Journal of Food Protection, Vol. 64, No. 2, 2001, Pages 240–245
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Mutagenicity and Identification of Mutagenic Compounds of Fumes Obtained from Heating Peanut Oil

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MS 00-141: Received 2 May 2000/24 July 2000

ABSTRACT

Since the fume of cooking oil has been reported to increase the risk of lung cancer, the objectives of this study were to evaluate the mutagenicity and to find the mutagens in the fumes of peanut oil heated to the smoke point. Peanut oil prepared from roasted peanut kernel showed a lower smoke point, less unsaturated fatty acids, more fume formation, and stronger mutagenicity than that from unroasted kernel. Further investigation of mutagenic compounds was performed by the Ames test and gas chromatography/mass spectrometry analysis. Among the 12 compounds identified from the neutral fraction of methanol extract, four compounds at a dose of 10 μg per plate were mutagenic to *Salmonella* Typhimurium TA98 and TA100 in the order of *trans-trans*-2,4-decadienal > *trans-trans*-2,4-nonadienal > *trans*-2-decenal > *trans*-2-undecenal. Results report the enal compounds formed as the mutagens in the fumes of peanut oil and indicate that inhaling cooking fumes might cause carcinogenic risk.

It has been reported that there is an increased risk of respiratory tract cancer among cooks and bakers *(5, 14)*. Since the cooking fume is believed to conduct this risk, many studies have focused on evaluating the mutagenicity and finding the mutagenic components in cooking fumes. The oil fumes from heated edible oils, including rapeseed oil, soybean oil, peanut oil, and lard, to a high temperature were noted to possess mutagenicity and genetic toxicity *(6, 18)*. Polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) are frequently reported as the most potent mutagens or carcinogens in the fumes of heated cooking oil *(6, 7)*. However, the oil fumes containing PAHs and/or HCAs in these studies were obtained at a temperature above 250°C *(13)*, which is not reasonable for daily home cooking. Because more fumes will be obviously produced while the cooking oil is heated to its smoke point and the actual home cooking is usually performed at a relatively lower temperature, it is interesting to investigate the mutagenicity and mutagens in oil fumes obtained at an appropriate cooking condition.

Oil prepared from roasted peanut (ROPO) contains a tasteful aroma and is popular in the Orient. Unfortunately, this oil is regarded as a "fume maker" because it has a smoke point around 100°C, which is 70°C lower than that of the soybean oil. Since the harms of the fumes from peanut oil are still unclear, peanut oil with a low smoke point temperature is a suitable material for investigating the mutagenicity and mutagens in the oil fumes. The objectives of this study were to evaluate the mutagenicity of fumes from peanut oil heated to a smoke point temperature and to identify the major mutagenic compounds in the fumes. This work may reveal new fume mutagens different to PAHs and HCAs and provide more information to understand the hazard to cooks exposed to fumes from edible oils.

MATERIALS AND METHODS

Materials. Peanut kernels (Tainan no. 11, a Spanish type) were purchased from the Sheng-gan Peanut Oil GMP Company (Yun-Lin County, Taiwan). NADP, methanol, *n*-hexane, acetone, ethyl acetate, dimethyl sulfoxide, and fatty acid methyl esters kit of standard were purchased from the Sigma Chemical Co. (St. Louis, Mo.). S9 was purchased from the Organon Teknika Co. (Durham, Switzerland). NADH was purchased from the E. Merck Co. (Darmstadt, Germany). 1-Pentanol, 2-heptenal, *n*-nonanal, *trans*-2-octenal, 1-heptanol, *n*-pentadecane, 1-octanol, *trans-trans*-2,4-nonanal, and *n*-heptadecane were purchased from the T.C.I. Co. (Tokyo, Japan).

Preparation of peanut oil. Peanut kernels were roasted at around 200°C for about 45 min and then pressed immediately to obtain the oil. The oil was kept at room temperature for 7 to 10 days; then its precipitate was removed with filtration. Oil prepared from unroasted peanut kernels (UROPO) was also examined. The UROPO was prepared by pressing freeze-dried kernels, and the oil was then centrifuged at 10,000 rpm ($1.69 \times 10^4 \times g$) for 30 min at 20°C. The supernatant oil was kept in brown glass vials at −25°C before analysis.

