Analysis of Trans Fatty Acid Content and Viscosity of the Repeteadly Used Frying Oil

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Abstract: The physicochemical properties of the repeatedly used frying oil were subjected to changes at frying temperature of 180°C and frying time of 10 minutes with five times frying repetitions. The changes included the formation of *trans* fatty acids and increased viscosity. The *trans* fatty acid content was analyzed by gas chromatography (GC) and the viscosity by "Brookfield DV-E Viscometer". The *trans* fatty acid content and the viscosity of the repeatedly used frying oil increased with the number of repeated uses and the trans fatty acid content increased with the viscosity. The higher the *trans* fatty acid, the higher the viscosity of the frying oil. The increased *trans* fatty acid content and viscosity indicated that a damage had occurred in the oil.

Keywords: *Trans* fatty acid, viscosity, deep frying, frying oil

1. Introduction

Oil is a mixture of fatty acid esters and glycerols in the form of liquid due to the low saturated fatty acid and high unsaturated fatty acid content (Winarno, 1986). Oil can be used as a frying medium for foods, for examples french fries, dough nut, potato chips, fried banana, fried fermented soybean, and powdered fried chicken available at many restaurants and hotels and even sold in the street. Frying oil is the end result of an oil purification process from various vegetable sources with function to conduct heat and add savory taste and calories to the fried food materials. Frying oil can be produced from various raw materials, including coconuts, copra, palm, soybean, corn , sunflower beans, olive beans, and others (Muchtadi, 2008).

Frying is a process of cooking food material by using hot fat or oil at high temperature (150°C – 190°C) and is one of the popular food preparation techniques because it can provide better color, taste, and texture for the fried foods. There are two frying methods: shallow frying and deep frying. Shallow frying that uses pan doesn't require a large volume of oil, so that the fried food is not submerged in the oil. In deep frying process, the food materials are fried submerged in the oil and the oil temperature can reach 200-205^{o}C, so that it needs large volume of oil. Deep frying process results in stability, quality, taste, color, and texture changes of the fried foods as well as the nutritional content. Deep frying enables heat transfer during the frying process from the hot oil into the cool product. It is this fact that makes this process occurs rapidly (Blumenthal, 1996; Krishnamurthy and Vernon, 1996; Choe and Min, 2007; Jana *et al*., 2011).

Trans fatty acids are unsaturated fatty acids containing at least one double bond in *trans* configuration. *Trans* fatty acid can be produced by partial hydrogenation of vegetable oils containing unsaturated fatty acid and heating process in oil purification process in industrial scale or occur naturally in ruminant animals by fatty acid biohydrogenation in the intestines. Trans fatty acid contained in the food can provide positive health effects, such as for coronary cardiac disease, insulin inhibition or diabetes, and pregnancy disorders. Trans fatty acids are abundant in margarine, butter, and frying oil. Trans fatty acid can be formed after 15 minutes of frying which is possibly caused by oil absorption (Wassel and Niall, 2007; Liu *et al*., 2008; Bhardwaj *et al.,* 2011; Thompson *et al.,* 2011; Hou *et al*., 2012).

Viscosity is a measure that can describe the physical nature of an oil. Viscosity can be used as an indicator in determining the damage in the oil structure. Viscosity of frying oil will subject to an increase with the frying length. The longer the frying time the higher the viscosity of the oil. During deep frying, viscosity will subject to changes due to temperature and frying time effects. During the frying process, complex changes occur from various chemical reactions including thermo-oxidation, hydrolysis, polymerization, and atomic breakdown, resulting in increased viscosity, dark color, increased foam formation, and decreased smoke point in the oil. The chemical reaction rate depends on oil compositions, temperature and length of frying, continuous frying or interrupted frying, food materials, and the freshness of the oil added into the used oil. Viscosity and heat transfer are factors influencing the quality of fried foods (Debnath *et al*., 2010; Rani *et al*., 2010).

2. Materials and Methods

2.1 Materials

This research was a laboratory experimental test with two treatments (used frying oil for bananas and used frying oil for chickens) and five frying repetitions with three treatment repetitions at 180^{o}C for 10 minutes. This research was conducted in Agricultural Product Technology Laboratory of Agricultural Faculty of Hasanuddin University Makassar and Integrated Laboratory of IPB Bogor. Materials used in this research included: a) commercial frying oil from "B" brand obtained from supermarket, wheat flour, seasoning powder, and food materials to fry including bananas and chickens which were divided in small pieces based on the general normal size sold by vendors, 250 g for one frying; b) chemicals used were standard solution, NaOH solution in methanol, BF_3 solution, saturated NaCl solution, hexane and anhydrous Na_2SO_4.

