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# Cytotoxicity Evaluation of Oil Fumes in Rat Hepatocytes Using a Model System for Deep-fat Frying

Fumiko KIMURA[1*], Chan Mo LI[2], Yasushi ENDO[3] and Kenshiro FUJIMOTO[4]

[1] *Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-amamiyamachi, Aoba-ku, Sendai 891-8555, Japan*

[2] *Tianjin Key Laboratory of Food Nutrition and Safety, College of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin 300222, China*

[3] *School of Bionics, Tokyo University of Technology, 1404-1 Katakura, Hachioji, Tokyo 192-0982, Japan*

[4] *Department of Food and Nutrition, Koriyama Women's University, 3-25-2 Kaisei, Koriyama, Fukushima 963-8503, Japan*

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**Although the carcinogenic effect of fumes produced during deep-fat frying has been well investigated, other toxic effects of these fumes have been little studied. In this study, a mixture of moisture and volatile compounds generated from a model system for deep-fat frying was collected for cytotoxicity evaluation using a series of connected ice and dry-ice traps. A system consisting of water-spraying-and-heating oil units was used as a model system for deep-fat frying. Chemical analysis of the trapped materials indicated that the main components were carbonyl compounds, and cytotoxicity evaluation revealed their cytotoxic activity on normal rat hepatocytes. The results of high-performance liquid chromatography analysis and cytotoxicity evaluation indicated that the main cytotoxic substances may be volatile aldehydes. The collection system used in this study could be useful to elucidate the toxicity of fumes generated from actual frying procedures.**

Keywords: frying, oil, cooking fumes, oxidation, hepatocyte

## Introduction

Deep-fat frying is a popular and useful cooking method in which foodstuffs are cooked using oil as the medium of heat transfer. Although frying improves the sensory properties of foods, it may cause health problems because high temperatures catalyze undesirable chemical reactions such as oxidation and decomposition of oil (Gerhard, 2000; Nawar, 1998; Clark and Serbia, 1991). Recently, epidemiological studies have revealed a positive relationship between lung cancer and exposure to cooking fumes resulting from frying (Yu *et al.*, 2006; Lam, 2005; Metayer *et al.*, 2002). Many polycyclic aromatic hydrocarbons have been detected in cooking fumes collected from both kitchen air and cooking trials, and are considered as carcinogens generated by frying procedures (Zyu *et al.*, 2003; Chen *et al.*, 2003, 2001; Tung *et al.*, 2001; Yang *et al.*, 2000). It is also well known that there are other toxic compounds in cooking fumes, such as aliphatic aldehydes (Fullana *et al.*, 2004a, 2004b; Fujisaki *et al.*, 2002; Svendsen *et al.*, 2002; Shahidi, 2001; Zhu *et al.*, 2001). However, the toxic effects of cooking fumes, other than carcinogenicity, have received little attention in research.

An ideal method for the evaluation of the toxicity of fumes would be an inhalation toxicity study. However, this method demands a substantial number of animals and background information. Therefore, the cytotoxic effect of fumes produced during deep-fat frying was first examined using rat hepatocytes. In contrast to sampling for chemical analysis, the fumes should be collected during deep-fat frying at substantial amounts and in a nontoxic solvent for cytotoxicity evaluation. The use of cold traps would be ideal for collecting volatile compounds for the assessment of cytotoxicity. However, water vapor generated from the fried material freezes on the inner side of the traps and interferes with the collection procedure. Therefore, a sampling train consisting of gas wash bottles filled with cold ethanol was used for this study. A system consisting of water-spraying-and-heating

*To whom correspondence should be addressed.
Email: f_kimura@biochem.tohoku.ac.jp

oil units (Fullana *et al.*, 2004a, 2004b; Fujisaki *et al.*, 2002; Dana *et al.*, 2003) was used as a model system for deep-fat frying to simplify the collection of volatile compounds from the deteriorated oil without the interference of water vapor.

In this study, volatile compounds generated from this system were collected into 2 types of traps arranged in a series. We then investigated the cytotoxicity of these compounds to normal cells (primary-cultured hepatocytes).