Physical and chemical analyses of peanut oil. The acid value, peroxide value, smoke point, and color of peanut oil were tested according to the American Oil Chemists Society methods (Cd 3a–63, Cd 8–5–53, Cc 9a–48, and Cd 13b–45, respectively) *(1)*. To determine the fatty acid content of the peanut oil, methyl esters of fatty acids were prepared according to the procedure of Bannon et al. *(4)* and analyzed by gas chromatography (GC). The GC system was a Hewlett Packard (HP) 5890 Series II CG equipped with a flame ionization detector. The stainless steel column (2 m × 2 mm inside diameter) was packed with 10% SP-2330 on 100/120 Chromosorb WAW (Supelco, Bellefonte, Pa.). The injection port temperature was 250°C; the detector was set at

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TABLE 1. *The physical and chemical properties of peanut oil prepared from ROPO and UROPO*

	POV[a] (meq/kg)	AV (mg/g)	Smoke point (°C)	Lovibond			Fatty acid composition (%)		
				R	Y	B	18:1	18:2	18:3
UROPO	0.26 A[b]	0.12 A	134.5 A	0.10 A	3.25 A	0.00 A	34.76 A	36.24 A	2.25 A
ROPO	0.67 B	0.42 B	97.5 B	5.05 B	20.71 B	0.05 B	32.89 B	34.87 B	1.52 B

[a] POV, peroxide value of oil determined by iodine titration; AV, acid value of oil determined by potassium hydroxide titration; Lovibond, the color of oil; R, red unit; Y, yellow unit; B, blue unit.
[b] Means in a column with the same letters are not significantly different ($P > 0.05$).

265°C. The initial column temperature was held at 50°C for 2 min, programmed to increase to 240°C at 2°C/min, and then held at that temperature for 30 min. The sample injection volume was 0.5 µl. The carrier gas was helium at 15 ml/min. Data were collected using a HP G1030A VL 24/66 ChemStation. The content of fatty acids was determined via a standard mixture of methyl esters. The relative content of each fatty acid was expressed as a percentage of the total peak areas. All determinations were performed in triplicate, and mean values were reported.

Collection and extraction of fumes of heating peanut oil. Peanut oil (90 g) was poured into an iron pot (diameter, 45 cm) and heated using an electric heater. The heating temperature was controlled at the smoke point ± 10°C (Table 1). The fumes from the heating oil were trapped with filter paper (capillary 3 µm, Unique Pretty Industries, Gary, Ind.) connected to a vacuum pump (MSA Escort ELF pump, Mine Safety Appliance Co., Pittsburgh, Pa.) placed 50 cm above the oil surface at a flow rate of 2 liters/min. Each filter paper was replaced with a new one after 30 s. Fifteen pieces of filter paper were used for each oil sample. The experiments were independently repeated three times. The collected condensates were separately extracted with 200 ml each of methanol, acetone, ethyl acetate, or *n*-hexane using a Soxhlet extractor for 24 h. The extracts were then dried by rotary evaporation at 40°C, and the yields of that were calculated. The extracts were sealed in brown glass vials and stored at −25°C before analysis.

Mutagenicity assay. The mutagenic effects of the fume extracts and the identified compounds were assayed according to the Ames test using *Salmonella* Typhimurium strains TA98 and TA100 *(15)*. The His^{+} revertants were counted after incubation at 37°C for 48 h. Each sample was assayed in triplicate plates per run, and data presented are mean ± SD of at least two experiments. In this mutagenicity test, the result was recognized as positive when the number exceeded twice the number of spontaneous revertants *(2)*.

Fractionation and identification of mutagenic compounds. Methanolic extracts from fumes of heating peanut oil were dissolved in 2 ml of *n*-hexane and then fractionated into acidic, neutral, and basic fractions according to the method of Ron and Louisa *(19)*. To the mixture, 0.1 N HCl was added to adjust the pH to 2.0, and then the aqueous and organic layers were separated. The aqueous layer was referred to as a basic fraction. The organic layer was then adjusted to pH 12 with 0.1 N NaOH, and the layers were separated. The second aqueous layer was referred to as an acidic fraction and the organic layer was referred to as a neutral fraction. These three fractions were then dried by rotary evaporation at 40°C, and the yield and mutagenicity were determined. Since the neutral fraction showed the stronger mutagenicity, the mutagenic compounds in this fraction were identified.

