Evaluation of Heat-Treated Frying Oils (Part V) Animal Study in Relation to Analytical Values of Heat-Treated Soybean Oils

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Synopsis

The objective of the study is to substantiate the meaning of P/AP values proposed previously[10] by showing correlations between the values and the observations made by animal feeding study.

Soybean oil was heated at 180°*C* by frying 2, 4 and 6 *kg* each of potatoes, chicken meat balls and sliced mackerel meat in 10, 20 and 30 *hr*, respectively; the oil samples are designated, respectively, as P-10, P-20, P-30, C-10, C-20, C-30, M-10, M-20 and M-30. A fairly good correlation was obtained between the amounts of the polar fraction formed by heating (determined by adsorption chromatography on a silica gel column) and P/AP values, r=0.79 at 233 nm and r=0.72 at 280 nm.

Male *Sprague-Dauley* rats, 7 animals/group, were fed each of the heated oils at a level of 10% for 35 - 50 days. The animals fed P-10 – P-20 and C-10 – C-30 showed a similar trend of growth retardation, whereas those fed M-10 and M-20 no retardation in an early stage of feeding and only those fed M-30 some retardation after 20 or more days of feeding. All the groups, except those fed M-10 and M-20, showed a significant difference ($p<0.05$) in body weight gains from the control group after 35 days. The P/AP values of P-10 – P-30 correlated well with the decreases in body weight gains of the animals fed the same oils, r=–0.75 at 233 nm and r=–0.72 at 280 nm. A significant difference ($P<0.05$) in liver weight/body weight was observed between the control group and the groups fed P-30, C-20 and M-30. The UV absorbance of the polar fraction of liver lipids (separated by TLC) from animals fed 20 *hr* and 30 *hr* heated oils, disregarding effects of food items used, was found to give significant differences of $p<0.05$ and <0.001 respectively, from that of the control group.

Discussions are given on deterioration of fats and oils in relation to yields of polar fractions and to P/AP values and also on comparison between the proposed method and those methods (GPC, LC and CC) recently examined by Billek et al.[5].

Introduction

The development of a simple and reliable method (or methods) of analysis to determine the point at which a batch of frying oil must be discarded has been awaited for more than a decade and of concern of those who are engaged in the control of the safe use of frying oils for human consumption. The subject matter has been reviewed by one of the authors[1] and recently by WALTKING et al.[2]. It may be said that a recent trend of research works

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with this respect is to measure directly concentrations of thermally oxidized products, some of which have been demonstrated to show toxicities in animals, by chromatographic means. A recent paper by ALEXANDER[3] includes a review on biological effects of oxidation products.

As one of simple analytical methods, FRITSCH et al.[4] have proposed recently the use of the so called "Food oil sensor", based on the measurement of dielectric constants of used fats. This method, however, require further studies as to establish its reliability. The German Society for Fat Research had made a recommendation in the quality assessment of used frying fats a level of 1.0% or more of petroleum ether-insoluble oxidized fatty acids (OFA) to augument sensory evaluation. Since the method of determination of OFA is time consuming, Billek et al. of the Unilever research laboratory, Hamberg, Germany,[5] conducted a survey for comparison of OFA values with the values obtained by gel permiation chromatography (GPC) developed by UNBEHEND et al.[6), 7] for the determination of plymeric triglycerides (PTG), by liquid chromatography (LC) developed by AITZETMÜLLER and GUHR[8] for the determination of total polor artefacts formed by heating, and by column chromatography (CC) developed by SEN GUPTA[9] for the determination of polor components. They reported that 1.0% of OFA corresponds to 15% (by peak area) of PTG, 28% (by peak area) of total artefacts, and 27% (by weight) of polor components but concluded that these methods are also time consuming and not very accurate in some cases of industrially used fats.

