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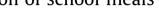
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# Analytical Methods

# Food types and frying frequency affect the lipid oxidation of deep frying oil for the preparation of school meals in Korea



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# 1. Introduction

Deep fat frying is widely used to prepare foods in school meal services of Korea. The frying oil is repeatedly heated to high temperatures (158-185 °C) in the presence of oxygen, moisture, prooxidants and antioxidants from foods being fried, resulting in lipid oxidation. This can lead to the deterioration of the organoleptic and nutritional characteristics of fried foods. Even with a careful control of all aspects of frying, including temperature, frying time and storage after use, the frying oil will be increasingly oxidised with repeated heating. In Korean school meal services, where the same food is usually fried in large amounts for a few hours per day, the frying oil must be discarded and replaced periodically in order to maintain the oil quality. Therefore, schools have set guidelines for oil use, including frying temperature, fryer selection, frying time, oil cooling and storage. However, the guidelines are just a recommendation for maintaining the quality of fried foods. Therefore, lipid oxidation products can be quantitatively and qualitatively different among schools. Given that lipid oxidation products exert cytotoxic and genotoxic effects (Esterbauer, 1993), repeated consumption of oxidised fat in the diet could pose a chronic threat to students' health.

## ABSTRACT

200 soybean oils used in school meals for deep frying were investigated to elucidate factors influencing lipid oxidation in the oils. The mean levels of moisture along with primary and secondary lipid oxidation products were significantly different among the oils used by the six schools. When comparing lipid oxidation products of frying oils used for four different food groups (vegetables, fish, meat or carbohydrate-rich foods), differences were found among them, with the values for the carbohydrate-rich group being the lowest. The vegetable group was higher in the contents of conjugated dienes and trienes, and lower for those of hydroperoxides and malondialdehyde. The mean values of malondialdehyde and *p*-anisidine value for the fish group were greater than those of the other groups. The levels of conjugated trienes and malondialdehyde increased with the frying frequency. These findings indicate that food types and frequency of frying play a role in determining the oil oxidation in deep fried foods in schools.

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Lipid oxidation is not a single reaction, but a series of reactions. Once oxidised, a series of breakdown products are produced, starting with primary oxidation products, such as free fatty acids, conjugated diene (CD) or conjugated triene (CT), and peroxides; secondary products, such as alcohol, aldehydes and ketones are produced subsequently. These oxidation stages progress at different rates depending on factors, including temperature (McClements & Decker, 2007), exposure time to high temperature (Juárez, Osawa, Acuña, Sammán, & Gonçalves, 2011; Naz, Siddiqi, Sheikh, & Sayeed, 2005; Silvagni, Franco, Bagno, & Rastrelli, 2012), light (Naz et al., 2005), oxygen (Naz et al., 2005), moisture, antioxidants (Chen & Ahn, 1998; Gertza, Klostermanna, & Kochharb, 2000; Naz, Sheikh, Siddiqi, & Sayeed, 2004; Naz et al., 2005), metals (Naz et al., 2005; Silvagni et al., 2012), fried food items (Naz et al., 2005) and oil type (Juárez et al., 2011; Naz et al., 2005). Primary oxidation processes in oil form hydroperoxides (ROOH), which are reflected by the peroxide value. Generally, the peroxide value is a measure of the extent to which the oil has undergone primary oxidation. However, the peroxide value reaches to a plateau and then decreases as peroxides are decomposed into secondary oxidation products, in particular, carbonyl compounds. Malondialdehyde (MDA) is one of the major aldehydes occurring in the secondary oxidation of lipids. Therefore, the oxidative status of deep frying oils must be evaluated considering both primary and secondary oxidation







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Lipid oxidation has been investigated in model systems in order to elucidate the contribution of each material (Chen & Ahn, 1998; Gertza et al., 2000; Juárez et al., 2011; Naz et al., 2004, 2005). However, the preparation of school meals differs from simulated frying conditions, as deep fryers in schools are usually operated at full capacity with continuous use for a few hours a day. Bouchon (2009) reported that foods fried between 150 and 180 °C absorbed 8 to 25 percent oil. In addition, lipid oxidation products in the oil accumulated in the foods at similar concentrations (Ghidurus, Turtoi, Boskou, Niculita, & Stan, 2010; Masson, Robert, Izaurieta, Romero, & Ortiz, 1999; Seppanen & Csallany, 2004). Therefore, lipid oxidation should be investigated under a variety of practical conditions.

The objectives of the study were as follows: (1) to determine lipid oxidation products (CD, CT, ROOH, and MDA) in soybean oils collected from deep fryers of six schools, and (2) to investigate the influence of frying frequency and food types on lipid oxidation in the oils, making it possible to assess – for the first time – the deterioration of soybean oils used in school meals for deep frying in Korea.