The neutral fraction was analyzed using the GC and GC/mass spectrometry (MS) system, consisting of a HP 5890 Series II GC and a HP 5972 mass selective detector (Hewlett Packard Co., Palo Alto, Calif.). The GC analyses were performed using a flame-ionization detector at 265°C and an injector temperature at 250°C. Separations were performed on a HP-Innowax fused-silica capillary column, 30 m (length) × 0.25 mm (inside diameter). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The oven temperature was programmed to increase from 50 to 240°C at a rate of 2°C/min, with an initial hold time of 2 min and a final hold time of 30 min. Mass selective detector conditions were as follows: capillary direct interface temperature, 200°C; ion source temperature, 200°C; ionization voltage, 70 eV; mass range, 30 to 500 amu (atomic mass unit); electron multiplier voltage, 2,000 V; and scan rate, 1.5 scans per s.

The GC and GC/MS methods were used as qualitative methods for the compounds in the neutral fraction at the same time. The retention index was calculated by applying the *n*-paraffins (C5 ~ C25) for reference retention times, and then the compounds were identified. The compounds in the neutral fraction of methanolic extracts of the fumes were identified using the retention index of the GC and based on computer matching of library spectra (the computerized databank adopted was NBS 75 K. L. Wiley 27K). The compounds of the neutral fraction were also confirmed by injecting the authentic compounds into GC/MS. A criterion of quality index above 95% (ions matching relative abundances of those in the authentic standard ±5%) was used for confirmation. To quantify each identified compound in the neutral fraction,

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TABLE 2. *Yield of extracts from fumes of heating peanut oil with various solvents*

	Yield (mg)[a]			
	Methanol	Acetone	Ethyl acetate	*n*-Hexane
UROPO	A[b] 301 ± 24.7 A[c]	A 280 ± 18.5 A	B 232 ± 8.2 A	B 239 ± 6.7 A
ROPO	A 970 ± 80.3 B	A 962 ± 82.5 B	B 847 ± 30.9 B	B 792 ± 25.4 B

[a] Based on 90 g of peanut oil and collected with 15 filter papers.
[b] Values within a row with the same letters are not significantly different ($P > 0.05$).
[c] Values within a column with the same letters are not significantly different ($P > 0.05$).

TABLE 3. *Mutagenicity of methanolic extracts from fumes of heating ROPO toward* Salmonella *Typhimurium TA98 and TA100 with or without a S9 mix*

Amounts (μg per plate)	His⁺ revertants per plate			
	Without S9		With S9	
	TA98	TA100	TA98	TA100
Spontaneous revertants	33 ± 5 A[a]	142 ± 11 A	33 ± 5 A	142 ± 11 A
1	35 ± 6 A	148 ± 11 A	38 ± 10 A	161 ± 13 A
5	37 ± 10 A	157 ± 16 A	49 ± 7 A	166 ± 9 B
10	42 ± 8 A	149 ± 14 A	48 ± 6 A	483 ± 13 C
50	637 ± 35 B	1,152 ± 41 B	592 ± 41 B	1,043 ± 54 D

[a] Values within a column with the same letters are not significantly different ($P > 0.05$).

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naphthalene was used as the internal standard. The quantitative result of each compound was calculated relative to that of the internal standard.

Statistical analysis. Statistical analysis were performed using SAS software *(20)*. Analyses of variance were performed by analysis of variance procedures. Significant differences ($P < 0.05$) between means were determined by Duncan's multiple range tests. Data shown are representative of three independent experiments.

