1 INTRODUCTION

We have been investigating the chemical properties of used frying oil (5-7). 5BCMF shows the chemical properties of “B grade recovered vegetable oil” (recovered oil, Miyoshi Oil & Fat Co., Ltd.) produced in Dec. 2004 (RO1), Apr. 2005 (RO2), and Apr. 2006 (RO3); frying oil in use at a croquette-frying stand in Osaka for 90 days (FO); fresh frying oil (MO); and that heated at 180\degree C for 20 h in the laboratory (HMO). All the oils were composed of soybean and rapeseed oils. Chemical properties of the oil became worse as

Abstract: Used frying oil recovered from food manufacturing companies in Japan and recycled often shows lower carbonyl (COV) and peroxide values (POV) than oil simply heated at 180\degree C for 20 h does. In this study the reasons for the low COV of oil used for deep-frying were investigated by employing model experiments and actual commercial frying. The results suggested that in actual frying, the factor most influencing the low COV was vaporization of carbonyl compounds, together with steam generated from water contained in frying foodstuffs. It was also suggested that the low levels of COV were attributable partly to inhibition by protein, amino acids exuded from frying foodstuffs, and starch, and slightly to the effects of natural antioxidants in fresh oil and frying foodstuffs, oil absorption by frying foodstuffs, and dilution of oil in use by fresh oil added between uses. On the other hand, the chemical properties of oil in a fryer and in batter coatings of deep-fried foods made with the former oil were checked. Content of polar compounds (PC) and color score were obviously worse in the oil extracted from batter coatings than in that in the fryer, but there were no differences in COV or content of triacylglycerol (TG) of the two oils.

Key words: COV, frying oil, deep-fry, vapor, antioxidant, protein, starch, amino acid, thermally oxidized oil

1 Carbonyl Compounds Vaporize from Oil with Steam during Deep-Frying

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\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
 & RO1 & RO2 & RO3 & FO & HMO & MO \\
\hline
Polar compounds (%) & 24.7 & 20.3 & 25.9 & 34.6 & 17.8 & 5.1 \\
Gardner color & 11 & 11 & 12 & 15 & 6 & 1 \\
AV & 2.0 & 2.8 & 2.1 & 3.8 & 0.1 & 0.1 \\
COV & 10.9 & 10.3 & 12.1 & 34.4 & 39.1 & 3.0 \\
POV (mEq/kg) & 26.2 & 23.5 & 28.4 & 43.7 & 76.9 & 6.6 \\
\hline
\end{tabular}
\caption{Properties of Used Frying Oils.}
\end{table}

(RO2), and Apr. 2006 (RO3); frying oil in use at a croquette-frying stand in Osaka for 90 days (FO); fresh frying oil (MO); and that heated at 180\degree C for 20 h in the laboratory (HMO). All the oils were composed of soybean and rapeseed oils. Chemical properties of the oil became worse as

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commercial frying proceeded, but the used (presumably for more than 20 h) oil often showed relatively low POV and COV, as shown in Table 1. One of the reasons for low POV seems to be that peroxides in heated oil are easily decomposed to corresponding alcohols and epoxides via alkoxy radicals, while natural antioxidants in oil should prevent oil from oxidation even at frying temperatures. In general, the COV of the heated oil increases with frying time macroscopically even though fresh oil is added between uses (described further later). And the COV of oil reached 40 after 20 h of heating at 180°C without frying foodstuffs. Thus, it may also be possible that some components exuded from frying foodstuffs remove or react with peroxides and carbonyl compounds to retard POV and COV increases of the oil. The present study investigated the contribution of these factors to keeping the COV of the used frying oil at a low level, in addition to studying effects of frying foodstuffs on the formation of polar compounds, which are closely related to the health damage from used frying oil.

2 EXPERIMENTAL

2.1 Materials

Fresh frying oil (The Nisshin Oil Mills, Ltd., Tokyo, Japan) had following fatty acid compositions and chemical properties: C16:0 12.3%, C18:0 3.4%, C18:1 32.2%, C18:2 45.3%, C18:3 6.8%, AV 0.1, COV 2.1, POV 4.8 mEq/kg, PC 4.7%, and Gardner color 2. All the amino acids, gluten from wheat, wheat flour (starch), and 2, 6-di-tert-butyl-p-cresol (BHT) were products of Nacalai Tesque, Inc., Kyoto. D-α-tocopherol (α-Toc) and 99% L-α-phosphatidyl choline from egg (lecithin) were purchased from Wako Pure Chemical Industries Ltd., Osaka and Funakoshi, Tokyo, respectively. Commercial breaded pork cutlets (80g pork) and oil used for deep-frying the cutlets were obtained from one of the campus restaurants at Kobe-Gakuin University over three weeks of July 2006; fatty acid compositions of typical frying oil showed C16:0 8.9%, C18:0 3.3%, C18:1 38.8 %, C18:2 40.2%, C18:3 6.5%, and others 2.3%.

