Color Deterioration of Oil during Frying

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Abstract: The food industries in Japan ban used edible oil in the amount of some hundred thousand tons every year. The color deterioration of the oil used for frying in the industries, and recovered on a large scale, was investigated. Virgin frying oil, whose mineral content was adjusted to that of recovered oil, was spiked with several components of fried foods (separately and in combination), then heated at 180°C for up to 70 h. From the change in oil viscosity, the apparent heating time of recovered oil was judged to be 20 h. It was found that in practice, starch, proteins, sugar, and pigments had little to do with the deterioration, whereas the amino acids, especially Cys, Met, Trp and the oil itself contributed to the deterioration. These amino acids seem to be exuded from foodstuffs during frying. The level of minerals in the oil affected the deterioration and viscosity increase of oil itself, in rate as well as in degree although the deterioration by amino acids was not affected much by mineral content. In conclusion, it is suggested that color deterioration of frying oil used in the Japanese food industry is attributable to (i) the amino-carbonyl reaction between thermally oxidized oil and amino acids exuded by fried stuffs, and (ii) coloring of oil itself influenced much by mineral content.

Key words: color deterioration, amino acid, amino-carbonyl reaction, recovered oil, used edible oil

1 Introduction

Annually, 2.5 million tons of edible oil is used in Japan - and some hundred thousand tons are banned from use - in the food industry, supermarkets, restaurants and so forth (1). Banned oil of light color is recovered and reused for paint, ink, fodder, etc. after simple refinement, and as a fatty acid source after hydrolysis. However, more than half of this oil is either discarded directly to nature or burned, because of the absence of appropriate methods for refining in spite of relatively low levels of deterioration. In order to make effective use of natural resources and to reduce environmental pollution, our previous papers (2, 3) proposed a practical method to refine recovered oil having Gardner color 11-12 (as triacylglycerol) to oil of Gardner color 3-5, with no rancid smell; and demonstrated that effective bleaching of recovered oil with hydrogen peroxide needed barium hydroxide refinement of oil to remove nitrogen compounds in advance.

There have been several papers on thermal color deterioration of oil. Free fatty acid produced by hydrolysis of oil was reported to be one of the causes (4). Particles of fried stuffs sticking to the surface of heated pans were found to lead to overheating and were broken down to small particles as coloring substances (5).
Phospholipids exuded from fried stuffs were another candidate for browning (6, 7). Burkhardt et al. studied color problems in safflower oil (8). The amino-carbonyl reaction, the major pathway for nonenzymatic browning of foods, is well known. Pokorny et al. (9) reported that a 1:10 mixture of oxidized lipids/protein was discolored by storing at 60°C as carbonyl group generated in the lipids reacted with amino group of protein. Koga et al. observed that glycine, alanine, and leucine discolored frying oil in their model experiment (10).

In our experiments, molecular distillation of used frying oil gave a viscous, dark brown residue and a slightly colored initial fraction as well. The brown fractions were located near the origin of thin layer chromatograms developed by hexane/diethyl ether. Thus, coloring substances in recovered oil are likely polar, oil-soluble and/or high in molecular weight.

In the present paper we investigated how virgin frying oil develops a brown color during industrial use, and which component in fried stuffs controls the color deterioration of oil. When foodstuffs are fried, the relation between oil volume and quantity of fried matter, and whether the food is floured/crumbed, are important for obtaining a good finished product. In tempura, for example, three pieces of 50 g prawns are dipped in wheat flour plus water (10 g wheat flour per piece), and fried in 0.6 L oil at 180°C for 1 min (10). A breaded and fried pork cutlet (a Japanese favorite), is made by powdering a 150 g pork cutlet with 3 g wheat flour, coating with 10 g beaten egg by dipping, followed by 9-10 g bread crumbs, and finally, deep frying at 170°C for 3-4 min. In the food industry, continuous frying is carried out at a larger scale than in the home. It is not known how long the recovered oil described above was heated and how much food in total was fried in a batch. Besides, additional virgin oil is added during frying, as fried stuffs absorb 5-25 wt% oil (11). Gradually, the oil temperature does not go up to 180°C despite heating, because of the increase in low-boiling substances derived from oil and other sources. The oil starts to smoke, acid value (AV) increases, and foaming and smell cause a decline in the quality of fried stuffs. Finally, the oil is no longer usable, and is banned. Thus, recovered oil is a mixture composed of several oil portions variously exposed to heat and food stuffs for varying lengths of time.

