Determination of Total Carbonyl Compounds in Aqueous Media

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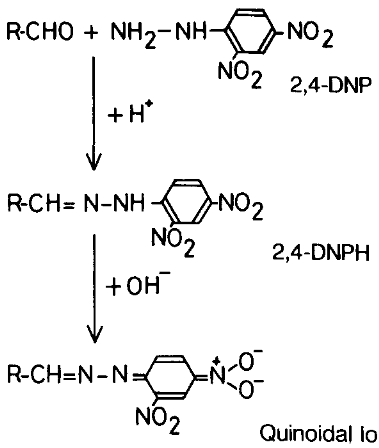
A method for the quantitative determination of total carbonyl compounds in aqueous media has been developed. The analysis is based on the reaction of 2,4-dinitrophenylhydrazine with aldehydes and the subsequent formation of the quinoidal ion under basic conditions. The aldehydes react with 2,4-dinitrophenylhydrazine in both ethanol and aqueous-ethanol media. The optimal wavelength to determine the total carbonyl compounds is 425 nm where the molar absorption coefficient of saturated aldehydes equals that of unsaturated aldehydes. The method can be used to determine all aldehydes in aqueous food media.

KEY WORDS: Aldehyde, carbonyl compound, carbonyl value, 2,4-dinitrophenylhydrazine, lipid peroxidation.

Various carbonyl compounds are formed during lipid oxidation in foods, and they are often major contributors to rancid and unpleasant flavors associated with oxidized oils (1). Carbonyl compounds also interact with other food components, resulting in further loss of food quality (2–5). Accordingly, the determination of total carbonyl compounds is important in estimating food quality.

The most reliable and widely used methods for measuring carbonyl compounds are the procedures of Henick *et al.* (6) and Kumazawa and Oyama (7). However, these methods use benzene, a nonpolar solvent, and are difficult to use with aqueous lipid media, such as milk or soymilk. Lappin and Clark (8) have reported a method that determines total carbonyl compounds in aqueous media based on the same principle, but in which methanol is used as the solvent. The procedure is shown in Scheme 1. In the first step, carbonyl compounds react with 2,4-dinitrophenylhydrazine (2,4-DNP) under acidic conditions to form 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives. In the second step, the 2,4-DNPHs are reacted with alkali to form the quinoidal ion, which causes the medium to turn wine-red. The total carbonyl content of the solution is then determined by measuring the absorption at 480 nm. Lappin and Clark (8) reported that the molar absorption coefficient for all aldehydes and ketones depends solely on the total number of carbonyl groups present in the medium. However, they did not fully consider the application of their method to an aqueous medium.



SCHEME 1

Preliminary experiments showed that with their method, aldehydes did not fully form 2,4-DNPH derivatives, and different types of aldehydes had different molar absorption coefficients at 480 nm. In this paper we have modified the method of Lappin and Clark (8) and tried to establish a more accurate method for determining total carbonyl compounds in aqueous media.

EXPERIMENTAL PROCEDURES

Materials. Pentanal and hexanal were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Heptanal and 2*E*-nonenal were from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). 2*E*-Hexenal, 4*Z*-decenal and 2*E*,4*E*-decadienal were from Aldrich Chemical Co., Inc. (Milwaukee, WI). Other chemicals were reagent-grade purchased from either Nacalai Tesque or Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methanol was rendered carbonyl-free as described by Lappin and Clark (8). The 2,4-DNPH derivatives of aldehyde standards were synthesized according to the procedure of Shriner *et al.* (9). Soybeans (*Glycine max* var. Tsuru-no-ko) were obtained from Mizuno Seedling Co. (Kyoto, Japan). Milk and soymilk were purchased from a local market in Nara, Japan.

Preparation of the soybean homogenate. Soybean seeds (5 g) were soaked overnight in water at 4°C. The soaked seeds were homogenized in 80 mL distilled water after removal of the seed coat.

Determination of total carbonyl compounds. Total carbonyl compounds were determined according to the procedure of Lappin and Clark (8) with methanol (original method) or ethanol (modified method) as the alcohol solvent. Acidic 2,4-DNP reagent was prepared by mixing 10 mL saturated 2,4-DNP alcohol solution and 350 μL concentrated HCl. A 1-mL sample of aldehyde (2% wt/vol in alcohol or aqueous solution) and 1 mL 2,4-DNP reagent were added to a test tube. The tube was stoppered and heated for 30 min at 50°C, cooled in an ice bath, and 5.0 mL of 10% KOH/80% alcohol solution was added. The tube was then centrifuged at 1300 × *g* for 20 min at 20°C, and the absorption spectrum (350–500 nm) was determined with a Shimadzu (Kyoto, Japan) UV-160 spectrophotometer. An alcohol solution of 2,4-DNPH derivative of each aldehyde was used as standard.