2.2 Chemical analyses

COV, AV, and Gardner color were measured according to the standard methods of the Japan Oil Chemists’ Society for analysis of fats, oils, and related materials. PC in oil was analyzed by a PC Tester (3M, Saint-Ouen l’Aumone, France). Triacylglycerol contents were determined by applying samples on Chromarods followed by development in a solvent mixture, hexane/diethyl ether/acetic acid, 50:10:1 v/v/v, and quantification with Iatroscan TLC/FID; triacylglycerol had an Rf value of 0.16.

2.3 Procedure

BHT, α-Toc., and lecithin, 500 ppm, were added to 30 mL fresh frying oil, respectively, and heated at 180°C for 20 h (10 h/day) by a hotplate stirrer IS-36H (Fine, Tokyo, Japan) with 6 hotplates. Agitation was done with a stirring bar at agitation speed scale 3, and the chemical properties COV, PC, TG, and Gardner color of oils obtained were determined.

Breaded pork cutlets were fried at 1500 on each sampling day, at the end of the operation at the campus restaurant, and 50 mL of oil used for deep-frying the cutlets was obtained during the first, the second, and the fourth weeks of July 2006. Each cutlet was frozen at −20°C immediately, and oil was extracted from milled batter coatings with hexane. Oil thus extracted and oil in the fryer were tested for COV, PC, TG, and Gardner color.

The fresh frying oil, 30 g, containing 500 ppm of amino acid; 7000 ppm of amino acid mixture, (Gly + Thr + Tyr, 1:1:1 wt/wt/wt), major amino acids exuded from frying foodstuffs; 7000 ppm of gluten as a representative protein of fried foods; and 7000 ppm of starch, was poured into a 50-mL beaker, respectively, and heated as described above. Agitation speed at scale 3 made added particles move freely. The amino acid amount, 500 ppm, correlated with the amount of amino acids exuded from frying foodstuffs, and 7000 ppm of gluten and starch, judged from the amount of fryer dregs collected at the end of operation at the campus restaurant; the dregs were composed of wheat starch and fragments of pork, chicken, beef, and other foods. After heating for 20 h, COV and PC were measured.

The fresh frying oil, 1234 g, was put in an electric fryer of type EP-D692 (Twinbird, Niigata, Japan, pan dimensions: 14 cm × 22.5 cm × 11 cm deep, cover dimensions: 19.5 cm × 29 cm, dimensions of filter on the cover to trap oil fume formed during deep-frying: 10 cm × 12.5 cm), and heated at 180°C. Forty-five pieces of 80 g pork breaded according to conventional methods using wheat flour, egg, and bread crumbs were deep-fried one by one in the oil in 3 h without replenishment of the fresh oil. Every 4 min, the cover of the fryer was opened, the cooked cutlet was taken out, and a raw cutlet was put in; the fryer was then covered again. The COV of leftover oil and oil recovered quickly with hexane from the filter (changed to a fresh one every 1 h during deep-frying), then desolvented under nitrogen stream, was determined.

One liter of the fresh frying oil was heated at 180°C under stirring in a 2-L separable four necked flask with two necks left open. After 10 h, brown viscous oily substance covering upper inside wall of the flask was recovered with ethanol, and ethanol removed by a rotary evaporator. Remaining oil and oil in the flask were analyzed on chemical properties.
3 RESULT AND DISCUSSION

3.1 Effects of antioxidants

Three antioxidants did not show a good inhibitory effect on COV increase (Fig. 1). But, in commercial frying antioxidants exuded from frying foodstuffs and contained in fresh oil repeatedly added to heated frying oil can not be ignored because antioxidants remain active for a few hours at 180°C.

However, α-Toc kept PC unchanged during up to 10 h of heating, and about 15% lower after 10 h than that of oil without antioxidant. Warner et al.11) fried potato chips in triolein containing 0 to 400 ppm γ-tocopherol for up to 6 h, and reported that the antioxidant significantly inhibited polar compound production in the triolein, although it disappeared rapidly. Gomez-Alonso et al.12) showed that the activity of phenolic antioxidants in virgin olive oil rapidly diminished during the first six deep-frying processes for preparing French fries, followed by rapid increases in the concentrations of polar compounds, oxidized triacylglycerol monomers, and dimeric and polymerized triacylglycerols.