Previously, URAKAMI et al.[10] have proposed a simple method of determination of UV absorbance of the polor fraction (PF) separated by thin layer chromatography (TLC), instead of weighing the fraction as did by AITZETMÜLLER[8]. Since the ultimate objective of the present study is to provide a suggestion for a safe use of frying oils in kitchens or in small restaurants, heating was simulated to actual frying practice by frying various food items. In a previous study[10] amounts of food items used were so small to give distinct differences among food items and the characteristics of heated oils depended much more on freshness of oils subjected to heating and on heating time. Therefore, in the present study large amounts of food items were used to see effects of components depleted from foods on characteristics of heated oils as well as on animal feeding experiments. LANG and VON JAN[11] reported that there are no correlations between chemical data and observations made in feeding study. NOLEN et al.[12] conducted a long term feeding experiments with food fried fats. They showed yields of PF and distillable non-urea adductable fraction (DNUF) of the fats but made no attempts to correlate these data with the results of animal studies. Therefore, the present study was designed to find some relations between P/AP values[10] and observations made by animal experiments such as body weight gains, liver weight/body weight, and liver lipids.

Experimental

1. Heat-treated soybean oils

Foods used for frying were chicken meat balls with a 3 *cm* in diameter and potatoes (*Danshaku*) and mackerel meat (with skin) cut or sliced into a size of 1 × 1 × 6 *cm*. A batch of 400 *g* of each food item was fried in 4 portions in a 2 *hr* period in 1 *kg* of soybean oil (purchased on the market and a canned product of a well known maker in the Kanto area) heated at 180°*C*. Heating was continued for 10 *hr*/day, the oil was allowed to stand overnight in the frying pan, and heating was repeated untill a desired length of heating time was reached. The total amounts of each food item fried in 10, 20 and 30 *hr* were 2, 4 and 6 *kg*, respectively. The oil samples thus prepared were filtered and placed in colored bottles, each was blanketed

with nitrogen gas, and stored in a freezer untill needed. These oils will be designated as P-10, P-20 and P-30 for potato fried ones, C-10, C-20 and C-30 for chicken fried, and M-10, M-20 and M-30 for mackerel fried.

2. Determination of polor fraction and analytical method

A weighed amount (25 – 100 *g*) of a heated oil dissolved in benzene (30 – 50%) was adsorbed on 500 *g*. of Wako gel C-300 packed in a glass column (7 × 40 *cm*) and developed with benzene. The silica gel somewhat colored with PF near the top of the column was removed and extracted with chloroform (C)) : methanol (M) 2:1 (*v/v*). The extract was monitored by TLC using hexan: diethyl ether 93:7 as a developing solvent and by spraying an iodine solution. If the extract was found to be contaminated with an apolor fraction, the column chromatography was repeated. The extract was concentrated under reduced pressure passing a stream of nitrogen gas and the concentrated was weighed.

The following analytical methods were used; AV by the standard method[13], P/AP at 233 nm and 280 nm by the method described in a previous paper[10], refractive index and viscosity with an Abbe refractometer and an Emillia viscometer, respectively. The measurement of UV absorbance of the PF of liver lipids separated by TLC was carried out essentially in the same manner as for heated oils[10]. Caution was excercised not to overexpose chromatograms to open air.

The fatty acid (FA) composition of the heated oils was analyzed by gas chromatography (GC) (HITACHI MODEL 163-6052) and the following conditions were used: a stainless steel column, 3 *mm* × 2 *m*, packed with a 5% DEGS coated on Celite 545 (60–80 mesh); column and injection temperature 185°*C* and 250°*C*, respectively; and nitrogen and hydrogen gas flow rate 45 *ml/min* and 40 *ml/min*, respectively. The identification of FA in mackerel fried oils was made by comparing the data obtained with those reported for mackerel oil[14].

2. Liver lipids

The liver excised at the termination of animal experiments (see the succeeding section) was homogenized at 0°*C* in less than 45 *sec* in C:M 2:1 (*v/v*). The supernatant obtained after centrifugation was concentrated under reduced pressure passing a stream of nitrogen gas. The concentrate was purified on a small column (1 *cm* × 10 *cm*) of Sephadex G-25 fine, and the eluate was concentrated in the same manner as described above. The lipids thus obtained were analyzed by TLC as mentioned in the preceeding section.