## Materials and Methods

*Experimental apparatus and sampling* The sample oil (Nisshin Salad Oil; Nisshin OilliO Group, Ltd., Tokyo, Japan) was purchased from a local supermarket. The oil was a mixture of soybean and rapeseed (canola) oils. The experimental apparatus used in this study is shown in Fig. 1. Approximately 200 g of oil was heated in a 500-mL Erlenmeyer flask to 180 ± 5 °C with continuous stirring. After preheating the oil for half an hour, water was dropped at a rate of 0.2 g/min through a Teflon tube. Moisture and volatile compounds generated from this apparatus were collected for 3 h using a sampling train that consisted of the cooling tube, ice traps, dry-ice traps, and an air pump (20 L/min). The details of the sampling train are as follows. The cooling tube was a Liebig condenser cooled by iced water. The ice traps consisted of a round-bottom flask, a cold trap, and a gas wash bottle with 20 mL ethanol, and were cooled by iced water. The dry-ice traps consisted of 2 gas wash bottles-one containing 20 mL ethanol and the other 10 mL ethanol-and 3 empty cold traps. These were cooled using dry-ice ether.

*Sample preparations* Water and volatile compounds trapped in ice traps were collected and extracted using a mixture of pentane and diethyl ether (1:1, v/v). The solvent was evaporated for 5 min under reduced pressure in an ice bath. Ethanol was added to the residue to bring up the volume to 50 mL (ice-trap fraction). The ethanol and volatile compounds in the dry-ice traps were collected and brought up to a volume of 50 mL with ethanol (dry-ice-trap fraction). They were stored at -40°C in a glass vial until analysis.

*Chemical analyses of volatile compounds* The acid value, the carbonyl value (butanol method), and the peroxide value were determined by employing the standard methods of the Japan Oil Chemists' Society (1996). The volatile carbonyl compounds were analyzed as their corresponding 2,4-dinitrophenylhydrazine (DNPH) derivatives by high-performance liquid chromatography (HPLC). In brief, the samples were allowed to react with 1.25% DNPH, 0.1 M HCl, and 90% ethanol solution overnight. The reaction mixture was injected into an HPLC system and separated in an octadecyl silica column in the reversed-phase mode. The column was eluted using a mobile phase gradient of 60/40 acetonitrile/water for 5 min, 60/40 to 70/30 acetonitrile/water linearly for 20 min, 70/30 acetonitrile/water to 100% acetonitrile linearly for 15 min, and 100% acetonitrile for 2 min. The DNPH derivatives were identified using a multiwavelength detector (JASCO MD-910; JASCO Corp., Tokyo, Japan) and quantified at 360 nm using external standards.

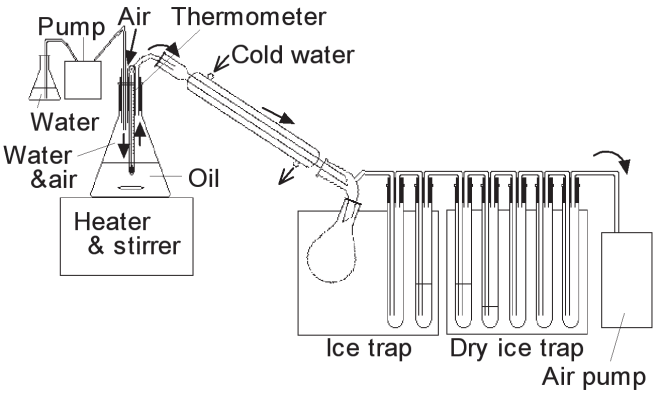


**Fig. 1.** System consisting of water-spraying-and-heating oil units and sampling train for the collection of moisture and volatile compounds.

*Animals* Male Wistar rats (234-325 g) were purchased from CLEA Japan, Inc. (Tokyo, Japan). The rats were anesthetized with pentobarbital (intraperitoneally, 50 mg/kg) prior to liver perfusion. All procedures were performed according to the Guidelines for Animal Experiments of Tohoku University (Decision of the University's President, March 24, 1988; revised on September 13, 1994).

