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Determination of aliphatic aldehydes as their thiazolidine derivatives in foods by gas chromatography with flame photometric detection

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Abstract

A selective and sensitive gas chromatographic method for the determination of saturated and unsaturated aliphatic aldehydes in foods has been developed. After extraction of the sample with acetonitrile, aldehydes were converted into their thiazolidine derivatives by the reaction with cysteamine, and then measured by gas chromatography with flame photometric detection. The calibration curves for aliphatic aldehydes in the range 20–2500 ng were linear and the detection limits at a signal-to-noise ratio of 3 were ca. 4–100 pg injected. Aliphatic aldehydes in foods could be selectively determined by this method without any interference from coexisting substances. Overall recoveries of aldehydes added to food samples were 82–111%. Analytical results for the determination of aliphatic aldehydes in various food samples are presented.

1. Introduction

Low-molecular-mass aldehydes, which have unpleasant pungent odors, are usually present at trace amounts in various complex materials such as foods, tobacco smoke, air and water pollution samples and physiological fluids. In fat-containing foods, these compounds are formed during maturation by enzymatic and nonenzymatic reactions, especially by oxidation of unsaturated fatty acids [1–4]. Rancidity, caused by lipid peroxidation, has long been recognized as a problem in the storage of fat and oils. Lipid peroxidation is also associated with numerous pathological conditions such as atherosclerosis, cardiovascular diseases, carcinogenesis, inflammatory disease, mammographic dysplasia, chronic gastritis, precancerous dysplasia and postischemic reperfusion injury [5–10]. Aldehydes formed during lipid peroxidation are shown to be highly cytotoxic and genotoxic [11–15] and to react with functional groups such as amino and sulphydryl groups of biomolecules, such as proteins, nucleic acids, glutathione, cysteine, lysine and coenzyme A [16–22]. Therefore, measurement of aldehydes in foodstuff is very important.

The determination of aliphatic aldehydes has been carried out by high-performance liquid chromatography (HPLC) or gas chromatography (GC). However, these methods have some inherent problems related to the difficulty in handling low-molecular-mass aldehydes because of their high water solubility, volatility and reactivity. HPLC analyses of aldehydes with

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ultraviolet (UV) [23–26] and fluorescence [27–29] detection require derivatization in order to increase the detection sensitivity. Although some of the derivatization methods are very sensitive, they require time-consuming preliminary clean-up of the sample to remove excess reagent and coexisting substances. Headspace GC analysis [30,31] of underivatized aldehydes presents a simple technique that measures volatile compounds in equilibrium with liquid or solid samples, but it requires a closed system to prevent loss of volatile aldehydes. On the other hand, GC methods based on the derivatization with 2,4-dinitrophenylhydrazine [32–34], methylhydrazine [35], benzyloxyamine [36], pentafluorobenzyloxyamine [37,38], N-benzylethanolamine [39], hydroxylamine/*tert.*-butyldimethylsilyltrifluoroacetamide [40,41] and morpholine [42] by flame ionization detection, nitrogen–phosphorus detection (NPD), electron-capture detection and GC–mass spectrometry with selected-ion monitoring have been reported. However, with some of these methods the aldehyde peaks are difficult to identify because of the inevitable formation of both *syn* and *anti* forms. Therefore, simultaneous determination of different aldehydes is almost impossible with these derivatization methods. Moreover, some of these methods require strong acidic conditions for derivatization that may cause undesirable reactions, such as decomposition of carbohydrates or proteins in the case of food samples. Recently, Shibamoto and co-workers [4,43–46] developed a new derivatization method of saturated aldehydes with cysteamine (2-aminoethanethiol) to form stable thiazolidine derivatives. This method gives only one derivative for each aldehyde with almost quantitative yield under mild conditions, and excess of the reagent (cysteamine) does not interfere with GC analysis. Furthermore, the resulting thiazolidine derivatives can be separated perfectly with fused-silica capillary columns and detected selectively with nitrogen–phosphorous detection (NPD). However, this method has not been applied to the determination of unsaturated aldehydes.

