A Modified Method to Estimate Total Carbonyl Compounds in Frying Oils Using 1-Butanol as a Solvent

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Abstract: A modified method has been developed for quantitative determination of total carbonyl compounds in frying oils without using benzene. The analysis is done by the reaction of 2, 4-dinitrophenylhydrazine (2, 4-DNPH) with aldehydes and ketones in 1-butanol (1-BuOH) for 20 min at 40°C. The optimal wavelength to determine the total carbonyl compounds is 420 nm where the 2, 4-dinitrophenylhydrazone (2, 4-DNP) derivatives from saturated and unsaturated aldehydes and ketones have the same molar absorption coefficient. When the modified method was applied for the estimation of total carbonyl compounds in frying oils by using 1-BuOH instead of benzene as a solvent, a good correlation with the conventional method was obtained. It was expected that the modified method could be substituted for the conventional method and be useful for evaluating the quality of frying oils.

Key words: carbonyl compound, carbonyl value, 2, 4-dinitrophenylhydrazine, frying oil, lipid peroxidation

1 Introduction

The determination of carbonyl compounds in frying oils is very important for evaluating the quality of frying fats and oils because carbonyl compounds formed during their thermal oxidation often contribute to rancid and unpleasant flavors, and reduce the nutritional value of fried foods. Therefore, the carbonyl value (CV: 2.5-4-1996) which represents the total carbonyl compounds in fats and oils has been adopted in the Standard Methods for the Analysis of Fats, Oils and Related Materials edited by the Japan Oil Chemists’ Society (1). In the
standard method, carbonyl compounds present in fats and oils react with 2, 4-dinitrophenylhydrazine (2, 4-DNPH) to form the corresponding 2, 4-dinitrophenyl-hydrazone (2, 4-DNP) derivatives, which are turned into quinoidals colored wine-red in alkaline solution. The total carbonyl compounds in solutions are then quantified by measuring the absorption at 440 nm. Henick et al. (4), Kumazawa and Oyama (5) and Miyashita et al. (6) also developed the method to quantify carbonyl compounds in fats and oils by using the spectrophotometer, but they use toxic benzene as a solvent. Moreover, the conventional methods are not appropriate for measuring lots of samples since a 50 mL-Erlenmeyer flask that takes up much space is used as a vessel.

We previously developed a method for the estimation of total carbonyl compounds in heated and frying oils by using 2-propanol as a solvent (2, 3). The modified method is safe, and it uses a smaller amount of solvent than the standard method, but it is necessary to remove endogenous carbonyl compounds from 2-propanol before the reaction.

We found that the commercial 1-butanol (1-BuOH) had less carbonyl compounds and required no additional treatment for purification. This paper describes a modified method for the estimation of total carbonyl compounds in frying oils with 1-BuOH as the solvent.

2 Experimental

2-1 Materials

Aldehydes and ketones such as n-heptanal, n-octanal, n-nonanal, n-decanal, 2-heptenal, 2-octenal, 2-nonenal, 2-decenal, 2, 4-heptadienal, 2, 4-nonenal, 2, 4-decadienal, 2-heptanone, and 2-octanone were of extra-pure grade and were purchased from Wako Pure Chemical Industries, Ltd. (Kyoto, Japan). 2, 4-Dinitrophenylhydrazine (2, 4-DNPH), potassium hydroxide (KOH), HCl and spectrophotometric analysis-grade 1-BuOH were purchased from Wako Pure Chemical Industries, Ltd. Vegetable oils such as soybean, rapeseed, palm, olive, corn and blended oils were provided by The Nisshin OilliO, Ltd. (Yokosuka, Japan). Frying vegetable oils were prepared by frying chicken nuggets and potato croquettes at 180°C.