4. Feeding study

The composition of the diet is shown in Table 1. The diet was prepared fresh weekly by incorporating fresh soybean oil (the product of Yoshihara Seiyu Co., Ltd. with AV 0.03 and IV 131.6) for the control group or heated oils.

Male albino rats (7 animals/group) of *Spraque-Dauley* strain weighing about 70 *g* (the fourth week after birth) were given the standard diet for 3 days previous to feeding the diets

Table 1 The composition of diet (%)

Sucrose	59.76
Casein	22.00
McCollum salt	4.00
Cellflour	3.00
Choline chloride	0.24
Vitamin mixture	1.00
Soybean oil*	10.00

* Fresh oil for the control group and the heated oils for the experimental animals.

containing the heated oils. The diets and water were given *ad libitum* and intake of the diets and their body weights were measured in every 2–3 days. The feeding of the potato and mackerel fried oils was continued for 35 days and that of the chicken fried oils for 35–50 days. At the end of the experimental periods the animals were fasted for 15 *hr*. before excising their livers under ether anesthesia. A few livers of each group were used for lipid extraction for measurement of UV absorbance of PF as described previously.

Results and Discussion

1. Characteristics of heat-treated oils

The canned soybean oil used for frying foods was not of high quality as indicated by its AV shown in Table 2. This accelerated thermal oxidation of the oil. POV and COV were

Table 2 Characteristics of heat-treated soybean oils

Food	Heating time (*hr*)	AV (S.E.)	cP 23°C	20 ^{n}D	P/AD 233 nm	P/AD 280 nm	PF weight(%)
Potato	0	1.15 (0.08)	60.5	1.4771	0.12	0.25	
	10	1.71 (0.03)	143.3	1.4801	1.85	2.42	10.3
	20	1.85 (0.18)	148.3	1.4802	2.06	2.51	10.9
	30	2.28 (0.29)	233.7	1.4819	2.34	3.91	14.6
Chicken	10	1.86 (0.17)	79.3	1.4774	1.08	1.23	5.4
	20	2.07 (0.12)	110.0	1.4782	2.45	1.88	13.7
	30	2.45 (0.12)	142.7	1.4782	1.62	2.30	15.2
Mackerel	10	1.59 (0.09)	63.1	1.4783	0.53	0.57	3.4
	20	1.81 (0.05)	69.3	1.4795	0.51	0.51	7.2
	30	1.98 (0.09)	74.1	1.4801	0.55	0.71	15.0

S.E. = the standard error of the mean.
PF = the polar fraction isolated by column chromatography.

not measured because they are known to vary by heat treatment. All the values shown in Table 2 increased by increasing heating time, except P/AP at 233 nm for C-30, which was however compensated by an increase at 280 nm. The mackerel fried oils showed distinctly smaller values compared with the others. This is attributed to depletion of considerable amounts of fish oil as oil volumes increased by 8, 20 and 40% in 10, 20 and 30 *hr* heating, respectively, and long chain FA were detected in these oils as shown in Table 4.

PF has been shown to contain acyclic and cyclic FA[15),16)] and some of them have been demonstrated to give adverse effects in animals and animal tissues[3)]. Furthermore, some of the voltatile compounds isolated from heated oils were toxic in nature[17)] and demonstrated to show some polarity on TLC plates[18)]. Thus, PF separated directly from heated oils by TLC contains both volatile and non-volatile toxic compound. The yields of PF shown in Table 2 differed with different food items used up to 20 *hr* of heating but reached a level of 15% after 30 *hr* of heating in all the cases. The low yields of PF with M-10 and M-20 may be considered to be the results of depletion of fish oil. Fairly good correlations were obtained between the yields of PF from the potato and chicken fried oils and their P/AP values, r=0.79 at 233 nm and r=0.72 at 280 nm. This suggests that the proposed method of determination of P/AP may well be used in place of weighing PF by LC[8)] or CC[9)].