In this work, we report a method for the determination of saturated and unsaturated aliphatic aldehydes as their thiazolidine derivatives by GC with flame photometric detection (FPD–GC). By using this method, the contents of these aldehydes in food samples, and the effects of heat treatment and UV irradiation on the formation of aliphatic aldehydes by lipid peroxidation of food oils were also studied.

2. Experimental

2.1. Reagents

Butanal (C_4) and acetaldehyde (C_2) were purchased from Nacalai Tesque (Kyoto, Japan) and E. Merck (Darmstadt, Germany), respectively. Propanal (C_3), isobutanal (i-C_4), pentanal (C_5), isopentanal (i-C_5), hexanal (C_6), heptanal (C_7), octanal (C_8), nonanal (C_9), decanal (C_{10}), *trans*-2-hexenal (C_{6-1}) and 2,4-hexadienal (C_{6-2}) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Each aldehyde was dissolved in 20% methanol to make a stock solution with a concentration of 2 mg/ml and used after dilution with 20% methanol to the required concentration (1–5 μg/ml). Phenyl sulphide (Tokyo Kasei Kogyo) as an internal standard (I.S.) was dissolved in ethyl acetate at a concentration of 0.5 μg/ml. Cysteamine (Nacalai Tesque) was dissolved in distilled water at a concentration of 2 mg/ml. All other chemicals were of analytical-reagent grade.

2.2. Preparation of samples

Food samples were purchased at local retail markets and were treated for analyses on the same day. Food oil samples (ca. 0.1 ml) and solid fat samples (ca. 0.1 g) were extracted twice with 0.5 ml of acetonitrile, and 0.1 ml of the combined extracts was used as the sample for derivatization. For solid samples containing lipid, e.g. cheese, chocolate and potatochips, an aliquot (ca. 1 g) was homogenized in 4 ml of acetonitrile with a Model LK-21 ultra-disperser (Yamato Kagaku, Tokyo, Japan). After centrifugation at 3000 *g* for 10 min, the precipitate was re-extracted with 4 ml of acetonitrile. The super-

natants were combined and 0.1 ml of the combined solution was used as the sample for derivatization.

2.3. Derivatization procedure

To the standard solution containing 20–2000 ng of each aldehyde or the sample prepared by the above method were added 0.2 ml of 0.2 mg/ml cysteamine and 0.1 ml of 0.1 *M* sodium hydroxide, and the total volume was made up to 1 ml with distilled water. The mixture was shaken with a Model SR-II shaker set (Taitec, Saitama, Japan) up and down at 3000 rpm for 10 min at room temperature and to the reaction mixture 0.5 ml of distilled water was added. After saturation with sodium chloride, the mixture was extracted with 0.2 ml of ethyl acetate containing 1 μg/ml phenyl sulphide (I.S.) and 1 μl of this extract was injected onto the FPD–GC system. The derivatization process is shown in Fig. 1.

2.4. Gas chromatography

GC analysis was carried out with a Shimadzu Model 12 A gas chromatograph equipped with FPD (S-filter). A fused-silica capillary column (J&W, Folsom, CA, USA) connected DB-17 (15 m × 0.53 mm I.D., 1.0 μm film thickness) and DB-210 (15 m × 0.53 mm I.D., 1.0 μm film thickness) was used. The operating conditions were as follows: column temperature, programmed at 6°C/min from 100 to 240°C; injection and detector temperature, 250°C; nitrogen flow-rate, 10 ml/min (flow-velocity, 76 cm/s). The peak height ratios against the I.S. were measured and the peak-height ratios against the I.S. were calculated.

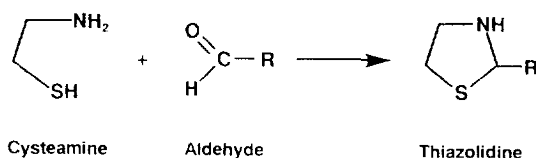

Fig. 1. Derivatization reaction of aldehydes. R = alkyl group.

2.5. Gas chromatography–mass spectrometry (GC–MS)

A Hewlett-Packard Model 5890A gas chromatograph was operated in conjunction with a VG Analytical Model 70-SE mass spectrometer and a VG-11-250J mass data system. A fused-silica capillary column containing cross-linked OV-1 (Quadrex, New Haven, CT, USA, 25 m × 0.25 mm I.D., 0.25 μm film thickness) was used. Column temperature: programmed at 8°C/min from 100 to 270°C; injection temperature, 280°C; ion-source temperature, 270°C; ionizing voltage, 40 eV; helium flow-rate, 8 ml/min.