2 Experimental

2·1 Materials

Five lots of “B grade recovered vegetable oil” (recovered oil; Miyoshi Oil & Fat Co., Ltd.), were collected during four different seasons for mineral content analysis, having AV 3-4; iodine value (IV) 113-118; water content 0.4-0.6%; Gardner color 11-12; and viscosity 65-75 mPa.s at 22°C. A representative fatty acid composition was reported in a previous paper (3): oleic and linoleic acids constituted about 80 wt% of the oil and highly unsaturated fatty acids, such as arachidonic acid, were not present at all. The chemicals and laboratory supplies used were: milk casein; gluten and starch from wheat; all the L-amino acids; potassium chloride; iron oxide black; calcium chloride dihydrate; sodium chloride; potassium dihydrogen phosphate; magnesium sulfate heptahydrate (Nacalai Tesque, Inc., Kyoto); egg albumin; bovine hemoglobin; β-carotene; sugar (Wako Pure Chemical Industries Ltd., Osaka); 99% L-α-phosphatidyl choline (Funakoshi, Tokyo); potato protein (Lysamine Bayer, Wuppertal-Barmen); soy protein (Ajinomoto, Tokyo); wheat flour; bread crumbs; and frying oil (Nisshin Flour Milling Inc., Tokyo).

2·2 Analyses

Carbonyl value (COV), AV, and Gardner color were measured according to Japan Oil Chemists’ Society standard methods for the analysis of fats, oils and related materials. The concentrations of sodium, phosphorus, iron, calcium, potassium, and magnesium in virgin and recovered oil were determined by atomic absorption spectrophotometry and ICP emission spectrophotometry. Viscosity was measured at 22°C. The content of polar compounds (PC) in oil was analyzed by a PC Tester (3M, USA). All experiments and measurements were done in triplicate.

2·3 Heating of Oils Having Different COV

5 g each of fresh virgin frying oils I (Gardner color 1, COV 4.3, AV 0.07, PC 4.2%) and II (Gardner color 1—, COV 4.4, AV 0.07, PC 4.2%) , and old virgin frying oil III (Gardner color 1—, COV 12.5, AV 0.76, PC 13.3%) , were poured in individual 15 mL tubes (i.d. 14 mm, length 100 mm) and heated at 180°C for 70 h with the top end open. Changes in color and viscosity were measured.
2.4 Effect of Minerals on Deterioration
To frying oil I were added 5.74 ppm potassium chloride, 2.07 ppm iron oxide black, and 36.8 ppm calcium chloride dihydrate, to make frying oil IA containing 15 ppm minerals in total. Separately, to frying oil I were added 200 ppm sodium chloride, 100 ppm potassium dihydrogen phosphate, 100 ppm iron oxide black, 50 ppm calcium chloride dihydrate, and 100 ppm magnesium sulfate heptahydrate, to make frying oil IB containing 225 ppm minerals in total. 5 g each of frying oils I, IA, and IB were also heated at 180°C for 70 h as above, and changes in color and viscosity were measured.

2.5 Effect of Lecithin on Oil Color Deterioration
Lecithin was chosen as an additive to estimate the effect of phospholipids contained in foodstuffs on deterioration. To 5 g each of frying oils I and IB were added 0, 250, 500, and 1,000 ppm lecithin respectively, and the oils were then heated at 180°C for 70 h as above. Changes in color and viscosity were again measured.

2.6 Substances Contributing to Color Deterioration
Bread crumbs; wheat flour; wheat starch; gluten; milk casein; egg albumin; soy protein; potato protein; bovine hemoglobin; 20 different amino acids; sugar; and β-carotene were each added to 5 g frying oil IA in the range of 100-100,000 ppm in 15 mL tubes, which were heated at 180°C for 40 h with the top end open. Changes in color and viscosity were measured as before. In addition, wheat flour; gluten; milk casein; egg albumin; and (gluten + sugar) respectively, were added to frying oil IA, mixed well with 100 μL water and heated in the same way.

