A Simple and Quick Determination of Aldehydes in Autoxidized Vegetable and Fish Oils

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A simple and quick method for the quantitative determination of aldehydes in oxidized oils was established. The analysis was based on the reaction of N,N-dimethyl-p-phenylenediamine (DPPD) with aldehydes in the presence of acetic acid. Reaction products were determined by visible absorption at wavelengths of 400, 460 and 500 nm. The errors of the DPPD method in the analyses of total aldehydes in authentic mixtures of various aldehydes were less than 5%, and this method could be used successfully to determine aldehyde contents in oxidized vegetable and fish oils. The reaction time of the DPPD method (10 min at 30°C) is much shorter than that of the conventional method that uses 2,4-dinitrophenylhydrazine as a reagent (30 min at 60°C). The simplicity of the procedure enables a quick determination of aldehyde contents in oxidized oil samples.

KEY WORDS: Aldehydes, autoxidation, colorimetric determination, edible oils, hydroperoxide decomposition, volatile compounds.

Major volatile carbonyls formed in the autoxidation of lipids are saturated and unsaturated aldehydes ranging in number of carbon atoms from 3 to 10. The aldehydes are mainly formed by homolytic β-scission on both sides of the hydroperoxy group (1,2). Although these volatile compounds are formed as relatively minor autoxidation products, they are the most important contributors to rancid and unpleasant flavors in oxidized oils (1,2), and some aldehydes are known to cause potential damage to biological systems (3,4). Therefore, the determination of aldehyde contents is important in judging the quality of oils, and many analytical methods for the determination of aldehydes have been proposed (5–8).

Of the better-known methods, the most widely used are those based on the colorimetric determination of the 2,4-dinitrophenylhydrazone (2,4-DNPH) in alkaline solution by Henick *et al.* (5). However, this method is time-consuming and difficult.

Herein, we report a simple and quick method for quantitative aldehyde determination by using N,N-dimethyl-p-phenylenediamine (DPPD) as a reagent.

EXPERIMENTAL PROCEDURES

Materials. All solvents were prepared by distillation of the reagent grade products. For the determination of aldehydes with the DPPD method, solvents were used without further purification, while solvents for 2,4-DNPH method were purified to a carbonyl-free state as described by Henick *et al.* (5). When the carbonyl-free solvents were not used, the absorbance of the blank solution for the 2,4-DNPH method was sometimes higher than 0.35, while the blank solution for the DPPD method had absorbances below 0.05. The high value for the 2,4-DNPH method was due to keto carbonyls as impurities in the solvents. On the other hand, keto carbonyls do not react with DPPD and solvents can be used directly for the DPPD method. All reagents were of analytical grade and purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Standard samples of alkanals, alkenals and alkadienals were obtained from Tokyo Kasei Co. (Tokyo, Japan). Methyl ethyl ketone and methyl nonyl ketone were purchased from Nacalai Tesque Inc. Oleic, linoleic and linolenic acids were prepared from olive, safflower and linseed oils, respectively, as described previously (9). Vegetable oils were obtained from Nacalai Tesque Inc. and sardine oil from an industrial plant (Hakodate, Japan). All spectrophotometric measurements were made on a Hitachi U-2000 spectrophotometer (Hitachi Seisakusho, Co., Tokyo, Japan) with a 1-cm cell.

Determination of aldehydes by the DPPD method. Hexanal, ocatanal, 2-hexenal, 2-heptenal, 2,4-decadienal and 2,4-heptadienal were used as calibration standards for measurements of the absorption spectrum and molar absorptivity. Ten mL of single aldehyde solution (0.4–8.0 μmol aldehyde/10 mL benzene) and 5 mL of reagent solution were pipetted into a 25-mL volumetric flask. The reagent solutions were prepared by dissolving 0.3% (w/v) of DPPD sulfate in glacial acetic acid/methanol (1:24, v/v). An ultrasonic vibrator was used for complete dissolution of the DPPD reagent. After stoppering and shaking, the flask was allowed to stand in a water bath at 30°C for 10 min. The absorption spectrum (360–650 nm) was then determined against a blank prepared in exactly the same manner, substituting for the sample solution with 10 mL of benzene.

The determination of three aldehyde classes (alkanal, alkenal and alkadienal) in authentic mixtures by the DPPD method was carried out on the basis of the molar absorptivities of each aldehyde class at absorption maxima 400, 460, and 500 nm.