2.6. Heat treatment and UV irradiation of food oil samples

Food oil (ca. 0.1 ml) was heated at 100°C in a Model TB-320 hot block bath (Advantec Toyo, Tokyo, Japan) or irradiated in a Model H-400 UV lamp (λ = 250–400 nm) house (Irie, Tokyo, Japan) from a distance of 40 cm for various times and then the aldehyde content in the sample was measured by the above method.

3. Results and discussion

Shibamoto and co-workers [44–46] developed a new derivatization method based on the reaction of aldehyde with cysteamine and applied to the determination of various volatile aldehydes in heated pork fat, cooking oils and automobile exhaust after headspace sampling. Although this method is sensitive and specific by using NPD, it is time-consuming because of the two-phase reaction with gaseous aldehyde and cysteamine solution, and has not yet been applied to unsaturated aldehydes. Therefore, we re-examined the reaction conditions of saturated and unsaturated aldehydes with cysteamine. The reaction of alde-

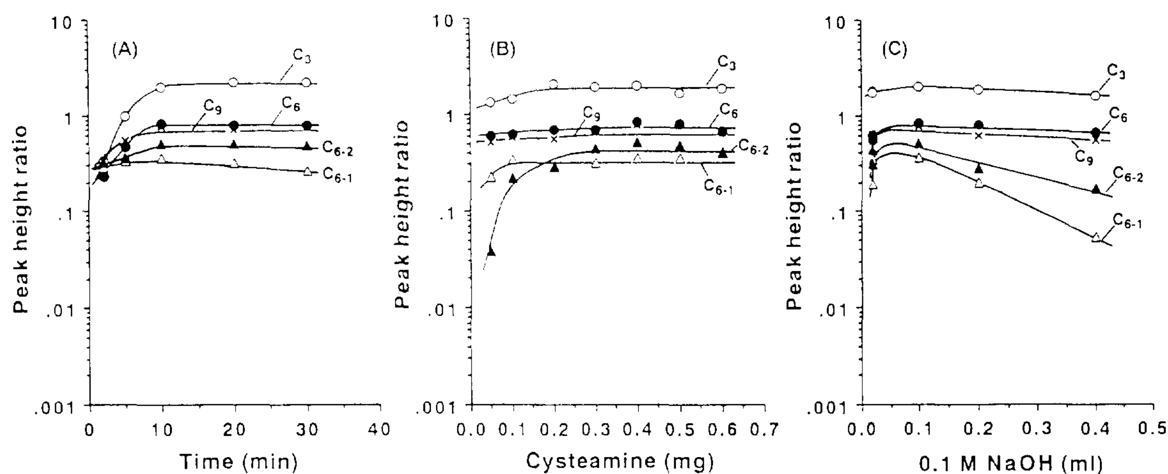

Fig. 2. Effects of (A) reaction time, (B) cysteamine and (C) sodium hydroxide on the formation of thiazolidine derivatives of aldehydes.