#### 2. Materials and methods

### 2.1. Chemicals

All of the reagents were of the highest grade commercially available. Isooctane and benzene were purchased from Fisher Scientific (Chicago, IL, USA). Cumene hydroperoxide, xylenol orange and MDA precursor 1,1,3,3-tetraethoxypropane were purchased from Sigma– Aldrich (St. Louis, MO, USA). Butylated hydroxy toluene (BHT) and ascorbic acid were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Trichloroacetic acid (TCA) and Karl Fisher reagent (Hydranal-Composite 5) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and Riedel-de Haën<sup>®</sup> (Seelze, Germany), respectively. The *p*-anisidine (4-methoxyaniline) and 2-thiobarbituric acid (TBA) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Ferrous sulphate (FeSO<sub>4</sub>) was obtained from Showa Chemical Industry Co., Ltd. (Tokyo, Japan). Distilled and deionized water were used for the preparation of all solutions.

#### 2.2. Sample collection

Oils used in school meal services were collected from deep fryers of six schools (designated as schools A to F) located in northeastern South Korea over June to August 2009. The meal count (the number of students eating school lunch per day) was between 410 and 1340 (Table 1). Schools were selected in order to obtain the comparative pairs for frying oil quality under similar conditions (e.g., same recipe), frying temperatures, frying time and oil capacity per batch. Table 1 shows detailed information on the oil samples and typical deep fat frying conditions conducted in Korean school meal services. To exclude the differences in cooking practice between schools, frying oils were collected for a second time from school F in June 2010. The oils collected in this study were all soybean oils. A total of 143 and 57 oil samples were obtained from the two collections, respectively. The oils from the deep fryer were collected in a 40 mL amber bottle with 0.1 g BHT between frying batches, and immediately transported to Kangwon National University and stored at -20 °C until analyses.

## 2.3. Determination of moisture

The moisture content of the frying oils was determined according to Karl Fischer titration, which can quantify trace amounts of water in a sample (Ruiz, 2004). The titration was performed using a Karl Fisher potentiometric titrator (702 SM Titrino, Metrohm, Flawil, Switzerland).

# 2.4. Determination of conjugated diene (CD) and conjugated triene (CT)

The oil (0.01 g) was weighed into a 25-mL volumetric flask and 10 mL of isooctane was added in order to dissolve the oil. The volume was made up to 25 mL with additional isooctane, and the absorbance was determined at 233 nm and 268 nm, respectively, using a UV–visible spectrophotometer (UV-1650; Shimazu, Kyoto, Japan). The results were expressed as the extinction values using the following equation:  $E_{1cm}^{1\%} = A_{\lambda}/(C_L \times l)$ , where *E* is the extinction value,  $A_{\lambda}$  is the absorbance measured at either 233 nm (for CDs) or 268 nm (for CTs),  $C_L$  represents the concentration of the oil solution in g/100 mL, and *l* is the path length of the cuvette in cm (Pegg, 2004a).

#### 2.5. Determination of hydroperoxides (ROOH)

Lipid hydroperoxides formed in the oils were spectrophotometrically measured according to the ferrous oxidation/xylenol orange method, which is based on the ability of lipid peroxides to oxidise ferrous ions at a low pH (Pegg, 2004a). The oil (0.1 g) was weighed into a glass tube and BHT (1% to oil) added to prevent further oxidation. The mixture was added to 9.9 mL chloroform/methanol 7:3 (v/v) and vortexed for 5 s before 50  $\mu$ L of 0.01 mol/L xylenol orange and 50  $\mu$ L of iron (II) chloride were added, and the samples vortexed again. Tubes that contained all the reaction solutions, except oil, were used as a blank. After allowing the reaction mixtures to stand for exactly 5 min at room temperature, the absorbances were determined at 560 nm using a UV-visible spectrophotometer (UV-1650; Shimazu, Kyoto, Japan). Lipid hydroperoxide in the oils was quantified by referencing a calibration curve, constructed with cumene hydroperoxide as an external standard.

#### 2.6. Determination of malondialdehyde (MDA)

MDA, a secondary oxidation product, was measured according to the TBA method (Pegg, 2004b). 0.1 g of the oil was accurately weighed into a 25-mL volumetric flask, and BHT (1% to oil) was immediately added to prevent further oxidation. Subsequently, the oil was added to 5 mL of 1-butanol and vortexed thoroughly for dissolution. The sample solution was added to 5 mL of 0.2% TBA in 1-butanol. After vortexing, the mixture was incubated at 95 °C in water bath for 2 h; then, it was quickly cooled under cold running tap water in order to stop the reaction. The resulting solution was allowed to stand at room temperature for 10 min in order to stabilize the chromogenic MDA-TBA complex; then, its absorbance was measured using a UV-visible spectrophotometer at 532 nm. The standard curve for the quantification of MDA was constructed by plotting the content of MDA against the absorbance of its complex using 1,1,3,3-tetraethoxypropane as an MDA precursor. The MDA content in the oil was determined by referencing to the calibration curve.

#### 2.7. Determination of p-anisidine value (p-AV)

The amount of non-volatile aldehydes (principally 2-alkenals) in the oils was measured using the *p*-anisidine test, which is the AOCS official method for determining the levels of the aldehydes (Tompkins & Perkins, 1999). 0.5 g of the oil sample was accurately weighed in a 25-mL volumetric flask and added to 10 mL isooctane for dissolution. The solution was made up to 25 mL with additional isooctane, and its absorbance (*A*<sub>b</sub>) at 350 nm was measured with the isooctane as a reagent blank. 5 mL of the solution was then

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