A Modified Method for the Estimation of Total Carbonyl Compounds in Heated and Frying Oils Using 2-Propanol as a Solvent

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ABSTRACT: A modified method without using benzene for quantitative determination of total carbonyl compounds in heated and frying fats and oils has been developed. The analysis is done by the reaction of 2,4-dinitrophenylhydrazine (2,4-DNP) with aldehydes and ketones in 2-propanol. The optimal wavelength to determine the total carbonyl compounds is 420 nm where the 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives from saturated and unsaturated aldehydes and ketones had the same molar absorption coefficient. The modified method for the estimation of total carbonyl compounds in heated and frying oils using 2-propanol instead of benzene as a solvent had a good correlation with the conventional method.

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KEY WORDS: Carbonyl compound, carbonyl value, 2,4-dinitrophenylhydrazine, frying oil, lipid peroxidation.

The determination of carbonyl compounds in frying fats and oils is very important for estimating the quality of frying oil because carbonyl compounds formed during thermal oxidation of fats and oils often contribute to rancid and unpleasant flavors and reduce nutritional values of the fried foods.

The most reliable and widely used methods for measuring carbonyl compounds are the procedures of Henick *et al*. (1) and Kumazawa and Oyama (2), which are recognized as the conventional methods. In the conventional method, carbonyl compounds react with 2,4-dinitrophenylhydrazine (2,4-DNP) to form the corresponding 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives that are converted to the quinoidals, which are colored wine-red in alkaline solution. The total carbonyl compounds in solutions are then quantified by measuring the absorption at 440 nm. Although some modified methods (3,4) to determine total carbonyl compounds in frying and oxidized oils have been proposed, these methods use benzene as the solvent, which is very toxic.

Yukawa *et al.* (5) developed a method for estimation of total carbonyl compounds in aqueous media such as milk and soy milk using ethanol as a solvent. However, their method is not applicable for frying and heated fats and oils since fats and oils may reduce the reactivity of 2,4-DNP with carbonyl compounds owing to their low solubility in ethanol.

This paper describes a modified method for the estimation of total carbonyl compounds in heated and frying oils using 2-propanol (2-PrOH) as the solvent and 2,4-DNP instead of the benzene used in the conventional method.

EXPERIMENTAL PROCEDURES

Materials. Aldehydes such as *n*-pentanal, *n*-hexanal, *n*-heptanal, *n*-octanal, *n*-nonanal, *n*-decanal, 2-pentenal, 2-hexenal, 2-heptenal, 2-octenal, 2-nonenal, 2-decenal, 2,4-heptadienal, 2,4-nonadienal, 2,4-decadienal, 2-heptanone, and 2-octanone were extra-pure grade, and they were purchased from Wako Pure Chemical Industries, Ltd. (Kyoto, Japan). 2,4-DNP, potassium hydroxide, triphenylphosphine, HCl, and high-performance liquid chromatography (HPLC)-grade 2-PrOH were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). 2-PrOH was refluxed with sodium borohydrate for 1 h and then distilled before the analysis to remove trace amounts of carbonyl compounds found in 2-PrOH. Vegetable oils such as high-oleic safflower, soybean, palm, and corn oils were provided by Ajinomoto Co. (Tokyo, Japan). Vegetable oils (250 g) were heated at 180°C with spraying water at a flow rate of 1.0 mL/min for 1 to 4 h. Frying soybean oils used for fried chicken were obtained from the Nisshin Oil Mills Ltd. (Yokosuka, Japan).