3.2 Changes of oil in batter coatings and fryer

The campus restaurant uses a fryer holding 18 L of frying oil and carries out frying in the manner shown in Fig. 2: on July 3, a Monday, 97 items in total, including breaded...
pork cutlets, potato croquettes, spring rolls, starched chicken, breaded beef cutlets, and breaded chicken cutlets, were deep-fried in fresh oil heated at 180°C between 8:45 and 15:00. Oil was cooled and filtered to obtain 140 g of dregs. Filtered oil was kept in a container overnight and used again on July 4 with 3 L of fresh oil added. Until July 7, frying was repeated in a similar manner. On July 10, deep-frying was started with 100% fresh oil. As the term-end examination period at the university started in the second week of July, the total number of fried products changed drastically and decreased gradually.

Changes in the COV and PC of frying oil sampled during the first week of July are shown in Fig. 3. From the second day on, frying oil was replenished with fresh oil so that concentrations of carbonyl compounds and polar compounds were diluted, resulting in sudden decreases of COV and PC. Although frying oil was used at 180°C for 6 h 15 min per day, COV increased by less than 1/day at the beginning of the week. On July 6 and 7, the increase became larger in spite of reduced production of fried foodstuffs. HMO, shown in Table 1, prepared by heating at 180°C at 10 h/day for two days without stirring, had a COV of 39.1, while, in Fig. 5, the stirred oil without any additions showed COV values of 10.8, 20.5, and 47.3 after 5 h, 10 h, and 20 h of heating, respectively. Thus, the increase of COV shown in Fig. 3 was obviously low. Macroscopically, COV and PC traced gradually increasing curves because the increase of each value was greater than the decrease by the replenishment of fresh oil. This proves the fact described in the introduction.

The result of oil extraction in batter coatings of breaded pork cutlets is shown in Table 2. Batter coatings of 80 g pork weighed 53-72 g and oil accounted for a quarter of the weight. The oil in batter coatings can contain lard exuded from pork in amounts up to 4 g, but frying oil nevertheless accounts for most of the oil.

PC and Gardner color were higher in oil from batter coatings than that from the fryer, as shown in Fig. 4. When frying foodstuffs full of water at ambient temperature are put in heated oil, numerous bubbles are formed, resulting in drastic oxidation of the oil due to great increases of oil surface. Much of the polar compounds thus formed probably could not diffuse but remained in the batter coatings. Phospholipids and sterols exuded from frying foodstuffs should be involved in the total amount of polar compounds.

The reason for oil browning during frying is attributed half to the amino-carbonyl reaction between amino acids and carbonyl compounds generated from oil. As a result of sudden heating, frying foodstuffs shrink and juice exuded from the tissue loses water vigorously. Most of the amino acids in the juice seem to have accumulated in the batter coatings and reacted with carbonyl compounds. As a result, the color of the oil in the batter coatings should deteriorate obviously, and some browning substances migrate into the oil in the fryer.

However, there were no remarkable differences in the TG and COV values of oil in batter coatings and in the fryer. Thus, it was confirmed that carbonyl compounds were not concentrated in batter coatings.

### 3.3 Effects of nutrients on COV

As shown in Fig. 5, the COV values of all the mixtures increased with time. Of amino acids added individually to oil at 500 ppm, Met inhibited the COV increase after 15 h of heating, and Gly, Leu, His, Ala, Val, and others accelerated it. Although Gly accelerated the increase, 7000 ppm of amino acid mixture composed of Gly, Thr and Tyr appeared to retard the increase up to 20 h of heating. However, 7000 ppm of free amino acids are not found often in oil used for deep-frying. Gluten and starch inhibited the increase of COV by 15-27% after 20 h. Protein denatures by reaction with oxidized oil, and the existence of a complex composed

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Fig. 4  Chemical Properties of Frying Oil in Batter Coatings and Fryer.