The results obtained in the present study may be compared with those reported by NOLEN el al.[12)] to see how the yields of PF are affected by heating time and by nature of fats used

Table 3 Analytical results of used fats reported by Nolen et al.[12)]

Fat	Frying time (*hr.*)	FFA (%, weight)	PF (%, volume)	DNUA
SOH-108	60	0.65 (0.02)[a]	13.6 (14.6)[b]	2.1
SOH-108 + MS	216	8.10 (0.02)	30.2 (32.5)	2.0
SOH- 70	84	1.30 (0.03)	20.1 (19.0)	2.0
CSO	49	0.45 (0.03)	14.2 (15.2)	2.3
Lard	116	2.30 (0.05)	23.4 (25.5)	1.7

a: The values for the fresh fats.
b: After 2 years of storage.
SOH-108 and SOH-70 mean soybean oil hydrogenated to IV 108 and 70, respectively. + MS means 1.6 ppm of methyl silicone was added. CSO designates cottonseed oil. FFA designates free fatty acids and determined by AOCS method. PF means the polar fraction measured by adsorptpion chromatography on silicic acid containing 4% methanol (M); eluted with M:benzene 1:49. DNUA means the distillable non-urea-adductable fraction.

for frying. Part of their results are summarized in Table 3. They fried 3.95 *kg* each of frozen potatoes, breaded scallops, and onion ring twice a day (5 *hr* /day) in 60 *kg* of fats heated at 182°*C*, replacing the absorbed fats with 2.47 *kg* of fresh fat daily. The yields of PF from the 49 *hr* heated cottonseed oil and from the 60 *hr* heated HSO-108 correspond approximately to those levels obtained from the 30 *hr* heated oils (Table 2), where no fresh oil was added. This indicates that the former two withstand 1.6–2 times as long heating time as that for the latter. The calculation of the rates of formation of PF, supposing they remain constant all through heating times, shows that the rate in HSO-108 is 1.62 times that of HSO-108 with added methyl silicone and heating time of the latter may be prolonged to 70.46 *hr* to give the same level of PF from HSO-108 alone. The comparison of the rate between HSO-70 (0.239/*hr*) and lard (0.202/*hr*) shows that the former is 1.18 times greater than the latter: concequently, heating lard for 99.5 *hr* would give a level of 20.1% of PF, which corresponds to heating HSO-70 for 84 *hr*. It is interesting to note that the level of DNUA, which is often used for animal experiments to observe toxicities, remain constant in all the cases. Therefore, estimation of DNUA would not be of value as an effective analytical means.

The results of GC analysis given in Table 4 show that the proportions of linoleic and

Table 4 Fatty acid compositions[a)] of the heat-treated soybean oils

Oil	Fatty acid[b)]											
	14:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:1	22:1	20:5
0		9.4				4.0	26.0	53.6	7.1			
P-10		13.4				4.6	26.7	49.4	6.0			
P-20		13.4				5.3	27.5	48.1	5.8			
P-30		13.6				7.8	29.8	44.0	4.6			
C-10		12.1	0.9			4.4	28.8	47.6	6.3			
C-20	0.4	13.4	1.4			4.9	30.8	43.6	5.3			
C-30	0.4	15.7	1.9			5.5	33.3	38.7	4.4			
M-10	1.3	11.4	0.9	0.2		4.3	23.3	44.5	5.4	3.7	3.8	1.1
M-20	3.0	11.0	1.8	0.5		4.0	23.0	35.6	6.0	6.4	6.5	2.1
M-30	3.2	12.8	2.8	1.0	0.5	4.2	22.6	28.9	3.9	3.9	8.8	2.6

a) Calculated by area normalization of peak areas and an average of 3 or more determinations.
b) Designated by carbon number: the number of unsaturation. P-, C- and M- designate potato, chicken and mackerel, respectively and 10, 20 and 30 heating time in hr.