Determination of total carbonyl compounds. 2,4-DNP solution was prepared by dissolving 50 mg 2,4-DNP in 100 mL 2-PrOH containing 3.5 mL concentrated HCl. Frying oil (0.04–1.0 g) was put into a 10-mL volumetric flask, and the flask was filled to volume with 2-PrOH containing triphenylphosphine (0.4 mg/mL) to reduce hydroperoxide formation. Standard aldehydes and ketones were weighed in a volumetric flask and dissolved in 2-PrOH at concentrations of 50 to 500 μM. Standard carbonyl compound solution or oil solution (1 mL) was put in a 15-mL test tube and then mixed with 1 mL 2,4-DNP solution. The test tube was stoppered and heated for 20 min at 40°C. It was cooled in water, and 8 mL of 2% KOH in 2-PrOH was added. The test tube was centrifuged at 2000 × *g* for 5 min at room temperature. The absorption spectrum (350–500 nm) of the upper layer was measured with a JASCO Ubest-35 spectrophotometer (Tokyo, Japan).

The Standard Methods for the Analysis of Fats, Oils, and Related Materials (2.5.4-1996) proposed by Japan Oil Chemists' Society (6) was used as a typical conventional method to determine total carbonyl compounds in oils.

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TABLE 1
Molar Absorption Coefficients of 2,4-Dinitrophenylhydrazone Derivatives of Carbonyl Compounds

	Wavelength (nm)			
Carbonyl compounds	410	415	420	425
			× 10^4	
n-Pentanal	1.298	1.395	1.471	1.606
n-Hexanal	1.203	1.352	1.424	1.476
n-Heptanal	1.307	1.409	1.489	1.613
n-Octanal	1.325	1.416	1.486	1.631
n-Nonanal	1.282	1.384	1.460	1.578
n-Decanal	1.257	1.350	1.427	1.559
2-Pentenal	1.190	1.379	1.574	1.869
2-Hexenal	1.235	1.432	1.631	1.916
2-Heptenal	1.233	1.437	1.646	1.974
2-Octenal	1.092	1.242	1.392	1.666
2-Nonenal	1.235	1.420	1.610	1.896
2-Decenal	0.996	1.147	1.297	1.535
2,4-Heptadienal	1.153	1.309	1.487	1.804
2,4-Nonadienal	1.009	1.169	1.350	1.648
2,4-Decadienal	0.900	1.135	1.288	1.407
2-Heptanone	1.375	1.451	1.534	1.663
2-Octanone	1.362	1.463	1.539	1.680
Average ± SD (× 10^4)	1.203 ± 0.130	1.341 ± 0.118	1.471 ± 0.117	1.678 ± 0.156
Average/SD × 100%	10.8	8.8	7.9	9.3

RESULTS AND DISCUSSION

At first, we tried to select the optimal wavelength to determine the carbonyl compound concentration in 2-PrOH. The conventional method uses benzene as a solvent and measures absorbance at 440 nm, while Yukawa *et al.* (5) reported that the optimal wavelength was 425 nm in ethanol. The optimal wavelength is probably solvent-dependent. Therefore, we measured absorption spectra (350–500 nm) after the incubation of 2,4-DNP with *n*-alkanal, 2-alkenal, and 2,4-alkadienal in 2-PrOH. As shown in Figure 1, absorption maximum varied by the source of the carbonyl compounds. The *n*-alkanals, such as *n*-hexanal, *n*-heptanal, and *n*-decanal, had absorption maxima at 430 nm. On the other hand, 2-alkenals, such as 2-hexenal, 2-heptenal, and 2-decenal, and 2,4-alkadienals, such as 2,4-heptadienals and 2,4-decadienals, had absorption maxima at 450 and 475 nm, respectively. The absorption maximum of aldehydes depended on their unsaturation but not on their carbon length. The 2-ketones, such as 2-heptanone and 2-octanone, had absorption maxima at 430 nm, similar to the *n*-alkanals. However, absorption spectra of saturated and unsaturated aldehydes and ketones in 2-PrOH crossed at 410–425 nm.