Determination of aldehydes in oxidized oils. One gram of each free fatty acid (oleic, linoleic and linolenic) and 10–30 grams of each edible oil (olive, soybean, linseed and sardine) were autoxidized individually by incubation at 50°C in the dark. Peroxide values (PVs) were determined by the colorimetric micromethod (10) for small samples or by the AOCS Official Method (11) for large samples. The aldehyde content was determined by the DPPD method presented here. The 2,4-DNPH method according to Henick *et al.* (5) also was used to determine the total carbonyls of oxidized vegetable and fish oils. Because hydroperoxides decompose to yield additional aldehydes (12,13) and react with DPPD to form color in the range from 500 to 800 nm (14,15), the hydroperoxides in the oxidized samples were reduced to the corresponding alcohols prior to carbonyl determination in both methods. The reduction was carried out with triphenylphosphine (TP) according to the method of Chiba *et al.* (13). Thus, when the DPPD method was used, the sample of 80 mg or less was weighed into a 25-mL volumetric flask, and 10 mL of benzene containing 2.1 mg of TP was pipetted into it.

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After standing for 30 min in the dark at room temperature, 5 mL of the DPPD solution was added, and then the flask was placed in a water bath at 30°C. After 10 min, the absorbances at 400, 460 and 500 nm were measured against a blank solution prepared from benzene containing TP in the same manner as the sample solution.

RESULTS AND DISCUSSION

Saturated and unsaturated aldehydes reacted with DPPD to form yellow or wine-red colors. Figure 1 illustrates the absorption spectrum of alkanal, alkenal and alkadienal. Each aldehyde showed one absorption maximum—400 nm for alkanal, 460 nm for alkenal and 500 nm for alkadienal. However, when methyl ethyl ketone and methyl nonyl ketone were incubated with DPPD at 30°C for 10 min in the presence of acetic acid, the solutions did not show any absorption in the range from 350 to 800 nm.

Figure 2 shows the rate of the color development in the reaction of 2-hexenal with DPPD reagent at 30°C. A 10-min incubation period was sufficient to bring the reaction to completion. Acetic acid was necessary for rapid color development. The rate of color development was not affected by the change of temperature in the range of 10°C to 40°C.

A good correlation (r=0.9999, n=6) was observed between absorbance at 460 nm and molar concentration in the concentration range of 0.243–1.415 μmol of 2-hexenal (Fig. 3). This linear relationship also existed for other aldehydes in the concentration range of 0.2–1.5 μmol. These results indicate that the reaction of each aldehyde with DPPD follows Beer's law.

The molar absorptivities at different wavelengths are given in Table 1. Analyses were run in triplicate on three different days and there was no significant variation in the results. The molar absorptivities at each wavelength were only dependent on the number of double bonds in an aldehyde molecule, but independent of its chainlength. The reaction of aldehyde with DPPD is suggested to be based on the formation of a Schiff base, as shown in Figure 4, and our results showed that the number of double bonds in R adjacent to the C=N bond influenced the absorption maximum and the molar absorptivity of reaction product.

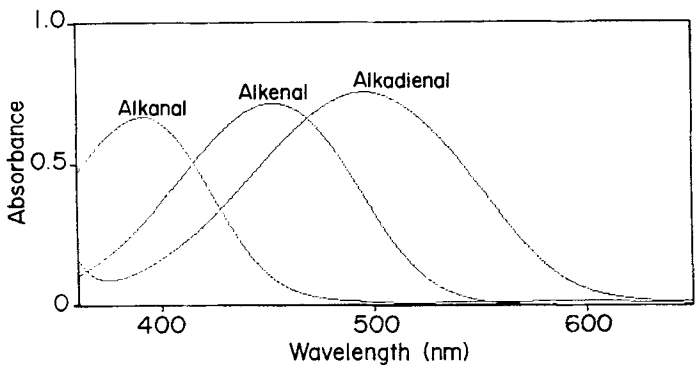

FIG. 1. Absorption spectra of reaction products of DPPD with three classes of aldehydes.

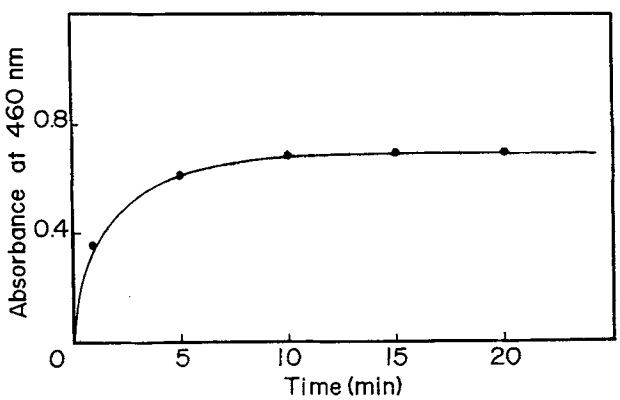

FIG. 2. Rate of color development in the reaction of 2-hexenal (0.657 μmol) with DPPD at 30°C.