3 Results and Discussion
3.1 Mineral Content of Recovered Oil
Recovered oil contains suspended brown particles presumably generated from denatured wheat flour, most of which precipitates when the oil is allowed to stand for some hours. The mineral content of this suspension, recovered oil supernatant and virgin oil is shown in Table 1. Virgin oil did not contain minerals in a detectable amount but the supernatant contained iron and potassium at the parts per million level. In the recovered oil suspension, some tens of ppm of minerals were detected. In this work iron (such as from surfaces of iron frying pans) was not studied for deterioration of oil. However, it is easily conjectured from the results above that elevated iron content induces rapid and intense color deterioration and a large viscosity increase of oil.

3.2 Effect of Carbonyl Group
The three virgin oils had similar Gardner color and viscosity, but frying oil III having COV 12.5 showed a rapid increase in color score within the first 20 h of heating, and in viscosity after 40 h although such highly oxidized virgin oil is not used for frying in Japan (Fig. 1). As recovered oil had viscosity below 75 mPa.s, it was conjectured that Japanese food manufacturers use frying oil apparently for 20 h at most.

3.3 Effect of Mineral Content
There was no difference in color deterioration and viscosity increase between oils with mineral content 0 and 15 ppm, but oil containing 225 ppm minerals showed rapid color deterioration after 10 h and a sudden increase in viscosity after 40 h (Fig. 2). Regardless of mineral content, the color score exceeded Gardner color 10 and the viscosity reached some hundred mPa.s after 40-70 h of heating. These results confirmed that recovered oil was different from frying oil that had simply been heated for a number of hours and that some other factors were needed for oil to reach properties of the recovered oil, Gardner color over 10 and viscosity below 75 mPa.s at the same time.

As mentioned above, the amount of frying oil in food factories decreases during continuous frying, so fresh oil is periodically added to maintain a certain oil vol-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mineral Content of Oils (mean ± SD).</th>
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<tbody>
<tr>
<td>Minerals</td>
<td>Frying oil I/II (ppm)</td>
</tr>
<tr>
<td>Na*</td>
<td>0</td>
</tr>
<tr>
<td>P**</td>
<td>0</td>
</tr>
<tr>
<td>Fe*</td>
<td>0</td>
</tr>
<tr>
<td>Ca**</td>
<td>0</td>
</tr>
<tr>
<td>K*</td>
<td>0</td>
</tr>
<tr>
<td>Mg*</td>
<td>0</td>
</tr>
</tbody>
</table>

* detection limit 1 ppm, ** detection limit 10 ppm.
ume. Meanwhile, the fried stuffs exude oil of their own, lipids, amino acids, proteins, starch, water, and so forth. Given an initial frying oil volume $A$, and that after $B$ h of frying, the volume decreases to $C$, then $(A-C)$ of virgin oil is added to continue frying. This addition is repeated $n$ times, until the oil is banned, resulting in the following equation for banned oil:

$$A(C/A)^{n+1} + (A-C) \cdot [C/A + (C/A)^2 + \cdots + (C/A)^n] = C$$

At the final stage (after final addition of $(A-C)$, oil is used for $B$ h and banned) initial portion of oil decreases to $A(C/A)^{n+1}$, and last added virgin oil, the most fresh portion, decreases to $(A-C)/C/A$, the second last added virgin oil to $(A-C)(C/A)^2$, and so on. Sum of all the portions makes $C$. For example, if $A = 20$ L, $B = 5$ h (frying time per day; virgin oil is added daily in the morning from the second day on), and $C = 17$ L, at the end of the 5th day the whole batch of oil is banned. From the equation above the following is calculated: 52.2% of oil is heated for 25 h and 38.6% for less than 15 h when banned. Another example is: $A = 200$ L, $B = 3$ h (addition of oil twice a day), $C = 180$ L, and on the end of the 10th day the oil is banned. In this case, 14% of the oil is heated 60 h and 65% for less than 30 h when banned. These two examples indicate that prolonged use of a batch of oil decreases the percentage of initial oil and that oil heated for a relatively short time constitutes the main part of oil.