RESULTS AND DISCUSSION

Physical and chemical properties of peanut oil. Table 1 shows the physical and chemical properties of ROPO and UROPO. The Lovibond color (R.Y.B. index) of ROPO was significantly ($P < 0.05$) higher that of UROPO. This means that the ROPO appeared to have a much darker color. The ROPO also had higher ($P < 0.05$) acid value and peroxide value than did UROPO. In addition, the amounts of oleic acid, linoleic acid, and linolenic acid in ROPO are lower than those in UROPO. It was believed that roasting at a high temperature contributed to the formation of partial free fatty acids and peroxides that might physically and chemically decrease the oil stability. Results were also in an agreement with the study of Shyu and Yen *(21)*. They concluded that the content of fatty acid in peanut kernels decreased with longer roasting time. Furthermore, the smoke point of ROPO (97.5°C) was 37°C lower than that of UROPO (134.5°C). The lower smoke point of ROPO could be more easily reached by regular cooking and could provide a risk for more fume production. The smoke point of the peanut oil was also highly related to whether the peanut kernels were roasted or not.

TABLE 4. *Content of acidic, neutral, and basic fractions of methanolic extracts from fumes of heating ROPO*

Fractions	Content (mg)[a]
Acidic	212.92 ± 23.2 A[b]
Neutral	100.13 ± 12.5 B
Basic	507.79 ± 68.8 C

[a] Based on 0.894-g methanolic extracts from fumes of heating peanut oil.
[b] Values within a column with the same letters are not significantly different ($P > 0.05$).

Yield of fume extracts. The total amount of fume particles collected from 90 g of heated ROPO and UPORO was 1,020 and 339 mg, respectively. Thus, the roasting process for peanut kernels enhanced the formation of fumes in the peanut oil after it was heated to the smoke point. In the present study, the fumes collected on filter papers were extracted using four solvents: methanol, acetone, ethyl acetate, or *n*-hexane, respectively. The yields of various solvent extracts of fumes from heated ROPO were significantly higher ($P < 0.05$) than those from heated UROPO (Table 2). The yields of extracts of fumes also depended on the polarity of the extraction solvent; they increased with an increase of the polarity of the solvent. However, there was no significant difference ($P > 0.05$) between methanol and acetone or between ethyl acetate and *n*-hexane. Because the yield of methanolic extracts was higher than those of other solvents, methanol was used as a solvent to extract oil fumes in later experiments.

Mutagenicity of fume extracts. The toxicity experiments of ROPO and UROPO fume extracts (50 μg per plate) showed no obvious toxicity ($P > 0.05$) toward *Salmonella* Typhimurium TA98 and TA100 either with or without S9 mix (data not shown). Therefore, the fume samples are not toxic to the strains under the test concentration.

Table 3 shows the mutagenic activity of ROPO fume extracts toward *Salmonella* Typhimurium TA98 and TA100. As shown in the results, the methanolic extracts of fumes exhibited mutagenicity ($P < 0.05$) toward both *Salmonella* Typhimurium TA98 and TA100 at a concentration of 50 μg per plate either in the presence or absence of the S9 mix. However, the His⁺ revertants for the UROPO fume extracts toward TA98 and TA100 were significantly lower ($P < 0.05$) than those for ROPO fume extracts at a same concentration of 50 μg per plate with or without adding the S9 mix (data not shown). Therefore, the mutagenic activity of extracts of fume from ROPO was stronger than that of fumes from UROPO. Although Chiang et al. *(8)* had reported that peanut oil fumes obtained at 250 ± 10°C showed mutagenicity toward *Salmonella* Typhimurium TA98 in the presence of the S9 mix, we demonstrated that peanut oil fumes obtained at a smoke point (97.5°C) of ROPO also showed mutagenicity (Table 3). Since PAHs noted as the mutagens in the study by Chiang et al. *(8)*

TABLE 5. *Mutagenicity of acidic, neutral, and basic fractions of methanolic extracts from fumes of heating ROPO toward* Salmonella *Typhimurium TA98 and TA100 without the S9 mix*

Amounts (μg/plate)	His⁺ revertants per plate					
	Acidic		Neutral		Basic	
	TA98	TA100	TA98	TA100	TA98	TA100
Spontaneous revertants	34 ± 5 A[a]	154 ± 9 A	34 ± 5 A	154 ± 9 A	34 ± 5 A	154 ± 9 A
1	32 ± 7 A	148 ± 11 A	51 ± 10 B	167 ± 13 A	38 ± 9 A	150 ± 13 A
10	36 ± 4 A	150 ± 6 A	1,027 ± 56 C	1,843 ± 64 B	89 ± 21 B	476 ± 23 B
50	48 ± 7 A	175 ± 12 A	2,278 ± 41 D	2,679 ± 48 C	589 ± 26 C	954 ± 29 C

