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Spectrophotometric Sequential Injection Analysis System for Estimating the Concentration of Lipid Hydroperoxides in Edible Oils

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Abstract

A sequential injection analysis (SIA) system with spectrophotometric detection was developed as an alternative method for estimating the concentration of lipid hydroperoxides, which are the primary products of the lipid peroxidation process. The lipid hydroperoxide quantification was based on a ferric thiocyanate method. Benzoyl peroxide was used to produce a standard calibration curve for the estimating the lipid hydroperoxide concentrations. The linear range was up to 0.5 mmol/L, for benzoyl peroxide standard, and the limit of detection (3S/N) was 0.015 mmol/L. The relative standard deviation, at 0.3 mmol/L, was 1.3% for 11 injections, and the recoveries were found to be in the range of 97.2–99.5%. The lipid hydroperoxide concentrations in eight edible oils determined using the proposed SIA system were significantly correlated with peroxide values obtained using a conventional American Oil Chemists' Society's method ($r = 0.987$, $n = 8$, $p < 0.01$).

Keywords Sequential injection analysis, lipid hydroperoxide, ferric thiocyanate method, peroxide value

1. Introduction

The quality of a fat or oil is defined and controlled by several physical and chemical parameters, such as its viscosity, color, turbidity, iodine value, *p*-anisidine value, saponification value. These parameters depend on the source of the oil (e.g., for a plant oil, the geographic, climatic, and agronomic growth variables affect the parameters), oil's processing and storage conditions. The fatty acid composition and the oxidative stability of a fat or oil are clearly of utmost importance to its qualities [1].

A fat or oil may be oxidized in different ways, including through autoxidation, photo-oxidation, thermal oxidation, or hydrolytic processes. All of these processes lead to the production of undesirable flavors and of products that are harmful to health.

The oxidation mechanism for an unsaturated lipid comprises three steps; initiation, propagation, and termination. Each step leads to the formation of intermediate and/or final products. Lipid hydroperoxides, which are the fundamental primary products of the lipid peroxidation process, can be changed into secondary products, such as peroxides, aldehydes, ketones, alcohols, hydrocarbons, esters, furans, and lactones [2–3]. These lipid peroxidation products are not only poisonous but also accelerate nutritional damage, increasing the rate at which fats and oils become rancid during the production, storage, and marketing processes [3–5].

Some parameters, such as the peroxide value (POV), the acid value and carbonyl value, can be used to evaluate the degree of lipid peroxidation in fats and oils. POV is one of the most important parameter, affecting the quality of fats and oils, for determining the amount of lipid hydroperoxides. It is often expressed in milliequivalents peroxide/kilogram of a fat and oil.

The iodometric titration method is an official method and is the most widely used method for determining POV [6–7]. It is based on the redox reaction between hydroperoxides in a fat or oil sample and potassium iodide (KI) under the acidic medium to form released iodine (I_2). The released iodine forms a complex with soluble starch, which acts as an indicator. The iodine is quantified by titrating with sodium thiosulfate. This method is simple and sensitive, but its accuracy depends on many parameters, such as reaction time, light, presence of oxygen, and temperature. Moreover, it is a batchwise method that requires rather large amounts of analytical sample and organic solvents (up to 5 g of a fat and oil sample and 50 mL of solvent per analysis).

To minimize the deleterious organic solvent, a Fourier transform infrared (FTIR) spectroscopic method has been developed to quantify peroxide in various samples, such as palm olein oil, crude palm oil, crude palm kernel oil, sunflower oil, olive oil, and rapeseed oil [8–12]. The FTIR method has significant advantages over the standard American Oil Chemists' Society's (AOCS) iodometric titration method in terms of the consumption of solvent and reagents and its rapidity. A chemometric approach based on partial least-squares analysis has been often used with the FTIR technique to construct a calibration model for predicting the POV in the samples. The disadvantages of this method are as follows: can be cumbersome, requires a skillful operator to construct the model, and has a relatively low degree of specificity.

Automatic systems that offer precise and accurate results, rapid yielding (giving high sample throughput) based on flow injection analysis, and several detection methods including spectrophotometry [2, 4, 5, 13], fluorometry [3], potentiometry

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with a triiodine electrode [14] and chemiluminescence [15–16], have been developed for determining the lipid hydroperoxides in oils and fats. A stable and continuous baseline is necessary when using a basic flow injection system. Thus, the reagents are consumed continuously, causing large amounts of waste generation.