### 3.4 Antioxidant Effect of Lecithin on Oil

It has been reported that lecithin contributes to the deterioration of oil color (6, 7) but we found no effects during up to 20 h of heating (Fig. 3). After 20 h, addition of 250 ppm lecithin inhibited deterioration, whereas 1000 ppm accelerated browning. Oil containing 500 ppm lecithin (12) was almost the same as oil without lecithin up to heating for 40 h, but the color score increased gradually after that. When frying oil IB containing lecithin in the same three concentrations was heated for 70 h, color deterioration was inhibited to about color score 2 in all samples, although the deterioration of the oil itself became drastic as shown in Fig. 2. Addition of lecithin did not influence oil viscosity before or after heating.

### 3.5 Major Coloring Substances

The results of heating frying oil IA containing various food components are summarized in Fig. 4. Amino acids were implicated in oil color deterioration. In Fig. 4, one of the most strongly coloring amino acids (Met) and one of the least (Glu) are indicated. Wheat starch and bread crumbs also contributed to coloring, but their effect was small even at levels over 300 times more than those of amino acids. In addition, it is hardly possible in practice for bread crumb or wheat flour scum to exceed 50,000 ppm in oil because this scum is scooped out of the oil from time to time. On the other hand, wheat flour contains 190 ppm free amino acids. If all of
Color Deterioration of Frying Oil

In the oil, 100 pieces of the tempura described above would have to be fried in 600 mL oil for it to reach 300 ppm amino acid, even assuming that tempura does not absorb oil at all. Because each piece of tempura absorbs 10 g oil on average, virgin oil has to be added during frying to cook, say, 100 pieces, and amino acids exuded in the oil are removed with the absorbed oil. Thus, it is plainly impossible for amino acid content in oil to reach 300 ppm solely from flour and bread crumbs.

Wheat flour contains 8 wt% gluten. Oil was browned to an extent similar to recovered oil by the addition of 20,000 ppm gluten, but such a high concentration of gluten in oil is even less likely than the case of wheat flour and bread crumbs discussed above.

Protein and water were added to frying oil and subjected to the thermal deterioration test. It was assumed that hydrolysis of protein would occur, but almost no difference was observed from when a combination of protein and oil was heated. Sugar and carbohydrate did not color oil much even though they browned or carbonized. As a combination of oil, gluten, sugar, and water did not differ in color from oil plus gluten under the testing conditions, it was concluded that sugar did not promote color deterioration. β-Carotene lost its color with increasing heating time, and hemoglobin did not contribute to coloring.

After 20 h and 40 h of heating, the viscosity of frying oil containing 300-100,000 ppm food component(s) described above rose to a little higher than 75 mPa·s and >130 mPa·s, respectively. From this information, it was also deduced that most recovered oil was heated only for about 20 h.

### 3.6 Amino Acid Browning

Table 2 shows that at 180℃, many amino acids browned frying oil to the level of recovered oil (Gardner color 11-12) within 5-20 h. In oil containing 15 ppm minerals, sulfur-containing amino acids, and structurally labile Trp produced strong browning. In addition, Gly, Ala, Val, Leu, Ile, Thr, Lys, Arg, Phe, and Pro browned oil containing 225 ppm minerals. Glu and Asp...
hardly browned oil. High mineral content tended to induce browning of oil in a shorter time and more intensively than low mineral content, but genuine color score increase by amino acid was not so different between the two mineral contents (see the color scores of oil itself for both mineral contents). Oxidation proceeds vigorously under high mineral content, and oxygen seems to attack unsaturated fatty acid moieties in triacylglycerols to brown oil, while amino acids react with already existing carbonyls first of all. The color generated by Trp in low mineral content oil faded slightly with heating time. Browning seems to be sum of many complex reactions of oil, amino acids, etc., all of which are influenced by heat, oxygen, and minerals.