Tools and equipments used in this research included: (1) frying kettle made of aluminum and wood stirrer; (2) analytical balance; (3) micro pipettes; (4) water bath; (5) Teflon-capped tube; (6) 10 μL syringe and (7) a gas chromatography set (Shimadzu GC-2010 plus, 007 series bonded phase fused silica capillary column no. C11804700336). This equipment was used to separate the *cis* fatty acid from *trans* configuration. The components were separated by evaporation, carried by inert gas and passed through a still column/phase in the form of non-volatile solid and liquid substances attached to inert support material. Column type: cyanopropil methyl sil (capillary column); column dimension: p = 60 m, Ө inner = 0.25 mm, 0.25 μm Film Thickness (AOAC, 1984; Fennema, 1996; Nielsen, 2009).

2.2 Methods

The fried food materials included bananas (kepok banana) and chickens (broiler chicken). Frying process was started by entering the fresh frying oil into the frying kettle in the volume of 3.5 liter. The kettle was then heated up to 180^{o}C. 250 grams of the food materials was fried until cooked during 10 minutes with minimum stirring to reduce the convection flow in the oil and oxidation reaction due to the aeration process. Frying repetitions were performed up to five times and in each of the frying repetition an oil sample (100 mL) was collected to analyze the *trans* fatty acid content and viscosity.

Analysis of *trans* fatty acid content and viscosity measurements were performed on two sample types: used frying oil for bananas and used frying oil for chickens. The frying oils used for frying repetition were the same frying oil (not changed and not added with new volume of fresh frying oil). There were five frying repetitions with same frying length. Analysis of trans fatty acid was performed by gas chromatography (GC) and the viscosity measurement by Brookfield DV-E Viscometer.

2.2.1 The measurement of trans fatty acid

Sample preparations were consisted of hydrolysis and esterification. Oil samples (20-30 mg) were weighted in a teflon-capped tube, and then added with 1 mL NaOH 0,5 N in methanol and heated in water bath for 20 minutes. 2 mL of BF_3 16% and 5 mg/mL of internal standard were then added and heated again for 20 minutes. After cooling process, 2 mL saturated NaCl and 1 mL hexane were added and the mixture was shaken thoroughly. The hexane layer was separated with the help of dropping pipette and entered into tube containing 0.1 g anhydrous Na_2SO_4 and left in place for 15 minutes. The liquid phase was separated and injected into gas chromatography, cyanopril methil sil column (capillary column). The equipment conditions were adjusted as follow: column dimension (p = 60 m, Ө inner = 0.25 mm, 0.25 μm Film Thickness); flow rate of N_2: 20 mL/minute; flow rate of H_2: 30 mL/minute; flow rate of air: 200-250 mL/minute; injector temperature: 200^{o}C; detector temperature: 230^{o}C; column temperature: early temperature 190^{o}C, late temperature 230^{o}C with rate of 10^{o}C/minute; ratio = 1:8; injection volume: 1 uL; linear velocity: 20 cm/sec. Analysis was started from injection if solvent (1 uL) into the column to obtain baseline values. When the carrier gas flow and heating system were perfect, the solvent peak will appear in less than 1 minute. After obtaining the baseline values, the procedure continued by injecting 5 uL standard mixture of FAME. When all the peaks appeared, the prepared samples were injected in the volume of 5 uL. Retention time and sample peaks were measured for each of the components compared to standard and calculated as follow:

$$C_x = (A_x\ R\ C_s)/(A_s)$$

Where,

C_x : Concentration of component x

C_s : Concentration of internal standard

A_x : Peak area of component x

A_s : Peak area of internal standard

R : Detector response against component x relative against standard.

2.2.2 Viscosity measurement

Viscosity measurements were performed using Brookfield DV-Viscometer by submitting 100 mL sample into a beaker glass and placed in rotary spindle 02 with speed of 100 rpm until a measurement stability achieved in display with sample temperature of around 30^{o}C (room temperature) (AOAC, 1995).

2.3 Data Analysis

The obtained data were analyzed statistically by ANOVA using SPSS version 17, according to complete random design with three repetitions.

3. Results and Discussion

3.1 The Test of Trans Fatty Acid of Frying Oil

Table 1 indicates that the *trans* fatty acid content of fresh oil (before used in frying process) was 0.085%. After used for frying banana in the first frying, there had been an increase in *trans* fatty acid content (0.097%) until fifth frying (0.112%). Accordingly, after they oil was used to fry chicken, an increase in trans fatty acid was observed starting from first frying (0.09%) until fifth frying (0.012%). Statistical test indicated significant difference ($P > 0.05$). This indicated that frying repetition can result in the formation of trans fatty acid in the used frying oil, the more frequent the oil used, the higher the formed trans fatty acid. This was in accordance with Sartika (2009) who suggested that repeatedly used frying oil can result in the presence of *trans* fatty acid in the used frying oil and in the fried foods. Bansal *et al*. (2009) suggested that *trans* fatty acid was observed in higher level in the used frying oil compared to fresh frying oil and the frying process results in more trans fatty acid (18:1). According to Hou *et al.* (2012), the fresh frying oil contain lower trans fatty acid level compared to the used frying oil and will increase with repeated uses.