The molar absorption coefficients of 2,4-DNPH derivatives from various aldehydes and ketones in 2-PrOH were estimated at 410, 415, 420, and 425 nm. Table 1 shows the molar absorption coefficients of 2,4-DNPH derivatives from six saturated aldehydes, nine unsaturated aldehydes, and two ketones at 410–425 nm. The molar absorption coefficients of 2,4-DNPH derivatives from alkadienals (2,4-nonadienal and 2,4-decadienal) were somewhat lower than those from alkanals and alkenals at the range of these wavelengths, whereas ketones (2-heptanone and 2-octanone) had similar molar coefficients as alkanals. The molar absorption coefficients of 2,4-DNPH derivatives from saturated and unsaturated aldehydes increased with measuring wavelength. The average at 425 nm was 1.68

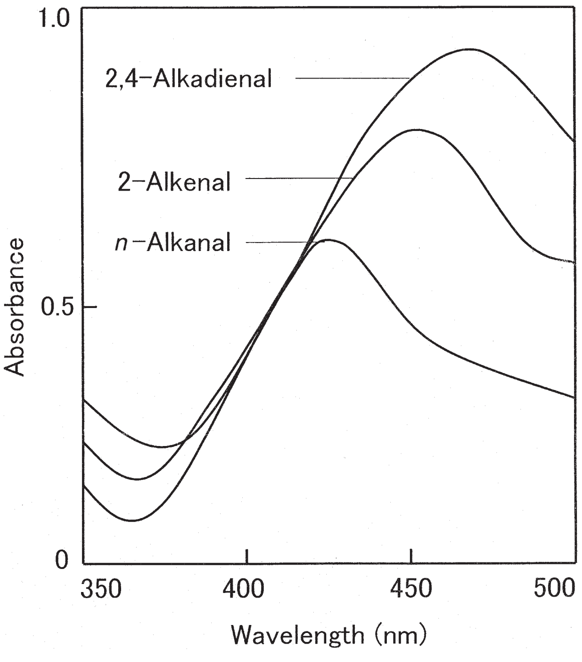

FIG. 1. Absorption spectra of 2,4-dinitrophenylhydrazone (DNPH) derivatives of *n*-alkanal, 2-alkenal, and 2,4-alkadienal.

× 10^4, and this average was higher than those at 420 (1.47 × 10^4), 415 (1.34 × 10^4), and 410 nm (1.20 × 10^4). However, the CV (coefficient of variation: average/SD × 100%) was 8% at 420 nm, and it was the lowest among the four wavelengths measured. Based on these observations, 420 nm was used as the wavelength in the modified method using 2-PrOH.

Absorption spectrum of frying oils after reaction with 2,4-DNP was measured to determine the carbonyl compound content in the frying oils. Figure 2 shows the absorption spectrum (350–500 nm) of heated soybean oil (CV = 22.2 meq/kg) after reaction with 2,4-DNP. An absorption maximum was observed at 470–480 nm. Similar absorption spectra were observed for heated corn, high-oleic safflower, and palm oils. These observations suggest that alkadienal was one of the main carbonyl compounds in heated vegetable oil. Therefore, we used 2,4-decadienal as a standard to estimate total carbonyl compounds in frying fats and oils because it is produced from linoleic acid in vegetable oils during the thermal oxidation. We confirmed 2,4-decadienal as major aldehydes in heated and frying vegetable oils by HPLC analysis (data not shown).

2,4-Decadienal in 2-PrOH solutions (50–500 µM) was reacted with 2,4-DNP, and the absorbance at 420 nm was measured. The absorbance at 420 nm increased linearly with an increase in the concentration of 2,4-decadienal (Fig. 3). A good correlation (r = 0.998) was observed between the absorbance at 420 nm and the molar concentration. The detection limit was 10 µM. Similar calibration curves were obtained for other aldehydes and ketones (data not shown). Moreover, the calibration curve was not affected by the presence of fats and oils. These results demonstrate that an accurate determination can be made at 420 nm using 2-PrOH.