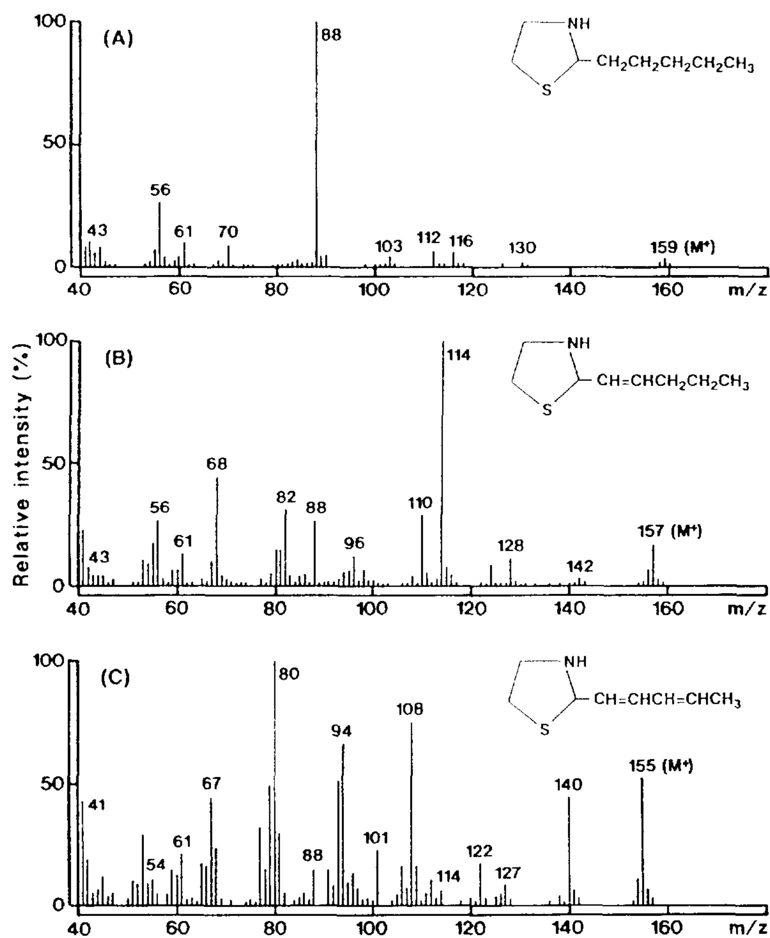

Fig. 3. Mass spectra of the thiazolidine derivatives of aldehydes. (A) Hexanal (C_6). (B) *trans*-2-hexenal (C_{6-1}), (C) 2,4-hexadienal (C_{6-2}).

hydes with cysteamine proceeds rapidly in aqueous alkaline media. As shown in Fig. 2A,B, with 0.4 mg of cysteamine the reaction is accomplished within 10 min at room temperature by shaking. Although saturated aldehydes readily reacted with 0.05 *M* sodium hydroxide, the derivatization yield of unsaturated aldehydes decreased by the excess of sodium hydroxide (Fig. 2C). It seems that unsaturated aldehydes are preferentially oxidized in stronger alkaline media. Subsequently, the thiazolidine derivatives produced by this reaction were extracted into the solvent and injected onto the GC system. Of the several solvents tested, ethyl acetate proved to be the most satisfactory for the rapid and quantitative extraction of thiazolidines. Although Shibamoto et al. used dichloromethane as extraction solvent, sampling of the organic layer was difficult because this solvent was found in the bottom layer. By using this improved method, preparation of the derivative could be performed within 15 min, and several samples could be treated simultaneously.

The structures of the thiazolidine derivatives of aldehydes were confirmed by GC–MS analysis. As shown in Fig. 3, a molecular ion peak $[M]^+$ was observed for each of the derivatives and other common ion peaks which were useful for structure elucidation, were $[M-15]^+$ (CH_3), $[M-88]^+$ (SCH_2CH_2NHCH) and *m/z* 88. As shown in Table 1, the derivatives of saturated aldehydes were stable under normal laboratory conditions, and no decomposition was observed during GC analysis. On the other hand, the