[a] Values within a column with the same letters are not significantly different ($P > 0.05$).

were unable to be formed at 97.5°C, different mutagens were believed to be produced in the present study. Dillon et al. *(9)* indicated that the products of oxidative degradation of oils, formaldehyde, and glutaraldehyde exhibited mutagenicity toward *Salmonella* Typhimurium TA100 and TA104 with or without the S9 mix. Qu et al. *(18)* also reported that fumes from heated rapeseed oil to 270°C had mutagenicity toward *Salmonella* Typhimurium TA98. They concluded that the mutagenicity of oil fumes was related to the amount of linoleic acid and linolenic acid in the oils; when the amounts of linoleic acid and linolenic acid were increased, the mutagenicity increased as well. This point of view corresponds to our results shown in Table 1 that the higher the acid value and peroxide value, the lower the unsaturated fatty acid in ROPO (due to partial oxidation).

All the solvent extracts showed mutagenicity toward TA98 and TA100 (data not shown). Because of the strongest mutagenicity the methanol extract showed, it was chosen for mutagen investigation in further experiments.

Fractionation and identification of mutagenic compounds. The methanolic extracts of fume from ROPO were fractionated into acidic, neutral, and basic fractions; then the three fractions were concentrated and quantified (Table 4). Among the three fractions, the basic fraction had the largest amount (507.79 mg), the acidic fraction was second (212.92 mg), and the neutral fraction had the smallest amount (100.13 mg). The mutagenicity of those three fractions was measured in the absence of the S9 mix, and results are presented in Table 5. The acidic fraction exhibited no mutagenicity to either TA98 or TA100, but the neutral and basic fractions had mutagenicity at a concentration of 10 μg per plate, especially the neutral fraction. Although the neutral fraction represented only 11.2% (Table 4) of the methanolic extracts, it had the strongest mutagenicity (Table 5). Therefore, we concluded that the major mutagenic components should be inside the neutral fraction. The gas chromatogram (GC) of the neutral fraction sample is shown in Figure 1.

When the neutral fraction was analyzed and identified by means of GC/MS, 12 major compounds were found and confirmed with authentic compounds (Table 6). Those 12 compounds, including three alcohols, five aldehydes, two dienals, and two alkanes, were 1-pentanol, 2-heptenal, *n*-nonanal, *trans*-2-octenal, *n*-heptanol, *n*-pentadecane, 1-octanol, *trans*-2-decenal, *n*-heptadecane, *trans*-*trans*-2,4-nonadienal, *trans*-2-undecenal, and *trans*-*trans*-2,4-decadienal. *trans*-*trans*-2,4-Decadienal had the highest amount (51.6%), and the amounts of *n*-nonanal, *trans*-undecenal, and *trans*-2-decenal were 13.2, 10.4, and 7.2%, respectively. Frankel *(10)* indicated that 2,4-decadienal was the main oxidation product of linoleic acid and that the quantity of 2,4-decadienal was obviously related to the flavor of fried

TABLE 6. *The compounds identified in neutral fraction of methanolic extracts from fumes of heating ROPO*

Peak no.	Compound[a]	RI (Innowax)[b]	Molecular weight	Content (%)
1	1-Pentanol	1,233	88	1.23
2	2-Heptenal	1,310	112	0.67
3	*n*-Nonanal	1,388	142	13.22
4	*trans*-2-Octenal	1,416	126	2.80
5	*n*-Heptanol	1,436	116	2.24
6	*n*-Pentadecane	1,485	212	2.35
7	1-Octenal	1,539	130	4.59
8	*trans*-2-Decenal	1,625	154	7.17
9	*n*-Heptadecane	1,674	240	1.90
10	*trans*-*trans*-2,4-Nonadienal	1,682	138	3.36
11	*trans*-2-Undecenal	1,734	168	10.42
12	*trans*-*trans*-2,4-Decadienal	1,805	152	51.60

[a] Identified by comparing the mass spectrum and retention time index with that of authentic compound.
[b] Calculated value using *n*-paraffins (C5 − C25) as references.