*Hepatocyte isolation and culture* Hepatocytes were isolated by a two-step collagenase perfusion method (Seglen, 1976). The cell viability measured by the trypan blue exclusion test was in excess of 80%. The cells were seeded at a density of $7 \times 10^5$ cells/mL in 96-well plates in 0.1 mL of a medium containing William's Medium E (W1878; Sigma-Aldrich Corp., MO, USA) with 10% fetal bovine serum, 0.292 mg/mL of glutamine, 100 units/mL of penicillin, and 0.1 mg/mL of streptomycin. The cells were incubated at 37°C in a 5% $CO_2$-humidified atmosphere for 4 h to allow cell attachment. The medium was then replaced with a serum-free medium containing William's Medium E (Sigma W1878) with 0.292 mg/mL of glutamine, 100 units/mL of penicillin, and 0.1 mg/mL of streptomycin. The serum-free medium was replaced daily until the cytotoxicity assay was performed.

*Cytotoxicity of volatile compounds on hepatocytes* The cytotoxicity of the ice-trap and dry-ice-trap fractions on hepatocytes was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The treatment was initiated approximately 2 d after cell plating. Cells were exposed to 0.1 mL of serum-free medium contain-

ing each fraction in a series of concentrations for 24 h. The medium was then replaced with 0.11 mL of the MTT working solution (1 mL of 5 mg/mL MTT in phosphate-buffered saline added to 10 mL of the serum-free medium). The plates were further incubated for 3 h. After the incubation period was complete, the working solution was replaced with 0.1 mL of 0.04 M HCl in 2-propanol and refrigerated overnight. The plates were read at 570 nm. Each sample was tested in 8 individual wells. The survival ratios were expressed as the percentage of an average absorbance of the exposed cells against that of the unexposed cells. An EC (effective concentration) 50 value was determined from the survival curves using probit analysis (GraphPad Prism Ver. 4, GraphPad Software Inc., San Diego, CA, USA).

*Statistical analysis* The sampling and subsequent chemical analysis and cytotoxicity assay were performed in triplicate. The difference between the means of the 2 fractions was assessed using Student's *t* test.

**Results and Discussion**

In this study, a system consisting of water-spraying-and-heating oil units was used as a model system for deep-fat frying. Using this model, we could develop a method to collect the mixture of volatile compounds and moisture without clogging the lines of the cold ice traps with condensed water vapor. The acid value of the deteriorated oil of the water-spraying-and-heating oil units after 3 h was 0.4 ± 0.0 mg KOH/g. The acid value of the oil indicates the level of free fatty acids generated during the frying procedure due to the hydrolysis of oil. The carbonyl value, which reflects the total carbonyl contents generated by the decomposition of the oxidized oil, was 20.7 ± 2.2 mmol/kg. These values agreed with those obtained for the oil that was used for potato frying for 3 h in our previous experiment (Li *et al.*, 2005). We assumed that the system used in our study could be a model system for deep-fat frying. The mixture of volatile compounds and moisture was collected using a two-step cooling system: ice traps and dry-ice traps. Using this apparatus, water was condensed in the ice traps without freezing.

*Chemical analysis of the ice-trap fraction* The water trapped by ice traps had a strong flavor, and oil drops floated on its surface. The main components in the pentane and diethyl ether extracts were acylglycerols and aldehydes, as determined by thin layer chromatography (TLC) (data not shown). The acid, carbonyl (butanol method) and peroxide values are shown in Table 1. In general, oil is oxidized and decomposed by generating carbonyl compounds or hydrolyzed by generating free fatty acids during the frying procedure. Therefore, we determined the carbonyl and acid values, which show the total carbonyl and free fatty acid contents, respectively. The carbonyl value was 6.45 mM, and the acid value was 0.09 mg KOH/L, which is equivalent to the value of 1.6 μM in the acid group. The concentration of the carbonyl groups was 1000-fold higher than that of the acid groups in the ice-trap fraction. The peroxide value indicates the amount of produced hydroperoxides, which are primary products of lipid autoxidation and are known to be toxic; it was too low (0.3 mEq/L) to exhibit toxicity. The carbonyl compounds of the ice-trap fraction were analyzed in more detail as their corresponding DNPH derivatives by HPLC (Table 2). The main carbonyls were identified as aldehydes. C3-C9 alkanals, C3-C11 2-alkenals, and C7-C10 2,4-alkadienals were detected as aldehydes in the ice-trap fraction. In particular, 2,4-decadienal, which is known to be a toxic and carcinogenic aldehyde (Feron *et al.* 1991), was shown to be the main component in this fraction.