2-2 Determination of Total Carbonyl Compounds

A 2, 4-DNPH solution was prepared by dissolving 50 mg 2, 4-DNPH in 100 mL 1-BuOH containing 3.5 mL concentrated HCl. Frying oil (20-200 mg) was put into a 10 mL-volumetric flask, and the flask was filled up with 1-BuOH. Standard aldehydes and ketones were weighed in a volumetric flask and dissolved in 1-BuOH at concentrations of 50 to 400 μM. One milliliter of standard carbonyl compound solutions or oil solutions were put in a 15 mL-test tube, and then were mixed with 1 mL 2, 4-DNPH solution. The test tube was stoppered and heated for 20 min at 40°C. Temperature and reaction time were varied to observe their effects on the reaction of 2, 4-DNPH with carbonyl compounds. The test tube was cooled to room temperature in a tap water. It was vigorously shaken after 8 mL of 8% KOH in 1-BuOH was added to it. It was centrifuged at 3,000 rpm for 5 min at room temperature. The absorption spectrum (420 nm) of the upper layer was measured with a spectrophotometer (ex. JASCO SSE-343).

The carbonyl value (2. 5. 4-1996) in “The Standard Methods for the Analysis of Fats, Oils and Related Materials” proposed by the Japan Oil Chemists’ Society (JOCS) was used as a typical conventional method to determine total carbonyl compounds in oils (1).

All data were expressed as the average for triplicate experiments. As for the collaborative study, the statistical analysis was performed by two-way ANOVA.

3 Results and Discussion

3-1 Absorption Spectra of Carbonyl Compounds Reacted with 2, 4-DNPH

At first we tried to select the optimal wavelength to determine the carbonyl compounds in 1-BuOH. The conventional method uses benzene as a solvent and measures absorbance at 440 nm, but the optimal wavelength may depend on the solvent. Therefore, we measured absorption spectra at wavelengths between 350 and 550 nm after the incubation of 2, 4-DNPH with n-alkanals, 2-alkenals and 2, 4-alkadienals in 1-BuOH. As shown in Fig. 1, the absorption maximum varied according to the carbonyl compound variety. n-Alkanal had an absorption maximum at 425 nm, while 2-alkenal and 2, 4-alkadienal had absorption maxima at 450 nm and 470 nm, respectively. The absorption maximum of aldehydes depended on their unsaturation but not on the
Determination of Carbonyl Compounds in Oil

2-Ketones such as 2-heptanone and 2-octanone had absorption maxima at 425 nm as n-alkanals, but the absorption spectra of saturated and unsaturated aldehydes and ketones in 1-BuOH crossed at 420 nm. These spectrophotometric characteristics in 1-BuOH were similar to those in 2-propanol (2).

The molar absorption coefficients of 2, 4-DNP derivatives from various aldehydes and ketones in 1-BuOH estimated at 420 nm are shown in Table 1. The molar absorption coefficient of 2, 4-DNP derivatives from n-heptanal was lower than those of other n-alkanals, 2-alkenals and 2, 4-decadienals, whereas that of 2-octanone was somewhat higher. But the molar absorption coefficients of 2, 4-DNP derivatives from saturated and unsaturated aldehydes and ketones were in the range 1.50 and 1.71 × 10^4 except for n-heptanal (1.32 × 10^4). The average was 1.62 × 10^4 and the standard deviation was 6 × 10^2. From these observations, the molar absorption coefficients of 2, 4-DNP derivatives of carbonyl compounds at 420 nm were almost the same and independent of the varieties of carbonyl compounds. We determined that the measuring wavelength was 420 nm for the modified method by using 1-BuOH as a solvent.