linolenic acid decreased in all the cases with concomitant increases in the proportions of oleic, stearic and palmitic acid, as generally observed in heated oils. The chicken fried oils show the presence of small amounts of myristic and palmitic acid and the mackerel fried oils that of heptadecanoic acid, heptadecenoic acid, and a number of long chain unsaturated FA in addition to those found in the chicken fried oils. These unsaturated FA affected P/PA values as discussed above and also the results of animal study as will be discussed in the succeeding seciton.

2. Feeding study

(1) Body weight gains and liver weights

It is well known that thermally oxidized fats give reduced body weight and increased liver weight in animals. The present study is to see correlations between these parameters and P/AP values or PF for heated oils.

Preliminary studies made earlier by feeding heat-treated oils and PF separated from them by silica gel CC on a large scale (eluted with benzene) showed no marked differences in animal growth curves. Thus, in the present sutdy the heated oils themselves were given to animals to elimate possible effects of benzene on the liver as benzene is known to give fatty livers.

Fig. 1 and 2 show the growth curves of the animals fed the diets containing the potato and chicken fired oils, respectively. Both are quite similar in that the retardation of growth begins

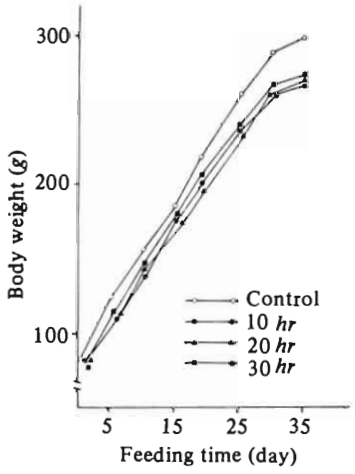

Fig. 1 Growth curves of animals fed the diet containing potato fried oils

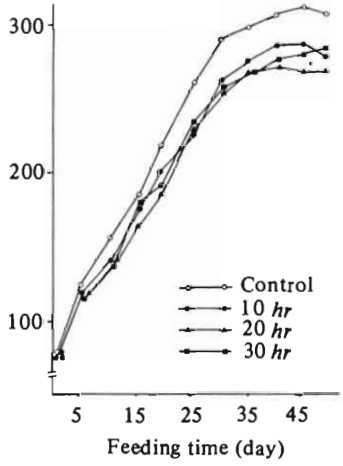

Fig. 2 Growth curves of animals fed the diet containing chicken fried oils

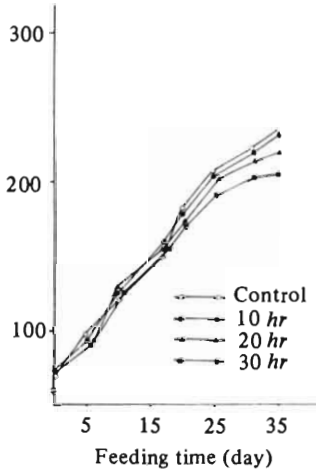

Fig. 3 Growth curves of animals fed the diet containing mackerel fried oils

after 5–10 days, a somewhat marked retardation after 30 days or more, and no differences depending on heating time up to 25 days. On the other hand, the growth curves of the animals fed the diet containing the mackerel fried oils as shown in Fig. 3 indicate no retardation in early days of feeding, except those given the 10 *hr* heated oil, which show no retardation all through the experimental period. It would be reasonable to assume that fish oil comprising of polyunsaturated FA present in the heated oils (Table 4) alleviated the growth retardation observed in the other cases (Fig. 1 and 2). The results of the latter (Fig. 3) may be considered to be reflected in the low values of P/AP and low yields of PF up to 20 *hr* of heating (Table 2).