Fig. 5  Effects of Amino Acids, Protein, and Starch on COV of Oil Heated at 180°C. Numbers in graphs show actual COV of control oil (None).
of protein and oxidized oil has been proven. Matsuo et al.\textsuperscript{13,14} observed a rapid POV decrease when oxidized fatty acid esters having a POV of about 2000 mEq/kg were stirred with various kinds of amines. Kikugawa et al.\textsuperscript{15} described the characteristics of the fluorescent substances formed in the \textit{in vitro} reactions of lipid oxidation products and the mechanisms of their formation. One mechanism for the production of these pigments is the reaction of malonaldehyde and proteins, producing conjugated Schiff bases. Monofunctional aldehydes alone can produce cross-links in protein molecules. Sayre et al.\textsuperscript{16} reported that bifunctional carbonyl compounds such as 4-hydroxy-2-nonenal, 4-oxo-2-nonenal, and acrolein, generated from oxidation of polyunsaturated fatty acids, readily bound to protein nucleophiles, and that modification by bifunctional aldehydes can also lead to intramolecular or intermolecular protein crosslinking.

3.4 Effects of nutrients on PC

The amino acid mixture, gluten, and Met inhibited PC increase, but starch did not show an obvious contribution (Fig. 6). It is very interesting that gluten kept PC at a low level especially during up to 5 h of heating. This is probably because polar compounds were rapidly trapped by gluten as long as the amount of polar compounds remained small. In commercial frying, gluten is likely to contribute greatly to inhibition as there are frequent chances for gluten to be introduced into oil.

3.5 COV of oil vaporized during deep-frying

Heated oil used for deep-frying breaded pork cutlets and remaining in the pan had Gardner color 6.5, COV 4.3, POV 1.5, PC 8.7, and TG 96.0%. The COVs of oils extracted from the filter are shown in Fig. 7: 43.6 mg, 36.5 mg, and 46.0 mg of oils were recovered during the first, second, and third hours of heating. It was obvious that the amounts of these recovered oils were not whole oil vaporized from oil during deep-frying, as the inside of the cover was covered by vaporized oil and the operator smelled the oil fumes all through deep-frying. The filter appeared to hold intact tri-

![Fig. 6](image-url) Effects of Amino acids, Protein, and Starch on PC of Oil Heated at 180°C. Numbers in graphs show actual PC of control oil (None).
Low COV of Used Frying Oil

acylglycerol, water, water-soluble volatile compounds, lipophilic oxidized compounds such as carbonyl compounds, and others. As hexane was used for the extraction, water-soluble carbonyl compounds were not involved in the determination. The COV of oil remaining in the pan increased slowly, but that of extracts showed a sudden increase after 2 h of heating. As carbonyl compounds were almost absent in the uncooked fresh breaded pork cutlets, carbonyl compounds should have originated from the frying oil.

3.6 COV of oil vaporized during simple heating

After 1 h of heating, yellow substances vaporized from oil started to cover upper inside wall of the flask, and smell of oil vapor became irritant gradually. At the end of the heating, the inside wall was covered by brown viscous oil. Chemical properties of the brown oil recovered with ethanol and the oil in the flask are shown in Table 3; PC of the brown oil was not determined because of the small amount. The brown oil had an extremely high COV and other, a far higher COV than that of recovered oil, about 10, as shown in Table 1; it cannot be denied that some oxidation of the oil proceeded on the wall, but such a high COV as more than 500 seems to be hard for the oil to reach in 10 h of exposure to air. When the findings above is considered together with the result from 3-5, it is clear that carbonyl compounds formed during deep-frying can vaporize independently from oil. But steam, if any, accelerated the vaporization. Thus, it was suggested that most of the carbonyl compounds occurring in heated oil vaporized out vigorously from the oil, together with high-temperature steam generated from the water content of frying foodstuffs, in the manner of steam distillation, which enables compounds to vaporize at a lower temperature than their boiling points, resulting in a small increase of COV of the remaining oil.

4 CONCLUSION

The low levels of COV in recovered oil appeared to be attributable partly to inhibition by protein and amino acids forming complexes with carbonyl compounds and slightly to effects from natural antioxidants in fresh oil and frying foodstuffs, oil absorption by frying foodstuffs, and dilution of oil in use by fresh oil added between uses. But the biggest contribution to the low COV was judged to be vaporization of carbonyl compounds from the heated oil with steam coming from frying foodstuffs. As a result, frying oil used for deep-frying had a lower COV than oil simply heated under similar heating conditions did.

In addition, a-Toc, gluten, and Met had potential inhibition capability against polar compound formation during frying.

PC and color score were obviously worse in the oil extracted from batter coatings than in that from the fryer, but there was no difference in COV and TG. Juice exuded from foodstuffs caused active formation of polar compounds and browning substances.

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References


