RESEARCH ON IDENTIFYING PAHS IN VEGETABLE OIL PRODUCED FROM SOME PLANTS IN OPEN ENVIRONMENT

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Summary. Some of vegetable oils were used to identify the Polycyclic Aromatic Hydrocarbons (PAHs). Contamination of PAHs in vegetable oils was due to technological processes or environmental sources. PAHs were detected in 3 vegetable oils, including raw coconut oil from Philippine, soya bean and olive-rapeseed oil from Vietnam. Concentration of PAHs was found in coconut oil, the maximum concentration was 22.048 μg/kg. PAHs' concentration in soya bean, olive-raspeseed oils was low, it was reduced during refining. Benzo(a)pyrene wasn't detected in all samples, the maximum concentration of other PAHs was 10.640 μg/kg. PAHs concentration in raw vegetable oils were higher than refined oils.

1. Introduction

The pollutants were transported into land and water through the air, and accumulated in plants. This was one of the reason that causes PAHs in some seeds and fruits - material for oil production. With the target of controlling food quality and explaining the appearance of PAHs in cooking oil, we have carried out a research to set up a method to identify PAHs quantity in some kinds of cooking oil, olive oil, soybean oil and coconut oil. The result shows that there were PAHs in some of them..

The relationship between planting environment and producing process with the appearance of PAHs in vegetable oil has been being explained.

2. Experimental

2.1. Materials, chemicals and instruments

The raw coconut oil, soya bean and olive-rapeseed oil was investigated. Standard solution of PAHs in cyclohexane was used. Solution standard of naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, fluorene, pyrene, benzo(a)anthracene, chrysene, benzo(bjka)fluoranthenes, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(123-cd)pyrene, dibenz(ah)anthracene, benzo(ghi)perylene containing 500 pg/àl. Phenanthrene–d10, perylene-d12 and benzo(e)pyrene-d12 was supplied by Supelco, containing 1 ng/àl each was prepared in cyclohexane. Internal standard solution of naphthalene-d8, acenaphthylene-d10, phenanthrene–d10, perylene-d12 and benzo(e)pyrene-d12, 0,1 àg/ml each was prepared in cyclohexane. All these

solutoins were stored at 5°C. The solvents for extract and clean up were cyclohexane, dimethylformamide, ethylacetate, n-hexane.

Silica gel, 70-230 mesh for column chromatography was removed impurities possibly present by heating overnight at 550°C. Before use, standardize by adding 10 % (v/v) of distilled water, after agitation allow to stand for 2 h.

Bio-Bead S-X3, 200-400 mesh was also used in cleaning proceduce.

The Hewllet-Parkard 5973 mass spectrometer interfaced with Agilent 6890 GC was used. HP-5, 30 m x 0,25mm fused silica column was used with helium as the carrier gas at 1.2ml/min, pressure 6.31psi. Splitless injection 250°C was employed. The oven temperature was programmed 70°C, 1 min, 5°C/min, 280°C, and keeping at 280°C until all components were eluted.

The mass spectrometer parameters used were as follows: electron impact energy 70 eV, source temperature 250°C, transfer line 280°C.

In the SIM mode, the ions monitored were m/e 136, 68 (naphthalene-d8), m/e 128, 129 (naphthalene), m/e 162, 164 (acenaphthylene-d10), m/e 151, 152 (acenaphthylene), m/e 153, 154 (acenaphthene), m/e 164, 166 (fluorene), m/e 186,188 (phenanthrene-d10), m/e 176,178 (phenanthrene, anthracene), m/e 200, 202 (fluoranthene, pyrene), m/e 226,228 (benzo(a)anthracene, chrysene), m/e 250, 252 (benzo(bjka)fluoranthenes, benzo(e)pyrene, benzo(a)pyrene, perylene), m/e 260, 264 (perylene-d12, benzo(e)pyrene-d12), m/e 276, 277 (indeno(123-cd)pyrene, benzo(ghi)perylene), m/e 278, 279 (dibenz(ah)anthracene), Figure 1.