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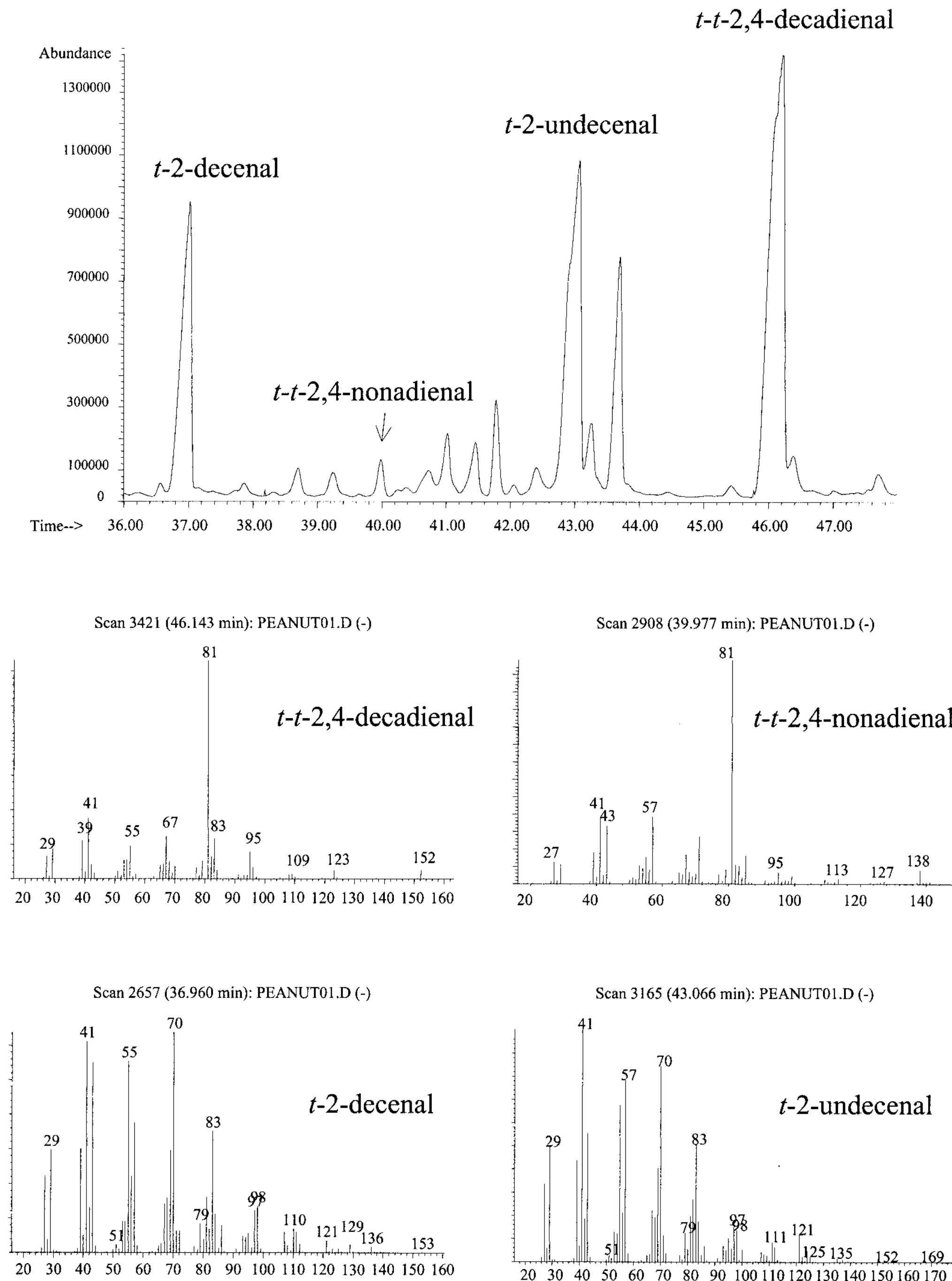

FIGURE 1. *GC of the fume sample and MS of the mutagens* trans-trans-*2,4-decadienal,* trans-trans-*2,4-nonadienal,* trans-*2-decenal, and* trans-*2-undecenal. Fume sample was prepared from the neutral fraction of methanolic extract obtained from the fumes of peanut oil.*

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foods. Pokorny *(17)* also pointed out that 2,4-decadienal, 2-undecenal, 2-decenal, and *n*-nonanal were found in vegetable oils and that 2,4-decadienal could be used as a good index to measure the oxidative stability of oils.