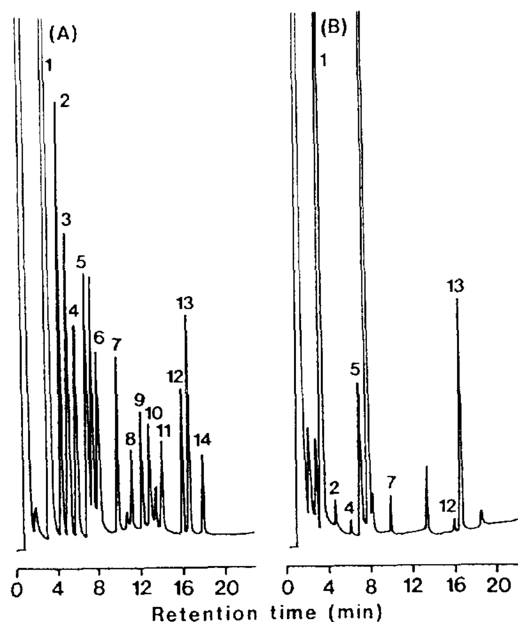

Fig. 4. Typical gas chromatograms obtained from standard and food sample. (A) Standard (containing 100 ng of C_2–C_8 and 500 ng of C_9, C_{10}, C_{6-1} and C_{6-2}), (B) sesame oil (10 *ml*). GC conditions are given in Experimental. Peaks: 1 = acetaldehyde (C_2), 2 = propanal (C_3), 3 = isobutanal (*i*-C_4), 4 = butanal(C_4), 5 = isopentanal (*i*-C_5), 6 = pentanal (C_5), 7 = hexanal (C_6), 8 = *trans*-2-hexenal (C_{6-1}), 9 = heptanal (C_7), 10 = 2,4-hexadienal (C_{6-2}), 11 = octanal (C_8), 12 = nonanal (C_9), 13 = phenyl sulphide (I.S.), 14 = decanal (C_{10}).

derivatives of unsaturated aldehydes were stable for 24 h but decomposed to below 60% of their original value over a 3-day period when these derivatives were stored as ethyl acetate solution at 4°C. This seems to be due to the oxidation of

Table 1
Stability of thiazolidines derived from aldehyde

Aldehyde	Storage time[a] (days)			
	0	1	2	3
Propanal (C_3)	2.02 ± 0.09	2.03 ± 0.12	2.04 ± 0.10	1.93 ± 0.07
Hexanal (C_6)	0.82 ± 0.02	0.82 ± 0.05	0.84 ± 0.04	0.84 ± 0.03
Nonanal (C_9)	0.76 ± 0.01	0.72 ± 0.02	0.79 ± 0.04	0.73 ± 0.02
trans-2-Hexenal (C_{6-1})	0.35 ± 0.02	0.32 ± 0.01	0.24 ± 0.01	0.20 ± 0.01
2,4-Hexadienal (C_{6-2})	0.52 ± 0.03	0.48 ± 0.03	0.42 ± 0.03	0.31 ± 0.02

[a] Samples were analysed immediately after derivatization and after 1, 2 and 3 days' storage as thiazolidine derivatives in ethyl acetate at 4°C. Each value, mean ± S.D. ($n = 4$), represents peak-height ratio against the I.S.

the unsaturated bonds. Thus, the samples must be analysed at least on the day after derivatization. The within-run C.V.s for the aldehydes were 1.3–7.1% ($n = 4$) and between-run C.V.s over a period of 4 days were 2.6–15.2% ($n = 4$). The derivatives were volatile and eluted as separate symmetrical peaks within 18 min by using connected DB-17 and DB-210 capillary columns (Fig. 4A). The derivatives gave an excellent FPD response and minimum detectable amounts of C_3,C_6, C_9, C_{6-1} and C_{6-2} (at a signal-to-noise ratio of 3 under the instrumental conditions used) were ca. 4, 10, 60, 100 and 100 pg injected, respectively. In order to test the linearity of the calibration curve, various amounts of aldehydes ranging from 20–500 ng (for C_3, C_4, i-C_4, C_5, i-C_5, C_6, C_7 and C_8) and 100–2500 ng (for C_9, C_{10}, C_{6-1} and C_{6-2}) were derivatized in a mixture and aliquots representing 0.1–2.5 ng (for C_3, C_4, i-C_4, C_5, i-C_5, C_6, C_7 and C_8) and 0.5–12.5 ng (for C_9, C_{10}, C_{6-1} and C_{6-2}) were injected onto the FPD–GC system. In each case, a linear relationship was obtained and the reproducibility was found to be satisfactory, when the peak-height ratios were measured with reference to the I.S. (Table 2). On the other hand, C_2 was detected with satisfactory sensitivity, but reproducibility was poor because of interference from background C_2 originating from the laboratory atmosphere and the reagents used.