*Chemical analysis of the dry-ice-trap fraction* When the dry-ice-trap fraction was subjected to TLC, the main component detected was aldehyde, and trace amounts of acylglycerols were also detected (data not shown). The carbonyl value indicated that the carbonyl content of the dry-ice-trap fraction was approximately 5 times higher than that of the ice-trap fraction. The composition of carbonyl compounds in the dry-ice-trap fraction is shown in Table 2. As was observed in the ice-trap fraction, the identified carbonyl compounds were aldehydes. Moreover, they also included relatively volatile

**Table 1.** Analytical values for the volatile compounds trapped by the sampling train.

| Analytical values | Ice-trap fraction | Dry-ice-trap fraction |
|---|---|---|
| Carbonyl value (mmol/L) | 6.45 ± 4.38* | 35.41 ± 6.37 |
| Acid value (mg KOH/L) | 0.09 ± 0.01 | 0.20 ± 0.07 |
| Peroxide value (mEq/L) | 0.34 ± 0.08 | 0.59 ± 0.38 |

Results are mean ± SD of triplicate trials.

* Significantly different by Student's *t* test ($P < 0.05$).

**Table 2.** Carbonyl components of the volatile compounds trapped by the sampling train analyzed by HPLC as their corresponding DNPH derivatives.

| Aldehydes (mM) | Ice-trap fraction | Dry-ice-trap fraction |
|---|---|---|
| Alkanals | | |
| butanal | n.d. | 16.6 ± 7.3 |
| pentanal | 0.3 ± 0.3 | 0.9 ± 0.3 |
| hexanal | n.d. | 5.2 ± 1.6 |
| heptanal | 0.2 ± 0.1 | 0.6 ± 0.2 |
| octanal | 0.4 ± 0.3 | 0.3 ± 0.1 |
| nonanal | 0.6 ± 0.4 | 1.3 ± 0.5 |
| Alkenals | | |
| acrolein | n.d | 4.7 ± 3.1 |
| 2-pentenal | 0.3 ± 0.2 | 0.5 ± 0.3 |
| 2-hexenal | 0.4 ± 0.2 | 1.0 ± 0.4 |
| 2-heptenal | 0.7 ± 0.7* | 5.2 ± 2.0 |
| 2-octenal | n.d. | 1.2 ± 0.6 |
| 2-nonenal | 0.2 ± 0.1 | n.d. |
| 2-decenal | 1.1 ± 0.5 | 0.6 ± 0.5 |
| 2-undecenal | 1.5 ± 0.5 | 0.5 ± 0.3 |
| Alkadienals | | |
| 2,4-heptadienal | 0.6 ± 0.3* | 6.2 ± 2.4 |
| 2,4-decadienal | 6.5 ± 2.5 | 4.2 ± 2.4 |

Results are mean ± SD of triplicate trials.
n.d., not detected.
*Significantly different by Student's *t* test ($P < 0.05$).

aldehydes such as acrolein and hexanal.

*Cytotoxicity of volatile compounds to hepatocytes* Optical microscopy revealed that 2,4-decadienal and the fractions obtained in the ice traps and dry-ice traps induced cell death. We used 2,4-decadienal as a positive control because it was known to be toxic and carcinogenic and it was the main component of both fractions, as shown in Table 2. Figure 2 shows the survival curve of hepatocytes exposed to 2,4-decadienal. The EC50 of 2,4-decadienal was 0.56 mM; this value was approximately consistent with that mentioned in a previous report (Feron, 1991). Figure 3 shows the typical survival curves of hepatocytes exposed to the ice-trap and dry-ice-trap fractions. The EC50 values (%) are shown in Table 3. The dry-ice-trap fraction inhibited the growth of normal hepatocytes by 0.2%. However, the ice-trap fraction demonstrated very weak cytotoxicity, and the survival ratios sharply decreased by more than 0.1%. The EC50 value was expected to be more than 1% for reference. Because the dry-ice-trap fraction included more aldehydes than the ice-trap fraction (Table 2), the higher toxicity of the dry-ice-trap fraction was thought to be due to the higher concentration of aldehydes. The EC50 value (mM) of the 2 fractions when converted to aldehyde equivalents showed no significant difference. These