In this study, a sequential injection analysis (SIA) system was developed for determining the lipid hydroperoxides in edible oils. This system used the well-established reaction; the oxidation of Fe(II) to Fe(III) by hydroperoxide and/or peroxide in the oil or fat samples. Subsequently, the colorimetric measurement of thiocyanate complex was used as the principle reaction [17–22]. In this system, benzoyl peroxide was selected as the standard peroxide for building the calibration curve that was used to determine the lipid hydroperoxide, because benzoyl peroxide is more stable, is easier to handle and reacts more rapidly than hydrogen peroxide [2].

2. Experimental

2.1 Chemicals

All of the chemical and reagent used in this study were of analytical grade, and they were used without further purification. Milli-Q water was purified using Auto Pure WQ501 (Yamato Scientific Co., Ltd., Tokyo, Japan) was used throughout the experiments.

A stock solution of 20% (w/v) SCN^- was prepared by dissolving 2 g of ammonium thiocyanate (Wako Pure Chemical Industries, Osaka, Japan) in 10 mL of MilliQ water. The working solution for each experiment was prepared by diluting the stock solution with MilliQ water to indicated concentration.

A stock solution of 12.5 mmol/L Fe(II) solution was prepared by dissolving 24.9 mg of iron(II) chloride tetrahydrate (Wako Pure Chemical Industries) in 10 mL of MilliQ water. The working Fe(II) solution for each experiment was prepared by diluting the stock solution with 3.5% HCl in 75% ethanol in proper concentration.

A standard benzoyl peroxide stock solution (10 mmol/L) was prepared by dissolving 16.5 mg of benzoyl peroxide (Sigma-Aldrich, St. Louis, MO, USA) in 99.7% isopropanol in the 10 mL volumetric flask. The working benzoyl peroxide solution for each experiment was prepared by diluting the stock solution with 5 mL of isopropanol to indicated concentrations.

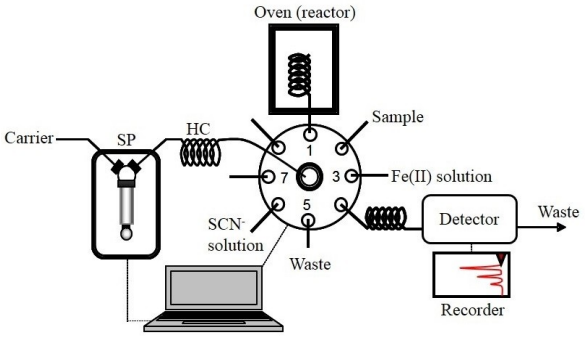

Figure 1. Proposed SIA system for determining the lipid hydroperoxides. Carrier, 80% isopropanol; SP, syringe pump; HC, holding coil.

2.2 Edible oil samples

Edible oil samples were purchased in local supermarkets and kept under refrigeration until they were analyzed. Before the analysis on the developed SIA system, each sample was diluted to reduce viscosity: 0.2 g of an oil sample was dissolved in 5 mL of isopropanol, and this solution was then diluted in four times its volume in isopropanol. The lipid hydroperoxide concentration in this solution was then determined.

2.3 AOCS official method Cd 8b-90 for the determination of lipid hydroperoxides

The POV (milliequivalents peroxide/kg: meq/kg) of each edible oil was determined according to the AOCS official method Cd 8b-90 [6] in order to compare the results obtained using our SIA system.

2.4 SIA system for determining the lipid hydroperoxides

A schematic of the proposed SIA system is shown in Figure 1. The system (MGC JAPAN Co., Ltd., Japan) consisted of a 2500 µL syringe pump, a holding coil (Teflon tube; 1.5 mm i.d., 1.5 m long), an eight ports selection valve, a reactor tube (Teflon tube; 0.8 mm i.d. , 4 m long), a mixing coil (Teflon tube; 0.8 mm i.d., 1 m long), a visible spectrophotometer S-3250 (Soma Optics, Tokyo, Japan), a recorder (Unicorder U-228; Pantos Nippon Denshi Kagaku, Kyoto, Japan), a reactor (Reactor 522; Flom, Tokyo, Japan) and a personal computer. An SIA MPV lite ever 2.55 for auto-pret system (MGC JAPAN Co., Ltd.) was used to provide the automatic control of this system.