RESULTS AND DISCUSSION

The method of Lappin and Clark (8) requires two steps (as shown in Scheme 1); one is 2,4-DNPH formation and the other is formation of the quinoidal ion. However, it was not clear whether the reported reaction conditions

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TABLE 1

Effect of Solvent on the Reactivity of Aldehydes and 2,4-Dinitrophenylhydrazine (2,4-DNP)

Solvent[a]		% Reaction[b]			
2,4-DNP	Aldehyde	Hexanal	4*Z*-Decenal	2*E*-Hexenal	2*E*,4*E*-Decadienal
MeOH	MeOH	70	68	99	103
	H_2O	102	95	98	95
EtOH	EtOH	97	102	98	100
	H_2O	96	99	97	97

[a]Aldehyde solution (alcohol solution or 2% aqueous alcohol solution) was reacted with acidic 2,4-DNP reagent. After mixing with alcoholic KOH solution, absorption at 480 nm was measured.

[b]Percent reaction calculated by comparing the absorption of the quinoidal ion of 2,4-dinitrophenylhydrazone (2,4-DNPH) with that of the same molar amount of previously synthesized 2,4-DNPH derivative.

were optimal for all carbonyl compounds. Therefore, we used the procedure of Lappin and Clark (8), aldehyde in methanol solution to form the quinoidal ion *via* 2,4-DNPH, to examine whether or not the reported reaction conditions are optimal. By absorption measurement at 480 nm, we compared the absorbances of quinoidal ions from aldehyde solutions that were obtained from 2,4-DNPH standards, and from this comparison we calculated percent reaction. The conjugated enals (α,β-unsaturated aldehydes), 2*E*-hexenal and 2*E*,4*E*-decadienal, reacted almost 100% (Table 1). However, for the saturated aldehyde, hexanal, only about 70% reaction was obtained (Table 1). Other saturated aldehydes behaved similarly (data not shown). The non-α,β-unsaturated 4*Z*-decenal did not react fully either, and the extent of the reaction was about 70%. These results suggested that α,β-unsaturated aldehydes react completely to form 2,4-DNPH, whereas other aldehydes do not (under the above conditions). However, the reaction of hexanal and 4*Z*-decenal was almost 100% when ethanol was used as the solvent (Table 1). The reaction of 2*E*-hexenal and 2*E*,4*E*-decadienal was also 100%.

To examine the application of this method to an aqueous medium, aqueous aldehyde solutions were used and aldehyde reactivity was determined (Table 1). All aldehydes reacted fully under those conditions. These results show that aldehydes react fully with 2,4-DNP reagent either in ethanol or in aqueous solution with ethanol as added solvent.

Wavelength. Lappin and Clark (8) reported that carbonyl compounds have the same molar absorption coefficient at 480 nm regardless of structure. However, we determined the molar absorption coefficient of several 2,4-DNPH aldehyde derivatives and found that they have different molar absorption coefficients at this wavelength (data not shown). To determine the optimal wavelength for the quantitation of aldehydes, the quinoidal ion in alkaline methanol solution was derived from the 2,4-DNPH derivatives of various aldehydes by the method of Lappin and Clark (8), and the absorption spectra (350–500 nm) were measured (Fig. 1A). The molar absorption coefficient of the aldehydes at 480 nm were different, and this suggested that 480 nm may not be the optimal wavelength. On the other hand, the absorption spectra of saturated and unsaturated aldehydes crossed at 390 nm (Fig. 1A). Therefore, determination at 390 nm is necessary to measure mixtures of different structural types of aldehydes when using methanol as the solvent.