Table 2
Linear regression data for aliphatic aldehydes

Aldehyde[a]	Regression line[b]		
	s	*b*	*r*
Propanal (C_3)	1.886	−3.466	0.9926
Isobutanal (i-C_4)	1.799	−3.450	0.9981
Butanal (C_4)	1.750	−3.523	0.9942
Isopentanal (i-C_5)	1.869	−3.660	0.9982
Pentanal (C_5)	1.778	−3.639	0.9975
Hexanal (C_6)	1.854	−3.795	0.9970
Heptanal (C_7)	1.842	−3.968	0.9972
Octanal (C_8)	1.782	−3.991	0.9960
Nonanal (C_9)	1.884	−5.202	0.9906
Decanal (C_{10})	1.818	−5.329	0.9905
trans-2-Hexenal (C_{6-1})	1.963	−5.748	0.9941
2,4-Hexadienal (C_{6-2})	2.129	−6.035	0.9977

[a] Range: 20–500 ng for C_3, i-C_4, C_4, i-C_5, C_5, C_6, C_7 and C_8; 100–2500 ng for C_9, C_{10}, C_{6-1} and C_{6-2}.
[b] $\log y = s \log x + b$; y = peak-height ratio, x = amount of aldehyde (ng), s = slope, b = intercept, r = correlation coefficient ($n = 15$).

For aldehyde analysis of foodstuffs, acetonitrile was used as extraction solvent. Aliphatic aldehydes were quantitatively extracted from food samples by extraction twice with 0.5 ml of

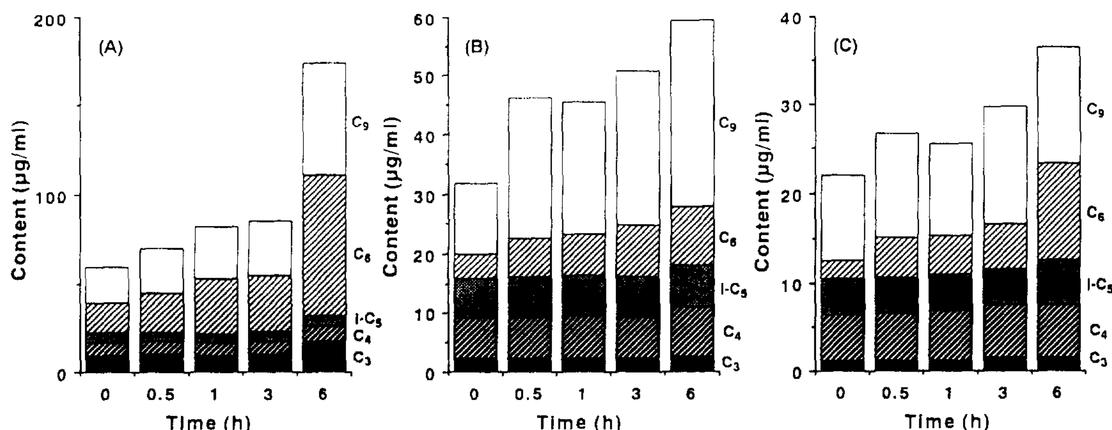

Fig. 5. Effect of heat treatment at 100°C on the formation of aldehydes in (A) salad oil, (B) sesame oil and (C) corn oil.

Table 3
Recoveries of aliphatic aldehydes added to food samples

Aldehyde[a]	Added	Amount found[b]		Recovery (%)
		Non-addition	Addition	
Sesame oil	(μg/ml)	(μg/ml)	(μg/ml)	
C_3	10	2.4 ± 0.1	10.6 ± 0.6	82
i-C_4	10	ND[c]	8.9 ± 0.4	89
C_4	10	6.9 ± 0.3	15.9 ± 0.9	90
i-C_5	10	6.7 ± 0.1	17.5 ± 1.3	108
C_5	10	ND	10.5 ± 0.6	105
C_6	10	4.0 ± 0.1	13.1 ± 0.5	91
C_7	10	ND	8.4 ± 0.2	84
C_8	10	ND	9.2 ± 0.4	92
C_9	50	11.9 ± 0.7	58.8 ± 1.7	94
C_{10}	50	ND	48.7 ± 1.3	97
C_{6-1}	50	ND	41.6 ± 1.1	83
C_{6-2}	50	ND	46.3 ± 1.8	93
Margarine	(μg/g)	(μg/g)	(μg/g)	
C_3	10	1.9 ± 0.2	11.6 ± 0.4	97
i-C_4	10	ND	10.2 ± 0.4	102
C_4	10	5.1 ± 0.4	15.8 ± 0.8	107
i-C_5	10	4.8 ± 0.3	15.2 ± 0.8	104
C_5	10	ND	10.3 ± 0.4	103
C_6	10	1.2 ± 0.1	12.0 ± 1.0	108
C_7	10	ND	10.4 ± 0.2	104
C_8	10	ND	10.3 ± 0.2	103
C_9	50	8.6 ± 0.8	53.1 ± 1.1	89
C_{10}	50	ND	50.6 ± 0.9	101
C_{6-1}	50	ND	49.2 ± 3.3	98
C_{6-2}	50	ND	55.5 ± 1.0	111

