

·研究简报·

鱼油挥发性成分分析和脱除方法

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摘要:采用改进的动态顶空技术捕集、浓缩鱼油挥发性成分,并通过GC-MS分析鉴定其中45个成分,结果表明,其中14个为饱和、不饱和醛类,8个为饱和、不饱和酮类。这些物质被认为是形成鱼油异味的主要成分。通过GC分析评价鱼油储藏过程中的感官变化和氧化,结果表明,GC分析的总峰面积能够指示鱼油的气味强度和氧化程度,并和感官检验结果一致。凹凸棒黏土处理鱼油取得较好脱色脱臭效果,其脱色脱臭效率分别为86.2%和84.1%。

关键词:鱼油;挥发性成分;感官检验;脱臭;黏土吸附

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鱼油富含高不饱和脂肪酸,具有抑制肥胖、抗癫痫和心率失常、防止血管纤维化等重要的生理功能,因此在过去十多年受到广泛关注^[1-4]。但是鱼油含有的不饱和脂肪酸易于氧化产生小分子醛、酮类物质^[5]。这些物质不仅改变鱼油的感官特性,并且对人体有潜在的危害。这是影响鱼油产业发展的关键因素之一^[6-7]。因此寻求脱除鱼油有害的、产生不良气味的挥发性成分方法,从而生产适合食品和医用的鱼油变得非常必要^[8]。

过氧化值(POV)、酸价(AV)、气味单位(FU)、核磁共振(NMR)等方法都被用于评价脂类物质的氧化程度或风味改变^[9]。但是这些方法各有优缺点。比如POV方法只适合评价植物或动物油的氧化程度,但对于含有高不饱和脂肪酸的鱼油并不适宜^[9]。测定FU方法繁琐、费时,并且氧化产物与鱼油气味及氧化稳定性之间的关系并不明确^[10-11],因此评价鱼油的质量需要更加简单实用的方法。本研究旨在分析鱼油的挥发性成分,了解这些成分与鱼油的氧化成分和臭味成分之间的关系,并在研究防止鱼油氧化方法的基础上^[12],探讨简便的除去鱼油中氧化产物的方法。

1 材料与方法

1.1 材料

日本鰵鱼(*Engraulis japonicus*)鱼油购于青岛鱼肝油有限公司。凹凸棒黏土采自江苏盩王山矿区,凹凸棒土含量70%~80%,典型分子式为 $Mg_3Si_4O_{10}(OH)_2 \cdot 4H_2O$,孔隙度为200~325目。

1.2 方法

1.2.1 鱼油挥发性成分的动态顶空—气相色谱分析(DHS-

GC) 鱼油挥发性成分通过DHS装置收集。3g鱼油加到DHS装置的烧瓶中,在65℃收集2h。载气为高纯氮气,流速100 mL/min。挥发性成分被吸附到装有250 mg Tenax TA(AR,60~80目,FARCO)的吸附柱上。用10 mL重蒸乙醚洗脱。洗脱液收集3 mL,以每管1 mL分装,并加入10 μ L 500×10^{-6} mg/L的 C_{28} 乙醚溶液作为内标,浓缩至20 μ L,取1 μ L进行GC分析。气相色谱仪:HP 5890 SII;色谱柱:SGE,AC-225,0.25 mm $\times$ 30 m;色谱条件:程序升温,40℃保留2 min,5℃/min升温到180℃,保留10 min;载气:高纯氮气,32 mL/min。

1.2.2 鱼油挥发性成分的气-质鉴定分析(GC-MS) GC分析的样品同时做GC-MS分析,GC部分条件完全一样。采用HP 5971 A MSD,质谱条件:离子源温度,250℃;离子化电压,70 eV;GC-MSD连接温度,200℃。质谱图在DEMO.L,NBSS4K.L,Wiley 138.L谱库检索进行挥发性成分的鉴定。

1.2.3 鱼油的超临界萃取(SCE) 将4 kg鱼油(A)加到超临界萃取设备(HA221-40-20,江苏省南通市,华安超临界设备有限公司)的萃取釜中,采用2级分离萃取法。萃取温度:35℃,萃取压力:15 MPa;一级分离温度:40℃,压力8 MPa;二级分离温度:35℃,压力6 MPa。萃取结束后,萃取釜、一级分离釜、二级分离釜鱼油分别记为B、C、D,另一氧化鱼油作为参照,记做E。样品A~E均在相同条件下保存,做感官分析、GC分析和POV测定。