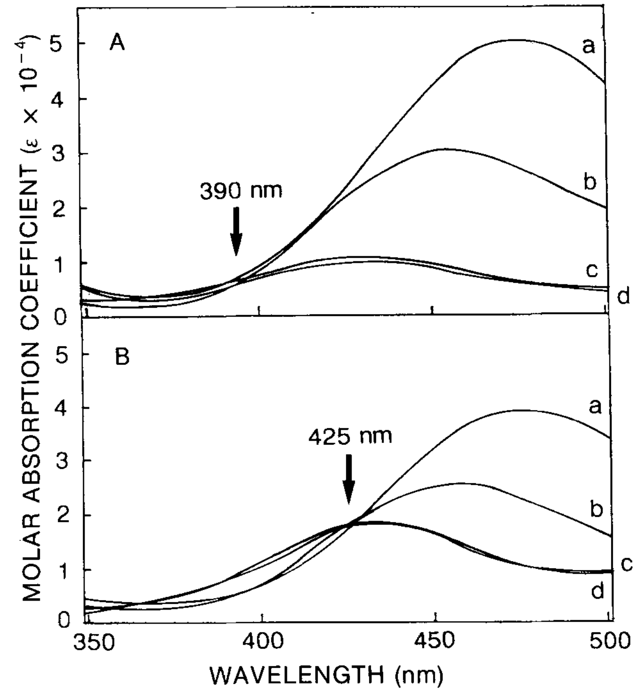

FIG. 1. **Absorption spectrum of 2,4-dinitrophenylhydrazone derivatives of aldehydes. (A) In methanol; (B) in ethanol; a, 2*E*,4*E*-decadienal; b, 2*E*-hexenal; c, 4*Z*-decenal; and d, hexanal.**

In the modified method, with ethanol solvent, the absorption spectra of saturated and unsaturated aldehydes crossed at 425 nm (Fig. 1B). Therefore, 425 nm is the optimal wavelength in the modified method with ethanol as solvent because the molar absorption coefficient at 425 nm was three times that at 390 nm. The use of ethanol as solvent therefore makes it possible to determine total carbonyl compounds more accurately, and with greater sensitivity, than when methanol is used as solvent.

Table 2 gives the molar absorption coefficients of various aldehyde 2,4-DNPHs with ethanol as the solvent. The

TABLE 2

Molar Absorption Coefficient of 2,4-DNPH Derivatives of Various Aldehydes[a]

Aldehyde	Wavelength (nm) 425	440	480
		ε × 10^{-4}	
Pentanal	1.76 (97)[b]	1.76 (97)	0.96 (97)
Hexanal	1.81 (100)	1.81 (100)	0.99 (100)
Heptanal	1.78 (98)	1.79 (99)	1.00 (101)
2*E*-Hexenal	1.82 (101)	2.37 (131)	2.18 (218)
2*E*-Nonenal	1.86 (103)	2.47 (137)	2.30 (230)
4*Z*-Decenal	1.75 (97)	1.78 (98)	1.04 (104)
2*E*,4*E*-Decadienal	1.74 (96)	2.64 (146)	3.97 (397)

[a]2,4-DNPH derivatives of aldehydes were reacted according to Lappin and Clark (Ref. 8) with ethanol as the solvent. Abbreviation as in Table 1.

[b]Numbers in parentheses are percentages. Percentages are normalized to hexanal.

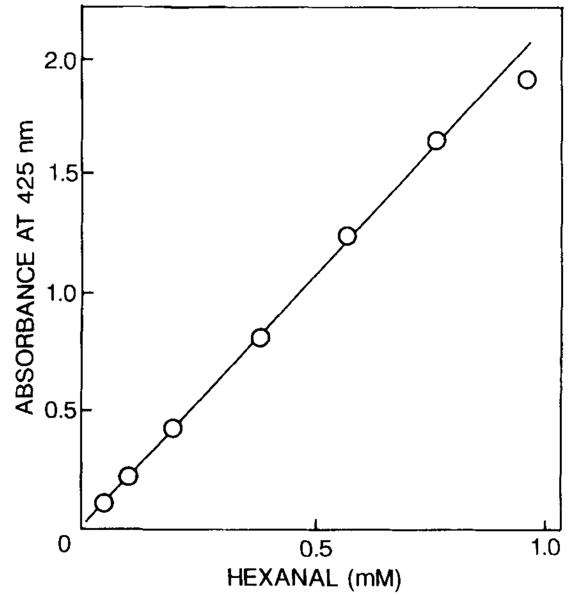

FIG. 2. Calibration curve of hexanal at 425 nm.

TABLE 3

Determination of Authentic Aldehyde Mixtures at Various Wavelengths[a]

	Aldehydes	Added		Determined wavelength (nm)		
				425	440	480
		(μM)			(μM)	
A	Hexanal	100				
	2*E*-Hexenal	100	300	303[b]	390	741
	2*E*,4*E*-Decadienal	100		(101)	(130)	(247)
B	Hexanal	50				
	2*E*-Hexenal	100	300	303	420	933
	2*E*,4*E*-Decadienal	150		(101)	(140)	(311)
C	Hexanal	100				
	2*E*-Hexenal	150	300	297	375	663
	2*E*,4*E*-Decadienal	50		(99)	(125)	(221)
D	Hexanal	150				
	2*E*-Hexenal	50	300	288	363	666
	2*E*,4*E*-Decadienal	100		(96)	(121)	(222)

[a] 2,4-Dinitrophenylhydrazone derivative of hexanal was used as standard.