[a] C_3 = propanal; i-C_4 = isobutanal; C_4 = butanal; i-C_5 = isopentanal; C_5 = pentanal; C_6 = hexanal; C_7 = heptanal; C_8 = octanal; C_9 = nonanal; C_{10} = decanal; C_{6-1} = *trans*-2-hexenal; C_{6-2} = 2,4-hexadienal.
[b] Mean ± S.D. (n = 4).
[c] Not detectable.

acetonitrile. Fig. 4B shows the chromatogram obtained from a food sample. Although background peaks originating from the laboratory atmosphere and the reagents used were observed between i-C_5 and C_5, and between C_{6-2} and C_8, aldehydes in the sample could be detected without any interference from coexisting substances. As shown in Table 3, the overall recoveries of aliphatic aldehydes added to several food samples were 82–111% and the relative standard deviations were 1.2–9.3% ($n = 4$). The aliphatic aldehyde contents in various food samples determined by this method are summarized in Table 4. It can be seen from our data that sardine oil and chocolate contained high concentrations of C_4, C_6, C_7 and C_9. Unsaturated aldehyde C_{6-1} was found in milk and chocolate. On the other hand, i-C_4, C_5, C_8, C_{10} and C_{6-2} were not detected at all in any of the sample investigated in this study. By using this improved method, production of aldehydes in salad oil (mixture of soybean oil and rapeseed oil), sesame oil and corn oil was measured during heat treatment and UV irradiation. As shown in Figs. 5 and 6, the total contents of aldehydes in these food oils increased by 1.3–1.9 and 2.3–5.0

Table 4
Aliphatic aldehyde contents in various foods from commercial sources

Sample	Aldehyde[a] content (μg/ml or g) (mean ± S.D., $n = 4$)						
	C_3	C_4	i-C_5	C_6	C_7	C_9	C_{6-1}
Sesame oil	2.4 ± 0.1	6.9 ± 0.3	6.7 ± 0.1	4.0 ± 0.1	ND[b]	11.9 ± 0.7	ND
Soybean oil	1.6 ± 0.1	5.4 ± 0.3	5.1 ± 0.3	1.8 ± 0.1	ND	8.1 ± 0.5	ND
Rapeseed oil	2.5 ± 0.1	5.3 ± 0.3	4.9 ± 0.3	3.6 ± 0.2	ND	10.6 ± 0.6	ND
Cottonseed oil	1.6 ± 0.1	5.3 ± 0.3	5.1 ± 0.3	2.3 ± 0.02	ND	10.0 ± 0.6	ND
Corn oil	1.3 ± 0.1	5.0 ± 0.2	4.0 ± 0.4	2.2 ± 0.1	ND	9.5 ± 0.2	ND
Olive oil	1.8 ± 0.1	5.5 ± 0.2	4.8 ± 0.3	3.5 ± 0.2	ND	12.6 ± 1.0	ND
Sardine oil	5.9 ± 0.1	6.2 ± 0.2	3.3 ± 0.2	6.5 ± 0.2	5.3 ± 0.2	23.7 ± 1.2	ND
Beef fat	2.7 ± 0.3	5.1 ± 0.3	5.4 ± 0.2	2.4 ± 0.2	ND	12.6 ± 1.7	ND
Lard	3.8 ± 0.2	5.5 ± 0.3	4.7 ± 0.3	2.6 ± 0.1	ND	9.0 ± 0.9	ND
Butter	2.1 ± 0.1	4.9 ± 0.2	5.1 ± 0.3	2.0 ± 0.2	ND	13.0 ± 0.8	ND
Margarine	1.9 ± 0.2	5.1 ± 0.4	4.8 ± 0.3	1.2 ± 0.1	ND	8.6 ± 0.8	ND
Mayonnaise	2.1 ± 0.3	3.8 ± 0.2	3.8 ± 0.4	2.2 ± 0.2	ND	10.6 ± 0.5	ND
Cheese	2.4 ± 0.1	4.6 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	ND	22.8 ± 1.3	ND
Egg yolk	2.7 ± 0.3	4.0 ± 0.2	3.5 ± 0.2	1.5 ± 0.1	ND	9.0 ± 0.2	ND
Fresh milk	3.0 ± 0.5	3.7 ± 0.4	5.1 ± 0.1	1.5 ± 0.1	ND	9.4 ± 0.5	17.8 ± 2.1
Chocolate	1.9 ± 0.2	4.4 ± 0.3	3.5 ± 0.4	5.1 ± 0.2	3.4 ± 0.1	19.1 ± 0.5	18.7 ± 0.8
Potatochips	2.0 ± 0.1	3.2 ± 0.2	4.1 ± 0.2	1.8 ± 0.1	ND	9.9 ± 0.9	ND