1.2.4 Friedman序和感官检验 10个感官检验成员用来评价以上A~E鱼油样品的气味特征。样品随机提供给评价成员,每个样品检测3次,每次检验中间间隔5 min。排序

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检测法用于分析不同样品气味差异显著性^[13];检验员根据样品的气味强度从弱到强进行排序,序号代表每个样品的得分。根据公式: $F = 12 \div [JP(P+1)] \times (R_1^2 + R_2^2 + \dots + R_p^2) - 3J(P+1)$ 计算 Friedman 序数和 F 值,并和查表得到的临界 F 值比较确定差异显著性。其中 J 是检验员数, P 为样品数, $R_1 \sim R_p$ 为每个样品的序数和。

1.2.5 黏土的活化以及鱼油脱臭处理 黏土与 1% HCl 以体积比 1:30 混合,煮沸保持 1 h。用蒸馏水洗至 pH 5,再于 150℃干燥 2 h,粉碎至 200 目。将活化好的黏土和鱼油按照质量比 1:20 混合,沸水浴 10 min,混合液冷却到室温后 3000 r/min 离心,上清液即为脱色、脱臭鱼油。原油 (POV 8.2 mmol/kg) 和脱臭后鱼油 (POV 2.8 mmol/kg) 分别做感官检验和 GC 分析。

1.2.6 POV 的测定 POV 测定采用 Takagi^[14] 改良的碘化锡比色法。

1.2.7 脱色率 (R_d) 的测定 黏土吸附法处理前后的鱼油加入 1 cm 的比色皿,在 510 nm 测定其吸光值,按照以下公式计算:

$$R_d = (A_0 - A_1) / A_0 \times 100$$

式中 A_0 为原油的吸光值; A_1 为脱色后鱼油的吸光值。

2 结果与分析

图 1 为鱼油挥发性成分 GC-MS 分析的总离子流谱图,从中可以辨认出 51 个峰,其中 45 个峰通过查谱库等方式得到确认,相应结果如表 1 所示。结果表明,被确认的 45 个化合物中,14 个是饱和或不饱和的醛类物质,8 个是饱和或不饱和的酮类物质,它们占有挥发性成分的 48.5% 左右,其碳数在 6 到 10 之间。此外还检测到一个含氮化合物和一个含硫化合物,其他化合物为 2 个酚类物质、2 个酸类物质、3 个酯类、1 个醇类以及 16 个饱和或不饱和的烃类化合物。

通过 SCE 处理的鱼油的各个组分的挥发性成分经过 GC 分析后挥发性成分的总峰面积如表 2 所示。由峰面积从小到大排列顺序为 B、C、A、D。根据感官检验的结果可知 (表 3),按照序数和 R_d 的排列,其气味强度由小到大也为 B、C、A、D。根据计算得到的 Friedman 序数和的值为 28.6,比查表得到的临界值 F_c 值大 ($F_c = 13.28, \alpha = 0.01$)。这说明以上几个样品的气味强度之间存在显著差异。也就是说,如果用 GC 分析得到的总峰面积代表鱼油的异味强度,可以得到和感官检验一致的结果。