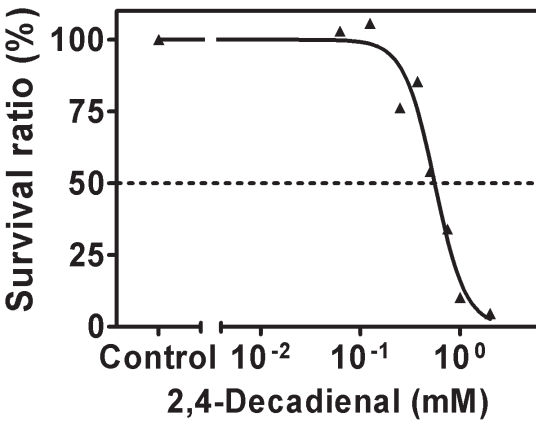


**Fig. 2.** Survival curve of hepatocytes exposed to 2,4-decadienal. Primary-cultured hepatocytes were exposed to serum-free medium containing 2,4-decadienal in a series of concentrations for 24 h. Survival ratios were expressed as percentages of the average absorbance of the exposed cells to that of the unexposed cells (control), and the survival curve was computed by probit transformation.

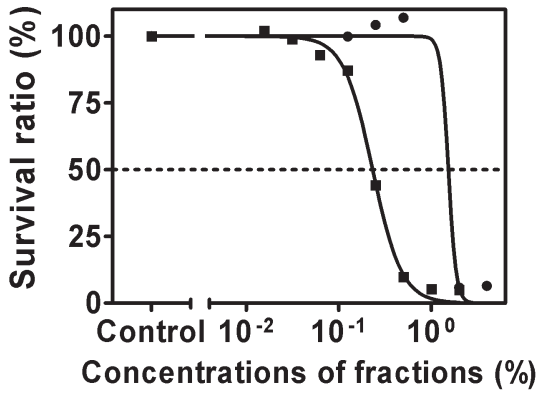


**Fig. 3.** Typical survival curves of hepatocytes exposed to ice-trap fraction (●) and dry-ice-trap fraction (■). Primary-cultured hepatocytes were exposed to serum-free medium containing each fraction in a series of concentrations for 24 h. Survival ratios were expressed as percentages of the average absorbance of the exposed cells to that of the unexposed cells (control), and the survival curve was computed by probit transformation.

**Table 3.** Cytotoxicity of volatile compounds to hepatocytes, expressed as EC50.

| | Ice-trap fraction | Dry-ice-trap fraction |
|---|---|---|
| EC50 (%) | 1.49 ± 0.05* | 0.19 ± 0.08 |
| Converted EC50[a] (mM) | 0.19 ± 0.03 | 0.09 ± 0.03 |

Results are mean ± SD of triplicate trials.
[a]EC50 value was converted to total aldehyde equivalent.
*Significantly different by Student's *t* test ($P < 0.05$).

values were lower than the EC50 (mM) of 2,4-decadienal but not far removed (same order of magnitude). Judging from the chemical analyses of ice-trap and dry-ice-trap fractions, the observed cytotoxicity would be induced by volatile compounds, including 2,4-decadienal, in both fractions, suggesting that low-volatility components as acylglycerols in ice-trap fraction would show no cytotoxicity.

The volatile compounds generated in the apparatus used in this study were successfully collected without the interference of frozen water vapor and shown to be cytotoxic to normal cells. The aldehyde contents, as shown by the results of the chemical analysis of volatile compounds and comparison with the cytotoxicity of a positive control (2,4-decadienal), indicated that the main cytotoxic substances among the volatile compounds were aldehydes. The collection apparatus used here would be useful to elucidate the toxicity of the mixture of water vapor and volatile components, although it would be necessary to confirm whether the nonvolatile compounds that exhibited toxicity escaped from the collection apparatus. Fumes from practical deep-fat frying may also contain other toxic compounds generated from the fried materials, which may not have been identified in this model system. In fact, several toxic compounds, such as modified aldehydes (Kawai *et al.*, 2006) and polycyclic aromatic hydrocarbons, have been detected in cooking fumes. Further research is required for practical deep-frying conditions using the collection apparatus.

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