### Table 1 Molar Absorption Coefficients of 2, 4-Dinitrophenyl-hydrazone Derivatives of Carbonyl Compounds.

<table>
<thead>
<tr>
<th>Carbon chain length</th>
<th>n-Alkanal</th>
<th>2-Alkenal</th>
<th>2, 4-Alkadienal (× 10^4)</th>
<th>2-Ketone</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.317</td>
<td>1.569</td>
<td>1.500</td>
<td>1.645</td>
</tr>
<tr>
<td>8</td>
<td>1.610</td>
<td>1.679</td>
<td>1.713</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.605</td>
<td>1.695</td>
<td>1.619</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.507</td>
<td>1.670</td>
<td>1.545</td>
<td></td>
</tr>
</tbody>
</table>

with 2, 4-DNPH was measured to characterize the carbonyl compounds contained in the frying oils. Figure 2 shows the absorption spectrum (350-550 nm) of frying soybean oil (CV = 7.8) after the reaction with 2, 4-DNPH. The absorption maximum was observed at 440 nm. Similar absorption spectra were observed for frying rapeseed and blended oils. These observations showed that alkenals were contained as main carbonyl compounds in frying vegetable oil. Therefore, we determined 2-decenal to be the standard aldehyde to estimate total carbonyl compounds in frying fats and oils, because it was produced from oleic acid in vegetable oils during the thermal oxidation (7).

### 3.3 Effects of Temperature and Reaction Time

Temperature and reaction time varied from 25 to 60°C and 10 to 60 min, respectively, to determine their effects on absorbance after the reaction with 2, 4-DNPH. The absorbance of blank, 2-decenal (200 μM) and frying soybean oil after the reaction with 2, 4-DNPH for 20 min depended on the temperature, as shown in Figure 3. The absorbance of blank and 2-decenal was increased at 60°C, whereas that of the soybean oil was slightly decreased at 60°C. Among tested temperatures, there was no difference between absorbance measured at 40 and 50°C.

Figure 4 shows the absorbance of blank, 2-decenal and frying soybean oil after different reaction times at 40°C. The absorbance did not remarkably change within the range 10 to 60 min. The reaction of carbonyl compounds with 2, 4-DNPH seemed to be rapid and independent of the reaction time.

From these results, the optimal temperature and reaction time were determined to be 40°C and 20 min.
3.4 Calibration Curve of 2-decenal

2-Decenal in 1-BtOH solutions (50-400 μM) was reacted with 2, 4-DNPH and the absorbance at 420 nm was measured. It increased linearly with the concentration of 2-decenal (Fig. 5). A close correlation ($r^2 = 0.999$) was observed between the absorbance at 420 nm and the molar concentration. The detection limit was 10 μM, because 2-decenal at the concentration below 10 μM had the same absorbance (0.28-0.29) as the control (0.282) after the reaction with 2, 4-DNPH. Similar calibration curves were obtained for other aldehydes and ketones. Moreover, the calibration curve was not affected by the presence of fats and oils. These results show that accurate determination can be made at 420 nm with the method using 1-BuOH.

3.5 Estimation of Total Carbonyl Compounds in Frying Oils

We measured total carbonyl compounds in different amounts of frying oil to determine the effects of amounts of frying oils on the CV. Frying soybean oil was measured at levels of 50, 100 and 200 mg/10 mL in the modified method. The total carbonyl compounds in frying soybean oil were estimated as the level (μmol) of the corresponding 2-decenal per gram of oil when 2-decenal was used as a standard aldehyde. As a result, the amounts of frying oil did not affect the CV, since 50, 100 and 200 mg/10 mL of soybean oil showed the same CVs of 11.6, 12.1 and 11.9 μmol/g, respectively.

3.6 Collaborative Study

The modified method was applied to different kinds of frying oils. Total carbonyl compounds were measured for 9 frying vegetable oils by 7 laboratories with the modified method. Three rapeseed oils (rapeseed-1, 2, 3), three soybean oils (soybean-1, 2, 3), two blended oils (blend-1, 2) and palm oil (palm) after frying were used for analytical sample oils. Table 2 shows the CV of frying vegetable oils estimated by different laborato-
Determination of Carbonyl Compounds in Oil