Comparison between used frying oil for banana and used frying oil for chicken indicated that the trans fatty acid content in used frying oil for chicken was higher than those in used frying oil for banana (Figure 1). A relatively high trans fatty acid content in the used frying oil for chicken was probably associated to the unsaturated fatty acids in the chicken were subjected to double bond breakdown and isomeration, resulting in higher trans fatty acid in the used frying oil for chicken. Statistical test indicated a not significant difference ($P < 0.05$).

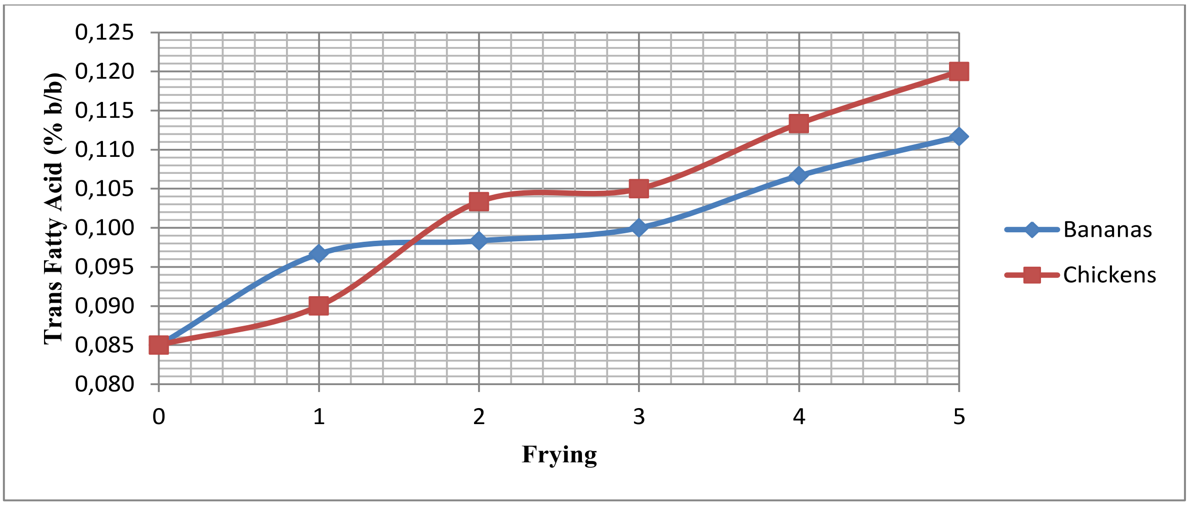

Figure 1. Trans Fatty Acid Content in the Used Frying Oil for Bananas and Chickens

Interrupted oil heating for several days results in faster destruction and decomposition and, when left overnight, will result in oil decomposition when heated again (Khomsan, 2003). *Trans* fatty acids are the results of hydrogenation from vegetable oils. According to Rani *et al.* (2010), the stability and physiochemical reactions in vegetable oils resulting in the formation of trans fatty acid occur at frying temperature (180°C) because the oil used in this research was a palm oil enabling the formation of trans fatty acid. Palm oil is widely used in the processing of industrial food, so it has the possibility of forming trans fatty acid and contribute to several human diseases including coronary disease, insulin inhibition or diabetes, pregnancy disorders, and increased total cholesterol, increased total triasilglicerol and LDL, and decreased HDL (Guzman *et al*., 1999; Fang *et al*., 2010; Filip *et al.,* 2010; Bhardwaj *et al.,* 2011).