2.5 Analytical process

Protocol sequence for determining the lipid hydroperoxides in oil samples are listed in Table 1. The analysis was started by operation of syringe pump to aspirate 100 µL of air via valve position 1 to a holding coil, followed by aspirating 40 µL of SCN^- solution via valve position 6, 30 µL of Fe(II) solution via valve position 3, and 100 µL of standard benzoyl peroxide solution or oil sample via valve position 2, 30 µL of Fe(II) solution, and 40 µL of SCN^- solution to the holding coil. After that, the aspirated solutions were dispensed through valve position 1 to a reactor by reversing a flow of the syringe pump. Three rounds of flow reversal were used to improve mixing of between analyte and reagents. The mixed solution was incubated at 60°C for 40 sec at the reactor. A 150 µL of air and mixed solution that was held in the line but outside the reactor was aspirated into a holding coil and dispensed to waste. After that, 150 µL of mixed solution was aspirated into the holding coil and dispensed toward a detector in order to detect the Fe(III)-thiocyanate complex at 500 nm. The residue of mixed solution in the line was sucked into the holding coil, followed by dispensing to waste. As a final step, it was necessary to clean the reactor to remove the remained mixed solution to prevent the contamination of reaction in a next cycle.

3. Results and Discussion

3.1 Optimization of the SIA system for determining the lipid hydroperoxides

Lipid hydroperoxide readily oxidizes Fe(II) to Fe(III), and this reaction was used as the principle to qualify the lipid hydroperoxide. It could be measured colorimetrically as the thiocyanate complex which has an absorption at 500 nm. Using the proposed SIA system (Figure 1), the effects of

Table 1. Protocol sequence of the proposed SIA system for estimating the concentration of lipid hydroperoxides.

Step	Operation	Valve position	Volume (μL)	Flow rate (μL/sec)
1	Aspiration of air	1	100	50
2	Aspiration of SCN^- to holding coil	6	40	50
3	Aspiration of Fe(II) to holding coil	3	30	50
4	Aspiration of standard or sample	2	100	50
5	Aspiration of Fe(II) to holding coil	3	30	50
6	Aspiration of SCN^- to holding coil	6	40	50
7	Dispensing aspirated zones to reactor and flow reversal at three times	1	-	-
8	Incubation of mixed solution 40 second	1	-	-
9*	Aspiration of mixed solution to holding coil	1	150	50
10	Dispensing solution to waste	5	Empty	500
11	Aspiration of mixed solution to holding coil	1	150	50
12	Aspiration of carrier to syringe	-	2350	500
13	Dispensing solution to detector	4	Empty	50
14**	Aspiration of solution to holding coil	1	500	100
15	Dispensing solution to waste	5	Empty	500
Cleaning step				
16	Aspiration of carrier to syringe	-	2000	500
17	Dispensing carrier to reactor	1	1,000	100
18**	Aspiration of solution to holding coil	1	2	50
19	Dispensing solution to waste	5	Empty	500

*This discarding step was the aspiration of the solution which was held in the line outside the reactor.
**The discarding step was the aspiration of the solution of the mixture at higher volume than the remaining solution to ensure complete removal.

different parameters, such as the aspiration sequence, the number of flow reversal, the concentrations of Fe(II) and SCN^- solution, the aspirated volume of sample, the reaction temperature, and the reaction time, were investigated.

As shown in Figure 2, three aspiration sequences were tested for determining the lipid hydroperoxides. As a result, sequence B was found to provide good sensitivity and required the shortest time to aspirate all of the reagents onto the holding coil. Therefore, the aspiration sequence B was selected for further experiments.

The solution mixing in the system initially occurred in the holding coil, but the solutions could not mix very well without additional processes. Between zero to four flow reversal steps were examined to determine the effectiveness of the number of flow reversal steps for improving the mixing of the solutions and increasing the yield of the product. Consequently, the peak height increased as the number of flow reversal steps increased, and the best sensitivity was found using three flow reversal steps. The signal was somewhat lower using four reversal steps than using three steps, because the mixed solution was diffused further in the carrier stream with each additional flow steps. Therefore, three flow reversal steps were chosen for the proposed SIA system.