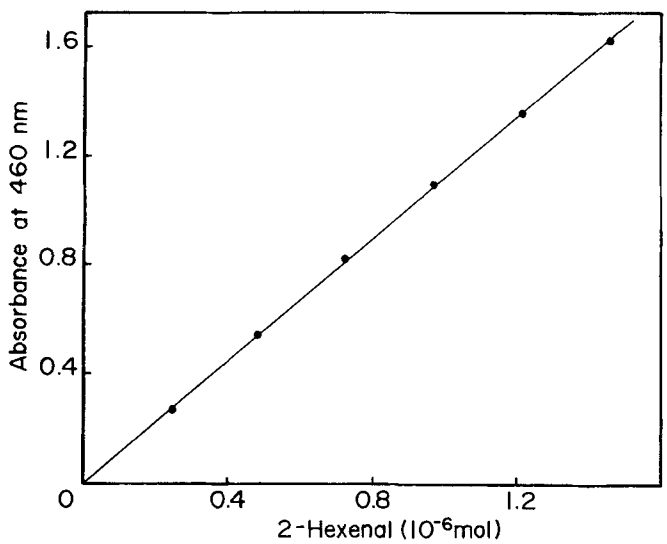

FIG. 3. Relationship between absorbance at 460 nm and concentration of 2-hexenal in the reaction with DPPD.

TABLE 1

Molar Absorptivity of the Reaction Product of Aldehydes with DPPD[a]

Aldehyde class	Aldehyde	Wavelength (nm) 400	460	500
Alkanal	Hexanal	1240	100	0
	Octanal	1270	110	0
	Average	1260	110	0
Alkenal	2-Hexenal	8210	15900	7490
	2-Heptenal	8090	15500	7350
	Average	8150	15700	7420
Alkadienal	2,4-Heptadienal	4850	18200	22500
	2,4-Decadienal	4360	16800	21200
	Average	4610	17500	21800

[a]The data are expressed as the averages from the results of three independent experiments. In each experiment, analyses were run in triplicate.

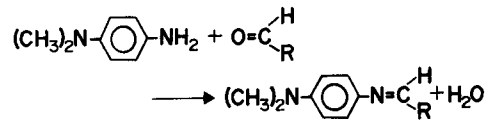

FIG. 4. Reaction of aldehyde with DPPD.

The DPPD makes a blue complex with aqueous methanol and peroxide accelerates complex formation (14,15). This color development is influenced by reaction time, temperature and light intensity (14,15). In preliminary work done in our laboratory, we tried to determine hydroperoxides in oxidized oils by addition of the DPPD reagent. However, the reaction products of DPPD with peroxides showed complex absorption in the range from 500 to 800 nm; it was difficult to obtain accurate values for peroxide contents because of the instability of the color development. On the other hand, the aldehyde reacted with DPPD to show a simple absorption in the range from 400 to 500 nm (Fig. 1), and the pigment formed in the reaction was stable.

As shown in Figure 1, the absorption of each of three classes of aldehydes overlapped with each other. Therefore, the following equations for the estimation of each aldehyde class in mixtures were developed by a three-by-three matrix inversion of the molar absorptivities shown in Table 1 (16):

$$\text{Alkanals } (\mu\text{mol}) = 11.90 \times A400 - 8.041 \times A460 + 3.937 \times A500$$

$$\text{Alkenals } (\mu\text{mol}) = 1.539 \times A460 - 1.236 \times A\ 500$$

$$\text{Alkadienals } (\mu\text{mol}) = -0.5240 \times A460 + 1.109 \times A500$$

Table 2 shows the application of the above equations to analysis of authentic aldehyde mixtures. Good agreement was found between the amount of aldehyde added and determined by the DPPD method. The percentage of errors for analyses of the total aldehydes by the DPPD method were 0.2–4.7%. These results demonstrated the successful application of the DPPD method to quantification of aldehydes in oxidized oils.

The determination of aldehydes in autoxidized oils by the DPPD method was preceded by the reduction of hydroperoxides to corresponding alcohols by TP (13). The absorption from hydroperoxides completely disappeared after TP reduction, but the characteristic absorption of the Schiff base remained unchanged. When authentic aldehyde solutions with and without TP were subjected to the DPPD method, both values for aldehyde contents were in good agreement. These results suggest that TP has no influence on the reaction of aldehydes with DPPD.