Table 1. Analysis results of trans fatty acid of used frying oil for bananas and chickens

Frying repetition treatment	Used fried oil for bananas (% $^b/_b$)	Used fried oil for chickens (% $^b/_b$)
Control	0,085**	0,085**
Frying 1	0,097*	0,090*
Frying 2	0,098*	0,103*
Frying 3	0,100*	0,105*
Frying 4	0,107ns	0,113ns
Frying 5	0,112ns	0,120ns

ns) not significant **) very significant *) significant

3.2 Viscosity Test of Frying Oil

In line with the analysis results for *trans* fatty acid in the used frying oil for bananas and chickens, the viscosity of the used frying oil for bananas and chickens also subjected to an increase. The viscosity of the fresh oil (370.6 cp) increased after used in frying bananas starting from the first frying (349.8 cp) until the fifth frying (392.0 cp). The oil viscosity after used in frying chickens also increased starting from the frying 1 (349.8 cp) until frying 5 (392.0 cp) (Table 2). Statistical test indicated a not significant difference ($P < 0.05$). This is in accordance to Blumenthal (1996) who suggested that frying oil viscosity is increased significantly with its uses in frying process. This increased viscosity is due to the formation of polymeric compounds in the oil caused by heating and oxidation. Increased oil viscosity is an indication of increased oil damage (Andarwulan *et al.,* 1997).

Table 2. Measurement results of viscosity of the used frying oil for bananas and chickens

Frying repetition treatment	Used fried oil for bananas (cp)	Used fried oil for chickens (cp)
Control	370,6^{ns}	370,6^{ns}
Frying 1	349,8**	349,8**
Frying 2	363,7**	370,6**
Frying 3	370,6**	377,7**
Frying 4	370,6**	392,0^{ns}
Frying 5	392,0 **	392,0^{ns}

^{ns}) not significant **) significant

The results of viscosity measurements in the used fried oil for bananas and chickens can be seen from Figure 2. In this figure it can be seen that an increase is relatively occurred with the frying oil uses until frying 5. When observed at every frying duration, the highest viscosity was observed in frying five treatments either for the used frying oil for bananas or used frying oil for chickens. Comparison between used frying oil for bananas and for chickens indicated that viscosity of the used frying oil for banana was higher. Statistical test indicated a significant difference ($P > 0.05$). This was due to the different components of the two materials used, and according to Rani et al. (2010) the oil viscosity will increase due to the fried food material degradation which result in dimers, trimers, polymers, epoxide, alcohole and hydrocarbon forms.

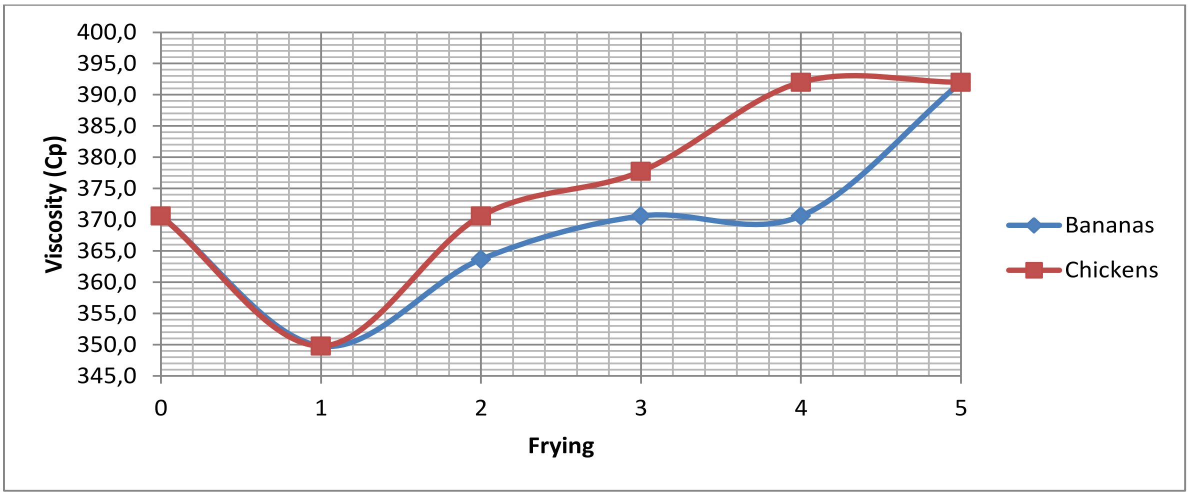

Figure 2. Viscosity of the used frying oil for bananas and chickens

Oil used in this study was a palm oil. According to Silva and Paul (1995), the viscosity of palm oil will increase due to the degradation during heating, so the viscosity of the used frying oil seemed increased. Oils that have subjected to heating and oxidation will have an increased viscosity as a result of polymeric compounds formation in the oil, whereas the oxidation is caused by the water exchange in the fried foods. Frying oil viscosity depends on frying conditions such as frying oil type, frying capacity, frying oil temperature, and frying length (Lioumbas *et al.,* 2012).

4. Conclusion

Trans fatty acid content and viscosity of the repeatedly used frying oil increased with the repeated uses of the oil, and the trans fatty acid content increased with the viscosity. The higher the *trans* fatty acid content, the higher the viscosity. The increased fatty acid content and viscosity indicated that damage has been occurred in the oil structure.

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