2.2. Clean up procedure

25 gram of vegetable oil in 250 ml separating funnel, add 2 ml internal standard solution was solved in 100ml cyclohaxane. The mixture was extracted by dimethylformamide : H_2O (9:2, v/v) three times with portions of 50 ml, 25 ml, 25 ml. The extract of sample was diluted with 100 ml of 1 % Na_2SO_4 solution. After adding 50 ml cyclohexane, the miture was extracted again with 25 ml of dimethylformamide : water (9:2; v/v). The extract was washed with distilled water and then dried with anhydrous sodium sulfate. The extract was concentrated on a rotary evaporator to 1 ml at 40°C.

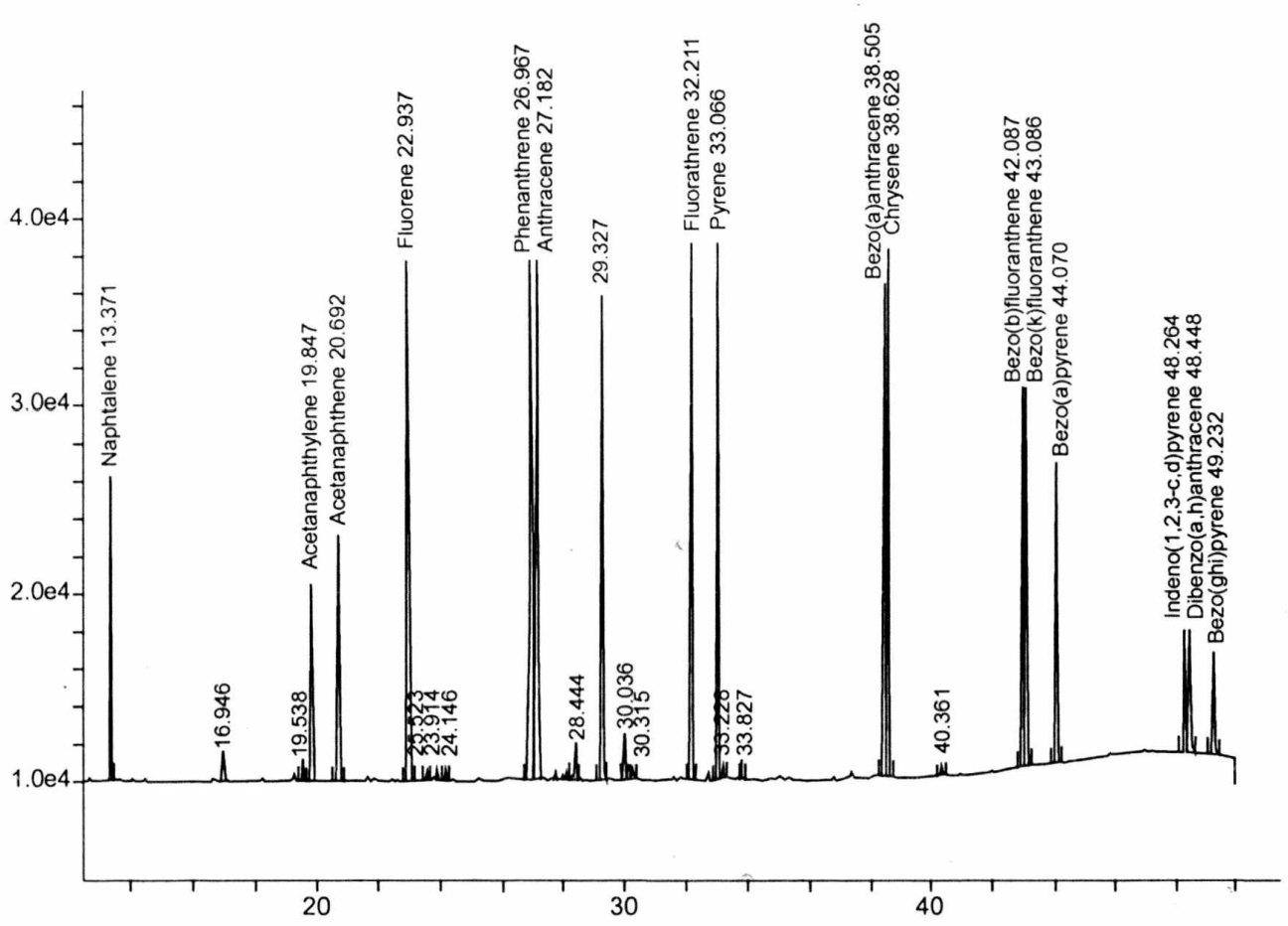
Chromatography column (500 mm x 0.6 mm i.d.) was packed a 5 gram of: silica gel (content 10 % of water). Load prepared extract on the column. 90 ml of cyclohexane was used as a solvent to clean up and elute PAHs from column. Discard 10 ml of first fraction (F1), and concentrate 80 ml of second fration to 2 ml (F2).