Sample aspiration volumes of 20 – 150 μL were tested using a 0.5 mmol/L standard benzoyl peroxide, and the peak height was found to increase as aspiration volume increased. Using a high sample aspiration volume, the amount of analyte used was also increased to enhance the Fe(III)–thiocyanate complex. From economic view, a 100 μL of sample aspiration volume was selected for further experiments.

The effect of reaction temperature on the coloration of the Fe(III)–thiocyanate complex was investigated between 50 and 70°C. The coloration of the Fe(III)–thiocyanate complex was found to be influenced by the increasing temperature, and for our SIA system, 60°C was selected as the reaction temperature.

The effect of reaction time, in the range of 0 – 60 sec, was investigated using a reaction temperature of 60°C. The peak height was found to increase as the incubation time increased up to 40 sec, and it increased slightly after that time. Therefore, a reaction time of 40 sec was selected for the proposed SIA system.

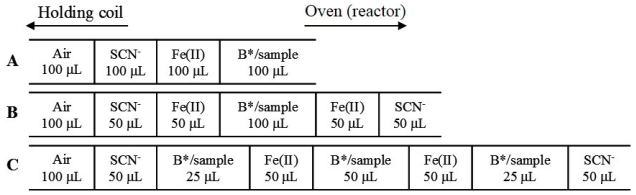

Figure 2. Aspiration sequences for the proposed SIA system.
*B: benzoyl peroxide

In the range of 0.6 – 4.4 mmol/L, the effect of the Fe(II) concentration on the coloration was investigated using the experimental conditions that had already been selected, as described above, except for the SCN^- concentration. The peak

height was found to reach a maximum and remain constant for the Fe(II) concentrations of over 3.1 mmol/L. Therefore, 3.1 mmol/L of Fe(II) was chosen.

Fe(II) aspiration volumes of 20 – 80 µL were used to determine the assay condition using the experimental conditions that had already been selected, as described above, except for the SCN^- concentration. The results of the study were expected to determine highest sensitivity that could be achieved using a low volume of Fe(II) solution. An Fe(II) aspiration volume of 60 µL was concluded to provide the best sensitivity in terms of the peak height. Over this volume, the peak height of signal decreased due to the effect of diffusion in the mixed solution.

Different SCN^- concentrations (2 – 8% (w/v)) were also tested using the experimental conditions that had already been selected. The peak height was found to increase as the SCN^- concentration increased up to 6%, and the sensitivity slightly ameliorated at higher concentrations. Therefore, the recommended SCN^- concentration for determining the lipid hydroperoxide using our system was 6%.

The effects of aspiration volume of SCN^- solution was also tested between 20 and 100 µL. The peak height increased as the volume of SCN^- solution was increased up to 60 µL, and the sensitivity remained constant at higher volume. Therefore, 60 µL of SCN^- solution was used for the determination of lipid hydroperoxides.

3.2. Analytical characteristics

As shown in Figure 3, the response signals, using benzoyl peroxide as the standard reagent to quantify the lipid hydroperoxides, were linearly increased over the 0 – 0.5 mmol/L in the proposed system using the selected conditions. The significant linearity was recognized between the concentration of benzoyl peroxide and the peak height ($r = 0.998$, $n = 6$, $p < 0.01$). The detection limit (defined as the concentration giving a signal to noise ratio of 3) was estimated to be 0.015 mmol/L. The reproducibility of the method was determined by analyzing a 0.3 mmol/L standard benzoyl peroxide 11 times. The relative standard deviation was 1.3%. It was possible to analyze 10 samples per hour (6 min per analysis cycle), including all of the steps (aspiration, incubation, detection, and cleaning).

3.3. Recoveries

Two kinds of oil samples, rapeseed and olive oils, were each spiked with 0.1 and 0.2 mmol/L of standard benzoyl peroxide and analyzed to determine the recoveries using the developed SIA system (Table 2). The recoveries were 97.2 – 99.5%. This shows that the SIA system provided appropriate recoveries for determining total lipid hydroperoxides in edible oils.