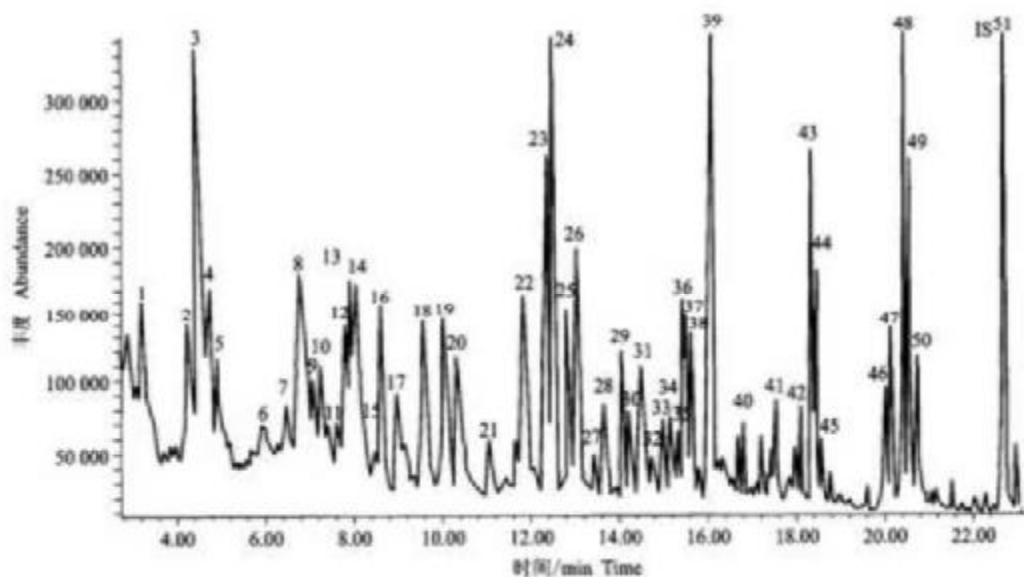


图 1 鱼油挥发性成分 DHS/GC/MS 分析的总离子流图

注: IS 为内标

Fig.1 Profile of total ionic current of crude fish oil volatiles by DHS/GC/MS

Note: IS—internal standard

表1 鱼油顶空挥发性成分

Tab. 1 Volatile compounds in the dynamic headspace of crude fish oil

峰号 No.	保留时间/min Retention time	保留指数 Retention index	化合物名称 Compound	峰面积/% Peak area
1	3.19	<1 000	二甲基二硫	1.25
2	4.23	1 000	1,3,5-辛三烯	1.39
3	4.39	1 025	己醛	6.04
4	4.69	1 040	1,2-二甲苯	1.93
5	4.86	1 048	2-戊烯醛	2.05
6	5.94	1 100	十一碳烷	1.29
7	6.47	1 120	苯乙烯	1.4
8	6.80	1 134	庚醛	4.81
9	7.09	1 145	2-己烯醛	1.05
10	7.24	1 151	未确定	1.31
11	7.64	1 167	2-甲基-1,3-丁二烯	0.8
12	7.79	1 173	未确定	2.38
13	7.92	1 178	未确定	1.08
14	8.06	1 183	己酸乙酯	4.48
15	8.49	1 200	十二碳烷	0.82
16	8.60	1 204	1,3,5-庚三烯	2.28
17	8.98	1 219	未确定	1.34
18	9.57	1 242	辛醛	2.76
19	10.04	1 260	2-甲基-2-环戊烯-1-酮	2.47
20	10.32	1 271	2,4-己二烯醛	2.69
21	11.08	1 300	十三碳烷	0.54
22	11.85	1 330	苯甲醛	2.64
23	12.37	1 351	2-壬酮	3.51
24	12.50	1 356	2,4-庚二烯醛	3.65
25	12.82	1 369	2-辛烯醛	1.69
26	13.03	1 377	2,4-庚二烯醛	2.93
27	13.43	1 393	辛酸乙酯	0.53
28	13.62	1 400	十四碳烷	0.89
29	14.07	1 417	1-氨基吡咯烷	1.02
30	14.22	1 423	3,5-辛二烯-2-酮	0.75
31	14.49	1 435	2,2-二甲基-3-己烯	1.71
32	14.54	1 437	5-甲基-2(H)-呋喃酮	0.81
33	15.01	1 455	2-癸酮	0.82
34	15.17	1 462	3,5-庚二烯-2-酮	0.55
35	15.35	1 469	苯乙酮	0.53
36	15.44	1 473	己酸	1.67
37	15.62	1 480	2-壬烯醛	1.42
38	15.65	1 481	2,4-辛二烯醛	1.23
39	16.13	1 500	十五碳烷	5.03
40	16.83	1 532	苯甲酸	0.78
41	17.56	1 565	2-十一酮	0.73
42	18.12	1 590	2-癸烯醛	0.74
43	18.40	1 603	癸酸乙酯	2.79
44	18.49	1 607	苯乙醇	1.56
45	18.60	1 611	苯酚	0.51
46	20.02	1 674	4-甲基苯酚	0.95
47	20.15	1 680	2,6,10,14-四甲基十五碳烷	1.71
48	20.46	1 693	未确定	3.83
49	20.61	1 700	十七碳烷	3.13
50	20.77	1 707	(E,E)-2,4-癸二烯醛	1.09
51	22.76	1 800	十八碳烷	6.63