Table 3 shows the application of the DPPD method for determinations of three aldehyde classes in autoxidized free fatty acids. A slight amount of alkadienals was detected in oxidized oleic acid. This acid has only one double bond in its molecule; therefore, the value for alkadienal is likely a calculation error. On the other hand, linoleic and linolenic acids have two or more double bonds and alkadienals were detected in the autoxidation of these fatty acids. Alkanals were the principal aldehydes in the autoxidation of the three fatty acids above, most likely because of the instability of alkenals and alkadienals. The unsaturated aldehydes are known to decompose during autoxidation (1,2).

Total aldehydes in oxidized vegetable and fish oils with various peroxide values are shown in Table 4. Total carbonyls were determined by the 2,4-DNPH method according to Henick *et al.* (5). Aldehyde and carbonyl contents in oxidized soybean and sardine oils increased with increasing PV in both methods. However, there were some differences in the values between DPPD and 2,4-DNPH methods. Two explanations could be proposed for these differences. The first is the difference in the reactivity of minor carbonyls with 2,4-DNPH and DPPD. The basic process for the formation of volatile carbonyls is homolytic β-scission of alkoxy radicals derived from hydroperoxides, and this cleavage mainly produces saturated and unsaturated aldehydes (1,2). However, it is also well known that oxidized unsaturated lipids produce keto carbonyls. These carbonyls did not react with DPPD but did react with 2,4-DNPH (17).

The second possible reason is the high temperature (60°C) and long reaction time (30 min) required in the

TABLE 2

Quantitative Determination of Authentic Aldehyde Mixtures by the DPPD Method[a]

Analysis no.	Aldehyde	Aldehyde added (μmol)	Aldehyde determined (μmol)	% Error
1.	Octanal	0.301	0.315	
	2-Heptenal	0.304	0.289	
	2,4-Decadienal	0.301	0.304	
	Total	0.906	0.908	(+0.2)
2.	Octanal	0.151	0.165	
	2-Heptenal	0.304	0.283	
	2,4-Decadienal	0.450	0.449	
	Total	0.905	0.897	(−0.9)
3.	Octanal	0.301	0.308	
	2-Heptenal	0.449	0.433	
	2,4-Decadienal	0.151	0.152	
	Total	0.901	0.893	(−0.9)
4.	Octanal	0.449	0.495	
	2-Heptenal	0.152	0.147	
	2,4-Decadienal	0.301	0.302	
	Total	0.902	0.944	(+4.7)

[a]The data are expressed as the average of triplicate analyses.

TABLE 3

Analysis of Aldehyde Content in Oxidized Free Fatty Acids by the DPPD Method[a]

Free fatty acid	Peroxide value (meq/kg)	Aldehyde determined (mmol/kg)			
		Alkanals	Alkenals	Alkadienals	Total
Oleic (%)	670	57.9 (84.3)	10.5 (15.3)	0.3 (0.4)	68.7 (100.0)
Linoleic (%)	1300	59.2 (56.3)	33.5 (31.8)	12.5 (11.9)	105.2 (100.0)
Linolenic (%)	2560	331.1 (75.8)	57.3 (13.1)	48.3 (11.1)	436.7 (100.0)

[a]The data are expressed as the averages of triplicate analyses.

TABLE 4

Analyses of Total Aldehydes and Total Carbonyls in Autoxidized Vegetable and Fish Oils[a]

Autoxidized oil	PV (meq/kg)	Aldehydes (mmol/kg) (by DPPD method)	Carbonyls (mmol/kg) (by 2,4-DNPH method)
Olive	228	11.5	22.6
Soybean	81	4.0	3.0
	130	7.7	5.7
	224	12.9	9.6
	413	31.9	21.5
Linseed	156	20.7	28.5
Sardine	31	7.6	5.8
	87	21.1	13.5
	157	41.4	22.2
	290	60.5	47.5
	510	115.0	90.3

[a]The data are expressed as the averages of triplicate analyses.

2,4-DNPH method. Peroxy-linked dimers and hydroperoxy epidioxides are known to be major secondary products during autoxidation of linoleate and linolenate (18). The peroxy linkage and epidioxy group are not reduced by TP; however, these endoperoxides may decompose to yield additional amounts of carbonyls under the reaction conditions of the 2,4-DNPH method (1,2,19).

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