisting of 22 carbons and a cis-configurated double bond on C-13[10]. Consumption of food rich in erucic acid has been found to have adverse effects on health. The main effect of erucic acid on health is the accumulation of triacylglycerol in the heart due to insufficient oxidation, which leads to the reduction of myocardial contractility [10-12]. n-hexadecanoic acid belongs to saturated fatty acid, which widely exists in nature and has been detected in many plants, such as *Peganum harmala* plant [13], *Hydrilla* [4] and *Elaeis guineensis* [14]. The main peak of salt is C16, which is consistent with the current research about aquatic plant *Hydrilla* [4]. The results indicated that the source of fatty acids from aquatic plants is closely related to the water quality of salt.
Phthalate esters (PAEs) such as DIBP, DBP and DEHP were found in the sample. PAEs have been widely used as plasticizers in products like plastic wrappers, toys, cosmetics, gaskets, plastic roofing systems, and furniture decoration materials [15]. Approximately 150 million tons of plastic products are consumed annually worldwide, and the global annual production of PAEs is nearly 6–8 million tons [16, 17]. PAEs exist widely in water and soil, and have gradually becoming a family of emerging environmental pollutants, which have frequently been detected in terrestrial and aquatic organisms such as mice, algae, zooplankton, fish, and even in human blood[18, 19]. Phthalates have been reported to affect multiple biochemical processes in humans and wildlife, and their effects include reproduction, damage to sperm [20], infertility [21], apoptotic responses [18] and so on. DBP and DEHP are two widely used plasticizers. Therefore, it should be pay attention to the influence of plasticizers on aquatic organisms in the future.

4. Conclusions
The organic matter analysis of salt showed that the polar substances in salt were complex, mainly including fatty acids, PAEs, and sulfur ring, in which erucic acid and PAEs should be paid more attention by food testing departments.

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