[a] C_3 = propanal; C_4 = butanal; i-C_5 = isopentanal; C_6 = hexanal; C_7 = heptanal; C_9 = nonanal; C_{6-1} = *trans*-2-hexenal.
[b] Not detectable.

fold during heat treatment and UV irradiation, respectively. Particularly C_6 and C_9 increased with time during both treatments. Increases of these aldehydes were approximately 1.4–5.0 fold for heat treatment (6 h) and 2.1–30.9 fold for UV irradiation (5 h). The levels of the other aldehydes were not affected by both treatments.

4. Conclusion

A convenient and reliable method for the determination of saturated and unsaturated aliphatic aldehydes in food samples has been established by modification of Shibamoto's method. Although this method is less sensitive than

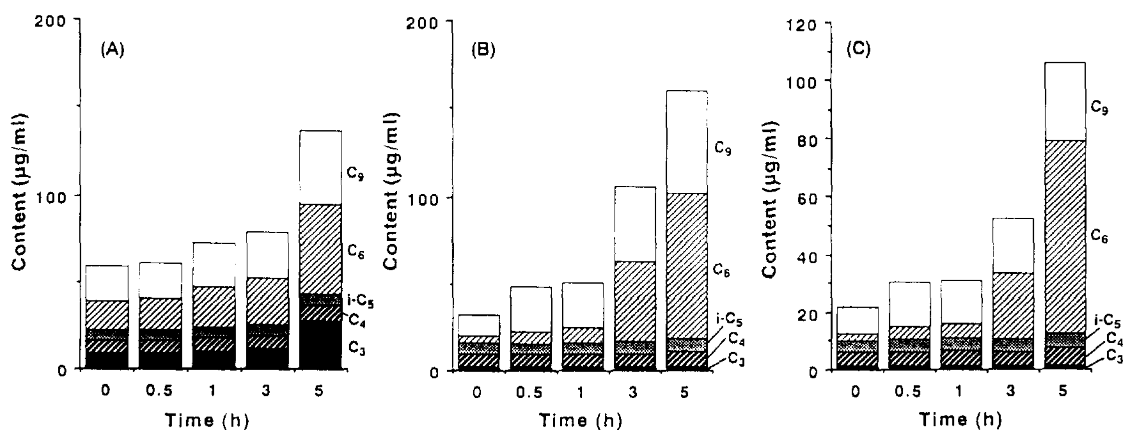

Fig. 6. Effect of UV irradiation on the formation of aldehydes in (A) salad oil, (B) sesame oil and (C) corn oil.

Shibamoto's method, this method is more simple and rapid, and sufficiently applicable for various foodstuffs. Moreover, with the present method food samples can be directly analysed without pre-treatment clean-up and without any interference from other coexisting substances. Therefore, we believe that this method provides a useful tool for routine analysis of foodstuffs.

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