[b] Numbers in parentheses indicate the percentage of total aldehyde added.

molar absorption coefficients at 480 nm of unsaturated aldehydes were two to three times that of saturated aldehydes. However, the molar absorption coefficient at 425 nm was almost the same, regardless of aldehyde type. Kumazawa and Oyama (7) reported that the absorption spectra of 2,4-DNPH derivatives of aldehydes crossed at 440 nm in benzene solvent. However, molar absorption coefficients at 440 nm for unsaturated aldehydes were about 1.5 times those of saturated aldehydes in the aqueous system. This difference is due to the solvent used for the measurement. These results demonstrate that accurate determination can be made at 425 nm in our method with ethanol as the solvent.

Calibration curve. Aqueous hexanal solutions (25–800 μM) were reacted by the modified method and the absorbance at 425 nm was measured. Good correlation was observed between the absorbance at 425 nm and the molar concentration (Fig. 2). A similar relationship was found with other aldehydes.

Determination of aldehyde mixture. Table 3 lists the results of our method in the analysis of authentic aqueous aldehyde mixtures. The samples contained hexanal as alkanal, 2*E*-hexenal as alkenal and 2*E*,4*E*-decadienal as alkadienal. The amount of aldehydes found at 440 nm and 480 nm were 1.2 to 3 times greater than the amount of aldehydes added. However, good agreement was found between the amount of aldehydes added with that determined at 425 nm.

Determination of carbonyl compounds in aqueous foods. Table 4 shows the application of the method to the

TABLE 4

Determination of Authentic Aldehyde Mixtures in Aqueous Food Media at 425 nm

	Aldehydes	Added		Determined[a] sample added			
				Control[b]	Milk	Soymilk	Soybean homogenate
		(μM)				(μM)	
A	Hexanal	100					
	2*E*-Hexenal	100	300	303	288	291	291
	2*E*,4*E*-Decadienal	100		(101)[c]	(96)	(97)	(97)
B	Hexanal	50					
	2*E*-Hexenal	100	300	303	288	291	291
	2*E*,4*E*-Decadienal	150		(101)	(96)	(97)	(97)
C	Hexanal	100					
	2*E*-Hexenal	150	300	297	279	282	282
	2*E*,4*E*-Decadienal	50		(99)	(93)	(94)	(94)
D	Hexanal	150					
	2*E*-Hexenal	50	300	288	271	274	271
	2*E*,4*E*-Decadienal	100		(96)	(90)	(91)	(90)

[a] The amount of aldehydes exogenously added was determined. 2,4-Dinitrophenylhydrazone derivative of hexanal was used as the standard.

[b] Control is authentic aldehyde mixture (see Table 3).

[c] Numbers in parentheses indicate the percentage of total aldehyde added.

analysis of authentic aqueous aldehyde mixtures in the presense of aqueous foods such as milk, soymilk or soybean homogenate. The amount of aldehyde mixtures exogenously added was determined. Good agreement was found between the amount of aldehydes added and that determined at 425 nm. These results suggest that other food components do not affect the formation of 2,4-DNPH or the subsequent color development by alkali, and demonstrate the successful application of this method to the determination of aldehyde mixtures in aqueous foods as well.

In this paper we have established a method for the determination of total carbonyl compounds in aqueous media. The method is applicable to several types of aldehydes found in aqueous foods.

REFERENCES

1. Frankel, E.N., in *Flavor Chemistry of Fats and Oils*, edited by D.B. Min, and T.H. Smouse, American Oil Chemists' Society, Champaign, 1985, p. 1.
2. Kanazawa, K., G. Danno and M. Natake, *J. Nutr. Sci. Vitaminol. 21*:373 (1975).
3. Gardner, H.W., *J. Agric. Food Chem. 27*:220 (1979).
4. Pokorný, J., W. Janitz, I. Víden, J. Velíšek, H. Valentová and J. Davídek, *Nahrung 31*:63 (1987).
5. Hidalgo, F.J., and J.E. Kinsella, *J. Agric. Food Chem. 37*:860 (1989).
6. Henick, A.S., M.F. Benca and J.H. Mitchell, Jr., *J. Am. Oil Chem. Soc. 31*:88 (1954).
7. Kumazawa, H., and T. Oyama, *Yukagaku (J. Jpn. Oil Chem. Soc.) 14*:167 (1965).
8. Lappin, G.R., and L.C. Clark, *Anal. Chem. 23*:541 (1951).
9. Shriner, R.L., R.C. Fuson and D.Y. Curtin, in *The Systematic Identification of Organic Compounds*, 4th edn., Wiley and Sons, New York, 1956, p. 219.

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