3.4. Application to edible oil samples

The proposed SIA system was applied to determine the lipid hydroperoxides in eight edible oils. Before the analysis using SIA system, a 0.2 g aliquot of each oil sample was dissolved in 5 mL of isopropanol, and then it was further diluted fourfold with isopropanol. The concentrations of lipid hydroperoxides are presented in Table 3, expressed in mmol benzoyl peroxide equivalent (eq.) per litter. The POV (meq/kg) was also determined using the AOCS method in order to compare the results (Table 3). Consequently, the results obtained using the SIA system and the AOCS method were linearly-related with a correlation coefficient of 0.987 ($n = 8$, $p < 0.01$).

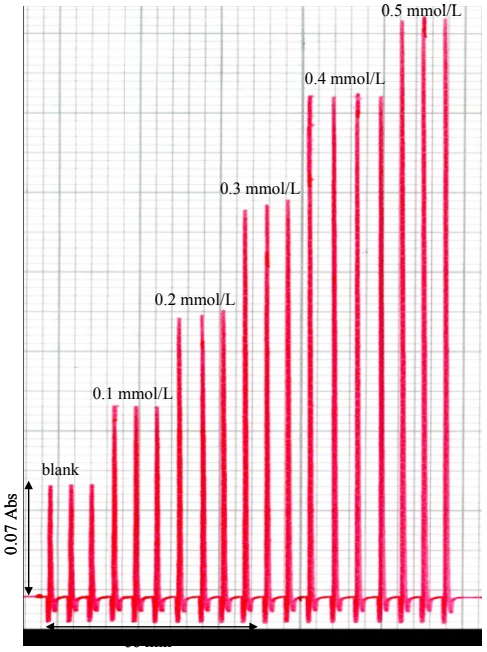

Figure 3. Typical response signals for benzoyl peroxide obtained using the proposed SIA system.

Table 2. Recovery tests of the lipid hydroperoxides in edible oils using the proposed SIA system (n = 4).

Edible oils	Lipid hydroperoxide (mmol benzoyl peroxide eq./L)			Recovery (%)
	Found	Spiked	Found in total	
Rapeseed	0.052 ± 0.001	0.1	0.152 ± 0.004	99.4 ± 3.6
		0.2	0.251 ± 0.007	99.5 ± 3.6
Olive	0.136 ± 0.002	0.1	0.234 ± 0.005	97.2 ± 4.9
		0.2	0.332 ± 0.004	98.0 ± 2.1

Table 3. Determination of the lipid hydroperoxides in edible oils using the proposed SIA system and the AOCS method.

Edible oils	SIA system (n = 4)	AOCS method (n = 2)
	Lipid hydroperoxide (mmol benzoyl peroxide eq./L)	POV (meq/kg)
Rapeseed	0.052 ± 0.001	1.1 ± 0.3
Soybean	0.065 ± 0.001	1.6 ± 0.1
Sesame	0.068 ± 0.001	1.8 ± 0.6
Olive	0.436 ± 0.002	7.2 ± 0.1
Grape seed	0.119 ± 0.001	2.2 ± 0.0
Corn	0.113 ± 0.002	2.4 ± 0.1
Perilla	0.426 ± 0.004	8.4 ± 0.1
Flaxseed	0.107 ± 0.001	1.6 ± 0.3

4. Conclusion

Lipid hydroperoxides, which are the primary products of the lipid peroxidation process, could be determined using a simple, rapid, and automatic SIA system with spectrophotometric detection. The concentrations of lipid hydroperoxides determined using the proposed SIA method correlated well with the POV determined using the AOCS official method ($r = 0.987$, $n = 8$, $p < 0.01$). The proposed system produces less organic waste than the conventional methods; the proposed SIA system used 240 μL of reagents for detection of lipid hydroperoxides and 4000 μL of career solution for moving of analyte and cleaning of line, whereas the ACOS method used approximately 55 mL of reagent per titration. In addition, the high reproducibility (RSD = 1.3%, n = 11) and high throughput (10 samples per hour) were obtained in the proposed SIA system. Thus, we concluded that the SIA system proposed here can be used as an alternative method for the routine determination of lipid hydroperoxides in edible oils.

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