Table 5 Result of feeding potato fried oils for 35 days

Oil	Body weight gains (g)	Leiver weight (g)	Liver weight/ Body weight (%)
Control	221.6±12.2	9.19±1.13	3.05±0.36
P-10	192.3±22.7[a)]	8.34±1.63	3.10±0.42
P-20	193.0±16.6[a)]	9.04±1.80	3.34±0.58
P-30	188.3±21.7[a)]	9.67±1.25	3.58±0.20[a), b)]

± designates S.E.. a) Significantly different from the control, $p<0.05$.
b) Significantly different from p-10, $p<0.05$. P-10, P-20 and P-30 mean heating time in hr.

Table 6 Result of feeding chicken fried oils for 35 and 50 days

Oil (days)		Body weight gain (g)	Liver weight (g)	Liver weight/Body weight (%)
Control	(35)	221.6±12.2		
	(50)	240.7	11.31±1.29	3.71±0.05
C-10	(35)	198.1±14.8[a)]		
	(50)	201.0±28.9	10.47±2.07	3.79±0.52
C-20	(35)	190.3±17.8[a)]		
	(50)	191.0±23.8	8.07±1.38	3.02±0.32[a), b), c)]
C-30	(35)	189.6±18.1[a)]		
	(50)	200.3±23.5	10.16±1.80	3.67±0.51

+designates S.E. a) Signifcantly different from the control, $p<0.05$. b) Significantly different from C-10, $p<0.05$. c) Significantly different from C-30, $p<0.05$. C-10, C-20 and C-30 mean heating time in hr.

Table 7 Result of feeding mackerel fried oils for 35 days

Oil	Body weight gain (g)	Liver weight (g)	Liver weight/Body weight (%)
Control	159.6±26.9	10.80±1.83	4.76±0.61
M-10	157.0±24.2	10.29±1.28	4.59±0.47
M-20	142.2±13.1	10.30±1.13	4.89±0.45
M-30	128.9±23.1[a)]	11.10±1.35	5.57±0.19[a), b), c)]

± designates S.E.. a) Significantly different from the control, $p<0.05$. b) Significantly different from M-10, $p<0.05$. c) Significantly different from M-20, $p<0.05$. M-10, M-20, and M-30 mean heating hr.

The results of body weight gains, liver weights and liver weight/body weight are shown in Table 5–7. A significant difference ($p<0.05$) was found between the control group and the groups fed p-10 ~ P-30, C-10 ~ C-30 (Table 5 and 6) and only the group fed M-30 (Table 7). Fairly good correlations were obtained between the decrease in body weight of animals fed the diet containing potato fried oils and P/AP of the same oils (Table 2), $r=-0.75$ at 233 nm and $r=-0.72$ at 280 nm. Similar calculations with the other cases gave $r \leqq -0.67$. A better correlation would be obtained, however, by increasing number of experiments and by limiting depletion of food components by breading or covering foods with flour paste as used in tempra (the Japanese style of fry).

A significant difference ($p<0.05$) was found in the liver weight/body weight between the control and the groups fed P-30, C-20 and M-30. The case of C-30 where no significant difference was found may be attributed to the presence of chicken fat in the oils fed (Table 4) in

an sufficient amount to mitigate the decreases in body weight observed in the other cases.

(2) Liver lipid

It has been shown by PERKINS et al.[19] that 44.5% of labeled linoleic acid in corn oil (randomly esterified with the acid) was converted to non-volatile oxidation products on heating the oil 24 *hr* at 200°*C* and the largest amount, 39.4%, of the radioactivity of the oil was incorporated into the polar lipids (chiefly phospholipids) of animal liver lipids. Recently, IWAOKA and PERKINS[20] have also shown the largest amount of incorporation of radioacitivity into the liver phospholipids in the sutdy of effects of cyclic monomers of methyl linolenate on the metabolism of acetate-1-^{14}C. PAULOSE and CHANG[21] and OHFUJI and KANEDA[22] isolated from heated oils toxic components that show absorbance neare 230 – 238 nm. Furthermore, PAIK et al.[23] reported that small molecular weight compounds contaninining carbonyl groups, which would show UV absorbance, were readily absorbed in animal organs and suspected of being involved in imparement of various organ tissues and mortality. Thus, a gross examination of the liver lipids was made in the same manner as described for the measurement of P/AP of heated oils. Since apolar fractions of liver lipids would be rather complicated by metabolic processes taking place in the tissue, only the absorbance of the PF was measured in the present study.