In this work, COV was measured using 2, 4-dinitrophenylhydrazine. A COV of 10 obtained from the method corresponds to about 25 mmol/kg of carbonyl groups. Amino acid at a concentration of 300 ppm yields about 2 mmol/kg on average. Thus, all frying oils employed had considerably more carbonyl groups than amino acid added, suggesting that amino-carbonyl reactions can occur in the oil.

Our heating conditions were relatively close to that of the food industry: oil was heated 10 h a day at 180°C. However, we heated food components in oil separately and in combination, so our components did not come from fried stuffs. The surface of oil exposed to air (5) in each tube was 0.308 cm²/g, and the oil depth at 180°C was 4 cm, both reasonable values for the heating experiment. In practice, fried stuffs usually bubble in oil, so thermal oxidation and hydrolysis are likely to be very vigorous. Nonetheless, recovered oil had a rather low viscosity, 65-75 mPa-s at 22°C, and a low AV range of about 3-4. Thus, our estimate of heating time for recovered oil, 20 h, may be too long.

The nitrogen content of recovered oil has been found to be 800 ppm (3). If this reflects protein content, several thousand parts per million of protein should exist in the oil. However, most of the nitrogen seemed to be distributed in various deterioration products. Our results revealed that 300 ppm amino acid was enough for browning oil, that is, less than 4% of the 800 ppm nitrogen was involved in browning. In this study oligopeptides, which might contribute to the color deterioration, were not investigated because of difficult availability, but free amino acids should react with carbonyls more easily than oligopeptides.

Given that phosphorus content in recovered oil (Table 1) is attributed to lecithin, 800 ppm lecithin was present in the oil. Up to 20 h of heating lecithin did not cause browning in the range of 0-1,000ppm.

To reach Gardner color 11-12 in 20 h, only amino acids were implicated. It is germane to ask where these most strongly implicated coloring substances, i.e., amino acids, come from. As protein is not hydrolyzed easily in oil (plus water), it was assumed that fried stuffs exuded amino acids (13). Tempura cuisine and breading fried foods seem to be good culinary techniques to avoid both loss of tasty amino acids from meat and oil deterioration.

### Table 2  
Color Deterioration of Frying Oil Containing 300 ppm Amino Acid at 180°C.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Color (Gardner)</th>
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<tbody>
<tr>
<td></td>
<td>Frying oil I A *</td>
</tr>
<tr>
<td>Gly</td>
<td>6 + 10 13 13</td>
</tr>
<tr>
<td>Ala</td>
<td>6 8 13 13</td>
</tr>
<tr>
<td>Val</td>
<td>8 + 13 13</td>
</tr>
<tr>
<td>Leu</td>
<td>8 12 13</td>
</tr>
<tr>
<td>Ile</td>
<td>8 12 13</td>
</tr>
<tr>
<td>Ser</td>
<td>8 + 13 13</td>
</tr>
<tr>
<td>Thr</td>
<td>7 - 10 14</td>
</tr>
<tr>
<td>Cys</td>
<td>7 11 16 16</td>
</tr>
<tr>
<td>Met</td>
<td>8 13+ 16 16</td>
</tr>
<tr>
<td>Glu</td>
<td>7 10 10</td>
</tr>
<tr>
<td>Asp</td>
<td>7 - 10 10</td>
</tr>
<tr>
<td>Gln</td>
<td>6+ 7+ 12 12</td>
</tr>
<tr>
<td>Asn</td>
<td>7 12 12 12</td>
</tr>
<tr>
<td>Lys</td>
<td>6+ 7 14+ 14</td>
</tr>
<tr>
<td>Arg</td>
<td>7 12 12 12</td>
</tr>
<tr>
<td>Phe</td>
<td>9 - 10 12 12</td>
</tr>
<tr>
<td>Tyr</td>
<td>7 12 12 12</td>
</tr>
<tr>
<td>Trp</td>
<td>13- 13 12+ 12</td>
</tr>
<tr>
<td>His</td>
<td>6 12 12 12</td>
</tr>
<tr>
<td>Pro</td>
<td>7- 8 14 14</td>
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</tbody>
</table>

* Frying oil I added 15ppm minerals, ** Frying oil I added 225ppm minerals,
Acknowledgments

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References