Bio-Bead S-X3 column (300 mm x 20 mm) was used to clean up again the F2 solution. The mixture of cyclohexane : ethylacetate (1:2, v/v) was used as a solvent to clean up and elute PAHs from column. Flow rate of the mixture of cyclohexane : ethylacetate (1:2, v/v) was 5 ml/min. Adding 3 ml of ethylacetate to F2 solution, load this solution on the column. Collect the 50 – 120ml fraction and concentrate on a

rotary evaporator to 5 ml. Tranfer a residue into a sample vial, dryness under nitrogen stream, and dissolve in 100 àl toluene. This solution was used to identify PAHs on GC-MS.

3. Results and discussion

A sample of raw coconut oil was produced in Philippine was analyzed to identify PAHs. The sample has not been refined, still has distinctive smell and colour of the input material.



Fi gure 1. Mass chromatograms by SIM mode of PAHs.

According to the table 1, the PAHs concentration in the sample was rather high, the concentration range was from not found to 22.048 μg/kg. Among group of PAHs, great attention has been paid to group of substances with high cancer potentiality as benzo(a)pyrene, benzo(a)anthracene, dibenz(a)anthracene. The concentration range was from not found to 9.478 μg/kg. Compared to the benzo(a)pyrene - permitted standard in Italian food, benzo(a)pyrene in raw coconut oil was 3 times higher. Moreover, the appearance of high PAHs concentration means the high risk of being cancer.

Table 1. Concentration of PAHs in vegetable oils (μg/kg)

Compounds	Raw coconut oil	Soya bean oil 1	Soya bean oil 2	Olive-rapeseed oil 1	Olive-rapeseed oil 2
Naphthalene	22.048	7.68	6.68	10.610	6.451
Acenaphthylene	4.041	-	-	0.122	0.401
Acenaphthene	-	-	-	0.101	-
Fluoranthene	9.478	1.25	0.89	0.027	-
Phenanthrene	2.460	0.29	-	-	-
Anthracene	2.460	0.35	-	-	0.012
Fluorene	2.372	1.58	2.76	-	-
Pyrene	0.439	-	-	-	-
Benzo(a)anthracene	0.615	3.34	-	-	-
Chrysene	-	-	-	-	0.123
Benzo(b)fluoranthene	0.176	-	-	-	-
Benzo(k)fluoranthene	0.088	-	-	-	-
Benzo(a)pyrene	-	1.11	5.67	0.058	-
Dibenz(a,h)anthracene	-	-	-	-	-
Benzo(ghi)perylene	-	4.89	2.03	0.079	0.237

Note : (-) not detected

The PAHs content in Philippine raw coconut oil was compared higher than that of German. However, benzo(a)pyrene in Germany raw coconut oil was very high, from 0.5 to 2.3 μg/kg. The analyzing result was completely relevant to the conclusion that raw coconut oil contains highest PAHs content.