The results are shown in Table 8. Since only a few animals liver were examined, average increases in intensities of absorbance were calculated at each heating time, disregarding

Table 8 UV absorbance[a] of the polar fraction separated from liver lipid

	Heating time (*hr*)	Potato		Chicken		Mackerel		Average (S.E.)
		1	2	1	2	1	2	
233 mm	Control	75.5	108.6	82.6	108.6	108.6	120.9	100.8 (7.2)
	10	83.8	87.8	119.5	153.9		135.2	116.0 (13.5)
	20	125.0	128.2	171.5	97.3	126.4	142.9	131.8 (10.0)[b]
	30	165.2	225.8	212.2	168.3	132.3	166.0	178.3 (14.1)[c]
280 mm	Control	106.9	97.0	100.7	97.0	97.0	112.5	101.9 (2.7)
	10	93.7	98.5	134.3	161.7		132.3	124.1 (12.6)
	20	119.7	176.9	192.8		129.0	154.8	154.6 (13.8)[b]
	30	189.4	211.7	218.4	173.5	136.0	153.1	180.4 (13.3)[c]

a) Absorbance/*g*. lipids

b) Significantly different from the control, $p<0.05$.

c) Significantly different from the control, $p<0.001$.

effects of food items used. The values increased at both wavelengths depending on heating time. Significiant differences $p<0.05$ and $p<0.001$ were found between the control animals and those fed the 20 *hr* and 30 *hr* heated oils, respectively.

(3) Safe use of frying oils

Extensive works are in progress to demonstrate detrimental or toxic effects in animals and animal organs, respectively, by giving concentrates rich in toxic components or ^{14}C-labeled toxic compounds[3]. In comparison to these observation, the effects observed in the present study are considered to be very mild and alleviated or completely eliminated by giving animals a high protein diet[24], α-tocopherol[25),26], a diet containing cellulose[19], or simply by giving diet containing heated oils every 2 or 3 days[27]. However, it does not mean that excessively heated oils be allowed to use for human consumption on the ground that no detrimental effects have been observed in animals. The components formed in heated oils are considered to give chronic effects as do some of food contaminats. We must be aware

of the fact that more of fried foods are consumed by general public in conjunction with ingestion of food contaminants as well as food additives present in processed foods. Therefore, it would be necessary to set a safe margin beyond which heat-treated oils must not be used.

The German Society for Fat Research recommends that fats and oils containing 1.0% of OFA are to be called "deteriorated" and the Public Health of Hamberg, Germany, recommends that fish fried oils are to be replaced completely with fresh one once a week according to the results reported (no injurious effects to animals) by LANG and VON JAN[11]. The JAS in Japan recommends AV 1.8, which corresponds to 0.9% of FFA, for the control of oils used by markers of fried instant noodles. AV, COV or FFA (used in the USA) commonly used are not directly related to toxic component produced in heated fats and oils. OFA, on the other hand, may have some relation to toxic components but the method of its determination is time consuming as has been pointed out by BILLEK et al.[5] It would be necessary to standardize animal experiments, for example, animal species, number of animals to be used, an amount of heated oils to be incorporated in diet, and parameters to be observed in providing a safety margin by analytical means. The present study indicates that decreases in body weight of animals is more sensitive than effects that appear in the liver.