The modified method and the conventional method (4) were used for the analysis of carbonyl compounds in 24 heated vegetable oil and 3 frying oil samples. Table 2 shows an example of the carbonyl values estimated using 2-PrOH and the conventional methods for heated oil samples. In both methods, the carbonyl values were estimated using 2,4-decadienal as a standard. As shown in Table 2, the carbonyl values using the modified method were the same as those using the conventional method for heated frying oils. Figure 4 shows the correlation curve obtained by the least squares method between carbonyl values of heated oils estimated by the two methods. The slope of the correlation curve was 0.995, and was almost equal to 1 (the expected value). There was a good correlation (r = 0.986) for carbonyl values estimated with the two methods at the range of 8 to 85 meq/kg for heated oils. These observations suggest that the 2-PrOH method could be substituted for the conventional method using benzene. There is an additional advantage of reduced solvent usage with the modified method.

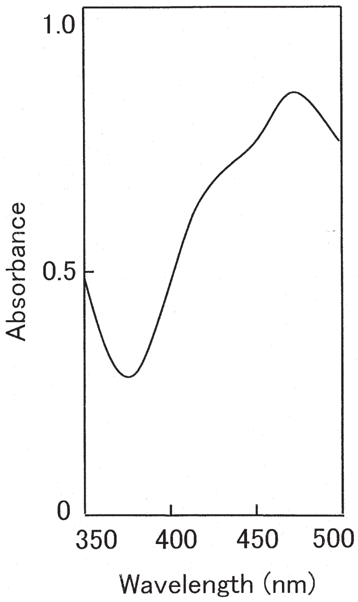

FIG. 2. Typical absorption spectrum of heated soybean oil after the reaction with 2,4-dinitrophenylhydrazine.

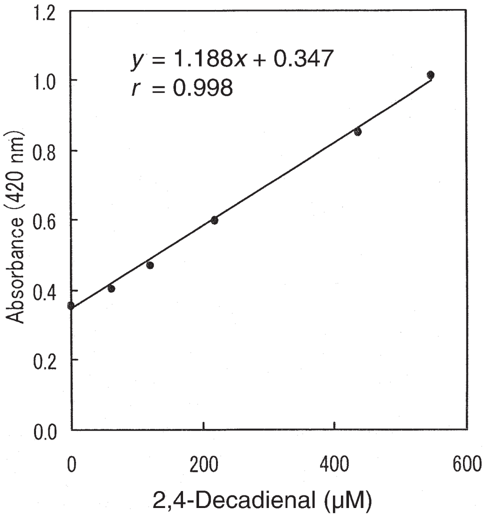

FIG. 3. Calibration curve of 2,4-DNPH derivative of 2,4-decadienal. See Figure 1 for abbreviation.

TABLE 2
Carbonyl Value of Heated and Frying Vegetable Oils Determined by 2-Propanol (2-PrOH) and Conventional Methods

	Carbonyl value (meq/kg)	
	2-PrOH	Conventional
Heated oil		
Soybean	10.9	12.1
	29.3	30.8
Palm	8.3	8.1
	17.3	17.2
High-oleic safflower	20.2	20.2
	40.0	38.9
	83.0	85.3
Corn	10.3	9.2
	20.9	19.4
Frying oil		
Soybean	23.0	22.7
	31.4	31.8
	52.7	54.3

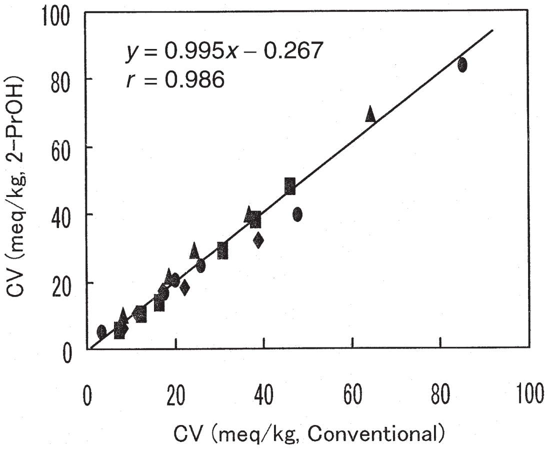

FIG. 4. Correlation curve between carbonyl values in heated soybean (■), high-oleic safflower (●), corn (▲), and palm (♦) oils estimated by the conventional and 2-propanol (2-PrOH) methods.

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