The CVs of rapeseed-1, rapeseed-2, rapeseed-3, soybean-1, soybean-2, soybean-3, blend-1, blend-2 and palm oils were 6.6 ± 0.5, 14.2 ± 1.2, 24.7 ± 1.9, 34.3 ± 2.4, 80.8 ± 5.5, 25.6 ± 2.7, 12.1 ± 1.3, 20.3 ± 2.1 and 83.4 ± 8.7 μmol/g, respectively, as shown in Table 3. The CV obtained by the modified method was higher than that by the conventional method (JOCS method). This is due to the different definition of CV. The CV is expressed as the level of the corresponding 2-decenal in oil (μmol/g) in the modified method, whereas it is expressed as the absorbance at 440 nm of oil after the reaction with 2, 4-DNPH in the JOCS method. The coefficient of variation (standard deviation/average × 100%) was 6.8-10.9% (8.9% as average) in the modified method. The standard deviation was somewhat higher with frying oil with high CV such as palm oil due to the small sampling. But no significant difference was observed between the CVs obtained by 7 laboratories, although they used different types of spectrophotometers. The modified method was found to be independent of the type of spectrophotometer, and it had considerable accuracy and reproducibility.

Figure 6 shows the correlation curve obtained by the least square method for the CVs of 11 sample oils including additional corn and olive oils estimated by the two methods. There was a good correlation ($r^2 = 0.992$) for the CV of frying oils estimated by the two methods. The CV of the oils with a high melting point such as palm oil could also be measured by the modified method. Hydroperoxide did not seem to affect the CV of frying oil because soybean-3 oil with a peroxide value (PV) of 20 did not always have a higher CV than the expected value. These observations showed that the modified method was hardly affected by the amount, variety or PV of the frying oils. The CV obtained by the modified method could be converted to the CV in the JOCS method by dividing by the slope (1.50) of the correlation curve of the CV obtained in the following two methods:

![Graph showing correlation curve between Carbyonyl Values of Frying Oil Estimated in the Conventional and Modified Methods.](image)

**Table 2** Carbonyl Value of Frying Oils Estimated in the Conventional and Modified Methods by 7 Laboratories.

<table>
<thead>
<tr>
<th>Frying oil</th>
<th>Conventional method$^a$</th>
<th>Modified method$^b$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Lab-1</td>
<td>Lab-2</td>
</tr>
<tr>
<td>Rapeseed-1</td>
<td>4.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Rapeseed-2</td>
<td>10.2</td>
<td>14.4</td>
</tr>
<tr>
<td>Rapeseed-3</td>
<td>18.1</td>
<td>25.7</td>
</tr>
<tr>
<td>Soybean-1</td>
<td>23.0</td>
<td>34.4</td>
</tr>
<tr>
<td>Soybean-2</td>
<td>54.7</td>
<td>85.1</td>
</tr>
<tr>
<td>Soybean-3$^e$</td>
<td>18.2</td>
<td>23.5</td>
</tr>
<tr>
<td>Blend-1$^e$</td>
<td>7.8</td>
<td>12.5</td>
</tr>
<tr>
<td>Blend-2$^e$</td>
<td>14.1</td>
<td>22.8</td>
</tr>
<tr>
<td>Palm</td>
<td>55.6</td>
<td>88.5</td>
</tr>
</tbody>
</table>

$^a$ Carbonyl value was expressed as absorbance at 440 nm per gram of oil.

$^b$ Carbonyl value was expressed as micromoles of the corresponding 2-decenal per gram of oil.

$^c$ AVG = average; SD = standard deviation.

$^d$ Frying oils had peroxide value below 5 except for soybean-3 (PV = 20).

$^e$ Blend-1 and 2 were mixtures of rapeseed, soybean and palm oils.
CV (JOCS method) = CV (modified method) / 1.50

or

= 0.67 × CV (modified method)

From these results, the modified method with 1-BuOH could be substituted for the conventional method with benzene and effective for evaluating the quality of frying fats and oils.

References