The mutagenicity of these compounds toward TA98 and TA100 without the S9 mix was evaluated using the authentic compounds. Among all 12 compounds, only *trans-trans*-2,4-decadienal, *trans-trans*-2,4-nonadienal, *trans*-2-decenal, and *trans*-2-undecenal exhibited marked mutagenicity toward both strains (Table 7). At a concentration of 10 μg per plate, the mutagenic activity of those four compounds was in the order of *trans-trans*-2,4-decadienal > *trans-trans*-2,4-nonadienal > *trans*-2-decenal > *trans*-2-undecenal. As shown in Table 6, these four compounds occupied 51.6, 3.4, 7.2, and 10.4% of the neutral fraction of the methanolic extracts of oil fumes, respectively.

Since it had the highest content (51.6%) and strongest mutagenicity in the neutral fraction, *trans-trans*-2,4-decadienal was regarded as an important mutagen in the fume extracts. In literature, *trans-trans*-2,4-decadienal has been reported to inhibit human erythroleukemia cell growth, to affect cell viability, to reduce the cellular glutathione level, and to be involved in the beginning of DNA fragmentation in vitro *(16)*. Therefore, the mutagenicity of cooking oil

TABLE 7. *Mutagenicity of* trans-trans-*2,4-decadienal,* trans-*2-undecenal,* trans-*2-decenal, and* trans-trans-*2,4-nonadienal toward* Salmonella *Typhimurium TA98 and TA100 in the absence of the S9 mix*

Sample (10 μg per plate)	His+ revertants per plate	
	TA98	TA100
trans-trans-2,4-Decadienal	178 ± 44 A[a]	3,853 ± 145 A
trans-trans-2,4-Nonadienal	312 ± 14 B	2,126 ± 52 B
trans-2-Decenal	256 ± 18 C	1,872 ± 31 C
trans-2-Undecenal	236 ± 19 C	1,011 ± 22 D
Spontaneous revertants	35 ± 3 D	148 ± 8 E

[a] Means in a column with the same letters are not significantly different ($P > 0.05$).

fumes and the existence of mutagens with cytotoxicity and carcinogenicity in the fumes indicated that inhaling cooking oil fumes might cause carcinogenic risk. Furthermore, *trans-trans*-2,4-decadienal is possibly formed due to the oxidative degrading of linoleic acid *(3)* and could be applied as a mutagenicity index of fumes of heated peanut oil. The addition of antioxidants might be a potential solution for reducing the content of mutagens and the mutagenicity in the oil fumes.

Most research concerning the mutagenicity of fumes of heated oil have focused on PAHs *(6, 8, 11, 12)* or HCAs *(7)*. However, only enal compounds in place of PAHs and HCAs were found as mutagens in this study. The low operation temperature is believed to contribute to the production of enals only in the fumes of peanut oil. Further investigation should be made to find the other mutagens in the acid and basic fractions and other solvent extracted. Additionally, the mechanisms of mutagen production, the relationship between temperature and the formation of enals or PAHs and HCAs, and the mutagenic enals content in cooking foods also should be studied.

CONCLUSION

Based on the results of the present study, the fumes collected from peanut oil heated to the smoke point showed mutagenicity, especially ROPO. Moreover, *trans-trans*-2,4-decadienal was found to be an important mutagenic compound in peanut oil fumes. Further research should be done on reducing the amounts of fumes and decreasing the mutagenicity of oil fumes. A study examining the application of an additional deguming process or additional antioxidants for the oil production from roasted peanut is currently under way.

ACKNOWLEDGMENT

This research work was supported in part by National Science Council, Republic of China, under grant NSC 88-2313-B005-047.

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