表2 超临界处理后鱼油挥发性成分 GC 分析的总峰面积

Tab.2 Total peak area of headspace volatiles of fractions obtained by SCE-CO₂

样品 Sample	鱼油编号 Fish oil No.			
	A	B	C	D
总峰面积 Total peak area	621 312	178 953	383 577	4 519 494
内标峰面积 Peak area of IS	22 099	20 621	34 911	27 621
挥发性成分总峰面积 Total peak area of volatiles	599 213	158 332	348 666	4 491 873
调整挥发性成分总峰面积 [*] Adjusted total peak area of volatiles [*]	599 213	169 680	220 708	3 593 856

注: * 挥发性成分总峰面积 ÷ 内标峰面积 × 原油(A)中内标峰面积,通过调整来消除因上样引起的误差。

Note: * Total peak area of volatiles ÷ peak area of IS in deodorized fish oil × peak area of IS in crude fish oil. It was used to eliminate the error caused by ejection technique.

表3 感官检验分数与统计结果

Tab.3 Ranks and the sum of ranks of fish oils by sensory evaluation

测定排序 Evaluation No.	鱼油编号 Fish oil No.				
	A	B	C	D	E
1	2	3	1	5	4
2	1	2	4	5	3
3	3	1	2	5	4
4	3	1	2	5	4
5	1	2	3	5	4
6	4	1	2	5	3
7	4	1	2	5	3
8	3	2	1	5	4
9	4	2	1	5	3
10	4	2	1	5	3
R _i	29	17	19	50	35

注: 气味由小到大得分依次为 1, 2, 3, 4, 5。

Note: The flavour from weakness to strongness follows the order of 1, 2, 3, 4 and 5.

根据计算 GC 峰面积可知, 经过黏土吸附处理后, 鱼油中总挥发性成分的 84.1% (R_i) 被黏土吸附而除去。对脱臭前后的鱼油经过感官检验分析表明(表 3), 未经处理的鱼油有强烈的刺激性气味, 而经过处理后鱼油只有很轻的类似青草气味, 这和新鲜的鱼油的味道比较接近。表 4 结果表明, 经过黏土吸附处理后的鱼油色素含量大大降低, 从感官上看, 处理前的鱼油是红棕色, 而处理后鱼油是浅黄色。结果表明黏土吸附法对鱼油脱色、脱臭都具有较明显的效果。

表4 黏土吸附法脱臭前后鱼油吸光值及脱色率

Tab.4 A₅₁₀ and R_d value of crude and deodorized fish oil by clay adsorption technique

样品 Sample	A ₅₁₀	R _d /%
原油 Crude oil	0.326	
脱臭鱼油 Deodorized oil	0.045	86.2

3 讨论

捕集时间、温度、载气流速等因素对动态顶空技术捕集效果有很大影响。根据鱼油高温易氧化分解的特点以及 Lin^[15]等人的文献报道, 本研究选择 65℃ 为 DHS 温度, 并分别在载气流速为 50 mL/min、100 mL/min、150 mL/min 下捕集 1 h、2 h、3 h。GC 分析结果表明, 当载气流速过小, 捕集时间短时, 因捕集到的挥发性成分浓度过低, GC 分析时信号小 (GC 谱图没给出), 出峰少; 当载气流速过大, 捕集时间长时, 收集到的挥发性成分浓度过高, 信号峰太多, 且多为平顶峰, 这两种情况都不利于对 GC 结果进行分析。为此, 根据预实验结果, 将在 65℃、100 mL/min 载气流速下捕集 2 h 作为本研究 DHS 条件。

通常认为碳数为 3—10 的小分子的醛、酮类分子具有较好的挥发性, 是鱼油储藏过程中因氧化产生, 并形成鱼油异味成分的关键性物质^[6, 16]。本研究证实这些小分子的醛、酮类物质和鱼油顶空挥发性成分中的其他组分一起形成了氧化鱼油特有的臭味。

鱼油中的挥发性醛、酮类物质是 PUFA 氧化产物, 其含量随着鱼油储藏时间的延长和氧化程度的增加而增加^[7, 16], 这些成分能够通过超临界萃取优先除去。本研究通过 GC 和感官检验分析超临界萃取处理的鱼油, 结果表明, 挥发性成分含量越高的鱼油, 异味强度也越大。此外, 鱼油 A 和 B、鱼油 C 和 D 组间的 POV 差别不大, 但其挥发性成分含量以及气味特征有明显差异。一些研究者用 GC 分析鱼油顶空气体中的一种或几种气味最强的挥发性成分来评价鱼油的氧化过程^[16, 17]和脱臭效率^[18], 但是确定何种成分对气味成分最强, 往往比较困难, 并且食品的某种感官特性是整个食品基质形成的^[19]。因此 GC 分析挥发性成分含量表示鱼油氧化程度和气味特征, 有一定的合理性。