Another factor to be taken into consideration is foaming property of heated oils in setting a margin of safety to meet actual frying practice. It was observed in a previous work[28] as well as in the present study that foaming of soybean oil occured in 10 – 25 *hr* of heating without addition of fresh oil. Soybean oils foamed considerably after 20 *hr* of heating and the potato fied oil showed P/AP at 233 nm 2.06 and PF 10.9% and that of the chicken fried oil 2.45 and 13.7%, respectively (Table 2). As has been discussed earlier in section 1 and as shown in Table 3, where fresh fats were added daily, the heating time of HSO-108 was extended to 60 *hr* and that of the same oil with added methyl silicone to 70 *hr* to give about the same level of PF (12.6%) as in the cases of P-20 and C-20 (Table 2). The animal study showed a significant difference ($p<0.05$) between the control animals and those fed the 20 *hr* heated oils (potato and chicken fry) in body weight gain (Table 7 and 9) and in UV absorbance of PF of liver lipids (Table 10). If these analytical values are taken tentatively to indicate a margin of safety, it is far more mild than the one presently in use in Germany because 1.0% of OFA corresponds to 27% by weight of PF[5]. Thus, soybean oil (initial AV 0.03) which has been heat-treated for a total of 6.6 *hr* without adding fresh oil (11 items of use in a period of four months by frying food items 35 *min* at a time and having P/AP at 233 nm 1.80 and AV 0.76)[28] is considered to withstand further use.

A survey made on use oils collected from homes and makers of fried products[29] showed that P/AP values of the former were less than 1.0 (0.87 after 7 times of use) but some of the latter considerably high values. Several examples may be cited here to show no correlation between P/AP and AV:4.0 (AV 8.06), 2.43(1.78), 3.0(0.53) from makers of satsuma-age (fried fish paste); 2.33(1.25) from a restaurant; and 2.30(1.60) and 1.46(7.15) from makers of fried soybean curd.

In conclusion, it may be said that the measurement of P/AP is simple enough to be of practical value for evaluation of heat-treated oils and less time consuming (2 – 3 samples, 0.2 *mg* each, can be analyzed in 1 – 2 *hr*)[10] than those methods cited earlier, although further substantiation of the present results remains to be done by applying the technique to wide varieties of used oils.

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V 加熱劣化油の評価

加熱劣化油の分析値と毒性との関連性

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要 約

本実験の目的は，先に提唱した P/AP 値の意義を動物実験の知見との関連を示すことにより証明することである．

大豆油はポテト，鶏肉ダンゴ，鯖肉片を各々につき，2*kg*，4*kg*，6*kg*を10，20，30時間，180°*C*でフライしながら加熱した．：試料油は，P-10，P-20，P-30，C-10，C-20，C-30，M-10，M-20，M-30 とする．加熱により生成した極性部量（シリカゲルカラムの吸着クロマトグラフィーにより測定）と P/AP 値の間にはかなり良好な相関関係 r =0.79(233 nm)，r =0.72(280 nm)が得られた．

S.D.系ラット(７匹/群)を10％レベルの各試料油で35～50日間飼育した．P-10～P-30とC-10～C-30で飼育したものは同様な成長遅延の傾向がみられた．一方，M-10，M-20で飼育したものは飼育の初期段階ではなく，M-30で飼育したもののみ，20日以上の飼育で多少の遅延があった．M-10，M-20を除くすべての飼育群は35日後，体重増加について対照群と有意差（$P<0.05$）を示した．P-10～P-30の P/AP 値は同じ試料油で飼育したラットの体重増加の減少との間にかなりよい相関を示した． r =−0.75(233 nm)，r =−0.72(280 nm)．

肝臓重量／体重については対照群と P-30，C-20，M-30との間に有意差（$P< 0.05$)があった．種物の影響を無視して，20*hr*，30*hr*加熱の試料油で飼育したラットの肝脂質の極性部の紫外吸収は対照群との間に，各々 $P<0.05$，$P<0.001$の有意差を示した．極性部収量及び P/AP 値と油脂の劣化との関連について，また最近，Billek 等が検討した方法（ GPC LC，CC ）と，我々の提案した方法との比較について考察を行った．