Two samples of refined soybean oil have been analyzed to find out PAHs. The PAHs concentration range was from not found to 7.68 μg/kg for each. Naphthalene was identified highest. Among 15 PAH substances we analyzed benzo(a)anthracene, benzo(a)pyrene, dibenz(a)anthracene, which were most poisonous, were not found.

Compared to the benzo(a)pyrene - permitted standard in Italian food, the benzo(a)pyrene concentration in the samples was under the standard. However, through first experiments, these two soybean oil samples contain some PAHs causing cancer as indeno (123-cd)pyrene with the concentration of 1.11 μg/kg in sample 1, and 5.57 μg/kg in sample 2.

The existence of PAHs in soybean oil samples was due to the accumulation in grains and the formation in the processing. Soybean plant was a herbaceous plant with short height, large and rough leaves easily absorbing PAHs deposited by polluted air. Besides, in processing, soybean with high protein is possibly converted

into PAHs. However, most PAHs were eliminated during the raw oil refining process.

We also carry out analyzing PAHs in two samples of olive oil. This was the material with high oil and low protein content. Though material was imported from temperate climate countries, the producing process was highly economically effective. The PAHs content in the samples was low, ranging from not found to 10.61 μg/kg. The samples contain some cancer-potential substances, as benzo(b)fluoranthene 0.123 μg/kg in sample 2 and indeno(123-cd)pyrene 0.058 μg/kg in sample 1. Benzo(a)pyrene was not found in both samples.

Our sample-analyzing results were showed that the PAHs concentration in olive oil were lowest. The reason can be that colza and olive - the material supply - grow and develop in an open environment with low PAHs concentration and that the components in these grains were less likely to be converted into PAHs.

4. Conclusion

Through research on identifying PAHs in vegetable oil, we have drawn out the following conclusions: PAHs content in Philippine raw coconut oil was high, from not found to 22.048 μg/kg. Benzo(a)pyrene concentration in the sample is 0.088 μg/kg, the total PAHs up to 45.415 μg/kg. PAHs content in the refined soybean sample was low, from not found to 7.68 μg/kg. Benzo(a)pyrene was not found in the two samples. Soybean oil does not contain PAHs as they are eliminated during refining process. PAHs content in olive and colza oil samples were quite low, the concentration range from not found to 10.61 μg/kg. Compared to the standard, the benzo(a)pyrene content in olive and cozal oil was permitted. PAHs content in raw oil was much more higher than in refined oil. Colour-washing method eliminates most poisonous substances that cause cancers. However, PAHs have been found through four refined oil samples. Producers should have methods to reject them completely from processed oil.

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NGHIÊN CỨU XÁC ĐỊNH CÁC HIDROCACBON THƠM ĐA VÒNG TRONG DẦU ĂN SẢN XUẤT TỪ QUẢ, HẠT CỦA MỘT SỐ LOÀI THỰC VẬT GIEO TRỒNG TRONG MÔI TRƯỜNG MỠ

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Về bản chất tự nhiên, các loại dầu thực vật đều không chứa các hidrocacbon thơm đa vòng (PAHs), chúng bị nhiễm bẩn các chất này trong các quá trình xử lý kỹ thuật hay từ môi trường. Chúng tôi đã tiến hành xác định PAHs trong ba loại dầu thực vật : dầu dừa của Philippin (thô), dầu nành và dầu oliu-hạt cải (đã qua tinh luyện) của Việt nam. Đã phát hiện ra hàm lượng cao của PAHs trong dầu dừa với giá trị cực đại là 22,048 μg/kg. Trong khi đó, hàm lượng của PAHs trong dầu nành và dầu oliu- hạt cải là thấp và hàm lượng này giảm khi được tinh chế. Benzo(a)–pyrene không tìm thấy trong cả ba loại dầu. Tóm lại, PAHs được tìm thấy trong cả ba loại dầu thực vật và hàm lượng của nó trong dầu thô cao hơn trong dâù đã được tinh chế.