一些吸附剂能优先吸附挥发性醛、酮类物质, 以及酸、醇类物质而用于鱼油的脱臭处理。本研究初步探讨凹凸棒黏土在鱼油脱臭、脱色方面的应用, 与传统的脱臭处理用的活性炭、硅藻土、白土等吸附剂相比, 凹凸棒黏土具有脱色、脱

臭效果好、成本低、且操作简单等特点。凹凸棒黏土是中国江苏、安徽等地特有的黏土,有较大储量,且安全无毒,可应用于食品业和医药业,用其处理鱼油不会引起污染。虽然将凹凸棒黏土用于鱼油的脱臭还有些问题需要解决,但本研究中其表现出的对鱼油良好的脱臭、脱色效果,因而具有较好工业应用前景。

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Analysis and removing of fish oil volatile-flavors

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Abstract: Fish oil has attracted much public attention in recent years because of its abundance of polyunsaturated fatty acids (PUFAs) with important bioactivities such as antiobesity activity, antiseizure and antiarrhythmic effects, and prevention of ventricular fibrillation. With the increasing awareness of health protection and further understanding of health benefits of fish oil on reducing cardiovascular risks and other diseases, there would be an increasing demand for fish oil products worldwide. But fish oil was highly susceptible to deterioration by oxidation processes because of its high level of PUFAs. The volatile oxidation breakdown products, such as aldehydes and ketones with low molecular weight, make great change to fish oil's sensory quality or even lead to potential harm to human body. It is one of the critical factors that limit further development of fish oil industry. So the development of processing to remove harmful components and undesirable odorous volatiles from crude oil becomes necessary for production of acceptable food-grade fish oil.

A number of methods were conventionally adopted or recently developed to evaluate the oxidative stability and flavor changes of lipids, but these methods had their disadvantages as well as advantages. For example, POV was applied to indicate the oxidative quality of vegetable and animal oils, but such a standard would not be applicable to fish oil because of its high content of unstable PUFAs. Moreover, there was a lack of information on the oxidation products relevant to flavor and oxidative stability. So evaluation of fish oil quality needs practical methods, especially those benefiting quality control in industrial production. Therefore the objective of this study was to analyze the headspace volatiles of fish oil, with the hope to have a good understanding of the formation of fish oil off-flavors and their relationship to fish oil oxidative products. A simple adsorption technique was also studied to remove fish oil off-flavors and produce fish oil for food grade.

A modified dynamic headspace method was applied for trapping and concentrating off-flavors in fish oil. Sampling temperature, sampling time and flow rate of carrier gas was 65 °C, 2 h, and 100 mL/min, respectively. Collected volatiles were separated by GC and identified by GC-MS. Temperature for GC analysis was programmed at initial temperature of 40 °C for 2 min, then to 180 °C at 5 °C/min, and held at 180 °C for 10 min. Parameters for MS detector were as follows: ion source temperature, 250 °C; ionization voltage, 70 eV; GC-MSD direct interface temperature, 200 °C. Forty-five compounds of fish oil headspace volatiles were identified, among which 14 compounds were saturated and unsaturated aldehydes, and 8 saturated and unsaturated ketones, which were most important odor compounds of fish oil during storage.

GC analysis was used to appraise fish oil sensory and oxidative change. Results showed that total peak area of headspace volatiles can indicate the flavor intensity and oxidation degree, which is supported by sensory evaluation. It is worth while to further study the application of GC analysis in quality evaluation of fish oil.

Attapulgitic clay, collected from mining area in Yongwang Mountain of Jiangsu Province, was tried in this study for fish oil deodorization. Activated clay was mixed with fish oil in weight proportion of 1:20, heated in boiling water and blended for 10 min. The mixture was cooled down and separated by centrifuge at 3 000 r/min. The supernatant was deodorized and decolorized fish oil. It showed that 84.1% of the total volatiles and 86.2% pigments of crude fish oil were removed. Attapulgitic clay adsorption technique is a simple and effective method for fish oil deodorizing and decolorizing, and is a promising method for large-scale production of fish oil and other lipid products.

Key words: fish oil; volatile flavors; sensory evaluation; deodorization; clay adsorption